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Neuronal Correlates of Brain-derived Neurotrophic Factor Val66Met Polymorphism and Morphometric Abnormalities in Bipolar Disorder

Koji Matsuo[1,2], Consuelo Walss-Bass[1], Fabiano G Nery[1,3], Mark A Nicoletti[4,5], John P Hatch[1,6], Benicio N Frey[7], Emel S Monkul[1,3], Giovana B Zunta-Soares[4], Charles L Bowden[1], Michael A Escamilla[1,8] and Jair C Soares*[,4,5]

[1]Department of Psychiatry, The University of Texas Health Science Center, San Antonio, TX, USA; [2]Division of Neuropsychiatry, Department of Neuroscience, Yamaguchi University Graduate School of Medicine, Yamaguchi, Japan; [3]Bipolar Disorder Research Program (PROMAN), Institute of Psychiatry, Department of Psychiatry, University of Sao Paulo Medical School, Sao Paulo, Brazil; [4]Department of Psychiatry, University of North Carolina, Chapel Hill, NC, USA; [5]Center of Excellence for Research and Treatment of Bipolar Disorder (CERT-BD), University of North Carolina, Chapel Hill, NC, USA; [6]Department of Orthodontics, The University of Texas Health Science Center, San Antonio, TX, USA; [7]Department of Psychiatry and Behavioural Neurosciences, McMaster University, Hamilton, Canada; [8]Department of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, TX, USA

The brain-derived neurotrophic factor (BDNF) Val66Met polymorphism has been proposed as a possible candidate for involvement in the pathophysiology of bipolar disorder (BD). To determine whether an association exists between the BDNF Val66Met genotype and morphometric abnormalities of the brain regions involved in memory and learning in BD and healthy subjects. Forty-two BD patients and 42 healthy subjects were studied. Interactions between BDNF Val66Met genotype and diagnosis in gray (GM) volumes were analyzed using an optimized voxel-based morphometry technique. Declarative memory function was assessed with the California Verbal Learning Test II. Left and right anterior cingulate GM volumes showed a significant interaction between genotype and diagnosis such that anterior cingulate GM volumes were significantly smaller in the Val/Met BD patients compared with the Val/Val BD patients (left $P=0.01$, right $P=0.01$). Within-group comparisons revealed that the Val/Met carriers showed smaller GM volumes of the dorsolateral prefrontal cortex compared with the Val/Val subjects within the BD patient ($P=0.01$) and healthy groups (left $P=0.03$, right $P=0.03$). The Val/Met healthy subjects had smaller GM volumes of the left hippocampus compared with the Val/Val healthy subjects ($P<0.01$). There was a significant main effect of diagnosis on memory function ($P=0.04$), but no interaction between diagnosis and genotype was found ($P=0.48$). The findings support an association between the BDNF Val66Met genotype and differential gray matter content in brain structures, and suggest that the variation in this gene may play a more prominent role in brain structure differences in subjects affected with BD.

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INTRODUCTION

Components of the limbic–thalamic–cortical and the limbic–striatal–pallidal–thalamic–cortical networks participate in mood regulation, cognitive processes, and behavior regulation. Specific abnormalities of the hippocampus, amygdala, and the cingulate, and prefrontal cortices have been reported in patients with mood disorders, and these regions have been implicated in the pathophysiology of mood disorders (Soares and Mann, 1997). The brain abnormalities in patients with bipolar disorder (BD) could potentially result from genetic factors, which regulate brain development or neurodegeneration. However, there is little information on the influence of specific genetic factors on these brain abnormalities.

The brain-derived neurotrophic factor (BDNF) polymorphism gene (Val66Met) has been proposed as a candidate for possible involvement in the abnormal mood regulation that characterizes mood disorders (Neves-Pereira

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*Correspondence: JC Soares, Department of Psychiatry, Center of Excellence for Research and Treatment of Bipolar Disorder (CERT-BD), Campus Box 7160, University of North Carolina, Chapel Hill, NC 27599-7160, USA, Tel: 919 966 8832, Fax: 919 966 7659,
E-mail: jsoares@med.unc.edu
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et al, 2002; Sklar *et al*, 2002). BDNF is highly expressed in the cortex, hippocampus, limbic structures, cerebellum, and the olfactory bulb (Huang and Reichardt, 2001; Vigers *et al*, 2000; Yan *et al*, 1997), where it is involved in basic neuronal functions, such as cell survival, axonal outgrowth, dendritic growth, and synaptic plasticity. Animal studies also support the association between BDNF and several treatments for mood disorders, including antidepressants, lithium, valproate, electroconvulsive therapy, and repetitive transcranial magnetic stimulation. All of these interventions influence the expression of BDNF messenger RNA or BDNF protein in the hippocampus and frontal lobe (Muller *et al*, 2000; Nibuya *et al*, 1995; Russo-Neustadt *et al*, 1999). In humans, a postmortem study showed that subjects with mood disorders who were taking antidepressants at the time of death had increased BDNF immunoreactivity in hippocampal tissue compared with unmedicated patients (Chen *et al*, 2001). Family-based association studies show that the Val66Met polymorphism is associated with the diagnosis of BD and the phenomenon of rapid cycling (Green *et al*, 2006; Lohoff *et al*, 2005) and early age of illness onset (Tang *et al*, 2008) in BD patients, albeit some other studies did not show this association (Gratacos *et al*, 2007; Kanazawa *et al*, 2007; Kunugi *et al*, 2004). Moreover, both BD and major depressive disorder (MDD) patients show decreased serum levels of BDNF (Cunha *et al*, 2006; Karege *et al*, 2004; Machado-Vieira *et al*, 2007) compared to healthy subjects, and there is an inverse correlation between BDNF levels and severity of the depressive (Karege *et al*, 2004; Machado-Vieira *et al*, 2007; Shimizu *et al*, 2003) and manic states (Cunha *et al*, 2006). A magnetic resonance study demonstrated that serum BDNF levels are positively associated with the concentration of N-acetyl aspartate, a marker of neuronal integrity, and choline, a marker of cell membrane turnover, in the anterior cingulate cortex (ACC) (Lang *et al*, 2007), suggesting that peripheral BDNF content may be a potential marker of cerebral cortical integrity.

