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DOI:

[10.1016/j.jad.2016.05.004](https://doi.org/10.1016/j.jad.2016.05.004)

*Document Version*

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*Citation for published version (APA):*

Yang, H., Li, L., Peng, H., Liu, T., Young, A. H., Angst, J., Ye, R., Rong, H., Ji, E., Qiu, Y., & Li, L. (2016). Alterations in regional homogeneity of resting-state brain activity in patients with major depressive disorder screening positive on the 32-item hypomania checklist (HCL-32). *Journal of Affective Disorders*. <https://doi.org/10.1016/j.jad.2016.05.004>

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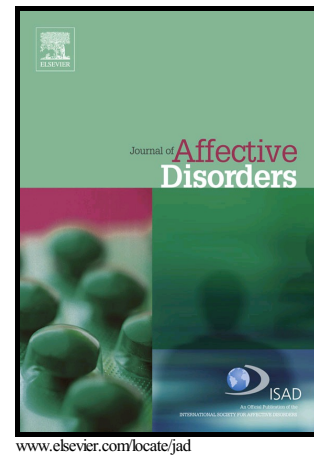
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## Author's Accepted Manuscript

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PII: S0165-0327(15)30980-0  
DOI: <http://dx.doi.org/10.1016/j.jad.2016.05.004>  
Reference: JAD8211

To appear in: *Journal of Affective Disorders*

Received date: 21 September 2015  
Accepted date: 9 May 2016

Cite this article as: Haichen Yang, Linling Li, Hongjun Peng, Tiebang Liu, Allan H. Young, Jules Angst, Rong Ye, Han Rong, Erni Ji, Yunhai Qiu and Lingjiang Li, Alterations in regional homogeneity of resting-state brain activity in patient with major depressive disorder screening positive on the 32-item hypomani checklist (HCL-32), *Journal of Affective Disorders* <http://dx.doi.org/10.1016/j.jad.2016.05.004>

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Alterations in regional homogeneity of resting-state brain activity in patients with major depressive disorder screening positive on the 32-item hypomania checklist (HCL-32)

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## ABSTRACT

### Background

Bipolar disorder (BD) is difficult to diagnose in the early stages of the illness, with the most frequent misdiagnosis being major depressive disorder (MDD). We aimed to use a regional homogeneity (ReHo) approach with resting-state functional magnetic resonance imaging (rs-fMRI) to investigate the features of spontaneous brain activity in MDD patients screening positive on the 32-item Hypomania Checklist (HCL-32).

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<sup>1</sup> Haichen Yang, Linling Li, Hongjun Peng contributed equally, they are co-first authors.

## Methods

Nineteen MDD patients screening positive (HCL-32+); 9 males;  $24.9 \pm 5.7$  years) and 18 patients screening negative (HCL-32-); 9 males;  $27.1 \pm 6.7$  years), together with 24 healthy controls (HC; 11 males;  $26.4 \pm 3.9$  years) were studied. ReHo maps were compared and an receiver operating characteristic (ROC) analysis was conducted to confirm the utility of the identified ReHo differences in classifying the patients.

## Results

The MDD versus HC showed different ReHo in many brain areas, especially in the frontal and parietal cortex. The HCL-32(+) versus HCL-32(-) showed significant increase of ReHo in the right medial superior frontal cortex, left inferior parietal cortex and middle/inferior temporal cortex, and decrease of ReHo in the left postcentral cortex and cerebellum. ROC analysis showed good sensitivity and specificity for distinguishing these two subgroups of MDD.

## Limitations

Recruited patients were all on antidepressants and standard mania rating scales were not used to assess their hypomanic symptoms.

## Conclusions

The rs-fMRI measurement of ReHo in distributed brain regions may be putative biomarkers which could differentiate subthreshold BD from MDD.

Keywords: Major depressive disorder, HCL-32, Resting-state fMRI, Regional homogeneity

## 1. Introduction

Bipolar disorder (BD) is a common, chronic and complex disease, which can cause psychosocial impairment and has both high mortality and morbidity rates (Angst, 1999; Kemp et al., 2008; Merikangas et al., 2007; Murray and Lopez, 1997). However, it is often difficult to make a correct early diagnosis of BD. Over one third of BD patients may wait 10 years or more before receiving an accurate diagnosis (Hirschfeld et al., 2003). Up to sixty-nine percent of BD patients may be initially misdiagnosed, with the most frequent misdiagnosis being major depressive disorder (MDD) (Hirschfeld et al., 2003). This is undoubtedly partly because a depressive episode is usually the first mood syndrome at the onset of BD and depressive

episodes are more frequent than manic/hypomanic episodes (Solomon et al., 2006) (Hirschfeld et al., 2003; Solomon et al., 2006).

MDD patients can be divided into positive (+) and negative (-) subgroups according to self-rating screening instruments for BD. The 32-item Hypomania Checklist (HCL-32) has been widely used as a screening instrument for BD in clinical psychiatry in the past decade (Yang et al., 2012; Yang et al., 2011). In the case of HCL-32, MDD patients (identified by the 4<sup>th</sup> edition of the Diagnostic and Statistical Manual of Mental Disorders; DSM-IV) can be stratified by HCL-32 scores according to the cutoff score between BD and MDD (Angst et al., 2005; Fornaro et al., 2013; Hu et al., 2012). This stratification thus results in one group of patients scoring positive for BD (HCL-32(+)) and another group scoring negative for BD (HCL-32(-)). Some researchers consider HCL-32(+) patients as subthreshold BD patients and HCL-32(-) patients as patients with “true” unipolar MDD (Fornaro et al., 2013; Hu et al., 2012). Follow-up studies have shown that the rates of diagnostic change to full-blown BD are significantly higher in HCL-32(+) patients than the rates in HCL-32(-) patients (Biederman et al., 2014; Holma et al., 2008).

