Efficacy of Cognitive Behavioral Therapy versus Selective Serotonin Reuptake Inhibitors at Increasing Peripheral Brain-Derived Neurotrophic Factor in Depressed Patients With or Without the Val66Met Polymorphism

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Abstract

Brain-derived neurotrophic factor (BDNF) is implicated in learning, memory, and behavioral processes. Low BDNF levels are associated with depressive symptoms, and selective serotonin reuptake inhibitors (SSRI) have been shown to significantly increase peripheral and brain BDNF levels. However, recent studies reveal that presence of the BDNF Val66Met polymorphism decreases BDNF expression and efficacy of antidepressants. Research on antidepressant treatment in rodents and humans suggests that drug therapy is not as effective at reducing symptoms and normalizing BDNF levels when the BDNF Val66Met polymorphism is present or when BDNF signaling pathways are impaired. These findings imply that the Val66Met polymorphism might serve as a biomarker for individuals unresponsive to antidepressants. This novel study will therefore assess for differential responses to cognitive behavioral therapy (CBT) and the SSRI paroxetine in individuals with and without the polymorphism. Participants diagnosed with major depressive disorder will be matched according to BDNF genotype and randomly assigned to receive CBT, paroxetine, or both CBT and paroxetine. Between-group differences of pre and post-treatment BDNF levels and depression scores will be analyzed to improve current understanding of the relationship between the Val66Met polymorphism and treatment type.

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The *Diagnostic and Statistical Manual of Mental Disorders* (DSM;APA, 2000) characterizes depression as having significantly impairing symptoms such as anhedonia, depressed mood, difficulties with sleep, fatigue, feelings of worthlessness or guilt, change in body weight, problems of mentation, and persistent thoughts of death. Anxiety, an associated feature of depression, can also cause significant impairment and distress, and several disorders are categorized with some form of anxiety as the defining feature (APA, 2000). The Global Burden of Disease project by the World Health Organization reported that depression is currently the second leading contributor to premature mortality and diminished quality of life for individuals between the ages of 15 to 44, and this estimate is expected to represent individuals of all ages by the year 2020 (World Health Organization [WHO], 2011). According to the National Institute of Mental Health, the prevalence of depression for adults in the United States is 6.7% with about 2% of this population presenting with symptoms classified as severe (Kessler, Chiu, Demler, &Walter, 2005). Such compelling statistics and the potentially incapacitating nature of anxiety and depression emphasize the need for further research on these topics. Studies that aim to elucidate the etiology of mental health problems and to lessen symptom severity can improve quality of life for many individuals.

Research addressing the biological mechanisms of psychological constructs is one way to gain a better understanding of mental health disorders. For instance, brain-derived neurotrophic factor (BDNF) and its receptor tyrosine kinase B (TrkB) are implicated in neurodegenerative diseases and neuronal functioning, synaptic plasticity, proliferation, turnover, and survival as

well as learning, memory, depression, and anxiety (Alexander et al., 2010; Gatt et al., 2008, 2009; Koponen et al., 2005; Lee & Kim, 2010; Saarelainen et al., 2003; Sairanen, Lucas, Ernfors, Castren, M., & Castren, E., 2005; Schofield et al., 2009). More specifically, studies have shown that low BDNF levels (Fujimura et al., 2002; Karege et al., 2002; Lee & Kim, 2010; Rasmusson, Shi, & Duman, 2002), BDNF-mediated neuronal plasticity, and the BDNF Val66Met polymorphism are all associated with depression (Koponen et al., 2005; Haapasalo et al., 2002; Saarelainen et al., 2003; Sairanen et al., 2005). Anti-depressant medications are effective at increasing BDNF and improving associated behavioral deficits (Gervasoni et al., 2005; Saarelainen, 2003). However, the efficacy of antidepressants such as selective serotonin reuptake inhibitors (SSRI) is inhibited in mice exhibiting either impaired BDNF signaling or the Val66Met polymorphism (Chen et al., 2006; Martinowich, Manji, & Lu, 2007; Sairanen et al., 2005). This potential limitation of antidepressant treatment in individuals with the BDNF met allele suggests that research is needed that compares the efficacy of traditional therapies such as cognitive behavioral therapy (CBT) with SSRIs. This study therefore examines the effectiveness of CBT versus SSRI treatment at increasing peripheral BDNF levels and alleviating symptoms in depressed patients with and without the Val66Met polymorphism.

Biology of BDNF

In order to provide a comprehensive understanding of this project, it is necessary to include a brief biological background. Neurotrophins are a growth-factor family of proteins widespread in the mammalian brain (Hwang et al., 2006). These proteins can impact memory and health as well as neuronal survival, synaptic plasticity, and response to injury (Teng, Felice, Kim, & Hempstead, 2010). The neurotrophin family includes the proteins neurotrophin 3, neurotrophin 4, nerve growth factor, and BDNF (Teng et al., 2010). BDNF is especially

significant because of its implications in mental health and its large abundance in the adult brain (Hwang et al., 2006).

Transcription of the BDNF protein by the BDNF gene (gene is denoted in italics as *Bdnf*) is complex as *Bdnf* may have as many as nine different promoters (Pruunsild, Kazantseval, Aid, Palm, & Timmusk, 2007). Antidepressants interact with some but not all of these promoter sites (Martinowich et al., 2007), and protein transcription facilitated by various promoters has different effects (Sakata et al., 2009). For instance, promoter III has been associated with epigenetic regulation (Martinowich et al., 2007), and promoter IV impacts GABAergic functioning (Sakata et al., 2009). Sakata et al. (2009) generated mice with a GFP-STOP cassette inserted into the exon IV locus in order to prevent translation but not transcription of BDNF protein from promoter IV. Interruption of translation rather than a promoter IV knockout was employed to avoid compensatory responses by other promoters (Sakata et al., 2009). This study revealed that promoter-IV transcribed BDNF protein is required for normal GABAergic transmission, cortical spike-timing dependent synaptic potentiation, and activity-dependent BDNF expression in the prefrontal cortex (Sakata et al., 2009).

