Aims: Neurodegenerative processes may be involved in the pathogenesis of tardive dyskinesia (TD), and a growing body of evidence suggests that brain-derived neurotrophic factor (BDNF) plays a role in both the antipsychotic effects and the pathogenesis of TD. BDNF and glycogen synthase kinase (GSK)-3β are important in neuronal survival, and thus abnormal regulation of BDNF and GSK-3β may contribute to TD pathophysiology. This study investigated the relationship between two polymorphisms, val66met in the BDNF coding region and -50T/C in the GSK-3β promoter, and susceptibility to TD among a matched sample of patients having schizophrenia with TD (n = 83), patients with schizophrenia without TD (n = 78), and normal control subjects (n = 93).

Methods: All subjects were Korean. The BDNF val66met and GSK-3β-50T/C genotypes were determined by polymerase chain reaction and restriction fragment length polymorphism analyses.

Results: Polymerase chain reaction analysis revealed no significant difference in the occurrence of the polymorphisms among the TD, non-TD, and control subjects, but a significant interaction was observed among the groups possessing BDNF val allele in compound genotypes (P = 0.001). We found that the schizophrenic subjects with the C/C GSK-3β genotype, who carry the val allele of the BDNF gene, are expected to have a decreased risk of developing neuroleptic-induced tardive dyskinesia (P < 0.001).

Conclusions: Our results demonstrate that the GSK-3β C/C genotype with the BDNF val allele is associated with patients having schizophrenia without TD. This study also suggests that the BDNF and GSK-3β gene polymorphisms work in combination, but not individually, in predisposing patients with schizophrenia to TD.

Key words: brain-derived neurotrophic factor, glycogen synthase kinase-3β, polymorphism, schizophrenia, tardive dyskinesia.

A MAJOR DIFFICULTY in treating patients with antipsychotic drugs is the possible development of tardive dyskinesia (TD) and extrapyramidal symptoms (EPS), such as parkinsonism, acute dystonia, and akathisia. TD is generally characterized by abnormal involuntary movements of the tongue, lips, face, trunk, and extremities. TD is an irreversible condition, and to date few effective treatment options exist. Although the prevalence of TD is greater among patients with schizophrenia who have been treated with antipsychotic medications, not all patients treated with antipsychotic medications develop TD. This may be partly attributable to biological factors, especially genetic differences, among patients with schizophrenia. Rosengarten et al. suggested that
genetic factors played a role in patients with TD who were treated with antipsychotic medications; other studies have indicated that some individuals may have a genetic predisposition to TD.\(^3,4\)

Antipsychotic drugs are generally accepted to alleviate psychotic symptoms by modifying the mesolimbic dopaminergic system, and EPS are the result of an altered striatonigral dopaminergic system.\(^5\) Several theories have been proposed to explain the pathogenesis of TD, including neurotoxicity and abnormal neurotransmitter regulation.\(^6\)

One hypothesis postulates that oxidative stress could have an important role in the development of TD, holding that treatment with neuroleptics increases radicals as a result of catecholamine metabolism.\(^7,8\)

GSK-3\(^\beta\) is an essential element of the apoptotic signaling cascade, induced by oxidative stress, which may be involved in the pathogenesis of TD.\(^9\) GSK-3\(^\beta\) also participates in neurodevelopment and in the regulation of neuronal plasticity and cell survival.\(^10\)

Recent evidence suggests that the regulation of GSK-3\(^\beta\) may be important in the pathogenesis of schizophrenia. In postmortem studies, patients with schizophrenia showed significantly lower levels of GSK-3\(^\beta\) mRNA, protein, and total GSK-3 enzyme activity.\(^11\)\(^\)\(^\)\(^13\) Recent evidence suggests that the brain-derived neurotrophic factor (BDNF), which is known to play a major role in neurogenesis and neuronal survival, has an important role in the pathogenesis of TD.\(^14\)\(^\)\(^\)\(^15\) Moreover, BDNF levels have been reported to be lower in patients with TD than those without the disease.\(^16\)

