Original article

Genomics and the future of psychopharmacology: MicroRNAs offer novel therapeutics

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MicroRNAs (miRNAs) are short, noncoding RNAs functioning as regulators of the transcription of protein-coding genes in eukaryotes. During the last two decades, studies on miRNAs indicate that they have potential as diagnostic and prognostic biomarkers for a wide range of cancers. Research interest in miRNAs has moved to embrace further medical disciplines, including neuropsychiatric disorders, comparing miRNA expression and mRNA targets between patient and control blood samples and postmortem brain tissues, as well as in animal models of neuropsychiatric disorders. This manuscript reviews recent findings on miRNAs implicated in the pathology of mood disorders, schizophrenia, and autism, as well as their diagnostic potential, and their potential as tentative targets for future therapeutics. The plausible contribution of X chromosome miRNAs to the larger prevalence of major depression among women is also evaluated.

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MicroRNAs: regulators of cellular communication

Since their discovery in the early 1990s, microRNAs (miRNAs), small (typically 21 nucleotides in length) noncoding RNAs, have become implicated as key posttranscriptional regulators of protein-coding RNAs in multicellular eukaryotes,[1] including fungi,[2] plants,[3] and animals.[4] A key feature of miRNAs is their high level of evolutionary conservation across species, compared with other noncoding RNAs, indicating their prominence for the physiology of multicellular organisms.[5] This evolutionary conservation has been instrumental for identifying miRNA-regulated genes and studying their contribution to human pathologies in animal models for human disorders.[6]

Among their many physiological functions, miRNAs are implicated in cell growth, differentiation, stress response and death,[7,8] apoptosis and autophagy,[9,10] cancer metastasis,[11] angiogenesis,[12] tissue repair,[13] and seemingly most physiological processes where their roles were explored. Besides cancer, where they are most often studied, miRNAs have been demonstrated to be implicated in the pathophysiology of insulin resistance and diabetes,[14,15] coronary artery disease,[16,17] stroke,[18,19] ischemic kidney injury,[20] and many further complex disorders.

In plants, miRNAs affect root physiology and, once released in extracellular vesicles, may even affect neighboring plants and fungi.[21] The idea that interspecies effects of miRNAs take place also in animals has been put forward as a hypoth-

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esis,[22] and preliminary evidence for their cross-species communication is emerging.[23,24] However, our understanding on cross-species effects of miRNAs is still sketchy and requires robust confirmation. Meanwhile, the concept that miRNAs function in intercellular communication between cells and tissues of animals has by now been established and exemplified by numerous studies. We now know that miRNAs shuffle in extracellular fluids, packaged inside small organelles, most notably inside exosomes.[25-27] Packaged in this manner, extracellular miRNAs are protected from exonucleases and may survive in body fluids for long periods.[28] Exosomal miRNAs are also present in human cerebrospinal fluid (CSF), and are suggested as biomarkers and/or drug targets in the context of neuropsychiatric disorders, such as for temporal lobe epilepsy.[29] Notably, neuronal secretion of exosome-packaged miRNAs is associated with neuronal depolarization,[30,31] as well as in cellular communication between neurons and glia.[32-34] A fine mouse model example of miRNA-facilitated glia-neuron communication is illustrated by findings that exosomal miR-124-3p secreted by microglial exosomes following traumatic brain injury inhibited neuronal inflammation and enhanced neurite outgrowth.[35]

MiRNAs seem to be promising drug targets for the development of future psychiatric therapeutics

Thus, in addition to being key regulators of gene transcription, miRNAs also function in tissue communication, in a manner somewhat reminiscent of the well-established mode of action of peptide and lipid hormones.[26,36] Exosomal miRNAs become active once they are taken up by host cells where they regulate mRNA translation into proteins.

MicroRNAs: regulators of neuroplasticity

A fascinating aspect of miRNAs is their emerging roles as regulators of brain neuroplasticity. Among the first clues for this role were observations that in neurons, miRNAs copurify with polyribosomes[37] and are located mostly in dendrites.[38] Further support for their role as regulators of neuroplasticity came from findings that both the induction of long-term potentiation (LTP) or long-term depression (LTD) regulates the expression of the same mouse hippocampal miRNAs with distinct expression dynamics for LTP or LTD.[39] Disruption of N-methyl-D-aspartate (NMDA) glutamate receptor signaling reduced the levels of a brain-specific miRNA, miR-219, in the mouse prefrontal cortex, and in vivo inhibition of miR-219 modulated behavioral responses of disrupted NMDA receptor transmission.[40] The Argonaute protein Ago2 associates with miRNAs for targeting specific mRNAs; rapid changes in Ago2 phosphorylation were observed following NMDA receptor stimulation, implicating miRNAs in NMDA-mediated dendritic spine morphogenesis.[41] Together, such findings elucidate the role of miRNAs in NMDA receptor-mediated changes in neuroplasticity and the dynamics of dendritic spines, the key mediators of learning in memory processes.[42]

