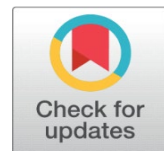# DIFFERENTIAL EXPRESSION OF MICRORNA IN GENERALIZED ANXIETY DISORDER AND ITS ASSOCIATION WITH SOCIAL SUPPORT

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ABSTRACT

To explore the differential expression of microRNA (miRNA, miR-) and its correlation with social support and personality in Generalized Anxiety Disorder (GAD) patients. A total of 80 GAD patients and 80 control participants were selected from our serial study samples to be tested for microarray analysis, and real time quantitative Polymerase Chain Reaction (qRT-PCR) verification was carried out in both patient group and control group. Correlation between miRNA expression and social support were also carried out. According to microarray analysis, 6 miRNAs were differentially expressed between patient group and control group, with miR-4505, miR-4484, miR-4674, miR-501-3p and miR-663 up-regulated, and miR-1301 down-regulated; qPCR verification revealed that 5 miRNAs (miR-1301, miR-4484, miR-4674, miR-501-3p, miR-663, miR-4505) were significantly up-regulated and the expression of miRNA-1301 down-regulated; the expression of miRNA-1301 was significantly related to social support in GAD patients. In conclusions, comprehensive factors, including miRNA regulation, social support may be involved into the aetiology of GAD.

Keywords: GAD; MicroRNA; Social Support; Personality

1. INTRODUCTION

Anxiety disorders are generally considered as a heterogeneous group of disorders that include acute stress disorder, agoraphobia, generalized anxiety disorder (GAD), obsessive-compulsive disorder (OCD), panic disorder (PD), phobias and posttraumatic stress disorder (PTSD). Domschke et al. (2021) and Baxter et al. (2013) found that anxiety disorders are the most prevalent mental disorders and a leading cause of disability, it was estimated the global prevalence of current anxiety disorders has been estimated at 7.3 % (4.8–10.9 %) and ranges from 5.3 % (3.5–8.1 %) in African cultures to 10.4 % (7.0–15.5 %) in Euro/Anglo cultures. Anxiety is thought to be a normal reaction to stress, It may help to deal with difficult situations by prompting one to cope with it. But when anxiety becomes excessive, it may fall under the classification of an anxiety disorder. Numerous factors play a role in the aetiology of anxiety disorders, such as cognitive and physiological factors, genetic heterogeneity as well as epigenetic or regulatory changes. Several animal and human studies have investigated the role of molecular mechanisms in anxiety disorders. Strell-Zimonyi et al. (2020) argued that the aetiology of GAD, PD, phobias and PTSD point to a complex interplay of genetics and the environment. But the molecular and cellular mechanism of anxiety disorders remain poorly understood.

Ebrahimi et al. (2020) reported that miRNAs are a large class of small noncoding RNAs of about 19–25 nucleotides in length in their mature form that act as posttranscriptional regulators of gene expression by either mRNA degradation or translational repression. The role that miRNAs play in synaptic plasticity and neuronal differentiation suggests that miRNAs may be involved in the aetiology of numerous psychiatric disorders. Segaran et al. (2021) argued that various miRNA expression studies have been conducted in Alzheimer's Disease, Depression, Schizophrenia. To date, there have been few studies of miRNAs in anxiety disorders and its association with social support and personality.

Ren et al. (2020) reported previous studies have shown that satisfaction with social support can have a direct effect on well-being and serve as a buffer between the negative health consequences of a stressor and mental health consequence. Tirone et al. (2021) obtained that 174 (73%) bereaved siblings (12-25 years) participated in a nationwide, long-term follow-up study in Sweden using an anonymous study-specific questionnaire, They conclude that bereaved siblings had a greater probability to report self-assessed anxiety if they perceived that their need for social support was not satisfied prior to and following death. Eilertse et al. (2013) found that information from both nurses and other health care professionals to families about the impact of social support may contribute to lessen the siblings' risk of anxiety.

Based upon all these studies, we are wondering if there is any relationship between miRAN expression and social support in GAD patients, in a further effort to shed new light on the aetiology of GAD.

