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Main Effects of Diagnoses, Brain Regions, and their Interaction Effects for Cerebral Metabolites in Bipolar and Unipolar Depressive Disorders

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Previous studies suggested patients with bipolar depressive disorder (BDd) or unipolar depressive disorder (UDd) have cerebral metabolites abnormalities. These abnormalities may stem from multiple sub-regions of gray matter in brain regions. Thirteen BDd patients, 20 UDd patients and 20 healthy controls (HC) were enrolled to investigate these abnormalities. Absolute concentrations of 5 cerebral metabolites (glutamate-glutamine (Glx), N-acetylaspartate (NAA), choline (Cho), myo-inositol (mI), creatine (Cr), parietal cortex (PC)) were measured from 4 subregions (the medial frontal cortex (mPFC), anterior cingulate cortex (ACC), posterior cingulate cortex (PCC), and parietal cortex (PC)) of gray matter. Main and interaction effects of cerebral metabolites across subregions of gray matter were evaluated. For example, the Glx was significantly higher in BDd compared with UDd, and so on. As the interaction analyses showed, some interaction effects existed. The concentrations of BDds' Glx, Cho, Cr in the ACC and HCs' mI and Cr in the PC were higher than that of other interaction effects. In addition, the concentrations of BDds' Glx and Cr in the PC and HCs' mI in the ACC were statistically significant lower than that of other interaction effects. These findings point to region-related abnormalities of cerebral metabolites across subjects with BDd and UDd.

Bipolar depressive disorder (BDd) is an aspect of bipolar disorder (BD), which is a severe illness with unpredictable cycles of depression and manic episodes[1–3]. Unipolar depressive disorder (UDd), which is a major depressive disorder (MDd), is a severe illness with negative mood[3,4]. In clinical practice, BDd patients are often misdiagnosed as UDd because the etiology is still unclear, and we know little about the underlying pathology of BDd and UDd[3,4]. Hence, it calls for unbiased and reliable diagnostic methods to distinguish BDd and UDd. Previous studies have examined biochemical abnormalities in subregions of gray matter in BDd and UDd patients using proton magnetic resonance spectroscopy (^{1}H-MRS)[5,6].

^{1}H-MRS offers a safe, noninvasive, and nonradioactive method[7]. It can help us obtain information about cerebral metabolites, including glutamate-glutamine (Glx), N-acetylaspartate (NAA), choline (Cho), creatine (Cr), and myo-inositol (mI). LCModel (version 6.3 1 A) is used to estimate and analyze metabolite concentrations from localized *in vivo* proton NMR spectra[8]. Alterations in cerebral metabolites have been demonstrated to serve as

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	ACC		mPFC		PC		PCC	
	Normlity	Homogeneity	Normlity	Homogeneity	Normlity	Homogeneity	Normlity	Homogeneity
Glx	0.526	0.198	0.051	0.801	0.811	0.801	0.999	0.837
NAA	0.851	0.898	0.000	0.047	0.418	0.047	0.157	0.789
Cho	0.000	0.001	0.069	0.851	0.369	0.851	0.025	0.133
mI	0.329	0.648	0.133	0.169	0.044	0.169	0.191	0.261
Cr	0.780	0.162	0.112	0.877	0.798	0.877	0.336	0.753

Table 1. The P-Value of Normlity Test and Homogeneity Test.

potential biomarkers that distinguish healthy, BDd, and UDd cohorts[9]. For example, Glu concentrations in the left Heschl's Gyrus and Planum Temporale of the superior temporal gyrus of patients with schizophrenia and BDd have been proved to be lower than healthy cohorts[10]. Comparing to schizophrenia patients and healthy cohorts, lower Glu, NAA, Cr and Inositol in patients with BDd suggested that a possible location-specific abnormality was existed in the dominant hemisphere of auditory cortices in patients with BDd[11]. As the main excitatory transmitter in the human brain, altered Glx was found in gray matter areas of patients with BDd[12,13]. NAA, considering close associations with the energetics of mitochondria and neuronal loss, has been found decreased in patients with BDd and UDd[14–16]. Cho is a membrane compound and increased choline may indicate increased membrane turn-over[17]. Many neuropsychiatric diseased are related with abnormal single transduction. Cho signaling may have impact on single transduction[14,16]. Hence, choline may have the potential to serve as a diagnostic biomarker in adolescent BDd and UDd[17]. As a putative marker of glial cells, the level of mI has been shown to be significantly reduced in UDd while increased in BDd paitents[7,14]. Cr plays a key role in maintaining energy stores, and its level is considered relatively constant[14,16,18], but recent research have report that Cr levels are not stable in some psychiatric populations[11,19].