Deficient neuroplasticity is recognized as a key underlying cause in the pathology of several neuropsychiatric disorders, in particular in mood disorders, schizophrenia, and autism spectrum disorder (ASD).[43] Thus, it is notable that aberrant regulation of brain miRNA transcription, as well as altered secretion of exosomal miRNAs implicated in neuron-glia communication, have been observed in these disorders. Below, examples for altered miRNA regulation in neuropsychiatric disorders are described, followed by discussion on the implications of such research findings for diagnostics and the development of future therapeutics addressing miRNA imbalance in these disorders. A review on miRNAs in addictive drug abuse is available for readers interested in additional coverage on miRNAs as regulators of neuroplasticity.[44]

MicroRNAs in major depressive disorder

Several miRNAs were suggested to be implicated in the etiology of major depressive disorder (MDD) and the response of patients to antidepressant therapeutics. Dysregulated serotonin signaling is considered as a major underlying factor in MDD, and selective serotonin reuptake inhibitors (SSRIs) have been, since the 1990s, the first-line MDD therapeutics. Thus, it is notable that miR-135, which showed upregulated expression in mouse raphe nuclei following administration of antidepressants,[45] was shown to regulate two mouse genes involved in serotonin signaling, the serotonin transporter (Slc6a4) and the serotonin 1a receptor (Htr1a). The same study reported lower expression levels of miR-135 in blood samples from depressed patients, with its levels being correlated with antidepressant drug efficacy.

Notably, miR-135, along with miR-191, was later shown to be required for the maintenance of NMDA receptor-dependent dendritic spine restructuring.[46] Among miRNAs reported as dysregulated in MDD, miR-1202 stands out as being a primate-specific miRNA (having no homologues in other mammals): lower levels of miR-1202 were found in both blood and postmortem brain tissues of MDD patients.[47] The same study showed that miR-1202 targets *GRM4*, encoding the metabotropic glutamate receptor-4, in agreement with the known implication of metabotropic glutamate signaling in MDD.

A genome-wide miRNA expression profiling study reported decreased expression of miR-221 and the nearly similar miR-222 following 21-day in vitro treatment of human cell lines with the SSRI drug paroxetine, while the expression levels of one of their target genes, *ITGB3*, were increased in the same paroxetine-treated cells.[48] In a subsequent study, the same team demonstrated the direct regulation of human *ITGB3* expression by miR-221, supporting the role of miR-221 in the response to SSRI drugs.[49] Indeed, elevated levels of miR-221 were observed in cerebrospinal fluid[50] and plasma samples[51] in independent studies of MDD patients. Lastly, a recent study reported on elevated miR-221 expression in the cerebrospinal fluid and serum of MDD patients and the hippocampus of mice exposed to chronic unpredictable mild stress. Moreover, miR-221 silencing by antisense oligonucleotides improved the behavioral symptoms of the chronic stress mouse model.[52] Thus, miR-221 appears to be a candidate target for future antidepressant drugs (see below in the section "Antagomirs as Psychiatric Therapeutics"). Both miR-221 and miR-222 genes are located on the human X chromosome; thus, these findings could be related to the higher prevalence of MDD in women (see below in "X chromosome miRNAs in neuropsychiatry").

MicroRNAs in bipolar disorder

Bipolar disorder (BD) is a common and disabling psychiatric disorder with a severe societal impact. The mood-stabilizing drug lithium is effective for reducing mania events in only about two thirds of patients, with many patients requiring a change in therapy during their treatment due to lack of efficacy or adverse events.[53] Compared with other common neuropsychiatric disorders, relatively little research has addressed the role of altered miRNA expression levels, or pre-miRNA mutations, in bipolar disorder. Indeed, genome-wide association studies on the genetics of bipolar disorder have largely been disappointing. Further research efforts and larger patient cohorts, ideally with longitudinal study designs that follow genome-wide gene expression during manic, depressive, and euthymic periods, are required for assessing genomic contribution to BD patients' well-being. Among the few published genome-wide association studies (GWAS) implicating miRNAs in BD etiology, it is notable that miR-137, discussed below as robustly associated with schizophrenia, was also found associated with BD.[54] In addition, a small study (29 BD and 29 control samples) reported elevated expression of miR-34a in postmortem cerebellar tissues from BD patients.[55] Among recent GWAS comparing lithium responder and nonresponder BD patients, a large study (N=2563 BD patients) did not identify polymorphisms in any protein-coding genes, or pre-miRNAs genes, as associated with clinical response to lithium medication. The only findings with genome-wide significance were in two long noncoding RNA genes (lncRNAs), AL157359.3 and AL157359.4, with unknown function.[56] These findings suggest that epigenomic effects, possibly including gut microbiome profiles and their consequent metabolome profiles, may have larger effects on BD clinical phenotypes rather than the common DNA polymorphic alleles. Indeed, a recent large human study demonstrated the robust effects of the gut microbiome and its associated plasma metabolome on quality of life and depression.[57] Such efforts should be helpful for addressing the complex epigenomics of further psychiatric disorders. In addition, DNA-sequencing studies, including DNA methylome and histone acetylation profiles, may yield better clues for the large phenotypic heterogeneity of bipolar disorder—a conclusion relevant for most neuropsychiatric disorders. A key issue to keep in mind is that, owing to their larger complexity, epigenomic studies with animal models may not be as informative for treating human neuropsychiatric disorders as GWAS or transcriptomic studies.

