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Original Research Article

Plasma miRNAs as potential biomarkers for schizophrenia in a Jordanian cohort

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ABSTRACT

Background: Schizophrenia (SZ), a complex and chronic neuropsychiatric disorder affecting approximately 1 % of the general population, presents diagnostic challenges due to the absence of reliable biomarkers, and relying mainly on clinical observations. MicroRNAs (miRNAs) signatures in a wide range of diseases, including psychiatric disorders, hold immense potential for serving as biomarkers. This study aimed to analyze the expression levels of specific microRNAs (miRNAs) namely miR-29b-3p, miR-106b-5p, and miR-199a-3p and explore their diagnostic potential for SZ in Jordanian patients.

Methods: Small RNAs (miRNAs) were extracted from plasma samples of 30 SZ patients and 35 healthy controls. RNA was reverse transcribed and quantified by real-time polymerase chain reaction (qRT-PCR). The expression levels of three miRNAs (miR-29b-3p, miR-106b-5p and miR-199a-3p) were analyzed. Receiver operating characteristic (ROC) curves analysis was performed to evaluate diagnostic value of these miRNAs. Target genes prediction, functional enrichment and pathway analyses were done using miRWalk and Metascape. STRING database was used to construct protein-protein network and identify hub genes.

Results: Notably, miR-106b-5p and miR-199a-3p were significantly upregulated ($p < 0.0001$), while miRNA-29b-3p was downregulated ($p < 0.0001$) in SZ patients compared to controls. The diagnostic potential was assessed through ROC curves, revealing substantial diagnostic value for miR-199a-3p (AUC: 0.979) followed by miR-106b-5p (AUC: 0.774), with limited diagnostic efficacy for miR-29b-3p. Additionally, bioinformatic analyses for the predicted target genes of the diagnostically significant miRNAs uncovered Gene Ontology (GO) terms related to neurological development, including morphogenesis, which is involved in neuron differentiation, brain development, head development, and neuron projection morphogenesis. These findings highlight a potential connection between the identified miRNAs and SZ pathophysiology in the studied Jordanian population. Furthermore, a protein-protein interaction network from the target genes identified in association with neurological development in the Gene Ontology (GO) terms deepens our comprehension of the molecular landscape of the regulated target genes.

Conclusions: This comprehensive exploration highlights the promising role of miRNAs in unraveling intricate molecular pathways associated with SZ in the Jordanian cohort and suggests that plasma miRNAs could serve as reliable biomarkers for SZ diagnosis and disease progression. Remarkably, this study represents the first investigation into the role of circulating miRNA expression among Jordanian patients with SZ, providing valuable insights into the diagnostic landscape of this disorder.

1. Introduction

Schizophrenia (SZ) is one of the most severe, complex mental disorders [1]. SZ is a brain dysfunction that can stimulate cognitive, emotional, mental, and behavioral disorders, which lead to problems in the patient's social life, work performance, self-interest, and usually causes comorbid diseases [2]. About 1~1.5 % of the world population with different ethnicities is affected by SZ, which accounts for around 20

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million people around the world [3]. Schizophrenic patients generally suffer from various clinical manifestations that are classified into positive, negative, and cognitive. Positive symptoms include delusions, disorganized speech, and hallucinations, while negative symptoms involve flat affect and speech defect, and cognitive symptoms such as working memory, impairments in attention, and executive functions [4]. These symptoms can have a negative impact on patients' functional independence and usually lead to social and occupational dysfunction.

SZ is considered a heritable disorder with an estimated heritability rate of approximately 80 %. However, there are more than 270 loci that have been reported so far to be associated with SZ, there is no single gene can explain the cause of the disease [4,5]. Like other psychiatric disorders, SZ is a multifactorial disorder, which involves the interplay between genetic, epigenetic, and environmental factors [6,7]. Although the diagnosis of SZ depends on the patient's signs and symptoms [7–9], nowadays scientists have focused on epigenetic markers such as such as DNA methylation, histone modification, and altered miRNAs expression that help in early SZ diagnosis [10,11].

MicroRNAs (miRNAs) are a type of small, (22–25 nucleotides), endogenous, and noncoding single-stranded RNAs that post-transcriptionally regulate the expression of targeted genes [12,13]. They can inhibit the expression of specific genes by promoting messenger RNAs (mRNAs) degradation or preventing their translation into proteins [14].

MicroRNAs are predominantly expressed in the nervous system, where they play a crucial role in the brain's development, function, and overall homeostasis [15,16]. Altered expression of miRNAs has been well documented and linked to various neuropsychiatric disorders, including Alzheimer's disease, major depressive disorder (MDD), bipolar disorder (BD), and SZ [15,17–21]. In the last decade, plenty of studies on miRNA have led to increasing interest is using miRNAs as valuable biomarkers for the diagnosis of human diseases, which may pave the way of developing miRNA-based therapies for the treatment of a variety of human conditions as well as prognosis prediction [22,23]. In the context of SZ, several studies have shown promising results about using miRNAs for therapeutic purposes as diagnostic and prognostic biomarkers [24–26].

