Brain-Derived Neurotrophic Factor Val/Met Polymorphism and Bipolar Disorder

Association of the Met Allele with Suicidal Behavior of Bipolar Patients

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Key Words

Bipolar disorder • Brain-derived neurotrophic factor • Suicide

Abstract

Background/Aims: The substitution of valine by methionine in the brain-derived neurotrophic factor (BDNF Val/Met) gene alters the intracellular trafficking and regulated secretion of BDNF. This study tested whether the BDNF Val/Met polymorphism is associated with bipolar disorder in Korean subjects, and whether clinical features vary according to genotype. **Methods:** The allelic and genotypic distributions of BDNF Val/Met were determined in a population of 169 bipolar patients and 251 normal controls. Between-genotype comparisons of clinical features were performed without a priori knowledge of the genotype of individual patients. **Results:** Allelic distributions did not differ significantly between bipolar patients and controls ($\chi^2 = 0.400$, $p = 0.821$). However, the rate of suicide attempts among the Val/Val (11.3%), Val/Met (28.8%) and Met/Met (38.9%) genotype groups were significantly different ($\chi^2 = 9.879$, $p = 0.007$). Relative to patients with the Val/Val genotype, those with the Met/Met genotype had a 4.9-fold higher risk of suicide attempts (95% CI, 1.7–14.7). **Conclusions:** These findings suggest that BDNF Val/Met is related to the suicidal behavior of bipolar patients, and may have clinical relevance as a biological indicator of bipolar patients at risk of suicide.

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Introduction

Neurotrophins provide necessary trophic support that leads to increased neuronal cell survival. Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, is critical for the survival, differentiation and growth of neuronal cells [1–3]. In the adult central nervous system, BDNF is responsible not only for synaptic plasticity and dendritic growth, but also for long-term memory formation [4, 5]. Thus, BDNF is involved in forming synapses and maintaining connections throughout the life cycle of neuronal cells. In addition, BDNF itself has direct antidepressant properties in animal models of depression, and protects neuronal cells against stress-induced neural damage [6]. These results have suggested that BDNF is a susceptibility gene for various psychiatric disorders, including schizophrenia [7], eating disorders [8, 9], obsessive compulsive disorder [10] and bipolar disorder [11, 12].

Of the known polymorphisms in the BDNF gene, a single nucleotide polymorphism (SNP) at nucleotide 196 (G/A), which leads to the substitution of methionine for valine in the region encoding the prodomain (Val/Met, rs6265), has been shown to have functional consequences [13–16]. The Met allele has been associated with impairments in intracellular trafficking and activity-dependent secretion of BDNF in neurons and neurosecretory cells [13–16]. These impairments are consistent with the functional deficits associated with the BDNF Met allele in

human subjects, including poorer episodic memory [15], reduced hippocampal volume [17, 18] and reduced gray matter volume in the dorsolateral prefrontal cortex [18].

Although the Val/Met polymorphism has functional effects, the biological relevance of this polymorphism to bipolar disorder remains unclear. Two family-based studies found that BDNF Val/Met has a significant association with bipolar disorder, with the Val allele showing increased susceptibility [11, 12]. Additional attempts to replicate these findings, however, have yielded inconsistent results [19]. In particular, a negative association between BDNF Val/Met and bipolar disorder was observed in Japanese and Han Chinese subjects [20–22], whereas a positive association was observed in predominantly Caucasian populations [11, 12, 23]. Moreover, the allele frequencies of BDNF Val/Met have been found to differ significantly between individuals of European and Asian descent [24], suggesting that the association of BDNF Val/Met with bipolar disorder should be assessed separately in different ethnic populations. To our knowledge, there have been no studies to date on the association of BDNF Val/Met with bipolar disorder in Koreans.

Instead of exerting a global influence in bipolar disorder, BDNF Val/Met may have a role in specific subgroups of bipolar patients or those with particular psychiatric symptom(s). For example, BDNF Val/Met was shown to be associated with childhood-onset affective disorder [25, 26] and rapid cycling [27, 28]. Moreover, this polymorphism may contribute to the development of particular clinical subphenotypes rather than bipolar disorder per se. For example, bipolar patients with the Met allele performed more poorly on the Wisconsin Card Sorting Test than bipolar patients homozygous for the Val allele [29–31], indicating that this polymorphism may be associated with different clinical variables commonly present in bipolar disorder. Thus, it is of interest to determine the association of BDNF Val/Met with clinical features that are frequently present in bipolar patients.

