Reduced but not Enhanced Default Mode Network Functional Connectivity in Major Depressive Disorder: Evidence from 25 Cohorts in the REST-meta-MDD Project

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ABSTRACT

Major Depressive Disorder (MDD) is one of the most common and disabling psychiatric disorders, but its neural pathophysiology remains largely unknown. Functional brain network studies in MDD have suffered from limited statistical power and high flexibility in data analyses. Here we launched the REST-meta-MDD Project of resting-state fMRI (R-fMRI) data of 1,300 patients with MDD and 1,128 normal controls (NCs) from 25 research groups in China. The data were preprocessed with a standardized protocol across all sites prior to aggregated group analyses. Our analyses focused on functional connectivity (FC) within the default mode network (DMN) which has been frequently reported to show increased FC in MDD. However, we found decreased instead of increased DMN FC in MDD compared to NCs. Specifically, this FC reduction only presented in recurrent MDD but not in first-episode drug naïve MDD. Patients with recurrent MDD even demonstrated decreases of DMN FC when directly compared with the first-episode drug naïve MDD. Decreased DMN FC was associated with medication usage in MDD but not illness duration or severity. Finally, exploratory analyses revealed alterations of local intrinsic activity in the MDD samples. The pooled R-fMRI metrics of the REST-meta-MDD Project provide an unprecedented opportunity to investigate the key neural underpinnings of MDD and call for longitudinal brain imaging studies to understand the effects of medications, illness duration and severity.

1. INTRODUCTION

Major Depressive Disorder (MDD) is the second leading-cause of disability world-wide, with 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.[2-4] An exception is the Enhancing Neuro Imaging Genetics through Meta-Analysis (ENIGMA) consortium which has meta-analyzed data of thousands of structural MRI scans from MDD patients and healthy controls.[5-7] The ENIGMA-MDD working group found a robust reduction in hippocampal volume[5] and cortical thinning in medial orbitofrontal cortex.[6] 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),[8] which is comprised of regions that are most active during rest and which has been linked to rumination.[9] Most MDD studies have found increased DMN FC in MDD, although decreased or unchanged DMN FC have also been reported. As shown in Supplementary Table S1, of 28 studies reporting DMN FC alterations in MDD, 19 found increases, five decreases, and four both increases and decreases. For example, the first study focusing on the DMN in MDD reported increased DMN FC using independent component analysis (ICA).[10] However, other ICA studies[11, 12] reported both increased and decreased DMN FC in

MDD. Using seed-based correlation, FC between the posterior cingulate cortex (PCC) and subgenual anterior cingulate cortex (sgACC) has been found to be increased[13, 14] and decreased[15] in MDD. MDD studies have also reported increased[16] and decreased[17] FC between PCC and inferior parietal lobe (IPL).

These inconsistencies may be due to small sample size and limited statistical power[2] but data analysis flexibility may also contribute.[18] MDD studies have used different multiple comparison correction methods, most likely inadequate.[19] Data analysis flexibility also impedes large-scale meta-analysis. For example, a meta-analysis on DMN FC in MDD could only include seven studies because seeds varied so much across studies.[8] 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, excessive flexibility in data analysis and the challenge of aggregating results across studies, we initiated the REST-meta-MDD Project. Building on the R-fMRI data processing pipeline DPARSF,[20] we implemented a standardized preprocessing protocol at local sites with only final indices provided to the consortium. We have obtained R-fMRI indices (including FC matrices) of 1300 patients with MDD and 1128 normal controls (NCs) from 25 cohorts in China. To the best of our knowledge, the REST-meta-MDD is the largest R-fMRI database of MDD in the world. 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)[20] to process their MDD R-fMRI data. In the initial phase, 25 research groups from 17 hospitals in China have agreed to share the final R-fMRI indices from patients with MDD and matched normal controls (please see Supplementary Table S2 for data composition; henceforth "site" refers to each cohort for convenience). All contributed data were from studies approved by local Institutional Review Boards. Data submitted to the consortium were fully deidentified and anonymized.

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. Measures of first-episode or medication status, illness duration, treatment responsiveness, family history, Hamilton Depression Scale (HAMD) and Hamilton Anxiety Rating Scale (HAMA) were also requested if they had been collected.

