

Study on the epigenetic methylation modification of bipolar disorder major genes

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Abstract. – OBJECTIVE: We aimed to study changes and possible roles of epigenetic modification of candidate genes in the pathogenesis of bipolar disorder, and provide the basis for clinical diagnosis and analysis.

PATIENTS AND METHODS: A total of 150 patients with bipolar disorder were enrolled in this study from January 2014 to June 2015; also, 50 healthy subjects were enrolled as control group. The patients were followed up for 18 months and followed up once every 3 months to review the methylation status. The methylation status was examined before and after treatment, and the patients were followed up every 3 months after treatment, and the follow-up period was 18 months.

RESULTS: Compared with the healthy control group, there were 2075 CpG island aberrant methylation points in patients with bipolar disorder, which can be divided into 24 categories. Log-Ratio > 0.5 was used as the positive criteria, and COMT and PPIEL were identified as the genes associated with bipolar disorder. Compared with the control group, the levels of COMT and PPIEL gene methylation in the observation group were significantly higher ($p < 0.05$). There was no significant difference in the methylation level of COMT and PPIEL gene between the two groups ($p > 0.05$) after 12 months of treatment.

CONCLUSIONS: The methylation level of COMT and PPIEL gene is closely related to bipolar disorder.

Key Words:

Bipolar disorder, Epigenetic modification, COMT gene, PPIEL gene.

Introduction

Bipolar disorder is the focus of prevention and treatment of mental illness in China. Epidemiological survey in the 1970s and 1980s showed that the prevalence of bipolar disorder in the western developed countries was 3.0-4.0%, and in the 1990s it was 5.5-7.8%, and the prevalence of bipolar disorder was 0.042%. 25-50% of bipolar disorder patients had suicidal behavior, 11% to 19% of bipolar disorder patients committed suicide; this is one of the major causes of death of adolescents¹. As the exact cause and pathogenesis of bipolar disorder can not be elucidated so far, leading to the early diagnosis and timely treatment of the disease can not be implemented, due to the early onset of clinical atypical, so the misdiagnosis rate is high and it takes an average of 8 years to be confirmed². Previous studies have attempted to use the results of neuropsychology, neurophysiology, biochemistry and brain imaging as diagnostic criteria for disease, but due to the level of technological development, these tests are not yet able to detect characteristic changes in bipolar disorder. This cannot be a biological indicator of bipolar disorder because of the inability to find the difference between the disease and other mental disorders such as depression, attention deficit hyperactivity disorder, posttraumatic stress disorder, etc. Looking for such indicators has become one of the key issues of bipolar disorder research³. Some scholars have suggested that epigenetic modification may be involved in the pathogenesis of complex diseases such as bipolar disorder. It is another genetic mechanism other than DNA mutation. The epigenetic mechanism mainly includes DNA methylation, histone modification and staining quality remodeling and other aspects, in which DNA methylation is the most profound mechanism of epigenetics research. We

Abbreviations

COMT: catechol-O-methyltransferase; PPIEL: peptidyl-prolyl isomerase E-Like; DNA: deoxyribonucleic acid; DSM-IV: diagnostic and statistical manual of mental disorder-IV.

used bipolar disorder patients as subjects, investigated the relationship between the methylation status of candidate genes and bipolar disorder, and understood the role and significance of epigenetics in the pathogenesis of bipolar disorder. We analyzed the methylation status of candidate genes and discussed changes and possible roles of epigenetic modification of candidate genes in the etiology of bipolar affective disorder, providing the basis for clinical diagnosis and analysis.

Patients and Methods

Patients

A total of 150 patients with bipolar disorder were enrolled in our hospital from January 2014 to June 2015. 50 healthy subjects were enrolled in this study as control group at the same time. In the observation group, there were 106 males and 44 females, aged 18-30 (24.31 ± 1.02) years, the duration was of 0-21 (10.32 ± 2.15) months, with 36 cases of family history. In the control group, there were 35 males and 15 females, aged 18-30 (23.93 ± 1.18) years, with 6 cases of family history. There was no significant difference of gender, age, course of disease, family history and other general clinical data between the two groups ($p > 0.05$). The above cases were confirmed by the hospital Ethics Committee Approval and patients and/or their families signed informed consent.