This study was designed to test whether the BDNF Val/Met polymorphism is associated with bipolar disorder in Korean subjects, and whether clinical features vary according to genotype.

Methods

Subjects

This study involved 169 Korean patients (75 males, 94 females; mean age 37.5 ± 12.3 years) diagnosed with type I and II bipolar disorders, as defined by DSM-IV criteria. All subjects were enrolled in the psychiatric department at a university tertiary hos-

pital (Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea). Diagnosis was confirmed using the Structured Clinical Interview for DSM-IV, and collateral information was collected from unstructured interviews of the subject and the family members, and from medical records. The control group consisted of 251 normal healthy individuals (108 males, 143 females; mean age 35.3 ± 11.2 years) with no history of psychiatric disorders, including suicide attempts. Written informed consent was obtained from all participants after explaining the aims and procedures of the study. This study was approved by the Ethics Committee of the Asan Medical Center.

Assessment of Clinical Features

Clinical information on bipolar patients was collected from direct interviews with individual patients and family members, and from medical records. Clinical features evaluated included: age of onset, psychotic symptoms, mixed episodes, rapid cycling, alcohol problems (abuse or dependence), any psychiatric family history within second-degree relatives, family history of bipolar disorder within second-degree relatives, history of suicide attempts and lethality of suicide attempts. The latter was assessed using a Lethality of Suicide Attempt Rating Scale [32]. If subjects had a history of more than 1 suicide attempt, the most lethal attempt was evaluated. All assessments were performed without a priori knowledge of the genotypic status of individual patients.

Genotyping of BDNF Val/Met

Venous blood (10 ml) was collected from each patient and normal controls, and genomic DNA was isolated from peripheral blood leukocytes using standard proteinase-K-RNase digestion procedures followed by phenol-chloroform extraction. BDNF Val/Met polymorphisms (rs6265) were identified by genotyping using a single-base primer-extension assay (ABI Prism SNaPshot Multiplex Kit; ABI, Foster City, Calif., USA) according to the manufacturer's recommendations. Genomic DNA flanking the SNP (rs6265) was amplified by PCR using the primers 5'-TGATGACCATCCTTTTCCTT-3' (forward) and 5'-CACTGGGAGTTCCAATGC-3' (reverse). Each PCR reaction utilized 10 ng genomic DNA, 0.5 pM of each oligonucleotide primer, 1 μ l 10 $\times$ PCR gold buffer, 250 μ M dNTP, 3 mM MgCl₂ and 0.25 unit i-StarTaq DNA polymerase (iNtRON Biotechnology, Sungnam, Kyungki-Do, Korea) in a total reaction volume of 10 μ l. The amplification protocol consisted of 1 cycle of denaturation at 95°C for 10 min, 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 1 min and extension at 72°C for 1 min, followed by a final extension at 72°C for 7 min. The PCR products were treated with 1 unit of shrimp alkaline phosphatase (Roche, Basel, Switzerland) and 1 unit of exonuclease I (USB Corporation, Cleveland, Ohio, USA) for 60 min at 37°C followed by 15 min at 72°C to purify the amplified products. A 1- μ l aliquot of each purified amplification product was added to a SNaPshot Multiplex Ready Reaction mixture containing 0.15 pM genotyping primer for the primer extension reaction, which consisted of 25 cycles at 96°C for 10 s, 50°C for 5 s and 60°C for 30 s. These reaction products were treated with 1 unit of shrimp alkaline phosphatase for 1 h at 37°C, followed by 15 min at 72°C to remove excess fluorescent dye terminators. A 1- μ l aliquot of each sample was added to 9 μ l of Hi-Di formamide (ABI), and incubated at 95°C for 5 min, followed by 5 min on ice. The reaction products were analyzed by electrophoresis using an ABI Prism 3730xl DNA analyzer, and results were interpreted using GeneScan analysis software (ABI).