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. DMN FC Analyses

After preprocessing (described in Supplementary Methods), time-series for the Dosenbach 160 functional regions-of-interest (ROIs)[21] were extracted. To validate the primary analyses, time series for Zalesky's random 980 parcellations[22] and the automated anatomical labeling (AAL) atlas[23] were also extracted. For each atlas, we defined the DMN ROIs as those which overlapped with the DMN delineated by Yeo et al.[24] The average FC (Fisher's r-to-z transformed Pearson's correlation coefficient between time-series of all pairs of ROIs) within the DMN ROIs was taken to represent DMN within-network FC and contrasted between MDD patients and controls. In supplementary analyses, we also compared DMN ROIs connection by connection to identify which pair of DMN ROIs contributed the most.

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

Beyond hypothesis-driven analysis of DMN FC, we also explored local abnormalities in MDD with voxel-wise amplitude of low frequency fluctuations (ALFF),[25] fractional ALFF (fALFF),[26] regional homogeneity (ReHo),[27] degree centrality (DC),[28, 29] and voxel-mirrored homotopic connectivity (VMHC).[30, 31] These metrics are 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 group 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 S1.

2.4.2. Statistical Analyses

We used the Linear Mixed Model (LMM) to compare MDDs with NCs while allowing the effect to vary across sites. LMM extents linear regression models to data that are collected and summarized in groups.[32] LMM describes the relationship between a response variable (here DMN FC or other brain indices) 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.

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 MDDs vs. 394 NCs from 5 sites, each site with more than 10 subjects per group) with the same LMM model. We also compared recurrent MDD patients with corresponding NCs (189 MDDs 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 same 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 S2 and S3). On average, each site contributed 52.4 ± 52.4 MDDs (range 13-282) and 45.1 ± 46.9 NCs (range 6-251). The majority of MDD patients were females (826 females vs. 474 males), as is typical in MDD studies. There were 562 first episode MDDs, including 318 FEDN MDD and 160 were currently on antidepressant treatment (medication status was unavailable for 84 first episode MDDs). Of 282 with recurrent MDDs, 121 were being treated with antidepressants and 76 were not (medication status was unavailable for 85 recurrent MDDs). Episodicity (first or recurrent) and medication status were unavailable for 456 MDDs.

3.2. Decreased DMN Functional Connectivity in Patients with MDD

By overlapping the Dosenbach 160 functional ROIs[21] with the Yeo DMN,[24] 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, Figure 2A). On subgroup analyses, FEDN MDDs did not differ significantly from NCs (T = -0.914, P = 0.361, Figure 2B), while DMN FC was significantly decreased in patients with recurrent MDD vs. NCs (T = -3.737, P = 0.0002, Figure 2C). We directly compared FEDN MDDs with recurrent MDDs (T = 2.676, P = 0.008, Figure 2D); the significantly lower DMN FC in recurrent MDD patients suggests they were the major contributor 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 (restricted to those with information on illness duration) 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 2 sites; unbalanced sample because sites with <10 subjects/group were excluded) in DMN FC (T = 1.140, P = 0.257, Figure 3A left panel). Similarly, 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 4 sites): T = 1.541, P = 0.124.

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

To test if decreased DMN FC in recurrent MDDs was associated with medication treatment, 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, Figure 3B). However, we cannot determine if this effect is due to short-term or long-term usage of medication, as only binary information on medication status was provided, not detailed medication history.

3.5. Association of DMN Functional Connectivity with Symptom Severity

The association between DMN FC and HAMD scores was tested on 689 MDD patients from 14 sites and was not significant (T = 1.346, P = 0.179).

3.6. Verification of Reproducibility

We tested several alternative analysis strategies to verify the reproducibility of our results (Table 1). 1) Beyond functional atlas (Dosenbach 160 functional ROIs), we also tested a structural atlas, i.e., AAL parcellation, analyzing DMN FC within 18 AAL ROIs. Conclusions remained unchanged. 2) We also tested finer-grade parcellations, e.g., Zalesky's random 980 parcellation (with 211 DMN ROIs). This confirmed our results again. 3) Beyond using LMM to summarize the diagnosis effect across sites, we also used meta-analysis to re-analyze our data. We calculated T values within each site, converted them into Hedge's *g*, and used a