Task-related functional magnetic resonance imaging (fMRI) studies suggest that MDD is associated with abnormalities of a complex series of brain regions (Fitzgerald et al., 2008). The resting-state fMRI (rs-fMRI) is a task-free technique which measures spontaneous brain activities as low-frequency fluctuations in blood oxygen level-dependent (BOLD) signals in the resting brain (Biswal et al., 1995). Instead of focusing on the neural responses under task conditions, rs-fMRI has been utilized to investigate the integration level of neural systems when a non explicit task is engaged (Fox and Raichle, 2007). This rs-fMRI technique has been demonstrated to be a powerful tool which reliably characterizes resting-state brain activities. However, it is important to take into account the abnormalities of neural activities at the baseline state when explaining findings obtained during the task-performing state. Additionally, rs-fMRI is simple and easier operationally compared to task-related fMRI.

The rs-fMRI data analyses includes functional connectivity and voxel-wise metrics of local brain activity. As shown in functional connectivity studies of MDD, affective disorders have been linked to alterations of the default mode network (DMN), the affective network (AN), the salience network (SN), and the cognitive control network (CCN) (Dutta et al., 2014). Thus, it is meaningful to investigate if localized dysfunction of specialized brain regions contributes to network-level abnormalities. Currently, local brain activity information can be assessed by rs-fMRI metrics including regional homogeneity (ReHo), amplitude of low frequency fluctuation (ALFF), fractional amplitude of low-frequency fluctuation (fALFF), voxel-mirrored homotopic

connectivity (VMHC) and so on (Zuo and Xing, 2014). ALFF was proposed to measure the intensities of the moment-to-moment low-frequency fluctuations in the fMRI time series (Yu-Feng et al., 2007). ALFF, as well as its normalized version fALFF, is a useful approach with which to map the voxel-wise spatial distribution or regional variation of low-frequency fluctuations across the entire brain. Abnormal baseline ALFF values have been identified in the depressive episode of BD within the prefrontal-limbic network (Liu et al., 2012a; Liu et al., 2012c). We applied an ReHo analysis to processed data in this study. According to one study about the test-retest reliability of voxel-wise rs-fMRI metrics, local functional homogeneity was one of the mostly reliable rs-fMRI metrics (Zuo and Xing, 2014).

The ReHo analysis was originally proposed to measure the degree of signal synchronization of fMRI time-courses (Zang et al., 2004). The hemodynamic characteristics of every voxel within a functional cluster are assumed to be similar and there is a dynamic synchronization of voxels within a cluster. The ReHo method can be used to assess the consistency of a conglomeration of BOLD time series. Therefore, ReHo reflects the temporal homogeneity of the regional BOLD signal, rather than its density and signal intensity (Zang et al., 2004). Abnormal ReHo is considered to be related to changes in the temporal aspects of spontaneous neural activity in the regional brain (Wu et al., 2010). ReHo abnormalities (either increase or decrease in ReHo value) are related to unbalanced local brain activity. In recent years, ReHo has been successfully used to investigate the brain function in healthy subjects (Kunisato et al., 2011; Luo et al., 2014) and clinical populations with psychiatric disorders (Chen et al., 2012; Guo et al., 2011; Liang et al., 2013; Liu et al., 2012e; Zang et al., 2004). For MDD, extensively distributed abnormal brain activities have been observed during resting-state and some clinical symptoms have been related to specific abnormal patterns of brain activities (Yao et al., 2009). Previously, regional ReHo differences have been tested as a differential diagnosis tool of BD and MDD (Liu et al., 2013a). However, it is not clear whether there are differences in brain activity between the two subgroups of MDD patients which are the subject of this study, i.e., HCL-32(+) patients and HCL-32(-) patients.

In this study, we focused on the whole brain ReHo comparison between these two MDD subgroups. We hypothesized that the HCL-32(+) MDD patients would be different from those HCL-32(-) MDD patients in resting-state brain function and such differences would be captured by the ReHo analysis. If present, any of these differences may be putative biomarker distinguishing HCL-32(+) patients from those patients with “true” MDD.

## 2. Methods

### 2.1 Subjects

All patients were recruited from outpatient departments and inpatient units at the department of Psychiatry, Second Xiangya Hospital of Central South University, from April 2012 to June 2013. The inclusion criteria for patients with MDD were: (1) age  $\geq 18$  and  $\leq 60$  years and ability to give voluntary informed consent; and that they (2) met the DSM-IV SCID (Structured Clinical Interview for DSM-IV) criteria for MDD and a current major depressive episode (First and Gibbon, 1997; First et al., 2002b); (3) had a total score of 17-item Hamilton Depression Rating Scale (HDRS)  $\geq 17$  (Hamilton, 1969); (3) satisfied criteria for undergoing magnetic resonance imaging (MRI) scanning based on a screening questionnaire; and (4) had not received electroconvulsive therapy (ECT) in the past four weeks. The diagnoses of MDD were made according to the SCID criteria by two experienced psychiatrists (First and Gibbon, 1997), both of whom had completed a 2-week training program before the diagnostic assessment. The inter-rater reliability of the SCID was tested and yielded satisfactory agreement (Kappa = 0.91). In total, 43 currently depressed MDD patients were enrolled in this study. The HCL-32, as well as HDRS and Hamilton Anxiety Rating Scale (HAMA) were administered after the patients completed the consent procedure. The HCL-32 is a self-report questionnaire which screens for hypomania (Angst et al., 2005). The multi-lingual hypomania checklist (HCL-32) has been developed and has been widely tested internationally. The Chinese version has acceptable psychometric reliability and validity in patients with BD (Yang et al., 2008; Yang et al., 2012; Yang et al., 2010; Yang et al., 2011). We used the cutoff of 12, which was the optimal discriminator between MDD and type II BD instead of 14, which was the optimal cutoff between MDD and all BD (Yang et al., 2012; Yang et al., 2011). In China, at the cutoff of 12 between BD and MDD, the sensitivity is 0.86, and the specificity 0.69 (Yang et al., 2012; Yang et al., 2011). The MDD patients with a HCL-32 score higher than or equal to 12 were defined in this study as HCL-32(+) patients, and those with a HCL-32 score lower than 12 as HCL-32(-) patients.