A human BDNF polymorphism gene, leading to a valine or methionine substitution at position 66 in the prodomain (Val/Met), plays a crucial role in memory formation. Healthy subjects with Val/Met BDNF also receive lower scores on cognitive tasks compared with Val/Val subjects (Egan *et al*, 2003; Hariri *et al*, 2003). In neuroimaging studies, healthy Val/Met subjects show smaller hippocampal (Bueller *et al*, 2006; Pezawas *et al*, 2004), parahippocampal (Nemoto *et al*, 2006) and dorsolateral prefrontal cortex (DLPFC) (Pezawas *et al*, 2004) volumes compared with Val/Val subjects. Psychiatric patients show associations between the BDNF Val66Met polymorphism and brain abnormalities in schizophrenia (Egan *et al*, 2003; Ho *et al*, 2006; Szeszko *et al*, 2005) and MDD (Frodl *et al*, 2007). Among BD patients, Met carriers showed greater volume loss within the temporal lobe over 4 years compared with Val/Val patients (McIntosh *et al*, 2007). We demonstrated that the Val66Met polymorphism affects creatine + phosphocreatine levels in the DLPFC of BD patients (Frey *et al*, 2007b). More specifically, Met carriers had lower creatine + phosphocreatine levels, which suggests impaired energy metabolism in the DLPFC of BD.

Despite the mounting evidence for BDNF abnormalities in BD, to our knowledge, the association between the Val66Met polymorphism, cerebral gray matter and memory in BD has not been investigated. The objective of this study was to examine the association between the BDNF Val66Met polymorphism and morphometric brain abnormalities in BD patients. We hypothesized that Met carrier subjects (Val/Met and Met/Met) would have smaller volumes of the hippocampus, ACC and DLPFC that contribute to memory and cognitive function compared with Val/Val homozygotes. We further hypothesized that the brain volume differences between Val/Val homozygotes and Met carriers would be larger among subjects with BD than among healthy comparison subjects.

METHODS

Subjects

Forty-two patients with BD and 42 healthy subjects were studied. This study was approved by the Institutional Review Board of The University of Texas Health Science Center at San Antonio. Written informed consent was obtained from all the participants after a complete description of the study was provided. The participants were recruited at hospitals and clinics and through advertisement broadcast in the community. All of the patients met DSM-IV-TR criteria for BD by the Structured Clinical Interview (SCID) for DSM-IV (First *et al*, 1996b). Healthy subjects were screened for DSM-IV axis I disorders by the SCID non-patient version (First *et al*, 1996a). Patients who had a history of electroconvulsive therapy or a substance use disorder within 6 months preceding the study were excluded. Healthy control subjects who had current or past axis I DSM-IV psychiatric disorders or had first-degree relatives with any Axis I psychiatric disorder were excluded. All participants were evaluated for handedness by the Edinburgh inventory (Oldfield, 1971). All participants also received laboratory tests and a physical examination to rule out physical illnesses. Any participant with current endocrinological disease, history of head trauma with loss of consciousness, current or previous neurological disease, family history of hereditary neurological disorders, or a current medical condition such as active liver disease, kidney problems, or respiratory problems was excluded. A senior psychiatrist (JCS) reviewed all clinical information and medical or neurological conditions and confirmed DSM-IV diagnostic criteria. Current mood states of the patients were evaluated using the 21-item Hamilton Rating Scale for Depression (HAM-D) (Hamilton, 1960) and the Young Mania Rating Scale (YMRS) (Young *et al*, 1978). Verbal and non-verbal general intellectual abilities were assessed using the Wechsler Test of Adult Reading (WTAR) (Holnack, 2001) and the Test of Non-verbal Intelligence-3 (TONI-3)(Brown *et al*, 1997).

MRI Acquisition

Brain images were collected on a Philips 1.5 T MR system (Philips Medical System, Andover, MA). Images were collected by means of an axial 3-dimensional T1-weighted field fast echo sequence (field of view 256 mm, view matrix 256×256, repetition time 24 ms, echo time 5 ms, flip angle 40°, slice thickness 1 mm).

