Neuroimaging studies suggest anterior-limbic structural brain abnormalities in patients with bipolar disorder (BD), but few studies have shown these abnormalities in unaffected but genetically liable family members. In this study, we report morphometric correlates of genetic risk for BD using voxel-based morphometry. In 35 BD type I (BD-I) patients, 20 unaffected first-degree relatives (UAR) of BD patients and 40 healthy control subjects underwent 3 T magnetic resonance scanner imaging. Preprocessing of images used DARTEL (diffeomorphic anatomical registration through exponentiated lie algebra) for voxel-based morphometry in SPM8 (Wellcome Department of Imaging Neuroscience, London, UK). The whole-brain analysis revealed that the gray matter (GM) volumes of the left anterior insula and right inferior frontal gyrus showed a significant main effect of diagnosis. Multiple comparison analysis showed that the BD-I patients and the UAR subjects had smaller left anterior insular GM volumes compared with the healthy subjects, the BD-I patients had smaller right inferior frontal gyrus compared with the healthy subjects. For white matter (WM) volumes, there was a significant main effect of diagnosis for medial frontal gyrus. The UAR subjects had smaller right medial frontal WM volumes compared with the healthy subjects. These findings suggest that morphometric brain abnormalities of the anterior-limbic neural substrate are associated with family history of BD, which may give insight into the pathophysiology of BD, and be a potential candidate as a morphological endophenotype of BD.

Keywords: genetic; insula; mood disorders; MRI; voxel-based morphometry
and third ventricle spaces, greater hyperintensities and smaller corpus callosum area compared with healthy comparison subjects. A recent meta-analysis of voxel-based morphometry (VBM) studies also demonstrated that BD patients have smaller bilateral insula and anterior cingulate cortex (ACC) volumes compared with healthy subjects. Structural MRI studies of adult and child family members of BD patients showed that the first-degree relatives had smaller GM volumes of the left anterior thalamus, caudate, ACC and striatum, and larger caudate, parahippocampus/hippocampus gyrus, left insula, left substantia nigra and left cerebrum compared with healthy control subjects. For white matter (WM), genetic family history of BD was associated with smaller anterior corpus callosum and bilateral frontal, left temporo-parietal and right parietal regions. Together these findings suggest that abnormalities of the anterior-limbic circuit are likely to be involved in the genetic risk for BD, but the association between genetic liability and morphometric change in BD remains unclear. The aim of this study was to test the hypothesis that unaffected first-degree relatives of BD patients (UAR) have regional morphometric abnormalities in the anterior-limbic circuit.

**Subjects and methods**

**Subjects**

In all, subjects with BD-I, 20 UAR subjects and 40 healthy control subjects with no first- or second-degree relatives with any axis I Diagnostic and Statistical Manual-IV (DSM-IV) psychiatric disorder were studied. The participants were recruited at hospitals and clinics and through advertisement broadcast in the community. This study was approved by the Institutional Review Boards of The University of Texas Health Science Center at San Antonio (UTHSCSA) and the University of North Carolina at Chapel Hill (UNC). Written informed consent was obtained from all the participants after a complete description of the study was provided. All of the patients met DSM-IV text revision criteria for BD-I by the Structured Clinical Interview for DSM-IV. The healthy and UAR subjects were screened for DSM-IV axis I disorders by the Structured Clinical Interview non-patient version. The UAR subjects had BD relatives who were given the diagnosis by psychiatrists in the community. 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- or second-degree relatives with any axis I psychiatric disorder were excluded.

