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Preliminary communication

Influence of BCL2 gene in major depression susceptibility and antidepressant treatment outcome

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ABSTRACT

Background: Our recent work indicated that low-expression of the anti-apoptotic protein B-cell/lymphoma 2 (Bcl-2) mRNA was observed among untreated major depressive disorder (MDD) patients, and the subsequent altered level of Bcl-2 was found to be close to the antidepressant treatment outcome. The primary aim of this present study was to examine whether a particular gene, encoding Bcl-2 (*BCL2*) confers risk to MDD, and likewise to investigate whether this gene acts as an indicator of antidepressant treatment outcome.
Methods: We enrolled 178 treatment-resistant depression (TRD) and 612 non-treatment-resistant depression (NTRD) patients as well as 725 healthy controls. In total, three selected tagging SNPs (tagSNPs) of *BCL2* (rs2279115, rs1801018 and rs1564483) were genotyped to test for possible association. Using TaqMan relative quantitative real-time polymerase chain reaction (PCR), we analyzed leukocytic expression of *BCL2* mRNA in 47 healthy subjects.
Results: Of the three SNPs, we observed no significant differences in genotype and allele frequencies between the MDD and control groups as well as between the TRD and NTRD groups. However, we found a significant association between the rs2279115C allele and TRD in males (corrected $P=0.048$) but not in females. Further real-time quantitative PCR analysis in healthy subjects revealed that the rs2279115 polymorphism significantly influenced *BCL2* mRNA expression ($P=0.03$).
Limitations: This is a preliminary investigation with relatively small sample size and cross-sectional design.
Conclusions: These initial findings strengthen the hypothesis that *BCL2* may play an important role in mediating the outcome of antidepressant treatment, a result that may further be confirmed by future genetic studies from large-scale populations that can overcome the limited sample size of this preliminary finding.

1. Introduction

Major depressive disorder (MDD) is a severe mental disorder with high levels of accompanying morbidity and mortality. Being prevalent in approximately 15% of the population worldwide, MDD is also among the leading causes of disability. While our current understanding of the etiology of MDD is fragmentary, family and twin studies have established that genetic factors are the most robustly validated, and consequently most likely, risk factors for the disorder (Zhang et al., 2013b). Accordingly, it is credible to speculate that identifying susceptibility genes may eventually lead to targeted "cure therapeutics" (Insel and Scolnick, 2006), giving impetus to identifying the underlying susceptibility genes and genetic factors associated with MDD.

To date, the accumulated neuropathological findings have elucidated several interesting findings regarding the brains of subjects with MDD: smaller brain tissue volumes (Gudmundsson et al., 2013), decreased neuropil (Cobb et al., 2013), and fewer neuronal progenitor cells (Boldrini et al., 2013). Though the pathophysiological basis of these supposed defects is unclear, the basic role of apoptosis – the physiological process of cell death that occurs as part of normal development – in MDD has attracted considerable attention (Wang et al., 2013a).

Apoptosis generally serves to eliminate excess neurons and maintain synaptic stability in brain, but growing evidence from neuroimaging and neuroimmunological studies imply that MDD can be accompanied by a combination of destructive autoimmune

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reactions and increased apoptotic activity (Eilat et al., 1999; Wang et al., 2013a). Fortunately, apoptosis can be prevented or even reversed through antidepressant treatments (Lucassen et al., 2004); by extension, this reality implicates apoptosis in the etiology of MDD.

The anti-apoptotic protein B-cell/lymphoma 2 (Bcl-2) inhibits most types of apoptotic and necrotic cell death, and also seems to act as a major regulator of neural plasticity and cellular resilience (Liu et al., 2013). Increased vulnerability to Bcl-2-related apoptosis induced by physiological stressors has been reported to contribute to the reductions in regional cerebral volumes, neurons and glial cells in MDD patients (Drevets et al., 2008). Our previous work similarly revealed that untreated MDD patients had lower levels of Bcl-2 mRNA expression than healthy controls (Hong et al., 2012). A preclinical study further indicated that BAG1, a Bcl-2-associated athanogene, plays a key role in affective resilience and in regulating recovery from both manic- and depressive-like behavioral impairments (Maeng et al., 2008). From a pharmacological perspective, the expression of Bcl-2 can be upregulated by antidepressant drugs, such as fluoxetine (a selective 5-hydroxytryptamine uptake inhibitor) and moclobemide (a monoamine oxidase-A inhibitor) (Chiou et al., 2006a, 2006b) *as well as atypical antipsychotics, such as quetiapine* (He et al., 2009)*, which has recently been approved for augmentation to antidepressant therapy in patients with MDD* (Bauer et al., 2013). Meanwhile, our previous study also indicated that the expression of Bcl-2 following antidepressant treatment appeared to have a specific relationship with treatment response. Acute antidepressant treatment can increase the mRNA expression of Bcl-2 only in non-treatment-resistant depression (NTRD) patients, but not among those with TRD (Hong et al., 2012). These findings collectively support the potential role of Bcl-2 in the etiology of MDD, and antidepressant treatment outcome.

