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Association of the Brain-derived Neurotrophic Factor Gene and Clinical Features of Bipolar Disorder in Korea

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Objective: Brain-derived neurotrophic factor (BDNF) plays an important role in cell survival, differentiation, and cell death as well as in neural plasticity. Recent studies have suggested that BDNF is involved in the pathogenesis of bipolar disorder. The aim of this study was to investigate the association of the genetic variations of the BDNF gene with bipolar disorder in Korea. We also studied the possible association of these genetic variants with clinical features.

Methods: The allelic and genotypic distributions of Val66Met polymorphism of the BDNF gene were analyzed using a polymerase chain reaction-based method in 184 bipolar patients and 214 controls. Analysis was performed to investigate an association of the Val66Met polymorphism of the BDNF gene and the clinical features in bipolar patients.

Results: No significant difference was found between bipolar patients and controls in the genotype and allele frequencies for the investigated BDNF polymorphism. However, the age of onset of bipolar disorder among the Val/Val (25.57), Val/Met (30.42) and Met/Met (32.45) genotype groups were significantly different (p=0.037).

Conclusion: This study suggests that Val66Met polymorphisms are unlikely to contribution to the genetic predisposition to bipolar disorder as a whole. But Val66Met polymorphism may be associated with age of onset of the disorder, further studies designed to investigate the relationship in a larger population may be warranted.

KEY WORDS: Bipolar disorder; Brain-derived neurotrophic factor; Polymorphism; Age of onset.

INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is involved in cell differentiation, cell survival, and cell death,[1] as well as in overall central nervous system development.[2,3] BDNF plays an important role in the maintenance of neuroplasticity and regulates neurotransmitter synthesis and synaptic activity.[4-6]

There is growing evidence that BDNF is involved in the pathophysiology of bipolar disorder. It has been reported to be a target for the action of mood-stabilizing agents and antidepressants.[7-13] Chen *et al.*[8] reported that levels of BDNF immunoreactivity were increased in postmortem hippocampal tissue among antidepressant-treated bipolar patients compared with antidepressant-untreated bipolar patients. In animal models, chronic administration of mood stabilizers was shown to up-regulate BDNF expression levels in rat frontal cortex.[14] In addition, neuroimaging studies have demonstrated that decreased BDNF levels could explain the structural brain changes observed in bipolar patients.[15]

A polymorphism that results in an amino-acid substitution of methionine for valine at codon 66 (Val66Met) of the BDNF gene may alter intracellular trafficking and regulated secretion of BDNF.[16] Two family-based studies involving relatively large samples found a significant association between this Val66Met polymorphism of BDNF and bipolar disorder.[17,18] Both studies reported overtransmission of the common Val allele to patients with bipolar disorder. In contrast, several studies have failed to confirm this association between BDNF and bipolar disorder.[19-21] In the Asian population in particular, there is no evidence for an allelic or genotypic association between Val66Met and bipolar disorders.[22-24]

The Val66Met polymorphism of BDNF may not play a major role in influencing susceptibility to bipolar disorder, but it may play a role in the specific clinical bipolar phenotype. For example, it has been shown that the Val66Met polymorphism of BDNF is associated with the suicidal behaviors of bipolar patients[25] and with rapid

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cycling.[26,27] Several researchers have also studied the association between BDNF and childhood-onset bipolar disorder.[28,29]

We performed this study to test whether the Val66Met polymorphism of BDNF is associated with bipolar disorder in Korean subjects. We also examined the role of this polymorphism, if any, in specific clinical features that commonly present in bipolar disorder.

METHODS

Subjects

A total of 184 Korean bipolar patients participated in this study. Patients were recruited from the Hallym University Sacred Heart Hospital, the Severance Mental Health Hospital, the Severance Hospital, and the National Health Insurance Corporation Ilsan Hospital. Diagnoses of bipolar disorder were made by a psychiatrist according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition.[30] Patients with history of seizure disorder, significant head trauma, mental retardation, or substance abuse (with the exception of alcohol abuse) were excluded from the study. The patients comprised 64 men and 120 women who were 41.5±13.6 years old (mean±standard deviation). The control group comprised 214 healthy college students with no history of psychiatric disorders (86 men and 129 women aged 23.7±2.5 years). All subjects provided written informed consent to participate in the study after they had received a full explanation of the aims of the study and the procedures involved. The study protocols were approved by the ethics committee of each hospital.

