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Morphologic Alterations in Amygdala Subregions of Adult Patients with Bipolar Disorder

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Objectives Previous studies have revealed inconsistent results on amygdala volume in adult bipolar disorder (BD) patients compared to healthy controls (HC). Since the amygdala encompasses multiple subregions, the subtle volume changes in each amygdala nucleus might have not been fully reflected in the measure of the total amygdala volume, causing discrepant results. Thus, we aimed to investigate volume changes in each amygdala subregion and their association with subtypes of BD, lithium use and clinical status of BD.

Methods Fifty-five BD patients and 55 HC underwent T1-weighted structural magnetic resonance imaging. We analyzed volumes of the whole amygdala and each amygdala subregion, including the anterior amygdaloid area, cortico-amygdaloid transition area, basal, lateral, accessory basal, central, cortical, medial and paralaminar nuclei using the atlas in the FreeSurfer. The volume difference was analyzed using a one-way analysis of covariance with individual volumes as dependent variables, and age, sex, and total intracranial volume as covariates.

Results The volumes of whole right amygdala and subregions including basal nucleus, accessory basal nucleus, anterior amygdaloid area, and cortico-amygdaloid transition area in the right amygdala of BD patients were significantly smaller for the HC group. No significant volume difference between bipolar I disorder and bipolar II disorder was found after the Bonferroni correction. The trend of larger volume in medial nucleus with lithium treatment was not significant after the Bonferroni correction. No significant correlation between illness duration and amygdala volume, and insignificant negative correlation were found between right central nucleus volume and depression severity.

Conclusions Significant volume decrements of the whole amygdala, basal nucleus, accessory basal nucleus, anterior amygdaloid area, and cortico-amygdaloid transition area were found in the right hemisphere in adult BD patients, compared to HC group. We postulate that such volume changes are associated with altered functional activity and connectivity of amygdala nuclei in BD.

Key Words Bipolar disorder · Magnetic resonance imaging · Amygdala · Basolateral nuclear complex · Image processing, computer-assisted · Lithium.

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Introduction

Despite the relatively low prevalence of bipolar disorder (BD), which is estimated as 0.8% for bipolar I disorder (BD I) and 1.1% for bipolar II disorder (BD II), the affected subjects are mostly working population, which in turn becomes a massive health burden to the society.[1)] In addition, BD seriously affects the quality of life of patients, and constitutes the major causes of premature mortality by leading to suicide and associated medical diseases.[2)3)] Yet the precise neurobiological mechanism of BD is not elucidated, but certain neuroimaging studies have suggested possible brain areas responsible for the pathophysiology of the disorder.[4)] Along with the findings of decreased volume in cortical regions such as the prefrontal cortex, orbitofrontal cortex, anterior temporal cortex, insula, and dorsal anterior cingulate cortex,[5-15)] subcortical areas such as amygdala and hippo-

campus showed significant volume changes in BD patients as well.[16)]

Along with the well-known function of processing unpleasant stimuli such as fear, the amygdala has a fundamental role in emotion regulation and processing.[17)18)] Impairment in emotion regulation and processing is one of the key symptoms of BD,[19)] suggesting the involvement of amygdala in the pathophysiology of BD.[20)] Erstwhile consistent results of functional studies support the supposition. Compared with the healthy control (HC) group, the left amygdala in BD subjects showed abnormal hyperactivity during mania,[21)] and in response to positive and negative facial expressions.[22)]

In pediatric BD patients, structural studies have shown reasonably consistent findings of smaller volume of the whole amygdala.[23-25)] In addition, within the same youth sample, the inverse correlation between the functional and the morphological alterations of the amygdala was validated.[26)] However, structural studies on the adult amygdala volume have produced contradictory results, reporting both volume reduction[23)27)28)] and volume enlargement[29-31)] in BD subjects.