Increasing evidence supports a link between GSK-3\(^\beta\) and BDNF, and suggests that GSK-3\(^\beta\) may be a negative regulator of BDNF. Foulstone et al. reported that BDNF inhibited GSK-3\(^\beta\) in cerebellar granule cells by increasing serine-9 phosphorylation.\(^17\) This is consistent with a recent finding that GSK-3\(^\beta\) is suppressed by BDNF-mediated signal transduction in human neuroblastoma SH-SY5Y cells.\(^18\)

Different cell types contain different levels of GSK-3\(^\beta\) mRNA, suggesting that it is regulated by diverse transcriptional regulators. Many regulatory elements have been identified in the region upstream of the start codon.\(^19\) Russ et al. detected several single nucleotide polymorphisms (SNP) in the same region.\(^20\) Of these, -50T/C occurs frequently and is particularly interesting because it is in the effective promoter region (nt -171 to +29) of the gene encoding GSK-3\(^\beta\). However, no functional study of -50T/C in GSK-3\(^\beta\) has yet been reported. A change in amino acids of val to met at position 63 of the BDNF precursor protein may affect the development of the precursor protein into a mature peptide. Egan et al. showed that the BDNF met allele was associated with poorer episodic memory and abnormal hippocampal activation.\(^21\)

In this study, we investigated the association between polymorphisms in the BDNF and GSK-3\(^\beta\) genes as risk factors for antipsychotic-induced TD in patients with schizophrenia. Additionally, we examined the effects of genetic polymorphism and gene–gene interactions of the two polymorphisms among Korean patients with schizophrenia and a normal control group.

**METHODS**

**Subjects**

In addition to our control group, we recruited 161 Korean men and women who had been diagnosed with schizophrenia based on the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I).\(^22\) All subjects with schizophrenia were inpatients or outpatients at psychiatric wards in Busan Paik Hospital, Dong Rae Mental Hospital, or Dong Suh Mental Hospital in Masan, South Korea.

The protocol for this study was approved by the ethics committee at Inje University Paik Hospital. Subjects provided informed consent and were notified that they could withdraw from the study at any time.

Reviews of the drug profiles and durations of medication for all subjects revealed that all subjects with schizophrenia had been taking antipsychotic medication for at least 3 months. We excluded subjects with major physical abnormalities or a history of psycho-stimulant drug abuse.

**Diagnosis of tardive dyskinesia**

This study used Abnormal Involuntary Movement Scale (AIMS) scores to diagnose TD according to previously developed criteria.\(^23\) Subjects scoring two or more two-point ratings, or one or more three-point ratings, in the first seven items of the AIMS were diagnosed with TD. All subjects were evaluated by two psychiatrists.

**Genotyping**

We collected blood samples at each clinical site. Genomic DNA was extracted from the blood using a
QIAamp blood kit (Qiagen, Valencia, CA, USA). Using previously developed methods, with minor modifications, the BDNF val66met and GSK-3β-50T/C genotypes were determined by the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis, with the restriction enzymes Eco72I (Roche Diagnostics GmbH, Mannheim, Germany) for BDNF and AluI (Takara, Tokyo, Japan) for GSK-3β. The BDNF genotypes were categorized as val/val, val/met, and met/met, and the GSK-3β genotypes as T/T, T/C, and C/C.

Statistical analysis
We used the χ²-test and two-tailed Fisher’s exact test to compare differences between groups with respect to genotype and allele frequency. P-values ≤0.05 were deemed statistically significant.

RESULTS

Demographic data
Table 1 presents the demographic data. The study involved a total of 254 subjects, 161 with schizophrenia and 93 normal controls. Of the subjects with schizophrenia, 83 met the criteria for TD. No significant difference in gender, age, education level, illness onset, illness duration, hospitalization duration, or daily dosage of antipsychotic medication was observed between subjects with and without TD.

