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ORIGINAL RESEARCH

Microribonucleic acid dysregulations in children and adolescents with obsessive–compulsive disorder

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Aim: Obsessive–compulsive disorder (OCD) is a disorder characterized by the presence of obsessions and/or compulsions. Although disorder etiology and pathogenesis remains unknown, several theories about OCD development have been proposed, and many researchers believe that it is caused by both genetic and environmental factors. In the current study, our aim was to investigate miRNA levels in OCD.

Methods: In the current study, we evaluated miR18a-5p, miR22-3p, miR24-3p, miR106b-5p, miR107, miR125b-5p, and miR155a-5p levels in child and adolescent OCD patients. The research sample consisted of a group of 23 OCD patients and 40 healthy volunteer controls.

Results: There was no significant difference in age and sex between the two groups ($P>0.05$). The levels of miR22-3p, miR24-3p, miR106b-5p, miR125b-5p, and miR155a-5p were significantly increased in the OCD subjects ($P\leq0.05$). There were no statistically significant differences in miR18a-5p or miR107 levels between groups ($P\geq0.05$).

Conclusion: There could be a close relationship between levels of circulating miRNAs and OCD. If we could understand how the signaling pathways arranged by miRNAs impact on central nervous system development, function, and pathology, this understanding could improve our knowledge about OCD etiology and treatment.

Keywords: OCD, micro RNA, miRNA, psychiatry, child psychiatry

Introduction

Obsessive–compulsive disorder (OCD) is a disorder characterized by the presence of obsessions and/or compulsions. Obsessions are recurrent intrusive thoughts, urges, or images that cause anxiety or distress, while compulsions are repetitive behaviors that are aimed at preventing or reducing anxiety or distress.[1] OCD may be chronic or have a periodic course, and significantly affects social and daily functions of patients. Although it was thought to be mostly evident in adulthood, studies in children and adolescents have shown a prevalence of OCD of 1%–4%.[2,3] Publications reporting onset of OCD before the age of 6 years and treatment notifications are available.[4,5] The coexistence of OCD and other psychiatric disorders is high. OCD is mostly associated with depression, tic disorder, and other anxiety disorders. More than 80% of patients diagnosed with OCD report that related symptoms began before the age of 18 years.[6]

In terms of symptoms, OCD is heterogenous in its clinical appearance.[7] Phenotypic differences suggest that genetic, environmental, psychological, social, and biochemical factors are involved in the etiology.[8,9] Genetic factors have been thought of as important in the development of OCD.[10] It is estimated that OCD symptom heritability is between 45% and 65% in children, and between 27% and 47% in adults.[11] Molecular genetic studies of OCD have usually focused on the monoamine pathway genes.[12–15]

These studies have implicated genes such as the catechol O-methyltransferase (*COMT*) gene, the serotonin 2β receptor (*5HT2B*) gene,[13] the serotonin transporter gene,[14] the serotonin 2α receptor (*5HT2A*) gene,[15] and the seven repeat of the dopamine D4 receptor (*DRD4*) gene.[16]

Several molecular genetic studies have aimed to determine the contributions of candidate genes to the pathophysiology of OCD. A study indicated that there was association of OCD hoarding type and a common single nucleotide polymorphism in the neurotrophin-3 receptor gene (NTRK3), which is regulated by micro-ribonucleic acids (miRNAs).[17] miRNAs are evolutionally conserved small noncoding RNAs that regulate approximately 30% of human protein coding gene expression at the post-transcriptional level, and they play important roles in a wide variety of biological functions.[18] miRNAs control gene expression by inhibiting translation or facilitating degradation of their target messenger RNAs (mRNAs). Computational predictions indicate that thousands of genes could be targeted by miRNAs in mammals.[19] miRNAs are important for maintaining homeostasis at the neuromuscular junction. They also play an important role in synaptic plasticity in the central nervous system (CNS), and are involved in memory and mental retardation.[20]