The amygdala participates in multiple domains of emotion and behavior, such as assigning emotional valence to neutral sensory inputs, social communication, reward learning, motivation, attention, perception, and explicit memory.[32-38)] The functional diversity of amygdala implies its structural heterogeneity as well. The amygdala encompasses multiple nuclei, distinct from one another by their unique microanatomy, functions and connections to other parts of the brain.[39)] Therefore, the subtle volume changes in each amygdala nucleus might have not been fully reflected in the measure of the total amygdala volume, consequentially causing discrepant study results.

Unlike the majority of the cerebral cortex, which almost completes its volume growth by the age of six, a study showed that amygdala enlarges by 40% and its neuronal number increases by 11% from adolescence to adulthood. More interestingly, the amount of neuron number increment differed from one nucleus to another, where the basal nucleus increased by 30%, the accessory basal nucleus by 17%, and the lateral nucleus by 3%, suggesting that each amygdala nucleus matures in different speed and pattern.[40)] Such developmental pattern of amygdala nuclei further necessitates the evaluation of each nucleus specifically in adult samples.

Thus, in the present study, we aimed to investigate changes in the volumes of amygdala nuclei in adult patients with BD. Our a priori hypothesis was that there might be a significant difference in the volume of each specific amygdala nucleus between patients with BD and HC. In addition, we also aimed to elucidate the association between the subtypes of BD, lithium treatment, clinical states, illness duration, and the volume of each specific amygdala nucleus.

Methods

Participants

Twenty-nine patients diagnosed with BD I, and twenty-six patients diagnosed with BD II were recruited from the outpatient clinics in Korea University Anam Hospital. A total of 55 BD patients were adults aged 19−64. Board-certified psychiatrists (K.-M. Han, E. Won, and B.-J. Ham) diagnosed patients with BD according to the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision (DSM-IV-TR) criteria, and an independent psychiatrist confirmed the diagnosis using the Structured Clinical Interview for DSM-IV-TR Axis I disorders. Following exclusion criteria were applied in recruitment: 1) primary comorbid diagnosis of any other major psychiatric illness (based on DSM-IV-TR criteria) on Axis I or Axis II within the last 6 months; 2) acute suicidal or homicidal idea necessitating admission; 3) serious or unstable medical illness; 4) primary neurological illness, such as cerebrovascular disease, Parkinson's disease, or epilepsy; 5) abnormal results on physical examination or laboratory tests; 6) pregnant or nursing women; and 7) any contraindication for magnetic resonance imaging (MRI). The duration of the illness was assessed by a board-certified psychiatrist using the life-chart methodology. We recruited 55 healthy volunteers aged between 19 and 64 years without a history of psychiatric illness from the community to build a control group. Board-certified psychiatrists confirmed the empty psychiatric history of the HC and applied the same exclusion criteria as the BD group. On the day of MRI scanning, 55 BD patients were assessed by psychiatrists using the 17-item Hamilton Depression Rating Scale (HDRS).[41)] Out of 55 BD patients, 37 patients were in euthymic state, as indicated by a HDRS score of 7 or lower, and 18 were in depressive state. Upon study enrollment, all 55 BD patients were receiving pharmacotherapy, of which 11 were taking lithium. The details of the patients are described in Table 1. The study protocol was approved by the Institutional Review Board of the Korea University Anam Hospital (2015AN0009), and all methods in this study were carried out in accordance with the approved guidelines and the Declaration of Helsinki. All subjects gave written informed consent to participate in the study after a full explanation and understanding of the study in accordance with the Declaration of Helsinki.