Table 1. BDNF Val/Met polymorphism: allele and genotype distributions between bipolar patients and normal controls

	Case (n = 169)	Control (n = 251)	χ^2	p
Val	186 (55.0)	268 (53.4)	0.400	0.821
Met	152 (45.0)	234 (46.6)		
Val/Val	53 (31.4)	77 (30.7)	0.220	0.640
Val/Met	80 (47.3)	114 (45.4)		
Met/Met	36 (21.3)	60 (23.9)		

Figures in parentheses are percentages.

Statistical Analysis

The genotype frequency was assessed for conformance to a Hardy-Weinberg equilibrium using a χ^2 test. Statistical differences in genotype and allele frequencies between bipolar patients and controls were evaluated by a χ^2 test or Fisher's exact test. Comparisons among BDNF Val/Met genotype groups were performed using Fisher's exact test for categorical data and ANOVA for continuous variables. Linear regression analysis was adapted to test the linearity between the number of Met alleles of individual patients and suicide lethality scores. All statistical analyses were performed with SPSS version 12.0. Statistical significance was defined as $p < 0.05$.

Results

BDNF Val/Met Allele and Genotype Frequencies in Bipolar Patients and Normal Controls

The BDNF Val/Met allele frequency and genotype distribution were compatible with a Hardy-Weinberg equilibrium, both in bipolar subjects ($\chi^2 = 0.321$, $p = 0.852$) and in normal controls ($\chi^2 = 1.919$, $p = 0.383$). There were no statistically significant differences in allele ($\chi^2 = 0.400$, $p = 0.821$) or genotype ($\chi^2 = 0.220$, $p = 0.640$) frequency between bipolar patients and normal controls (table 1).

Comparisons of Clinical Features according to BDNF Val/Met Genotypes

Val/Val, Val/Met and Met/Met bipolar patient groups were not significantly different with respect to age, sex, proportions of bipolar type I and II patients, or the duration of the disorder (table 2). The only clinical features that were significantly different among the 3 genotype groups were suicide attempt and suicide lethality (table 2). Of the 169 bipolar patients, 43 (25.4%) had a history of previous suicide attempt(s). The rate of suicide attempts among the Val/Val (11.3%), Val/Met (28.8%)

Fig. 1. Comparisons of frequency of suicide attempters according to genotype of BDNF Val/Met.

and Met/Met (38.9%) genotype groups were significantly different ($\chi^2 = 9.879$, $p = 0.007$). Relative to patients with the Val/Val genotype, those with the Met/Met genotype had a 4.9-fold higher risk of suicide attempts (95% CI, 1.7–14.7; fig. 1). A comparison of the allelic distribution of the Val/Met polymorphism in normal controls and bipolar subjects with a history of suicide attempt(s) showed a significantly higher frequency of the Met allele in suicidal bipolar subjects than in normal controls (59.3 vs. 46.6%; $\chi^2 = 4.733$, $p = 0.035$).

Discussion

Although we did not observe a significant association between bipolar disorder and the BDNF Val/Met polymorphism, we found a significant association between this polymorphism and the suicidal behavior of bipolar patients; to our knowledge, this study is the first to report such an association. In agreement with our findings, a recent meta-analysis of 11 case-control studies [19], an analysis of 3,062 Caucasian subjects in the UK [27] and additional studies in Asian subjects [20–22] showed no

Table 2. Comparisons of clinical features according to BDNF Val/Met genotype groups