MicroRNAs in schizophrenia

While DNA polymorphisms in mature miRNA sequences are extremely rare (apparently being lethal during embryonic development), pre-miRNAs (pre-miRs) genes may harbor polymorphic alleles in sequences removed during the processing of pre-miRs to mature miRNA. Such noncoding pre-miR variants may modulate the posttranscriptional regulation of gene expression and thereby be associated with

diseases; for example, single nucleotide polymorphisms in pre-miR-27a, pre-miR-196a2, pre-miR-423, and pre-miR-618 were strongly associated with breast cancer risk.[58] In neuropsychiatric disease research, the miR-137 gene locus was associated with increased schizophrenia risk with genome-wide significance; moreover, miR-137 targets the genes *CSMD1*, *C10orf26*, *CACNA1C*, and *TCF4* which are among schizophrenia-associated risk genes.[59] Expressing noncoding variants of miR-137 schizophrenia-associated single nucleotide polymorphism alleles in human neurons led to increased miR-137 expression and gain-of-function, resulting in impaired vesicle release in vitro, along with deficits in hippocampus-dependent learning and memory in mice.[60] The same study demonstrated that sequestering endogenous miR-137 by a "sponge" construct (compared with an empty vector backbone as control) corrected the anomalous synaptic phenotypes induced by these miR-137 variants. Indeed, high plasma levels of miR-137 (along with two additional miRNAs, miR-22-3p, miR-92a-3p) were suggested as potential diagnostics for schizophrenia, while bioinformatic analyses of their target genes indicated their association with synaptic structure, function, and plasticity,[61] in agreement with the central role of altered neuroplasticity in schizophrenia. Altogether, over one hundred reports associating the miR-137 gene locus or altered miR-137 expression in schizophrenia were published. Indeed, this single miRNA gene seems to be among the most promising targets for future development of molecular schizophrenia therapeutics, owing to its capacity to regulate an entire gene network implicated in its etiology (see a comprehensive recent review on miR-137 in schizophrenia by Sakamoto and Crowley in ref 62). Nonetheless, searches of the US Patent Office website, as well as the ClinicalTrials.gov website (both performed in February 2019) did not identify any miRNA or anti-miRNA patent applications or clinical trials studies in the context of schizophrenia. Such clinical studies are likely to take place once genomic medicine therapeutics will become better established.

MicroRNAs in autism spectrum disorder

Autism spectrum disorder (ASD) is characterized by neurodevelopmental dysfunctions leading to social function deficits, stereotypy, and restrictive patterns of interest, which are central for its diagnosis, as well as several comorbidities such as high anxiety, poor intellectual capacities, and epilepsy. The majority of ASD cases seem to arise due to single nucleotide mutations, deletions, and copy number variants (CNVs) in ASD-risk genes arising de novo in gametes or very early during embryonic development, while only a minority are heritable. Moreover, epigenomics seems to play a key role in ASD, more than in other neuropsychiatric disorders. The genetic heterogeneity of ASD is immense, apparently surpassing any other human disorder, with hundreds of genes and thousands of common variants estimated to contribute to ASD, although the effect size of individual loci is small. This situation poses great challenges for prenatal and early postnatal ASD diagnosis, as well as for patient stratification for clinical trials and drug target identification for the development of future ASD therapeutics.[63] Nonetheless, many ASD-associated mutations have the shared effect of converging on biological pathways crucial for correct brain circuitry during embryonic and early postnatal development, including synaptic function, neuronal activity, neuroplasticity, neuronal cell adhesion proteins, and chromatin remodeling during neurogenesis.