MRI data acquisition

MRI scans were acquired parallel to the anterior commissure–

Table 1. Demographic and clinical characteristics of patients with BD and HC

Characteristics	BD (n = 55)	HC (n = 55)	p value (t, χ^2)
Age (years)	33.29 ± 11.32	35.18 ± 12.75	0.413 (t = -0.822)
Sex (female/male)	24/31	28/27	0.567 (χ^2 = 0.584)
Education level			
Elementary and middle school	0	2	
High school or college/university	51	47	0.278 (χ^2 = 2.563)
Above graduate school	4	6	
HDRS-17 score	6.31 ± 4.87	1.80 ± 2.01	< 0.001 (t = 6.344)
BD I/BD II	29/26	NA	NA
Euthymic (or remission) state/depressive state	37/18	NA	NA
Duration of illness (months)	45.29 ± 64.38	NA	NA
Drug-treated patients (n)	55	NA	NA
Medication, n			
SSRI	8		
SNRI	2		
NDRI	7		
NaSSA	1		
Combination of AD	0		
Lithium	7	NA	NA
AED	38		
Lithium + AED	4		
AED + AED	1		
AP	25		
Combination of AP	23		
TICV (mm^3)	1444253.31 ± 131053.36	1448157.16 ± 177530.28	0.896 (t = -0.131)

Data are mean ± standard deviation for age, HDRS-17 scores, and illness duration. The p values for distribution of sex and education level were obtained using a chi-squared test. The p values for comparisons of age and HDRS scores were obtained using an independent t-test. BD : bipolar disorder, HC : healthy controls, HDRS-17 : 17-item Hamilton Depression Rating Scale, BD I : bipolar I disorder, BD II : bipolar II disoder, SSRI : selective serotonin reuptake inhibitor, SNRI : serotonin and norepinephrine reuptake inhibitor, NDRI : norepinephrine-dopamine reuptake inhibitor, NaSSA : noradrenergic and specific serotonergic antidepressant, AD : antidepressants, Combination of AD : combinations of two or more types of antidepressants, AED : anti-epileptic drugs, AP : antipsychotics, Combination of AP : combinations of two or more types of antipsychotics, TICV : total intracranial cavity volume

posterior commissure line using a 3.0 T Siemens Trio whole-body imaging system (Siemens Medical Systems, Iselin, NJ, USA), using T1-weighted magnetization-prepared rapid gradient-echo with the following parameters: 1900 ms repetition time, 2.6 ms echo time, 220 mm field of view, 256 × 256 matrix size, 1 mm slice thickness, 176 coronal slices without gap, 0.86 × 0.86 × 1 mm^3 voxels, 16° flip angle, number of excitations = 1.

Image processing

The volumes of nine amygdala nuclei (lateral nucleus, basal nucleus, accessory basal nucleus, central nucleus, medial nucleus, cortical nucleus, anterior amygdaloid area, cortico-amygdaloid transition area, and paralaminar nucleus) and the whole amygdala were calculated from T1 images of all participants using the automated procedure for volumetric measures in the FreeSurfer 6.0 development version (Massachusetts General Hospital, Boston, MA, USA; http://surfer.nmr.mgh.harvard.edu). The automatic segmentation method for the nine amygdala nuclei developed by Saygin et al.[39] uses a statistical atlas of the amygdala and the surrounding tissue. The automated segmentation method by Saygin et al.[39] labeled nine amygdala nuclei by scanning postmortem specimens at high resolution, and created an atlas using a recently developed atlas-building algorithm based on Bayesian inference. Details of the technical aspects of this procedure have been described in a previous publication of Saygin et al.[39] In brief, preprocessing of T1 images was performed using the FreeSurfer pipeline. The preprocessing procedures included skull-stripping, bias field correction,[42] automated Talairach transformation of each subject's native brain,[43][44] intensity normalization, and segmentation of subcortical structures.[43] After the above-mentioned procedures were performed, preprocessed T1 images were fed to the automatic segmentation algorithm of amygdala implemented in the FreeSurfer, and the volumes of each amygdala nuclei and the entire amygdala were calculated.[39] The demeaned value for the total intracranial cavity volume (TICV), measured manually,[45] was

used as a covariate to normalize regional brain volumes. Our T1 imaging outputs in all processes were visually inspected by two trained independent researchers (W.-S. Tae and J. Kang) to ensure the quality of the analyses.