BDNF is first produced as a propeptide precursor in the endoplasmic reticulum (Mowla et al., 1999). ProBDNF is then folded in the trans-Golgi, placed into secretory vesicles, and directed to either the constitutive (passive) release or the regulated (active or induced) release pathway (Mowla et al., 1999). ProBDNF can be protealytically cleaved to make mature or mBDNF (Friedman & Greene, 1999), and both proBDNF and mBDNF have significant albeit opposing biological functions (Volosin et al., 2006). The main receptors associated with BDNF are TrkB and p75 neurotrophin receptor ($p75^{NTR}$). As described below, it is partially through

these receptors that proBDNF and mBDNF elicit various responses on brain and behavior (Volosin et al., 2006; Klein et al., 1991).

Clinical Implications of BDNF

Neurotrophin hypothesis of depression. The neurotrophin hypothesis of depression emphasizes the interplay between antidepressant medications, neurotrophic regulation, and behavior. Early studies with rodents used stress as a model for depression. Stress, strongly implicated in the biopsychosocial model of depression, decreased hippocampal *Bdnf* mRNA in rodents exposed to foot shock, social defeat, and maternal stress (Rasmusson et al., 2002; Roceri, Hendriks, Racagni, Ellenbroek, & Riva, 2002). In humans, BDNF was significantly decreased in peripheral blood cells of depressed patients who had recently attempted suicide (Gonul et al., 2005; Karege et al., 2002; Lee & Kim, 2010). However, treatments such as antidepressant administration and electroconvulsive shock therapy increased expression of *Bdnf* and its receptor TrkB in the rodent hippocampus and cortex (Nibuya, Morinobu, & Duman, 1995; Russo-Neustadt, Bear, Huan, & Cotman, 2000). Similarly, exogenous BDNF infused into the hippocampus resulted in anti-depressant like behaviors in mice (Shirayama, Chen, Nakagawa, Russell, & Duman, 2002). A study by Koponen et al. (2005) further revealed that transgenic mice over-expressing the full-length BDNF receptor TrkB had less behavioral despair as evidenced by increased latency to immobility in the forced swim test (Koponen et al., 2005).

In humans, antidepressant treatment increased BDNF immunoreactivity in the anterior hippocampus (Chen et al., 2001). Frozen postmortem hippocampal sections of individuals treated with antidepressants at time of death were compared to hippocampal sections of individuals who had not received medications. The treated tissues were stained with antibody

markers for BDNF, and more BDNF was seen in the medicated versus the non-medicated patients (Chen et al., 2001).

In addition, studies have shown that BDNF mediates response to antidepressant treatment. Saarelainen et al. (2003) produced mice with inhibited TrkB signaling. In the forced swim test (used to screen for reactivity to antidepressant medication in mice), mice with inhibited TrkB functioning were unresponsive to antidepressant treatment. Koponen et al. (2005) also modified BDNF signaling in mice, but they created mice with overexpression of TrkB receptor and activity. In the forced swim test, these mice performed similarly to wild-type mice injected with the antidepressant fluoxetine. Furthermore, comparison of the transgenic mice with wild-type, fluoxetine-treated mice in the forced swim test revealed no significant effects (Koponen et al., 2005). These results suggest that increased BDNF activity yields behavioral changes similar to antidepressant treatment and that normal BDNF signaling is necessary to elicit antidepressant-induced responses.

Yin-Yang hypothesis. The research described above is consistent with a majority of the studies on BDNF and mental health. However, it should be pointed out that the literature also contains contradictions. While the previous findings imply that impaired BDNF functioning would lead to behavioral deficits, the research is not so straightforward.

For instance, there is a large amount of literature that associates impaired GABAergic function to Alzheimer's disease (AD) (Lanctot, Hermann, Mazzotta, Khan, & Ingber, 2004; Rissman & Mobley, 2011). As the study by Sakata et al. (2009) revealed that disruption of the BDNF pathway negatively affected GABAergic transmission, it would seem that individuals with unusual BDNF function might be at higher risk of developing AD. However, a comparison of 375 Finnish subjects exhibiting a BDNF polymorphism with 460 controls without the

polymorphism found no significant correlations between atypical BDNF genotype and AD (Hwang et al., 2006).

Studies on animals have also revealed seemingly contradictory findings. The interaction between anti-depressant medications and *Bdnf* expression as well as the behavioral data on mice with overexpression of the BDNF receptor or exogenous BDNF infusion suggests that impaired BDNF signaling would result in depressive-like symptoms. However, Saarelainent et al. (2003) tested mice that were genetically altered to express reduced TrkB signaling. These mice, when compared to wild-type mice, performed comparably in the Morris water maze but did show deficits in long-term memory. Thus, behavioral data on the transgenic mice was inconclusive with regards to the association between BDNF impairment and depressive behavior.

One proposed explanation for these inconsistencies is the Yin-Yang Hypothesis. Martinowich et al. (2007) explained that most studies on transgenic rodents have led to a general overexpression or underexpression of the *Bdnf* gene. However, the authors noted that BDNF actually has 2 forms, proBDNF and mBDNF, and these forms have different functions. Whereas mBDNF and its receptor TrkB lead to long-term potentiation (LTP), proBDNF facilitates long-term depression (LTD) via the receptor $p75^{NTR}$ (Martinowich et al., 2007). The authors stated that inhibiting the *Bdnf* gene would likely restrict activity of both proBDNF and mBDNF and therefore show little or inconsistent results. This argument, in conjunction with the data on BDNF, emphasizes the significance of the neurotrophin hypothesis of depression as well as the need for further research.

Val66Met BDNF polymorphism. Another consideration when examining the BDNF research is the Val66Met polymorphism. A single nucleotide polymorphism (SNP) refers to a small genetic variation that is limited to only one nucleotide in a chain (Human Genome Project,

2008). An SNP on the *Bdnf* gene results in a valine to methionine substitution at amino acid 66 (Martinowich et al., 2007). A substitution of one Met allele is referred to as $Bdnf^{Val/Met}$, and two Met alleles are represented as $Bdnf^{Met/Met}$ (Martinowich et al., 2007). The Val66Met polymorphism is a functional polymorphism that has significant clinical implications including presentation of depressive and anxiety symptoms, information processing ability, resilience, episodic memory, and acute stress (Alexander et al., 2010; Cathomas et al., 2010; Gatt et al., 2008, 2009; Hwang et al., 2006; Tapia-Arancibia, Givalois, & Arancibia, 2004; Schofield et al., 2009).

