Twenty-Five Cohorts of Major Depressive Disorder in China: Evidence for Reduced Default Mode Network Functional Connectivity in Recurrent Depressive Patients

Chao-Gan Yan, Ph.D.[1,2,3,4,*], Xiao Chen, M.A.[1,2], Le Li, Ph.D.[1,2], Francisco Xavier Castellanos, M.D.[4,5], Tong-Jian Bai, M.D.[6], Qi-Jing Bo, M.D.[7], Guan-Mao Chen, M.A.[8], Ning-Xuan Chen, M.A.[1,2], Wei Chen, M.D.[9], Chang Cheng, M.D.[10], Yu-Qi Cheng, M.D.[11], Xi-Long Cui, M.D.[10], Jia Duan, M.D.[12], Yi-Ru Fang, M.D.[13], Qi-Yong Gong, M.D.[14], Wen-Bin Guo, M.D.[10], Zheng-Hua Hou, M.D.[15], Lan Hu, M.D.[16], Li Kuang, M.D.[16], Feng Li, M.D.[7], Kai-Ming Li, M.D.[14], Tao Li, M.D.[17], Yan-Song Liu, M.D.[18], Zhe-Ning Liu, M.D.[19], Yi-Cheng Long, M.A.[19], Qing-Hua Luo, M.D.[16], Hua-Qing Meng, M.D.[16], Dai-Hui Peng, M.D.[13], Hai-Tang Qiu, M.D.[16], Jiang Qiu, Ph.D.[20], Yue-Di Shen, M.D.[21], Yu-Shu Shi, M.D.[22], Tian-Mei Si, M.D.[23], Chuan-Yue Wang, M.D.[7], Fei Wang, M.D.[12], Kai Wang, M.D.[6], Li Wang, M.D.[23], Xiang Wang, M.D.[10], Ying Wang, M.D.[8], Xiao-Ping Wu, M.D.[24], Xin-Ran Wu, B.A.[20], Guang-Rong Xie, M.D.[10], Hai-Yan Xie, M.D.[25], Peng Xie, M.D.[26,27,28], Xiu-Feng Xu, M.D.[11], Hong Yang, M.D.[22], Jian Yang, M.D.[24], Jia-Shu Yao, M.D.[9], Shu-Qiao Yao, M.D.[10], Ying-Ying Yin, M.D.[15], Yong-Gui Yuan, M.D.[15], Ai-Xia Zhang, M.D.[29], Hong Zhang, M.D.[24], Ke-Rang Zhang, M.D.[29], Lei Zhang, Ph.D.[1,2], Zhi-Jun Zhang, M.D.[30], Ru-Bai Zhou, M.A.[13], Yi-Ting Zhou, M.D.[17], Jun-Juan Zhu, M.D.[13], Chao-Jie Zou, M.D.[11], Xi-Nian Zuo, Ph.D.[1,2,3], Jing-Ping Zhao, M.D.[19,*], Yu-Feng Zang, M.D.[31,32,*]

[1]CAS Key Laboratory of Behavioral Science, Institute of Psychology, Beijing, China; [2]Department of Psychology, University of Chinese Academy of Sciences, Beijing, China;

[3]Magnetic Resonance Imaging Research Center and Research Center for Lifespan Development of Mind and Brain (CLIMB), Institute of Psychology, Chinese Academy of Sciences, Beijing, China; [4]Department of Child and Adolescent Psychiatry, NYU School of Medicine, New York, NY, USA; [5]Nathan Kline Institute for Psychiatric Research, Orangeburg, NY, USA; [6]Anhui Medical University, Hefei, Anhui, China; [7]Beijing Anding Hospital, Capital Medical University, Beijing, China; [8]The First Affiliated Hospital of Jinan University, Guangzhou, Guangdong, China; [9]Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China; [10]The Second Xiangya Hospital of Central South University, Changsha, Hunan, China; [11]First Affiliated Hospital of Kunming Medical University, Kunming, Yunnan, China; [12]Department of Psychiatry, First Affiliated Hospital, China Medical University, Shenyang, Liaoning, China; [13]Department of Psychiatry, Shanghai Jiao Tong University School of Medicine, Shanghai, China; [14]Huaxi MR Research Center, West China Hospital of Sichuan University, Chengdu, Sichuan, China; [15]Department of Psychosomatics and Psychiatry, Zhongda Hospital, School of Medicine, Southeast University, Nanjing, Jiangsu, China; [16]Department of Psychiatry, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China; [17]Mental Health Center, West China Hospital, Sichuan University Chengdu, Sichuan, China; [18]Department of Clinical Psychology, Suzhou Psychiatric Hospital, The Affiliated Guangji Hospital of Soochow University, Suzhou, Jiangsu, China; [19]The Institute of Mental Health, Second Xiangya Hospital of Central South University, Changsha, Hunan, China; [20]Faculty of Psychology, Southwest University, Chongqing, China; [21]Department of Diagnostics, Affiliated Hospital, Hangzhou Normal University Medical School, Hangzhou, Zhejiang, China; [22]Department of Radiology, The First Affiliated Hospital, College

of Medicine, Zhejiang University, Hangzhou, Zhejiang, China; [23]National Clinical Research Center for Mental Disorders (Peking University Sixth Hospital) & Key Laboratory of Mental Health, Ministry of Health (Peking University), Beijing, China; [24]The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an Central Hospital, Xi'an, Shaanxi, China; [25]Department of Psychiatry, The Fourth Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang, China; [26]Institute of Neuroscience, Chongqing Medical University, Chongqing, China; [27]Chongqing Key Laboratory of Neurobiology, Chongqing, China; [28]Department of Neurology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China; [29]First Hospital of Shanxi Medical University, Taiyuan, Shanxi, China; [30]Department of Neurology, Affiliated ZhongDa Hospital of Southeast University, Nanjing, Jiangsu, China; [31]Center for Cognition and Brain Disorders, Institutes of Psychological Sciences, Hangzhou Normal University, Hangzhou, Zhejiang, China; [32]Zhejiang Key Laboratory for Research in Assessment of Cognitive Impairments, Hangzhou, Zhejiang, China.

***Corresponding authors:**

Chao-Gan Yan, Ph.D.

CAS Key Laboratory of Behavioral Science, Institute of Psychology, Beijing, China

16 Lincui Road, Chaoyang District, Beijing 100101, China

Tel: +86-10-64101582

Fax: +86-10-64101582

E-mail: ycg.yan@gmail.com

Jing-Ping Zhao, M.D.

The Institute of Mental Health, Second Xiangya Hospital of Central South University, Changsha, Hunan, 410011, China

E-mail: zhaojingpingcsu@163.com

Yu-Feng Zang, M.D.

Center for Cognition and Brain Disorders, Institutes of Psychological Sciences, Hangzhou Normal University, Hangzhou, Zhejiang, 310015, China

E-mail: zangyf@gmail.com

Xiao Chen and Le Li contributed equally to this work.