2. MATERIALS AND METHODS

Participants

Between Aug. 2018 and Jun. 2020, 80 GAD patients fulfilling the criteria as defined by the Diagnostic and Statistical Manual 5th edition (DSM-Ⅴ) were prospectively recruited from No.904 Hospital of the Chinese PLA. Clinical diagnoses of the patients were made by at least two consultant psychiatrists, and the diagnoses were further confirmed by an additional experienced clinical psychiatrist. All patients were first visitors to the clinics and prior to any clinical treatment for GAD, or in the absence of anxiolytics medication within at least three months. No patient had history of severe medical diseases, other psychiatric disorders, structural brain disorders, mental retardation, unstable psychiatric features and movement disorders. Also, patients who had brain injury causing traumatic amnesia longer than 24 hours and who received blood transfusion within a month or electroconvulsive therapy within 6 months, were excluded from the study.

In addition, 80 healthy controls without any family history of major psychiatric disorders within the last three generations were recruited. Similarly, all healthy controls were without any history of blood transfusion or severe traumatic event within a month. Patients and healthy controls were matched in gender, age and ethnicity. All individuals recruited in the study were provided with written informed consent. The study was approved by local Institutional Review Boards.

Measuring instruments

Social Support Scale (SSS) had 20 items, covering 3 dimensions, namely subjective support, objective support and utility of social support. The Cronbach's α coefficient was 0.874. Higher scores indicate less social support.

MiRNA microarray expression profiling

Total RNAs from three GAD patients and three controls were used for miRNA microarray profiling. MiRNA expression was measured by Affymetrix miRNA 3.0 array (Affymetrix, Santa Clara, CA, USA) containing probes for a total of 723 human miRNAs. The sample labeling, microarray hybridization and washing were performed based on the manufacturer's standard protocols. The scanned images were analyzed using Expression Console software (version1.3.1, Affymetrix). Screening was carried out based on the criteria: Fold change value≥2.0 and P valus≤0.05 for those miRNAs with biological duplication, and on the criteria: Fold change value≥ 2.0 for those miRNAs without biological duplication.

Real-time quantitative reverse-transcription PCR (qRT-PCR)

Total RNAs were extracted from the purified plasma using Trizol reagent (Invitrogen®, USA) for quantitative detection of miRNA. Complementary DNA was synthesized using the Reverse Transcription TaqMan MicroRNA Reverse Transcription Kit and miRNA-specific stemloop primers (Applied Biosystems, inc., USA, P/N: 4366596) according to the manufacturer's instructions. The 5 × RT primers (miRNA-specific stem-loop primers) and 20 × miRNA-specific PCR primer/probe mix were supplied by the TaqMan MicroRNA Assays (Applied Biosystems, Inc.) based on the miRNA sequences obtained from the miRBase database. After normalized to RNU48, the expression levels of miRNAs were calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

Wilcoxon rank sum test was used to compare the expression levels of miRNA between SZ and healthy controls. Spearman correlation test was carried out for testing the correlation of miRNA expression levels with social support score and PDQ score. All data were processed by DataAssist v3.0 and SPSS v17.0 (Chicago, IL, USA). $P<0.05$ was considered statistically significant.

3. RESULTS

Clinical characteristics of the patients

All the patients and controls were of Han nationality, and there were no differences in age, sex or residential locations between MDD patients and healthy controls($P＞0.05$).

MiRNA microarray expression profiling

Using six blood samples in microarray profiling, 6 miRNAs were identified with significantly different expression levels in GAD patients compared with controls (fold change≧2; $P<0.05$). Of these, miR-1301 were down-regulated and miR-4484, miR-4674, miR-501-3p, miR-663, miR-4505 were up-regulated (Table 1).

Table 1 Differentially expressed miRNA screened by microarray analysis in GAD group			
miRNA	FC (abs)	Regulation	p value
miR-1301	2.3241158	down	0.043555733
miR-4484	2.6544758	up	0.027691107

miR-4674	5.235361	up	0.033227257
miR-501-3p	2.603456	up	0.020094587
miR-663	3.5586042	up	0.030692855
miR-4505	5.31572	up	0.013972622

qPCR verification

The expression levels of 6 miRNAs were compared between GAD group and control group. The results indicated that 5 out of the 6 miRNAs, including miR-4484, miR-4674, miR-501-3p, miR-663, miR-4505 were up-regulated, had significantly increased expression levels in GAD group than those in control group and miR-1301 were down regulated (Table 2).