Hwang et al. (2006) genotyped 110 Chinese older adults (mean age of 75) with and without depression. This study revealed a significant increase of the Met allele in depressed comparison to non-depressed patients (Hwang et al., 2006). Interestingly, the authors reported that in their previous study on younger adults, they found no significant differences and suggest that there might be an interaction of the polymorphism with age (Hwang et al., 2006). This explanation is supported by other studies that have revealed age-related changes in BDNF levels and associated behaviors (Adlard et al., 2005; Mata, Thompson, and Gotlib, 2010). In a study of adolescent girls, physical exercise was associated with fewer depressive symptoms for girls with the met allele but not for girls without the met allele (Mata et al., 2010).

Other research has made apparent additional mediating roles of the Val66Met polymorphism in memory, stress-reactivity, and depression (Alexander et al., 2010; Cathomas et al., 2010; Gatt et al., 2008, 2009; Schofield et al., 2009). Accounting for the fact that *Bdnf* has multiple potential polymorphisms, Calthomas et al. (2010) examined memory performance and genotypes of 333 healthy Swiss young adults. Of the 55 SNPs on the *Bdnf* gene and the adjacent *Bdnfos* gene, the Val66Met polymorphism on the *Bdnf* gene most significantly influenced

memory. Val66Met was associated with lower free recall of words with positive emotion in comparison to the Val/Val (typical) genotype. Schofield et al. (2009) further examined the association between Val66Met and memory impairments in healthy individuals. They examined the relationship between memory and the presence or absence of the Val66Met poymorphism (either with one Met allele, V/M, or with two met alleles, M/M). There were 475 participants with a mean age of 34 (Schofield et al., 2009). Using the California Verbal Learning Test, it was found that M/M subjects had more recall errors due to intrusive words than V/M or V/V subjects (Schofield et al., 2009).

Because of the connection between memory and mood (Gatt et al., 2009), a number of studies have explored the relationship of BDNF Val66Met polymorphism to depression and anxiety (Alexander et al., 2010; Gatt et al., 2008). Using the electroencephalogram (EEG) as an endophenotypic (internal phenotype observable via testing) marker of depression, Gatt et al. (2008) compared depressive traits in 305 healthy Caucasian V/V, V/M, or M/M individuals. This study demonstrated that M/M participants exhibited alterations in traits associated with depression including lower EEG alpha power, less neuronal excitability, and poorer working memory (Gatt et al., 2008). In a later study by several of these same authors, it was revealed that interaction of the BNDF Val66Met polymorphism and early life stress were reflected in poorer working memory, less amygdala and hippocampal volume, and elevated heart rate (Gatt et al., 2009). Structural equation path modeling (SEM) further showed that this interaction predicted depression, anxiety, and neuroticism (Gatt et al., 2009). These findings were supported by a recent study by Alexander et al. (2010) that reported a significant effect of the BDNF Val66Met polymorphism on stress reactivity.

Val66Met BDNF polymorphism and antidepressants. The impact of BDNF and of the BDNF Val66Met polymorphism on depressive traits and biological functioning suggests an important role for antidepressants capable of increasing BDNF levels. As previously mentioned, human and animal studies have shown that antidepressants can upregulate BDNF levels in humans and rodents (Chen et al., 2001; Nibuya et al., 1995; Russo-Neustadt et al., 2000). However, anti-depressant treatment was only effective for those groups without inhibited *Bdnf* signaling (Koponen et al., 2005; Saarelainen et al., 2003). Because these studies examined impaired BDNF pathways as a result of under-expression of the *Bdnf* gene rather than as a result of the Val66Met polymorphism more commonly seen in humans, Chen et al. (2006) sought to create a mouse model of the human polymorphism. Using Mendelian breeding techniques and a $BNDF_{Met}$ knock-in allele (with transcription regulated by endogenous promoters), the authors developed $BDNF^{Met/Met}$ mice and $BDNF^{+/Met}$ that were compared to both BDNF knock-out mice ($BDNF^{+/-}$) and BDNF wild type (WT) mice (Chen et al., 2006). The $BDNF^{Met/Met}$ mice revealed similar characteristics to knockout mice from this and earlier studies as well as similarities to humans with the Val66Met polymorphism (Chen et al., 2006). For instance, $BDNF^{Met/Met}$ mice had impaired activity-dependent BDNF secretion, decreased hippocampal volume, and poorer hippocampus-dependent memory (Chen et al., 2006). Of especial significance is that the $BDNF^{Met/Met}$ mice were unresponsive to SSRI fluoxetine (Chen et al., 2006). Thus, these findings indicate that Val66Met polymorphism might inhibit the efficacy of SSRI treatment.

However, according to a report by Tsai, Hong, and Liou (2010), human studies have yielded mixed results. Homozygous patients for Val/Val or Met/Met often showed differential response when compared to heterozygous individuals (Tsai et al., 2010). Unexpectedly, Met66 allele carriers displayed better rather than worse response to antidepressant treatment in some

studies (Tsai et al., 2010). Possible explanations that the authors provide include variations in types of antidepressants administered and variations in methods of assessment (Tsai et al., 2010). The authors also pointed out that *Bdnf* expression might be a matter of balance. While studies generally focus on increasing or decreasing overall *Bdnf* expression, behavioral deficits might be a reflection of a BDNF imbalance rather than simply a BDNF deficiency (Tsai et al., 2010). This notion of balance reflects the Yin-Yang hypothesis described earlier in this paper.