	Val/Val (n = 53)	Val/Met (n = 80)	Met/Met (n = 36)	Statistics	p values
Age, years	36.5 ± 11.6	37.9 ± 12.8	37.1 ± 12.3	F = 0.199	0.820 ^a
Female sex	27 (50.9)	48 (60.0)	19 (52.8)	$\chi^2 = 1.232$	0.576 ^b
Bipolar type I:bipolar type II	41:12	60:20	26:10	$\chi^2 = 0.353$	0.845 ^b
Duration of illness, years	9.4 ± 8.7	8.9 ± 8.3	10.8 ± 8.9	F = 0.562	0.571 ^a
Age of onset, years	27.6 ± 8.8	28.9 ± 11.6	25.7 ± 8.4	F = 1.211	0.301 ^a
Psychotic symptoms	21 (39.6)	33 (41.3)	17 (47.2)	$\chi^2 = 0.568$	0.790 ^b
Mixed episode	14 (26.4)	22 (27.5)	12 (33.3)	$\chi^2 = 0.611$	0.736 ^b
Rapid cycling	4 (7.5)	6 (7.5)	5 (13.9)	$\chi^2 = 1.470$	0.526 ^b
Alcohol problems	6 (11.3)	11 (13.8)	4 (11.1)	$\chi^2 = 0.231$	0.911 ^b
Any psychiatric family history	21 (39.6)	19 (23.8)	9 (25.0)	$\chi^2 = 4.102$	0.126 ^b
Family history of bipolar disorder	6 (11.3)	7 (8.8)	2 (5.6)	$\chi^2 = 0.819$	0.685 ^b
Suicide attempt	6 (11.3)	23 (28.8)	14 (38.9)	$\chi^2 = 9.879$	0.007 ^b
Lethality of suicide attempt (0–10), score	2.83 ± 1.4	4.00 ± 2.1	4.89 ± 2.3	F = 4.257	0.045 ^c

Where indicated, results are presented as means ± SD. Figures in parentheses are percentages.

^a ANOVA. ^b Fisher's exact test. ^c Linear regression for testing trend.

significant association between bipolar disorder and BDNF Val/Met. However, this polymorphism has been shown to be associated with particular subgroups of bipolar patients, including childhood-onset [25, 26] and rapid cycling [27, 28]. In addition to its association with specific phenotypes, bipolar patients with the Met allele showed poorer performance on the Wisconsin Card Sorting Test than bipolar patients homozygous for the Val allele [29–31]. These results indicate that the BDNF Val/Met polymorphism is related to particular clinical features commonly appearing in bipolar disorder, rather than to bipolar disorder per se. Thus, it may be premature to conclude that this polymorphism plays no role in the etiopathogenesis of bipolar disorder or the development of its clinical manifestations.

An analysis of the associations between the Val/Met polymorphism and specific clinical features that usually accompany bipolar disorder showed that suicide attempt was significantly associated with the Met allele. This allele also exerted a dosage effect on the lethality of suicidal behavior: suicidal behavior became more lethal as the number of Met alleles increased. These results suggest that clinical feature(s) may vary according to the genotypic status of BDNF Val/Met, even among patients who fall within the same bipolar disorder diagnostic boundary.

In contrast to our finding of an association between BDNF Val/Met and suicidal behavior in patients with bi-

polar disorder, a previous study has reported that this genotype did not have a significant effect on suicidal history in patients with mood disorder [20]. Bipolar patients, however, are at higher risk of suicide and their suicidal behavior has a higher lethality [33] than that of patients with other psychiatric disorders [34]. Suicidal patients may represent a distinct subgroup of bipolar patients [35], possibly with a different genetic background [36]. Patients with bipolar disorder have a lifetime suicide risk of 19%, and 25–50% of these patients attempt suicide at least once during the course of their illness [34, 37]. Of our bipolar patients, 25% reported a history of suicide attempt(s), which is within the reported range.

BDNF may play a role in suicidal behavior. For example, BDNF levels in the hippocampus and prefrontal cortex were significantly lower in suicide victims who were drug-free compared with nonsuicide controls, regardless of diagnosis [38, 39]. In addition, depressive patients who attempted suicide had lower plasma concentrations of BDNF than depressive patients without a history of suicide attempts [40]. Furthermore, lithium, which is used to reduce suicide risk, increases BDNF levels [41, 42]. Although there is little direct evidence to indicate how the Met allele might lead to suicidal behavior, previous results suggest several hypotheses. For example, bipolar patients with the Met allele may have an impairment in the regulated secretion of BDNF [14, 15] that alters BDNF levels, as observed in suicidal patients. In addition, the

Met allele has been associated with genetic predispositions to anxiety and depressive disorders, as evidenced by the increase in antidepressant-resistant anxiety-related behaviors in BDNF Met/Met mice [16]. These results suggest that the suicidal behavior of bipolar patients may be due to an increased vulnerability to anxiety associated with the Met allele, since comorbid anxiety is a known risk factor for suicide in bipolar disorder [43].