At the molecular level, the gene encoding Bcl-2 (*BCL2*) is located at chromosome 18q21, which was previously reported to be positive linkage with MDD (LOD=3.75) (Camp et al., 2005). Accordingly, we hypothesized that *BCL2* may be a promising candidate gene associated with MDD. In this study, we first used a sample of MDD patients to examine whether the *BCL2* gene is associated with MDD susceptibility. Second, we investigated whether this gene could predict the antidepressant treatment outcome of MDD patients.

2. Materials and methods

All procedures for this study were reviewed and approved by the Institutional Review Boards of the Shanghai Mental Health Center and other participating institutions. This study was strictly performed in accordance with the Declaration of Helsinki. Written informed consent was provided by each participant prior to any procedures related to this study being performed.

2.1. Subjects

For the current study, we recruited 790 MDD patients. All patients were inpatients and outpatients from our "OPERATION" (OPtimized trEatment stRAtergies for Treatment-resIstant depressiON) study, "OPERATION-ECMA" (OPERATION Extended-Climbing Mountain Action plan) study, and "CARE-SSD/MDD" (Construct A Rough Evaluation index system for subsyndromal symptomatic depression and major depressive disorder) study. The sample characteristics have been described previously (Zhang et al., 2013a). Briefly, patients between the ages of 18 and 65 years with a diagnosis of MDD based on the criteria of the DSM-IV for MDD (and not as a secondary to any other Axis I disorder) were screened for the study. To be eligible, they had to meet the stage II TRD criteria described by Rush et al. (2006). *Stage II TRD in this study was determined retrospectively in 451 patients and prospectively in 339 ones by one of the following*: (1) According to patients' medical records, they had a failed response to 2 or more adequate treatments from different classes of antidepressants (including selective reuptake inhibitors SSRIs, SNRIs or tricyclic antidepressants TCAs) in the current depressive episode; (2) Patients who did not respond to an initial adequate antidepressant treatment based on their medical records were treated with an antidepressant from a different class. If they had a smaller than 50% reduction in the 17-item Hamilton Rating Scale for Depression (HRSD-17) total score after a 6-week treatment, they were considered to have stage II TRD; (3) Those who did not take any antidepressant according to their previous treatment and family histories and other clinical considerations for 6 weeks. Those who failed the initial 6-week treatment were treated with a second antidepressant from a different class for an additional 6 weeks. If they failed the second treatment again, they were also considered to have stage II TRD. In addition to meeting the criteria for stage II TRD, participants also had to have a baseline HRSD-17 total score of 17 or greater. HRSD-17 assessments were conducted by two experienced psychiatrists independently (inter-rater reliability, Kappa=0.84) to assess the depressive symptoms of MDD patients, and the outcome measure was the occurrence of TRD. A set of screening laboratory tests and a physical examination before recruitment were completed to ensure all participants were physically healthy for the study and could be included in our present analysis.

Patients were excluded for several different criteria: if they had a lifetime diagnosis of bipolar disorder, schizoaffective disorder, schizophrenia, or other psychotic disorders; if they had an imminent risk for suicide or homicide judged by a research psychiatrist; if they had any medical contraindication to antidepressants or other psychotropic medication; if they had an unstable general medical condition or a condition that required the combination treatment of an antidepressant and any other psychotropic medication (including typical/atypical antipsychotic agents, mood stabilizers, anticonvulsants, and stimulants); *if they dropped out with any reason*; or if they had modified electroconvulsive therapy with one month of the study screening. In addition, female patients were excluded if they were pregnant, planning to become pregnant, or breast-feeding during the study period.

Control subjects (N=725) were enrolled from among the hospital staff and students of the School of Medicine in Shanghai who had been interviewed by a specialized psychiatrist using the Structured Clinical Interview for DSM-IV-TR Axis I Disorders-Patient Edition (SCID-P) (Wang et al., 2012, 2013b). Subjects with any psychiatric disorder and chronic physical disease were excluded from our analysis.

2.2. SNP selection and genotyping

The human *BCL2* gene located in chromosome 18q21, spans a region of 195 kb with 3 exons. We chose three common tagSNPs of the from a Chinese Han population, including rs2279115, rs1801018 and rs1564483 (Xu et al., 2013). These SNPs were selected as they are the most frequently studied SNPs located in the functional region of the 5′-promoter, exon-2 and 3′-untranslated region (UTR) of *BCL2*.

Venous peripheral blood samples were collected from all participants in 5 mL EDTA vacuum tubes. Genomic DNA was isolated from peripheral blood using a Tiangen DNA isolation kit (Tiangen Biotech, Beijing, China) according to standard procedures, and then stored at −80 °C until genotyping. After quality assessment, DNA samples (250 ng/each) were genotyped using the TaqMan SNP Genotyping Assay (Applied Biosystems, Foster City,