As mentioned above, cerebral metabolites are considered biomarkers for distinguishing BDd and UDd patients, and the ROIs are associated with them as well. A series of studies on the neurobiology of BDd and UDd were done within the last decades. Most of them simply focused on independently comparing cerebral metabolites among various groups in each brain region[20–22]. Some studies conducted an interaction analysis on between the effect of diagnosis by brain region on the Glx/Cr level or the Glu/Cr level[23]. However, to our knowledge, few studies have comprehensively examined the relationships of cerebral metabolites' absolute concentration in the various subregions of gray matter among 3 cohorts. Based on the literature reviewed above, we hypothesized that abnormalities of metabolites in multiple sub-regions of gray matter in brain region might be happened in patients with BDd and UDd. And then the main effects of diagnoses, brain regions, and their interaction for cerebral metabolites in patients with BDd and UDd would be presented when comparing to HC.

Patients and Methods

Patients Selection. Our study was approved by the Shantou University psychiatric research ethics committee (see the attach file of the project approved document). All our clinical trials adhered to the protocols of Shantou University psychiatric research ethics committee. The methods applied in this study were closely performed according to the guide of the proposal of applying multiple neuroimaging technology to diagnose bipolar and unipolar patients (see the attach file of the proposal of project).

In this context, we mainly analyzed acquired data of 5 cerebral metabolites from 4 brain regions in gray matter among three independent cohorts, including 13 un-medicated patients with BDd, 20 un-medicated patients with UDd, and 20 healthy controls (HC). Patients with BDd and UDd were enrolled from the outpatient clinic at Shantou University Mental Health Center, Shantou, China, from August 2014 to July 2015. Twenty HC were recruited volunteers. Demographic and clinical characteristics of the 3 cohorts are compared in Table 1 (See Supplementary Appendix).

DSM-IV criteria, which were based on the Structured Clinical Interview for DSM-IV Patient Edition (SCID-P), were used to confirm BDd and UDd patients with a score of 17 or greater on the 17-item Hamilton Depression Rating Scale (HDRS). Written informed consent was obtained from all subjects. Patients were excluded if they declined to sign the informed consent. In addition, we excluded participants who were younger than 18 years or older than 60 years, who had a history of neurological disorders, a serious medical condition, or a ferrous implant or pace maker, and those who were pregnant or breastfeeding. All participants were right-handed.

Cerebral Metabolites and Subregions of Gray Matter. MRS with a 2-dimensional multi-voxel technique (2D MRSI) was performed to measure simultaneously cerebral metabolites from multi-voxels within a single slice. In this study, 4 subregions were measured in gray matter, including the medial frontal cortex (mPFC), anterior cingulate cortex (ACC), posterior cingulate cortex (PCC), and parietal cortex (PC) (see Figs 1 and 2 in Supplementary Appendix)[24]. All of the regions of interest (ROIs) were located in gray matter areas. These ROIs are essential to mood regulation and cognitive processes.