The ability of miRNAs to affect the activity of all biological pathways may underlie some of the difficulties associated with linking psychiatric disorders to specific causative genes.[21] The involvement of multiple signaling pathways in psychiatric disease complicates both the investigation of the underlying biological causes and efforts to identify effective therapies. Focusing on the roles of miRNAs in psychiatric diseases may lead not only to an explanation of the dysregulation of multiple pathways but also to novel therapies that can target entire gene networks. Perkins et al examined the expression of 16 miRNAs in the prefrontal cortex in subjects with schizophrenia or schizoaffective disorder and found decreased expression of 15 miRNAs in patients with schizophrenia.[22] However, although miRNAs have been shown to be particularly abundant in the brain, their role in the development and activity of the nervous system remains largely unknown. In the present study, we evaluated miR18a-5p, miR22-3p, miR24-3p, miR106b-5p, miR107, miR125b-5p, and miR155a-5p levels in children and adolescents with OCD. In selecting miRNAs for the current study, previous literature pointing out the potential underlying neurobiology (enzyme, carrier molecule, receptor, etc) of the disease was reviewed, and potential most disease related miRNAs from the miRNA database (http://mirbase.org/) were chosen. Table 1 shows the features of the studied miRNAs.

Methods

The research sample consisted a group of 23 patients from Harran University Faculty of Medicine Research Hospital, Child and Adolescent Psychiatry Clinic, who were referred to the clinic for the first time and were diagnosed with OCD, and 40 healthy volunteer controls. All patients were diagnosed with OCD by a child psychiatrist according to the *Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision* (DSM-IV-TR)[23] diagnostic criteria, and they were treatment-naive. Patients with a history of cardiovascular disorders, epilepsy, diabetes mellitus, psychotic disorders, pervasive developmental disorders,

Table 1 Some of the features of the studied miRNAs

miRNA	Rationale for inclusion in the current study	Previous studies' findings	References
miR18a-5p	Believed to be involved in DNA damage in OCD	Altered in DNA damage response	24
miR22-3p	Believed to regulate four candidate genes: *BDNF, MAOA*	Found to be altered in panic disorder	25
miR24-3p	Believed to be related to oxidative stress, which is a potential neurobiological mechanism in OCD	Found to be altered in oxidative DNA damage and lipid peroxidation	26
miR106b-5p	Believed to be related to oxidative stress, which is a potential neurobiological mechanism in OCD	Downregulated in resveratrol treatment that has antioxidant properties	27
miR107	Believed to be related to minimal brain change in OCD	Altered in traumatic brain injury and neurodegenerative diseases	28
miR125b-5p	Believed to be related to hypoxia in OCD	Altered in high altitude sickness	29
miR155a-5p	Believed to be related to inflammation which is a potential neurobiological mechanism in OCD	Altered in inflammation	30

Abbreviations: BDNF, brain-derived neurotrophic factor; OCD, obsessive–compulsive disorder.

or severe head injury were excluded. The healthy controls were recruited from a healthy child outpatient unit. After complete description of the study to the subjects, written informed consent was obtained from the parents, and assent was obtained from the children and adolescents. All of the study procedures were performed in accordance with the Declaration of Helsinki. The ethics committee of the Harran University Medical School approved the trial. Also, a semi-structured form was used to detect several sociodemographic and clinical variables of the patients. The final patient and control groups displayed similar distributions in age and sex. The current study was funded by the Harran University Board of Scientific Research Projects (funding number 12010). The current study was a cross-sectional study, and blood samples were taken once from both controls and OCD patients.

Total RNA was extracted from peripheral whole blood using Tri Reagent® (Sigma-Aldrich Co, St Louis, MO, USA). Reverse transcriptase (RT) reactions contained 5 µL of extracted total RNA, 50 nM stem-loop RT primer, 1× RT buffer, 0.25 mM each of deoxynucleotide triphosphates, 50 units of modified moloney murine leukemia virus RT (Thermo Fisher Scientific, Waltham, MA, USA), 25 units of RiboLock™ RNase inhibitor (Thermo Fisher 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, CA, USA), Real-time polymerase chain reaction (RT-PCR) conditions were sustained for 30 minutes at 16°C, 30 minutes at 42°C, 5 minutes at 85°C, and then samples were held at 4°C. Quantitative-comparative computed tomography (ΔΔCT) RT-PCR was performed in an ABI Prism® 7500 RT-PCR system (Applied Biosystems), using SDS version 2.0.6 software (Applied Biosystems). The specific primers and fluorogenic ZNA™ probes for the miRNAs were designed using Primer Express 3.0 software (Applied Biosystems), and these primers and probes are listed in Table 2. The stem-loop hsa-miR26b-5p was used as control according to the Applied Biosystems application note cms_044972.[31] The mixed RNAs generated from the control group were used as a single reference sample. Primers and probes were purchased from Metabion International AG (Basel, Switzerland). The 25 µL PCR mixture included 3 µL RT-PCR product, 12.5 µL of 2× Taq-Man® 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 pre-incubation conditions at 50°C for 2 minutes and at 95°C for 10 minutes, followed by 40 cycles