Treatment Options

Use of SSRI. Many of the studies described thus far have used antidepressants to increase expression of BDNF and to improve behavioral deficits. Rodent studies have employed an array of medications including SSRIs, norepinephrine selective reuptake inhibitors (NESRI), monoamine oxidase inhibitors (MAOI), and tricyclic agents (TCA) (Duman & Monteggia, 2006; Lee & Kim, 2010). The success of antidepressant treatment in rodent models as well as the wide application of antidepressant medications in the clinical setting at increasing BDNF has led to antidepressant treatment in humans to correct abnormal BDNF expression (Tsai et al., 2010). However, such studies on humans have used tricyclics much less than other medication types (Duman & Monteggia, 2006; Tsai et al., 2010). A compilation of treatments used in studies up to 2006 revealed that SSRIs and NESRIs were the most common medications administered in studies with humans (Duman & Monteggia, 2006). In addition, many of the studies on human patients offer several choices of medications in order to accommodate a wide range of people. For instance, Gonul et al. (2005) administered either extended-release venlafaxine (average 180mg/day) or SSRIs (100mg/day sertraline, 32mg/day fluoxetine, 30mg/day paroxetine, or 40mg/day citalopram) to patients aged 35.5 ± 8.1 with a current depressive episode of 13.1 ± 9.7 weeks. Treatment was continued for an average of 8 weeks. For depressed patients with a mean

age of about 40 years, Gervasoni et al. (2005) administered 20-40mg/day paroxetine with some bipolar patients also receiving lithium sulfate, clomipramine, or venlafaxine. Treatment was administered until complete remission was reached with a range of about 6-30 weeks.

Use of CBT. As previously stated, treatment targeting abnormal BDNF expression has mainly employed antidepressant medications. However, literature on depression states that cognitive behavioral therapy is effective at treating and preventing depression in both older and younger adults (Bhar et al., 2008; David & Szentagotai, 2005; Konnert, Dobson, and Stelmach, 2009; Laidlaw, Thompson, & Thomson, 2004). In addition, research has shown that CBT is as effective as pharmacological treatment at improving depressive symptoms (Bhar et al., 2008). CBT improves coping strategies and reduces negative thinking patterns (Beck, Rush, Shaw, & Emery, 1979). Its goal-oriented, structured, and time-limited nature make it a useful therapy in the research setting (Beck et al., 1979). CBT is designed to be implemented both during therapy sessions and outside of sessions through the use of homework assignments. Homework compliance has been shown to be a positive predictor of therapy outcome (Coon & Thompson, 2003). Because many of the BDNF studies previously described were performed on animals, CBT was not a practical option. However, in order to increase the external validity of the present findings in animals, studies with humans are necessary, and research on other treatment forms is especially important. CBT might therefore be a useful alternative to SSRI treatment at increasing BDNF expression and reducing depressive traits.

Bhar et al. (2008) administered either cognitive therapy (CT) or pharmacotherapy (PT) to 180 individuals diagnosed with major depressive disorder according to the Structured Clinical Interview for DSM-IV Axis I Disorders-Patient Version, the Hamilton Revised Scale for Depression, and the Beck Depression Inventory-II (BDI-II). Participants were randomly

assigned to either the CT or PT treatment groups. The BDI-II was given at baseline and again throughout the following 16 weeks. Patients in the CT group attended therapy sessions in accordance with the treatment manuals by Beck et al. (1979). Patients in the PT group were given paroxetine so that levels were gradually increased to 50mg/day by week 6. For unresponsive patients, paroxetine treatment was increased. The results showed that there were no significant differences between patients in the PT and CT groups, and these results were reported to be the same as those from an earlier study by Beck et al. in 1984 (as reported by Bhar et al., 2008).

In a study of older adults, Konnert et al. (2009) examined the use of brief CBT interventions to improve and prevent depressive symptoms. Participants were 60 years and older, at risk for depression (as determined by Geriatric Depression Scale (GDS) scores and the SCID-IV-R), and functioning well cognitively (score of 21 or above on the Mini State Mental Examination (MMSE)). Participants were randomly assigned to either the Treatment as Usual (TAU) or the CBT group. The CBT intervention was adapted from a study by Clarke et al. (1995) with only minor variations (Konnert et al., 2009). Therapy was done in groups of four to six and met twice each week for one hour. Homework assignments were adapted to meet the needs of the geriatric population, and group leaders/therapists were doctoral level psychology students of the CBT orientation (Konnert et al., 2009). The authors reported that CBT effectively treated and prevented depressive symptoms.

Rationale

Antidepressant treatment, while extremely beneficial, is not always efficacious (Chen et al., 2006; Martinowich, Manji, & Lu, 2007; Sairanen et al., 2005). One explanation for patient unresponsiveness may be the BDNF Val66Met polymorphism. Recent findings indicate that this

polymorphism might have mediating effects on antidepressant medication (Chen et al., 2006; Martinowich, Manji, & Lu, 2007; Sairanen et al., 2005). A possible alternative to SSRI is CBT (Bhar et al., 2008; Beck et al., 2005; Konnert et al., 2009; Laidlaw et al., 2004). However, research comparing the efficacy of SSRI with CBT is needed as most studies involving the BDNF Val66Met polymorphism have focused on antidepressant medications alone. Furthermore, despite recent findings that BDNF expression varies with age (Adlard et al., 2005; Hwang et al., 2006; Mata, et al., 2010), current studies have not examined differences in older versus younger adults. Should CBT prove comparably effective or more effective at increasing BDNF levels and at improving symptoms in mildly to moderately depressed patients, the clinical implications would be broad. CBT is a short-term form of treatment that can be useful not only in treating symptoms but in preventing them as well (Bhar et al., 2008; Beck et al., 2005; Konnert et al., 2009; Laidlaw et al., 2004).

This study will therefore compare CBT and SSRI treatment for individuals suffering from mild to moderate depression. Independent variables will be the presence or absence of the BDNF Val66Met polymorphism and treatment type (CBT, Paroxetine, or a combination of the two). Dependent variables will be the comparison of BDI and GDS scores obtained post-treatment to scores obtained pre-treatment. An additional dependent variable will be the comparison of BDNF levels following treatment to those recorded prior to treatment. The hypotheses for this study are listed below.

H I: CBT will significantly increase serum levels of BDNF in depressed patients.

H II: The effects of CBT will be comparable to SSRIs at significantly increasing serum levels of BDNF in depressed patients.

H III: CBT will be more effective at increasing serum levels of BDNF in depressed patients without the BDNF Val66Met polymorphism when compared to participants with the polymorphism.

H IV: CBT will be more effective at increasing serum levels of BDNF in depressed patients with the V/M polymorphism when compared to participants with the M/M polymorphism.