Image analysis. Preprocessing was performed using SPM2 software (Wellcome Department of Imaging Neuroscience, London, United Kingdom) running under Matlab 7.1.0 (MathWorks, Natick, MA). The images were preprocessed following the optimized voxel-based morphometry (VBM) protocol (Good *et al*, 2001). First, we created a customized anatomical T1 template and prior probability images from the sample of all 84 participants. All original images were manually aligned on the anterior commissure–posterior commissure line. The customized T1 template was created by averaging the images of all the participants and prior probability images by an optimized VBM script (http://dbm.neuro.uni-jena.de/vbm/). The original images were analyzed using this own template. The extracted segmented images were normalized with the own gray matter (GM) template. Deformation parameters were applied to the original images followed by a second segmentation step in stereotactic space. This procedure automatically removed non-brain tissues including scalp tissue, skull and dural venous sinus. Finally, the segmented images were modulated by Jacobian determinants derived from the spatial normalization. Images were obtained in $1 \times 1 \times 1$ mm resolution by the optimized VBM script. These images were smoothed with an 8 mm Gaussian filter.

Genotyping Procedure

Blood samples were collected from all subjects and identified by codes, and DNA was subsequently extracted. BDNF Val66Met genotype was determined using the Taqman 5′, with a Peltier Thermal Cycler and the ABI (Applied Biosystems, Foster City, CA) 7900 Sequence Detection System. The 5′ nuclease assay was used, which allows direct detection of the polymerase chain reaction product by the release of a fluorescent reporter. We used two fluorescent probes, one for each single nucleotide polymorphism allele, that hybridize to the target sequence in the assay. Each probe consists of an oligonucleotide with a 5′-reporter fluorescent dye (either 6-carboxy-fluorescine or VIC™) and a 3′ non-fluorescent quencher. Genotyping error rate for this assay was determined by replicate genotyping of sample, and was <0.005. The genotyping was performed with the clinical status of the subjects masked, and genotypes were scored using the SDS2.1 software program from ABI. When the probe is intact, the proximity of the reporter and quencher results in the suppression of fluorescence. As the Taq polymerase cleaves the probe with its 5′ to 3′ nuclease activity, the reporter dye is separated from the quencher, resulting in increased fluorescence. The fluorescence intensity was read and quantified by the ABI Prism 7900HT, allowing for immediate availability of the genotype information. Primers and probes for each single nucleotide polymorphism were obtained using the custom assay-by-demand service offered by ABI. Eighteen randomly selected short tandem repeat markers (DXS993, D9S285, D4S392, D21S252, D20S117, D19S571, D16S515, D14S276, D10S1653, D11S904, D7S510, D7S507, D6S422, D5S641, D3S1569, D2S396, D2S168, and D12S368) were genotyped as control markers to test for possible stratification effects.

Neuropsychological Testing

We administered the California Verbal Learning Test II (CVLT-II)(Delis *et al*, 2000) to evaluate verbal learning and recall declarative memory. Subjects listened to a list of 16 items belonging to four categories presented at a rate of one item per second and were then requested to repeat as many as they can remember. This procedure was repeated in five trials and the score for each trial was recorded. The total score (CVLT-II total) is the sum of the five trials. An interference trial with a different list of 16 items is then performed, and the subjects are asked to recall this new list. This test is followed by subjects being asked to recall the items in the initial list (CVLT-II short delay free) and then again after they are provided with the categories as cues (CVLT-II short delay cued). After 20 min of performing a distracter task, the subjects are asked to recall the initial items spontaneously (CVLT-II long delay free) and again after being provided with the categories as cues (CVLT-II long delay cued).

Statistical Analysis

For image analysis, we used SPM2 software that implemented a General Linear Model. We selected three *a priori* regions of interest (ROIs) based on previous neuroimaging studies: DLPFC, ACC, and hippocampus. We identified these regions using Talairach Daemon (Lancaster *et al*, 2000) and automated anatomical labeling (Tzourio-Mazoyer *et al*, 2002) through WFU PickAtlas version 2 (Maldjian *et al*, 2004; Maldjian *et al*, 2003). Total brain volumes were calculated by the optimized VBM script. Age, sex (Good *et al*, 2001; Sowell *et al*, 2003) and total brain volumes were treated as nuisance variables in SPM2. We analyzed the interaction between genotype and diagnosis. Within-group comparisons were also done in SPM2 to test the difference between the ROI volumes of the Val/Val and Met carriers in the healthy and BD subject groups. The resulting set voxel values for each contrast constituted an SPM *t* statistic (SPM {t}). Significant results were reported at $P<0.05$ corrected for multiple comparisons at both cluster and voxel levels. At the voxel level, we applied the small volume False Discovery Rate correction in SPM and extent threshold = 50 voxels to confirm our hypothesis. All results are presented as Talairach coordinates, which we obtained by applying Brett's transformation (www.mrc-cbu.cam.ac.uk/Imaging/Common/mnispace.shtml) to the MNI coordinates output by SPM2.