Statistical analyses

For group differences in demographic and clinical characteristics, age, HDRS scores, and TICV were analyzed using t-tests, and the distributions of sex and education level were analyzed using the chi-square test. Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 24.0 (IBM Corp., Armonk, NY, USA). For main analysis, first, we compared the volumes of each amygdala subregion and the whole amygdala according to the diagnostic groups (BD group vs. HC group), the subgroups of BD patients determined by the subtypes (BD I vs. BD II), and lithium treatment [lithium-treated patients with bipolar disorder (Li+) vs. bipolar disorder patients without taking lithium treatment (Li-)]. Automatically calculated data for the volumes of each amygdala subregion and the whole amygdala obtained from the FreeSurfer were analyzed using a one-way analysis of covariance with individual volumes as dependent variables, and age, sex, and TICV as covariates. Illness duration and HDRS score were used as additional covariates when comparing amygdala subregion volumes according to lithium treatment. For multiple comparisons, we applied the Bonferroni correction: $p < 0.05/20$ (18 subregions and 2 total volume in bilateral amygdala) = 0.0025. As a secondary analysis, we investigated the correlation of illness duration and HDRS score with amygdala subregion volumes in BD patients using a two-tailed Pearson's partial correlation analysis with age, sex, and TICV as covariates.

Results

Demographic and clinical characteristics

The data regarding age, sex, education level, HDRS score, and TICV of BD and HC groups, duration of illness, subtype of BD, mood state and treated medication are shown in Table 1. The BD group and the HC group did not differ significantly in terms of age, sex, education level, and TICV, but had different HDRS scores ($t = 6.344$, $p < 0.001$). The mean duration of the illness in patients with BD was 45.29 ± 64.38 months.

Table 2. Amygdala subregion volume difference between patients with BD and HC

Amygdala subregions	BD (mm^3)		HC (mm^3)		BD vs. HC	
	Mean	SD	Mean	SD	$F_{(1, 105)}$	p value
Left hemisphere						
Lateral nucleus	719.24	81.74	742.89	112.59	2.607	0.109
Basal nucleus	435.60	43.70	456.54	59.54	7.518	0.007
Accessory basal nucleus	258.67	26.15	271.86	33.71	7.500	0.007
Anterior amygdaloid area	67.30	8.84	70.18	10.23	3.710	0.057
Central nucleus	54.28	8.94	56.82	10.77	3.336	0.071
Medial nucleus	26.47	5.05	27.38	5.66	1.122	0.292
Cortical nucleus	27.11	4.55	27.54	4.79	0.148	0.701
Cortico-amygdaloid transition area	155.41	22.03	164.19	27.91	3.448	0.066
Paralaminar nucleus	41.82	5.53	43.29	6.29	2.154	0.145
Whole amygdala	1785.89	174.64	1860.67	245.98	5.589	0.020
Right hemisphere						
Lateral nucleus	723.07	84.43	751.93	91.44	3.767	0.055
Basal nucleus	447.72	45.10	478.87	58.16	17.206	6.83.E-05*
Accessory basal nucleus	277.76	27.40	300.83	38.62	20.332	1.70.E-05*
Anterior amygdaloid area	67.74	8.09	74.58	9.64	19.629	2.32.E-05*
Central nucleus	52.56	7.86	54.97	8.10	3.259	0.074
Medial nucleus	27.39	5.01	28.52	5.49	1.683	0.197
Cortical nucleus	30.82	3.71	32.66	5.12	5.927	0.017
Cortico-amygdaloid transition area	161.24	18.21	178.56	29.90	16.895	7.86.E-05*
Paralaminar nucleus	41.68	5.42	43.45	5.95	3.544	0.063
Whole amygdala	1829.99	178.29	1944.39	224.07	14.293	2.60.E-04*

The F and p values were obtained using one-way analysis of covariance with the adjustment for age, sex, and total intracranial cavity volume as covariates. The Bonferroni correction was applied : $p < 0.05/20 = 0.0025$. * : Significant amygdala subregion volume differences. BD : bipolar disorder, HC : healthy controls, SD : standard deviation