at 95°C for 15 seconds at 60°C for 90 seconds. All reactions were run in triplicate. Relative expression of miRNA was calculated using the 2-ΔΔCT method. The 2-ΔΔCT method allows the calculation of the relative expression, which is the most common way to express this type of result. Higher ΔΔCT values indicate lower expression rates.

The data were processed and analyzed using the statistical package SPSS 11.5 for Windows. Normality of the 2-ΔΔCT values was checked by using the Shapiro–Wilk test. In the case of non-normal distribution, 2-ΔΔCT values were expressed as medians, first quartiles (25th percentile), and third quartiles (75th percentile), and the comparison between groups was performed using the Mann–Whitney *U*-test. There are issues with using the Mann–Whitney *U*-test, especially in the context of gene expression data, as outliers may affect the results, and we are usually quite safe using the Mann–Whitney *U*-test. This test rank transforms and smoothes the data by reducing the effect of outliers. A box-plot graph was used to represent data distribution of miR18a-5p, miR22-3p, miR24-3p, miR106b-5p, miR107, miR125b-5p, and miR155a-5p variables according to the groups. Significant differences (two-tailed *P*-values) of <0.05 were regarded as significant.

Results

This study included 23 OCD subjects with a mean age of 10.60±2.06 years (range, 7–16 years) and 40 control subjects with a mean age of 11.25±2.93 years (range, 7–17 years). No statistically significant differences were detected in age and sex between the two groups ($P>0.05$) (see Table 3).

The levels of miR22-3p, miR24-3p, miR106b-5p, miR125b-5p, and miR155a-5p were significantly more in the OCD subjects compared with controls ($P≤0.05$). There were no statistically significant differences in miR18a-5p and miR107 levels between groups ($P≥0.05$) (see Table 4).

Discussion

To the best of our knowledge, this is the first study to evaluate miRNA levels in OCD subjects; therefore, we have chosen to discuss our findings in relation to other psychiatric disorders. We found increased miR22-3p, miR24-3p, miR106b-5p, miR125b-5p, and miR155a-5p levels in OCD patients compared with controls. In a study examining the relationship between miR18a and endogenous glucocorticoid receptor protein expression in rats, Uchida et al and Vreugdenhil et al suggested that levels of miR18a could be an important susceptibility mechanism for stress-related disorders.[32,33]