All participants underwent 2D MRSI exam of the brain as well as a structural MRI scan. A 15-mm axial slice was used to cover all of the ROIs in gray matter. ^{1}H MRSI acquisitions were conducted on a 3 T GE Signa MR scanner (General Electric Medical Systems, USA) scanner equipped with a standard 8-channel head coil array, and the spectroscopic data were obtained from the slice with a nominal voxel size of 2.8 ml. Concentrations of 5 cerebral metabolites were determined in the 4 volume-localized spectrums.
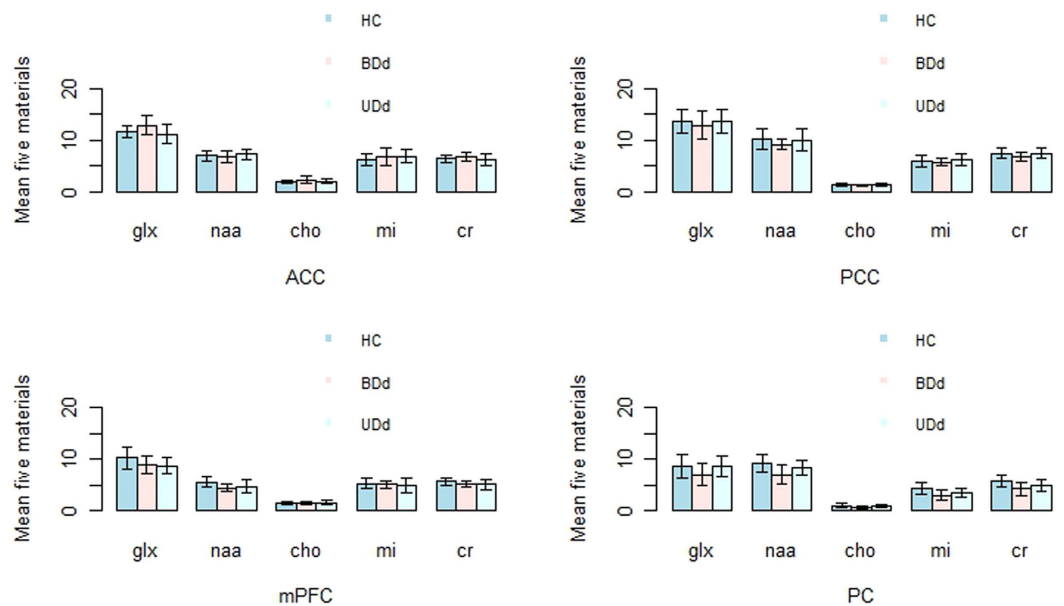
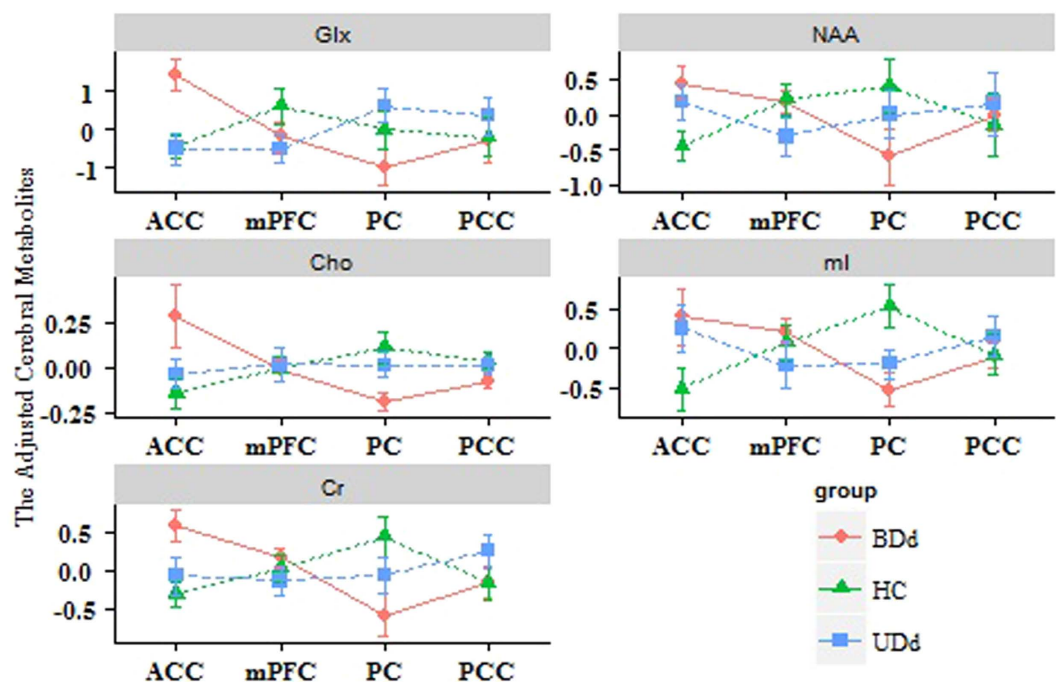**Figure 1. Concentrations of Metabolites in Different Brain Regions from the 3 Controls.**

Figure 2. Diagnosis by brain region interaction effect of adjusted cerebral metabolites.