random effect meta-model to test the difference (using R package "metansue", https://www.metansue.com/). Results were almost the same, although the difference between FEDN MDDs and recurrent MDDs only showed a trend to significance (Z = 1.732, P = 0.083). 4) We also tested whether using global signal regression (GSR) mattered, and we found similar results except the difference between FEDN MDDs and recurrent MDDs became non-significant (T = 0.974, P = 0.331) and the medication effect became a trend (T = -1.891, P = 0.060). 5) For head motion control, although we already used the Friston-24 model at the individual level and a motion covariate at the group level for the primary analyses, we also used scrubbing (removing time points with framewise displacement (FD)[33] > 0.2mm) to verify results. All results remained the same under 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 analysis searching for local abnormalities to demonstrate the potential of this project's shared voxel-wise R-fMRI metric maps. Since ReHo demonstrated the highest test-retest reliability among the commonly used R-fMRI indices,[34] we present ReHo abnormalities in MDD and include the results of 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. To avoid unacceptable family-wise error rates, 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 setup achieves a two-tailed voxel $p < 0.001$ and

cluster $p < 0.05$, and maintains the family-wise error rate under 5%.[4, 19] When we compared all 848 MDDs with 794 NCs, left dorsolateral prefrontal cortex (DLPFC) showed increased ReHo in MDD, while bilateral primary motor cortex showed decreased ReHo (Figure 4A). Interestingly, left DLPFC only showed significantly increased ReHo in FEDN MDDs (Figure 4B) but not in recurrent MDD (Figure 4C). By contrast, bilateral primary motor cortex only showed significantly decreased ReHo 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 directly compared.

4. DISCUSSION

Using the unprecedentedly large sample provided by the REST-meta-MDD Project we found decreased instead of increased FC within the DMN in MDDs compared with NCs. However, this effect was not significant in FEDN MDDs, but only in recurrent MDDs. Recurrent MDDs even demonstrated decreased DMN FC when directly compared to FEDN MDDs. Furthermore, we revealed that the decreased DMN FC in recurrent MDDs was associated with medication usage and not with illness duration nor severity. Exploratory analysis of local abnormalities demonstrated increased ReHo in left DLPFC in FEDN MDD, and decreased ReHo in bilateral primary motor cortex in recurrent MDD.

Our primary results contradict a substantial number of previous studies which reported increased DMN FC in MDD (see 19 of 28 studies in 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. A possible reason of the inconsistent findings of previous studies is the heterogeneity of analysis strategies. In the current study, we applied a standardized analysis protocol to all sites, thus removing variations in analysis method. 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 the discrepancy in DMN FC findings, as east Asians have a lower lifetime prevalence of MDD,[2, 4] have more somatic symptoms and fewer psychological symptoms in MDDs,[1] and even have different risk genes for MDD.[35] 3) The measure of overall DMN FC (averaged across all pairs of within-DMN connections) might be over-simplified and insensitive to possibly pair-wise increase of DMN FC in MDDs. However, we tested pair-wise within-DMN connections in supplementary analyses (see Supplementary Results and Supplementary Figure S2) but did not find even a single pair of significantly increased within-DMN connection in MDDs. Large sample studies across ethnicities will be needed to clarify the pattern of DMN FC in MDD.

We found decreased DMN FC only in recurrent MDD patients. In a large-scale MDD structural abnormality study, the ENIGMA MDD working group[36] found a robust reduction in hippocampal volume only in recurrent MDD patients and not in first episode MDD patients. The hippocampus is a key DMN structure, thus this large-sample anatomic finding partly supports our functional findings of reduced DMN FC in recurrent MDDs. We initially considered that our findings in recurrent MDD might reflect illness duration. However, direct comparisons did not reveal any effects of illness duration, whether in all MDDs or only in

FEDN MDD. In an early study of MDD and DMN FC, Greicius et al.[5] reported that DMN FC (especially at sgACC) was positively correlated with the length of current depressive episode. However, later studies that directly tested the relationship between MDD illness duration and DMN FC found no significant correlations. For example, Guo et al.[10] found no significant correlation between illness duration and DMN FC (indexed by network homogeneity). Studies have also found no significant correlation between illness duration and DMN homotopic connectivity,[12] DMN FC variability[37] as well as DMN deactivation.[38] Taking these results together, we conclude that illness duration appears to have minimal effect on DMN FC. However, our comparison of illness duration was cross-sectional; future longitudinal studies are needed to confirm how DMN FC changes over the life course.