Table 2 Comparison of miRNA expression levels between GAD group and control group(ΔCT)

miRNA	GAD group	Control group	Z	P
miR-1301	11.09±2.72	9.61±3.05	2.718	0.007
miR-4484	-0.74±3.84	1.57±3.02	-3.426	0.001
miR-4674	2.89±6.00	5.45±4.15	-2.285	0.023
miR-501-3p	8.88±3.17	10.46±2.32	-2.883	0.004
miR-663	2.61±4.83	4.64±3.60	-2.399	0.016
miRNA-4505	8.35±3.27	10.48±2.61	-3.451	0.001

Correlation between miRNA expression and social support score

Spearman correlation analysis results revealed that significant correlation were found between the expression level of miR-1301 and total score of social support and the score of support utilization (Table 3).

Table 3 Correlation analysis between miRNA expression levels and social support(r)

Items	miR-1301	miR-4484	miR-4674	miR-501-3p	miR-663	miR-4505
Total score	-0.47*	-0.359	-0.237	-0.268	-0.307	-0.208
Objective support	-0.384	-0.415	-0.237	-0.309	-0.244	-0.253
Subjective support	-0.422	-0.202	-0.174	-0.081	-0.321	-0.364
utilization	-0.573**	-0.415	-0.272	-0.431	-0.305	-0.277

Note: *P＜0.05, **P＜0.01

4. DISCUSSION

In previous studies, Song et al. (2014) and Zhou et al. (2021) have identified that circulating miRNAs were associated with human schizophrenia and major depressive disorder. Malan-Müller et al. argued it has been increasingly acceptable that aberrant expressions of certain miRNAs also have a significant role in underlying pathophysiology of GAD, even less studies on correlation between miRNA expression profiling in GAD patients and social support.

In this study, 6 miRNAs (miR-1301, miR-4484, miR-4674, miR-501-3p, miR-663, miR-4505) have been identified by microarray analysis and qPCR analysis, In studies of Menezes et al. (2019) and Arzua et al. (2021) reported that miRNAs, as genetic biomarkers, are related to genes that play a fundamental role in synaptic plasticity, neurogenesis, mood control, brain ageing, immune-inflammatory processes and mitochondrial respiratory chain, and these neuro-process may furtherly induce pathology of GAD. In addition, the expression level of miR-1301 was significantly and negatively correlated to the total social support score and score of social support utilization, which maybe suggests a close connection between miRNA expression and GAD molecular and cellular mechanism. On one hand, Qi et al. (2020), Abbas et al. (2019) and Zhou et al. (2020) reported that social support has great impact upon the mental health of individuals, attracting tremendous attention demonstrated by many studies on children and adults. Initial community and school violence exposure and witnessing IPV were both positively associated with initial levels of anxiety. Further, Kennedy et al. (2009) found that gender, initial family social support, and change in family social support significantly moderated the effect of change in community and school violence exposure on anxiety. There was no evidence that social support was more 'protective' in areas of greatest social fragmentation, Fagg et al. (2008) note that while being in employment with more social inerpersonal relationship was associated with better mental health in this sample. Ren and Li (2020). found that social support was negatively related to anxiety, In this study, the △CT value of miR-1301 was negatively related to the total social support score and score of social support utilization, indicating that individuals who had better social support had lower expression of miR-1301. GAD patients had differentially expressed miR-1301 but those of GAD patients who had better social support had lower expression of miR-1301, suggesting that social support has negative relation with anxiety, and there are interactions between social support, anxiety and miR-1301 expression.

Hoffart et al. (2021) and Fitzpatrick et al. (2020) noted that GAD patients have usually demonstrated to be over-concerned with negative stimulating events and poorly regulated inhibition, which may suggest that GAD patients have difficulty in regulating negative emotions. Social support refers to emotional and material connection with relatives, friends, colleagues and relevant organizations. Weiss et al. (2021) confirmed that effective social support could substantially down regulate the effects of negative life events, thus serving a positive role in maintaining good mental health. Based upon these studies mentioned above, we can say that the development of GAD probably results from the interaction between social support, individual's cognition, endocrinology and also the molecular genetics such as miR-1301 expression.

5. CONCLUSION

In conclusion, comprehensive factors, including miRNA regulation, social support may be involved into the aetiology of GAD. We can say that the development of GAD probably results from the interaction between social support, individual's cognition, endocrinology and also the molecular genetics such as miR-1301 expression.

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