ABSTRACT

Background: Major Depressive Disorder (MDD) is common and disabling, but its neural pathophysiology remains unclear. Functional brain network studies in MDD have largely had limited statistical power and data analysis approaches have varied widely. The REST-meta-MDD Project of resting-state fMRI (R-fMRI) addresses these issues. **Methods:** The 25 research groups in China composing the REST-meta-MDD Project contributed R-fMRI data of 1,300 patients with MDD and 1,128 normal controls (NCs). The data were preprocessed locally with a standardized protocol prior to aggregated group analyses. We focused on functional connectivity (FC) within the default mode network (DMN), frequently reported to show increased FC in MDD. **Outcomes:** We found decreased instead of increased DMN FC when comparing 848 MDDs with 794 NCs from 17 sites after data exclusion. We found FC reduction only in recurrent MDD, not in first-episode drug-naïve MDD. Decreased DMN FC was associated with medication usage but not with MDD duration. DMN FC was also positively related to symptom severity but only in recurrent MDDs. Exploratory analyses also revealed alterations of local intrinsic activity in MDD. **Interpretation:** We confirmed the key role of DMN in MDD but found reduced rather than increased FC within the DMN. Future studies should test whether decreased DMN FC mediates treatment response. The REST-meta-MDD model can be generalized to longitudinal MDD studies and to other psychiatric disorders. **Funding:** National Key R&D Program of China, National Natural Science Foundation of China and Chinese Academy of Sciences.

RESEARCH IN CONTEXT

Evidence before this study

We conducted a literature search in PubMed on July 2, 2018, using the keywords *(((default mode network) OR default network) AND depress*) AND connect**. We also manually searched the reference sections of empirical and review articles for their citations. Original studies were included for review if they 1) included patients with current major depressive disorder (MDD), 2) used resting-state fMRI (R-fMRI), 3) made comparisons to matched healthy controls (HCs), and 4) reported within-DMN connectivity on measures of functional connectivity. Systematic review yielded 38 studies (Supplementary Table S1). Of these, 18 found increases in DMN FC in MDD, eight reported decreases, seven found both increases and decreases, and five no significant changes. Thus, consensus on DMN FC in MDD was lacking.

Added value of this study

We created the REST-meta-MDD model and accumulated the largest MDD R-fMRI sample to date (1300 MDDs and 1128 NCs). With this unprecedented database, we addressed the issues of limited statistical power and data analytic heterogeneity in the literature. We found decreased instead of increased DMN FC in MDD compared to NCs. Specifically, FC reduction was only present in recurrent MDD and not in first-episode drug-naïve MDD. Decreased DMN FC in MDD was associated with current medication treatment but not with MDD duration.

Implications of all the available evidence

These findings suggest that DMN functional connectivity remains a prime target for understanding the pathophysiology of MDD, with particular relevance to revealing mechanisms of effective treatments, as the greater the medication benefit, the more DMN FC was reduced. Further, the REST-meta-MDD model can generalize to large-scale collaborative MDD longitudinal studies, and to other neuropsychiatric disorders.

1. INTRODUCTION

Major Depressive Disorder (MDD) is the second leading-cause of disability world-wide, with point prevalence exceeding 4%.[1] The pathophysiology of MDD remains unknown despite intensive efforts, including neuroimaging studies. However, the small sample size of most MDD neuroimaging studies entails low sensitivity and reliability and results unlikely to reflect true effects.[2,3] An exception is the Enhancing Neuro Imaging Genetics through Meta-Analysis (ENIGMA) consortium which has meta- and mega-analyzed data of thousands of structural MRI scans from MDD patients and healthy controls.[4,5] The ENIGMA-MDD working group found a robust reduction in hippocampal volume[4] and cortical thinning in medial orbitofrontal cortex.[5] However, this approach does not consider communication among brain regions, i.e., functional brain networks.

Imbalanced communications among functional brain networks have been reported in MDD using resting-state functional connectivity (FC), which detects synchronized activity among anatomically distinct networks. MDD studies have focused on the default mode network (DMN), which is comprised of regions that are most active during rest and which has been linked to rumination.[6] Most MDD studies have found increased DMN FC in MDD, although decreased or unchanged DMN FC have also been reported. Previous meta-analyses revealed increased DMN FC in MDD,[6,7] although based on few studies, e.g., Hamilton et al. only included seven because of seed variation across studies.[6] As summarized in Supplementary Table S1, of 38 studies examining DMN FC alterations in MDD, 18 found increases, eight decreases, seven both increases and decreases, and five no significant

changes. For example, the first study focusing on the DMN in MDD reported increased DMN FC using independent component analysis (ICA).[8] However, other ICA studies[9,10] found both increased and decreased DMN FC in MDD. As demonstrated in Supplementary Figure S1, a voxel-wise meta-analysis of 32 studies showed increased orbitofrontal DMN FC and decreased dorsal medial prefrontal cortex (dmPFC) / posterior DMN FC in MDD. These complex results may have contributed to prior inconsistent reports.

Inconsistencies may be due to small sample size and limited statistical power[2] but data analysis flexibility may also contribute.[11] MDD studies have used different multiple comparison correction methods, most likely inadequate.[12] Data analysis flexibility also impedes large-scale meta-analysis. Moreover, clinical characteristics such as number and type of episodes, medication status and illness duration vary across studies, further contributing to heterogeneous results.

To address limited statistical power and analytic heterogeneity, we initiated the REST-meta-MDD Project. Building on the R-fMRI data processing pipeline DPARSF,[13] we implemented a standardized preprocessing protocol at local sites with only final indices provided to the consortium. We obtained R-fMRI indices (including FC matrices) corresponding to 1300 patients with MDD and 1128 normal controls (NCs) from 25 cohorts in China. To our knowledge, the REST-meta-MDD is the largest R-fMRI database of MDD in the world (ongoing R-fMRI cohort studies in MDD have not yet published on this scale; see Supplementary Table S2). We used linear mixed models to identify abnormal FC patterns

associated with DMN across cohorts, and investigated whether episode type, medication status, illness severity and illness duration contribute to abnormalities.

2. MATERIALS AND METHODS

2.1. Contributions

Initial contributions were requested from researchers who used DPARSF (software developed by CGY and YFZ)[13] to process their MDD R-fMRI data. Twenty-five research groups from 17 hospitals in China agreed to share final R-fMRI indices from patients with MDD and matched normal controls (see Supplementary Table S3 for data composition; henceforth "site" refers to each cohort for convenience) from studies approved by local Institutional Review Boards. Data submitted to the consortium were fully deidentified and anonymized. See Supplementary Methods for how subject privacy was protected.

2.2. Phenotypic Data

Consortium members met on March 25th, 2017 to establish the collaboration; all agreed to include diagnosis, age at scan, sex and education in the "base" phenotypic protocol. When collected systematically, measures of first-episode or recurrent MDD (if a patient's prior episode and current episode were diagnosed as MDD based on ICD10 or DSM4), medication status, illness duration, treatment responsive, family history, Hamilton Depression Rating Scale (HAMD, with 17 items) and Hamilton Anxiety Rating Scale (HAMA) were also provided.