Analyses of amygdala subregion volumes in patients with BD and HC

The whole right amygdala volume [$F_{(1, 105)}$ = 14.293, p = 2.60 × 10^{-5}; BD = 1829.99 ± 178.29 mm^3, HC = 1944.39 ± 224.07 mm^3], and the subregions including basal nucleus [$F_{(1, 105)}$ = 17.206, p = 6.83 × 10^{-5}; BD = 447.72 ± 45.10 mm^3, HC = 478.87 ± 58.16 mm^3], accessory basal nucleus [$F_{(1, 105)}$ = 20.332, p = 1.70 × 10^{-5}; BD = 277.76 ± 27.40 mm^3, HC = 300.83 ± 38.62 mm^3], anterior amygdaloid area [$F_{(1, 105)}$ = 19.629, p = 2.32 × 10^{-5}; BD = 67.74 ± 8.09 mm^3, HC = 74.58 ± 9.64 mm^3], and cortico-amygdaloid transition area [$F_{(1, 105)}$ = 16.895, p = 7.86 × 10^{-5}; BD = 161.24 ± 18.21 mm^3, HC = 178.56 ± 29.90 mm^3] in the right amygdala were significantly smaller for the HC group in BD patients after the Bonferroni correction (Table 2).

Association between the amygdala subregion volumes and subtypes of BD and lithium treatment

We investigated association of the subtypes of BD with amygdala subregion volumes in the BD I and BD II subgroups, using the same statistical methods as those used in the analysis of BD and HC groups (Table 3). We found a significant volume difference in right paralaminar nucleus [$F_{(1, 50)}$ = 5.576, p = 0.022 ; BD I = 42.66 ± 5.64 mm^3, BD II = 40.59 ± 5.06 mm^3]. However, this finding was not significant after the Bonferroni correction.

We also investigated associations between lithium treatment and the amygdala subregion volumes, using the same above statistical methods, but including illness duration and HDRS as additional covariates (Table 4). We found significant volume difference in the medial nucleus [$F_{(1, 48)}$ = 4.269, p = 0.044; Li+ = 29.97 ± 6.66 mm^3, Li- = 26.75 ± 4.36 mm^3], although this finding was not significant after the Bonferroni correction.

Association of amygdala subregion volumes with clinical states and illness duration

As secondary analysis, we investigated correlations between the duration of illness and the amygdala subregion volumes using partial correlation analysis adjusting for age, sex, and TICV (Table 5). However, we could not find any significant correlations (all, p > 0.05). We also explored correlations between HDRS scores and the amygdala subregion volumes using the same statistical methods as those used in the illness duration analysis. We found a significant negative correlation (r = -0.311,

Table 3. Amygdala subregion volume difference between patients with BD I and BD II

Amygdala subregions	BD I (mm^3)		BD II (mm^3)		BD I vs. BD II	
	Mean	SD	Mean	SD	$F_{(1, 50)}$	p value
Left hemisphere						
Lateral nucleus	708.88	70.37	730.78	92.86	0.102	0.750
Basal nucleus	431.36	41.88	440.33	46.00	0.006	0.940
Accessory basal nucleus	255.12	24.71	262.62	27.61	0.197	0.659
Anterior amygdaloid area	66.56	9.03	68.12	8.73	0.005	0.946
Central nucleus	54.89	10.48	53.61	6.99	2.147	0.149
Medial nucleus	26.24	4.95	26.73	5.25	0.009	0.925
Cortical nucleus	26.57	4.33	27.73	4.80	0.417	0.521
Cortico-amygdaloid transition area	151.67	21.66	159.59	22.10	1.089	0.302
Paralaminar nucleus	41.30	5.57	42.40	5.54	0.181	0.673
Whole amygdala	1762.58	159.05	1811.90	190.28	0.117	0.733
Right hemisphere						
Lateral nucleus	727.46	82.42	718.18	87.99	1.572	0.216
Basal nucleus	451.37	42.68	443.66	48.17	2.679	0.108
Accessory basal nucleus	277.97	25.51	277.52	29.89	0.770	0.384
Anterior amygdaloid area	67.44	8.02	68.07	8.32	0.026	0.873
Central nucleus	53.14	8.60	51.91	7.07	1.403	0.242
Medial nucleus	27.68	5.34	27.07	4.70	1.001	0.322
Cortical nucleus	30.62	3.73	31.04	3.75	0.083	0.774
Cortico-amygdaloid transition area	161.26	17.35	161.22	19.48	0.367	0.547
Paralaminar nucleus	42.66	5.64	40.59	5.06	5.576	0.022*
Whole amygdala	1839.60	172.19	1819.26	187.68	2.163	0.148