In this study, we focus on three miRNAs (miR-29b-3p, miR-106b-5p, and miR-199a-3p) to find a possible association with SZ. These miRNAs were previously studied in postmortem brain tissues and blood. MiR-199a-3p and miR106b-5p are known to be involved in the CNS differentiation and proliferation processes [27–29], and miR-29b has a protective function in neurons and is important in brain maturation regulation [30]. Alteration of the expression of these miRNAs have been linked with neurodevelopmental and psychotic disorders including SZ [17,31–36]. Both miR-106b-5p and miR199a-3p were found to upregulated in SZ patients compared to controls [27,32,37–39], whereas miRNA29b-3p was found to be negatively correlated with ZS [32].

Based on numerous studies demonstrating the importance and distinct roles of aforementioned miRNAs in psychiatric disorders, we aimed to investigate the expression profiles of miR-29b-3p, miR-106b-5p, and miR-199a-3a in the plasma of SZ patients and healthy controls and whither they can be used as biomarkers for early diagnosis of SZ patients in Jordanian population.

2. Methods

2.1. Study participants characteristics

A total of 60 participants were enrolled in this study and divided into two groups: (1) SZ-diagnosed patients diagnosed with SZ from psychiatric clinics at King Abdullah University Hospital (KAUH) and Princes Basma Teaching Hospital. (2) Sex- and age-matched healthy volunteers. Exclusion criteria for all participants included medical conditions that may display psychotic SZ-like symptoms, such as epilepsy, metabolic disturbance, brain lesions, and limbic encephalitis, or others like stroke, multiple sclerosis, and dementia. Moreover, individuals with drug-induced psychosis, acquired brain injuries, and intellectual disabilities were also excluded. A pre-tested questionnaire containing information related to age, gender, medication, clinical and family history of SZ.

2.2. Blood sampling and miRNA isolation from plasma

Peripheral blood was collected in EDTA tubes from all participants and centrifuged at 1200 g for 10 min at 4 °C. The plasma was transferred into Eppendorf tubes, followed another round of centrifugation at 10,000×*g* for 10 min at 4 °C, which was then stored at −80 °C until miRNA extraction and further analysis.

2.3. miRNAs extraction and cDNA synthesis

The miRNeasy Serum/Plasma Kit (cat.no 217184), (Qiagen) was used to extract miRNAs from plasma samples according to the manufacturer's instructions. The quantity and quality of miRNA samples were evaluated by Nanodrop (Qiagen, CA, USA) and stored at −80 °C until further use. The average RNA yield was 25 μg per 500 μl of plasma sample. Extracted miRNAs were reverse transcribed to complementary DNA (cDNA) using Mir-X™ miRNA First-Strand Synthesis and TB Green® qRT-PCR kit (Takara, Japan).

2.4. Quantitative reverse transcription polymerase chain reaction qRT-PCR

Each Quantitative PCR (qPCR) reaction was prepared using Mir-X™ miRNA First-Strand Synthesis and TB Green® qRT-PCR kit (Takara, Japan) according to the manufacturer's instructions. qPCR using Applied Biosystems, 7500. The cycling conditions were 95 °C for 10 s followed by 40 cycles at 95 °C for 5 s and 60 °C for 25 s. Primers were designed from miRBase database (https://mirbase.org/) and ordered from Macrogen company (Table 1).

Data were normalized with *C. elegans* miR-39 (*cel*-miR-39). The 2^{-ddCT} method was applied to compare the relative expression levels of tested miRNAs. Samples with cycle threshold (Ct) values > 35 were excluded from the analyses.

2.5. In silico target genes identification and bioinformatic analysis

The target genes associated with has-miR-106b-5p and has-miR-199a-3p, which held prognostic significance, were identified through the miRWalk (version 3.0) (http://mirwalk.umm.uni-heidelberg.de/). To ensure accuracy, only the predicted genes present in both TargetScan (http://www.targetscan.org/vert_72/) and miRDB (https://mirdb.org/) databases were considered as the final set of target genes.

2.6. Gene enrichment analysis

The Mayaan laboratory database (https://maayanlab.cloud/Enrichr/) was then employed to explore gene ontology (GO). This resource was instrumental in conducting comprehensive analyses, including biological processes, gene ontology characteristics, and pathway enrichment studies for the predicted target genes identified through the MirWalk server [40]. To affirm our analysis, the Metascape (https://metascape.org/) tool was applied to analyze and interpret large-scale

Table 1
Sequences of miRNA primers for qRT-PCR.

miRNA	miRBase accession	Primer sequence (5′-3′)
miR-199a-3p	MIMAT0000232	ACAGUAGUCUGCACAUUGGUUA
miR-29b-3p	MIMAT0000100	UAGCACCAUUUGAAAUCAGUGUU
miR-106b-5p	MIMAT0000680	UAAAGUGCUGACAGUGCAGAU
cel-miR39-3p	MIMAT0000010	UCACCGGGUGUAAAUCAGCUUG

datasets. This powerful computational analysis platform enabled us to predict potential biological functions and pathways associated with the identified target genes [41]. P < 0.05 was considered as significant.