2.3. Individual-Level Image Processing

Individual-level imaging processing was performed at each site using the standardized DPARSF processing parameters on individual R-fMRI data and 3D T1-weighted images. Neuroimaging analysts from each site took a two-day onsite DPARSF training course on May 13-14, 2017 at the Institute of Psychology, Chinese Academy of Sciences to harmonize data analyses.

2.3.1. Preprocessing

As detailed in Supplementary Methods, preprocessing included slice timing correction, realignment, segmentation, nuisance regression, spatial normalization and temporal filtering, while adapting to distinct data acquisition parameters (e.g., TRs and slice timing parameters). We did not include global signal regression (GSR) in primary analyses, but did in supplementary analyses. We also confirmed results using head motion scrubbing.

2.3.2. DMN FC Analyses

After preprocessing, time-series for the Dosenbach 160 functional regions-of-interest (ROIs)[14] were extracted. To validate primary analyses, time series for Zalesky's random 980 parcellations were also extracted. For each, we defined the DMN ROIs as those overlapping with the DMN delineated by Yeo et al.[15] The average FC (Fisher's r-to-z transformed Pearson's correlation between time-series of all ROI pairs) within DMN ROIs was defined as DMN within-network FC for patient-control contrasts. In supplementary analyses, we also compared DMN ROIs connection-by-connection.

2.3.3. Exploratory Analyses of R-fMRI Metrics for Local Abnormalities

We explored local abnormalities in MDD with voxel-wise amplitude of low frequency fluctuations (ALFF), fractional ALFF (fALFF), regional homogeneity (ReHo), degree centrality (DC), and voxel-mirrored homotopic connectivity (VMHC)[16] as detailed in Supplementary Methods.

2.4. Group-Level Image Processing

After individual-level imaging processing performed locally at each site, individual-level imaging metrics (i.e., the ROI time-series and R-fMRI indices) together with phenotypic data were uploaded to the data center at the Institute of Psychology, Chinese Academy of Sciences through the R-fMRI Maps Project (http://rfmri.org/maps) platform.

2.4.1. Sample Selection

From 1300 MDDs and 1128 NCs, we selected 848 MDDs and 794 NCs from 17 sites for statistical analyses. Exclusion criteria (e.g., incomplete information, bad spatial normalization, bad coverage, excessive head motion and sites with fewer than 10 subjects in either group) and final inclusions are provided in Supplementary Methods and Supplementary Figure S2.

2.4.2. Statistical Analyses

We used the Linear Mixed Model (LMM) to compare MDDs with NCs while allowing site-varying effects. LMM extends linear regression models to data collected and summarized

in groups.[17] LMM describes the relationship between a response variable (e.g., DMN FC) and independent variables (here diagnosis and covariates of age, sex, education, and head motion), with coefficients that can vary with respect to grouping variables (here site). We utilized MATLAB's command fitlme (https://www.mathworks.com/help/stats/fitlme.html) to test the model:

y ~ 1 + Diagnosis + Age + Sex + Education + Motion + (1 | Site) + (Diagnosis | Site)

which yields T and P values for the fixed effect of Diagnosis. Cohen's d effect size was computed as $d = \frac{T(n_1 + n_2)}{\sqrt{df}\sqrt{n_1 n_2}}$.[18]

2.4.3. Subgroup Analyses

We also examined sub-group effects: several sites reported whether patients with MDD were in their first episode (and drug-naïve) or recurrent. We compared first episode drug-naïve (FEDN) MDD patients with corresponding NCs (232 FEDN MDDs vs. 394 NCs from 5 sites). We also compared recurrent MDD patients with corresponding NCs (189 recurrent MDD vs. 427 NCs from 6 sites). To directly compare FEDN MDD patients with recurrent MDD patients (119 FEDN MDDs vs. 72 recurrent MDDs from 2 sites), we replaced Diagnosis with FEDN or recurrent status in the LMM model.

2.4.4. Analyses of Effects of Illness Duration, Medication, and Symptom Severity

As the distribution of illness duration was skewed (most were brief), we contrasted the terciles with longest and shortest illness durations instead of Diagnosis in the LMM model. To test medication effects, we replaced Diagnosis with medication on or off in the LMM model. Finally,

to test symptom severity effects, we replaced Diagnosis with the 17-item HAMD total score regressor in the LMM model.

3. RESULTS

3.1. Sample Composition

Twenty-five research groups from 17 hospitals contributed 25 previously collected datasets of 2428 individuals (1300 MDDs and 1128 NCs) (Figure 1 and Supplementary Tables S3-5). On average, each site contributed 52·0±52·4 MDDs (range 13-282) and 45·1±46·9 NCs (range 6-251). Most MDD patients were female (826 vs. 474 males), as is typical in MDD studies. There were 562 first episode MDDs, including 318 FEDN MDD and 160 scanned while receiving antidepressants (medication status was unavailable for 84 first episode MDDs). Of 282 with recurrent MDD, 121 were scanned while receiving antidepressants and 76 were not being treated with medication (medication status was unavailable for 85 recurrent MDD). Episodicity (first or recurrent) and medication status were unavailable for 456 MDD patients.

3.2. Decreased DMN Functional Connectivity in Patients with MDD

By overlapping the Dosenbach 160 ROIs[14] with the Yeo DMN,[15] we identified 33 DMN ROIs. Mean DMN within-network FC (averaged across 33*32/2=528 connections) was compared between 848 MDDs and 794 NCs (see Sample Selection in Supplementary Methods) with the LMM. MDD patients demonstrated significantly lower DMN within-network FC than NCs (T=-3·762, P=0·0002, d=-0·186, Figure 2A). On subgroup analyses, FEDN MDDs did not differ significantly from NCs (T=-0·914, P=0·361, d=-0·076, Figure 2B), while DMN FC was

significantly decreased in patients with recurrent MDD vs. NCs (T=-3·737, P=0·0002, d=-0·326, Figure 2C). We directly compared FEDN MDDs with recurrent MDDs (T=2·676, P=0·008, d=0·400, Figure 2D); significantly lower DMN FC in recurrent MDD patients suggests they were the major contributors to decreased DMN FC in MDD.

3.3. Reduced DMN Functional Connectivity Was Not Associated with Illness Duration

We found that recurrent MDDs but not FEDN MDDs demonstrated reduced DMN FC. This could reflect illness duration or medication history. We first tested the effect of illness duration in FEDN MDDs to reduce medication confounds. The tercile with longest illness duration (≥12 months, 70 MDDs from 2 sites) did not differ significantly from the tercile with shortest illness duration (≤3 months, 48 MDDs from the same 2 sites) in DMN FC (T=1·140, P=0·257, d=0·214, Figure 3A). Similarly, when exploring in the entire sample, the tercile with longest illness duration (≥24 months, 186 MDDs from 4 sites) did not differ significantly from the tercile with shortest illness duration (≤6 months, 112 MDDs from the same 4 sites): T=1·541, P=0·124, d=0·184 (Figure 3B).