Inclusion Criteria

All patients met the following criteria: (1) in line with the bipolar disorder diagnostic criteria of American Department of Mental Disorders Diagnostic and Statistical Manual Fifth Edition; (2) 18-30 years; (3) patients were first onset; (4) patients had written informed consent.

Exclusion Criteria

Exclusion criteria: (1) patients had a history of alcohol and drug dependence; (2) patients had brain diseases and endocrine diseases history; (3) patients had dysfunction after the examination of blood, heart and liver and kidney; (4) pregnant and lactating women; (5) subjects who retreated from the research.

Inclusion Criteria of Control Group

Healthy subjects in control group met the following criteria: (1) gender, age, and education were matched with patients; (2) right hand; (3) subjects with normal intelligence who were able

to conduct neuropsychological testing; (4) subject with no color blindness, weak color, deafness, stuttering and other disease that affected the cognitive test; (5) subjects with no head trauma with a history of more than 5 min of mental disorders; (6) subjects without neurological disease. Subjects with mental disorders of DSM-IV diagnostic criteria in line with three generations were excluded.

Method

Before treatment, 8-10 ml of peripheral blood was prepared to examine the patients. All patients were treated with emotional stabilizers and other drugs (Hunan Pharmaceutical Co., Ltd., Shaoyang, China). Patients with bipolar disorder after treatment were observed continuously, and patient's condition changes were observed; the bipolar disorder patients were followed up for 1 year and a half, and the methylation status of patients with bipolar disorder was re-examined every 3 months. Venous blood was collected to examine methylation status. 10 ml of peripheral blood was collected from all subjects. Peripheral blood mononuclear cells were isolated by Ficoll density gradient centrifugation and DNA was extracted. According to the potential methylation sensitive susceptibility, genes for bipolar disorder were screened by bioinformatics analysis. Data source was PubMed, the potential methylation susceptibility gene was screened by methylation inhibition assay, and the methylation status of the promoter region of two groups subjects was analyzed by methylation gene chip technique. The data were analyzed by R language, this part of the experiment was outsourced to the Beijing Genomics Institute (Beijing, China).

Statistical Analysis

SPSS 19.0 (SPSS Inc., Chicago, IL, USA) was used to store and process the original data. The mean \pm standard deviation and percentage were used to represent the measurement and count data. t -test and X^2 test were used to analyze the differences between the two groups. $p < 0.05$ indicated that the difference was statistically significant.

Results

Gene Chip Results

Gene chip results showed that, compared with the healthy control group, there were 2075 CpG

Table I. Results of gene chip.

GO Term	%	GO Term	%
GO:0009987	0.1306	GO:0050896	0.0366
GO:0007582	0.1296	GO:0032502	0.0352
GO:0065007	0.0708	GO:0051179	0.0275
GO:0050789	0.0602	GO:0043226	0.0265
GO:0005623	0.0545	GO:0051234	0.0251
GO:0044464	0.0545	GO:0048518	0.0198
GO:0032501	0.0530	GO:0005215	0.0193
GO:0005488	0.0511	GO:0044422	0.0154
GO:0008152	0.0482	GO:0048519	0.0130
GO:0003824	0.0439	GO:0060089	0.0125
GO other items	0.0448	GO:0032991	0.0111
GO:0030234	0.0067	GO:0002376	0.0101

methylation differential loci in patients with bipolar disorder, which were divided into 24 groups (Table I).

Methylation Specific Polymerase Chain Reaction

In this study, gene-functional analysis was used to determine $\log\text{-Ratio} > 0.5$ as the positive criterion. COMT and PPIEL were identified as genes related to bipolar disorder. Methylation-specific polymerase chain reaction (MS-PCR) results showed that, compared with the control group, the methylation level of COMT and PPIEL gene in observation group was significantly higher than that in control group ($p < 0.05$) (Table II).

Table II. Methylation level of promoter region of COMT and PPIEL gene.

Groups	CpG island methylation rate	
	COMT	PPIEL
Observation group	0.55 ± 0.12	0.61 ± 0.14
Control group	0.43 ± 0.16	0.46 ± 0.15

Note: Compared with the control group, $*p < 0.05$.