In genotyping, the allele frequencies and genotype distribution of BDNF were compared with Hardy–Weinberg equilibrium. Fisher's exact test was applied to compare the Val66Met genotypes with regard to the control markers.

The CVLT data were analyzed using ANOVA for repeated measures with one within subjects factor with seven levels (t1 to t5, short recall and long delay recall) and two between subjects factors each with two levels (diagnosis, ie, BD patients *vs* healthy comparison subjects, and genotype, ie, Val/Val *vs* Met carrier) using SPSS software (SPSS Inc., Chicago, IL).

RESULTS

Demographic and Clinical Data

The genotype distribution was 24 (57%) Val/Val and 18 (43%) Val/Met subjects in the BD group and 29 (69%)

Val/Val and 13 (31%) Val/Met subjects among the healthy subjects. The distribution was not significantly different between the two groups ($P=0.37$). No subject with the Met/Met allele participated in the study. One control marker (D3S1569), out of 18 tested, was unequally distributed between the Val/Val and Val/Met subjects ($P=0.04$), which is similar to what one would expect to find by chance for this number of markers. The remaining 17 control markers were equally distributed between Val/Val and Val/Met subjects ($Ps>0.1$). There was no significant difference between Val/Val and Val/Met subjects with regard to the distributions of sex, age, years of education, verbal IQ, non-verbal IQ, or handedness ($Ps>0.05$) (Table 1). For the BD patients, the mean age of onset of illness was 16.6 ± 6.7 years (mean $\pm$ SD), and the mean length of illness was 18.3 ± 11.0 years. The mean number of episodes was 26.4 ± 25.7 for 18 of the patients; the number of episodes was 'too numerous to count' in the remaining 24 patients. The mean HAM-D score was 14.5 ± 8.9, and the mean YMRS score was 7.9 ± 6.7 years. Twelve patients were depressed, four were manic, four were mixed, and 22 were euthymic. Thirteen patients were unmedicated and 29 were taking psychiatric medications including mood stabilizers ($n=22$), antidepressants ($n=21$) and atypical antipsychotropics ($n=12$) at the time of the study. Thirty-four patients were diagnosed as having bipolar I disorder, and eight patients had bipolar II disorder. Twenty-two patients had one or more comorbid anxiety disorders including panic disorder ($n=5$), generalized anxiety disorder ($n=7$), post traumatic stress disorder ($n=4$), obsessive–convulsive disorder ($n=4$), and specific phobia ($n=7$). No patients met the diagnostic criteria for substance abuse or dependence disorder within 6 months preceding the study. There was no statistical difference between Val/Met and Val/Val BD patients with regard to age of onset ($F_{1,39}=2.8$, $P=0.11$), length of illnesses ($F_{1,39}=2.0$, $P=0.17$), medication use ($\chi^2=0.17$, $P=0.68$), family history of mood disorders ($\chi^2<0.01$, $P=0.97$), number of episodes of the 18 patients who identified a specific number of episodes ($F_{1,15}=0.4$, $P=0.54$), HAM-D score ($F_{1,38}=0.63$, $P=0.43$) or YMRS score ($F_{1,39}=1.23$, $P=0.27$).

Imaging Data

The interaction between genotype and diagnosis was significant in the analyses of both left and right ACC GM volumes. The differences of the regional ACC GM volumes between Val/Val and Val/Met subjects were larger for the BD patients compared with the healthy subjects (Talairach and Toronoux coordinates of the voxel of maximum statistical significance; left ACC, $x=-7$, $y=13$, $z=25$, $t=3.55$, $k=66$, $P=0.011$; right ACC, $x=12$, $y=25$, $z=23$, $t=3.24$, $k=133$, $P=0.013$) (Figure 1). Within-group ROI analysis showed that the Val/Met carriers had significantly smaller ACC and DLPFC GM volumes compared with the Val/Val subjects in BD patients and healthy groups (Figure 2). The healthy comparison subjects group also showed that the Val/Met subjects had significantly smaller left hippocampus GM volumes compared with the Val/Val subjects (Figure 2). To assess the potential impact of race/ethnic group on our results, we performed a 3-way ANOVA including factors representing genotype, diagnostic group, and race/ethnic group (coded white and non-white). The three-way interaction was not statistically significant for left or right ACC ($F_{1,76}=1.15$, $P=0.29$; $F_{1,76}=1.16$, $P=0.69$, respectively), but the diagnosis by genotype interaction remained significant for both left and right ACC ($F_{1,76}=5.69$, $P=0.02$; $F_{1,76}=5.03$, $P=0.03$, respectively). This result suggests that the interaction between BDNF genotype and diagnostic group was not moderated by race/ethnic group.