Healthy controls (HC) were recruited by poster or advertisement in local newspapers. Subjects were excluded if they had: (1) a current or past psychiatric diagnosis; (2) organic brain disease; (3) a history of head trauma resulting in loss of consciousness longer than 10 minutes; (4) a history of substance or alcohol dependence within the 12 months before assessment; (5) a first degree family history of any major psychiatric disorders, dementia, or mental retardation. The non-patient

version of the SCID was used to ensure that the healthy subjects had no history of psychiatric or neurologic illness (First et al., 2002a). In total, 26 healthy subjects were enrolled in this study.

All the MDD patients and healthy subjects enrolled in this study were right-handed, as measured by the Edinburgh Inventory (Oldfield, 1971). This study was approved by the Institutional Review Board of the Second Xiangya Hospital. Subjects provided both oral and written informed consent prior to any study-related procedures. The MRI scanning was performed for each patient within one week of the clinical assessment.

## 2.2 MRI image acquisition

MRI scanning was performed on a 3.0 T Philips 3T magnetic resonance system (Philips, Best, Netherlands) in the Second Xiangya Hospital of Central South University. Subjects were in the supine position with their heads snugly fixed by a belt and foam pads to minimize head motion. The scanning session included: (1) localization; (2) a 3-dimensional high-resolution T1-weighted anatomical image (TR = 1900 ms, TE = 2.53 ms, TI = 900 ms, FA = 9°, FOV = 240×240 mm<sup>2</sup>, matrix = 256×256, slice number = 176, voxel size = 0.9×0.9×1 mm<sup>3</sup>) was acquired; (3) rs-fMRI images were acquired with 36 axial slices, TR = 3000 ms, TE = 30 ms, FA = 90°, thickness/gap = 4/0 mm, FOV = 240 mm × 240 mm, and matrix = 64 × 64. The fMRI scanning lasted for 9 min, and subjects were asked to keep their eyes closed and not to think about anything in particular.

## 2.3 MRI data analysis

Imaging preprocessing and ReHo analysis was conducted using a MATLAB toolbox called Data Processing Assistant for Resting-State fMRI (DPARSF, the State Key Laboratory of Cognitive Neuroscience and Learning, Beijing Normal University, Beijing, China) (Chao-Gan and Yu-Feng, 2010). Between-group comparisons were carried out with the SPM8 toolbox (<http://www.fil.ion.ucl.ac.uk/spm>).

The first 10 volumes of the resting-state images were discarded because of magnetic saturation effects. The remaining 170 images were corrected for the acquisition delay between slices and for the head motion through slice timing and motion correction. Subjects who had more than 2 mm maximum displacement and 2° of angular motion during the whole resting fMRI scanning were excluded from the subsequent analysis. 6 MDD patients and 2 healthy subjects were excluded because of excessive head motion. As described in previous studies, head motion can significantly influence rs-fMRI metrics and results derived (Van Dijk et al., 2012).

Therefore, volume-level the mean framewise displacements (FD) were computed (Power et al., 2012). As results, MDD and HC did not differ in mean FD (independent two-sample t-test:  $t(59) = -0.718$ ,  $p = 0.476$ ; MDD:  $0.16 \pm 0.03$  mm; HCs:  $0.19 \pm 0.03$  mm). And HCL-32(+) and HCL-32(-) did not differ in mean FD (independent two-sample t-test:  $t(35) = -1.075$ ,  $P = 0.290$ ; HCL-32(+):  $0.14 \pm 0.02$  mm; HCL-32(-):  $0.19 \pm 0.05$  mm). Each individual structural image was first coregistered with the mean functional image and then segmented into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) using a unified segmentation algorithm. A local brain template was generated using diffeomorphic anatomical registration through exponential liealgebra (DARTEL). In order to reduce the potential effects of physiological process and motion, 26 nuisance covariates (including signals from WM, CSF and Friston 24 motion parameters were regressed out (Power et al., 2012; Van Dijk et al., 2012). The functional images were then temporally band-pass filtered (0.01-0.08 Hz) to reduce the effect of low-frequency drifts and high-frequency noise (Biswal et al., 1995; Lowe et al., 1998). Normalization and smoothing was performed later after ReHo maps calculation in original space.

For each individual, a ReHo map was generated which included the Kendall's coefficient of concordance (KCC) of each voxel within its nearest neighbors (26 voxels) in a voxel-wise analysis (Chao-Gan and Yu-Feng, 2010; Zang et al., 2004). To reduce the influence of individual variations in the KCC value, standardization of ReHo maps were performed by dividing the KCC of a given voxel by the averaged KCC of the whole brain. Then the standardized ReHo maps were spatially normalized to the Montreal Neurological Institute (MNI) template and resampled at a resolution of  $3 \times 3 \times 3$  mm<sup>3</sup> using the normalization parameters estimated during unified segmentation, and finally smoothed with a Gaussian kernel of 4 mm full-width at half-maximum to reduce noise. The resulting ReHo maps were entered into second level analyses for between-group comparisons.

## 2.4 Statistical analysis

Subjective characteristics were analyzed by SPSS (IBM SPSS Statistics for Windows; Armonk, NY: IBM Corp). Chi-square tests were performed to assess the differences in gender and two sample t-tests were performed to assess the differences in FD, age, age of onset, education, HAMA, HDRS and HCL-32 scores. Mann-Whitney U test was applied to compare disease duration between HCL-32(+) and HCL-32(-).