Statistical Analysis. *Group Difference Analysis of Cerebral Metabolites across Brain Regions.* We compared the 5 cerebral metabolites among 3 independent cohorts in 4 brain regions and analyzed the differences to assess their diversities. We initially checked normality and homogeneity assumptions using normality and homogeneity of variance tests, respectively (the results see Table 1). When the assumptions were satisfied, analysis of variance (ANOVA) was used to compare the differences among the 3 groups. If difference among groups existed, Tukey's HSD test was then used to determine which groups had significant differences among the cerebral metabolites and the degree of the difference between groups was calculated. As long as a hypothesis was unsatisfied, the Kruskal-Wallis test, a nonparametric method, was used to assess the differences among groups. Wilcoxon test was preceded to identify the significant difference between two groups.

	ACC		PCC		mPFC		PC	
	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value
Glx	4.469	**0.016**	0.833	0.659	4.293	**0.019**	2.981	0.060
NAA	0.813	0.449	1.496	0.473*	5.264	**0.008**	6.033	**0.004**
Cho	2.68	0.262*	3.097	0.213	0.246	0.783	7.829	**0.020***
MI	1.234	0.300	0.759	0.474	2.748	0.253*	7.879	**0.001**
Cr	1.153	0.324	1.763	0.182	2.753	0.073	7.296	**0.002**

Table 2. The Results of ANOVA Analysis. *Kruskal-Wallis H test was used.

Metabolites	Inter-group	ACC		mPFC		PC	
		Diff	P-value	Diff	P-value	Diff	P-value
Glx	HC-BDd	—	0.109	—	0.090	—	—
	HC-UDd	—	0.561	−1.59	**0.023**	—	—
	BDd-UDd	−1.76	0.012	—	0.957	—	—
NAA	HC-BDd	—	—	−1.11	**0.015**	−2.08	**0.003**
	HC- UDd	—	—	−0.87	**0.033**	—	0.338
	BDd- UDd	—	—		0.820	—	0.079
Cho	HC-BDd	—	—	—	—	0.780	**0.005**
	HC- UDd	—	—	—	—	—	0.368
	BDd- UDd	—	—	—	—	—	0.992
MI	HC-BDd	—	—	—	—	−1.40	**0.001**
	HC- UDd	—	—	—	—	−0.87	**0.026**
	BDd- UDd	—	—	—	—	—	0.332
Cr	HC-BDd	—	—	—	—	−1.57	**0.001**
	HC- UDd	—	—	—	—	−0.90	**0.049**
	BDd- UDd	—	—	—	—	—	0.265

Table 3. Analysis of Multiple Comparisons Among Groups. - insignificant difference, p-value > 0.05.

Interaction Effect of Diagnosis by Brain Region. The aim of our study was to find out whether an interaction of effect of each adjusted cerebral metabolites of 4 brain regions and 3 diagnoses cohort existed separately. After the normality test for 5 cerebral metabolites (the results see the Supplementary Appendix), we found that NAA and Cho were unsatisfied with the assumptions. We do Box-Cox transformation for NAA and Cho. And then a linear regression model of each of these 5 adjusted cerebral metabolites (the detailed method See Supplementary Appendix) was used to determine whether an interaction effect of each adjusted cerebral metabolite of 4 brain regions and 3 diagnosis cohorts existed. In addition, an interaction diagram plot, which clearly illustrated the combined effect of the 3 cohorts and brain regions, was drawn using the ggplot2 of R software.

Results

Cerebral Metabolites and Subregions of Gray Matter.
Figure 1 depicts the distribution of the 5 cerebral metabolites in 4 brain regions for the 3 independent cohorts. "Mean ± SD" was used to describe variability within controls.

Group Difference Analysis of Cerebral Metabolites in 4 Subregions.
Group differences in 5 cerebral metabolites are displayed in Table 2. Multiple comparison analysis on the above selected significant cerebral metabolites was performed, and the results were shown in Table 3.

ACC. In the 3-group difference analysis, there was no statistically significant group difference except for Glx (see Table 2). Table 3 suggests that the BDd group had higher Glx levels in the ACC when compared with the UDd group.