2.7. Protein-protein interaction network analysis

A protein-protein interaction (PPI) network involving genes that were associated with GO terms that linked to neuronal development was established using the Search Tool for the Retrieval of Interacting Genes (STRING database, https://string-db.org/). This database is designed to facilitate the exploration and analysis of functional interactions among proteins. The interactions within the network were curated with a confidence score of >0.40, ensuring the reliability of the identified protein associations. This approach allowed for a comprehensive examination of the interconnections and functional relationships among the genes that are involved in gene ontology terms associated with neurodevelopment. Providing valuable insights into their roles within cellular processes and pathways [42].

3. Statistical analysis

Comparison of the relative transcript expression of miRNAs normality assumption of 2^-ΔΔCt values were analyzed by using Unpaired *t*-test with Welch's correction using the Statistical Package for Social Sciences (SPSS) software (Version 22, IBM Inc). Furthermore, to address the concerns of multiple testing, we applied the Benjamini-Hochberg correction method to our Mann-Whitney *U* test results, ensuring a more accurate and reliable statistical analysis. A p-value less than 0.05 was considered statistically significant. A GraphPad Prism version (9.4.1) was used to build the graphs. Receiver-operating characteristics (ROC) curve analysis was performed for each miRNA to evaluate the diagnostic value. We also employed a bootstrapping analysis to assess the stability and reliability of the Area Under the Curve (AUC). using a custom Python script (publicly accessible at https://github.com/AbdulmalekAZ/miRNA-ROC-Script.git). The bootstrapping procedure consists of randomly selecting 85 % of the cases and controls, repeated 1000 times.

4. Results

4.1. Relative expression level of miR-29b-3p, miR-106b-5p and miR-199a-3p in patients and controls

SZ patients displayed a significant down-regulation of miR-29b-3p, compared to controls (p < 0.0001), while both miR-106b-5p and miR-199a-3p were higher than that of controls with (p < 0.0001) (Fig. 1). The p-value based on correction by Benjamini-Hochberg correction method to our Mann-Whitney *U* test results are shown in the Supplementary Table S2.

4.2. ROC curve analysis

To evaluate whether tested miRNAs have diagnostic ability in SZ patients, we performed ROC curve analysis. The results showed significant AUCs in miR-106b-5p (AUC = 0.774, 95 % C.I.: 0.645 to 0.874, the sensitivity was 0.75 and the specificity was 0.75) (Fig. 2A) and miR-199-3p (AUC = 0.979, 95 % C.I.: 0.903 to 0.999, the sensitivity was 1.0 and the specificity was 0.83) (Fig. 2B). On the other hand, differences in miR-29b-3p expression between SZ patients and controls reveals poor diagnostic outcomes, with an (AUC = 0.058) (Fig. 2C) rather having a low expression of miR-29-3p might be an indicator of having SZ. The ROC showed that the AUC of both miRNAs combined was 0.73, the sensitivity was 0.91 and the specificity was 0.53 (Fig. 2D). The sensitivity and specificity of miR-199a-3p for the diagnosis of SZ were higher compared with that in miR-106b-5p for the diagnosis of SZ. In addition, the specificity of each miRNA alone was higher compared with that of combined miRNAs. AUCs validation by a bootstrapping analysis using a custom Python script confirmed our results (Supplementary Fig. S2). This method enabled a thorough re-evaluation of the AUC values, enhancing the validity of our findings in response to the results yielded by our initial analysis.

4.3. Predicted target genes identification for miR-106b-5p and miR-199a-3p

The analysis identified a total of 265 unique target genes for both miRNAs. Specifically, 234 predicted targets for miR-106b-5p and 31 for miR-199a-3p. Of note, 6 genes were commonly targeted by both miRNAs. The Supplementary Fig. S1 shows the visualization produced by the miRWalk server and The Supplementary Table S1 depicts the target genes in each category.

4.4. Gene enrichment analysis of the diagnostically significant miRNAs

To understand how miR-106b-5p and miR-199a-3p are involved in various biological processes, we used miRWalk 3.0 to identify the potential targets of these miRNAs. Subsequently, the identified target genes were subjected firstly to Enrichr to explore the Molecular Function (MF), Cellular compartments (CC), and Biological Processes classification of the identified genes (Fig. 3). A special emphasis on BP was placed to uncover any biological process involved in neuronal development because of its relation to SZ. The former classification uses an algorithm called "GOrilla" to perform biological processes enrichment. This algorithm is based on a hypergeometric test, which is a statistical test that is used to determine whether two groups of items are more likely to share certain characteristics than would be expected by chance. While the latter uses a custom algorithm called "GO2Enrich" to perform biological processes enrichment. This algorithm considers the hierarchical structure of the Gene Ontology (GO) terms, and it also considers the size of