Table 2 Primer/probe sequences of the miRNAs analyzed by quantitative RT–PCR

miRNA name	Gene ID*	NCBI reference sequence number**	Primer/probe sequence
hsa-miR26b-5p	407017	NR_029500.1	hsa-miR-26b-5p-RT 5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGCACTGCATACGACACCTAT-3′
			hsa-miR-26b-5p-F 5′-GCCGCTTCAAGTAATTCAGG-3′
			hsa-miR-26b-5p-PR 5′-FAM-TG(pdC)ATA(pdC)GA(pdC)A(pdC)CTATCC-ZNA4-BHQ-1-3′
hsa-miR18a-5p	406953	NR_029488.1	hsa-miR-18a-5p-RT 5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGCACTGCATACGACCTATCT-3′
			hsa-miR-18a-5p-F 5′-GCCGCTAAGGTGCATCTAGTG-3′
			hsa-miR-18a-5p-PR 5′-FAM-TG(pdC)ATA(pdC)GA(pdC)CTAT(pdC)TGC-ZNA4-BHQ-1-3′
hsa-miR22-3p	407004	NR_029494.1	hsa-miR-22-3p-RT 5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGCACTGCATACGACACAGTT-3′
			hsa-miR-22-3p-F 5′-GCCGCAAGCTGCCAGTT-3′
			hsa-miR-22-3p-PR 5′-FAM-TG(pdC)ATA(pdC)GA(pdC)A(pdC)AGTT(pdC)T-ZNA4-BHQ-1-3′
hsa-miR24-3p	407012	NR_029496.1	hsa-miR-24-3p-RT 5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGCACTGCATACGACCTGTTC-3′
			hsa-miR-24-3p-F 5′-GCCGCTGGCTCAGTTCAG-3′
			hsa-miR-24-3p-PR 5′-FAM-TG(pdC)ATA(pdC)GA(pdC)CTGTTCCT-ZNA4-BHQ-1-3′
hsa-miR106b-5p	406900	NR_029831.1	hsa-miR-106b-5p-RT 5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGCACTGCATACGACATCTGC-3′
			hsa-miR-106b-5p-F 5′-GCCGCTAAAGTGCTGACAGT-3′
			hsa-miR-106b-5p-PR 5′-FAM-TG(pdC)ATA(pdC)GA(pdC)ATCTGCAC-ZNA4-BHQ1-3′
hsa-miR107	406901	NR_029524.1	hsa-miR-107-RT 5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGCACTGCATACGACTGATAG-3′
			hsa-miR-107-F 5′-GCCGCAGCAGCATTGTACAGGG-3′
			hsa-miR-107-PR 5′-FAM-TG(pdC)ATA(pdC)GA(pdC)TGATAG(pdC)C-ZNA4-BHQ-1-3′
hsa-miR-125b-5p	406911	NR_029671.1	hsa-miR-125b-5p-RT 5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGCACTGCATACGACTCACAA-3′
			hsa-miR-125b-5p-F 5′-GCCGCTCCCTGAGACCCTAAC-3′
			hsa-miR-125b-5p-PR 5′-FAM-TG(pdC)ATA(pdC)GA(pdC)T(pdC)A(pdC)AAGT-ZNA4-BHQ1-3′
hsa-miR155-5p	406947	NR_030784.1	hsa-miR-155-5p-RT 5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGCACTGCATACGACACCCCT-3′
			hsa-miR-155-5p-F 5′-GCCGCTTAATGCTAATCGTGAT-3′
			hsa-miR-155-5p-PR 5′-FAM-TG(pdC)ATA(pdC)GA(pdC)A(pdC)C(pdC)(pdC)TAT-BHQ-1-3′
			miR-Universal-R 5′-GTGCAGGGTCCGAGGTAT-3′

Notes: The substitution of C-5 propynyl-dC (pdC) for dC is an effective strategy to enhance base pairing. Using these base substitutions, duplex stability and melting temperatures are raised by C-5 propynyl-C 2.8°C per substitution. Zip nucleic acid (ZNA) probes provide broad flexibility in assay design and represent an effective alternative to minor groove binder (MGB)- and locked nucleic acid (LNA)-containing oligonucleotides.[34,41]
Abbreviations: ID, identification; NCBI, National Center for Biotechnology Information; RT–PCR, reverse transcription polymerase chain reaction.

Table 3 Sociodemographic and clinical characteristics of OCD patients

	OCD patients (n=23)	Controls (n=40)	P-value
Sex: male/female (n)	15/8	26/14	0.987*
Age: mean ± SD (years)	10.60±2.06	11.25±2.93	0.374**
Age range (years)	7–16	7–17	

Notes: *χ^2 test; **Student's *t*-test.
Abbreviations: OCD, obsessive–compulsive disorder; SD, standard deviation.

Recent studies also reported an increase in the hypothalamic–pituitary–adrenal axis activity in OCD patients.[35,36]

Muiños-Gimeno et al reported that miR22 was associated with panic disorder and that it regulated several candidate genes and related pathways involved in anxiety.[25] In the Muiños-Gimeno et al study, miR22 was the miRNA with the highest number of functional targets, including the neurotrophic factor brain-derived neurotrophic factor and monoamine oxidase A genes, which are also potential targets in the neurobiology of OCD.[37,38] A study performed in Turkey indicated that of OCD patients, 9.5% have co-morbid panic disorder.[39] Moreau et al reported dysregulation of miR22 in schizophrenia and bipolar disorders.[40] In our previous study, we found significantly decreased miR18a-5p, miR22-3p, miR24-3p, miR106b-5p, and miR107 levels and increased miRNA155a-5p levels in attention deficit hyperactivity disorder (ADHD) patients.[41]

