The Promise of Predictive Biomarkers for Antipsychotic Efficacy: A Review of Peripheral microRNAs to Evaluate Schizophrenia Treatment Response

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ABSTRACT

With the ongoing evolution in genetics, recent evidence highlights the role of circulatory microRNA (miRNA) for schizophrenia. The objective of this article is to explore the role of blood/serum miRNA expression in schizophrenia management and to review the expression of different miRNAs before and after treatment with antipsychotics. miRNAs can help increase the accuracy of diagnosis, identify patients at risk of developing schizophrenia, and possibly predict drug response. The collective evidence from this review showed that several miRNAs are promising candidates for schizophrenia diagnosis, management, and prognosis.

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Schizophrenia is a clinical syndrome that involves a range of cognitive, behavioral, and emotional dysfunctions, which vary significantly among patients.[1] Schizophrenia is one of the leading causes of disability worldwide.[2] The median incidence of schizophrenia is 1.5 per 10,000 people, and the rate ratio for males: females is 1.4:1.[3] While the global prevalence of schizophrenia has been reported to range from 0.33% to 0.75%, in the United States the estimated prevalence of schizophrenia and related psychotic disorders ranges between 0.25% and 0.64%.[3,4] The age at onset is typically early to mid-20s for males and the late 20s for females, with slightly more males than females developing the disorder.[1,3] Genetics is shown to have a strong influence in the development of schizophrenia. First-degree relatives of a schizophrenia patient have an increased risk of approximately 10% compared to 3% in second-degree relatives.[5] With the etiology and pathogenesis still evolving and under investigation, the diagnosis of this complex disease at present is mainly based on clinical symptoms. Schizophrenia patients may experience delusions, hallucinations, disorganized speech, grossly disorganized or catatonic behavior, or negative symptoms. According to the *DSM-5* diagnostic criteria for schizophrenia, at least 2 of these symptoms must occur for 6 months, with active symptoms exhibiting for at least 1 month.[1] It is a clinical diagnosis that depends primarily on self-reports from patients, collateral information from family members, mental state examination, and clinical interviews. Due to the lack of objective laboratory tests, misdiagnosis is common.[6]

Standard laboratory tests are not yet available to diagnose psychiatric disorders, as most can be attributable to poor understanding of their biological mechanisms or difficulty in routinely accessing brain tissue in live diseased patients.[7,8] The lack of tests highlights the role of biological markers, which can aid in the understanding of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.[9] Biomarkers have been shown to help researchers observe the disease's biological state, can be measured accurately, and have reproducible results.[10]

Several research studies[10–17] have shown the important role that microRNA (miRNA) plays in various human diseases, and growing evidence has shown that miRNAs are valuable biomarkers in disease pathogenesis, diagnosis, treatment efficacy, and prognosis prediction. The objective of this article is to review and summarize the most important findings of experimental investigations aimed at identifying the role of human miRNAs in schizophrenia management. We also aimed to review the expression of different miRNAs before and after treatment with

Clinical Points

- Several miRNAs are promising candidate biomarkers for schizophrenia diagnosis, management, and drug response.
- Schizophrenia diagnosis might be enhanced by use of peripheral miRNAs as biomarkers.
- In rare situations, use of peripheral miRNAs as biomarkers can expedite the diagnosis, allowing for quicker intervention, particularly in rural areas with few mental health resources.

antipsychotics, which could serve as a biomarker and help in determination of therapeutic intervention measures and pharmacologic targets.