3.4. Medication Effect and Reduced DMN Functional Connectivity in MDD Patients

To further examine medication treatment effects, we contrasted first episode MDDs on medication (115 MDDs from Site 20) with FEDN MDDs (97 MDDs from Site 20) and found significantly reduced DMN FC (T=-2·629, P=0·009, d=-0·362, Figure 3C). When directly comparing first episode MDDs on medication with NCs (102 first-episode MDDs on medication vs. 266 NCs from 2 sites), we found a non-significant trend (T=-1·614, P=0·108,

d=-0·188). While FEDN MDDs showed higher DMN FC than recurrent MDDs as shown in Section 3.2, first-episode MDDs on medication and recurrent MDDs did not differ (102 first-episode MDDs on medication vs. 57 recurrent MDDs from 2 sites: T=0·548, P=0·585, d=-0·091). This convergent evidence suggests that medication treatment might account for our overall finding of reduced DMN FC in MDD. However, we could not address whether currently unmedicated recurrent MDDs had been treated with antidepressants in prior episode(s). We were also unable to examine treatment duration, as only binary information on medication status was provided.

3.5. Association of DMN Functional Connectivity with Symptom Severity

The association between DMN FC and HAMD scores was tested on 734 MDD patients (excluding patients with HAMD scores below 7) from 15 sites and was not significant (T=1·591, P=0·112, r=0·059). The effect of symptom severity was not significant in FEDN MDDs (N=197, 3 sites; T=-0·158, P=0·874, r=-0·011), but significant in recurrent MDDs (N=126, 4 sites; T=2.167, P=0·032, r=0·194).

3.6. Reproducibility

We assessed reproducibility through several strategies (Table 1). 1) Using a finer-grade parcellation with 211 DMN ROIs confirmed our results, except that the effect of symptom severity in recurrent MDDs became insignificant (T=1·264, P=0·209, r=0·115). 2) Beyond LMM, we also performed meta-analyses: within-site T-values were converted into Hedge's *g*, and entered in a random effect meta-model (using R package "metansue",

https://www.metansue.com/). Results were almost the same, although the difference between FEDN MDDs and recurrent MDDs became a trend (Z=1·732, P=0·083, d=0·251), and symptom severity in recurrent MDDs became insignificant (Z=1·304, P=0·192, r=0·119). 3) We also tested whether global signal regression (GSR) mattered. With GSR, we found similar results except the difference between FEDN MDDs and recurrent MDDs became insignificant (T=0·974, P=0·331, d=0·145) and the medication effect became a trend (T=-1·891, P=0·060, d=-0·261), as did the symptom severity in recurrent MDDs (T=1·741, P=0·084, r=0·157). This overall confirmation is important since global signal has been viewed as reflecting spurious noise,[19] and its standard deviation differed significantly between MDDs and NCs (T=-2·662, P=0·008, d=-0·131). 4) For head motion control, despite already incorporating the Friston-24 model at the individual level and a motion covariate at the group level in primary analyses, we also used scrubbing (removing time points with framewise displacement (FD)[20] > 0·2mm) to verify results. All results remained the same using this aggressive head motion control strategy.

3.7. Exploratory Findings of Local Abnormalities in MDD Patients

Although the main scope of the current study was to investigate DMN FC in MDD, we also performed exploratory analyses searching for local abnormalities to illustrate the potential value of the shared voxel-wise R-fMRI metric maps. Since ReHo demonstrated the highest test-retest reliability among the commonly used R-fMRI indices,[16] we present ReHo abnormalities in MDD and include other indices in Supplementary Results. We applied the LMM model to each voxel, and then used Gaussian random field (GRF) theory correction to

correct for multiple comparisons across voxels. We set a strict threshold for GRF correction for each tail, with voxel p<0·0005 (Z>3·29) and cluster p<0·025. This achieves two-tailed voxel p<0·001 and cluster p<0·05 and maintains the family-wise error rate under 5%.[3,12] Comparing all 848 MDDs with 794 NCs, ReHo was increased in left dorsolateral prefrontal cortex (DLPFC) in MDD, and it was decreased in bilateral primary motor cortex (Figure 4A). Among subgroups, left DLPFC ReHo was significantly increased in FEDN MDDs (Figure 4B) but not in recurrent MDD (Figure 4C). By contrast, ReHo in bilateral primary motor cortex was only significantly decreased in recurrent MDDs (Figure 4C) but not in FEDN MDDs (Figure 4B). However, FEDN MDDs and recurrent MDDs did not differ significantly in ReHo when compared directly.

4. DISCUSSION

We created a model for building consortium and aggregate large-scale functional brain imaging data for depression, which can be generalized to other psychiatric disorders. Using an unprecedentedly large sample, we found decreased instead of increased FC within the DMN in MDDs compared with NCs. However, this effect was only significant in recurrent MDD whether vs. controls or patients with FEDN MDD. Furthermore, decreased DMN FC in recurrent MDD was associated with being scanned on medication rather than illness duration. DMN FC was also positively related to symptom severity but only in recurrent MDDs. Exploratory analyses revealed increased ReHo in left DLPFC in FEDN MDD, and decreased ReHo in bilateral primary motor cortex in recurrent MDD.

Our primary results contradict the prevailing notion that DMN FC is increased in MDD[6,7] (see Supplementary Table S1). Several factors may account for this discrepancy. 1) Prior studies have also reported decreased DMN FC in MDD (see Supplementary Table S1), in line with our findings. In our voxel-wise meta-analysis of 32 studies (Supplementary Figure S1), we found both increases (orbitofrontal DMN FC) and decreases (dmPFC / posterior DMN FC) in MDD. Prior inconsistent results may also reflect heterogeneous analysis strategies. We applied a standardized analysis protocol across sites, thus removing analytic variations. 2) Most studies reporting increased DMN FC in MDDs were conducted in Caucasian samples, while our study was performed in purely Chinese population. Ethnic differences may contribute to discrepant findings, as east Asians have a lower lifetime prevalence of MDD,[1] more somatic symptoms and fewer psychological symptoms in MDDs,[21] and differ in MDD risk genes.[22] 3) Our measure of averaged DMN FC might be insensitive to possible pair-wise increases in MDD DMN FC. However, pair-wise tests but did not reveal even a single pair of significantly increased within-DMN connection in MDDs (see Supplementary Results and Supplementary Figure S3). Large sample studies will be needed to clarify the pattern of DMN FC in MDD across ethnicities.