The serotonergic system is also related to suicidal behavior. Suicidal behavior runs within families independently of the transmission of psychiatric illness itself and is linked to serotonergic dysfunction [44, 45]. BDNF modulates the activity of many neurotransmitter systems, including the serotonergic system [46, 47]. A deficit in BDNF is also associated with behavioral alterations, including the development of aggressiveness and hyperphagia, which are associated with a decrease in serotonin concentrations [48–50]. These findings suggest that the contribution of BDNF to suicidal behavior implied by our results may be mediated by the serotonergic system; however, additional experimental and clinical studies will be required to confirm this speculation. One subject that warrants further investigation is the interaction of BDNF with other genes and genetic variants associated with susceptibility to suicidal behavior, such as 5-HTTLPR (serotonin transporter gene-linked promoter region). It is likely that multiple genes are involved, each of which may make only a minor contribution to mood disorder and suicidal behavior.

Ethnic differences have been observed in the genotype and allele frequencies of BDNF Val/Met. For example, our control group had a higher frequency of the Met allele (46.6%) than that in Western populations (19.0% in Caucasian citizens of the UK) [27]. However, the Met allele frequency we observed is similar to that in other Asian populations [20–22]; in Japanese subjects, the Met allele frequencies were between 41 and 48% [21, 22]. Significant differences in BDNF Val/Met genotype frequency have been observed in populations in Japan, the USA and Italy [24]. These differences may influence the results of case-control studies of genetic associations, and lead to inconsistencies between reports based on different populations.

Our study had several limitations. First, our failure to observe a significant association between the BDNF Val/Met and overall bipolar patients may be due to an insufficient number of subjects. In addition, the association between BDNF Val/Met and suicidal behavior of bipolar patients could possibly reflect a type I error due to multiple comparison of clinical variables; however, our sta-

tistical association ($p = 0.007$) remained significant after correction ($p = 0.049$). Second, the retrospective assessment of clinical features, including history of suicide attempt(s), is subject to recall bias. However, we tried to minimize the impact of bias by supplementing patient recall with collateral information from all available medical records and from interviews with close relatives. Third, there might have been confounding variables not tested in this study that contributed to the association between BDNF Val/Met and the suicidal behavior of bipolar patients. For example, depressive and mixed states have been associated with a high risk of suicide in bipolar patients [35]. Since most suicide attempts by bipolar patients occur during depressive or mixed states [51], bipolar patients carrying the Met allele may be more vulnerable to depressive symptoms as well as suicidal behavior. This is supported by evidence of an association between the Met allele and geriatric depression [52], post-stroke depression [53] and the risk of depression, the latter through interactions with the serotonin transporter gene [54–56]. Although the Val allele has been associated with susceptibility to bipolar disorder [11, 12], this speculative relationship should be validated by a systematic assessment of the severity of depressive symptoms in bipolar disorder patients. The fourth limitation of our study was that we did not evaluate other SNP of the BDNF gene that might be involved in BDNF production and function. Therefore, we cannot exclude associations between other polymorphism(s) in regulatory or coding regions of BDNF and bipolar disorder. Furthermore, the association between BDNF Val/Met and the suicidal behavior of bipolar patients may have resulted from another unknown functional polymorphism(s) in linkage disequilibrium with BDNF Val/Met.

In conclusion, our findings suggest that the BDNF gene may be associated with susceptibility to suicidal behavior of bipolar patients. Due to genetic and phenotypic heterogeneities in psychiatric disorders, including bipolar disorder, patients satisfying the same formal bipolar disorder diagnostic criteria may present with distinct clinical features that have different genetic bases. Our results may have clinical implications in that they suggest that BDNF Val/Met may be a biological indicator for bipolar patients at risk of suicide. Prospective studies on a larger patient population are required to validate this association.

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