ReHo maps of all subjects were entered into a two-sample test contrasting the

MDD group with the HC group. Then another two-sample t-test was done contrasting the HCL-32(+) versus the HCL-32(-). Voxels with a P value  $< 0.005$  and cluster size  $> 13$  were considered to show significant difference, which was equal to a corrected threshold of  $P < 0.05$ , determined by the Monte Carlo stimulation (the AlphaSim program in DPARSF toolbox). The Parameters were: single voxel  $P = 0.005$ , 1000 simulations, FWHM = 4 mm, cluster connection radius  $r = 5$  mm. Results of group analyses were displayed by superimposing the T statistic maps on a standard high-resolution T1-weighted brain template using MRICroN software (<http://www.mricro.com>).

Brain regions displaying significant differences between the HCL-32(+) and the HCL-32(-) groups were identified as regions of interest (ROIs) and the mean ReHo values were extracted for each subject. We correlated the mean ReHo values of these five ROIs with the demographic measures, including disease duration, HAMA scores, and HDRS score. Correlation analyses between mean ReHo values and HCL-32 scores were performed for HCL-32(+) group and HCL-32(-) group separately.

We also performed an receiver operating characteristic (ROC) analysis to examine whether these ROIs could be used as biomarkers to discriminate the HCL-32(+) group from HCL-32(-) group as the ROC analysis has been commonly used to summarize the sensitivity and specificity of a biomarker for distinguishing subject groups (Liu et al., 2012d; Liu et al., 2013b).

### 3. Results

#### 3.1 Demographic and clinical characteristics

Twenty-four patients were defined as HCL-32(+) because their HCL-32 scores were equal to or higher than 12. The HCL-32 scores of 19 patients were lower than 12 and they were defined as HCL-32(-). Five HCL-32(+) patients, one HCL-32(-) patient and two healthy subjects were excluded because of excessive head motion. Eventually, there were 19 patients in HCL-32(+) group, 18 patients in HCL-32(-) group and 24 subjects in HC group. All patients with MDD were taking antidepressants at the time of fMRI scanning. In HCL-32(+) group, 16 patients were being treated with selective serotonin reuptake inhibitors (SSRIs), and 3 with venlafaxine. In HCL-32(-) group, 16 patients were being treated with SSRIs, and 2 with venlafaxine. The demographic and clinical characteristics of all subjects included in the final data analysis are presented in Table 1.

The MDD group and the HC group did not differ significantly in gender, age,

education level (gender:  $X(1) = 0.046$ ,  $p = 0.830$ ; age:  $t(59) = -0.312$ ,  $p = 0.756$ ; education:  $t(59) = -1.954$ ,  $p = 0.055$ ). The MDD group had statistically higher HAMA and HDRS score than HC group (HAMA:  $t(59) = 17.667$ ,  $p = 0.000$ ; HDRS:  $t(59) = 25.063$ ,  $p = 0.000$ ). Besides, these two subgroups were matched for gender, age, education level, disease duration and age of onset (gender:  $X(1) = 0.026$ ,  $p = 0.873$ ; age:  $t(35) = -1.089$ ,  $p = 0.284$ ; education level:  $t(35) = 1.440$ ,  $p = 0.159$ ; disease duration:  $U = 163$ ,  $p = 0.807$ ; age of onset:  $t(35) = -1.504$ ,  $p = 0.141$ ). There was no significant difference of HAMA and HDRS score between these two subgroups (HAMA:  $t(35) = 1.222$ ,  $p = 0.230$ ; HDRS:  $t(35) = 0.368$ ,  $p = 0.715$ ). HCL-32 score of HCL-32(+) group was significantly higher than HCL-32(-) group ( $t(35) = 12.468$ ,  $p = 0.000$ ).

### 3.2 ReHo profiles

Compared to HC group, the MDD group showed significant increased and decreased ReHo values in many brain regions, such as the bilateral frontal cortex, bilateral postcentral cortex, bilateral superior temporal cortex (STC), left inferior occipital cortex (IOC), bilateral middle cingulate cortex (MCC), angular gyrus, precuneus, parahippocampal gyrus, insular and cerebellum (Table 2).

Significant ReHo differences derived from comparison between HCL-32(+) and HCL-32(-) are showed in Table 3 and Fig. 1. HCL-32(+) patients showed significantly increased ReHo values in the right medial superior frontal cortex (MSFC), left inferior parietal cortex (IPC) and left middle/inferior temporal cortex (MTC/ITC), and decreased ReHo in the left postcentral cortex and cerebellum.

### 3.3 ROI-wise ReHo analysis

Mean ReHo values of those five above-mentioned clusters (MSFC, IPC, MTC/ITC, postcentral cortex and cerebellum) were extracted. For all MDD patients, mean ReHo value of IPC showed positive correlation with disease duration ( $r = 0.381$ ,  $p = 0.020$ ). No other significant correlation was identified. By ROC curves analyses (as shown in Table 4 and Fig. 2), the areas under the curves (AUC) of these five ROIs were good (0.830 – 0.871).

## 4. Discussion

MDD is a serious condition with disrupted neural network underlying mood and cognition processing (Zhang et al., 2011). In this study, compared to healthy subjects, MDD patients exhibited abnormal resting-state brain activities in the MSFC, IPC, STC,

angular gyrus, precuneus, parahippocampal gyrus, insular and cerebellum. Most of these brain regions are considered to belong to the default mode network (DMN) which is one of the most commonly recognized resting-state networks in the brain. DMN is relevant to the origin and experience of mood and psychotic symptoms (Pomarol-Clotet et al., 2012; Whitfield-Gabrieli and Ford, 2012). Evidence has showed that DMN plays a key role in neural activities mediating MDD (Sheline et al., 2010; Zhou et al., 2010).