We searched peer-reviewed published articles using PubMed, PubMed Central, and MEDLINE databases. A wide range of key terms were used, such as *blood*, *miRNA*, *schizophrenia*, *biomarkers*, and *antipsychotics*. The search process uncovered 8 peer-reviewed articles published from 2010 to 2020 with a single filter that included only human studies (Table 1).[10–17] We excluded articles that did not mention miRNAs associated with schizophrenia and alteration of expression with antipsychotics, which is the topic of interest.

miRNAs AND SCHIZOPHRENIA

After introduction in the early 1990s, miRNAs became a special focus due to their unique functions and mechanism of action. miRNAs are present in various tissues and body fluids such as blood, cerebrospinal fluid, urine, and saliva.[18] So far, more than 2,500 human miRNAs are reported in miRBase version 22.[19] miRNAs are a class of short noncoding RNAs consisting of 20 to 22 nucleotides in length. They undergo a series of steps within the nucleus to form primary miRNA transcribed by RNA polymerase II, which is further processed by Drosha (RNase III enzyme), ultimately creating pre-miRNA. This pre-miRNA is transported to the cytoplasm and undergoes further processing to form a miRNA duplex containing mature miRNA by exportin-5, where it encounters a further transformation by Dicer (RNase III protein). It then changes into a miRNA duplex that includes the mature miRNA.[20] After unwinding from this duplex, the mature single-stranded miRNA is incorporated into a multiprotein complex RNA-induced silencing complex, wherein it assumes the job of inducing the silencing of messenger RNAs (mRNAs).[21] A single miRNA can regulate the expression of more than one messenger RNA, and each messenger RNA can be regulated by multiple miRNAs.[22]

Approximately ~50% of all protein-coding genes are regulated by miRNA, and studies[23] suggest that they contribute to the regulation of nearly all cellular processes. The mutations of underlying miRNA biogenesis are associated with the pathophysiology of neuropsychiatric disorders like schizophrenia.[24] Researchers have noticed neuropathologic differences in the miRNA expression in postmortem studies of schizophrenia.[25] In contrast to other blood molecules, miRNAs have several unique properties, such as they are cell or tissue specific and steady in the RNase-rich blood environment, are stable in extremes of pH, and can sustain prolonged storage at room temperature and have freeze-thaw cycles.[26,27] Peripheral miRNAs are easy to access through noninvasive and straightforward processes and are readily identified by quantitative reverse transcription-polymerase chain reaction (qRT-PCR).[28] Use of peripheral miRNA as biomarkers could help improve the diagnosis of schizophrenia. In some cases, miRNAs can also fast track the diagnosis, which allows earlier interventions, especially in remote areas where access to mental health services is limited.[15]

ALTERATION IN miRNA BIOMARKER EXPRESSION WITH ANTIPSYCHOTICS

Antipsychotics are the medication of choice in managing the symptoms of schizophrenia, and depending on the symptoms, it could be either typical/first-generation or atypical/second-generation antipsychotics.[29] The world's first antipsychotic, chlorpromazine, was introduced into clinical practice in France in 1952.[30] Thereafter, atypical/second-generation antipsychotics were introduced in 1990 in the United States, and since then atypical antipsychotics have been used as first-line treatment for schizophrenia because of their safety side effect profile, which includes decreased extrapyramidal symptoms and increased efficacy on negative symptoms.[29] Few studies were conducted to understand the effect of antipsychotic medications on miRNAs before and after taking them in patients with schizophrenia.

To discover the role of miRNAs as diagnostic and prognostic biomarkers, Wei et al[15] used Solexa sequencing, TaqMan Low-Density Assay to profile circulating miRNAs in plasma (n = 351, scz = 164, ctl = 187). They observed 8 upregulated miRNAs, and on validation (n = 775, scz = 400 ctl = 213, non-scz disorder = 162) with qRT-PCR, they found upregulation of miR-130b and miR-193a-3p in patients with schizophrenia compared to control subjects. They also observed suppressed levels of miR-130b and miR-193a-3p on follow-up after 1 year of treatment with antipsychotics.[15]

To understand the role of miRNA as a therapeutic marker in schizophrenia, a study[10] (n = 235, scz = 105, ctl = 130) using microarray and qPCR found decreased expression of miR-132, miR-134, miR-1271, miR-664(∗), miR-200c, and miR-432 levels in patients with schizophrenia before taking antipsychotics compared to control subjects (Table 2). The study also found increased expression of miR-132 ($P < .01$), miR-664(∗) ($P < .05$), and miR-1271 ($P < .05$) after treatment compared to the expression level prior to taking medications.[10]

