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Brief report

MiRNA-206 and BDNF genes interacted in bipolar I disorder

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ABSTRACT

Background: Several lines of evidence have suggested that has-mir-206 (miRNA-206) may regulate brain-derived neurotrophic factor (BDNF) protein synthesis. The primary aim of this study was to determine whether miRNA-206 gene (*MIR206*) may confer susceptibility to bipolar disorder type I (BD-I) and treatment response to mood stabilizers. Also, we intended to verify the hypothesis that a potential interplay of *MIR206* and *BDNF* may influence the genetic risk for BD-I and treatment response.
Methods: The *MIR206* rs16882131 and *BDNF* rs6265 polymorphisms were genotyped in 280 BD-I patients and 288 healthy controls. Treatment response to lithium and valproate was retrospectively determined.
Results: No association was observed in the individual polymorphism with regards to risk of BD-I and treatment response. Our results showed a significant gene to gene interaction between the *MIR206* rs16882131 and *BDNF* rs6265 polymorphisms that contribute to BD-I susceptibility and treatment response. Further analysis showed a significant interaction between *MIR206* and *BDNF* on treatment score ($F_{3,\ 138}=8.61$, $P=0.046$), and individuals with *MIR206* T/T+TC and *BDNF* A/A genotypes had a significantly lower mean treatment score than those with *MIR206* CC and *BDNF* A/A+A/G as well as those with *MIR206* CC and *BDNF* G/G genotypes ($P=0.018$ and 0.013, respectively).
Limitation: This is a preliminary investigation with relatively small sample size.
Conclusion: Our findings provide initial evidence of the gene-to-gene interaction of *MIR206* and *BDNF* in regards to the risk for BD-I as well as treatment response to mood stabilizers.

1. Introduction

There is evidence of brain-derived neurotrophic factor (BDNF) in the etiology of BD and the mechanism being mood stabilizing medication's efficacy (Post, 2007). A recent meta-analysis concluded that BDNF levels are consistently reduced during manic and depressive episodes and recover after treatment for acute mania (Fernandes et al., 2011). At the molecular level, though a number of genetic studies supported the functional polymorphism rs6265 (also known as Val66Met) within the gene encoding BDNF (*BDNF*) to be related to BD susceptibility and treatment response (Fan and Sklar, 2008), this association yields inconsistent results when tested against different populations, including Han Chinese (Hong et al., 2003; Tang et al., 2008; Xu et al., 2010). One possible explanation for these observed discrepancies is that clinical heterogeneity may lead to genetic heterogeneity (Lee et al., 2012b). Accordingly, our recent study differentiated BD into type I (BD-I) and type II (BD-II); however, the results showed no association between the rs6265 polymorphism with either BD-I or BD-II (Wang et al., 2012).

MiRNAs are a class of small, approximately 22-nucleotide non-coding RNA molecules that play an important role in the transcriptional and post-transcriptional regulations of gene expression (Bosia et al., 2013). Several recent lines of evidence have indicated that has-mir-206 (miRNA-206) may potentially regulate BDNF protein synthesis by interfering with BDNF mRNA translation and transfection of a miRNA-206 precursor repressed endogenous

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BDNF mRNA levels (Lee et al., 2012a; Miura et al., 2012; Radzikinas et al., 2011). The gene encoding miRNA-206 (*MIR206*) is located on the chromosome 6p, a linkage region that has been implicated in BD (Abou Jamra et al., 2007; Schulze et al., 2004). Accordingly, in this study we conjectured that *MIR206* may be a potential susceptibility gene for BD. To our knowledge, no genetic study addressing the association of *MIR206* with BD has been done to date. The primary aim of this study is therefore to determine whether *MIR206* may influence the susceptibility to BD as well as treatment response to mood stabilizers, such as lithium and valproate, among Han Chinese. In order to avoid clinical and genetic heterogeneities, the recruitment of patients was restricted to the individuals with BD-I. Considering the potential involvement of miRNA-206 in the regulation of BDNF mRNA expression, we hypothesized that the potential interaction effect of *MIR206* and *BDNF* may influence the genetic risk for BD and treatment response, and accordingly investigated this possibility.

2. Methods

2.1. Subjects

The Ethics Committee of Shanghai Mental Health Center reviewed and approved the study protocol. Written informed consent was obtained from each subject before any study-related procedures were performed. Males and females between 16 and 65 years old that also met DSM-IV criteria for BD-I ($n=280$) were enrolled, totaling 124 males and 156 females. The mean age for all participants was 34.4 ± 13.6 years, with a mean age at first episode of 26.0 ± 9.6 years. In total, 288 age- and gender-matched healthy controls were included (131 males and 157 females) with a mean age of 33.8 ± 10.4 years. All subjects were of Han Chinese origin.

