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Research report

Association of brain-derived neurotrophic factor DNA methylation and reduced white matter integrity in the anterior corona radiata in major depression

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ABSTRACT

Considerable evidence suggests a crucial role for the epigenetic regulation of brain-derived neurotrophic factor (BDNF) in the pathophysiology of major depressive disorder (MDD). However, the relationship between *BDNF* DNA methylation and white matter (WM) integrity in MDD has not yet been investigated. In the current study, we examined the association between the DNA methylation status of the *BDNF* promoter region and WM integrity in MDD. Sixty patients with MDD and 53 healthy controls underwent T1-weighted structural magnetic resonance imaging (MRI), including diffusion tensor imaging (DTI), to assess their WM integrity. *BDNF* DNA methylation at 4 CpG sites of the promoter region was also measured.

As compared to healthy controls, the MDD group demonstrated reduced fractional anisotropy (FA) in the bilateral anterior and posterior corona radiata (ACR and PCR), genu of the corpus callosum, and the bilateral posterior thalamic radiations. We observed a significant inverse correlation between the DNA methylation of the *BDNF* promoter region and the FA of the right ACR in MDD patients.

Our findings demonstrate a relationship between methylation of the *BDNF* promoter region and the integrity of the ACR, a key structural component of the emotional and cognitive control network involved in the pathophysiology of MDD. This correlation suggests that *BDNF* DNA methylation may contribute to structural WM changes in MDD patients.

1. Introduction

Major depressive disorder (MDD) is one of the most common psychiatric illnesses and inflicts a major socioeconomic burden on mental health and society. It is projected to become the leading cause of disease burden worldwide by 2030 (Lepine and Briley, 2011). In recent decades, intensive effort has been undertaken to elucidate the neurobiological basis of MDD, which has led to several important working models of the disease. Among them, the "neurotrophic hypothesis of depression" proposes that alterations in several neurotrophic factors can precipitate MDD by modulating synaptic connectivity and neurotransmission (Alexopoulos et al., 2010). One major neurotrophic factor, brain derived-neurotrophic factor (BDNF), is well-known to have an important role in neuronal plasticity, differentiation, survival, and function (Dwivedi, 2009). Numerous genetic investigations of the *BDNF* gene have linked this gene to dysfunctions in intracellular trafficking, activity-dependent release of the BDNF protein, and ultimately to impaired neuronal function (Chen et al., 2004; Egan et al., 2003). Polymorphisms in this gene are known to be correlated with various MDD-related phenotypes including vulnerability to MDD, as well as responses to antidepressant treatments (Dwivedi, 2009).

In recent years, studies have suggested an important interplay between genetic and environmental factors in the pathophysiology of MDD (Surtees et al., 2006). Epigenetic regulatory processes have now come into the spotlight to explain the mediating role of social environmental factors, such as childhood adversity or stressful life events, on gene expression (Carballedo et al., 2012b). Epigenetic regulation of the *BDNF* gene expression by DNA methylation or histone acetylation is thought to be a key mechanism by which gene-environment interactions contribute to MDD (Boulle et al., 2012). Specifically, increased cytosine-guanine

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(CpG) methylation at the *BDNF* promoter region has been associated not only with MDD (Fuchikami et al., 2011), but also borderline personality disorder (Perroud et al., 2013), post-stroke depression (Kim et al., 2013), antidepressant treatment response (Tadic et al., 2014), and suicide (Kang et al., 2013). Correspondingly, animal studies have shown an inverse correlation between CpG methylation of the *BDNF* promoter region and *BDNF* gene expression in neuronal cells (Martinowich et al., 2003). Hence, it has been proposed that CpG methylation status is a good candidate for the biological marker of MDD, and is deeply involved in the pathophysiology of MDD (Fuchikami et al., 2011).

Several studies have reported that the *BDNF* genotype (Val66-Met polymorphism) can influence brain structure, particularly regional gray matter volume (Gerritsen et al., 2012) and WM integrity (Carballedo et al., 2012a), and that this may influence the dysfunctional limbic-cortical network activity found in MDD. Cumulative evidence also suggests the potential of *BDNF* DNA methylation to influence the interplay between genetic variants and environmental factors in the development of MDD (Boulle et al., 2012). The above has led to the presumption of a potential central role of epigenetic regulation of *BDNF* expression on brain morphological changes. Although studies have postulated that environmental factors might impact brain morphologic changes via epigenetic mechanisms (Carballedo et al., 2012b), there have not been studies to date showing a direct correlation between epigenetic gene regulation and structural brain alterations. With current methodological advances combining genetic and structural brain imaging studies, the influence of the *BDNF* together with its epigenetic regulation can be comprehensively explored to elucidate the etiology of MDD.