A large genome-wide miRNA expression profiling in postmortem brains from individuals with ASD and controls identified several miRNAs, in particular higher expression levels of miR-21-3p (as well as miR-23a-3p, miR-103a-3p, miR-143-3p and additional miRNAs) in ASD.[64] Bioinformatics analysis of the latter study findings indicated that miR-21-3p, the second most upregulated miRNA in ASD postmortem cerebral cortex, is widely expressed in several human brain regions throughout early brain development and targets several protein-coding genes already known as implicated in ASD risk. Notable among these miR-21-3p regulated genes is *DLGAP1*, coding for a brain-specific protein localized at the post-synaptic densities of glutamatergic neurons, for which knockout mice exhibited altered postsynaptic densities and reduced sociability.[65]

Decreased levels of melatonin, a pineal gland hormone implicated in the circadian biological clock and synthesized mostly at night, along with elevated levels of the melatonin precursor N-acetylserotonin, were reported in individuals with ASD. In the context of the immense genetic heterogeneity of ASD, the commonly altered melatonin pathway may point toward tentative diagnostics and novel shared therapeutic pathways. In agreement with this notion, increased levels of miR-451, a miRNA targeting *ASMT*, the gene coding for acetylserotonin O-methyltransferase, the key enzyme in the melatonin synthesis pathway,

were found in a study of 239 ASD individuals compared with their first-degree relatives and age-matched controls.[66] Lastly, a recent GWAS found a miR-873-5p variant with reduced binding affinity for several ASD risk genes, as associated with ASD.[67] Considering the high genetic heterogeneity of ASD and the relatively small cohorts so far studied for miRNA expression levels in ASD, larger studies are required for validation of these miRNAs as putative ASD biomarkers. Eventually, blood or saliva expression data for several miRNAs and mRNAs may need to be integrated into a single reliable prediction tool for establishing the much-needed ASD diagnostics and prognostics for children with developmental delays.

X-chromosome miRNAs and neuropsychiatric disorders

A large sex bias has been well-established for several autoimmune disorders, in particular rheumatoid arthritis and systemic lupus erythematosus, that predominantly affects women. Several dysregulated miRNAs were suggested as associated with the higher prevalence of rheumatoid arthritis among women.[68] Likewise, the prevalence of MDD, the most common psychiatric disorder, is higher among women compared with men. Depression is nearly twice more common among women, and this sex bias is even higher among adolescents.[69] Differential levels of sex hormones are among key suspects for this sex bias of MDD[70]; stress associated with modern urban lifestyles, and, in particular, expectations of women to lead independent careers while raising children, is another contributing factor.[71] Yet there could also be a genetic contribution to this sex bias: women (like all mammals) have two X chromosomes, compared with a single X chromosome in men. One of the female X chromosomes is silenced very early during embryo development by a process known as X chromosome inactivation (XCI); this process assures X chromosome gene dosage compensation in female mammals. This silencing process is imperfect, requiring the silencing machinery to function throughout life, with some genes "escaping" XCI. Indeed, a study measuring the extent of escape from XCI in fibroblasts from healthy women according to their relative expression levels of heterozygous X chromosome alleles estimated that about 15% of X chromosome genes escaped XCI to some degree, with additional 10% of genes showing variable patterns of inactivation.[72] Genes escaping XCI may include X chromosome miRNAs. For example, five X chromosome miRNAs were suggested as being implicated in lupus,[73] an autoimmune disorder also more prevalent among women (and often comorbid with MDD).

The key regulator of XCI is the long-noncoding *XIST* gene, which initiates chromosomal silencing at one of the two X-inactivation centers on the X chromosome. The mechanism by which one X chromosome is selected for silencing remains unclear, while a recent study showed that this process coincides with genome dynamics and the onset of global regulatory programs during early differentiation.[74] It could well be that dysregulated XCI is implicated in the higher prevalence of some disorders among women. It has been hypothesized that elevated expression of X chromosome miRNAs due to escape from XCI may (at least partially) explain the sex bias of disease burden.[75] Indeed, miR-221 and miR-222, discussed above in the context of MDD, as well as in rheumatoid arthritis, are both located on the human X chromosome (at Xp11.3). Of note, the human X chromosome has higher miRNA density compared with the average for human autosomes, a situation shared with other mammals (but not with non-mammal vertebrates).[76] *Supplementary Table I* (in the online version of this article) lists 117 miRNA genes on the human X chromosome (according to the NCBI Gene server [https://www.ncbi.nlm.nih.gov/gene/; February 2019]).

Antagomirs as psychiatric therapeutics?