Analyses of genotype and allele frequencies in the BDNF and GSK-3β genes
No deviation from Hardy–Weinberg equilibrium was observed in either the BDNF or GSK-3β genotypes (all P > 0.05).

Compound genotypes for the BDNF val66met and GSK-3β-50T/C polymorphisms
We observed no significant differences in the val/met, val/met, or met/met BDNF genotype distributions according to the three GSK-3β genotypes between the normal group and the schizophrenic group with TD or without TD (Table 2). However, our analysis showed the differences in the val or met allele frequencies according to the GSK-3β genotypes among the three groups (χ² = 18.719, d.f. = 4, P = 0.001, Table 3). Our results suggest that the schizophrenic subjects with the C/C GSK-3β genotype, who carry the val allele of the BDNF gene, are expected to have a decreased risk of developing neuroleptic-induced TD (23.7% vs 13.3%, OR = 0.101, 95% CI = 0.021–0.480, P = 0.001, Fisher’s exact test).

Table 1. Demographic characteristics of subjects having schizophrenia with and without TD

<table>
<thead>
<tr>
<th>Variable</th>
<th>With TD (n = 83)</th>
<th>Without TD (n = 78)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male/Female (73/10)</td>
<td>Male/Female (68/10)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>41.3 ± 6.0</td>
<td>38.8 ± 7.5</td>
</tr>
<tr>
<td>Education (years)</td>
<td>10.2 ± 3.3</td>
<td>9.8 ± 4.6</td>
</tr>
<tr>
<td>Onset of illness (years)</td>
<td>11.8 ± 6.3</td>
<td>13.0 ± 7.1</td>
</tr>
<tr>
<td>Duration of neuroleptization (months)</td>
<td>84.6 ± 63.3</td>
<td>87.7 ± 79.9</td>
</tr>
<tr>
<td>Chlorpromazine (daily dosage, mg)</td>
<td>448.7 ± 528.5</td>
<td>596.3 ± 405.6</td>
</tr>
</tbody>
</table>

TD, tardive dyskinesia

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DISCUSSION

Our data revealed no difference in genotype or allele frequencies of the BDNF val66met polymorphism among normal controls, patients with TD, and patients without TD, or between the control group and subjects with schizophrenia. A report by Liou et al. demonstrated that neither the val66met genotypes nor the allelic distribution was associated with TD. However, they found a positive association between those heterozygous for the BDNF val/met genotype and orofacial TD symptomatology, and

Table 2. Compound genotypes for the BDNF val66met and GSK-3β-50T/C polymorphisms

<table>
<thead>
<tr>
<th>BDNF val/met</th>
<th>Control ( n = 93 )</th>
<th>Schizophrenia ( n = 161 )</th>
<th>Schizophrenia with TD ( n = 83 )</th>
<th>Schizophrenia without TD ( n = 78 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>v/v</td>
<td>v/m</td>
<td>m/m</td>
<td>v/v</td>
</tr>
<tr>
<td>GSK-3β-50T/C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>N: 4, 8, 1</td>
<td>N: 14, 29, 8</td>
<td>N: 14, 22, 16</td>
<td>N: 4, 7, 1</td>
</tr>
<tr>
<td></td>
<td>%: 4.3, 8.6, 1.1</td>
<td>%: 15.1, 31.2, 8.6</td>
<td>%: 15.1, 31.2, 8.6</td>
<td>%: 15.1, 31.2, 8.6</td>
</tr>
<tr>
<td></td>
<td>%: 30.6, 24.2</td>
<td>%: 16.0, 12.1</td>
<td>%: 30.6, 24.2</td>
<td>%: 30.6, 24.2</td>
</tr>
<tr>
<td>C/C</td>
<td>N: 33, 25</td>
<td>N: 59, 42</td>
<td>N: 33, 22</td>
<td>N: 33, 22</td>
</tr>
<tr>
<td></td>
<td>%: 17.7, 13.4</td>
<td>%: 19.3, 13.3</td>
<td>%: 17.7, 13.4</td>
<td>%: 17.7, 13.4</td>
</tr>
</tbody>
</table>