CVLT Performance

Figure 3 illustrates that the Val/Met BD showed the poorest performance on the CVLT compared to the Val/Val BD patients and the healthy subjects. There was a significant main effect for diagnosis ($F_{1,\ 69}=4.39$, $P=0.04$), but not for genotype ($F_{1,69}=0.82$, $P=0.63$) or for the interaction between diagnosis and genotype ($F_{4.1,283.3}=0.86$, $P=0.48$). We conducted exploratory Pearson correlation analyses with Bonferroni correction to examine the association between the CVLT data and the regional GM volumes of

Table 1 Demographic and Clinical Characteristics of Participants

	Patients with BD		Healthy subjects		*P*-value
	Val/Val ($n=24$)	Val/Met ($n=18$)	Val/Val ($n=29$)	Val/Met ($n=13$)	0.37
Age, mean (SD), years	36.1 (9.3)	35.2 (9.7)	35.4 (10.5)	34.2 (10.4)	0.96
Female, numbers (%)	19 (79.1)	14 (77.8)	19 (65.5)	8 (61.5)	0.53
Years of education, mean (SD)	14.1 (2.2)	13.8 (2.2)	16.6 (3.1)	16.0 (2.9)	<0.01
TONI (SD)	20.6 (7.5)	18.8 (9.1)	17.4 (6.1)	22.3 (8.9)	0.34
WTAR (SD)	39.8 (8.4)	36.1 (7.6)	39.4 (9.4)	34.8 (10.8)	0.33
Handedness, right numbers (%)	23 (95.8)	17 (94.4)	26 (89.7)	12 (92.3)	0.35
Ethnicity, Caucasian numbers (%)	13 (54.2)	10 (55.6)	9 (31.0)	6 (46.2)	0.26
Age of onset, mean (SD), years	15.1 (4.8)	18.5 (8.6)	NA	NA	0.11
Illness duration, mean (SD), years	19.9 (10.8)	16.2 (11.1)	NA	NA	0.17
HAM-D, mean (SD)	15.3 (9.4)	13.4 (9.4)	NA	NA	0.43
YMRS, mean (SD)	8.9 (7.4)	6.7 (5.6)	NA	NA	0.27

HAM-D, Hamilton Depression Rating Scale; TONI, Test of Nonverbal Intelligence III; WTAR, Wechsler Test of Adult Reading; YMRS, Young Mania Rating Scale.

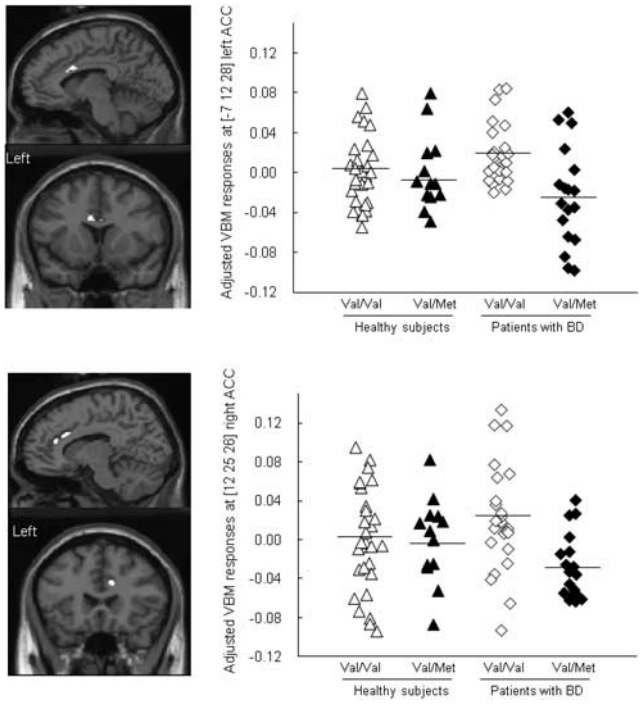


Figure 1 Scatterplots show the adjusted VBM responses of left and right ACC as a function of BDNF genotype and diagnosis at the Talairach coordinates. These regions showed an interaction between genotype and diagnosis, and the regional ACC GM volume differences between Val/Met and Val/Val subjects were larger for the BD patients compared to the healthy subjects. Horizontal lines represent the mean adjusted VBM response of each group. The T1-weighted template image showed the voxels with statistical significance for left and right ACC. The statistical thresholds showed were the extent thresholds = 50 voxels and $P_{uncorrected} < 0.005$ for the ROI analysis.

left and right ACC that showed the interaction between genotype and diagnosis in the Val/Val, the Val/Met, the BD, and the healthy subjects, respectively. No significant correlations were observed in any group (adjusted $Ps > 0.1$).