We found decreased DMN FC only in recurrent MDD patients, which may account for our results in whole-group analysis, as its effect size is nearly double (d=-0·326 vs. -0·186). Similarly, ENIGMA MDD[4] found a robust reduction in hippocampal volume (a key DMN node) only in recurrent MDD and not in first episode MDD. We considered illness duration effects, as recurrent MDDs had significantly longer illness duration than FEDN MDDs (Z=6·419,

p<0·001). However, direct comparisons did not reveal effects of illness duration. An early study in MDD[8] found that DMN FC was positively correlated with current depressive episode duration. However, this relationships has not been confirmed by subsequent studies[10 23 24]. We conclude that illness duration is likely unrelated to DMN FC. However, longitudinal studies are needed to determine whether DMN FC changes over the course of depressive episodes.

Decreased DMN FC in recurrent MDD appeared related to antidepressant medication treatment. We confirmed that first episode MDDs scanned while on medication had decreased DMN FC than FEDN MDD. This result aligns with studies of antidepressants on DMN FC in MDD,[25] dysthymia,[26] and in healthy individuals.[27] In MDD, antidepressant treatment (with either paroxetine, venlafaxine, duloxetine or citalopram) for 12 weeks reduced posterior DMN FC.[25] In patients with dysthymic disorder, 10 weeks of duloxetine treatment reduced DMN FC.[26] In healthy individuals, duloxetine for 2 weeks reduced DMN FC and improved mood.[27] Our finding of medication-associated reduction in DMN FC is consistent with prior results and suggests antidepressant medications may alleviate depressive symptoms by reducing DMN FC. This medication effect (effect size d=-0·362) might be also the key to the contradiction between our finding of reduced DMN FC in MDD and prior meta-analyses. This effect was observed in a cross-sectional sample, so it has to be confirmed by longitudinal medication follow-up designs.

We did not find significant associations between DMN FC and symptom severity in all MDDs

nor in FEDN MDDs. However, symptom severity was positively correlated with DMN FC in recurrent MDDs. This is consistent with a prior report[28] which found a positive correlation between DMN FC in a specific frontal subcircuit and illness severity in MDDs (half treated with medication). Our finding may reflect medication effects in recurrent MDDs (effects was even stronger in recurrent MDDs on medication: N=40, 2 sites; T=3.268, P=0·003, r=0·489): the greater the medication benefit (indicated by lower HAMD score), the more DMN FC was reduced. Again, this hypothesis needs to be further confirmed by longitudinal medication follow-up designs.

To expand our investigation beyond DMN and the dependence of analyses on ROIs specified a priori, we conducted exploratory analyses with local R-fMRI metrics. Results included significantly increased ReHo in DLPFC and decreased ReHo in primary motor area in MDDs compared with NCs. The increase in DLPFC ReHo was attributable to abnormalities in FEDN MDDs whereas decreased primary motor area ReHo was attributable to recurrent MDD (see Figure 4). Our results in motor cortex align with a previous meta-analysis[29] of ReHo studies. However, that meta-analysis reported increased ReHo in medial PFC, instead of DLPFC as we found. A recent direct comparison of classical coordinate-based meta-analyses and direct aggregated analyses with multi-site datasets[30] found that results from meta-analyses are less reliable. Additional results from other local metrics also revealed local functional abnormalities in MDD (see Supplementary Results, Supplementary Figures S4~S7), which may be useful for identifying local neuromodulation targets for treating MDD if replicated independently.

Study limitations include an exclusively Chinese sample, thus generalization to other populations is unknown. In the future, we plan to invite international MDD researchers to join the REST-meta-MDD Project to include cross-ethnicity samples with the goal of identifying ethnicity/culture-general and ethnicity/culture-specific abnormal brain patterns in MDD. Second, our retrospective cross-sectional sample cannot address longitudinal effects, such as response to treatment. We plan to encourage the REST-meta-MDD consortium to perform coordinated prospective longitudinal studies. Third, medication treatment was a binary variable; future studies should quantify cumulative doses and include non-pharmacologic treatments. Finally, our findings require independent replication.[11] To improve transparency and reproducibility, the analysis code has been openly shared at https://github.com/Chaogan-Yan/PaperScripts/tree/master/Yan_2018. Upon publication, the R-fMRI indices of the 1300 MDD patients and 1128 NCs will be openly shared through the R-fMRI Maps Project (LINK_TO_BE_ADDED). These data derivatives will allow replication, secondary analyses and discovery efforts while protecting participant privacy and confidentiality. Future independent efforts could examine other networks beyond DMN (e.g., salience network or executive control network), dynamic FC, MDD subtypes, and data mining with machine learning algorithms.

In summary, based on the largest R-fMRI database of MDD, we confirmed the key role of the DMN in MDD, identifying a reduction of DMN FC in recurrent MDD patients. This reduction appears to reflect medication usage rather than illness duration. These findings suggest that

the DMN should remain a prime target for further MDD research, especially to determine whether reducing DMN FC mediates symptomatic improvement.

DATA SHARING STATEMENT

Immediately upon publication, the aggregated data of this study (deidentified anonymized R-fMRI indices of 1300 MDD patients and 1128 NCs) will be made available through The R-fMRI Maps Project (http://rfmri.org/maps) to interested investigators. The investigators should sign a data access agreement and submit a data use proposal, which will be reviewed by the REST-meta-MDD consortium.

DECLARATION OF INTERESTS

All the authors declare no competing financial interests.

ACKNOWLEDGEMENTS

This work was supported by the National Key R&D Program of China (2017YFC1309902), the National Natural Science Foundation of China (81671774, 81630031 and 81371488), the Hundred Talents Program of the Chinese Academy of Sciences, Beijing Municipal Science & Technology Commission (Z161100000216152 and Z171100000117016), Department of Science and Technology, Zhejiang Province (2015C03037).

REFERENCES

1. Ferrari AJ, Charlson FJ, Norman RE, et al. Burden of Depressive Disorders by Country, Sex, Age, and Year: Findings from the Global Burden of Disease Study 2010. *PLOS Medicine* 2013; **10**(11): e1001547.

2. Button KS, Ioannidis JPA, Mokrysz C, et al. Power failure: why small sample size undermines the reliability of neuroscience. *Nat Rev Neurosci* 2013; **14**(5): 365-76.

3. Chen X, Lu B, Yan CG. Reproducibility of R-fMRI metrics on the impact of different strategies for multiple comparison correction and sample sizes. *Hum Brain Mapp* 2018; **39**(1): 300-18.

4. Schmaal L, Veltman DJ, van Erp TG, et al. Subcortical brain alterations in major depressive disorder: findings from the ENIGMA Major Depressive Disorder working group. *Mol Psychiatry* 2016; **21**(6): 806-12.

5. Schmaal L, Hibar DP, Samann PG, et al. Cortical abnormalities in adults and adolescents with major depression based on brain scans from 20 cohorts worldwide in the ENIGMA Major Depressive Disorder Working Group. *Molecular psychiatry* 2017; **22**(6): 900-9.

6. Hamilton JP, Farmer M, Fogelman P, Gotlib IH. Depressive Rumination, the Default-Mode Network, and the Dark Matter of Clinical Neuroscience. *Biological psychiatry* 2015; **78**(4): 224-30.