Clinical Assessments

We collected clinical information regarding bipolar disorder via direct interviews with individual patients and family members, and from medical records. Clinical features including age at onset, polarity at onset, any psychiatric family history, and suicidal history were evaluated.

Genotyping

We used venipuncture to collect blood samples, and DNA was isolated using standard techniques. The genotyping was screened using a single base primer extension assay using ABI PRISM SNaPshot Multiplex kits (ABI, Foster City, CA, USA) according to the manufacturer's recommendations. Briefly, the genomic DNA flanking the single-nucleotide polymorphism (SNP) of interest was amplified using polymerase chain reaction (PCR) with forward primer 5'-TGATGACCATCCTTTTCCTT-3' and reverse primer 5'-CACTGGGAGTTCCAATGC-3' and with standard PCR reagents in a 10 μl reaction volume containing 10 ng of genomic DNA, 0.5 pM of each oligonucleotide primer, 1 μl of 10×PCR buffer, 250 μM dNTP (2.5 mM each), and 0.25 units of i-StarTaq DNA Polymerase (5 units/ μl; iNtRON Biotechnology, Seongnam, Korea). The PCR reactions were carried out as follows: 1 cycle of 10 min at 95°C; 35 cycles of 95°C for 30 sec, 55°C for 1 min, and 72°C for 1 min; and 1 cycle of 72°C for 10 min. After amplification, the PCR products were treated with 1 unit each of shrimp alkaline phosphatase (SAP; Roche, Besel, Switzerland) and exonuclease I (USB Corporation, Cleveland, OH, USA) at 37°C for 75 min and 72°C for 15 min to purify the amplified products. A 1 μl aliquot of the purified amplification products was added to a SNaPshot Multiplex Ready reaction mixture containing 0.15 pmol of genotyping primer for the primer extension reaction. The primer extension reaction was carried out for 25 cycles of 96°C for 10 sec, 50°C for 5 sec, and 60°C for 30 sec. The reaction products were treated with 1 unit of SAP at 37°C for 1 hr and 72°C for 15 min to remove excess fluorescent dye terminators. A 1 μl aliquot of the final reaction sample containing the extension products was added to 9 μl of Hi-Di formamide (ABI). The mixture was then incubated at 95°C for 5 min, placed on ice for 5 min, and then analyzed by electrophoresis (ABI Prism 3730 xl DNA analyzer; ABI). The data were analyzed using GeneMapper software (version 4.0; Applied Biosystems, Carlsbad, CA, USA).

Statistical Analysis

Statistical differences in the genotype distributions and allele frequency between patients and controls were tested using the chi-square test. Comparisons among BDNF Val/Met genotype groups were conducted using the chi-square test for categorical data and one-way analysis of variance for continuous variables. The level of statistical significance was set at $p < 0.05$. All tests were two-tailed.

RESULTS

The demographic characteristics of the subjects are given in Table 1. The mean age and education level differed significantly between the two groups, but there was no difference in the gender distribution.

The BDNF Val66Met polymorphism genotype distributions and allele frequencies of subjects are presented in Table 2. The genotype (χ^2=1.33, p=0.52) and allele

Table 1. Demographic characteristics of the subjects

	Bipolar patients (n=184)	Controls (n=214)	*p* value
Age (year)	41.51±13.56	23.73±2.51	<0.01*
Sex (male/female)	64/120	83/129	0.37†
Education (year)	12.76±4.17	15.53±0.95	<0.01*

Values are presented as mean±standard deviation or number. *Independent *t*-test, †chi-square test.

Table 2. BDNF Val66Met polymorphism genotype distributions and allele frequencies in bipolar patients and normal controls

	Bipolar patients (N=184)	Controls (N=214)	*p* value*
Val/Val	66 (35.9)	73 (34.1)	
Val/Met	90 (48.9)	99 (46.3)	0.515
Met/Met	28 (15.2)	42 (19.6)	
Val allele	222 (60.3)	245 (57.2)	0.378
Met allele	146 (39.7)	183 (42.8)	

Values are presented as number (%). *Chi-square test.

(χ^2=0.78, p=0.38) frequencies did not differ significantly between the bipolar patients and the normal controls. The Val66Met genotype distributions in these two groups complied with Hardy-Weinberg equilibrium.

Differences in clinical variables in the various BDNF Val66Met genotype groups are presented in Table 3. Val/Val, Val/Met, and Met/Met bipolar patient groups did not differ significantly with regard to polarity at onset, any psychiatric family history, or suicidal history. The only clinical feature that did differ significantly among the three genotype groups was age at disorder onset.