Methods

Participants

A sample of 500 participants will be recruited from the general population via newspaper and other advertisements as well as from assisted living facilities and nursing homes. Monetary compensation will not be offered, but transportation to the treatment facility will be provided. In addition, patients will receive either CBT or SSRI (paroxetine) to treat existing symptoms of a major depressive disorder (MDD). The participants will have the following inclusion criteria: (a) age range 20 to 80 years, (b) any ethnicity, (c) male or female, (d) fluent in English, (e) healthy, (f) good cognitive functioning, and (g) diagnosis of a major depressive episode with onset in the last month. Exclusion criteria will include: (a) substance abuse history, (b) cognitive impairment, (c) brain injury, (d) neurological disorders, (e) serious medical conditions, (f) nonresponse to paroxetine in the preceding year, and (g) any other conditions that would prevent treatment with antidepressant medication or interfere with participation in therapy sessions. A more detailed breakdown of the targeted enrollment population can be found in the appendix.

Measures

Measures listed below are in the order that they will be employed. Urine toxicology will exclude participants with substance use, and the Modified Mini Mental State Examination (3MS) will be used to exclude participants with cognitive impairments. The SCID, BDI, and GDS will

be employed to determine presence and severity of a current major depressive episode. Polymerase Chain Reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) will be used to assess genotype and serum levels of BDNF protein.

Urine toxicology screen. Urine toxicology testing is one of the most common forms of drug testing due to ease of collection and sensitivity of tests (Moeller et al., 2008). In comparison to blood, substances can be detected in the urine for longer amounts of time ("Tests for Drugs," 2003). Both immunoassay and gas chromatography-mass spectrometry will be used in this study.

Immunoassay uses antibodies to detect the presence of drugs and metabolites (Moeller et al., 2008). This type of assay is readily accessible, relatively inexpensive, and has been standardized in test kits (Moeller et al., 2008). One study reported that the Roche DAT immunoassay drug screen kit had a sensitivity and specificity of greater than 94% (Crooks & Brown, 2010). However, immunoassay may produce false-positive results. In comparison, GC-MS is more expensive and time-consuming than immunoassay. In GC-MS, separation of the drug from the sample occurs as the sample is propelled through a column by inert gas (GC). After separation, the drugs are eluted from the column and analyzed by a mass spectrometer. Electrons are used to ionize the sample and to reveal a data set that is unique to each drug (GC/MS, 2011). This process produces results that are more accurate than immunoassay and is considered the standard for confirmation of positive results (Moeller et al., 2008). In one study that compared the detection of marijuana metabolites in the urine using GC-MS and immunoassay, it was found that GC-MS was able to detect the presence of the drug for twice as long following initial use (Huestis, Mitchell, & Cone, 1995). Thus, immunoassay will be used as

an initial screen, and GC-MS will be used as confirmation for those tests that yield positive results.

Modified mini mental state examination (3MS). The 3MS is a modified version of the Mini Mental Status Exam (MMSE), but the range of scores is increased by 70 points with a cutoff of around 78 (Tombaugh, McDowell, Kristjansson, & Hubley, 1996). Like the MMSE, the 3MS examines cognitive functioning and changes in cognition in order to screen for dementia. Certain subtests (i.e. attention, visuospatial items, and delayed recall) appear on both the MMSE and the 3MS, with the 3MS including additional yet similar categories such as word fluency, delayed word recall, similarity testing, and date and place of birth (Folstein, Folstein, & McHugh, 1975; Tombaugh et al., 1996). Despite these commonalities, the MMSE has been reported to have a ceiling effect and to be less effective at identifying mild cognitive impairment (Blais & Baity, 2005). Internal consistency for the 3MS was found to be 0.82 in a group of patients with no cognitive deficits and 0.88 in a group patients diagnosed with Alzheimer's disease (Blais & Baity, 2005). In a group of patients admitted for acute treatment in a psychiatric inpatient setting, it was found that the 3MS had a coefficient alpha (internal consistency) of 0.72, and that the MMSE had an alpha score of 0.56. In a nonclinical population, the alpha for the 3MS was reported to be 0.82, and the alpha for the MMSE was 0.62 (Tombaugh et al., 1996). Sensitivity of the 3MS was found to be 0.926 (Tombaugh et al., 1996). In addition, it was reported that sensitivity and specificity were higher for the 3MS when compared to the MMSE (Teng et al., 1990 as cited by Tombaugh et al., 1996).

Structured clinical interview for DSM-IV-Axis I disorders, research version, patient edition, with psychotic screen, abridged version (SCID-I RV/P). The SCID-I is a semi-structured interview used as a diagnostic tool to determine the presence of Axis-I DSM-IV

disorders (Biometrics, 2011). The SCID is considered to be the "gold standard" of semi-structured diagnostic interviews (Lobbesteal, Leurgans, & Arntz, 2010, p.76). It has various modules, and researchers can choose to use certain modules based on the needs of the study. The SCID is designed for use with a wide variety of English-speaking adults including inpatients, community populations, and the elderly. The research version is similar to the clinical version but is longer and contains more disorders, subtypes, and specifiers. The patient edition is used for individuals already being seen in a clinical setting or to rule out psychotic disorders (Biometrics, 2011). In this study, the patient edition is being used to exclude participants with psychotic and other disorders and to identify those with MDD. In a study of nonpsychotic young adults, Zanarini et al. (2000) reported reliability for the SCID-I to be 0.80. However, the website for the SCID warns that when diagnosing disorders such as MDD, psychometric properties might be lower (Biometrics, 2011). Using a sample population that included men and women from the general community and from inpatient settings, Lobbesteal, Leurgans, & Arntz (2010) found that interrater agreement for major depression had a kappa value of 0.66. Basco et al. (2000) reported that when using the SCID-I to diagnosis MDD, the results were as follow: sensitivity of 84%, specificity of 91%, positive predictive value of 0.70, and negative predictive value was 0.96. This study included 210 subjects of various ethnicities that were recruited from a community mental health center (Basco et al., 2000).