7. Kaiser RH, Andrews-Hanna JR, Wager TD, Pizzagalli DA. Large-Scale Network Dysfunction in Major Depressive Disorder: A Meta-analysis of Resting-State Functional Connectivity. *JAMA Psychiatry* 2015; **72**(6): 603-11.

8. Greicius MD, Flores BH, Menon V, et al. Resting-state functional connectivity in major depression: abnormally increased contributions from subgenual cingulate cortex and thalamus. *Biological psychiatry* 2007; **62**(5): 429-37.

9. Zhu X, Wang X, Xiao J, et al. Evidence of a dissociation pattern in resting-state default mode network connectivity in first-episode, treatment-naive major depression patients.

Biological psychiatry 2012; **71**(7): 611-7.

10. Guo W, Liu F, Zhang J, et al. Abnormal default-mode network homogeneity in first-episode, drug-naive major depressive disorder. *PLoS ONE* 2014; **9**(3): e91102.

11. Poldrack RA, Baker CI, Durnez J, et al. Scanning the horizon: towards transparent and reproducible neuroimaging research. *Nat Rev Neurosci* 2017; **18**(2): 115-26.

12. Eklund A, Nichols TE, Knutsson H. Cluster failure: Why fMRI inferences for spatial extent have inflated false-positive rates. *Proc Natl Acad Sci U S A* 2016.

13. Yan CG, Zang YF. DPARSF: A MATLAB Toolbox for "Pipeline" Data Analysis of Resting-State fMRI. *Front Syst Neurosci* 2010; **4**: 13.

14. Dosenbach NU, Nardos B, Cohen AL, et al. Prediction of individual brain maturity using fMRI. *Science (New York, NY* 2010; **329**(5997): 1358-61.

15. Yeo BT, Krienen FM, Sepulcre J, et al. The organization of the human cerebral cortex estimated by intrinsic functional connectivity. *Journal of neurophysiology* 2011; **106**(3): 1125-65.

16. Zuo XN, Xing XX. Test-retest reliabilities of resting-state FMRI measurements in human brain functional connectomics: a systems neuroscience perspective. *Neurosci Biobehav Rev* 2014; **45**: 100-18.

17. West BT, Welch KB, Galecki AT. Linear mixed models: a practical guide using statistical software: CRC Press; 2014.

18. Rosenthal R, Rosnow RL. Essentials of behavioral research: Methods and data analysis (3rd ed.). New York, NY: McGraw-Hill; 1991.

19. Power JD, Plitt M, Laumann TO, Martin A. Sources and implications of whole-brain fMRI

signals in humans. *Neuroimage* 2016.

20. Jenkinson M, Bannister P, Brady M, Smith S. Improved Optimization for the Robust and Accurate Linear Registration and Motion Correction of Brain Images. *NeuroImage* 2002; **17**(2): 825-41.

21. Ryder AG, Yang J, Zhu X, et al. The cultural shaping of depression: somatic symptoms in China, psychological symptoms in North America? *Journal of abnormal psychology* 2008; **117**(2): 300-13.

22. Long H, Liu B, Hou B, et al. The long rather than the short allele of 5-HTTLPR predisposes Han Chinese to anxiety and reduced connectivity between prefrontal cortex and amygdala. *Neurosci Bull* 2013; **29**(1): 4-15.

23. Guo W, Liu F, Dai Y, et al. Decreased interhemispheric resting-state functional connectivity in first-episode, drug-naive major depressive disorder. *Prog Neuropsychopharmacol Biol Psychiatry* 2013; **41**: 24-9.

24. Wise T, Marwood L, Perkins AM, et al. Instability of default mode network connectivity in major depression: a two-sample confirmation study. *Translational psychiatry* 2017; **7**(4): e1105.

25. Li B, Liu L, Friston KJ, et al. A treatment-resistant default mode subnetwork in major depression. *Biological psychiatry* 2013; **74**(1): 48-54.

26. Posner J, Hellerstein DJ, Gat I, et al. Antidepressants normalize the default mode network in patients with dysthymia. *JAMA Psychiatry* 2013; **70**(4): 373-82.

27. van Wingen GA, Tendolkar I, Urner M, et al. Short-term antidepressant administration reduces default mode and task-positive network connectivity in healthy individuals during rest.

Neuroimage 2014; **88**: 47-53.

28. Davey CG, Harrison BJ, Yucel M, Allen NB. Regionally specific alterations in functional connectivity of the anterior cingulate cortex in major depressive disorder. *Psychological medicine* 2012; **42**(10): 2071-81.

29. Iwabuchi SJ, Krishnadas R, Li C, Auer DP, Radua J, Palaniyappan L. Localized connectivity in depression: A meta-analysis of resting state functional imaging studies. *Neuroscience & Biobehavioral Reviews* 2015; **51**: 77-86.

30. Jia X-Z, Zhao N, Barton B, et al. Small effect size leads to reproducibility failure in resting-state fMRI studies. *bioRxiv* 2018.

TABLES

Table 1. Verification results of default mode network (DMN) within-network functional connectivity (FC) in MDD with multiple alternative analysis strategies. Linear Mixed Effect (LME) model or meta-analytic model was utilized on different parcellations in different statistical comparisons (the effects of age, sex, education level, head motion and scanning site were controlled).

	Dosenbach 160 functional ROIs (LME)		Zalesky random 980 parcellations (LME)		Dosenbach 160 functional ROIs (meta)		Dosenbach 160 functional ROIs (LME & GSR)		Dosenbach 160 functional ROIs (LME & Scrubbing)	
	T	*P*	*T*	*P*	*Z*	*P*	*T*	*P*	*T*	*P*
All MDDs vs. NCs (848 vs. 794)	-3 762	0 0002	-3 179	0 002	-4 057	0 00004	-4 373	0 0001	-3 818	0 0001
FEDN MDDs vs. NCs (232 vs. 394)	-0 914	0 361	-0 561	0 575	-0 658	0 511	-0 585	0 559	-0 990	0 322
Recurrent MDDs vs. NCs (189 vs. 427)	-3 737	0 0002	-3 356	0 0008	-3 702	0 0002	-4 382	0 0001	-3 836	0 0001
FEDN MDDs vs. Recurrent MDDs (119 vs. 72)	2 676	0 008	3 284	0 001	1 732	0 083	0 974	0 331	2 527	0 012
Long duration FEDN MDDs vs. Short duration FEDN MDDs (70 vs. 48)	1 140	0 257	1 116	0 267	1 089	0 276	0 522	0 603	1 169	0 245
Long duration MDDs vs. Short duration MDDs (186 vs. 112)	1 541	0 124	1 361	0 175	1 386	0 166	1 334	0 183	1 552	0 122

On medication vs. FEDN (115 vs. 97)	-2 629	0 009	-2 293	0 023	-2 568	0 010	-1 891	0 060	-2 504	0 013
Correlation with HAMD in all MDDs (*N* = 734)	1 591	0 112	1 181	0 238	0 754	0 451	0 448	0 654	1 765	0 078
Correlation with HAMD in FEDN MDDs (N = 197)	-0 158	0 874	0 540	0 590	-0 676	0 499	-0 163	0 871	-0 167	0 868
Correlation with HAMD in recurrent MDDs (N = 126)	2 167	0 032	1 264	0 209	1 304	0 192	1 741	0 084	2 446	0 016

Abbreviations: FEDN, First Episode Drug Na ve; LME, Linear Mixed Effect; global signal regression; DMN, Default Mode Network.