All participants were evaluated for handedness by the Edinburgh inventory. All participants also received laboratory tests and a physical examination to rule out physical illnesses. No 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 participated in the study. A senior psychiatrist (JCS) confirmed DSM-IV text revision diagnostic criteria of the patients after reviewing all clinical information and medical or neurological conditions. Current mood states of the patients were evaluated using the Young Mania Rating Scale and the 21-item Hamilton Rating Scale for Depression (HAM-D) at UTHSCSA or Montgomery–Aasberg Depression Scale (MADRS) at UNC. Severity of depressed state was defined as follows: HAM-D, 0–7 or MADRS, 0–8 as euthymic; HAM-D, 8–15 or MADRS, 9–17 as mild; HAM-D, 16–27 or MADRS, 18–34 as moderate and HAM-D, ≥28 or MADRS, ≥35 as severe. Manic state was defined based on Young Mania Rating Scale scores using the following cut points: 13+ as mania, 9–12 as hypomania and 0–8 as not clinically significant. Anxiety state was assessed by the Hamilton Anxiety Rating Scale. The Barratt Impulsiveness Scale version 11, was used to evaluate trait impulsivity. Scoring yields a total score and three subscale scores derived by factor analysis: cognitive/attentional (rapid shifts and impatience with complexity), motor (impetuous action) and non-planning (lack of future orientation). Higher scores indicate higher impulsivity.

**MRI acquisition**

Brain images were collected at the two sites (UTHSCSA and UNC). The images at UTHSCSA were acquired on a Siemens 3T trio scanner (Siemens, Washington, DC): axial three-dimensional T1-weighted magnetization-prepared rapid acquisition gradient echo, slice thickness = 0.8 mm, repetition time = 22 ms, echo time = 3 ms, flip angle = 13° and field of view = 320 mm. The images at UNC were acquired on a Siemens 3 T Allegra scanner (Siemens): axial axial three-dimensional T1-weighted magnetization-prepared rapid acquisition gradient echo, slice thickness = 0.8 mm, repetition time = 17.5 ms, echo time = 4 ms, flip angle = 8° and field of view = 256 mm.

**Image analysis**

Preprocessing was performed using DARTEL (diffeomorphic anatomical registration through exponentiated lie algebra) for voxel-based morphometry in SPM8 software (Wellcome Department of Imaging Neuroscience) running under Matlab R2007b, 7.5.0 (MathWorks, Natick, MA, USA). All original images were manually aligned on the anterior–posterior commissure line. T1-weighted images were segmented and imported into a form that could be used by the main DARTEL algorithm. The procedure of segmentation automatically removed non-brain tissues including scalp, skull and dural venous sinus. A template was created by using the imported images. The segmented images were normalized to the Montreal Neurological Institute space using the template and were smoothed with an 8 mm Gaussian filter.
**Statistical analysis**

We used SPM8 software to implement a general linear model analysis. A whole-brain analysis was used, the model comprising a factorial design with diagnosis as a factor with three levels (BD-I, UAR and the healthy volunteers) and center as a factor with two levels (UTHSCSA and UNC) with age, sex, years of education and handedness as covariates and with total GM and WM volumes as nuisance variables, based on previous multicenter VBM studies. A preliminary voxel-wise F-test was performed with the threshold set at \(P < 0.001\) without correction and an extent threshold \(k\) value > 50 voxels. The regions of interests (ROIs) that met this threshold then were tested by small volume corrections with significance defined as family-wise error set at \(P < 0.05\). For the ROI analysis, a priori ROIs were defined as the following regions comprising anterior-limbic structures: ventromedial and dorsolateral prefrontal cortex, anterior cingulate, striatum, hippocampus and amygdala. These regions were identified using automated anatomical labeling via WFU_PickAtlas version 2.4 (http://www.nitrc.org/projects/wfu_pickatlas/) (Maldjian et al. 27, 28 and Lancaster et al. 29) on SPM8. The mean GM and WM volumes of regions that reached significance by small volume correction were extracted by MarsBar and were tested by analysis of variance, and multiple comparisons corrected by the Bonferroni method to compare the three diagnoses using SPSS for Windows statistical software, version 15.0 (SPSS, Chicago, IL, USA). To examine possible medication effects, we also tested for volume differences of these regions among the unmedicated BD-I, the medicated BD-I, the UAR and the healthy subjects if the analysis of the combined group of medicated and unmedicated BD-I patients differed from the other groups. To do this, the data were analyzed using a main effects analysis of covariance model. Fixed factors representing diagnostic group (unmedicated BD-I, medicated BD-I, UAR and healthy subjects), center (UTHSCSA or UNC) and handedness (left, right or mixed) were included. We also adjusted for covariates representing gender, age and years of education. The primary hypotheses concerning pairwise differences among the adjusted diagnostic group means on each brain area of interest were tested with Bonferroni’s adjusted multiple comparisons. Regarding the mean GM and WM volumes of regions extracted by MarsBar within the BD-I patients, we performed an exploratory analysis of variance for the differences among mild, moderate, severe depression and mixed mood states using analysis of variance. To evaluate the possible influence of clinical variables, we computed correlations with the age of illness onset, the length of illness, anxiety score and Barratt Impulsiveness Scale total score using Pearson’s correlation coefficients. We also used a multiple regression model to assess the association of these clinical features to brain volumes while statistically controlling age, sex, years of education, center and handedness as covariates and total GM and WM volume as a nuisance variable in the whole-brain analysis. All results were presented as Montreal Neurological Institute coordinates.

