

## Investigation of Dysregulation of Several MicroRNAs in Peripheral Blood of Schizophrenia Patients

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**Objective:** The prevalence of schizophrenia is 1%, and it is a debilitating disorder that often results in a shortened lifespan. Peripheral blood samples are good candidates to investigate because they can be easily drawn, and they are widely studied in psychiatric disorders. MicroRNAs are small non-coding RNA transcripts. They regulate the expression of genes by binding to the 3'-untranslated region (UTR) of mRNAs and pointing them to degrade. In this study, we aimed to investigate the expression of miR-9-5p, miR-29a-3p, miR-106-5p, miR-106b-5p, miR-107, miR-125a-3p, and miR-125b-3p in schizophrenia patients and healthy controls.

**Methods:** We collected blood samples from 16 patients with schizophrenia and 16 healthy controls. MicroRNAs were measured with reverse transcriptase polymerase chain reaction.

**Results:** Schizophrenia patients showed statistically significant upregulation of five microRNAs: miR9-5p ( $p=0.002$ ), miR29a-3p ( $p<0.001$ ), miR106b-5p ( $p=0.002$ ), miR125a-3p ( $p<0.001$ ), and miR125b-3p ( $p=0.018$ ).

**Conclusion:** Our results increased the value of the miR106 and miR29 families as potentially and consistently dysregulated in psychiatric disorders. Our results should be considered preliminary, and they need confirmation in future studies with larger sample sizes.

**KEY WORDS:** Blood; MicroRNAs; Schizophrenia; Genes.

### INTRODUCTION

The prevalence of schizophrenia is 1%, and it is a debilitating disorder that often results in a shortened lifespan. Many studies, including neuroimaging, genetic, or biochemical parameters, have been performed in an attempt to identify the neurobiology of schizophrenia.<sup>1-3</sup> Because of the mentioned burden of schizophrenia in patients' lives, researchers focused on identifying markers that represent disease status and prognosis. Peripheral tissues like plasma, serum, and lymphocytes are easily accessible, and are thus convenient to investigate.<sup>4</sup> In this context, many studies have been done to find peripheral markers like se-

rum interleukins, leptin, oxidative stress parameters, and antioxidant enzyme activity for psychiatric disorders.<sup>5-9</sup>

MicroRNAs are approximately 22-nucleotide-long, small non-coding RNA transcripts. They bind to the 3'-untranslated region of mRNAs and induce degradation of pointed mRNA. The interaction between microRNAs and mRNAs usually results in decreased gene expression in a cell.<sup>10,11</sup> Although microRNAs usually decrease the translation of mRNA, they rarely increase translation.<sup>12</sup> With this mechanism, microRNAs have the potential to regulate more than 10,000 genes in the cell.<sup>13</sup> Previous studies have shown the importance of microRNAs for several psychiatric disorders. Perkins *et al.*<sup>14</sup> found that miR-9 and miR-29 were downregulated and miR-106 was upregulated in the prefrontal cortex of schizophrenia patients. MicroRNA analysis of the dorsolateral prefrontal cortex and superior temporal gyrus in schizophrenia patients revealed upregulation of miR-107 and

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**Table 1.** Importance of selected microRNAs depending on previous literature

microRNA	Psychiatric disease	Tissue	Result	Reference
miR-106	Depression	Plasma	Upregulated	Liu <i>et al.</i> <sup>16)</sup>
miR-106b-5p	Attention deficit and hyperactivity disorder	Plasma	Downregulated	Kandemir <i>et al.</i> <sup>17)</sup>
miR-9-3p miR-29a miR-106b	Schizophrenia	Prefrontal cortex	Downregulated	Perkins <i>et al.</i> <sup>14)</sup>
miR-107	Alzheimer disease	Cortical tissue	Upregulated	Wang <i>et al.</i> <sup>18)</sup>
miR-107 and miR-125	Schizophrenia	Prefrontal cortex, superior temporal gyrus	Downregulated	Beveridge <i>et al.</i> <sup>15)</sup>
miR-125b		Mice brain	Upregulated	Beveridge <i>et al.</i> <sup>15)</sup>
			Related with FMRP	Edbauer <i>et al.</i> <sup>19)</sup>

FMRP, fragile X mental retardation protein.

miR-125.<sup>15)</sup> Furthermore, miR-106 was reported to be upregulated in the plasma of patients with depression and downregulated in the plasma of attention deficit and hyperactivity disorder (ADHD) patients.<sup>16,17)</sup> In Table 1,<sup>14-19)</sup> several miRNAs that we studied and their relevance with psychiatric disorders are shown.

Depending on increasing data about the importance of microRNAs in psychiatric disorders, we aimed to investigate different expression profiles of miR-9-5p, miR-29a-3p, miR-106a-5p, miR-106b-5p, miR-107, miR-125a-3p, and miR-125b-3p in the plasma of schizophrenia patients and healthy controls.