This study focused on the investigation of the differences between two MDD subgroups in brain activities during resting-state. We found that the HCL-32(+) group showed significant increase of ReHo in the right MSFC, left IPC, left MTC/ITC and significant decrease of ReHo in the left postcentral cortex and cerebellum compared to the HCL-32(-) group. ROC analysis was performed to summarize the sensitivity and specificity of these five ROIs for distinguishing these two subgroups of MDD. The AUC values of these five ROIs were 0.845, 0.830, 0.848, 0.871 and 0.871 respectively. The discrimination results of ROC analysis was commonly considered excellent for AUC values between 0.9 and 1, good for AUC values between 0.8 and 0.9, fair for AUC values between 0.7 and 0.8, poor for AUC values between 0.6 and 0.7 and failed for AUC values between 0.5 and 0.6 (El Khouli et al., 2009; Ludemann et al., 2006; Obuchowski, 2003). According to these criteria, these five ROIs showed good performance to distinguish HCL-32(+) patients from HCL-32(-) patients.

The MSFC plays a role in the integration of emotional and cognitive processes by incorporating emotional biasing signals or markers into decision making processes (Bechara et al., 1994) and has extensive reciprocal connections to the limbic structures (Ongur and Price, 2000). The MSFC has been suggested to be related to the emotional regulation impairment in BD (Phillips et al., 2008). BD patients exhibited increased functional connectiv

ity between MSFC and amygdale while viewing sad stimuli compared with HC (Versace et al., 2010). And the effective connectivity between MSFC and amygdale has been shown to differentiate BD from MDD (de Almeida et al., 2009). These results suggested that MSFC was one of the most important brain areas underlie the affective disturbance in BD (Vargas et al., 2013). In this study, HCL-32(+) patients versus HCL-32(-) patients exhibited increased ReHo in the right MSFC. So, it was demonstrated again that MSFC might be used to differentiate BD from MDD.

The IPC lies at a key location in the brain, at the junction of the auditory, visual, and somatosensory cortex, with which it is highly connected. The bilateral IPC was consistently identified in the DMN and was considered to be implicated in emotion

processing (Canli et al., 2004). For MDD, the IPC was proposed as one of the “core nodes” of the dysfunctional intrinsic organization (Zhou et al., 2010). However, the IPC has also been found to be involved in a wide range of different processes, ranging from action, memory, and language to mathematical problem solving and social cognition (Rolls, 1999). Comparing with HC, increased ReHo values in the IPC have been observed in both BD and UD, but no difference was identified when comparing with each other (Liang et al., 2013). Then the authors suggested that the ReHo abnormalities in IPC might be attributed to learning disabilities with parietal dysfunction because that depressed patients usually had low level of education compared with normal subjects (Liang et al., 2013; Shippee et al., 2011). We observed increased ReHo in the IPC for MDD patients comparing with HC, as well as for HCL-32(+) compared with HCL-32(-). The HCL-32(+) and HCL-32(-) patients showed no statistical difference on the level of education. Besides, mean ReHo value of IPC showed positive correlation with disease duration. So, the different ReHo values between these two subgroups might be attributed to distinguishing features of emotion processing dysfunction. Besides, the postcentral cortex in the parietal lobe showed lower ReHo values in HCL-32(+) patients compared with HCL-32(-) patients. The postcentral cortex is conceived as primary somatosensory cortex, and receives the bulk of the thalamocortical projection from the sensory input fields. BD patients (in depressive state) showed significant lower ReHo and ALFF in left postcentral cortex compared to HC (Liang et al., 2013; Liu et al., 2012a). However, more straightforward evidences are needed to interpret the implication of the abnormal brain activities in the postcentral cortex.

Besides, the HCL-32(+) patients exhibited significantly higher ReHo values in the MTC/ITC than those of HCL-32(-) patients. Previous studies found no difference of local region activity in MTC/ITC for BD compared with HC or UD (Liang et al., 2013; Liu et al., 2012b; Liu et al., 2012c; Liu et al., 2012d). But functional connectivity between MTC and other emotion regulation related brain regions, such as prefrontal cortex, has been found increased in patients with MDD (Zhou et al., 2010) and this abnormal activity might be resulted from negative experiences or represent a dynamic prediction of expected future coactivations as a results of the overall negative expectations of depressed patients (Fox et al., 2006). The ITC is also involved in the processing of working memory (Axmacher et al., 2008), and complex emotional visual stimuli (Geday et al., 2007). It has also been reported that the ITC demonstrated structural (Wu et al., 2011) and functional abnormalities, which correlated with self-reported anxiety (Kroes et al., 2011). In this study, we identified higher ReHo values in HCL-32(+) patients and this issue still awaits in-depth explanation with

future confirmation.

In consistent with this study, increased ReHo in the posterior lobe of cerebellum has been observed in MDD (Liu et al., 2010). There are evidences from neuroimaging studies that the cerebellum plays a role in the emotional and cognitive processing of negative stimuli (Schraa-Tam et al., 2012). The important role of the cerebellum in the cerebellar cognitive affective syndrome has been described in healthy subjects (Schmahmann, 2004; Schmahmann and Sherman, 1998). And the cerebellum has been previously identified as relevant to depression because of the increase in resting anterior cerebellar activity and the abnormal responses of cerebellar activation to both positive and negative affect (Fitzgerald et al., 2008). Cerebellar abnormalities with increased ReHo have been observed in BD compared with UD (Liu et al., 2012d), but no ReHo difference was found in another study (Liang et al., 2013). In this study, HCL-32(+) patients versus HCL-32(-) patients showed decreased ReHo in cerebellum. These inconsistencies may be attributed to several confounding factors of enrolled patients, such as depression severity, diverse medication.