Analysis of Methylation of COMT and PPIEL Gene in Patients During Follow-up

The results of this study showed that the level of methylation of COMT and PPIEL gene in observation group was not significantly different from that of healthy control group at 12 months after treatment ($p > 0.05$), and it was stabilized after 12 months (Table III).

Discussion

Mental illness is an important disease affecting human life and health^{4,5}. The majority of mental illness, including the diagnosis of bipolar disorder, has not yet an objective standard for biological diagnosis. The clinical pathology is still the basis for the diagnosis of bipolar disorder, depending on the doctor's subjective judgments. Symptomatic treatment is still the only measure of improving the symptoms of bipolar disorder. Some patients are not treated in time, and this leads to recurrent disease. In the early treatment, early diagnosis must be firstly solved; only an early treatment was established in a strict, reliable, and highly sensitive diagnosis basis, which could be effective⁶. Therefore, to find and develop

Table III. Results of COMT and PPIEL gene methylation analysis in patients with follow-up.

Groups	End of treatment	3 months after treatment	6 months after treatment	9 months after treatment	12 months after treatment	15 months after treatment	18 months after treatment
COMT	0.51 ± 0.21	0.49 ± 0.19	0.46 ± 0.08	0.47 ± 0.17	0.45 ± 0.13	0.42 ± 0.09	0.43 ± 0.18
PPIEL	0.53 ± 0.14	0.51 ± 0.11	0.52 ± 0.32	0.49 ± 0.16	0.48 ± 0.21	0.45 ± 0.19	0.46 ± 0.22

biologic diagnostic indicators of bipolar disorder, so as to provide an objective diagnostic basis when the clinical manifestations are very early or when the symptoms are not obvious or atypical, is the basis of a timely and effective treatment of patients^{7,8}. Epigenetic is considered to be “the focus of life science in the new century”, which in essence means that the expression of genes depends on the degree of chromatin compression, which in turn depends on the reversal regulation of DNA methylation and histone modification⁹. The process of epigenetics, especially DNA methylation and histone modification, recorded the interaction of body and the environment, and reflected the pathological marks on the individual after interaction¹⁰. From the perspective of epigenetics to study the bipolar disorder will be a fruitful area, and existing research has also demonstrated the importance of this research direction^{11,12}. The current view is that the occurrence of bipolar disorder or other complex disease, is not just the candidate genes showed significant variation, but its epigenetic modification have undergone heritable changes¹³. Genetic factors control the susceptibility of the individual to the disease, but epigenetics ultimately determine the occurrence and phenotype of the disease¹⁴, in other words, this means that individuals with the same epigenetic characteristics may be the high-risk population¹⁵. At present, epigenetic is considered as the biological marker that closed to the etiology of bipolar disorder, and is another genetic mechanism than DNA mutations¹⁶. Epigenetic mechanisms include DNA methylation, histone modification, chromatin remodeling and other aspects, in which DNA methylation is the most profound mechanism of epigenetics research. DNA methylation plays a vital role in normal cell development, gene expression patterns, and genome stability, and it is considered as the third mechanism of regulating gene expression except deletion and mutation¹⁷. The etiology and pathogenesis of bipolar disorder have been an important research topic in the field of neuroscience. The genetic study of bipolar disorder suggests that the disease is a polygenic disease but does not follow the Mendelian genetic law¹⁸. The heritability of bipolar disorder in the population is 70-85% and the concordance rate of identical twins is more than 50%¹⁹. Bipolar disorder is a kind of mental disease caused by genetic and environmental factors. The results of this study showed that the methylation level of COMT and PPIEL gene in observation group was significantly higher than that in control group, and

the methylation level of COMT and PPIEL gene was almost normal after one year of treatment, which was similar to that of previous study²⁰. The COMT and PPIEL genes were the important candidate genes for biphasic disorder, and could regulate the level of dopamine, and were closely related to bipolar disorder. So, the low methylation of COMT and PPIEL gene promoter region may be the one pathogenesis of bipolar disorder.

Conclusions

The results of this work show that the level of methylation of COMT and PPIEL genes were closely related to the biphasic disorder, but this research did not analyze the acetylation of bipolar disorder and candidate gene, so it needs to be further studied.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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