The F and p values were obtained using one-way analysis of covariance with the adjustment for age, sex, and total intracranial cavity volume as covariates. * : Significant amygdala subregion volume differences. BD I : bipolar I disorder, BD II : bipolar II disorder, SD : standard deviation

Table 4. Amygdala subregion volume difference between Li+ (n = 11) and Li- (n = 44)

Amygdala subregions	Li+ (mm^3)		Li- (mm^3)		Li+ vs. Li-	
	Mean	SD	Mean	SD	$F_{(1, 48)}$	p value
Left hemisphere						
Lateral nucleus	741.38	78.61	713.70	82.44	0.663	0.419
Basal nucleus	446.31	45.72	432.92	43.30	0.777	0.382
Accessory basal nucleus	269.32	25.90	256.00	25.82	3.125	0.083
Anterior amygdaloid area	70.90	9.60	66.40	8.52	1.550	0.219
Central nucleus	53.65	8.47	54.44	9.14	0.195	0.661
Medial nucleus	27.38	4.78	26.24	5.15	0.233	0.631
Cortical nucleus	29.25	4.53	26.58	4.44	3.981	0.052
Cortico-amygdaloid transition area	163.15	18.19	153.48	22.66	1.708	0.198
Paralaminar nucleus	42.10	6.40	41.75	5.38	0.004	0.947
Whole amygdala	1843.45	159.56	1771.51	176.98	1.465	0.232
Right hemisphere						
Lateral nucleus	728.50	83.93	721.71	85.47	0.246	0.622
Basal nucleus	446.35	61.34	448.07	40.97	0.000	0.992
Accessory basal nucleus	279.16	37.80	277.41	24.70	0.112	0.739
Anterior amygdaloid area	68.91	9.34	67.45	7.84	0.278	0.600
Central nucleus	51.40	8.50	52.85	7.77	0.281	0.599
Medial nucleus	29.97	6.66	26.75	4.36	4.269	0.044*
Cortical nucleus	31.89	4.23	30.55	3.57	1.707	0.198
Cortico-amygdaloid transition area	158.30	19.11	161.98	18.13	0.288	0.594
Paralaminar nucleus	41.17	6.13	41.81	5.30	0.203	0.654
Whole amygdala	1835.65	220.38	1828.57	169.15	0.103	0.749

The F and p values were obtained using one-way analysis of covariance with the adjustment for age, sex, total intracranial cavity volume, illness duration, and Hamilton Depression Rating Scale score as covariates. * : Significant amygdala subregion volume differences. Li+ : lithium-treated patients with bipolar disorder, Li- : bipolar disorder patients without taking lithium treatment, SD : standard deviation

p = 0.025) between the volume of right central nucleus and the HDRS score, which was not significant after the Bonferroni correction.

Discussion

In this study, we investigated the volume change of amygdala nuclei in BD patients. Compared to HC group, we observed significant volume decrements in the basal nucleus, accessory basal nucleus, anterior amygdaloid area, and cortico-amygdaloid transition area in the right amygdala, and the whole right amygdala even after the Bonferroni correction.