Beck depression inventory-II (BDI-II). The BDI-II will be used in this study for individuals under the age of 65. The BDI-II is a 21-item self-rating questionnaire with four options for each item ranging from "0/not present" to "3/severe" (Beck, Rush, Shaw, & Emery, 1979). This questionnaire is used to assess symptom severity of depression (Beck et al., 1979). A score of 17-20 is considered to be on the border between mild and clinical depression, 21-30

indicates moderate depression, and 31 and over suggests severe depression (Beck et al., 1979). Alpha coefficients show a reliability of 0.92 to 0.93 in outpatient samples, and test reliability over a one-week period was 0.93 (Beck, Steer, & Brown, n.d). Item to total correlation varied based on the item measured (0.27 for loss of sexual interest to 0.74 for self-dislike) (Beck et al., n.d).

Geriatric depression scale (GDS). The GDS is a 30-item scale designed to measure symptom severity of depression in older adults. Unlike the BDI-II, the GDS does not include physical symptoms and is presented in a yes/no format (Montorio & Izal, 1996). The yes/no format is easier for older adults to fill out, and the lack of physical symptoms reduces confounds due to normative age-related physical changes (Montorio & Izal, 1996). Scores range from 0-30, and a cutoff of 11 or more resulted in sensitivity of 84% and specificity of 95% (Yesavage et al., 1983). A cutoff of 14 or more yielded an 80% sensitivity rate and a 100% specificity rate (Yesavage et al., 1983). Validity was reported to be 0.78 when compared to the BDI, and test-retest reliability was reported as 0.94 (Montorio & Izal, 1996). However, it was found that the GDS was not valid in elderly populations with dementia. To avoid this complication, individuals with dementia will be excluded with the SCID-I/NP before participants are asked to complete the GDS.

Polymerase chain reaction (PCR). Polymerase chain reaction (PCR) is a method of quickly amplifying deoxyribonucleic acid (DNA) sequences. Samples are first denatured with high temperatures. The sample is then cooled to allow primers to bind to target DNA sequences. The temperature is raised once again, allowing enzymes to instigate synthesis of new complementary DNA strands. These new strands are once again denatured, and the process is

repeated. Each cycle of PCR produces increasingly more amplified sequences (Invitrogen, 2011). In this study, genotyping will be done as described by Mata, Thompson, & Gotlib (2010).

Enzyme-linked immunosorbent assay (ELISA). ELISA will be used to determine amounts of BDNF levels in serum. BDNF-ELISA assays can be purchased in kits from companies such as R&D Systems and Abcam. Protocols vary depending on the kit used but are derived from the same principles. The following procedure is based on a protocol by Abcam (2011). Diluted antigen-containing samples are placed onto plates that have been coated with a capture antibody. Alternatively, plates can be coated manually with capture Ab before samples are added. Next, a blocking solution is added to the plate to prevent non-specific binding of the sample to the plate. The sample solution is then added, and antigen present in the sample binds to the capture Ab. The plate is then washed to remove any excess, unbound antigen. Antibodies are added to bind to the antigen. These antibodies are linked to enzymes. In the final step of the ELISA, a substrate is added that binds to the enzyme-linked Ab. This binding of substrate and enzyme produces a color change, and fluorescence can then be read and quantified with a microplate reader.

Demographic Questionnaire. A brief questionnaire will be created regarding basic demographic data on each participant. Information such as age, ethnicity, race, level of education, financial status, and marital status will be recorded.

Procedure

Planning and collaboration. Since this study involves the administration of antidepressant medication, collaboration with medical doctors will be necessary. In order to raise interest in this study, short presentations geared towards the medical and research communities will be organized. Two potential institutions that will be approached include the

San Mateo Medical Center and Stanford University. The San Mateo Medical Center is listed as a potential collaborator, because it has a residency program for psychiatrists and rotations in both geropsychiatry and neurology. Likewise, Stanford is a research oriented institution that has a school of psychiatry and an associated medical school. In addition, Mata, Thompson, and Gotlib, referenced earlier in this paper, are affiliated with Stanford University and have a research interest in the mediating effects of the BDNF genotype on depression.

Recruitment. Participants diagnosed with Major Depressive Disorder and those with depressive symptoms will be recruited from the general population via newspaper and other advertisements. Older adults will also be recruited from nursing homes and assisted living facilities. Contact with these facilities will first be made through supervisors and directors. The supervisors will then explain to the residents that a researcher on aging and health is interested in speaking with them about possible participation in a mental health study. If the individual shows interest in speaking with a researcher, the details of the study will be further explained. Because this study targets a large age range and a diverse population, recruitment will include individuals throughout the Bay Area. A breakdown of the targeted enrollment is included in the Appendix. Throughout the recruiting process, demographic information will be reviewed in order to ensure representation of targeted participants.

Informed Consent. Informed consent will be fully explained and obtained from patients. The consent form will include but not be limited to a description of the nature of the study, the time to completion of study (three to four months), the randomized nature of the study, and the procedures used. The informed consent will also clearly state the participant's right to withdraw from the study at any time, the legal rights of the participant, and contact information should the participant have questions or concerns regarding the study. Patients will be informed

that monetary compensation will not be provided. However, transportation to the treatment facility will be available, and patients will receive either SSRI, CBT, or a combination of both SSRI and CBT. It will also be explained to the patient that should continuation in the study prove problematic, the patient will still be able to receive treatment. For instance, if a patient is not responding to the treatment group that he or she has been randomly assigned to, then the patient can choose to receive additional treatment. However, data on these patients will not be included in the study.

Screening of Participants. After informed consent has been obtained from individuals interested in participating in the study, various screening procedures will occur. Urine samples will be collected in order to exclude individuals with substance use. Similar to other studies, participants with substance use, brain injury, neurological disorders, serious medical conditions, and cognitive impairment (3MS score of under 80) will be excluded (Hwang et al., 2005; Konnert et al., 2009; Lee & Kim, 2010; Mata, et al., 2010). These exclusion criteria present possible confounds to the present study either because they might limit efficacy of CBT treatment or because they might complicate treatment with antidepressants. Eligible participants will be those who are diagnosed with MDD according to the SCID-I RV/P and to scores on the BDI and GDS. Participants with scores of 14 to 20 on the GDS or those with scores of 17 to 28 on the BDI will be included (Beck, Steer, & Brown, n.d; Montorio & Izal, 1996; Yesavage et al., 1983). These scores reflect symptoms severity ranging from mild to moderate (Beck et al., 1979). Beck et al. (1979) reported that CBT sessions and total duration of therapy should be altered based on symptom severity. Thus, the mild to moderate range has been chosen to maintain uniformity in the sample population and in the treatment administered.

