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Longitudinal associations between *BDNF* promoter methylation and late-life depression

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ABSTRACT

Reduced brain-derived neurotrophic factor (BDNF) function has been suggested as a risk factor for late-life depression. BDNF secretion is influenced by epigenetic (DNA promoter methylation) and genetic (val66met polymorphism) profiles. We investigated the independent and interactive effects of *BDNF* methylation and val66met polymorphism on late-life depression. In total, 732 Korean community residents aged ≥65 years were evaluated, and 521 of them without depression at baseline were followed up 2 years later. Depression was determined using the Geriatric Mental State Schedule, and depression severity was evaluated with the Geriatric Depression Scale. Demographic and clinical covariates were obtained. The effects of *BDNF* methylation and polymorphism on the diagnosis of depression were investigated using a multivariate logistic regression model, and the relationships between *BDNF* methylation and depression severity were evaluated using partial correlation tests. Higher *BDNF* methylation was independently associated with the prevalence and incidence of depression and severe depressive symptoms. No significant methylation-genotype interactions were found. *BDNF* promoter methylation could be a proxy biomarker for depression late in life.

1. Introduction

Depression is the most common psychiatric disorder among elderly people (Alexopoulos, 2005). Late-life depression is associated with an elevated risk of suicide and functional and cognitive impairments, which confer a risk of dementia (Alexopoulos and Kelly, 2009; Dintz et al., 2013). Considering these negative consequences of geriatric depression, understanding the etiology of depression is an important step toward early detection and effective treatment of depression in the elderly population.

Studies have suggested that late-life depression has a complex and multifactorial etiology. The complex interplay between biological factors (e.g., hypothalamic-pituitary-adrenal axis dysfunction, neurodegenerative changes, and vascular and genetic mechanisms) and psychosocial factors (e.g., stressful life events and decreased social support) reportedly contributes to the occurrence of late-life depression (Belmaker and Agam, 2008; Kim et al., 2007; Smith et al., 2007). Among those etiologies, there is growing interest in brain-derived neurotrophic factor (BDNF) as a neurobiological mechanism of depression. BDNF is critical for the growth, survival, and differentiation of neuronal cells and for neuronal plasticity (Hu and Russek, 2008; Huang and Reichardt, 2001). Several studies that assessed BDNF levels in patients with depression found that plasma BDNF levels were significantly lower in geriatric depressed patients compared with controls (Chu et al., 2012; Laske et al., 2010; Shi et al., 2010). Genetic studies have shown that *BDNF* val66met polymorphisms are associated with an increased risk of depression in elderly patients (Hwang et al., 2006; Talyor et al., 2007). Additionally, studies on the association between *BDNF* val66met polymorphisms and brain parameters such as hippocampal volume and white-matter hyperintensities have suggested that the met allele is associated with decreased BDNF secretion (Egan et al., 2003), conferring a risk of depression (Kanellopoulous et al., 2011; Talyor et al., 2011).

BDNF expression is also regulated by epigenetic chromatin remodeling in gene promoter regions. In the central nervous system, DNA methylation of cytosines in cytosine-guanine (CpG)

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dinucleotides is regarded as the representative component of broader epigenetic modification at a given locus (Hochberg et al., 2010). Increased CpG methylation at promoter regions of the *BDNF* gene is reportedly correlated with the decreased synthesis of BDNF in neurons (Martinowich et al., 2003). Such studies focused on the adult population and showed that increased *BDNF* methylation was correlated with depression in the general population (Fuchikami et al., 2011) and with suicidal ideation in patients with major depression (Kang et al., 2013a). Based on these findings, we hypothesized that the *BDNF* DNA methylation status is associated with depression in elderly individuals. To our knowledge, no investigation has been conducted on the role of *BDNF* promoter methylation and its interaction with *BDNF* polymorphism in the higher prevalence and burden of depression among older adults. This study aimed to investigate whether *BDNF* promoter methylation and val66-met polymorphism are independently or interactively associated with the prevalence and incidence of depression in late life using data from a longitudinal study of an older Korean community population.

2. Materials and methods

2.1. Study overview and participants

This analysis was performed using data from a community-based prospective survey of late-life psychiatric morbidity carried out in Kwangju, South Korea (Kim et al., 2007, 2013c). All community residents aged ≥65 years within 2 geographic catchment areas (1 urban, 1 rural) were identified from the national registration lists and approached to participate at baseline. Attempts were made to follow up with all the participants 2 years later (mean [standard deviation {SD}] interval, 2.4 [0.3] years). Depression was examined at baseline and at follow-up; all other data used in this study were obtained at baseline. After all the participants had been provided a complete description of the study, written informed consent was obtained at each examination. This study was approved by the Chonnam National University Hospital Institutional Review Board.