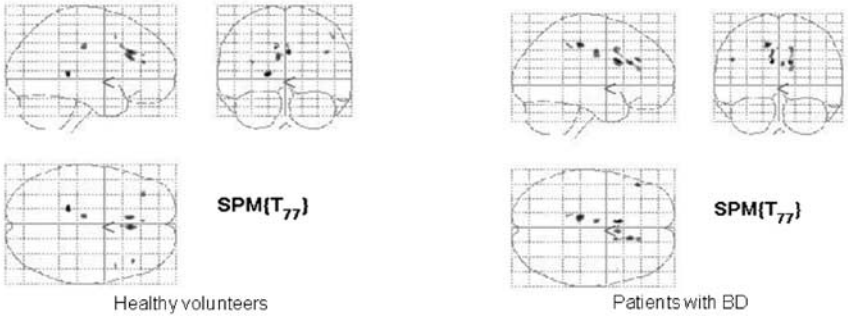
DISCUSSION

We found an association between the BDNF Val66Met polymorphism and GM volumes in the ACC and DLPFC, brain regions, which have been linked in other studies to the pathogenesis of BD. The Val/Met carriers had smaller ACC GM volumes compared to the Val/Val carriers in the BD patients and the healthy subjects (Figure 2). The difference in size of the regional ACC volumes, between Val/Val carriers and Val/Met carriers, was larger in the BD patients than in the healthy subjects (Figure 1). This suggests that, in the context of BD, the BDNF Val/Met genotype may play a heightened role in determining the size of the ACC GM. The Val/Met healthy subjects also had smaller hippocampal volumes than the Val/Val healthy subjects, although this difference was not seen in BD subjects. These results replicate previous reports of smaller structural brain volumes among Met carriers (Egan *et al*, 2003; Pezawas *et al*, 2004) and suggest that variation in the expression of the BDNF genotype may play a role in determining structural differences in these brain regions, which are relevant to memory, cognitive function and mood regulation. The variation in this gene may play a more prominent role in brain structure differences in subjects affected with BD.

The ACC contributes to working memory (Callicott *et al*, 1998; Kondo *et al*, 2004; Ragozzino and Kesner, 2001) and executive functions including attention, inhibition, and resolution of cognitive conflict in executive processes (Fletcher and Henson, 2001; Luks *et al*, 2002; Smith and Jonides, 1999). There are strong anatomical and functional connections between the ACC and hippocampus. The ACC connects with the hippocampus, OFC, amygdala, periaqueductal gray, nucleus accumbens, hypothalamus, and anterior insula (Bush *et al*, 2000). The ACC plays a crucial role, along with hippocampus, in the storage and retrieval of remote spatial memories (Maviel *et al*, 2004; Teixeira *et al*, 2006). The BDNF Val66Met polymorphism has been implicated in memory and cognitive functions that are impaired in subjects with mood disorders (Bath and Lee, 2006). Our results may provide evidence that the Val66Met polymorphism may play a critical role in ACC.

Bath and Lee (2006) proposed that the BDNF Val/Met polymorphism, ie, carriers of the Met allele compared to those with Val/Val alleles, mediates some processes involved in the brain volume reduction reported in BD patients, particularly the hippocampus. At the molecular level, the Met substitution alters the intracellular trafficking and the activity-dependent (but not the constitutive) secretion of BDNF (Chen *et al*, 2004). The implicated processes may impact brain volume through decreased dendritic complexity, fewer neuronal and supporting cells, increased cell death, or decreased neurogenesis during embryological development or over the lifespan (Bath and Lee, 2006). This hypothesis may extend to the present findings and explain the smaller ACC volumes observed in our Val/Met subjects compared with Val/Val subjects. However, this does not imply that this genetic variant directly regulates ACC volume reduction. It should be noted that this genetic variation may impact on some other neural circuit affecting the ACC function or regulate some other genes directly relevant to the ACC volume reduction. Nonetheless, the findings of this study suggest that the presence of the BDNF Val66Met genotype predicts smaller volumes of brain structures relevant to working memory and further suggest that variation in this gene may play an even more prominent role in brain structure differences in subjects affected with BD.

We replicated the finding of smaller hippocampal and DLPFC GM volumes among Val/Met subjects compared with the Val/Val healthy subjects (Hariri *et al*, 2003; Pezawas *et al*, 2004). Previous studies in animals and humans demonstrate an association between BDNF genotype and hippocampus morphology, function, and neurochemistry. Restricted BDNF mutant mice without BDNF in the dorsal cortex and hippocampus failed to learn a hippocampus-dependent memory task (Gorski *et al*, 2003). Moreover, knockout mice with reduced BDNF expression in the cortex and hippocampus showed approximately 70% reduction of BDNF protein level in the hippocampus accompanied by impairment in hippocampal-dependent learning and long-term potentiation (Monteggia *et al*, 2004). In humans the Val/Met genotype



	Talairach coordinate[1]	*t*	*k*	*p*
Healthy volunteers (Val/Val > Val/Met)				
Left DLPFC	(-33, 39, 16)	3.06	57	0.028
Right DLPFC	(42, 29, 26)	3.05	61	0.032
Left ACC	(-7, 25, 22)	3.22	211	0.011
Right ACC	(3, 27, 26)	3.34	271	0.009
Left hippocampus	(-18, -36, 6)	3.60	269	0.007
Patients with BD (Val/Val > Val/Met)				
Left DLPFC	(-49, 33, 26)	3.15	113	0.017
Left ACC	(-7, 13, 25)	3.55	191	0.011
Right ACC	(12, 25, 23)	3.12	173	0.013

[1]Talairach and Toronoux (1988) coordinates of the voxel of maximal statistical significance
DLPFC Dorsolateral prefrontal cortex, ACC Anterior cingulate cortex

Figure 2 Glass brain maps show the Talairach coordinates that showed significant volume differences between Val/Met and Val/Val groups. The left map represents healthy subjects and the right represents BD patients. The table under the figure displays the results including *t*, *k*, and *P* values.