**Results**

**Demographic data**

Table 1 summarizes the clinical and demographic characteristics of the participants. Mean age, years of education, handedness and distribution by sex were not significantly different between the BD, UAR and healthy participants. There was a significant

<table>
<thead>
<tr>
<th></th>
<th>BD</th>
<th>UAR</th>
<th>HC</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (s.d.), years</td>
<td>40.8 (9.2)</td>
<td>46.2 (10.7)</td>
<td>41.6 (9.1)</td>
<td>0.12</td>
</tr>
<tr>
<td>Female, numbers (%)</td>
<td>77.1</td>
<td>75.0</td>
<td>60.0</td>
<td>0.23</td>
</tr>
<tr>
<td>Years of education, mean (s.d.), years</td>
<td>14.5 (2.5)</td>
<td>15.1 (2.1)</td>
<td>15.8 (2.8)</td>
<td>0.10</td>
</tr>
<tr>
<td>Handedness, right (%)</td>
<td>79.4</td>
<td>89.5</td>
<td>76.3</td>
<td>0.44</td>
</tr>
<tr>
<td>Center, SA number (%)</td>
<td>8 (22.9)</td>
<td>10 (50.0)</td>
<td>31 (77.5)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td><strong>BIS11(_A), mean (s.d.)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>59.3 (13.5)</td>
<td>42.3 (5.6)</td>
<td>40.2 (5.6)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Cognitive/attentional</td>
<td>14.2 (4.0)</td>
<td>8.7 (2.0)</td>
<td>8.1 (1.9)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Motor</td>
<td>19.8 (4.9)</td>
<td>15.7 (2.3)</td>
<td>14.6 (2.2)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Non-planning</td>
<td>25.5 (6.0)</td>
<td>17.8 (3.0)</td>
<td>17.7 (3.3)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td><strong>Volumes, mean (s.d.) cm(^3)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM</td>
<td>608.5 (59.6)</td>
<td>620.7 (49.8)</td>
<td>643.3 (12.2)</td>
<td>0.07</td>
</tr>
<tr>
<td>WM</td>
<td>444.7 (65.9)</td>
<td>447.5 (43.4)</td>
<td>463.0 (57.1)</td>
<td>0.36</td>
</tr>
<tr>
<td>GM + WM</td>
<td>1053.2 (120.8)</td>
<td>1068.1 (87.8)</td>
<td>1106.4 (126.6)</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Abbreviations: BD, bipolar disorder patients; BIS11\(_A\), Barratt Impulsiveness Scale version 11\(_A\); GM, gray matter; HC, healthy control subjects; SA, San Antonio; UAR, unaffected first-degree relatives; WM, white matter.