Baseline Measure. Prior to starting treatment, blood and saliva samples will be collected from all participants. ELISA on blood samples will be used to determine baseline BDNF serum levels. As described by Gervasoni et al. (2005), blood samples (5mL) will be collected in anticoagulant-free tubes at consistent times of day for all groups. In order to activate platelets, samples will be left at room temperature for one hour and then at 4°C for one hour. Samples will then be centrifuged at 4°C and 2,000 g for ten minutes. Samples will be stored at -20°C until assay by ELISA, and all samples will be analyzed within one month of collection. ELISA will be done using a basic ELISA kit and results will be read using a microplate reader. Repeated trials will be done to ensure test-retest stability. (Gervasoni et al., 2005).

Saliva samples will be used for genotyping and for categorizing subjects as having either the V/V, V/M, or M/M BDNF genotype (Mata et al., 2010). As previously stated, genotyping will be based on procedures described by Mata, Thompson, & Gotlib (2010). Saliva will be collected with the Oragene Kit (DNA Genotek, Ottawa, Ontario, Canada). Polymerase Chain Reaction (PCR) will be performed using the primers 5-ACT CTG GAG AGC GTG AAT GG-3 and 5-ACT ACT GAG CAT CAC CCT GGA-3. The amplified product will then be digested with PmaCI restriction enzyme and separated on agaraose gels (Mata et al., 2010).

Group Assignment. Based on the genotyping results, participants will separated into 3 groups: V/V genotype, V/M genotype, or the M/M genotype. Each of these groups will be further separated by age: younger adults (ages 20-65) and older adults (ages 65-80). At this point there will be 6 groups. Participants in each group will be randomly assigned to receive either SSRI (paroxetine), CBT, or a combination of both SSRI and CBT.

Treatment. Treatment procedures will be adapted from earlier studies of mildly to moderate depressed patients receiving paroxetine and/or CBT. Protocols for paroxetine

treatment are based on recommended dosage by PEPID Software (Version 3.2.2.). Protocols for CBT will follow the format described by Beck et al. (1979) with adaptations for working with older adults (Thompson, Coon, Gallagher-Thompson, Summer, & Koin, 2001). All treatments will begin at the same time.

Paroxetine treatment. Paroxetine treatment has been chosen because of its frequent use in previous studies and its well-known efficacy at improving depressive symptoms (Duman & Monteggia, 2006; Gervasoni et al., 2005; Gonul et al., 2005; Tsai et al., 2010). Paroxetine will be prescribed by medical doctors, and dosage will be adjusted according to age, treatment response, and symptom severity (PEPID, Version 3.2.2). Patients in this group will meet with a psychiatrist for short sessions (~20 minutes) each week to review symptoms, dosage, and any other relevant concerns. A likely course of treatment would be the following for younger adults: initial dose of 10mg/day or 20mg/day taken by mouth that can be weekly increased by 10mg/day or 20mg/day (depending on tolerance on drug and treatment effects) with a limit of 50mg/day (PEPID, Version 3.2.2). For older adults a likely course of treatment would be: initial dose of 10mg/day taken by mouth to be gradually increased as needed with a limit of 40mg/day (PEPID, Version 3.2.2). Total treatment time will be 16 weeks. This time frame is based on previous studies with efficacious results (Bhar et al., 2008; Coon & Thompson, 2003; DeRubeis et al., 2005; Thompson et al., 2001).

CBT treatment. CBT treatment will be conducted over 16 to 20 individual sessions total. Treatment will be tailored to address the needs of the patient. Patients will meet with a therapist for 60-minute sessions twice weekly for the first four weeks and once weekly for the remainder of the study. The range of sessions allows for some flexibility in treatment plans in accordance with the guidelines set forth by Beck et al. (1979). Therapy will be provided by doctoral level

clinicians or students trained in the CBT orientation and work with both older and younger adults. CBT will be carried out according to procedures documented by Beck et al. (1979). Patients will be taught to identify and challenge automatic negative thoughts and to adopt a more realistic viewpoint. In order to ensure uniformity of treatment, all sessions will be video-recorded and spontaneously reviewed. Homework will be used for all individuals.

Modifications for older adults will be based on those employed by Coon & Thompson (2003) and by Thompson, Coon, Gallagher-Thompson, Summer, and Koin (2001). These techniques include slower presentation of materials, repetition, use of more elaborative explanations, more practice during sessions, and increased modeling of behaviors (Coon & Thompson, 2003; Thompson et al., 2001). In addition, homework assignments will be tailored to meet the needs of older adults. The following techniques will be used for all older adults as necessary and for any younger adults as seems beneficial. For instance, older adults might be given shorter and more frequent homework assignments. Older adults will also be given an organized and very structured set of materials to help them remember which assignments need to be completed and the instructions for those assignments. Key points and concepts will be included in these materials in a concise and straightforward manner. For adults in assisted living facilities and nursing homes, the staff will also be instructed as to the nature of the study and the homework requirements so that staff will be able to help patients complete assignments if necessary. Additional options available to patients will include audio-taping sessions for later review and developing memory aids specific to the patient. For example, contextual cues could be incorporated to help patients remember assignments by designating a specific time and location for homework completion. Techniques such as these have been chosen based on their

successful implementation in past studies and in clinical application (Coon, Thompson, & Gallagher-Thompson, 2007; Thompson et al., 2001).

CBT and Paroxetine Treatment. Patients in the combined treatment group will receive both paroxetine and CBT in accordance with the above procedures. During treatment days, patients will meet first with psychiatrists to address medication and dosage concerns. The patient will meet with the CBT therapist later in the day. However, special care will be taken to ensure that older adults will be treated earlier in the day to avoid excess fatigue.