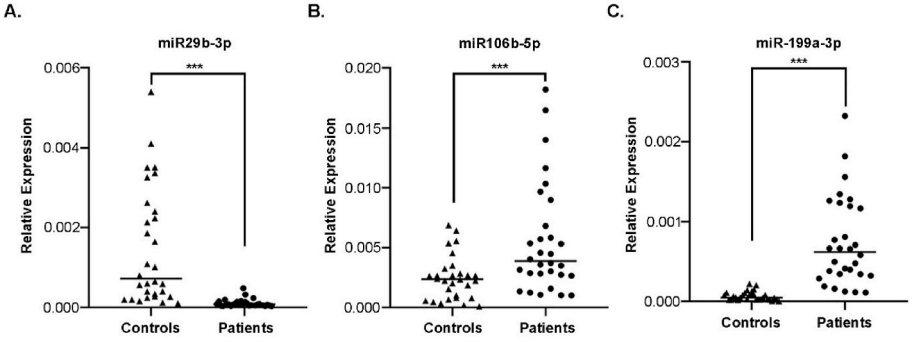

Fig. 1. Differential expression of plasma miRNAs in SZ patients compared to healthy controls. A. miR29b-3p relative expression. B. miR106b-5p relative expression. C. miR199a-3p relative expression. *cel*-miR-39 was used for normalization. *** denotes significance versus control group at p < 0.0001.

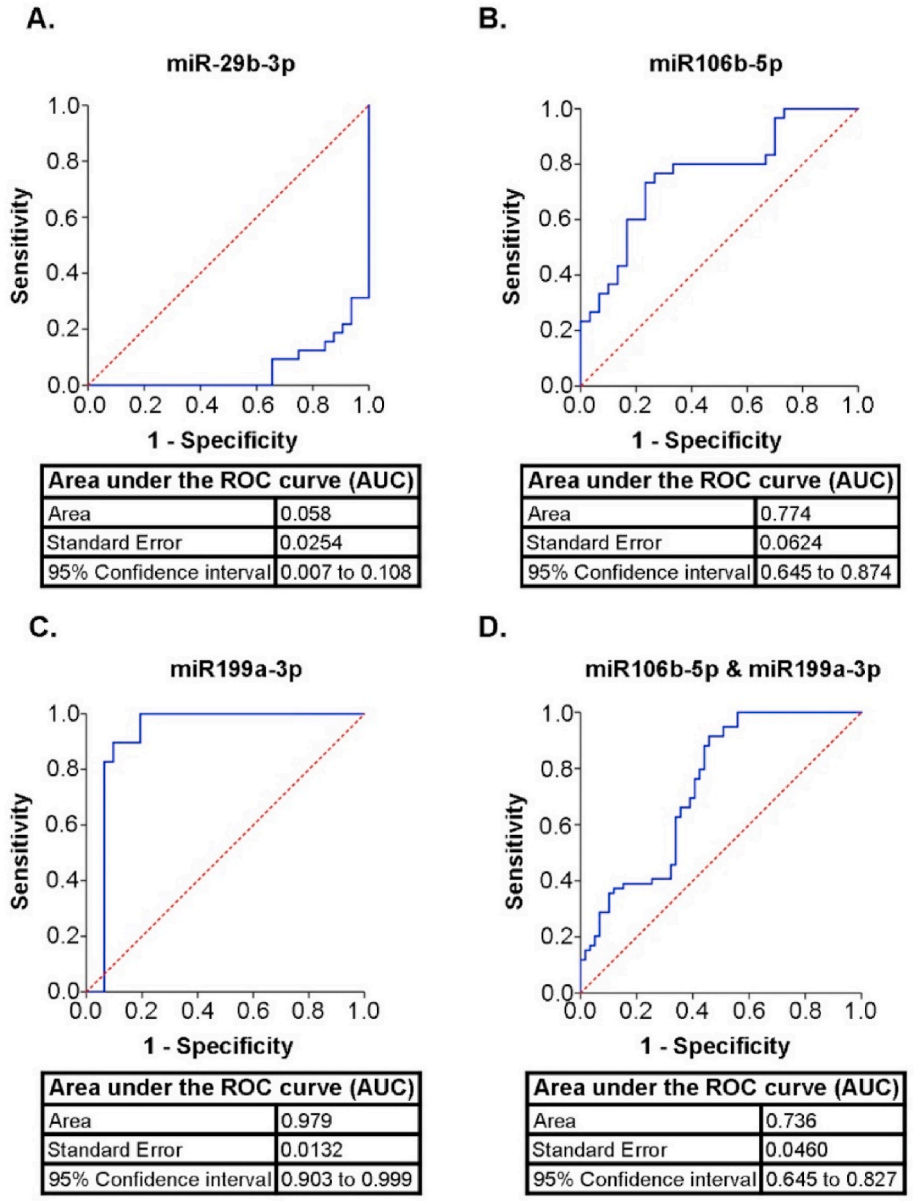

Fig. 2. ROC curve analysis illustrates the diagnostic potential of the measured parameters. A) ROC curve of miR-29b-3p, miR-106b-5p and miR-199a-3p. B) ROC curve of the combined miRNAs.