*Pearson \(\chi^2\)-test. The other characteristics were analyzed using analysis of variance.
difference for the center where the MRI was performed ($P<0.01$). Six patients had one or more comorbid anxiety disorders, including panic disorder ($n=2$), post-traumatic stress disorder ($n=3$) and generalized anxiety disorder ($n=1$). In all, 21 patients were unmedicated during the 2 weeks immediately before the study, and the remaining 13 patients were receiving psychiatric medications, including mood stabilizers including lithium, antidepressants and atypical antipsychotics at the time of the study. In all, 10 patients were remitted, 4 patients had mild depression, 13 patients had moderate depression and 2 patients were in a mixed state, according to DSM-IV text revision criteria. The mean ± s.d. HAM-D score was 18.6 ± 9.3 (range 2–31) for the BD-I patients, the mean ± s.d. MADRS score was 19.0 ± 15.0 (range 0–48) and the mean ± s.d. Young Mania Rating Scale score was 3.7 ± 4.1 (range 0–15). The mean age of onset was 19.6 ± 10.3 years (range 7–55), and the mean length of illness was 21.3 ± 11.5 years (range 0–39). Four UAR subjects had a parent with BD, eight had a sibling with BD and nine had a child with BD. The BD-I patients had higher Barratt Impulsiveness Scale version 11A scores compared with the UAR and healthy subjects for total ($F_{(2, 80)}=38.4$, $P<0.01$), cognitive/attentional ($F_{(2, 85)}=43.7$, $P<0.01$), motor ($F_{(2, 86)}=20.5$, $P<0.01$) and non-planning ($F_{(2, 82)}=31.0$, $P<0.01$).

**Imaging data**

There were no significant differences amongst the BD-I, UAR and healthy control subjects for total brain volume, total GM or total WM volumes (Table 1). The whole-brain analysis and small volume correction revealed that there was a significant main effect of diagnosis on the GM volumes of left anterior insula and right inferior frontal gyrus (Figure 1). In the two regions, there was also a significant main effect of center in the left insular GM volumes ($x = -38$, $y = 8$, $z = 16$; $F = 17.00$, $k = 71$, $P_{FWE} < 0.01$) and was not a significant interaction center and diagnosis in the whole-brain analysis. The left insular GM volumes with the significant effects of diagnosis and center extracted by MarsBar were compared. The result was that the main effect of diagnosis ($F_{2, 80} = 6.93$, $P<0.01$) was significant, but the main effect of center ($F_{1, 80} = 0.01$, $P = 0.92$) and the interaction between diagnosis and center ($F_{2, 80} = 0.77$, $P = 0.49$) were not significant, suggesting that left anterior insular GM volumes were associated with diagnosis but did not depend on center. The pairwise multiple comparison analyses revealed that the BD-I patients and the UAR subjects had smaller left anterior insular GM volumes compared with the healthy subjects ($P < 0.01$, $P = 0.02$, respectively; Figure 2), and the BD-I patients had smaller right inferior frontal gyrus volumes compared with the healthy subjects ($P < 0.01$; Figure 3). The unmedicated BD-I patients had smaller GM volumes of the left anterior insular and the left middle frontal gyrus compared with the healthy subjects ($P = 0.04$, 0.03, respectively; Figures 2 and 3). For WM, there was a significant main effect of diagnosis on the medial frontal gyrus volumes (Figure 1), with non-significant effects of center and diagnosis by center interaction. The UAR subjects had smaller WM volumes of the right medial frontal gyrus compared with the healthy subjects ($P = 0.05$; Figure 4).

The BD-I patients showed a significant inverse correlation between the duration of illness and the right inferior frontal GM volumes ($r = -0.35$, $P = 0.05$). The BD-I patients also showed a positive correlation between the right inferior frontal GM volumes and both the cognitive/attentional Barratt Impulsiveness Scale version 11A scores ($r = 0.47$, $P = 0.01$) and the anxiety scores and ($r = 0.36$, $P = 0.04$). There were no significant regional GM or WM volume differences among the BD-I patients classified according to current mood state. A voxel-wise F-test revealed that mood state, medication, anxiety scores and impulsivity scores were not significantly correlated with the GM volumes of left anterior insular and right inferior frontal gyrus or the WM volume of right medial frontal gyrus.

For the ROI analysis, there was not a significant main effect of diagnosis in the ROI GM or WM volumes, except for the regions that showed significance in the whole-brain analysis.

**Discussion**

The primary results of this study were that the BD-I patients, in particular the unmedicated patients, and the UAR subjects had smaller left insular GM volumes compared with the controls, and the BD-I patients had...
smaller right inferior and left medial frontal GM volumes compared with the UAR and control subjects. This was the case whether or not the patients were medicated. More chronic BD-I patients showed smaller right inferior frontal GM volumes. The UAR subjects had smaller right medial frontal WM volumes compared with the healthy subjects. These finding are in agreement with the evidence that the anterior fronto–limbic circuit is involved in the pathophysiology of BD, and further suggest that the left insular GM volumes are associated with BD genetic liability and also that the right inferior frontal GM volumes are associated with BD manifestation and progression.