The direct silencing of specific miRNA using carrier-conjugated oligonucleotides antisense nucleotide sequences, termed "antagomirs," is emerging as a promising research tool, with a potential to lead to novel therapeutics. A fine example in the context of brain disorders is a study demonstrating the outcome of in vivo silencing of miR-134 in a mouse model of temporal lobe epilepsy.[77] In this study mice received intraventricular injection of locked nucleic acid 3′ cholesterol-conjugated antagomirs targeting miR-134 (Ant-134) resulting in significant knockdown of hippocampal miR-134 within 12 h post injection, remaining below control levels for at least 1 month, and recovering to control levels after 2 months. Brains from mice injected with this antagomir displayed normal gross anatomy with no evidence of hippocampal neuronal death, while confocal microscopy indicated reduced hippocampal CA3 spine density. Seizures evoked by intra-amygdala kainite injections 24 h following Ant-134 injections were reduced, supporting its therapeutic

anti-epileptic potential. Another example for the therapeutic potential of antagomirs in brain disorders is illustrated by a study demonstrating protective effects of intravenous injection of antagomiR-1 on a rat brain ischemia model. Rats injected IV with antagomiR-1 followed by middle cerebral artery occlusion and examined 24 hours later showed fewer neurologic deficits, smaller infarct volumes, reduced brain edema, and lower BBB permeability compared with saline control mice.[78] Together, such studies indicate that in vivo antagomir injections, in some cases even IV injections, have the potential to be developed as neuropsychiatric disorder therapeutics. Still lacking is knowledge about long-term effects of such antagomir treatments; yet, given the low in vivo stability of injected oligonucleotides, one can be optimistic that only minor long-term effects, if any, are expected in long-term follow-up studies. At the time of writing this review (February 2019) no antagomir studies in animal models of schizophrenia or ASD had been published. Hopefully in the not-too-distant future, antagomir-137 oligonucleotides will be studied in schizophrenia mouse models, and possibly developed as novel schizophrenia therapeutics. The potential of such therapeutics, if proven safe, given the unique capacity of miRNAs to control entire gene pathways, seems to be promising.[79] Developing antagomirs as neuropsychiatric therapeutics will take many years for assuring their safety, but appears to be worth the effort.

Conclusions

Recent research findings indicate that the expression levels of certain miRNAs deserve further exploration as tentative diagnostic biomarkers for neuropsychiatric disorders including major depressive disorder, bipolar disorder, schizophrenia, and autism spectrum disorder. Moreover, miRNAs seem to be promising drug targets for the development of future psychiatric therapeutics. Understanding the role of dysregulated miRNAs in aberrant neuronal signaling, neuron-glia communication, and neuroplasticity in such disorders will allow earlier and more accurate diagnosis as well as improved precision medicine for affected individuals. ■

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References

1. Ambros V. MicroRNAs: tiny regulators with great potential. *Cell.* 2001;107(7):823-826.
2. Lee HC, Li L, Gu W, et al. Diverse pathways generate microRNA-like RNAs and Dicer-independent small interfering RNAs in fungi. *Mol Cell.* 2010;38(6):803-814.
3. Hake S. MicroRNAs: a role in plant development. *Curr Biol.* 2003;13(21):851-852.
4. McManus MT, Sharp PA. Gene silencing in mammals by small interfering RNAs. *Nat Rev Genet.* 2002;3(10):737-747.
5. Yates LA, Norbury CJ, Gilbert RJ. The long and short of microRNA. *Cell.* 2013;153(3):516-519.
6. Artzi S, Kiezun A, Shomron N. miRNAminer: a tool for homologous microRNA gene search. *BMC Bioinformatics.* 2008;9:39.
7. Ambros V. MicroRNA pathways in flies and worms: growth, death, fat, stress, and timing. *Cell.* 2003;113(6):673-676.
8. Baehrecke EH. miRNAs: micro managers of programmed cell death. *Curr Biol.* 2003;13(12):R473-475.
9. Xu J, Wang Y, Tan X, Jing H. MicroRNAs in autophagy and their emerging roles in crosstalk with apoptosis. *Autophagy.* 2012;8(6):873-882.
10. Su Z, Yang Z, Xu Y, Chen Y, Yu Q. MicroRNAs in apoptosis, autophagy and necroptosis. *Oncotarget.* 2015;6(11):8474-8490.
11. Gilam A, Conde J, Weissglas-Volkov D, et al. Local microRNA delivery targets Palladin and prevents metastatic breast cancer. *Nat Commun.* 2016;7:12868.
12. Icli B, Wara AK, Moslehi J, et al. MicroRNA-26a regulates pathological and physiological angiogenesis by targeting BMP/SMAD1 signaling. *Circ Res.* 2013;113(11):1231-1241.
13. Icli B, Wu W, Ozdemir D, et al. MicroRNA-135a-3p regulates angiogenesis and tissue repair by targeting p38 signaling in endothelial cells. *Faseb J.* 2019:fj201802063RR.
14. Latreille M, Hausser J, Stutzer I, et al. MicroRNA-7a regulates pancreatic beta cell function. *J Clin Invest.* 2014;124(6):2722-2735.
15. Fu X, Dong B, Tian Y, et al. MicroRNA-26a regulates insulin sensitivity and metabolism of glucose and lipids. *J Clin Invest.* 2015;125(6):2497-2509.
16. Jansen F, Schafer L, Wang H, et al. Kinetics of circulating microRNAs in response to cardiac stress in patients with coronary artery disease. *J Am Heart Assoc.* 2017;6(8). doi:10.1161/JAHA.116.005270.
17. Kanuri SH, Ipe J, Kassab K, et al. Next generation MicroRNA sequencing to identify coronary artery disease patients at risk of recurrent myocardial infarction. *Atherosclerosis.* 2018;278:232-239.
18. Wu J, Fan CL, Ma LJ, et al. Distinctive expression signatures of serum microRNAs in ischaemic stroke and transient ischaemic attack patients. *Thromb Haemost.* 2017;117(5):992-1001.
19. Ma Q, Li G, Tao Z, et al. Blood microRNA-93 as an indicator for diagnosis and prediction of functional recovery of acute stroke patients. *J Clin Neurosci.* 2018;62:121-127.
20. Wei Q, Sun H, Song S, et al. MicroRNA-668 represses MTP18 to preserve mitochondrial dynamics in ischemic acute kidney injury. *J Clin Invest.* 2018;128(12):5448-5464.
21. Pandey P, Wang M, Baldwin IT, Pandey SP, Groten K. Complex regulation of microRNAs in roots of competitively-grown isogenic Nicotiana attenuata plants with different capacities to interact with arbuscular mycorrhizal fungi. *BMC Genomics.* 2018;19(1):937.
22. Igaz I, Igaz P. Possible role for microRNAs as inter-species mediators of epigenetic information in disease pathogenesis: is the non-coding dark matter of the genome responsible for epigenetic interindividual or interspecies communication? *Med Hypotheses.* 2015;84(2):150-154.
23. Perge P, Nagy Z, Decmann A, Igaz I, Igaz P. Potential relevance of microRNAs in inter-species epigenetic communication, and implications for disease pathogenesis. *RNA Biol.* 2017;14(4):391-401.