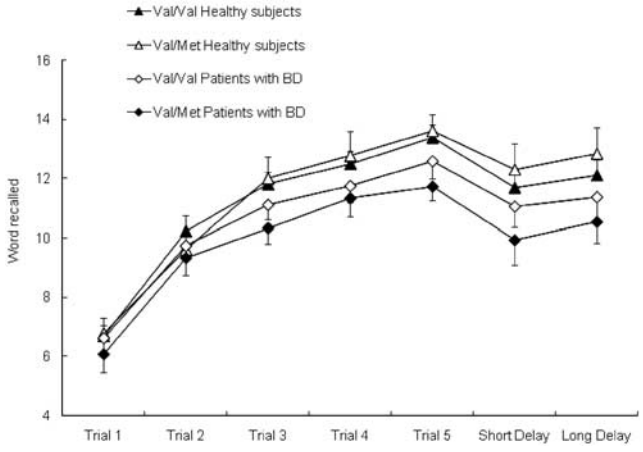


Figure 3 The number of recalled words on the CVLT test for the BD patients and healthy subjects with Val/Met and Val/Val. Error bars indicate SE.

is associated with poor performance on a hippocampus-dependent memory task compared to subjects with the Val/Val genotype (Egan *et al*, 2003; Hariri *et al*, 2003). Furthermore, healthy Val/Met subjects show hippocampal hypoactivation compared with healthy Val/Val subjects during a declarative memory (Hariri *et al*, 2003) and a working memory task (Egan *et al*, 2003). A magnetic resonance spectroscopy study also demonstrates abnormal neurochemical activity in Val/Met BD patients: Val/Met BD patients show lower creatine + phosphocreatine (Cr + PCr) levels compared with Val/Val BD patients in the prefrontal cortex involved in the brain circuit of working memory (Frey *et al*, 2007b).

The current study found no interaction between genotype and diagnosis and no difference between the Val/Met and the Val/Val BD patients with regard to hippocampal volumes. A postmortem study demonstrated reduced pyramidal cell size in the hippocampus of BD patients compared with healthy control subjects (Liu *et al*, 2007). Most previous *in vivo* studies also showed no difference in hippocampal volumes between BD patients and healthy subjects (Frey *et al*, 2007a), although a few studies have shown smaller hippocampal volumes in BD patients (Bearden *et al*, 2007; Frazier *et al*, 2005). Lithium and valproate, primary mood stabilizers for the pharmacological treatment of BD patients, increase BDNF concentration in the rat hippocampus (Frey *et al*, 2006). Furthermore, psychiatric patients treated with antidepressants at the time of death showed higher hippocampal BDNF expression compared to those not treated with antidepressants (Chen *et al*, 2001). Antidepressant administration increases BDNF mRNA in the hippocampus (Nibuya *et al*, 1995) and acutely increases BDNF receptor (Trk B) signaling in the cerebral cortex (Saarelainen *et al*, 2003). Concerning the effects of antidepressant use on brain volume change, antidepressants increase the proliferation of hippocampal cells, and these new cells mature and become neurons (Malberg *et al*, 2000). Lithium administration for 4 weeks also increases the whole brain gray matter volume in BD patients (Moore *et al*, 2000). Sixty-nine percent of our BD sample were taking antidepressants and/or mood stabilizers at the time of the study. The potential compensatory effects of these

medications on hippocampal volume size may in part explain why the differences in hippocampal volumes between the BD patients and healthy subjects did not reach statistical significance. It may also explain why differences between Val/Met and Val/Val carriers in hippocampal volume were not observed. We did show evidence that the Val/Met genotype influences hippocampal volume in the healthy control sample, where healthy subjects with a Val/Met genotype had smaller hippocampal volume than healthy Val/Val carriers.

The CVLT performance of the BD patients was worse than that of the healthy subjects, as we had previously reported (Bearden *et al*, 2006), but that of the Val/Met subjects was similar to that of the Val/Val subjects in the BD and the healthy subjects, respectively. The difference between BD and healthy subjects is consistent with prior studies (Bearden *et al*, 2006; Clark *et al*, 2002; van Gorp *et al*, 1998), and the results suggesting no difference between the genotypes also support a recent report on CVLT (Egan *et al*, 2003). However, the performance of Val/Met BD patients on the Wisconson Card Sorting Test reportedly is inferior to that of Val/Val BD patients (Rybakowski *et al*, 2003; Rybakowski *et al*, 2006). The Wisconsin Card Sorting Test measures working memory and executive functions involving frontal cortex, whereas the CVLT assesses complex cognitive processes and declarative memory involving the hippocampus and frontal regions. A recent study also reports an association between CVLT performance and the catechol-*O*-methyl transferase rs165599 genotype in BD patients (Burdick *et al*, 2007). Although the CVLT is associated with hippocampal volume (Banos *et al*, 2004; Tischler *et al*, 2006) and frontal function (Baldo *et al*, 2002), the relationship between ACC function and memory processing measured by CLVT is unclear. Therefore, other neurocognitive tasks that activate ACC function (Gehring and Willoughby, 2002; Matsuo *et al*, 2007) are warranted to determine whether the BDNFVal66Met polymorphism participates in working memory processing related to ACC function in BD patients.