All BD-I patients were recruited from those who were admitted to the Division of Mood Disorders at Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine between November 2006 and October 2010. Clinical diagnosis was made by two psychiatrists: one attending psychiatrist and one chief psychiatrist. Each patient was independently interviewed by both clinicians, and only patients diagnosed with the same Axis I disorders by the two psychiatrists were recruited. All diagnoses were further confirmed with an Extensive Clinical Interview and a Structured Clinical Interview for DSM-IV Axis I Disorders, Patient Version (SCID-P) by a research psychiatrist. The Extensive Clinical Interview contains items to assess demographics, mental status, suicidal severity, as well as other variables of interest. Control subjects were recruited from the students and staff at the Shanghai Mental Health Center, who were then interviewed by a research psychiatrist according to the SCID-P (Wang et al., 2012; Zhang et al., 2013). All subjects with any other psychiatric disorders or chronic physical disease were excluded from this study.

2.2. Treatment response assessment

Treatment response was retrospectively determined among the patients who were first treated with mood stabilizers (lithium or valproate) monotherapy during previous hospitalization(s), because lithium- and valproate-induced *BDNF* promoter activation could be part of the molecular mechanisms underlying the therapeutic effects in BD (Yasuda et al., 2009). This process was completed by a trained research assistant reviewing the appropriate medical records and using a scale described previously (Grof et al., 2002). The scale was developed specifically for retrospective evaluation of long-term treatment response in research subjects with BD. More details have been described in the previous literature (Wang et al., 2012).

2.3. Genotyping

Genomic DNA was extracted from peripheral blood according to standard laboratory procedures (Blood Genomic DNA Extraction Kit, Tiangen, Beijing, China). The sequence of miRNA-206 gene and ± 500 bp was acquired from UCSC Genome Bioinformatics (http://genome.ucsc.edu/), and then imported into Primer 3 Software Version 0.4.0 (Rozen and Skaletsky, 2000) to select the primers: upstream primer 5′-ATG CAC AAA AAC AGC AGC AG-3′, downstream primer 5′-GAA AAA CCT TTG GGG GAA AG-3′. Gene sequencing was performed on an ABI PRISM 3130 Genetic Analyzer with Data Collection 3.1 (Applied Biosystems, Foster City, CA, USA). Sequencing data was identified by using GenalysWin2.8.3b (Takahashi et al., 2003). Genotyping of rs6265 was performed with TaqMan genotyping assay which has been reported in our previous study (Wang et al., 2012). Primers and probes were all purchased from Applied Biosystems. All genotypes were independently confirmed by two researchers, and in the event of any discrepancy or ambiguous genotype result occurring, a resolution was made by repeating the genotyping. Ten percent of the samples were later randomly selected to duplicate genotyping, and no genotype errors were found.

2.4. Statistical analyses

The SHEsis software (Shi and He, 2005) was used to analyze genotype and allele distribution comparisons in case-control and treatment response analyses. We also used Haploview 4.2 (Barrett et al., 2005) to estimate Hardy–Weinberg equilibrium (HWE) and pairwise linkage disequilibrium (LD) estimation. When examining the effects of gene-to-gene interaction between *MIR206* and *BDNF* for the risk of BD-I, analyses were performed by comparing *major allele homozygotes* with pooled heterozygote and homozygote carriers of the *minor* allele, taking into account the relatively low frequency of the *minor* homozygote genotype (Benedetti et al., 2012). This approach was justified by our previous observations (Lu et al., 2012; Yi et al., 2011). Logistic regression was used to control the effect of the possible covariates of age and gender. The treatment scores between genotype groups were assessed with one-way ANOVA for comparison. All statistical analyses were carried out by using the SPSS 17.0 (SPSS, Inc., Chicago, IL, USA). Criterion for statistical significance was set at $\alpha=0.05$ and results were two-tailed.

3. Results

3.1. MiRNA-206 gene sequencing

Gene sequencing revealed that there were two polymorphisms located at -214 bp and -155 bp of upstream in *MIR206*, which corresponds respectively to rs16882131(C/T) and rs62408583 (A/C) in the NCBI SNP database (dbSNP) (http://www.ncbi.nlm.nih.gov/projects/SNP/). After linkage disequilibrium analysis, we found that rs16882131 and rs62408583 were complete linkage disequilibrium (disequilibrium coefficient $r^2=1.0$) among the studied samples. Subsequently, only rs16882131 C/T was selected for further association analysis.

3.2. Association analysis of MIR206 and BDNF with BD-I

For either *MIR206* rs16882131 or *BDNF* rs6265 polymorphism, the genotype distributions were consistent with the Hardy–Weinberg