Chen et al[16] used microarray analysis on blood mononuclear cells from 125 schizophrenia patients prior to taking any antipsychotics and from control subjects and found 9 miRNAs with differential expression on validation

Table 1. Characteristics of Studies on microRNAs (miRNAs)

Study	Conclusion
Shi et al (2012)[11]	miR-181b, miR-219–2-3p, miR-346, miR-195, miR-1308, miR-92a, miR-17, miR-103, and let-7g are the key players to reflect schizophrenia illness status and may serve as candidate biomarkers for diagnosis of schizophrenia. This study also found that the risperidone significantly improved the serum miR-346 level of schizophrenia and thus may not be an effective drug in regulating the serum miR-346 level of schizophrenia.
Liu et al (2013)[12]	Compared with baseline, the expression levels of miR-365 and miR-520c-3p were significantly downregulated after 1 year of risperidone treatment ($P < .001$). There were no significant correlations between the clinical symptoms and the expression levels of these 2 miRNAs ($P > .05$).
Song et al (2014)[13]	miRNA-181b, miRNA-30e, miRNA-34a, and miRNA-7 are probably involved in the pathogenesis of schizophrenia, and the significant downregulation of miRNA-181b expression predicts improvement of negative symptoms to treatment and thus can serve as a potential plasma molecular marker for antipsychotic responses.
Sun et.al (2015)[14]	miR-30e, miR-181b, miR-34a, miR-346, and miR-7 combined as a panel are potentially useful noninvasive biomarkers for schizophrenia diagnosis. Markers miR-132, miR-181b, miR-30e, and miR-432 are potential indicators for symptomatology improvements, treatment response, and prognosis for schizophrenia patients.
Yu et al (2015)[10]	After antipsychotic treatment, there was a marked increase in miR-132, miR-664(*), and miR-1271 levels in schizophrenia patients compared with the levels before treatment. miR-132 is a potential and superior biomarker in peripheral blood that will allow discrimination of schizophrenia patients from healthy controls.
Wei et al (2015)[15]	The upregulation of miR-130b and miR-193a-3p is a state-independent biomarker for schizophrenia, and these 2 miRNAs could be used to develop a diagnostic tool for schizophrenia.
Chen et al (2016)[16]	Serum miR-21 expression strikingly decreased in patients after antipsychotic treatment. The change of miR-21 expression was negatively correlated with improvement of positive, general psychopathology, and aggressiveness symptoms.
Liu et al (2017)[17]	Antipsychotic treatment resulted in the elevation of EGR1 and miR-30a-5p but the reduction of NEUROD1. Receiver-operating characteristic analysis showed that the EGR1-miR-30a-5p-NEUROD1 axis possessed significantly greater diagnostic value than miR-30a-5p alone. The data suggest EGR1-miR-30a-5p-NEUROD1 axis might serve as a promising biomarker for diagnosis and treatment monitoring for those patients in an acute psychotic state.

Abbreviations: EGR1 = early growth response protein 1, NEUROD1 = neurogenic differentiation factor 1.

Table 2. Summary of microRNAs (miRNAs) Differentially Expressed Before and After Taking Antipsychotics

miRNA	Before Treatment	After Treatment
miR-132	Downregulated	Upregulated
miR-664	Downregulated	Upregulated
miR-1271	Downregulated	Upregulated
miR-132	Upregulated	Downregulated
miR-181b	Upregulated	Downregulated
miR-432	Upregulated	Downregulated
miR-30e	Upregulated	Downregulated
miRNA-21	Upregulated	Downregulated
miR-30a-5p	Downregulated	Upregulated
miR-346	Upregulated	Upregulated
miR-365	Upregulated	Downregulated
miR-520c-3p	Upregulated	Downregulated

by qRT-PCR (scz = 82, ctl = 43). When these 9 miRNAs were compared in patients before and after taking antipsychotics, they found miRNA-21 decreased expression and negatively associated clinical symptomatology.[16] On the other hand, in a study by Gardiner et al,[31] miRNA-21 was found to be significantly downregulated in patients with schizophrenia compared to control subjects. Also, in a study by Miller et al,[32] miRNA-132 was significantly downregulated in the prefrontal cortices of patients with schizophrenia compared to controls.