#### 5. Limitations

There are several limitations to be noted regarding this study. First, the number of depressive episodes and ratings of standard mania scales were not recorded and therefore we couldn't make complete description about all the clinical features as well as their correlation with brain activity. Second, the antidepressants and relative larger age range might have impacts on the neuronal activity of the resting brain (Fang et al., 2015). Third, the results would be more persuasive if the ROC analysis could be performed with another group of patients (Kriegeskorte et al., 2009). Forth, BD patients were not enrolled to perform direct comparison of BD and HCL-32(+). Last but not least, follow-up tests were not included and more straight evidence could be provided with longitudinal study design, considering that 8.9% subjects with previous unipolar MDD switched to BD type II and 2.8% to BD type I after a 5-year follow-up (Holma et al., 2008).

#### 6. Conclusions

In summary, we found that the HCL-32(+) patients showed significant increase of ReHo in the right MSFC, left IPC, left MTC/ITC and significant decrease of ReHo in the left postcentral cortex and cerebellum compared to HCL-32(-) patients. According to the results of ROC analysis, the alterations of ReHo in these five brain regions showed good performance to differentiate HCL-32(+) patients from HCL-32(-) patients. The features of ReHo in these regions may be regarded as putative biomarkers which

could help clinicians to differentiate depressed patients with subthreshold bipolarity from the “true” UD patients. This exploratory study will encourage future in-depth investigations, such as functional connectivity analysis and task-based fMRI studies, to provide complementary information about the features of spontaneous brain activity in HCL-32(+) patients.

#### Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Fig. 1. ReHo differences between HCL-32(+) and HCL-32(-) (two-sample t-tests, thresholded at  $p < 0.05$ , AlphaSim corrected). Upper panel presents brain areas exhibiting significant higher ReHo value in HCL-32(+) than HCL-32(-) (axial view), including the left inferior parietal cortex (A), right medial superior frontal cortex (B) and left middle/inferior temporal cortex (C). Lower panel presents brain areas exhibiting significant lower ReHo value in HCL-32(+) than HCL-32(-) (coronal view), including the left postcentral cortex (D) and left cerebellum (E). The magnitudes and directions of the t score are presented by warm color (HCL-32(+) > HCL-32(-)) or cool color (HCL-32(+) < HCL-32(-)).

Fig. 2. Receiver operating characteristic (ROC) curves for HCL-32(+) and HCL-32(-). ROI 1: the right medial superior frontal cortex; ROI 2: the left inferior parietal cortex; ROI 3: the left middle/inferior temporal cortex; ROI 4: the left postcentral cortex; ROI 5: the left cerebellum.

Table 1. Demographic and clinical characteristics.

	HCL-32(+)	HCL-32(-)	P-value <sup>1</sup>	MDD	HC	P-value <sup>2</sup>
Gender (M/F)	9M/10F	9M/9F	0.873 <sup>a</sup>	18M/19F	11M/13F	0.830 <sup>a</sup>
Age (years)	24.9 ± 5.7	27.1 ± 6.7	0.284 <sup>c</sup>	26.0 ± 6.2	26.4 ± 3.9	0.756 <sup>c</sup>
Age range	18 - 35	18 - 45	—	—	21 - 37	—
EDU (years)	14.1 ± 2.4	12.8 ± 3.0	0.159 <sup>c</sup>	13.5 ± 2.7	15.0 ± 3.3	0.055 <sup>c</sup>
DD (months)	19.0(36.0)	24.0(23.2)	0.807 <sup>b</sup>	—	—	—
Age of Onset (years)	22.0 ± 5.6	24.8 ± 5.7	0.141 <sup>c</sup>	23.4 ± 5.7	—	—
HAMA	28.5 ± 5.1	26.4 ± 5.3	0.230 <sup>c</sup>	27.5 ± 5.2	8.5 ± 0.9	0.000 <sup>c</sup>
HDRS	31.8 ± 4.3	31.3 ± 5.0	0.715 <sup>c</sup>	31.6 ± 4.6	6.9 ± 1.8	0.000 <sup>c</sup>
HCL-32	16.5 ± 2.9	6.3 ± 2.0	0.000 <sup>c</sup>	—	—	—
On SSRIs	16	16	—	—	—	—
Venlafaxine	3	2	—	—	—	—

Data are expressed as mean ± SD or median(IQR); MDD: major depressive disorder; HCL-32: 32-item Hypomania checklist score; HCL-32(+): the MDD patients with HCL-32 score higher or equal to 12; HCL-32(-): the MDD patients with HCL-32 score lower than 12; HC: healthy control; EDU: years of education; DD: disease duration; HAMA: Hamilton Anxiety Rating Scale; HDRS: Hamilton Depression Rating Scale; SSRIs: selective serotonin reuptake inhibitors; P-value<sup>1</sup>: derived from statistical tests between HCL-32(+) and HCL-32(-); P-value<sup>2</sup>: derived from statistical tests between MDD and HC; <sup>a</sup>: Indicates P values for Pearson Chi-square test; <sup>b</sup>: Indicates P-values for Mann-Whitney U test; <sup>c</sup>: Indicates P values for independent two-sample t-test.

Table 2. Brain regions exhibiting different regional homogeneity of MDD patients compared to HC.