Control group, schizophrenia with TD, and schizophrenia without TD group versus BDNF val66met genotypes in GSK-3β-50T/C genotypes with: GSK-3β-50T/T \( (\chi^2 = 2.723, \text{d.f.} = 4, P = 0.605) \); GSK-3β-50C/C \( (\chi^2 = 3.326, \text{d.f.} = 4, P = 0.505) \). Control group and schizophrenia versus BDNF val66met genotypes in GSK-3β-50T/C genotypes with: GSK-3β-50T/T \( (\chi^2 = 2.022, \text{d.f.} = 2, P = 0.904) \); GSK-3β-50C/C \( (\chi^2 = 2.055, \text{d.f.} = 2, P = 0.358) \); GSK-3β-50C/C \( (\chi^2 = 1.750, \text{d.f.} = 2, P = 0.417) \).

Table 3. Distribution of compound GSK-3β T/C genotypes according to BDNF val and met allele

<table>
<thead>
<tr>
<th>BDNF val/met</th>
<th>Control ( n = 93 ) (%)</th>
<th>Schizophrenia ( n = 161 ) (%)</th>
<th>Schizophrenia with TD ( n = 83 ) (%)</th>
<th>Schizophrenia without TD ( n = 78 ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>val allele</td>
<td>met allele</td>
<td>val allele</td>
<td>met allele</td>
</tr>
<tr>
<td></td>
<td>v/v</td>
<td>v/m</td>
<td>m/m</td>
<td>v/v</td>
</tr>
<tr>
<td>GSK-3β-50T/C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>16 (8.6)</td>
<td>10 (5.4)</td>
<td>28 (16.0)</td>
<td>20 (12.4)</td>
</tr>
<tr>
<td>T/C</td>
<td>57 (30.6)</td>
<td>45 (26.2)</td>
<td>87 (52.9)</td>
<td>89 (55.1)</td>
</tr>
<tr>
<td>C/C</td>
<td>33 (17.7)</td>
<td>25 (15.4)</td>
<td>59 (36.6)</td>
<td>39 (24.2)</td>
</tr>
</tbody>
</table>

Values for combinations of the BDNF val (met) allele and GSK-3β T/T (T/C or C/C) genotype were expressed relative to the frequency of BDNF val/met and val/met (val/met and met/met) genotype in subjects carrying the GSK-3β T/T (T/C or C/C) genotype.

BDNF, brain-derived neurotrophic factor; GSK, glycogen synthase kinase; m/m: met/met; TD, tardive dyskinesia; v/m: val/met; v/v: val=val;
determined that dyskinetic movements of the trunk and extremities were not associated with this polymorphism. Thus, TD exhibited in various body regions may have a different underlying pathophysiological mechanism. A meta-analysis of case–control studies for an association between BDNF val66met and psychiatric disorders confirmed an association of the val/met and met/met genotypes with eating disorders and substance-related disorders, and of homozygous met/met carriers with schizophrenia.27 Additionally, met allele carriers have been associated with reduced hippocampal volumes in major depression,28 while the frequency of the val allele was significantly elevated in bipolar disorder.29 Although the val66met polymorphism has been shown to be as functional as the met allele, the biological relevance of the val allele in TD remains unknown.