FIGURE LEGENDS

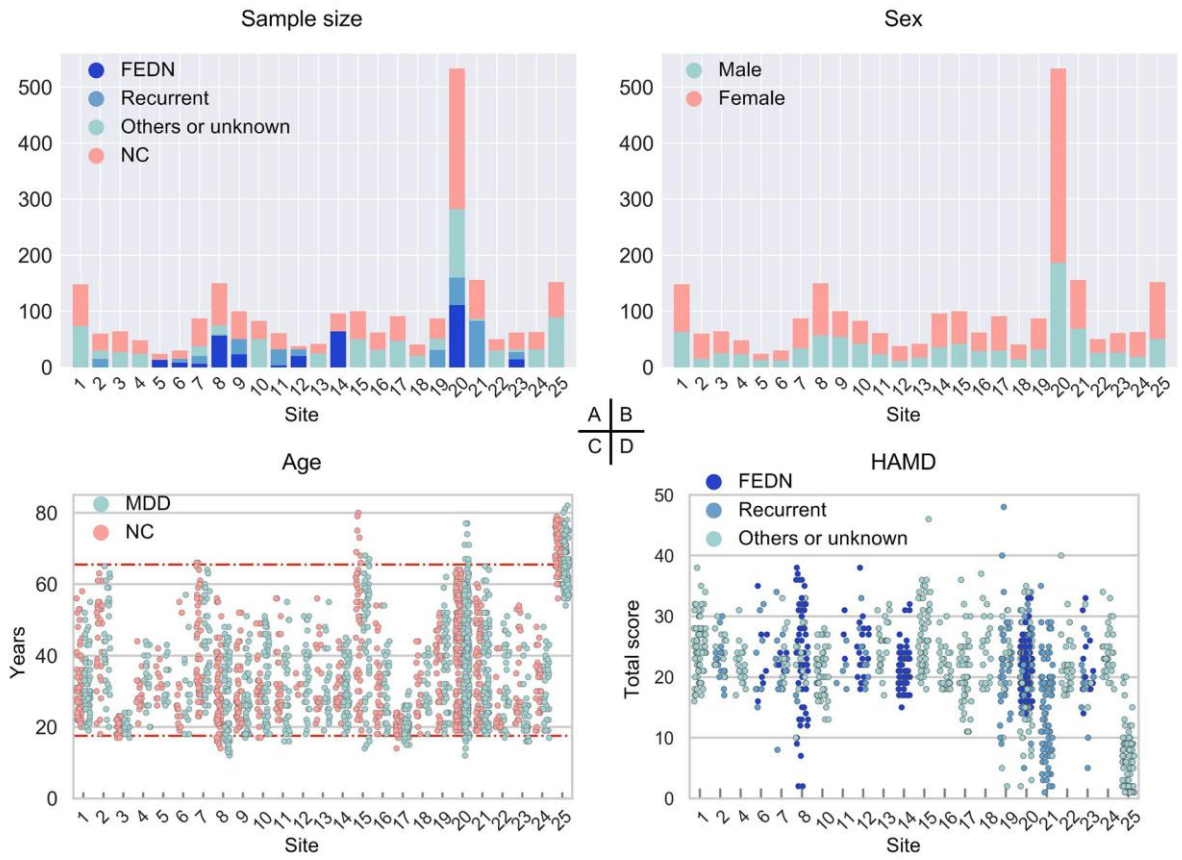

Figure 1. REST-meta-MDD sample characteristics. (A) Total number of participants per group for each contributing site. The MDD patients were subdivided into first-episode drug-naïve (FEDN), recurrent and others/unknown types. (B) Number of male subjects and female subjects for each site irrespective of diagnostic group. (C) Age (in years) for all individuals per site for the MDD group and NC group. The two horizontal lines represents ages 18 and 65, the age limits for participants chosen for imaging analysis. (D) The score of Hamilton Depression Rating Scale (HAMD) for the MDD patients. Of note, some sites did not provide HAMD information.

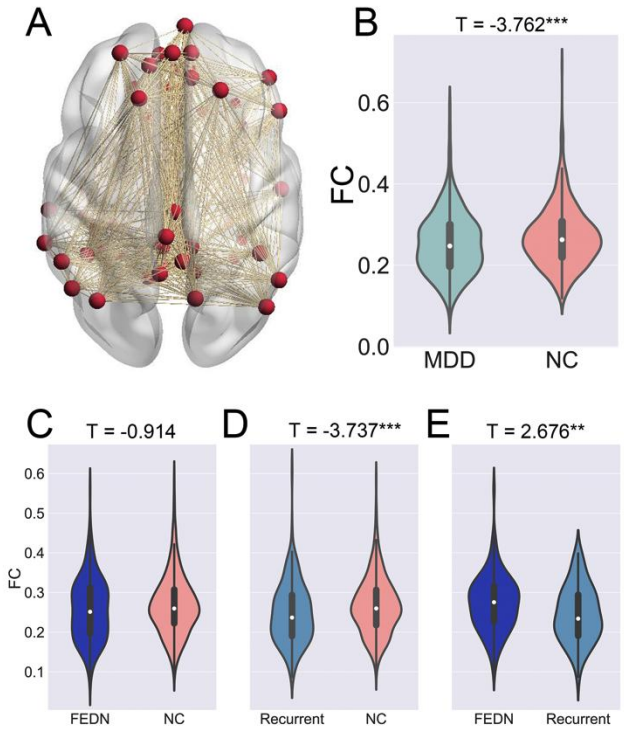

Figure 2. Decreased DMN functional connectivity in MDD patients. Mean DMN within-network FC was averaged across 33*32/2=528 connections as shown in A. The violin figures show the distribution of mean DMN within-network FC between MDD group and NC group (B), between first episode drug naïve (FEDN) MDD group and NC group (C), between recurrent MDD group and NC group (D), and between FEDN MDD group and recurrent MDD group (E). Of note, for each comparison, only sites with sample size larger than 10 in each group were included. The T values were the statistics for these comparisons in Linear Mixed Model analyses. **, $p < 0{\cdot}01$; ***, $p < 0{\cdot}001$.