To our knowledge, this is the first study to compare the amygdala subdivision volume of BD patients with HC group in adult sample. One study on youth with BD showed reductions in specific areas of left and right amygdala, which correspond to basolateral and superficial subregions, compared to HC group.[46] The results may be consistent with our results, but the patient group was limited to pediatric familial BD and the analytic method was different from the present study since radial distances were measured via statistical surface modeling analysis.[46]

Previous studies on amygdala subregions have investigated the amygdala structure by dividing it into 2−4 parcellations. Four subregions, namely lateral, basolateral, basomedial, and centromedial, were compared in their degree of atrophy between schizophrenia and psychotic BD patients, to suggest a differential biomarker.[47] In another attempt to distinguish the morphologic changes of amygdala in BD and schizophrenia, volume, total neuron numbers, neuronal size, and density of neurons were analyzed in specific amygdala subregions, localized as lateral, basal, accessory basal, and cortical nuclei.[48] Youth with BD were investigated on three subregions, which are basolateral, superficial, and centromedial.[46] Each subregion of such studies correspond to a cluster of numeral nuclei of our division, which implies higher sensitivity of our study in detecting structural abnormalities in the amygdala.

According to the study by Sah et al.,[49] each amygdala nucleus can be classified as either basolateral group, superficial group, or centromedial group. The basal and accessory basal nucleus belong to the basolateral group, which functions mainly

Table 5. Correlation of illness duration and HDRS score with amygdala subregion volumes in patients with bipolar disorder

Amygdala subregion volumes	Illness duration		HDRS score	
	r	p value	r	p value
Left hemisphere				
Lateral nucleus	-0.263	0.060	-0.259	0.064
Basal nucleus	-0.219	0.119	-0.076	0.594
Accessory basal nucleus	-0.161	0.253	-0.020	0.885
Anterior amygdaloid area	-0.213	0.130	-0.216	0.124
Central nucleus	-0.161	0.255	-0.053	0.712
Medial nucleus	-0.153	0.277	-0.102	0.474
Cortical nucleus	-0.007	0.960	0.018	0.900
Cortico-amygdaloid transition area	-0.204	0.146	-0.042	0.767
Paralaminar nucleus	-0.270	0.053	0.021	0.884
Whole amygdala	-0.271	0.052	-0.169	0.230
Right hemisphere				
Lateral nucleus	0.019	0.895	-0.246	0.079
Basal nucleus	-0.072	0.610	-0.099	0.485
Accessory basal nucleus	-0.031	0.826	-0.184	0.191
Anterior amygdaloid area	-0.223	0.113	-0.003	0.986
Central nucleus	0.142	0.314	-0.311	0.025*
Medial nucleus	-0.014	0.924	-0.200	0.154
Cortical nucleus	-0.012	0.932	-0.166	0.241
Cortico-amygdaloid transition area	-0.099	0.484	-0.064	0.653
Paralaminar nucleus	-0.131	0.355	0.011	0.936
Whole amygdala	-0.037	0.797	-0.214	0.128

A two-tailed Pearson's partial correlation analysis was performed controlling for age, sex, and total intracranical cavity volume. * : Significant correlations. HDRS : Hamilton Depression Rating Scale

in integrating, coordinating, and processing the received sensory inputs.[49)50)] In a study of youth with BD I, abnormal functional connectivity was detected between the amygdala basolateral subregion and the brain areas crucial for emotion processing and regulation.[51)] Such report implies meaningful contribution of the basolateral subregion to the pathophysiology of BD, which corresponds with our result of reduced volume of the basal and accessory basal nucleus in patients with BD. In addition, the lateral nucleus, which has a major role in receiving and assigning emotional valence to the environmental information, extensively projects to the basal and accessory basal nucleus.[33)38)52-58)] The basal and accessory basal nucleus then make internuclear and intranulcear connections, and projects to cortical and subcortical areas as well.[49)59-63)] Therefore, it is plausible to expect that deformations in these nuclei contribute to the maladaptive pattern of emotional perception in BD.