No reported study has previously evaluated GSK-3β polymorphisms in terms of an association with TD. In this study, we investigated the occurrence of a GSK-3β gene polymorphism among Asian subjects having schizophrenia with and without TD and a control group, and between patients with schizophrenia and normal controls. We found that the GSK-3β gene promoter variants had no significant influence on susceptibility to TD in subjects with schizophrenia. In addition, the distribution of the GSK-3β genotypes and alleles did not differ significantly between patients with schizophrenia and controls. The effects of the GSK-3β -50T/C polymorphism on the activity of GSK-3β is unknown. Low activity of GSK-3β has been detected in the brains of subjects with schizophrenia compared to those of control subjects.12 Research has shown that a high dose of haloperidol (1 mg/kg) or risperidone (2 mg/kg) increased the total GSK-3 protein level in the striatum of rats.30 Thus, high doses of both drugs likely saturate dopaminergic D2 receptors in the striatum, causing EPS in humans and inducing catalepsy in rats. However, a 25 mg/kg dose of clozapine, a drug that rarely causes EPS, did not affect the total GSK-3 protein level in the striatum of rats.30 Accordingly, development of TD may be attributable to an abnormality in GSK-3β due to the dopaminergic supersensitivity due to high doses of antipsychotics.

The -50T/C region of the GSK-3β human promoter contained no putative transcription factor binding site in a deletion analysis.19 However, very low expression was found when the region between nt −171 and +29 was deleted, indicating that an essential promoter element had been removed. Ultimately, functional studies are necessary to elucidate whether GSK-3β genotypes are correlated with brain levels of GSK-3β mRNA and protein. Benedetti et al. reported that the better improvement in the recurrence of mood episodes after lithium treatment was found in carriers of the C variant, while homozygotes for the T allele showed a worse response pattern.31 Moreover, C allele carriers with bipolar disorder showed a superior response to lithium augmentation.32 In another study, the GSK-3β C/C genotype showed a later onset of bipolar illness and better acute effects of total sleep deprivation treatment on perceived mood,33 while the T/T genotype has been associated with an increased risk of late-onset Alzheimer’s disease.34 These studies revealed that carriers of the C allele may have a superior protective effect in psychiatric disorders.

Our study revealed a significant association of the C/C GSK-3β genotype and val allele of the BDNF gene between a group of subjects having schizophrenia with TD and a group without TD, consistent with the hypothesis that the interaction of the two polymorphisms with schizophrenia may explain the abnormal involuntary movements related to antipsychotic drug exposure in some patients. Our results showed, for the first time, that compared to the TD group, homozygosity for the GSK-3β C/C genotype was over-represented in the non-TD group carrying the BDNF val allele. Recent studies have demonstrated that the met allele of BDNF is associated with psychiatric disorders. However, the BDNF met allele in the compound GSK-3β genotype was not statistically significant between patients with TD and those without TD. The BDNF val allele in an interaction between BDNF and GSK-3β gene polymorphisms may be related to not developing TD in patients with schizophrenia. BDNF can inhibit GSK-3β through increased serine-9 phosphorylation. Neural plasticity and survival by inhibition of GSK-3β is regulated through the activity of BDNF-mediated signaling.35 Thus, the association between the GSK-3β C/C genotype and the BDNF val allele in the non-TD group may be explained by the C/C genotype lowering GSK-3β activity, relative to C/T or T/T, probably via the BDNF val allele. Further research is necessary to determine whether an interaction between BDNF val/met and GSK-3β -50T/C genotypes is correlated with protein levels of BDNF and GSK-3β in patients with and without TD.

This study had several limitations. First, as it was a retrospective study, completely controlling demo-
graphic variables was not possible. Second, our sample size was relatively small and may have lacked statistical power. Analyzing a combination of genotypes requires many cells in a contingency table, and our study resulted in relatively small counts per cell.

In conclusion, we found similar polymorphic variants of the BDNF and GSK-3β genes among subjects having schizophrenia with TD, those without TD, and normal controls. Nevertheless, we found evidence for an additive effect of these two genes and for an association between TD and these polymorphisms. Our results suggest that a combination of the genetic variations in the BDNF and GSK-3β genes may play a major role in the development of TD. This association needs to be analyzed among subjects with other psychiatric illnesses and in other ethnic groups to establish whether it is a useful marker for TD induced by antipsychotic medications.

ACKNOWLEDGMENTS
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