each GO term. This allows Metascape to identify more relevant and specific biological processes that are enriched in the gene list. The result showed that predicted targets of two miRNAs are involved in a wide range of biological processes related to cell morphogenesis, including cell adhesion, protein phosphorylation, and cell projection morphogenesis. This suggests that these miRNAs could have a central role in coordinating the different aspects of cell morphogenesis. Some of the key genes regulated by these miRNAs include those involved in the development of the shape of neurons, hence affirming their involvement in diseases that might be associated with neurological development. Therefore, a special emphasis was placed on GO terms that are linked to neurons, brain, and head development. As evident with Metascape BP gene enrichment, the following terms are of special interest; cell morphogenesis involved in neuron differentiation, brain development, head development, and neuron projection morphogenesis. Moreover, other findings also include the response of cells to cyclic nucleotides, the development of tubes, and the regulation of cellular catabolic processes. These genes are essential for the development of complex tissues and organs, such as the brain and blood vessels. Overall, the results of this gene enrichment analysis suggest that these two miRNAs could play a critical role in neuronal cell morphogenesis and the development of complex tissues and organs.

4.5. *Protein-protein interaction network analysis*

In the provided protein-protein (PPi) interaction network in Fig. 3, genes in the aforementioned GO terms that were of special interest were subjected to make a PPi that is comprised of 34 nodes and 17 edges, the average node degree of 1 implies a sparse network where proteins have limited direct interactions. Despite this sparsity, the network exhibits a moderate level of clustering (average local clustering coefficient of 0.387), indicating the formation of small, interconnected groups among proteins. The expected number of edges in a random network of the same size is 6; however, the observed 17 interactions yield a remarkably low p-value of 0.000399, indicating a significant deviation from randomness (Fig. 4). This statistical significance suggests that the

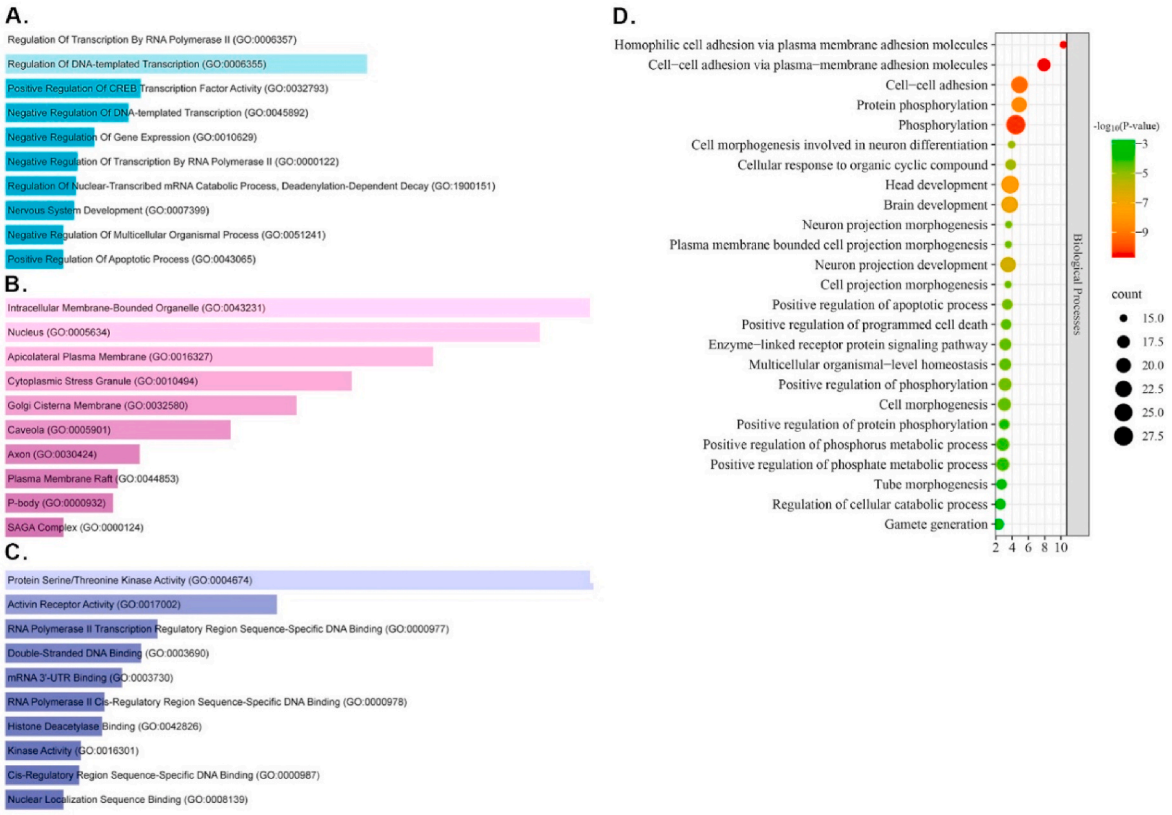

Fig. 3. Gene enrichment analysis of the predicted target genes associated with miR-106b-5p, and miR-199a-3p. Top 10 enriched gene ontology (GO) in the following classes: molecular function (MF) (A), cellular component (CC) (B), and biological process (BP) (C) as per Enricher webserver. Enrichment of BP as per Metascape (D).

proteins in this network are significantly more interconnected than expected by chance alone. In biological terms, the observed enrichment in interactions strongly suggests that the proteins, derived from genes associated with Gene Ontology terms related to neuronal development, play a pivotal role in specific pathways or functional modules. This finding underscores a non-random and biologically relevant network structure, affirming their involvement in SZ. Further detailed analysis of these proteins and their interactions could unveil crucial insights into the underlying biological processes governing this network (see Fig. 4).