Some methodological limitations of the present study should be noted. As the association between the BDNF Val66Met polymorphism and memory has been observed in schizophrenia, MDD, and neurodegenerative diseases, the finding of this study may not be specific to BD. Studies of patients with schizophrenia showed that Met carriers have poorer spatial and verbal memory performance (Egan *et al*, 2003; Ho *et al*, 2006) and smaller hippocampal (Egan *et al*, 2003; Szeszko *et al*, 2005), parahippocampal and supramarginal gyrus volumes, which modulate visuo-motor control (Ho *et al*, 2006), than Val/Val carriers. Met carriers with MDD (Frodl *et al*, 2007) also have smaller hippocampal volumes compared with Val/Val carriers. As the sample size was relatively small for a genetic analysis, this may be the reason for the lack of association between BDNF polymorphism and hippocampal volumes in BD patients. Next, the interpretation of the interaction between the BDNF polymorphism and brain volumes may be modest as the sample is affected by some potentially confounding factors such as medication, ethnicity, and subtype of bipolar disorder. As noted above, antidepressants and mood stabilizers may alter brain volumes in BD (Malberg *et al*, 2000). These factors make it difficult to attribute observed brain volume differences between BD and healthy subjects specifically to the Val66Met polymorphism. The ethnicity is mixed in this study. Stratification of ethnicity is crucial for genetic studies. The samples in this study were not ideally matched with respect to ethnic/racial background although the interaction between the BDNF genotype and the diagnostic group was not moderated by race/ethnic group. Stratification by ethnicity is crucial for genetic studies. The prior studies of the BDNF polymorphism with BD patients demonstrated that Caucasians showed significant associations between Val66Met polymorphism and BD (Neves-Pereira *et al*, 2002; Sklar *et al*, 2002) but Asians did not (Hong *et al*, 2003; Kunugi *et al*, 2004; Nakata *et al*, 2003). Studies with substantial number of ethnically homogeneous subjects would be required to confirm that the main results of this study still exist. Other genes such as 5HTTLPR (Frodl *et al*, 2004; Hickie *et al*, 2007; Taylor *et al*, 2005) and DISC1 Ser704Cys polymorphism (Hashimoto *et al*, 2006) may potentially mediate the association between the Val66Met polymorphism and brain structural change in patients with mood disorders, and these polymorphisms were not analyzed in this study. As in any association study involving genotypes, undetected population stratification between 'cases' and 'controls' (in this study, between Val/Met carriers and Val/Val carriers) can lead to false positive or negative results. Our control markers, however, suggest that the Val/Met and Val/Val carriers are reasonably matched, as only one of the 18 control markers showed a statistical difference in genotypes between the Val/Met and Val/Val group, about what one would find by chance in two properly matched genetic samples. Regarding BD subtype, the results of this study may be confounded although there was no significant difference in ACC volumes between bipolar I and II patients and little evidence for a neurobiological difference between bipolar I and II has been reported (McGrath *et al*, 2004). Then, we did not evaluate the personality or character traits of the participants. Serum BDNF levels are inversely correlated with neuroticism and directly correlated with conscientiousness in mentally healthy individuals (Lang *et al*, 2004); however, recent studies did not find any association between BDNF Val66Met polymorphism and personality traits, as assessed by the Temperament and Character Inventory, in female healthy subjects (Rybakowski *et al*, 2007; Tsai *et al*, 2004). Personality differences between BD and healthy individuals may confound the results of this study. Additional studies are needed to determine if BDNF serum levels are correlated with the BDNF gene-related brain volume differences. Finally, the distribution of the Val/Val *vs* the Val/Met genotype was not significantly different between the BD and healthy subjects. Some association studies showed that BD patients have the Val allele at significantly higher frequencies compared with healthy subjects (Lohoff *et al*, 2005; Tang *et al*, 2008). The equal distribution in this study may be due to the sample size. Our sample included 42 healthy and 42 BD subjects whereas some previous studies used much larger samples (eg, 375 BD and 208 healthy subjects (Tang *et al*, 2008) and 621 BD and 998 healthy subjects (Lohoff *et al*, 2005)). The subjects analyzed in this study were completers for the MRI, neuropsychological and blood tests protocols, and some subjects dropped out. Therefore, the gene distribution of our sample may have some bias due to subject attrition.

In summary, we examined the relationship between BDNF Val66Met polymorphism and brain volume differences between BD and healthy individuals. We report the novel finding that the Val/Met carriers showed smaller ACC GM volumes compared to the Val/Val carriers and that this genotype-based difference was larger in the BD patients than in the healthy control subjects.

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DISCLOSURE/CONFLICT OF INTEREST

Emel S Monkul serves as a consultant for Eli Lilly, Brazil. The other authors declare that they do not have any commercial or financial involvements that might present an appearance of a conflict of interest in connection with the submitted article.

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