PCC. According to the normality and homogeneity analysis, all cerebral metabolites except NAA satisfied the assumptions. Then, group difference analysis was performed in a manner identical to that for the ACC. Table 2 shows that there were no significant group differences for any metabolites in the PCC.

mPFC. From Table 2, we found that significant group differences existed for both Glx and NAA in the mPFC. Table 3 showed that the Glx and NAA levels of the UDd group and the NAA level of the BDd group were lower than those of the HC group.

PC. In addition to Glx, all considered metabolites had significant group differences (see Table 2). From Table 3, we found that PC levels of NAA, Cho, mI, and Cr of the BDd group, as well as the level of Cr of the UDd group were lower when compared with the healthy group.

	Coefficient	P-value	Metabolite
BDd:ACC	1.408	0.012	Glx
BDd:ACC	0.189	0.012	Cho
BDd:PC	−0.187	0.013	Cho
HC:ACC	−0.519	0.044	mI
HC:PC	0.531	0.039	mI
BDd:ACC	0.579	0.033	Cr
HC:PC	0.441	0.043	Cr
BDd:PC	−0.585	0.031	Cr

Table 4. Interaction Effect of Adjusted Cerebral Metabolites. Note: insignificant interaction effects (P-value > 0.05) were not displayed in Table 4.

Interaction Effect of Diagnosis by Subregion of Gray Matter. The interaction effect of brain regions and the 3 diagnosis groups was tested to see whether there were differences in each cerebral metabolite among specific brain regions across 3 groups. Table 4 presents the results of 3 diagnosis groups (BDd, UDd, HC), 4 brain regions, and interactions of the 5 independently adjusted cerebral metabolites. The interaction plot in Fig. 2 clearly illustrates the interaction effects.

Glx. From results in Table 4 and Fig. 2, we found that there was an interaction effect for the cerebral metabolite, Glx. For example, the interaction effect of BDd: ACC indicated that the adjusted Glx level for BDd patients were measured more from the ACC region (estimate = 1.408) than that of other interaction effects in Table 4. It was also demonstrated by the P-value of unadjusted Glx (P-value = 0.009) in Fig. 2.

NAA. From Table 4, Fig. 2, we found that there were interaction effects existed in the ACC and PC regions for 3 diagnosis groups. For example, the level of BDds' NAA was measured less in PC region than that of other interaction effects.

Cho. Interaction effects of Cho were displayed in Table 4 and Fig. 2). The interaction of BDd:ACC indicated that the adjusted Cho level for BDd patients were discovered more from the ACC region (estimate = 0.189) than that of other interaction effects. The interaction of BDd:PC indicated that the adjusted Cho level for BDd patients were less found from the PC region (estimate = −0.187) than that of other interaction effects.

mI. In Table 4, The interaction of HC:ACC indicated that the adjusted mI level for HC were measured less from the ACC region (estimate = −0.519) than that of other interaction effects. In addition, the interaction of HC:PC suggested that the adjusted mI level for HC was found more from the PC region (estimate = 0.531) than that of other interaction effects. Figure 2 also proved that the interaction effects existed for HCs' levels of mI in the ACC and PC regions.

Cr. The interaction of BDd:ACC indicated that the adjusted Cr level for BDd patients were measured more from the ACC region (estimate = 0.579) than that of other interaction effects. However, the adjusted Cr level for BDd patients were less found from the PC region (estimate = −0.585). Moreover, the interaction of HC:PC indicated that the adjusted Cr level for HC were discovered from the PC region (estimate = 0.411) (see Table 4). Figure 2 also shows the interaction effects of Cr.

Discussion

All participants are un-medicated subjects in our study. Medications have major influence on the level of brain biochemical metabolites[25]. For example lithium have been found that it has significant effects on Glu/Cr and Glx/Cr concentrations[26,27] and cho membrane transport[28]. The effect of antidepressant treatment might cause NAA levels increased significantly on medial frontal cortex in MDD patients[29]. Therefore, in order to better understand the brain metabolism changes in the subregions of gray matter on patients with BDd and UDd, participants with un-medicated BDd patients and UDd patients were enrolled in this study. The methodology of using a ratio of one cerebral metabolism compared with other metabolite was applied in earlier studies, just like Cho/Cr ratios[2,13,30], NAA/Cr ratios[2,6], or Glx/Cr ratios[2], etc However, they could not accurately reflect the change of cerebral metabolite concentrations[30]. Recent results revealed that using ratio rather than absolute concentration levels of metabolites was a technical limitation[23]. Hence, we combined the 2D-MRSI (magnetic resonance spectroscopy imaging) and LCModel software[21,22] in gray matter areas to measure the absolute concentration.