2.2. Evaluation of depression

Depression was assessed using the community version of the Geriatric Mental State (GMS B3) diagnostic schedule (Copeland et al., 1986). This is a fully structured diagnostic instrument for evaluation of mood disorders, with an accompanying computerized algorithm widely used in international epidemiological research with older people. The GMS B3, which takes approximately 30–40 minutes, was administered by specifically trained interviewers. Its accompanying computerized algorithm (AGECAT) provides likelihoods of individual diagnoses on a 0-to-5 scale. The GMS B3 was translated into Korean according to a formal standardization process (Kim et al., 2003). The GMS B3 and AGECAT are designed to define current depression in the last month at a level of severity warranting clinical intervention. As in other studies, participants rated as AGECAT ≥3 were considered likely to have depression. The GMS AGECAT depression criteria encompass both moderate and severe symptomatologies and, therefore, define a broader syndrome than that defined by the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition (major depression). The prevalence of depression at baseline was measured. To analyze the incidence of depression, the sample was restricted to those without depression at baseline, and depression at follow-up was treated as a binary dependent variable. Additionally, depression severity was measured with the Geriatric Depression Scale (Yesavage et al., 1982–1983) at both baseline and follow-up.

2.3. BDNF *promoter methylation and genotyping*

The DNA methylation status of the *BDNF* promoter was investigated using blood samples obtained from the subsample of participants who provided consent for this procedure. The correlation between gene expression in peripheral tissue and physiologically meaningful gene expression in the brain is not clear. However, it has been shown that BDNF may cross the blood-brain barrier and that postnatal platelet BDNF shows changes similar to those in the brain (Karege et al., 2005), suggesting parallel changes in BDNF at the blood and brain levels. Furthermore, many previous studies have suggested an association between the *BDNF* methylation status in peripheral blood and psychiatric symptoms such as depression (Devlin et al., 2010; Fuchikami et al., 2011). DNA was extracted from venous blood using standard procedures. The *BDNF* promoter region used to analyze the methylation status is presented in Fig. 1. These data have been deposited in the GenBank (accession number: BankIt1568919 BDNF JX848620). A CpG-rich region of the promoter lying between −612 and −463 relative to the transcriptional start of exon VII, that included 9 CpG sites, was analyzed, as in other studies (Devlin et al., 2010; Roth et al., 2009). This region was chosen because it has been reported to correspond to an analogous region in rat *BDNF* that is differentially methylated and associated with *BDNF* messenger RNA (mRNA) expression (Roth et al., 2009) and because it has been investigated in relation to antenatal depression (Devlin et al., 2010). Genomic DNA (1 μg) was extracted from leukocytes using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer's suggested protocol. The DNA then underwent bisulfite treatment using the EpiTech Bisulfite Kit (Qiagen) according to the manufacturer's protocol. A 118-bp fragment of the *BDNF* promoter was amplified by polymerase chain reaction (PCR) from bisulfite-treated DNA using the forward and reverse primers designated in Fig. 1. PCR conditions were 95 °C for 15 minutes, followed by 45 cycles of 95 °C for 15 seconds, 57 °C for 30 seconds, and 72 °C for 15 seconds, with a final extension of 5 minutes at 72 °C. PCR products were sequenced using the PSQ 96M Pyrosequencing System (Biotage) according to the manufacturer's protocol using the sequencing primers designated in Fig. 1. The methylation percentage at each CpG region was quantified using Pyro Q-CpG software, version 1.0.9 (Biotage). Because the *BDNF* promoter methylation percentage at CpG sites 2, 6, and 8 was 100% in all participants, these 3 sites were excluded from the analyses. The individual methylation percentages at the remaining 6 CpG sites and their average values were used in the analyses. For genotyping, PCR and PCR-based restriction fragment length polymorphism assays were performed. The primer sequences used were as follows: forward primer 5′-ACTCTGGAGAGCGTGAATGG-3′ and reverse primer 5′-ACTACTGAGCATCACCCTGGA-3′. The amplification conditions were predenaturation at 95 °C for 5 minutes, followed by 40 cycles of denaturation at 95 °C for 30 seconds, 62 °C for 30 seconds, and 72 °C for 30 seconds, post-elongation at 72 °C for 5 minutes, and a final maintenance step at 4 °C. The PCR products were digested at 37 °C with the corresponding restriction enzyme (*Eco*72I), and gel electrophoresis was used to detect the 196G (*val*, 99- and 72-bp fragments) and 196A (*met*, 171-bp fragment) alleles. The genotype was categorized as *val*/*val*, *val*/*met*, or *met*/*met*.

2.4. Demographic and clinical characteristics

Demographic and clinical characteristics potentially associated with depression were considered. Data on age, sex, years of