5. Discussion

Schizophrenia (SZ) is one of the most severe, complex mental disorders with unknown etiology. Like other complex disorders, a combination of genetic and environmental factors plays a role in its development, including the alteration of gene expression via epigenetic mechanism [43,44].

Most of the identified miRNAs are expressed in the mammalian brain, playing a crucial role in neurodevelopmental signaling, synaptic plasticity, and adult neuronal activity [45,46]. Disruptions in either the miRNA biogenesis pathway or the function of a single miRNA have the potential to induce neurological deficits in both human subjects and animal models [47–51].

In the context of SZ, there is compelling evidence pointing to the central role of miRNA dysregulation in the pathogenesis SZ. Initial findings from postmortem brain studies indicate the dysregulation of multiple miRNAs in individuals diagnosed with SZ [52–54]. Furthermore, the sensitivity of brain miRNA levels to environmental factors linked to an elevated risk of SZ adds another layer to the evidence highlighting the central role of miRNA dysregulation in the pathogenesis of this disorder [55].

miRNAs are found as freely circulated miRNAs in various body fluids including serum, plasma, cerebrospinal fluid, urine, and saliva, or encapsulated inside exosomes that are selectively released by cells and play a crucial role in intercellular communication [25]. Freely circulated miRNAs and exosome miRNAs have emerged as promising biomarkers for several diseases such as cancer, atherosclerosis, cardiovascular diseases, autoimmune disorders, metabolic disorders, and neurodegenerative diseases including SZ, depression, Alzheimer's disease, bipolar disorder, and Parkinson's disease [25,56]. Both exosome and plasma miRNAs have been well reported as diagnostic and therapeutic biomarkers for SZ in recent years [24,26,57], however, exosome miRNAs tend to be more favorable because they are more stable and present in a high level within the exosome than other body fluids.

In Jordan, the rising number of individuals with SZ underscores the urgency for improved diagnostic strategies. Presently, the diagnosis primarily relies on symptoms and physical signs. Genetic, protein and biochemical biomarkers are not incorporated into the diagnostic process for SZ due to the limited access to neuronal tissue and many other factors. Thus, utilizing the easily accessible blood/plasma is of crucial interest when dealing with neurodevelopmental/degenerative diseases. To address this limitation, plasma levels of miRNAs stand out as promising candidates for such biomarkers due to their involvement in neurodevelopmental processes and their potential to reflect the underlying pathophysiological changes.

Integrating miRNA expression analysis extracted from peripheral blood (plasma or serum) into diagnostic protocols could provide a more thorough and objective approach to identifying SZ, offering the prospect of improved early detection and targeted interventions. Therefore, in this study, we aimed to analyze the expression of selected miRNAs: miR-29b-3p, miR-106a-5p and miR-199a-3p and their possible diagnostic value for SZ.

The selection of these specific miRNAs was informed by their distinct roles in neuronal development and their association with neurological