The insular cortex has efferent and afferent projections with the frontal cortex, anterior cingulate cortex, parietal lobe, temporal lobe, basal nuclei, amygdala, dorsal thalamus and other limbic areas in primates and humans. The region has a key role in self-relevant feelings, empathy and uncertainty prediction. A review PET and functional MRI studies of emotional activation concluded that the insular cortex is activated in response to cognitively emotional tasks induced by internally generated

**Figure 2** The mean left anterior insular gray matter volumes were significantly different among the three diagnostic groups. The cross-hair represents the coordinate with maximum threshold. BD, patients with bipolar disorder; HC, healthy control subjects; med, medicated; UAR, unaffected first-degree relatives of BD; unmed, unmedicated.

**Figure 3** The mean right inferior frontal gray matter volumes were significantly different among the three diagnostic groups. The cross-hair represents the coordinate with maximum threshold. BD, patients with bipolar disorder; HC, healthy control subjects; UAR, unaffected first-degree relatives of BD.
The left insula activates the processing of neutral and sad faces compared with baseline and the processing of angry faces compared with neutral faces, whereas right insular activates the processing of faces showing disgust compared with baseline and deactivates the processing of angry faces compared with disgust faces. These findings from anatomical and functional neuroimaging studies indicate that insular cortex is involved in the neural substrates of emotional processing and participates in mood regulation per se. In turn, cumulative evidence suggests that patients with mood disorders have abnormal insular cortex volumes and functions. A recent meta-analysis of VBM studies showed that BD patients have bilaterally smaller insular and perigenual anterior cingulate GM volumes compared with healthy control subjects. Depressed BD patients showed greater glucose metabolism in the right anterior insula compared with healthy controls, and more severely depressed BD patients had lower glucose metabolism of the left anterior insula. Further, depressed patients had lower activation in the left insula in response to negative versus neutral stimuli than healthy comparison subjects at baseline and 2 weeks after a serotonin–norepinephrine reuptake inhibitor (venlafaxine) treatment. The finding was supported by an animal study showing that antidepressant administration reduced depression-like behaviors and glucose metabolism of insular/piriform cortex in rats. Although the neuropathological abnormality of the insular cortex in patients with BD remains unclear, this region has a critical role as a neural substrate for mood regulation and is involved in the pathophysiology of BD. However, to our knowledge, there is only one previous study showing abnormal insular volume in family members of BD patients. Kempton et al. demonstrated that left insula, left cerebellum and left substantia nigra showed significant differences because of the diagnosis (BD patients, their unaffected and affected first-degree relatives and controls). The BD patients and UAR subjects also had larger volumes of the left insula than healthy subjects. Their findings were in contrast with ours. They did not discuss why BD patients and first-degree relatives of BD had larger insular volumes. On the basis of cumulating evidence that patients with BD have small insular volumes, we speculate that if the insular GM volume is associated with genetic risk for BD, then first-degree relatives of BD would have somewhat smaller insular GM volumes compared with healthy subjects, and maybe also would have volumes intermediate to those of BD patients and healthy subjects. The results of this study support this speculation. Although the reason why the two studies had contrasting results is unclear, the findings would imply that insula is likely to be involved in the morphometric pathophysiology of familial and genetic liability for BD.