## METHODS

### Participants

Sixteen patients diagnosed with schizophrenia according to the the Diagnostic and Statistical Manual of Mental Disorders 4th edition, text revision (DSM-IV-TR) criteria and admitted to the Psychiatry Clinic of Harran University Medical Faculty Hospital were included in our study. An adult psychiatrist validated the diagnoses of the patients. We excluded patients with comorbid psychiatric or medical illnesses. Sixteen healthy volunteers were included in the control group of our research. Written and verbal informed consent was obtained from patients and healthy volunteers. All of the procedures of the study were executed according to the principles of the Helsinki Declaration, and the Harran University Ethical Committee approved it (date:10.01.2012, no:76).

### RNA Extraction

Total RNA was extracted from Peripheral whole blood using Tri-Reagent (Sigma, Taufkirchen, Germany).

### Reverse Transcriptase Polymerase Chain Reactions (RT-PCR)

RT-PCR contained 5 µl of extracted total RNA, 50 nM stem-loop RT primer, 1× RT buffer, 0.25 mM each of dNTPs, 50 units of modified M-MuLV Reverse Transcriptase (Thermo Scientific, Vilnius, Lithuania), 25 units of RiboLock RNase inhibitor (Thermo Scientific) and nuclease-free water to a total reaction volume of 15 µl. The reaction was performed on an automated Thermal Cycler (Veriti, Applied Biosystems, Foster City, CA, USA). RT-PCR conditions for 30 minutes at 16°C, 30 minutes at 42°C, 5 minutes at 85°C and then held at 4°C.

### Quantitative-Comparative CT ( $\Delta\Delta$ CT) Real-time PCR

$\Delta\Delta$ CT Real-time PCR was performed in an ABI Prism 7500 Real-Time PCR System using the SDS 2.0.6 software (Applied Biosystems). The specific primers and fluorogenic ZNA<sup>TM</sup> probes for the microRNAs were designed using Primer Express 3.0 software (Applied Biosystems) and defined in the article of Kandemir *et al.*<sup>17)</sup> The hsa-miR-26b-5p was used as an endogenous control microRNA. The mixed RNAs generated from the control group was used as a Reference RNA sample. Primers and probes were purchased from Metabion International (Martinsried, Germany). The 25 µl PCR included 3 µl RT-PCR product, 12.5 µl of 2X TaqMan Universal PCR Master Mix (Applied Biosystems), 900 nmol of each primer (Primer F and Universal Primer R) and 200 nmol TaqMan probe. The reactions were incubated in a 96-well plate of preincubation at 50°C for 2 minutes and at 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds and at 60°C for 90 seconds. All reactions were run in triplicate.

### Statistical Analysis

The data were processed and analyzed using the SPSS software package for Windows (version 16.0; SPSS Inc.,

Chicago, IL, USA). Normality assumption of  $2^{-\Delta\Delta CT}$  values was checked by Shapiro-Wilk test. Since the assumption of normality was not met, the comparisons between groups were performed using Mann-Whitney *U*-test. Descriptive statistics for  $2^{-\Delta\Delta CT}$  values were expressed as mean, standard deviation, median, first quartile (25th percentile) and third quartile (75th percentile). Significant differences (two-tailed *p*) less than 0.05 were regarded as significant. Parametric variables (age) compared with Student *t*-test. Chi-square test used to compare gender between groups.

## RESULTS

We found no significant difference between the groups regarding gender and age, as shown in Table 2.

All of the patients were in active psychotic episode. The clinical characteristics of the patients were as follows: Three patients were drug-naïve and first episode, and 13 patients were previously on atypical antipsychotic treatment. The average duration of illness was  $7.5 \pm 1.7$  years. Routine biochemical examinations of patients were within the normal range.

We compared the microRNA expression levels of the schizophrenia group with controls. Compared with the controls, as shown in Table 3, schizophrenia patients showed statistically significant upregulation of five microRNAs: miR9-5p ( $p=0.002$ ), miR29a-3p ( $p < 0.001$ ), miR106b-5p ( $p=0.002$ ), miR125a-3p ( $p < 0.001$ ), and miR125b-3p ( $p=0.018$ ).

**Table 2.** Sociodemographic traits of the groups

	Schizophrenia (n=16)	Controls (n=16)	<i>p</i> value
Age (yr)	34.6±9.2	32.5±8.4	0.501
Gender (F/M)	8/8	8/8	1.0

Values are presented as mean±standard deviation or number only.

F, female; M, male.

Two groups are similar in terms of age and gender. We found no significant difference among them.