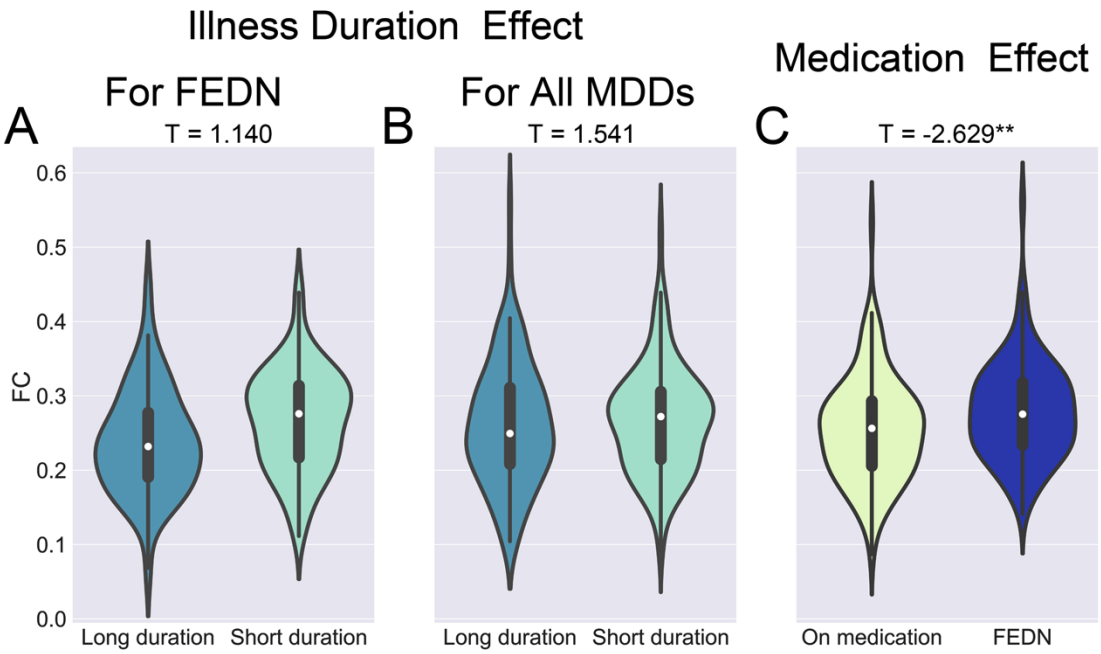

Figure 3. The effects of illness duration and medication on decreased DMN functional connectivity in MDD patients. The violin figures show the distribution of mean DMN within-network FC for first episode drug naïve (FEDN) MDDs with long vs. short illness duration (A), for pooled MDDs with long vs. short illness duration (B), and for first episode MDDs with vs. without medication usage (C). The T values are the statistics for these comparisons in Linear Mixed Model analyses. **, $p < 0{\cdot}01$.

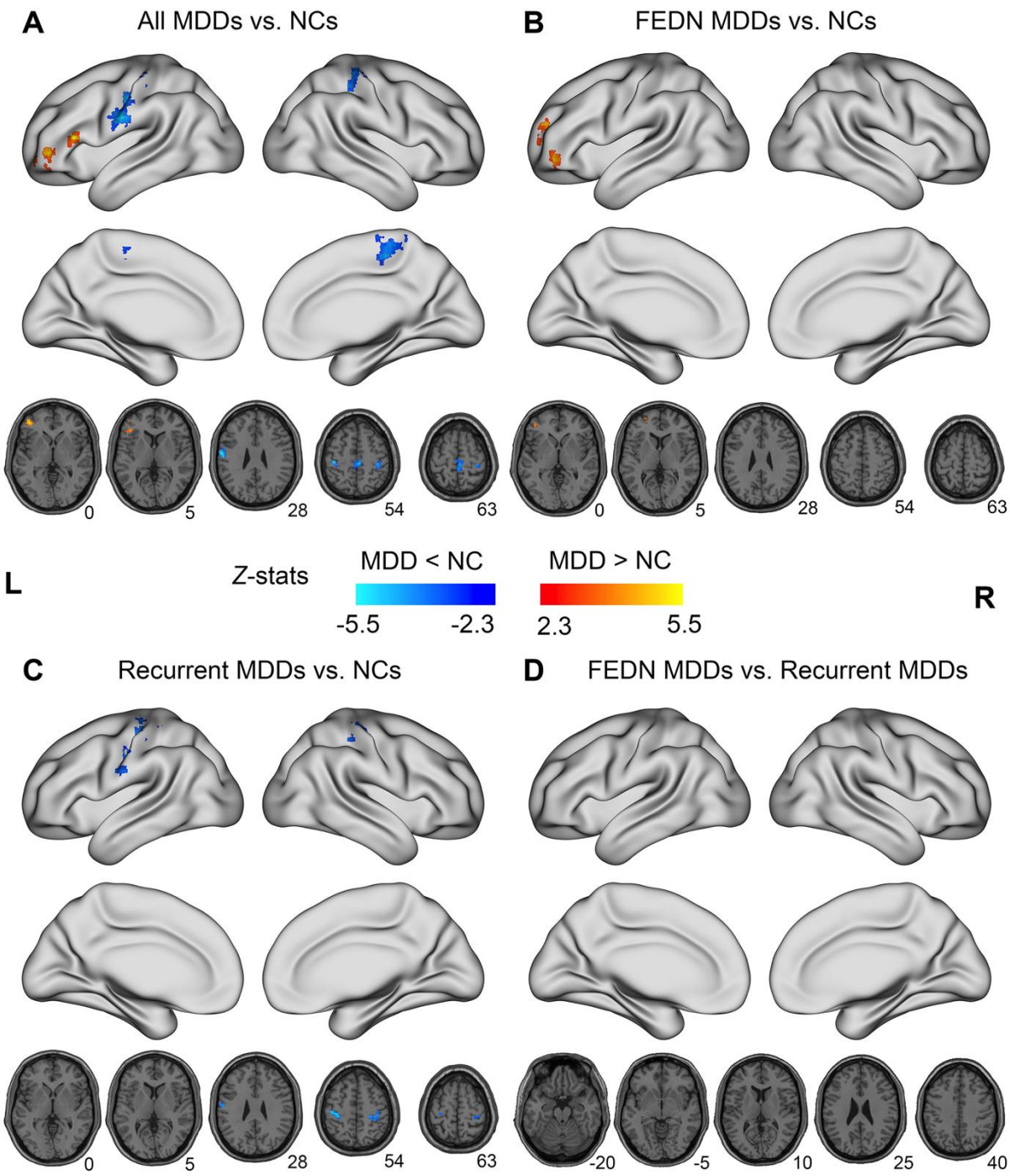

Figure 4. Regional homogeneity (ReHo) abnormalities in MDD patients. Significant group differences between (A) all individuals with MDD and NCs, (B) first episode drug naïve (FEDN) MDDs and NCs, (C) recurrent MDDs and NCs, (D) FEDN and recurrent MDDs were depicted. Gaussian random field (GRF) theory correction was employed to control family-wise error rates (voxel-level $p < 0{\cdot}0005$; cluster-level $p < 0{\cdot}025$ for each tail, two-tailed). L, Left hemisphere; R, right hemisphere.