This study also showed that the BD-I patient subjects had smaller right inferior frontal GM volumes compared with the control subjects, the patients with a longer course of the illness had smaller GM volumes of this region and the UAR had comparable GM volumes of this region to the healthy subjects. Previous VBM studies demonstrated that BD patients had smaller GM density in right inferior frontal gyrus, left anterior cingulate gyrus, left medial frontal gyrus and right precentral gyrus compared with healthy subjects. BD patients with family history of BD also showed smaller GM density in the right inferior frontal gyrus and the left and right lateral orbital gyri. These two studies evaluated chronic BD patients (the duration of illness, 18.1 ± 11.0 years and 15.4 ± 10.0 years, respectively). In contrast, the first-episode patients with BD did not show abnormal GM volumes of ventrolateral prefrontal cortex including inferior frontal gyrus, although an MRI meta-analysis of first-episode BD patients excluded the prefrontal region from the analysis because there were too few quantitative MRI studies and/or the data presented did not meet the criteria for analysis. Further, the chronic BD patients had...
smaller volumes of bilateral inferior frontal gyrus compared with the first-episode BD patients. The volume of the left inferior frontal gyrus in BD patients was inversely correlated with the illness duration. These findings suggest that the inferior frontal GM region is associated with the course of the disease. Our finding of the BD patients and UAR subjects may contribute to expanding the evidence that volume changes of this region relate to BD progression and manifestation.

The right inferior gyrus has a role in inhibition control. Subjects with larger damage of the right inferior gyrus had more blunted inhibition response, but this association was not observed in subjects with damage of the middle frontal gyrus or superior frontal gyrus. Functional MRI studies showed increasing activation of the right inferior gyrus was associated with inhibition response. Converging evidence of neurocognitive studies suggests that BD patients have impairment of inhibition control. A meta-analysis study of BD and UAR subjects demonstrated that response inhibition, set shifting, executive function, verbal memory and sustained attention deficits are common features for BD and UAR, and that response inhibition is assumed to be the most prominent endophenotype of BD. Moreover, pediatric BD patients showed less activation of right ventral prefrontal cortex, adjacent to inferior frontal gyrus and bilateral striatal cortex on failure of inhibitory response. The result of this study that the BD-I patients with higher cognitive/attentional impulsiveness had larger right inferior GM volumes supports these findings. However, studies of adult BD patients did not show any association between abnormal inhibition/impulsiveness and inferior gyrus volume but did show increased activation in left middle frontal gyrus during an inhibition task, and that smaller GM volumes of the left rostral ACC correlated with higher impulsiveness. Although more investigations of the neural correlates of abnormal impulsiveness in BD are needed to settle the inconsistency, these findings indicate that BD patients may have different neural substrates of emotional regulation of impulsiveness and inhibition control than healthy subjects.

We found small WM volumes of the medial frontal gyrus, which lies close to the ACC, in the UAR subjects. The ACC contributes to executive functions, including attention, inhibition and resolution of competitive cognitive conflict in executive processes, and is assumed to be involved in the pathophysiology of BD. A meta-analysis of VBM studies demonstrated smaller bilateral ACC volumes in the BD compared with healthy subjects. Cognitive and executive dysfunction in UAR subjects has been noted in meta-analyses of neuropsychological studies. Previous MRI studies demonstrated that small WM volumes of the anterior medial frontal region are associated with genetic risk of BD, and small GM volumes of the ACC were found in the first-degree relatives of BD. The findings of this study further support the hypothesis that medial frontal cortex, including ACC, is a candidate structural endophenotype of BD.

Although the reason why the BD patients did not show abnormal medial WM volume in our study is unknown, medication effects may have had some influence on the result because the medicated patients were taking mood stabilizers including lithium, second generation antipsychotics and antidepressants, and the unmedicated BD patients had a history of medication use at the time of their study participation. Accumulating evidence suggests that lithium and mood stabilizers are associated with increased GM volumes, but the effects of medications on WM volumes remains unclear. In fact, the medicated BD patients had larger GM volumes of the left insular cortex compared with the unmedicated BD patients, but medial WM volumes were not different in this study. We cannot exclude the possibility that medication might be masking the effects of BD illness on GM and WM volumes in our study. Future studies of medication-naive BD patients will be warranted to resolve this question.