24. Liang H, Zen K, Zhang J, Zhang CY, Chen X. New roles for microRNAs in cross-species communication. *RNA Biol.* 2013;10(3):367-370.
25. Villarroya-Beltri C, Gutierrez-Vazquez C, Sanchez-Cabo F, et al. Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs. *Nat Commun.* 2013;4:2980.
26. Gurwitz D. Exosomal microRNAs in tissue crosstalk. *Drug Dev Res.* 2015;76(6):259-262.
27. Kalluri R. The biology and function of exosomes in cancer. *J Clin Invest.* 2016;126(4): 1208-1215.
28. Rayner KJ, Hennessy EJ. Extracellular communication via microRNA: lipid particles have a new message. *J Lipid Res.* 2013;54(5):1174-1181.
29. Raoof R, Jimenez-Mateos EM, Bauer S, et al. Cerebrospinal fluid microRNAs are potential biomarkers of temporal lobe epilepsy and status epilepticus. *Sci Rep.* 2017;7(1):3328.
30. Goldie BJ, Dun MD, Lin M, et al. Activity-associated miRNA are packaged in Map1b-enriched exosomes released from depolarized neurons. *Nucleic Acids Res.* 2014;42(14):9195-9208.
31. Chivet M, Javalet C, Laulagnier K, Blot B, Hemming FJ, Sadoul R. Exosomes secreted by cortical neurons upon glutamatergic synapse activation specifically interact with neurons. *J Extracell Vesicles.* 2014;3:24722.
32. Bahrini I, Song JH, Diez D, Hanayama R. Neuronal exosomes facilitate synaptic pruning by up-regulating complement factors in microglia. *Sci Rep.* 2015;5:7989.
33. Wang S, Cesca F, Loers G, et al. Synapsin I is an oligomannose-carrying glycoprotein, acts as an oligomannose-binding lectin, and promotes neurite outgrowth and neuronal survival when released via glia-derived exosomes. *J Neurosci.* 2011; 31(20):7275-7290.
34. Fruhbeis C, Frohlich D, Kramer-Albers EM. Emerging roles of exosomes in neuron-glia communication. *Front Physiol.* 2012;3:119.
35. Huang S, Ge X, Yu J, et al. Increased miR-124-3p in microglial exosomes following traumatic brain injury inhibits neuronal inflammation and contributes to neurite outgrowth via their transfer into neurons. *Faseb J.* 2018;32(1):512-528.
36. Mirra P, Raciti GA, Nigro C, et al. Circulating miRNAs as intercellular messengers, potential biomarkers and therapeutic targets for Type 2 diabetes. *Epigenomics.* 2015;7(4):653-667.
37. Kim J, Krichevsky A, Grad Y, et al. Identification of many microRNAs that copurify with polyribosomes in mammalian neurons. *Proc Natl Acad Sci U S A.* 2004;101(1):360-365.
38. Kye MJ, Liu T, Levy SF, et al. Somatodendritic microRNAs identified by laser capture and multiplex RT-PCR. *Rna.* 2007;13(8):1224-1234.
39. Park CS, Tang SJ. Regulation of microRNA expression by induction of bidirectional synaptic plasticity. *J Mol Neurosci.* 2009;38(1):50-56.
40. Kocerha J, Faghihi MA, Lopez-Toledano MA, et al. MicroRNA-219 modulates NMDA receptor-mediated neurobehavioral dysfunction. *Proc Natl Acad Sci U S A.* 2009;106(9):3507-3512.
41. Rajgor D, Sanderson TM, Amici M, Collingridge GL, Hanley JG. NMDAR-dependent Argonaute 2 phosphorylation regulates miRNA activity and dendritic spine plasticity. *Embo J.* 2018;37(11).
42. Attardo A, Fitzgerald JE, Schnitzer MJ. Impermanence of dendritic spines in live adult CA1 hippocampus. *Nature.* 2015;523(7562):592-596.
43. Goto Y, Yang CR, Otani S. Functional and dysfunctional synaptic plasticity in prefrontal cortex: roles in psychiatric disorders. *Biol Psychiatry.* 2010;67(3):199-207.
44. Kenny PJ. Epigenetics, microRNA, and addiction. *Dialogues Clin Neurosci.* 2014;16(3): 335-344.
45. Issler O, Haramati S, Paul ED, et al. MicroRNA 135 is essential for chronic stress resiliency, antidepressant efficacy, and intact serotonergic activity. *Neuron.* 2014;83(2):344-360.
46. Hu Z, Yu D, Gu QH, et al. miR-191 and miR-135 are required for long-lasting spine remodelling associated with synaptic long-term depression. *Nat Commun.* 2014;5:3263.
47. Lopez JP, Lim R, Cruceanu C, et al. miR-1202 is a primate-specific and brain-enriched microRNA involved in major depression and antidepressant treatment. *Nat Med.* 2014;20(7):764-768.