DISCUSSION

In the present study the allelic and genotypic distributions of the Val66Met polymorphism of BDNF in bipolar disorder were studied with the aim of verifying their roles in the genetic susceptibility to bipolar disorder. The results provide no support for an association between bipolar disorder and the Val66Met polymorphism of BDNF. However, we did find a significant association between this polymorphism and the age at disorder onset.

The age at the onset of bipolar disorder may be a potentially important marker of a more familial and more severe form of the disorder.[31] In agreement with our finding, it has been reported that bipolar patients with the Val/Val genotype experience an earlier onset of the illness (on average by 11 years) than those with the Val/Met genotype.[32] Similarly, Skibinska *et al*.[20] reported a significant association with the Val66Met polymorphism in a subgroup of bipolar patients with early-onset disease, but there was no association between the presence of the Val66Met polymorphism and bipolar disorder overall. In addition, a recent study performed in China demonstrated that the frequency of the Val allele in the Val66Met polymorphism was significantly increased in the subgroup of patients who were younger at the onset of bipolar disorder.[33] Recent evidence also suggests that decreased BDNF gene expression plays a role in the pathophysiology of pediatric bipolar disorder.[34] Therefore, our results suggest that the BDNF gene may be a modifier of age at onset of bipolar disorder.

Table 3. Differences of clinical variables in BDNF Val66Met genotype groups

	Val/Val (n=66)	Val/Met (n=90)	Met/Met (n=28)	*p* value
Age at onset (year)	25.57±8.35	30.42±12.91	32.45±13.46	0.037*
Polarity at onset				0.746†
Manic episode	34 (69.4)	46 (63.0)	17 (68.0)	
Depressive episode	15 (30.6)	27 (37.0)	8 (32.0)	
Any psychiatric family history	15 (30.6)	21 (30.0)	5 (21.7)	0.710†
Suicide history	4 (7.4)	10 (13.0)	4 (16.0)	0.461†

Values are presented as mean±standard deviation or number (%). *One-way analysis of variance, †chi-square test.

Several genetic studies have shown a significant association between the BDNF Val66Met polymorphism and several psychiatric disorders.[35-37] In patient with schizophrenia, the BDNF Val66Met polymorphism was found to be associated with age at onset.[38] The Val66Met genotype may be involved in the expression of psychiatric phenotype in psychiatric disorders by modulating the activity of many neurotransmitter systems.[4,39,40]

Many studies have analyzed samples of different ethnic backgrounds; significant differences in BDNF Val66Met genotype frequency have been reported between populations from Japan, USA, and Italy.[41] There are also reports of allelic or genotypic association between Val66Met and bipolar disorder in Caucasians than in other ethnic groups, and so the BDNF gene possibly represents a prominent genetic risk factor in this ethnic group. Differences in more general genetic backgrounds or local differences in and around the BDNF gene may lead to inconsistent results in different populations.

Our study has several limitations. The first of them is the use of multiple comparisons. Although Bonferroni's correction would be more appropriate, this was not applied since this study was an explorative approach to a ge-

netically complex trait for which the relationship between genotype and phenotype has not been established, and thus such corrections might inappropriately increase the likelihood that real effects will be missed (type II error rates). Second, we did not use structured clinical interview for normal control screening. Therefore, it might be possible that some subjects with psychiatric problems were included in control group. However, because this kind of potential misclassification usually decreases the statistical power rather than increase, the type I error in this study might not be increased. Third, our failure to find a significant association between the Val66Met polymorphism of BDNF and bipolar disorder may be due to the small sample. Fourth, there was significant difference between the mean ages of the patient and control groups. The mean age of the control group was 23.73 years, and so it is possible that some members of that group would develop bipolar disorder after the completion of our investigation, which may have affected our results. Fifth, we focused on single markers that do not cover the entire region of the BDNF gene. Two recent genome-wide association studies found that four other SNPs were significantly associated with bipolar disorder.[42,43] Finally, clinical features based on retrospective reports are subject to recall bias. However, we tried to minimize this bias by obtaining collateral information from all available medical records and from interviews with close relatives.

In conclusion, we found no association between the Val66Met polymorphism of BDNF and bipolar disorder. However, we have shown that the BDNF gene can represent a genetic risk factor for an earlier onset of bipolar disorder. Further investigations with larger samples are necessary to confirm the validity of these results. Studies designed to increase our understanding of these associations and to find new candidate genes arising from the genome-wide association studies are needed.

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