Liu et al[17] witnessed significant downregulation of transcription factor (early growth response protein 1 [EGR1]) and miR-30a-5p and upregulation of neurogenic differentiation factor 1 (NEUROD1) using RT-qPCR in peripheral blood mononuclear cells in schizophrenia patients (n = 38) compared to control subjects (n = 50). However, patients who underwent treatment with antipsychotics had increased expression of EGR1 and miR-30a-5p and decreased expression of transcription factor NEUROD1.

To explore the effect of antipsychotics on miRNAs, Sun et al[14] analyzed 10 miRNAs in plasma by qPCR in 148 subjects (scz = 61, ctl = 62, 6-week drug t/t group = 25) and observed significantly increased expression (area under the curve: 0.713, sensitivity: 35.5%, specificity: 90.2%) of a cluster of miRNAs such as miR-30e, miR-181b, miR-34a, miR-346,

and miR-7. In the treatment group, they found notably decreased expression levels of miR-132, miR-181b, miR-432, and miR-30e, which were associated with improved symptoms in the patients.[14]

On the other hand, Song et al[13] observed significant downregulation of miR-181b after treatment (n = 40, scz = 20, ctl = 20) ($P < .05$) compared to the level before treatment. This change was significantly correlated to symptomatic improvement ($P < .001$).[13]

Shi et al[11] quantified 9 miRNAs using qRT-PCR and semi-nested qRT-PCR and found the expression levels of miR-181b, miR-219–2-3p, miR-1308, let-7g, miR-346, and miR-92a were considerably higher in schizophrenia patients compared to healthy controls. Also, the expression levels of miR-195 and miR-17 were significantly downregulated in schizophrenia patients compared to healthy controls. In comparison, the expression level of miR-346 was significantly higher in the risperidone treatment group than in healthy controls.[11] Gardiner et al[31] showed the downregulation of miR-181b in peripheral blood mononuclear cells of patients with schizophrenia compared to control subjects, which is inconsistent with Shi et al,[11] Song et al,[13] and Sun et al.[14]

Liu et al[17] quantified expression of 7 miRNAs in plasma using qRT-PCR in patients with schizophrenia before and after taking antipsychotics (n = 40). They[17] observed decreased expression levels of miR-365 and miR-520c-3p after antipsychotic treatment ($P < .001$), but there was no correlation with the symptoms ($P > .05$). Overall, these research studies have acknowledged that the peripheral miRNA patterns would have value as biomarkers for schizophrenia disorder.

Although the results of these studies are promising, one of the limitations of our review is the sample size of the studies we included. Large-sample, longitudinal studies are needed in this field.

In summary, miR-664, miR-1271, and miR-30a-5p were downregulated in patients with schizophrenia before they started taking antipsychotics and upregulated after the intake of antipsychotic medications. In contrast, miRNAs such as miR-181b, miR-432, miR-30e, miRNA-21, miR-346, miR-365, and miR-520c-3p were upregulated in patients with schizophrenia before taking antipsychotics and downregulated after intake of antipsychotics. Yu et al[10] found that miR-132 was downregulated before antipsychotics intake and upregulated after antipsychotics in patients with schizophrenia, which contrasts with Miller et al whose results were opposite.[32] This expression of different miRNAs before and after treatment with antipsychotics could serve as a biomarker and help in determination of therapeutic intervention measures and pharmacologic targets.

CONCLUSION

Although the role of miRNAs in the diagnosis, management, and prognosis of schizophrenia is not yet understood, collective evidence from this review shows that several miRNAs are promising candidate biomarkers in this regard. They can help increase the accuracy of diagnosis, identify patients at risk of developing schizophrenia, and possibly predict drug response. Further large-scale controlled clinical studies are required to determine the sensitivity and specificity of biomarkers, which could be a turning point for schizophrenia management.

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