Another factor potentially contributing to decreased DMN FC in recurrent MDD patients is antidepressant medication usage in a large proportion of patients. We directly tested this possibility by comparing first episode MDDs on medication to FEDN MDDs and found medication usage indeed was associated with decreased DMN FC in MDD patients. This result is supported by previous studies exploring the effect of antidepressants on DMN FC in MDD,[39] dysthymia,[40] and in healthy individuals.[41] Li et al.[42] found 12 weeks of antidepressant treatment (with either paroxetine, venlafaxine, duloxetine or citalopram) reduced posterior DMN FC in MDD patients. Posner et al.[40] found 10 weeks of duloxetine treatment reduced DMN FC in patients with dysthymic disorder. In healthy individuals, van Wingen et al.[41] found 2 weeks of duloxetine administration significantly reduced DMN FC and improved mood. Focusing on mechanisms of how antidepressants might change DMN activity, previous

studies have reported that serotonin receptor binding in DMN key regions (PCC and medial prefrontal cortex) can modulate the introspective functioning of the DMN;[42] tryptophan depletion[41] and selective serotonin reuptake inhibitor administration[43] can also influence DMN activity. The current finding of medication-associated reduction in DMN FC further supports those prior results and suggests a possible mechanism of antidepressant medications is to alleviate depressive symptoms in MDD by reducing DMN FC. Again, this effect was observed in a cross-sectional sample, so it has to be confirmed by longitudinal medication follow-up designs.

To expand our investigation beyond DMN and partly overcome the dependence of previous analysis on a priori information (ROI specification), we further conducted exploratory analyses with local R-fMRI metrics and found some substantial alterations in MDD patients. For example, we found significantly increased ReHo in DLPFC and decreased ReHo in the primary motor area in MDDs compared with NCs. Further comparisons showed that the increase in DLPFC ReHo was attributable to abnormalities in FEDN MDDs while the decrease in primary motor area ReHo was attributable to recurrent MDD (see Figure 4). Our results are in line with several previous studies.[45-47] Specifically, a previous meta-analysis[44] of ReHo studies reported decreased ReHo in motor areas in MDDs, which is consistent with our current finding. However, that meta-analysis reported increased ReHo in medial prefrontal cortex, instead of DLPFC as we found. Recently, Jia et al.[45] directly compared the classical coordinate-based meta-analysis and direct aggregated analysis with multi-site datasets and found that results from classical coordinate-based meta-analysis are less reliable than direct

analysis on aggregated large samples. Thus, the discrepancy between our results and previous meta-analysis call for further studies on local R-fMRI abnormalities in MDD. Besides, results from other metrics also highlight MDDs' local impairments (see Supplementary Results, Supplementary Figures S2~S5), which may shed light on future studies to identify local targets for neuromodulation of MDD.

The current study has several limitations. First, the MDD and control samples were all Chinese, thus we do not know if our findings will generalize to other populations. In the next step, we will expand the REST-meta-MDD Project to include cross-ethnicity samples by inviting international MDD researchers to join. With cross-ethnicity/culture samples, we can identify the ethnicity/culture-general and ethnicity/culture-specific abnormal brain patterns in MDD. Second, our retrospective cross-sectional sample cannot directly answer questions regarding longitudinal effects (e.g., illness duration or medication usage). We plan to encourage the REST-meta-MDD consortium to perform coordinated prospective longitudinal studies to further investigate brain development and remission across the MDD course. Finally, reproducibility is still a challenge for brain imaging,[46] thus the current findings require independent replication. To improve the transparency and reproducibility of the current study, we have openly shared the analysis code at https://github.com/Chaogan-Yan/PaperScripts/tree/master/Yan_2018. Furthermore, 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) upon acceptance of this manuscript. This large amount of data will allow replication, secondary analyses and discovery efforts.

In summary, based on the largest R-fMRI database of MDD, we identified the key role of DMN in MDD, showing a reduction of DMN FC in recurrent MDD patients. This reduction appears to reflect medication usage rather than illness duration. The current findings suggest that the DMN should remain a prime target for further MDD research, especially for improving treatment (e.g., medication or other non-medication treatment).

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).

CONFLICT OF INTEREST

The authors declare no competing financial interests.

Supplementary information is available at MP's website.

REFERENCES

1. Ferrari AJ, Charlson FJ, Norman RE, Patten SB, Freedman G, Murray CJL *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, Nosek BA, Flint J, Robinson ESJ *et al.* Power failure: why small sample size undermines the reliability of neuroscience. *Nat Rev Neurosci* 2013; **14**(5)**:** 365-376.