We observed significant volume decrement in the cortico-amygdaloid transition area and the anterior amygdaloid area as well. The superficial group, which comprises the cortico-amygdaloid transition area, is expected to play a crucial role in social communication.[64)] Functional connections with the anterior insula and inferior frontal gyrus, which are associated with the perception of emotional face expressions, assist the role of the superficial group in the processing of socially salient inputs.[65-67)] Specifically, the cortico-amygdaloid transition area is thought to participate in the assessment of negative emotions, such as anger.[68)] Therefore, abnormality in the cortico-amygdaloid transition area is likely to contribute to the deficient ability in facial emotion interpretation and social skills encountered in BD patients. Since little is revealed about the connections and functions of the anterior amygdaloid area, along with its association with other amygdala nuclei, further investigation should be conducted to elucidate its role in the pathophysiology of BD.

It is worth notice that the volume reductions were one-sided, biased to the right hemisphere. When stimulated electronically, the right amygdala produced negative emotions, while the left amygdala induced either positive or negative emotions, which indicates functional differences between left and right amygdala.[69)] In pediatric samples, the results were not consistent in its hemispheric specialization.[46)51)] Due to the shortage of data in adult BD samples, further studies are required to adequately explain such one-sidedness.

Since functional studies showed significant differences be-

tween BD I and BD II,[70)71)] meaningful result was anticipated on our investigation of comparing amygdala subregion volumes of each BD subtype. However, coherent with inconsistent results of former structural MRI studies comparing structural anomalies between BD subtypes,[72)] we could not find any significant amygdala volume differences between BD I and BD II patients. Starting with our study, further investigations with larger samples are needed to elucidate the role of amygdala in each BD subtype.

Considering the heterogeneity of our BD group, which is comprised of both depressive and euthymic patients, we investigated the correlation between the amygdala volume and the clinical state and illness duration. Although we could not find any significant finding, in context of a previous finding which implicated smaller amygdala volume in depressive BD patients compared to HC group,[73)] the possibility of the association between the clinical states and the amygdala volume must be taken into account in further studies.

In our study, there was no association between lithium treatment and amygdala subregion volume. Previous study results with larger samples indicate larger amygdala volume in lithium treatment group.[24)74)] This implies that our result may be changed when the sample is expanded.

There are several limitations to consider throughout the study. First, within BD subjects, the drug-naïve group was not analyzed separately from the drug-treated group. The BD group in our study is heterogeneous in its treated medication, including antidepressants, lithium, antiepileptic drugs, and antipsychotics. Although the impact of such medication in amygdala volume is not clear, there were some related reports. A negative correlation between neuron somata size and lithium and chlorpromazine exposure was suggested,[48)] and lithium showed some neuroprotective function in the amygdala.[75)] On such grounds, we cannot overlook the possibility of the confounding effects of pharmacological treatment. Second, because of the cross-sectional design of this study, we could not elucidate clear causal relationship between the morphologic changes in the amygdala and lithium treatment, clinical states, and illness duration. Finally, although the total intracranial volume was adjusted, the amygdala volume can have innate variation and have been affected by any disruptions during development. Interaction with psychosocial factors were not investigated as well. Despite such limitations, we demonstrated significant difference in the specific amygdala nuclei volume between the BD patients and HC group. Although our explanations remain at the level of postulation and are deficient in precise neurobiological grounds of the smaller amygdala nuclei volume in BD subjects, this study advocate the amygdala nuclei as intimately related to the pathophysiology of BD. Hence, further studies should be conducted on the amygdala nuclei in BD patients.

In summary, we observed significant volume decrements of the basal nucleus, accessory basal nucleus, anterior amygdaloid area, and cortico-amygdaloid transition area in the right hemisphere in adult BD patients, compared to HC. We postulate that such volume changes are associated with altered functional activity and connectivity of amygdala nuclei in BD. Additional investigations are needed to confirm our hypothesis, but we believe that our findings have significant meaning in extending the research domain to the specific amygdala nuclei and providing additional neurobiological evidence regarding the associations between BD and the structural changes of the brain in patients.

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Conflicts of interest

The authors have no financial conflicts of interest.

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