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Genetic variants in the promoters of let-7 family are associated with an increased risk of major depressive disorder

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ABSTRACT

Background: Let-7 family plays a critical role in the pathogenesis of major depressive disorder (MDD). Genetic polymorphisms in the promoters of miRNA may influence individual's susceptibility to diseases. The purpose of this study was to investigate the association between rs10877887 and rs13293512 polymorphisms in the promoters of let-7 family and the risk of MDD.

Method: Polymerase chain reaction-restriction fragment length polymorphism and DNA sequencing assays were used to analyze the rs10877887 and rs13293512 polymorphisms in 237 MDD patients and 296 controls.

Results: We found that the rs10877887 CC genotype was associated with an increased risk of MDD (CC vs. TT: OR=1.73, 95% CI, 1.04−2.86, P=0.03, and CC vs. TT/TC: OR=1.74, 95% CI, 1.08–2.80, P=0.02, respectively). Similarly increased risk was also observed for the rs13293512 (CC vs. TT: OR=1.83, 95% CI, 1.12–2.99, P=0.015; CC vs. TT/TC: OR=1.84, 95% CI, 1.20–2.81, P=0.005; and C vs. T: OR=1.32, 95% CI, 1.03–1.68, P=0.03, respectively). Stratification analysis showed that patients with the rs13293512 TC and CC genotypes had a 2.29 and 2.56-fold increased risk of MDD recurrence after treatment (TC vs. TT: 95% CI, 1.23–4.25, P=0.008; CC vs. TT: 95% CI, 1.25–5.23, P=0.009, respectively).

Limitations: Relatively small sample size and hospital-based study design may influence the results.

Conclusions: Our findings suggest that the rs10877887 and rs13293512 polymorphisms may be related to the development of MDD.

1. Introduction

Major depressive disorder (MDD) is a devastating psychiatric condition characterized by a low mood, loss of interest or pleasure in daily life and high suicide rates (Al-Harbi, 2012). The prevalence of MDD is very high in both developed and developing countries, with an estimated 10–15% of the population affected annually (Al-Harbi, 2012; Lepine and Briley, 2011). Previous studies have suggested that aberrant neuronal plasticity and neural remodeling play crucial roles in the pathophysiology of MDD (Krishnan and Nestler, 2008). Besides biological factors, psychological, social and genetic factors have also been indentified to be related to the development of MDD (Caspi et al., 2003; Kendler et al., 2003; Monroe et al., 2007).

MicroRNAs (miRNAs) are a cluster of 18–25 nt non-coding RNAs with a function of gene regulation by modulating messenger RNA degradation and/or translation (Mathonnet et al., 2007). To date, about 2588 miRNAs have been identified in human genome using microarray technology and 20–40% miRNAs are determined during brain development (Miska et al., 2004; Sempere et al., 2004). Recent studies have indicated that miRNAs are involved in the pathophysiology of various of diseases, including MDD and/or suicidal behavior (Dwivedi, 2014; Fan et al., 2014; Serafini et al., 2014). Smalheiser et al. (2012) reported that miRNA expression is globally down-regulated in prefrontal cortex of depressed suicide victims. Maussion et al. reported that an increase of hsa-miR-185* expression regulates a truncated form of tropomyosin-related

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kinase B in frontal cortex of suicide completers (Serafini et al., 2014). Moreover, several studies have shown that the expression of let-7 family members is altered in acute stress, chronic stress, and depressed patients during antidepressant treatment (Bocchio-Chiavetto et al., 2013; Dwivedi, 2014; Meerson et al., 2010; Rinaldi et al., 2010). Animal experiment also showed that mmu-let-7a* is up-regulated and rno-let-7e* is down-regulated in learned helpless rats (Smalheiser et al., 2011). These findings indicate that let-7 family members may participate in the pathogenesis of MDD.

Currently, two polymorphisms (i.e., rs10877887 and rs13293512) in the promoters of let-7 family have been identified, and the rs10877887 polymorphism may serve as a prognostic biomarker for the overall survival of hepatocellular carcinoma (Xie et al., 2013). Since genetic polymorphisms in the promoters of miRNAs may influence the risk of several diseases (Gao et al., 2013; Li et al., 2012, 2013a, 2013b; Liu et al., 2012; Xu et al., 2011), we hypothesized that the two polymorphisms may be associated with the risk of MDD. The main purpose of the present study was to investigate the relationship of the two polymorphisms and the susceptibility to MDD. Because the association with stress and anxiety raises the question of links with the cholinergic system and with increased pulse rate (Shenhar-Tsarfaty et al., 2014), the influence of the two polymorphisms on pulse rate was also evaluated.