3. Ioannidis JP. Why most published research findings are false. *PLoS medicine* 2005; **2**(8)**:** e124.

4. 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)**:** 300-318.

5. Schmaal L, Veltman DJ, van Erp TG, Samann PG, Frodl T, Jahanshad N *et al.* Subcortical brain alterations in major depressive disorder: findings from the ENIGMA Major Depressive Disorder working group. *Mol Psychiatry* 2016; **21**(6)**:** 806-812.

6. Schmaal L, Hibar DP, Samann PG, Hall GB, Baune BT, Jahanshad N *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)**:** 900-909.

7. Thompson PM, Stein JL, Medland SE, Hibar DP, Vasquez AA, Renteria ME *et al.* The ENIGMA Consortium: large-scale collaborative analyses of neuroimaging and genetic data. *Brain imaging and behavior* 2014; **8**(2)**:** 153-182.

8. 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)**:** 224-230.

9. Andrews-Hanna JR, Smallwood J, Spreng RN. The default network and self-generated thought: component processes, dynamic control, and clinical relevance. *Ann N Y Acad Sci* 2014; **1316**(1)**:** 29-52.

10. Greicius MD, Flores BH, Menon V, Glover GH, Solvason HB, Kenna H *et al.* Resting-state functional connectivity in major depression: abnormally increased contributions from subgenual cingulate cortex and thalamus. *Biological psychiatry* 2007; **62**(5)**:** 429-437.

11. Zhu X, Wang X, Xiao J, Liao J, Zhong M, Wang W *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)**:** 611-617.

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

13. Zhou Y, Yu C, Zheng H, Liu Y, Song M, Qin W *et al.* Increased neural resources recruitment in the intrinsic organization in major depression. *Journal of affective disorders* 2010; **121**(3)**:** 220-230.

14. Berman MG, Peltier S, Nee DE, Kross E, Deldin PJ, Jonides J. Depression, rumination and the default network. *Soc Cogn Affect Neurosci* 2011; **6**(5)**:** 548-555.

15. Wu M, Andreescu C, Butters MA, Tamburo R, Reynolds CF, 3rd, Aizenstein H. Default-mode network connectivity and white matter burden in late-life depression. *Psychiatry Res* 2011; **194**(1)**:** 39-46.

16. Kim SM, Park SY, Kim YI, Son YD, Chung US, Min KJ *et al.* Affective network and default mode network in depressive adolescents with disruptive behaviors. *Neuropsychiatr Dis Treat* 2016; **12:** 49-56.

17. Chen Y, Wang C, Zhu X, Tan Y, Zhong Y. Aberrant connectivity within the default mode network in first-episode, treatment-naive major depressive disorder. *Journal of affective disorders* 2015; **183:** 49-56.

18. Poldrack RA, Baker CI, Durnez J, Gorgolewski KJ, Matthews PM, Munafo MR *et al.* Scanning the horizon: towards transparent and reproducible neuroimaging research. *Nat Rev Neurosci* 2017; **18**(2)**:** 115-126.

19. 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.

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

21. Dosenbach NU, Nardos B, Cohen AL, Fair DA, Power JD, Church JA *et al.* Prediction of individual brain maturity using fMRI. *Science (New York, NY* 2010; **329**(5997)**:** 1358-1361.

22. Zalesky A, Fornito A, Harding IH, Cocchi L, Yucel M, Pantelis C *et al.* Whole-brain anatomical networks: does the choice of nodes matter? *Neuroimage* 2010; **50**(3)**:** 970-983.

23. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N *et al.* Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* 2002; **15**(1)**:** 273-289.

24. Yeo BT, Krienen FM, Sepulcre J, Sabuncu MR, Lashkari D, Hollinshead M *et al.* The organization of the human cerebral cortex estimated by intrinsic functional connectivity. *Journal of neurophysiology* 2011; **106**(3)**:** 1125-1165.

25. Zang YF, He Y, Zhu CZ, Cao QJ, Sui MQ, Liang M *et al.* Altered baseline brain activity in children with ADHD revealed by resting-state functional MRI. *Brain Dev* 2007; **29**(2)**:** 83-91.

26. Zou Q-H, Zhu C-Z, Yang Y, Zuo X-N, Long X-Y, Cao Q-J *et al.* An improved approach to detection of amplitude of low-frequency fluctuation (ALFF) for resting-state fMRI: Fractional

ALFF. *J Neurosci Methods* 2008; **172**(1)**:** 137-141.