Brain areas	MNI Coordinates						
			X	Y	Z	T	N
MDD > HC							
Superior Frontal Cortex (Medial)	L	BA 8, 9	-3	36	45	4.37	39
	R	BA 10	3	60	9	3.31	31
Middle Frontal Cortex	R	BA 10, 46	27	54	24	4.38	89
Inferior Frontal Cortex (Orbital)	L	BA 47	-39	24	-3	4.24	122
	R	BA 47	45	36	-12	3.90	14
Inferior Frontal Cortex (Triangular)	L	BA 45	-48	45	12	3.05	32
Inferior Parietal Cortex	R	BA 40	60	-30	30	4.77	64
Angular Gyrus	R	BA 39	48	-63	36	3.54	44
Precuneus	L/R	BA 23,7	6	-51	27	4.75	77
ParaHippocampal Gyrus	L	BA 37	-27	-33	-9	3.73	32
Insular	R	BA 47	39	15	3	3.60	26
Cerebellum	L		-18	-75	-30	4.54	142
	R		15	-75	-30	4.61	82
MDD < HC							
Postcentral cortex	L	BA 4	-45	-9	30	3.65	97
	R	BA 43	66	-6	27	5.04	271
Superior Temporal Cortex	L	BA 22	-57	-15	6	4.80	210
	R	BA 22,48	60	-9	-3	3.81	107
Inferior Occipital Cortex	L	BA 19	-51	-72	-9	3.81	22
Middle Cingulate Cortex	L/R	BA 24	3	0	45	4.23	29

x, y, z, coordinates of peak voxel in the MNI space; N: cluster size; MDD: major depressive disorder; HC: healthy control; BA: broadmann's area.

Table 3. Brain regions exhibiting different regional homogeneity of HCL-32(+) group compared to HCL-32(-) group.

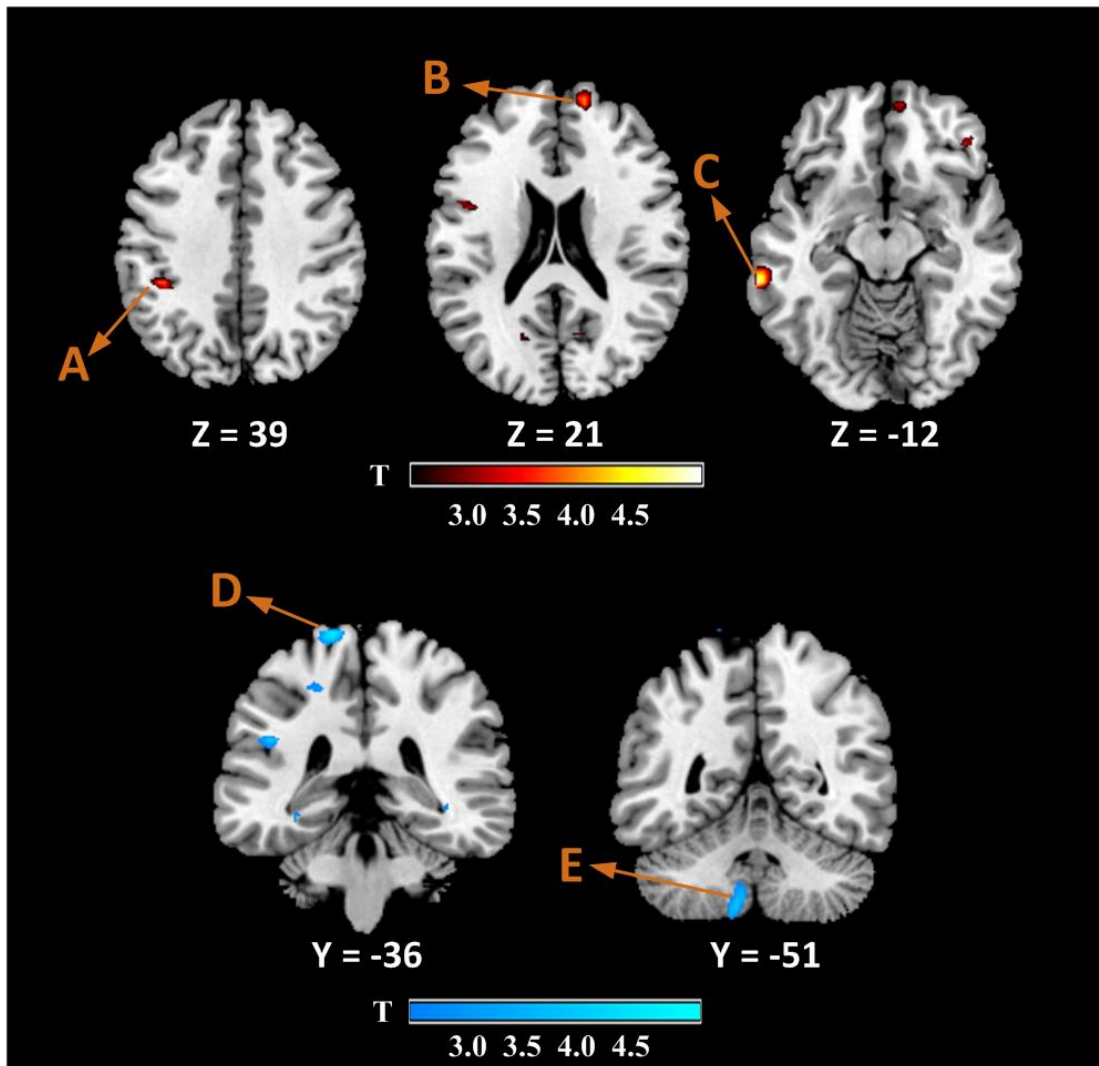
Brain areas	Side	MNI Coordinates					
		X	Y	Z	Peak T	N	
HCL(+) > HCL(-)							
Superior Frontal Cortex (Medial)	R	BA10	15	57	21	3.96	32
Inferior Parietal Cortex	L	BA 40	-42	-36	39	3.82	19
Middle/Inferior Temporal Cortex	L	BA 20,21	-66	-36	-12	4.90	26
HCL(+) < HCL(-)							
Postcentral Cortex	L	BA 4	-15	-36	78	4.74	42
Cerebellum	L		-9	-51	-51	3.99	23