## DISCUSSION

The main findings of our study are the upregulation of five microRNAs (miR9-5p, miR-29a-3p, miR106b-5p, miR125a-3p, and miR125b-3p) in schizophrenia patients. As previously noted, several dysregulated microRNAs in the plasma of schizophrenia patients were miR-181b, miR219-2-3p, miR346, miR195, miR1308, miR92a, miR17, miR103, let-7g, miR181b, miR30e, miR34a, and miR7.<sup>20,21</sup> Our study adds five potential new microRNAs to this field. After searching databases, we found that these five microRNAs are related to several important genes for schizophrenia (Table 4).<sup>22</sup> miR-9 is related to the glycogen synthase kinase 3B interactin protein (GSKIP), which modulates GSK3B signaling, and important for schizophrenia.<sup>23</sup> miR-29 targets the glutamate receptor 3 (GRIA3) gene, which is also associated with schizophrenia.<sup>24</sup> HTR2A and SLC17A7 (known as the vesicular glutamate transporter 1 [VGLUT1]) are targets for miR-106b, and HTR2A is involved in epigenetic disturbances in schizophrenia.<sup>25</sup> miR125a targets the GRIP2 gene, which is altered in the superior temporal cortex of schizophrenia patients.<sup>26</sup> Glutamate receptor hypo-

**Table 3.** Comparison of microRNA levels between schizophrenia patients and healthy controls

MicroRNA level	Control (n=16)	Schizophrenia (n=16)	<i>p</i> value*
miR9-5p	0.54 (0.37-0.95)	4.10 (2.41-5.38)	0.002*
miR29a-3p	0.0009 (0.0005-0.0039)	0.0326 (0.0219-0.0514)	<0.001*
miR106a-5p	1.2584 (0.5469-3.9152)	3.8572 (2.7856-6.7942)	0.089
miR106b-5p	0.9353 (0.5019-1.6489)	8.3803 (3.6318-32.6446)	0.002*
miR107	6.3747 (0.8042-8.0077)	6.5119 (4.6031-17.3886)	0.724
miR125a-3p	0.5108 (0.2495-1.6409)	24.3688 (14.0268-30.0851)	<0.001*
miR125b-3p	0.1454 (0.0844-0.3232)	0.5433 (0.2974-1.4554)	0.018*

Values are presented as median (interquartile range).

\*Statistically up regulated in schizophrenia patients compared to the healthy controls.

**Table 4.** Predicted target genes and pathways for selected microRNAs\*

	Target gene(s) <sup>22,*</sup>	Pathway	Disorder	Reference
miR-9	GSKIP	GSK3	Schizophrenia	Emamian <sup>23</sup>
miR-29	GRIA3	Glutamate	Schizophrenia	Magri <i>et al.</i> <sup>24</sup>
miR-106b	HTR2A, SLC17A7	5HT <sub>2</sub> receptor, Glutamate	Schizophrenia	Abdolmaleky <i>et al.</i> <sup>25</sup>
miR-125a	GRIP2	Glutamate	Schizophrenia	Bowden <i>et al.</i> <sup>26</sup>

\*Significantly upregulated in this study.

function and decreased levels of glutamate have been reported in schizophrenia.<sup>27-29)</sup> Upregulation of microRNAs usually results in decreased gene expression. In this regard, increased microRNA levels that target glutamatergic pathways may result in decreased glutamatergic transmission. We think that the examination of possible interactions between microRNAs and glutamatergic pathways could be helpful for further understanding of schizophrenia.

We found miR-106b-5p to be upregulated in schizophrenia patients. In their study, Liu *et al.*<sup>16)</sup> compared 14 healthy controls with 16 depressed patients and found that miR106-5p and four other microRNAs were upregulated in the plasma of depressed patients. Additionally, miR106b-5p was found to be downregulated in children with ADHD.<sup>17)</sup> We believe that combining our results with previous findings increases the likelihood that the miR-106 family is a potential plasma marker for psychiatric disorders.

Several studies investigated miR-9, miR-29, miR-106, and miR-125 in schizophrenia. Evaluating these studies together reveals that the same microRNA may be expressed differentially in diverse brain regions. For instance, Perkins *et al.*<sup>14)</sup> found that miR-9 was downregulated in the prefrontal cortex of individuals with schizophrenia, while Beveridge *et al.*<sup>15)</sup> found miR-9 to be upregulated in the superior temporal gyrus. Another point is that the same microRNAs may be expressed diversely between brain tissue and peripheral tissues. Although a study noted that miR-107 was upregulated in the dorsolateral prefrontal cortex, Gardiner *et al.*<sup>30)</sup> found that miR-107 was downregulated in peripheral blood mononuclear cells.

The limitations of our study are its small sample size, its cross-sectional design, and the limited number of microRNA types analyzed.

In brief, we found five microRNAs that were upregulated in schizophrenia patients. These microRNAs are predicted to be targeting important genes for schizophrenia. Evaluating our results with previous data increases the importance of the miR-106 family as a potential plasma marker for psychiatric disorders. While investigating microRNA in psychiatric disorders, authors should keep in mind that microRNAs are affected by age, diet, exercise, and acute or chronic diseases. Strict exclusion criteria should be determined to provide a homogeneous study population. Although we found upregulation of five microRNAs in the plasma of schizophrenia patients, these findings should be considered preliminary and specific to plasma, because microRNA expressions

may alter in different tissues, and even in different regions of the same tissue.

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