2. Materials and methods

2.1. Subjects

The case-control study included 237 patients with MDD and 296 healthy controls, who were recruited from the Mental Hospital of Jining and the Mental Health Center of Yunnan Province between September 2013 and November 2014. All the subjects were Chinese Han population. The study was approved by the ethics committee of the university. After being informed of the study's purpose, all the subjects signed a full written consent. The patients were diagnosed by DSM-IV, and the ratings of symptom severity were evaluated using the 24-item version of the Hamilton rating scale for depression (HAMD-24). The exclusion criteria of the patients were as follows: medical or neurological illnesses, acute or chronic infections, abnormal thyroid function, neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, and other psychiatric disorders, such as comorbidity for substance abuse, cognitive impairment, poor ability to participate to evaluate and current pregnancy or feeding. Clinical data was obtained from medical record, including age, gender, age of onset, HAMD score, pulse rate, depressive episode, family history, suicide attempt, and first-episode patients (yes or no). The mean age of cases (90 males and 147 females) was 41.5 ± 16.4 years. The controls were healthy volunteers without psychiatric conditions, who came to the hospital for physical examination during the same period. The exclusion criteria for healthy controls were the same used for patients. The mean age of the controls (120 males and 176 females) was 40.3 ± 12.5 years (Table 1). The control subjects were frequency matched to cases based on age, gender, ethnicity, and living area.

2.2. Genotyping

2–3 ml of peripheral blood was collected in tubes containing EDTA and genomic DNA was extracted using a DNA isolation kit according to the manual instructions (Bioteke, Beijing, China). The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) procedure was used to genotype the rs10877887 polymorphism. The primers were: 5′–AACCAGTTGGTGTCTGACTGC-3′ (forward) and 5′–CCACCGCTCTGAAGAGAGAA-3′ (reverse). PCR was performed under the following conditions: 95 °C for 2 min, 35 cycles of 95 °C for 30 s, 61 °C for 30 s, 72 °C for 30 s, and 72 °C for 10 min. The PCR product was digested 1 h at 55 °C with *Fau* I (New England Biolabs, Ipswich, MA, USA). After digestion, the heterozygote CT genotype was indicated by bands at 137, 106, and 31 bp, while the TT genotype was indicated by a band at 137 bp and the CC genotype was indicated by bands at 106 and 31 bp. DNA sequencing was used to confirm the genotyping results. The rs13293512 polymorphism was analyzed by DNA direct sequencing.

Table 1
Characteristics of the study population.

Variables	Controls, $n=296$	Patients with MDD, $n=237$
Age (years)	40.3 ± 12.5	41.5 ± 16.4
Gender (%)		
Male	120 (40.5)	90 (38.0)
Female	176 (59.5)	147 (62.0)
Age of onset (years)		35.9 ± 14.3
HAMD score		33.6 ± 8.8
Pulse rate		72.5 ± 10.4
Depressive episode (%)		
Severe		106 (44.7)
Mild/moderate		131 (55.3)
Family history (%)		
Positive		40 (16.9)
Negative		197 (83.1)
Suicide attempt (%)		
Yes		100 (42.2)
No		137 (57.8)
First-episode patients (%)		
Yes		100 (42.2)
No		137 (57.8)

MDD, major depressive disorder; HAMD, Hamilton rating scale for depression.

2.3. Statistical analyses

Data analyses were done using the SPSS 13.0 statistic software (SPSS Inc, Chicago, IL, USA). The rs10877887 and rs13293512 genotype frequencies were obtained by direct counting. Hardy–Weinberg equilibrium was tested by χ^2 test. The rs10877887 and rs13293512 genotype distributions in cases and controls were examined using χ^2 test, and the correlation between the two polymorphisms and MDD risk was assessed using odds ratios (ORs) and 95 % confidence intervals (CIs). Quanto software (version 1.2.3) was used to calculate the statistical power. A P value of less than 0.05 was considered as statistically significant.

3. Results

The genotype frequencies of the two polymorphisms in control subjects and patients with MDD are presented in Table 2. The genotype distribution in the control group did not deviate from Hardy–Weinberg equilibrium (rs10877887: $P=0.40$ and rs13293512: $P=0.28$, respectively). A significantly different distribution of the rs10877887 CC genotype was found between MDD patients and controls. The OR was 1.73, with a 95% CI of 1.04–2.86 and P value of 0.03. Similarly, a significantly different frequency of the rs10877887 between MDD patients and controls was observed in a recessive model (OR=1.74, 95% CI, 1.08–2.80, $P=0.02$). Although there was a trend for the rs10877887 allele distribution, the difference was not significant ($P=0.07$). For the rs13293512 polymorphism, the CC genotype and C allele frequencies in the case group was significantly higher than those in the control group (CC vs. TT: OR=1.83, 95% CI, 1.12–2.99, $P=0.015$; CC vs. TT/TC: OR=1.84,