48. Oved K, Morag A, Pasmanik-Chor M, Rehavi M, Shomron N, Gurwitz D. Genome-wide expression profiling of human lymphoblastoid cell lines implicates integrin beta-3 in the mode of action of antidepressants. *Transl Psychiatry.* 2013;3:e313.
49. Oved K, Farberov L, Gilam A, et al. MicroRNA-mediated regulation of ITGB3 and CHL1 is implicated in SSRI action. *Front Mol Neurosci.* 2017;10:355.
50. Wan Y, Liu Y, Wang X, et al. Identification of differential microRNAs in cerebrospinal fluid and serum of patients with major depressive disorder. *PLoS One.* 2015;10(3):e0121975.
51. Enatescu VR, Papava I, Enatescu I, et al. Circulating plasma micro RNAs in patients with major depressive disorder treated with antidepressants: a pilot study. *Psychiatry Investig.* 2016; 13(5):549-557.
52. Lian N, Niu Q, Lei Y, Li X, Li Y, Song X. MiR-221 is involved in depression by regulating Wnt2/CREB/BDNF axis in hippocampal neurons. *Cell Cycle.* 2018:1-11.
53. Papiol S, Schulze TG, Alda M. Genetics of lithium response in bipolar disorder. *Pharmacopsychiatry.* 2018;51(5):206-211.
54. Duan J, Shi J, Fiorentino A, et al. A rare functional noncoding variant at the GWAS-implicated MIR137/MIR2682 locus might confer risk to schizophrenia and bipolar disorder. *Am J Hum Genet.* 2014;95(6):744-753.
55. Bavamian S, Mellios N, Lalonde J, et al. Dysregulation of miR-34a links neuronal development to genetic risk factors for bipolar disorder. *Mol Psychiatry.* 2015;20(5):573-584.
56. Hou L, Heilbronner U, Degenhardt F, et al. Genetic variants associated with response to lithium treatment in bipolar disorder: a genome-wide association study. *Lancet.* 2016;387(10023): 1085-1093.
57. Valles-Colomer M, Falony G, Darzi Y, et al. The neuroactive potential of the human gut microbiota in quality of life and depression. *Nat Microbiol.* In press. doi:10.1038/s41564-018-0337-x.
58. Morales S, Gulppi F, Gonzalez-Hormazabal P, et al. Association of single nucleotide polymorphisms in Pre-miR-27a, Pre-miR-196a2, Pre-miR-423, miR-608 and Pre-miR-618 with breast cancer susceptibility in a South American population. *BMC Genet.* 2016;17(1):109.
59. Kwon E, Wang W, Tsai LH. Validation of schizophrenia-associated genes CSMD1, C10orf26, CACNA1C and TCF4 as miR-137 targets. *Mol Psychiatry.* 2013;18(1):11-12.
60. Siegert S, Seo J, Kwon EJ, et al. The schizophrenia risk gene product miR-137 alters presynaptic plasticity. *Nat Neurosci.* 2015;18(7):1008-1016.
61. Ma J, Shang S, Wang J, et al. Identification of miR-22-3p, miR-92a-3p, and miR-137 in peripheral blood as biomarker for schizophrenia. *Psychiatry Res.* 2018;265:70-76.
62. Sakamoto K, Crowley JJ. A comprehensive review of the genetic and biological evidence supports a role for MicroRNA-137 in the etiology of schizophrenia. *Am J Med Genet B Neuropsychiatr Genet.* 2018;177(2):242-256.
63. Fernandez BA, Scherer SW. Syndromic autism spectrum disorders: moving from a clinically defined to a molecularly defined approach. *Dialogues Clin Neurosci.* 2017;19(4):353-371.
64. Wu YE, Parikshak NN, Belgard TG, Geschwind DH. Genome-wide, integrative analysis implicates microRNA dysregulation in autism spectrum disorder. *Nat Neurosci.* 2016;19(11): 1463-1476.
65. Coba MP, Ramaker MJ, Ho EV, et al. Dlgap1 knockout mice exhibit alterations of the postsynaptic density and selective reductions in sociability. *Sci Rep.* 2018;8(1):2281.
66. Pagan C, Goubran-Botros H, Delorme R, et al. Disruption of melatonin synthesis is associated with impaired 14-3-3 and miR-451 levels in patients with autism spectrum disorders. *Sci Rep.* 2017; 7(1):2096.
67. Williams SM, An JY, Edson J, et al. An integrative analysis of non-coding regulatory DNA variations associated with autism spectrum disorder. *Mol Psychiatry.* In press. doi:10.1038/s41380-018-0049-x.
68. Khalifa O, Pers YM, Ferreira R, et al. X-Linked miRNAs Associated with gender differences in rheumatoid arthritis. *Int J Mol Sci.* 2016;17(11). pii: E1852.
69. Sullivan PF, Neale MC, Kendler KS. Genetic epidemiology of major depression: review and