27. Zang Y, Jiang T, Lu Y, He Y, Tian L. Regional homogeneity approach to fMRI data analysis. *NeuroImage* 2004; **22**(1)**:** 394-400.

28. Buckner RL, Sepulcre J, Talukdar T, Krienen FM, Liu H, Hedden T *et al.* Cortical hubs revealed by intrinsic functional connectivity: mapping, assessment of stability, and relation to Alzheimer's disease. *J Neurosci* 2009; **29**(6)**:** 1860-1873.

29. Zuo XN, Ehmke R, Mennes M, Imperati D, Castellanos FX, Sporns O *et al.* Network Centrality in the Human Functional Connectome. *Cereb Cortex* 2012; **22**(8)**:** 1862-1875.

30. Anderson JS, Druzgal TJ, Froehlich A, DuBray MB, Lange N, Alexander AL *et al.* Decreased interhemispheric functional connectivity in autism. *Cerebral cortex* 2011; **21**(5)**:** 1134-1146.

31. Zuo XN, Kelly C, Di Martino A, Mennes M, Margulies DS, Bangaru S *et al.* Growing together and growing apart: regional and sex differences in the lifespan developmental trajectories of functional homotopy. *J Neurosci* 2010; **30**(45)**:** 15034-15043.

32. West BT, Welch KB, Galecki AT. *Linear mixed models: a practical guide using statistical software*. CRC Press2014.

33. 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)**:** 825-841.

34. 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:** 100-118.

35. Ryder AG, Yang J, Zhu X, Yao S, Yi J, Heine SJ *et al.* The cultural shaping of depression: somatic symptoms in China, psychological symptoms in North America? *Journal of abnormal psychology* 2008; **117**(2)**:** 300-313.

36. Long H, Liu B, Hou B, Wang C, Li J, Qin W *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.

37. Guo W, Liu F, Dai Y, Jiang M, Zhang J, Yu L *et al.* Decreased interhemispheric resting-state functional connectivity in first-episode, drug-naive major depressive disorder. *Prog Neuropsychopharmacol Biol Psychiatry* 2013; **41:** 24-29.

38. Wise T, Marwood L, Perkins AM, Herane-Vives A, Joules R, Lythgoe DJ *et al.* Instability of default mode network connectivity in major depression: a two-sample confirmation study.

Translational psychiatry 2017; **7**(4)**:** e1105.

39. Rodriguez-Cano E, Alonso-Lana S, Sarro S, Fernandez-Corcuera P, Goikolea JM, Vieta E *et al.* Differential failure to deactivate the default mode network in unipolar and bipolar depression. *Bipolar disorders* 2017; **19**(5)**:** 386-395.

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

41. Posner J, Hellerstein DJ, Gat I, Mechling A, Klahr K, Wang Z *et al.* Antidepressants normalize the default mode network in patients with dysthymia. *JAMA Psychiatry* 2013; **70**(4)**:** 373-382.

42. van Wingen GA, Tendolkar I, Urner M, van Marle HJ, Denys D, Verkes RJ *et al.* Short-term antidepressant administration reduces default mode and task-positive network connectivity in healthy individuals during rest. *Neuroimage* 2014; **88:** 47-53.

43. Kunisato Y, Okamoto Y, Okada G, Aoyama S, Demoto Y, Munakata A *et al.* Modulation of default-mode network activity by acute tryptophan depletion is associated with mood change: a resting state functional magnetic resonance imaging study. *Neurosci Res* 2011; **69**(2)**:** 129-134.

44. Matthews SC, Simmons AN, Strigo IA, Arce E, Stein MB, Paulus MP. Escitalopram attenuates posterior cingulate activity during self-evaluation in healthy volunteers. *Psychiatry Res* 2010; **182**(2)**:** 81-87.

45. 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.