In the current study, we found increased miR155a-5p levels in patients with OCD compared with controls. A previous study reported miR155 dysregulation in patients with depression.[42] Additionally, increased miR155 levels were reported in patients treated with lithium[43] and increased miR155a-5p levels in ADHD patients.[41] Rodriguez suggested that miR155 plays a key role in the homeostasis and function of the immune system.[30] It was demonstrated that a wide spectrum of miR155–regulated genes, including cytokines, chemokines, and transcription factors.[30] miR155 was indicated to be induced in lipopolysaccharide-stimulated human monocytes.[44] There has been mounting evidence that immune mechanisms may be directly implicated in the pathophysiology of some subtypes of OCD, such as pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections. Clinical observation of the elevated frequency of obsessive–compulsive symptoms in patients with rheumatic fever, a post-streptococcal autoimmune disease, provoked the study of immune parameters in OCD.[45] Konuk et al found that pro-inflammatory cytokine levels increased in OCD.[46] miR155 might therefore play role in inflammation in OCD.

Dysregulation of miR106b and miR24 levels has been reported in some psychiatric disorders including schizophrenia, bipolar disorder, and autism.[22,40,47–49] Researchers found that miR24 levels were increased in acute pancreatitis.[50] It may be that miR24 levels are increased secondary to inflammation. Sarachana et al found that miR106b and miR107 levels were associated with autism.[51]

In the present study, we observed increased miR125b levels in OCD subjects. Eipper-Mains et al reported an association of miR125 levels and addiction disorders in mice.[52] Additionally, miR125-5p levels have been studied in autism, Huntington's disease, and Alzheimer's disease.[51,53–55] miR125-5p levels were also increased in hypoxia,[29] which might be released during cellular damage. Understanding the signaling pathways affected by miRNAs in CNS development, function, and pathology could lead to improved therapies for treating heterogenic diseases.

Target genes for each miRNA and their potential neurobiological mechanisms are listed in Tables 1 and 2. We think that DNA damage, oxidative stress, hypoxia, and inflammation-related molecular mechanisms may be dysregulated in OCD. A study has revealed that aberrant expression of circulating miRNAs suggests potential diagnostic biomarkers of disease.[56] There are 17 studies of neuropsychiatric disorders such as schizophrenia, bipolar disorder, and Alzheimer's

Table 4 Comparison of miRNA levels

	Controls		Patients with OCD		P-value*
	Median	25%–75%	Median	25%–75%	
hsa-miR18a-5p	2.0884	1.2391–3.4683	2.2334	0.5514–8.0329	0.898
hsa-miR22-3p	0.7023	0.2737–1.9575	9.0235	1.1827–46.7572	**0.001**
hsa-miR24-3p	1.2362	0.5471–2.0238	2.1497	1.5132–6.9911	**0.004**
hsa-miR106b-5p	1.8210	1.1544–2.7165	7.0767	2.6346–18.3542	**<0.001**
hsa-miR107	1.9392	0.8819–4.5595	1.9309	0.4692–5.6560	0.775
hsa-miR125b-5p	1.6689	0.8125–4.5344	7.2907	2.4899–40.1506	**0.002**
hsa-miR155-5p	0.3331	0.1660–0.9977	5.4671	1.6614–8.2755	**<0.001**

Notes: *Mann–Whitney *U*-test. Bold font indicates significant differences (*P*-values) of 0.05 were regarded as significant.
Abbreviation: OCD, obsessive–compulsive disorder.

dementia showing peripheral miRNA changes, reflecting the CNS pathology in those diseases.[57] On the other hand, since psychiatric disorders including OCD are syndromes that not only affect the brain but also most of the body, peripheral changes are expected as well. For example, in OCD peripheral oxidative stress, biomarkers are shown to be altered, as in most of the other neuropsychiatric disorders;[58] however, no blood test for OCD has yet been developed.

Conclusion

Our present study had several limitations, including the sample size, cross-sectional design, and the limited number of miRNA types. Moreover, the lack of previous studies in the literature regarding OCD and miRNAs prevents the comparison of our findings with previous studies. Despite these limitations, we believe our findings provide important groundwork for future studies.

In conclusion, understanding the dysregulation of circulating miRNA levels may improve our knowledge about OCD etiology and treatment. Our results should be regarded as preliminary until they are replicated.

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Disclosure

The authors report no conflicts of interest in this work.

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