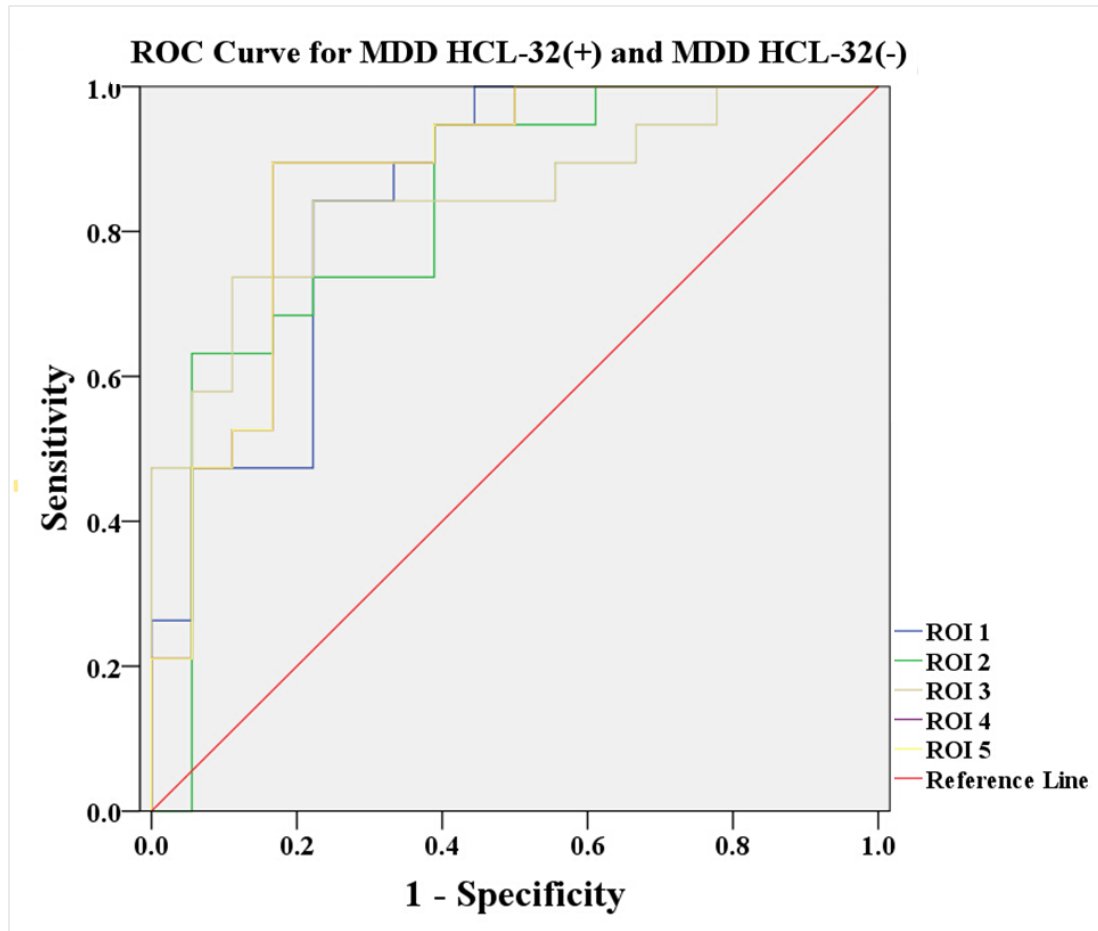
x, y, z, coordinates of peak voxel in the MNI space; N: cluster size; BA, broadmann's area; HCL-32(+): the MDD patients with HCL-32 score higher or equal to 12; HCL-32(-): the MDD patients with HCL-32 score lower than 12.

Table 4. ROC curves analyses between HCL-32(+) and HCL-32(-)

Brain regions	AUC	Cut-off value	Sensitivity	Specificity
MSFC (R)	0.845	1.05 <sup>a</sup>	84% (16/19)	78% (14/18)
IPC (L)	0.830	1.19	74% (14/19)	78% (14/18)
MTC / ITC(L)	0.848	1.09	84% (16/19)	78% (14/18)
Postcentral Cortex (L)	0.871	0.87	90% (17/19)	78% (14/18)
Cerebellum (L)	0.871	0.71	84% (16/19)	83% (15/18)

ROC: receiver operating characteristic; HCL-32(+): the MDD patients with HCL-32 score higher or equal to 12; HCL-32(-): the MDD patients with HCL-32 score lower than 12; AUC: area under the curve; <sup>a</sup>: using this cut-off value, the mean ReHo value of right MSFC could correctly classify 16 of 19 HCL-32(+) patients and 14 of 18 HCL-32(-) patients, yielding a sensitivity of 84% and a specificity of 78%. The means of other cut-off values were similar.





## HIGHLIGHTS

- This is the first paper that the whole brain ReHo comparison analysis was examined between two MDD subgroups, which were divided based on HCL-32 scores by search the Pubmed of American National Library. It was also confirmed by the author (Jules Angst) of HCL-32.
- The HCL-32 has been widely used as screening tool for BD in clinical psychiatry for the last 10 years. However, until now we did not know the different features in resting-state brain function of the patients positive for the HCL-32 (MDD HCL-32(+)) compared to the patients negative to HCL-32 (MDD HCL-32(-)).
- We found that MDD HCL-32(+) showed significant increase of ReHo in right MSFC, left IPC, left MTC/ITC and significant decrease of ReHo in left postcentral cortex and left cerebellum compared to MDD HCL-32(-) patients. The changes in these brain areas differentiated HCL-32(+) patients from HCL-32(-) patients and may be putative biomarkers which differentiate bipolar depression from unipolar depressive disorder.