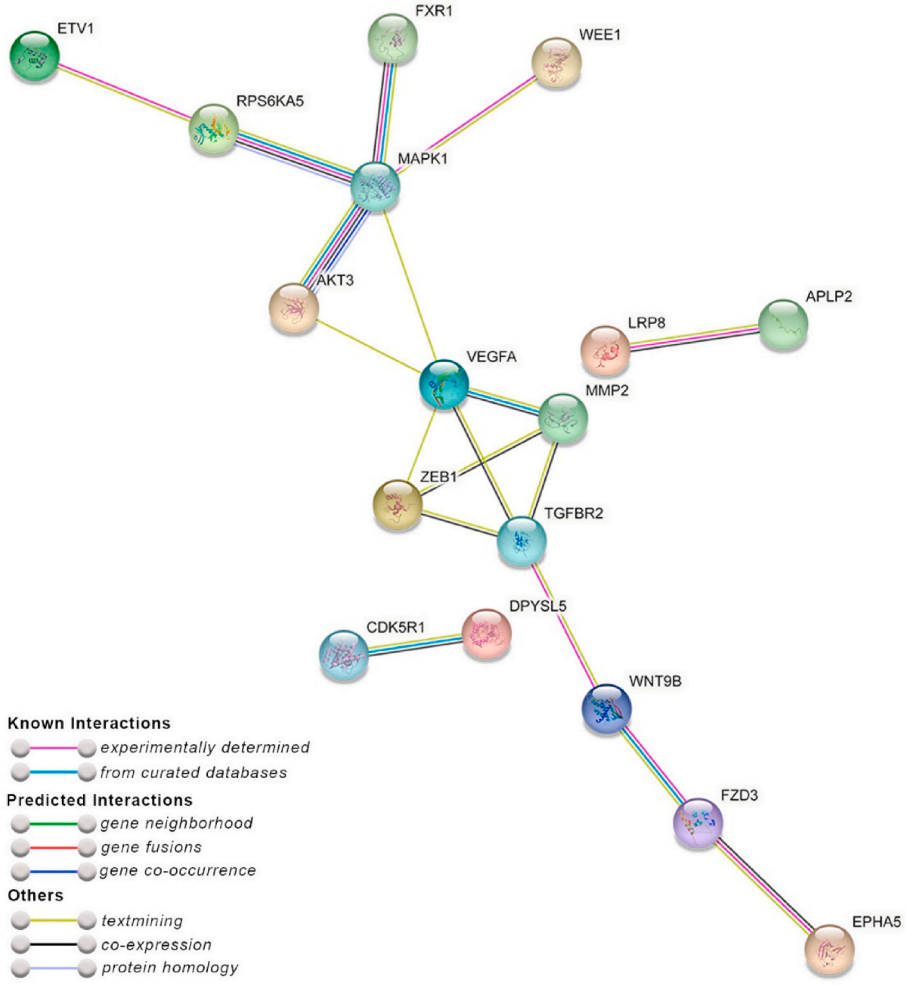

Fig. 4. PPi of the genes that are associated with GO terms relevant to schizophrenia. Target gene interactions; Number of nodes: 33, number of edges: 18, average node degree: 1, average local clustering coefficient: 0.387, expected number of edges: 7, PPI enrichment p-value: <0.001.

diseases. For example, miR-29b-3p was found to be a pivotal molecule activated during neuronal maturation, playing a crucial role in suppressing the translation of the BH3-only family of proteins. This mechanism crucial for protecting against apoptosis, marking a significant instance where a mammalian miRNA specifically inhibiting programmed cell death in healthy neurons. Therefore, maintaining high levels of miR-29b-3p in mature neuronal tissue is essential for long-term survival [58].

Furthermore, in Rat models of autism spectrum disorder (ASD) induced by valproic acid, miR-29b-3p was present in circulating exosomes, causing autistic phenotype in mice by suppressing insulin-like growth factor 1 (IGF-1) in the medial prefrontal cortex (mPFC) [59]. Enriched with miR-29b-3p, these exosomes crossed the blood–brain barrier, resulting in behavioral abnormalities. ASD phenotypes were rescued by inhibition directed at IGF-1 or miR-29b-3p. Importantly, when administered to the mPFC, serum exosomes from human ASD donors, containing an abundance of miR-29b-3p, triggered ASD behaviors in mice. Thus, affirming the role that miR-29b-3p plays in neurogenesis [60]. Also, the miR-29b-3p is known to target the glutamate receptor 3 (*GRIA3*) gene and plays a critical role in glutamate neurotransmitter system, neuronal maturation and survival, calcium signaling and iron overload [61–65] and it could be a promising therapeutic target for the treatment of depression disorders [63]. Moreover, this miRNA has been reported to be associated with SZ susceptibility [66].

In a related context, miR-199a-3p, along with hsa-miR-373-5p, have been identified as a potential biomarker for schizophrenia, as noted by Pala et al. [36]. Moreover, studies led by Michael P. Geaghan have shown a significant enrichment of miRNA binding site variants (MBSVs) in psychiatric disorders, including schizophrenia. This suggests that miRNA dysregulation may play a role in the pathophysiology of these disorders [67]. Further supporting this notion, research by Mehmet A. Camkurt and colleagues found miR-106b-5p to be dysregulated in schizophrenia patients, adding another layer of understanding to the complex relationship between miRNAs and neuronal disorders [27] found miR-106b-5p to be dysregulated in individuals with schizophrenia.

In this study, the qRT-PCR findings revealed a significant downregulation of miRNA-29-3p compared to the control group ($p < 0.0001$). In contrast, both miR-106-5p and miR-199-3p exhibited elevated expression levels in SZ relative to the controls ($p < 0.0001$). Despite the limited research investigating plasma miRNAs levels in SZ for diagnostic purposes, there has been a focus on investigating miRNAs from postmortem prefrontal cortex tissue in relation to SZ. Perkins et al. examined the miRNA profiles in dorsolateral prefrontal cortex (DLPFC) of 13 individuals diagnosed with SZ, utilizing a tailored microarray for analysis. Comparing these profiles to those of 21 individuals without psychiatric conditions, the study unveiled notable variations in expression [68]. Specifically, 15 miRNAs exhibited differential expressions, with 14 showing downregulation including miRNA-29-3p and one miRNA (miR-106b-5p) demonstrated upregulation in SZ patients, which are in line with our findings. Like Perkins et al. work, Smalheiser et al. also

showed upregulation of miR-106b-5p in the DLPFC of patients with SZ [69,70]. These findings together with Mehmet et al. findings in plasma samples and Camkurt et al. in peripheral whole blood of SZ patients are in line with ours [27,71]. This miRNA has been proposed as a biomarker for acute stroke related to neuronal impairment [72], epilepsy [73], Alzheimer's disease [74], and other neuropsychiatric disorders [75]. Other studies demonstrated the contribution of miR106b-5p in microglial polarization and activation, as well as neuroinflammation and it was proposed as a powerful therapeutic target for neuropathic pain or depression [76–78]. Moreover, in the animal model, the antagomir to miR-106-5p showed neuroprotective effects [79].