Efficacy of Study. In order to assess the efficacy of the study, BDNF serum levels will tested again post-treatment. Higher BDNF compared to baseline levels will indicate successful implementation of treatment modalities. In addition, patients will complete the BDI-II or the GDS (according to age). These scores will be compared to initial scores. Data analysis will be a 2x3 factorial ANVOA.

Length of Study. The planning and recruitment phase of the study is estimated to take three months. The treatment phase will take three to four months. Data analysis will take about one month. The overall estimated time of the project is seven to eight months.

Discussion

Ethical Consideration, Benefits, and Risks

This study has potential benefits as it may reveal useful information concerning the efficacy of treatment modalities in younger and older adults with and without the BDNF Val66Met polymorphism. In addition, participants in the study will receive treatment for their symptoms. However, there are several risks involved in this type of study. Ethical considerations include unresponsiveness to treatment methods, adverse reactions to medication, and proper attainment of informed consent. In an attempt to prevent unresponsiveness to

treatment, patients that have taken paroxetine in the past year with no benefits will be excluded from the study. Patients that show no response to medication during the course of the study will be given the option to switch to CBT; however, the data on these patients will not be included in the analysis of study results. In addition, the informed consent will warn patients of any possible side effects of taking paroxetine and will provide patients with contact information to a medical doctor should any concerns arise. For older adults, extra caution will be needed to ensure that patients fully understand the informed consent and how to access additional information.

Inclusion of Women and Minorities

Women and minorities will be included in this study. Our Planned Enrollment Form is based on the racial breakdown of participants seen at Palo Alto University and on review of the literature. The recruitment of minorities is approximately 25% in this study. However, we will continue to make efforts to increase the presence of minority subjects in our studies.

Study Limitations

External Validity. Based on the ethnic and gender makeup of past studies in this research area, it is expected that the majority of subjects will be Caucasian. Thus, results from this study can be generalized to the Caucasian population, but caution will need to be taken for application to other ethnic groups.

The inclusion of only English-speaking participants also limits the external validity of this study. Encompassing non-English speakers is beyond the scope of this study due to the expense of a multi-lingual study and the complications of incorporating measures normed on such diverse populations. However, should the results of this research be clinically significant, repetition with other populations should be considered.

Since the treatments in this study include only Paroxetine and CBT, it will not be possible

to generalize the findings of the study to include other treatment options. As certain individuals in the population may be unable to partake in treatment with CBT or Paroxetine and as others many not respond well to either of these treatments, future studies on additional treatment options might be necessary.

Internal Validity. The present study will be limited by its design. Because of the need to separate individuals with the BDNF Val66Met polymorphism from individuals without the polymorphism, a truly randomized study will not be possible. The results of this study will therefore provide a correlational understanding rather than a causal explanation.

The individual experiences of the participants in the study could also lessen internal validity. Participants in the study might present with depressive symptoms that would have remitted without treatment. Due to the limited time period of the study, such a remission would most likely be due to specific changes in circumstances (history threat) rather than personal maturation. Thus, it will be necessary to carefully assess for a diagnosis of MDD and to exclude those participants whose symptoms are caused by known and time-limited life stressors or to other potentially confounding diagnoses such as Adjustment Disorder. The background data collection on each participant and the use of previously described assessment procedures will be used to minimize personal history threats.

As attrition is a potential limitation of this study, it is necessary to address ways in which to lessen attrition threats. Collecting background information on the participants and including only those individuals with similarly assessed levels of depression (mild to moderate) should lessen attrition threats by excluding participants that vary significantly from one another. In other words, since the study will begin with a sample group that contains few extreme variations, attrition of certain individuals alone should not result in skewed data. Furthermore, the use of a

large sample size should also help to offset losses due to attrition.

Current technology may also limit the implications of this study. Past research has indicated that hippocampal BDNF levels may be more sensitive to antidepressant treatments (Chen et al., 2001) than peripheral BDNF levels (Gervasoni et al., 2005; Gonul et al., 2005). However, current technology only can only assays hippocampal BDNF levels in postmortem subjects. Thus, statistically insignificant results might reflect a limitation of current technology rather than a lack of significant treatment effects.

Clinical Implications

The lifetime prevalence of depression in the general population is 10% to 25% for women and 5% to 12% for men (APA, 2000). In addition, MDD is associated with increased risk of mortality (APA, 2000). Although prevalence of MDD is lower in older adults, with community samples showing a prevalence of about 1% to 5%, risk of depression rises substantially for elderly individuals with physical health problems (Hybels & Blazer, 2003). However, benefits of current treatments are controversial. Pharmacological treatment may have adverse side effects, and certain clinical therapies may not be accessible to all patients. Thus, research on better and more efficacious treatments is necessary.

This study aims to address current limitations in MDD treatment by identifying patients whose genetic predispositions might make them more amenable to various treatment modalities. Current research suggests that biological markers such as the BDNF Val66Met polymorphism may prove useful in selecting patients that are more responsive to specific treatments. However, these studies have focused mostly on animals and the use of antidepressants to increase BDNF levels while largely ignoring alternative treatment options such as CBT.

In this novel study on humans, CBT will therefore be compared to antidepressant medication in order to determine relative efficacy at increasing peripheral BDNF levels in patients with and without the BDNF Val66Met polymorphism. If significant differences in treatment response are found between these two groups, then this study would have many clinical applications. A simple cheek swab could be used to detect presence of the BDNF Val66Met polymorphism, and the results could be used to help identify the best course of treatment. Such a selection process would minimize both the financial costs and psychological distress that can accompany a more trial and error approach to treatment options. Patient-specific therapeutic benefits could be estimated prior to engaging both time and financial resources in a potentially ineffective modality.

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Appendix

Targeted/Planned Enrollment Table

Total Planned Enrollment: 500

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	23	23	46
Not Hispanic or Latino	230	230	460
Ethnic Category: Total of All Subjects *	250	250	500
Racial Categories			
American Indian/Alaska Native	0	0	0
Asian	27	27	54
Native Hawaiian or Other Pacific Islander	8	8	16
Black or African American	19	19	38
White	196	196	392
Racial Categories: Total of All Subjects *	250	250	500

* The “Ethnic Category: Total of All Subjects” must be equal to the “Racial Categories: Total of All Subjects.”