Novel neuroimaging techniques using diffusion tensor imaging (DTI) and Tract-Based Spatial Statistics (TBSS) have made it possible to detect disease-related microstructural abnormalities on the white matter (WM) tracts of MDD patients in a more sensitive and accurate manner by measuring tissue fractional anisotropy (FA) or mean diffusivity (Murphy and Frodl, 2011). These novel tools have further enabled researchers to assess microstructural changes in WM due to both genetic and environmental factors in MDD patients (Murphy et al., 2012). Numerous DTI studies have indicated that WM tracts involved in cortico-limbic circuit of emotional regulation demonstrated impaired integrity in MDD patients, for example, superior longitudinal fasciculus, genu of corpus callosum (GCC), uncinated fasciculus, internal and external capsule, cingulum, and anterior corona radiata (ACR) (Murphy and Frodl, 2011). Several recent studies using DTI in MDD patients have reported that the *BDNF* genotype can impact WM integrity of above-mentioned brain regions. (Alexopoulos et al., 2010; Carballedo et al., 2012b). However, there have been no studies to date examining the link of *BDNF* DNA methylation changes with WM structural changes in MDD patients.

In the current study, we used DTI and TBSS to study the relationship of epigenetic *BDNF* DNA methylation with structural alterations in the WM tracts of MDD patients. Our *a priori* hypothesis was that decreased integrity in WM tracts related to cortico-limbic circuit abnormalities in MDD patients would be associated with increased methylation of CpG sites at the *BDNF* promoter regions.

2. Experimental procedures

2.1. Participants

A total of 60 patients with a diagnosis of MDD were recruited from the outpatient psychiatric clinic of Korea University Anam Hospital located in Seoul, South Korea. Inclusion criteria for the MDD patient group consisted of an ongoing major depressive episode with a minimum score of 8 on the 17-item Hamilton Depression Rating Scale (HDRS-17). The exclusion criteria were as follows: (1) comorbidity with any other major Axis I or Axis II psychiatric illness within the last 6 months based on the DSM-IV criteria; (2) MDD with psychotic features; (3) comorbidity with another serious medical condition or a primary neurological condition such as cerebrovascular disease, Parkinson's disease, or epilepsy; (4) any contraindication for MRI. Board- certified psychiatrists confirmed the diagnosis of all the recruited patients using the Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I) and assessed the disorder durations using the Life-Chart Methodology. Fifty three age- and sex-matched healthy controls were recruited by community advertisements. Board-certified psychiatrists confirmed the absence of past or present psychiatric disorder in these subjects.

The age of participants in both the MDD patient and control subject groups ranged from 20 to 64 years. All participants were right-handed according to the Edinburgh Handedness Inventory (Oldfield, 1971). The severity of depressive symptoms was assessed using the HDRS in both groups on the day of the MRI scans. In accordance with the Declaration of Helsinki, all the subjects gave informed consent to participate in the study. The protocol of this study was reviewed and approved by the ethics committee of the Korea University Anam Hospital. During the time of the study, 47 patients were receiving selective serotonin reuptake inhibitors (SSRIs) and 5 patients were receiving serotonin-norepinephrine reuptake inhibitors (SNRIs). Twenty six of the patients received only one type of anti-depressant whereas 28 of the patients received combinations of two or more types. Six patients were medication-naïve.

2.2. Analysis of BDNF DNA methylation

Peripheral blood samples were obtained from 60 patients with MDD and 53 healthy controls for analysis of *BDNF* DNA methylation. The DNA methylation of the *BDNF* promoter region was 4 CpG sites in CpG-rich region of the promoter between −694 and −577 (relative to the transcriptional start, CpG1 = −675, CpG2 = −682, CpG3 = −686, CpG4 = −688). The *BDNF* promoter region was analyzed in previous studies investigating association between *BDNF* DNA methylation status and MDD or suicide (Roth et al., 2009; Devlin et al., 2010; Kim et al., 2013; Kang et al., 2013). It is well known that this region is correspondent with an analogous region in rat Bdnf, and that the methylation status of rat Bdnf was correlated with expression of Bdnf mRNA (Devlin et al., 2010). The *BDNF* DNA methylation was analyzed as previously described using the bisulfite pyrosequencing method (Kang et al., 2013). PCR and sequencing primers were designed using the Pyrosequencing Assay Design Software v2.0 (Qiagen). The PCR reaction was carried out in a volume of 20 μl with 20 ng or more converted DNA, 2.5 μl of 10 × Taq buffer, 5 U Hot/Start Taq polymerase (Enzynomics, Korea), 2 μl of each 2.5 mM dNTP mixture, 1 μl of 10 pmol/μl Primer-S, and 1 μl of 10 pmol/μl biotinylated-Primer-As. The amplification was carried out according to the general guidelines suggested for pyrosequencing: denaturation at 95 °C for 10 min, followed by 45 cycles at 95 °C, 55 °C, and 72 °C, each for 30 s, followed by a final extension cycle at 72 °C for 5 min. The PCR reaction (2 μl) was confirmed by electrophoresis in a 2% Agarose gel and visualized by ethidium bromide staining. ssDNA templates were prepared from 16to 18 μl of biotinylated PCR products using streptavidin Sepharose® HP beads (Amersham Biosciences, Sweden) and following the PSQ 96 sample preparation guide for multichannel pipettes. Fifteen picomoles of the respective sequencing primers were used for analysis. Sequencing was performed on a PyroMark ID