Several studies have shown that miR199a-3p plays an important role in brain, as well as cell proliferation and survival processes, where the altered expression is associated with various neurodevelopmental and psychiatric disorders such as SZ [80], Parkinson's disease [81], major depression disorder (MDD) [82] and Alzheimer's disease [83]. In Perkins et al. study, the influence of antipsychotic treatment on miRNA expression was investigated [68]. A comparative analysis was conducted of 179 miRNAs between rats treated with haloperidol and those left untreated. There results revealed a high expression level of three miRNAs in the haloperidol-treated rats, notably miR-199a-3p, which was replicated in our study that SZ had a significantly higher expression of the same miRNA. Moreover, Wei et al. verified that miR-193a-3p upregulated significantly in plasma of SZ patients and suggested that it could be used as biomarkers for SZ [80]. Beveridge et al. found that miR-199a-3p was upregulated in superior temporal gyrus (STG) [84], both studies are consistent with our findings. On the other hand, Gardiner et al. reported that miR199a-3p is downregulated in the peripheral blood mononuclear cells (PBMCs) using human miRNA array matrices [85], which is not consistent with our findings that show a significantly high level of miR199a-3p in SZ patients however the findings of Gardiner et al. showed high false discovery rate (FDR) and were not validated by qRT-PCR.

The predicted target genes of miRNAs with prognostic significance demonstrated associations with various biological processes. While not directly linked to neuronal development, these processes are implicated in phosphorylation and the regulation of apoptotic and programmed death activities within the cell. These observations align with the outcomes reported in a previous study [86] and support the idea that the aforementioned processes are involved in the development of SZ.

While considering the limitation of postmortem data and the potential lack of direct correlation between plasma levels and those in neuronal tissue, these findings should be further validated in blood (plasma and serum). Our study corroborates the findings of Perkins et al. aligning with the significant upregulation of miR-106b-5p in SZ patients. The parallel discovery in DLPFC further emphasizes the relevance of miRNA dysregulation in the pathophysiology of SZ. Additionally, our research supports the notion of increased miR-199a-3p expression associated with antipsychotic treatment of SZ, mirroring the observations in haloperidol-treated rats [68]. This collective evidence underscores the potential significance of miRNAs, particularly miR-106b-5p and miR-199a-3p, as key players in both the pathogenesis of SZ and the response to antipsychotic interventions.

In this study, we established the potential of miRNAs as biomarkers for SZ. The integration of miRNA biomarkers into routine clinical practice involves addressing various aspects, including the development of diagnostic tools, assessment of performance across diverse and larger populations, and consideration of factors such as cost-effectiveness and accessibility. Future studies in this field should focus on bridging the gap between biomarker discovery and clinical application. Additionally, investigations should explore the integration of miRNA biomarkers within existing diagnostic frameworks, aiming for seamless adoption in real-world clinical settings.

While our inclusion and exclusion criteria aimed to establish a well-defined study cohort, there are several limitations. First, the number of participants is relatively small. Therefore, as a next step, a large cohort study is needed to confirm our results further. Next, all patients in this study were on medications, which may alter the expression of circulated miRNAs, therefore, enrolling newly diagnosed patients to determine the expression level of miRNA before and after treatment and in several time points of the disease to further confirm the diagnostic value of the tested miRNAs.

6. Conclusion

We may have discovered a model for diagnosing SZ by specific dysregulation of selected miRNAs (upregulated: miR-106b-5p and miR-199a-3p and downregulated: miR-29b-3p) that could act as possible biomarkers for diagnosing and disease management. This diagnostic tool reported in our study could help in early diagnosis of SZ and in pharmacological intervention. So, it contributes to decreasing the burden it places on both patients and society. However, to transition from promising findings to practical application, rigorous validation in larger sample sizes is imperative. This process involves assessing the consistency and reproducibility of the biomarker's performance across diverse populations and conditions. By confirming the robustness of these miRNAs through thorough validation, we can establish a solid foundation for their use as dependable diagnostic markers, ultimately enhancing the effectiveness of early diagnosis and targeted therapeutic interventions in SZ management.

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Ethics approval and consent to participate

Ethical approval was obtained from the Institutional Review Board (IRB) committee (2020/15) of the Jordan University of Science and Technology (JUST). An informed consent was obtained from all participants before enrollment in the study.

Availability of data and materials

The data generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Mohammad Shboul: Writing – review & editing, Writing – original draft, Supervision, Formal analysis. **Amal Bani Domi:** Methodology, Data curation. **Abdulmalek Abu Zahra:** Writing – original draft, Data curation. **Aws G. Khasawneh:** Resources, Investigation, Data curation. **Reem Darweesh:** Methodology.

Declaration of competing interest

The authors declare that they have no competing interests.

Acknowledgments

Not applicable.

Appendix A. Supplementary data

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