meta-analysis. *Am J Psychiatry.* 2000;157(10): 1552-1562.
70. Chhibber A, Woody SK, Karim Rumi MA, Soares MJ, Zhao L. Estrogen receptor beta deficiency impairs BDNF-5-HT2A signaling in the hippocampus of female brain: A possible mechanism for menopausal depression. *Psychoneuroendocrinology.* 2017;82:107-116.
71. Beutel ME, Brahler E, Wiltink J, et al. New onset of depression in aging women and men: contributions of social, psychological, behavioral, and somatic predictors in the community. *Psychol Med.* 2018:1-8.
72. Carrel L, Willard HF. X-inactivation profile reveals extensive variability in X-linked gene expression in females. *Nature.* 2005;434(7031): 400-404.
73. Hewagama A, Gorelik G, Patel D, et al. Overexpression of X-linked genes in T cells from women with lupus. *J Autoimmun.* 2013;41:60-71.
74. Pollex T, Heard E. Nuclear positioning and pairing of X-chromosome inactivation centers are not primary determinants during initiation of random X-inactivation. *Nat Genet.* 2019;51(2):285-295.
75. Pinheiro I, Dejager L, Libert C. X-chromosome-located microRNAs in immunity: might they explain male/female differences? The X chromosome-genomic context may affect X-located miRNAs and downstream signaling, thereby contributing to the enhanced immune response of females. *Bioessays.* 2011;33(11):791-802.
76. Guo X, Su B, Zhou Z, Sha J. Rapid evolution of mammalian X-linked testis microRNAs. *BMC Genomics.* 2009;10:97.
77. Jimenez-Mateos EM, Engel T, Merino-Serrais P, et al. Silencing microRNA-134 produces neuroprotective and prolonged seizure-suppressive effects. *Nat Med.* 2012;18(7):1087-1094.
78. Talebi A, Rahnema M, Bigdeli MR. Effect of intravenous injection of antagomiR-1 on brain ischemia. *Mol Biol Rep.* doi:10.1007/s11033-018-04580-y.
79. Issler O, Chen A. Determining the role of microRNAs in psychiatric disorders. *Nat Rev Neurosci.* 2015;16(4):201-212.