Other methodological limitations of this study should be noted. The sample size is fairly small, particularly for the UAR subjects. In this cross-sectional study, we were unable to assess the actual degree of risk for developing BD among the UAR subjects. Also, it is noteworthy that the mean age of the UAR subjects in this study was greater than the mean onset age for BD according to epidemiological findings. Longitudinal studies of younger unaffected offspring of a parent with BD will be necessary to determine if the presence of structural brain abnormalities actually predict BD onset, as shown by Pantelis et al., in their study of schizophrenia. This study combined subjects from two centers (UTHSCSA and UNC) without prospective harmonization of the two MRI scanners. If prospective harmonization of multiple scanners is carried out, then the analysis cannot extract and directly test the effect of scanner model on the results. Thus, we adopted a factorial experimental design that permitted the extraction and quantification of between center variance in brain volumes. Using this approach, we attempted to control not only scanner specific effects but also other potential center-related differences, such as sample characteristics and study personnel. This analysis supported the conclusion that neither the main effect of center nor the center by diagnosis interaction was a significant determinant of the regional brain volumes. Clinical features of the BD-I subjects, including mood state, anxiety and impulsivity, also were not associated with brain volumes. Taken together, these findings suggest that diagnosis is the best explanation for the volume differences between groups.

We did not directly examine the BD-affected relatives of the UAR subjects, so we relied on indirect information obtained by interviewing the UAR subjects. Most of these BD-affected relatives our UAR subjects were diagnosed and treated by their commu-
nity psychiatrists, but they were not diagnosed by our Structured Clinical Interview interview. We, therefore, were not able to assess, for example, whether these BD-affected relatives had bipolar I or II disorder. This study was similar to two previous ones\(^1\),\(^2\) with respect to study design and participants, yet produced somewhat different results. McDonald et al.\(^1\),\(^2\) developed a formula for a genetic liability scale that was derived from a polygenic multifactorial liability threshold model, and used a multiple regression model to estimate the association between the genetic liability and brain structure variation in optimized VBM in SPM99. They included BD-I patients with a history of psychosis and their relatives without psychosis, some of whom met criteria for a lifetime DSM-IV axis I disorder. McIntosh et al.\(^1\) included BD-I patients with family history of mixed disorders (schizophrenia and BD) and BD, and unaffected relatives of patients with mixed disorders and BD and performed the following comparisons: BD patients versus healthy subjects, unaffected relatives versus healthy subjects and BD patients versus unaffected relatives in SPM99. This study compared affected BD-I patients with and without psychosis versus psychologically healthy (that is, no lifetime axis I disorders) first-degree relatives of BD-I patients with or without psychoses (for example, schizophrenia). We performed a voxel-wise F-test to test the effect of diagnosis in optimized VBM in SPM8 and multiple comparisons of the regional volumes with Bonferroni’s correction in SPSS. It is suggested that SPM8 has a superior algorithm for segmentation and normalization of pre-processing compared with older versions of SPM. These differences in study design, study participants, statistical analyses and SPM version may potentially explain the different results between the present findings and previous ones. Another limitation is that we used the HAM-D to assess severity of depression at UTHSCSA and the Another limitation is that we used the HAM-D to assess severity of depression at UTHSCSA and the Another limitation is that we used the HAM-D to assess severity of depression at UTHSCSA and the Another limitation is that we used the HAM-D to assess severity of depression at UTHSCSA and the MADRS at UNC. We addressed this issue by classifying the depressive states of subjects into four categories according a method used in a previous study.\(^2\) There also was a large difference in the distribution of subjects according to diagnosis between the two clinical centers. Although the MRI equipment used at the two centers was manufactured by the same company, we included treatment center as a covariate in the statistical analyses of VBM. Elevated anxiety among the BD patients may affect the results for the anterior insula because the anterior insula has a role of the processing of anxiety.\(^5\) However, we showed no relationship between severity of anxiety and the right insular GM volumes in the BD patients. Thus, anxiety is an unlikely explanation for these results.

This goal of this study was to elucidate the association between genetic liability for BD, as it is related to family history, and morphometric correlates by comparing BD patients versus a sample of unaffected relatives of BD patients. We identified several critical brain regions that may be vulnerable due to genetic liability, and these may be implicated in BD pathophysiology and disease progression. The regions comprise the anterior limbic circuit that is relevant to mood regulation and is involved in the pathophysiology of BD. Our findings may provide insight into a potential candidate for morphological endophenotype of BD.

**Conflict of interest**

The authors declare no conflict of interest.

**Acknowledgments**

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