Most studies focused on certain cerebral metabolite concentrations in single voxel sections (such as ACC[31,32] and mPFC[32,33]) among groups. Researchers utilized multi-voxel technique to obtain multiple 1H-MRS spectra and evaluate multiple brain areas, just like we measured multi-voxel sections in gray matter areas. Also, we analyzed the main effect of diagnoses, brain regions for cerebral metabolites in BDd and UDd patients. Abnormalities of the 5 cerebral metabolites in different gray matter were existed in BDd and UDd patients. In the ACC region, we observed a significantly higher concentration of Glx when comparing BDd patients with UDd patients. That is, elevated glutamate in the ACC was associated with BDd[22,34,35] and reduced Glx in the ACC was associated with patients with UDd. These findings were congruent with several previous reports[22,34–38]. These alterations of Glx suggested that a primary abnormality was presented in the ACC brain region between these two groups. In the PCC region, there were no significant group differences among 5 metabolites. The interpretation of this result is

stillFZ difficult because studies on the PCC area are still few[39,40]. In addition, we found that the concentrations of Glx and NAA were reduced in UDd patients as well as NAA concentration was reduced in BDd patients in the mPFC region when compared to the HC cohort. This result was in accordance with prior studies demonstrating changes of NAA concentration in patients with BDd[41,42]. In contrast to the HC cohort, decreased NAA, Cho, mI, and Cr in patients with BDd and decreased Cr in the UDd cohort were found in the PC region in our studies. These findings are in agreement with previous studies[22]. Reductions of NAA, Cho, mI and Cr concentration in PC region may serve as some functional signals for neuronal damage or impaired function in patients with BDd. With the interaction effect analyses, Glx concentrations in patients with BDd always were measured lower in the ACC region. The changes of Glx concentrations in mPFC of patients with UDd were higher than that of other interaction effects. This result was confirmed with the result of main effects analyses. In a recent study, lower Cho/Cr was reported in the bilateral ventral prefrontal white matter of patients with MDD[43]. We found that Cho concentrations in the PC region of patients with BDd always were lower than that of other interaction effects. Our finding in turn, was a good evidence of that previous study. As the present study showed, the HCs' mI concentrations in ACC were higher and in PC region were lower than that of other interaction effects. Some postmortem study have similar reports[44,45]. The Cr level for BDd patients in PC regions were lower than that of other interaction effects. It coincided with the earlier research about decreased Cr concentrations were seen in the left hippocampus of BD patients[46,47]. The interaction effects analyses of diagnosis by brain region for five cerebral metabolites are our preliminary research work. It is difficult for us to mine and interpret the clinical significance of differences at present. More clinical trials are needed to further validated and explained.

Some possible methodological limitations of the current study should be taken into consideration. Unable to find a good way to distinguish glutamate from glutamine, creatine and phosphocreatine signals accurately is the first limitation. The second limitation is that the sample size was relatively small in our study. An additional limitation is that the brain metabolite changes are not measured before and after treatment of BDd and UDd participants.

In summary, the findings of this study indicate abnormalities of cerebral metabolites in subregions of gray matter in UDd and, in particular, BDd. In the follow-up experiments, a larger number of subjects must be enrolled and longitudinal study of BDd and UDd participants may be introduced to see whether there are brain metabolite changes before and after treatment. In addition, this investigation will have to be validated in clinical trials.

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Author Contributions

Hai-Zhu Tan wrote the main manuscript text. Hui Li designed the clinical trial, Chen-Feng Liu, Xiao-Bo Guo, Can-Hong Wen and Hai-Zhu Tan analyzed the data. and Ji-Tian Guan and Zhi-Wei Shen prepared Figures 1–2 in Supplementary Appendix. Shao-Min Ou designed the database for depression Disorder. Yin-Nan Zhang, Jie Zhang and Chong-Tao Xu and Hui Li collected the data. Ren-Hua Wu is the instructor for clinical trail. Xue-Qin Wang is the instructor for statistical analysis.

Additional Information

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