46. Jia X-Z, Zhao N, Barton B, Burciu R, Carriere N, Cerasa A *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)		AAL atlas (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*	*T*	*P*	*Z*	*P*	*T*	*P*	*T*	*P*
All MDDs vs. NCs (848 vs. 794)	-3.762	0.0002	-2.869	0.004	-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	-0.623	0.489	-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	-3.669	0.0003	-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	3.084	0.002	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	0.750	0.455	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	0.544	0.587	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	-2.695	0.008	-2.293	0.023	-2.568	0.010	-1.891	0.060	-2.504	0.013
Correlation with HAMD (*N* = 689)	1.346	0.179	0.978	0.329	0.898	0.370	0.759	0.448	0.091	0.928	1.530	0.126

Abbreviations: FEDN, First Episode Drug Naïve; LME, Linear Mixed Effect; AAL, Automated Anatomical Labeling; GSR, 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, 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 for age 18 and 65, within which were the participants chosen for imaging analysis. (D) The score of Hamilton Depression Rating Scale (HAMD) for the MDD patients. Of note, some sites didn't provide HAMD information.

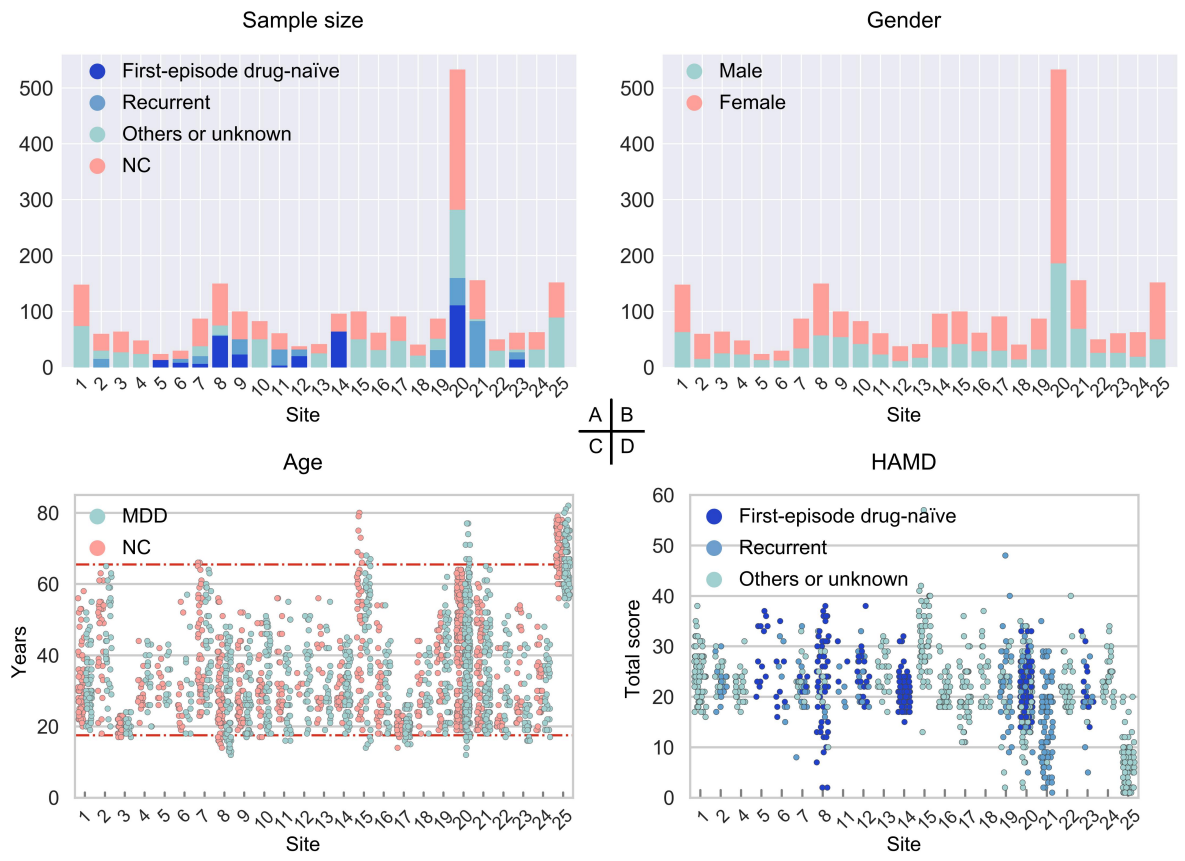

Figure 2. Decreased DMN functional connectivity in MDD patients. The violin figures show the distribution of mean DMN within-network FC for each contributing site, between MDD group and NC group (A), between first episode drug naïve (FEDN) MDD group and NC group (B), between recurrent MDD group and NC group (C), and between FEDN MDD group and recurrent MDD group (D). 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. **, $p < 0.01$; ***, $p < 0.001$.

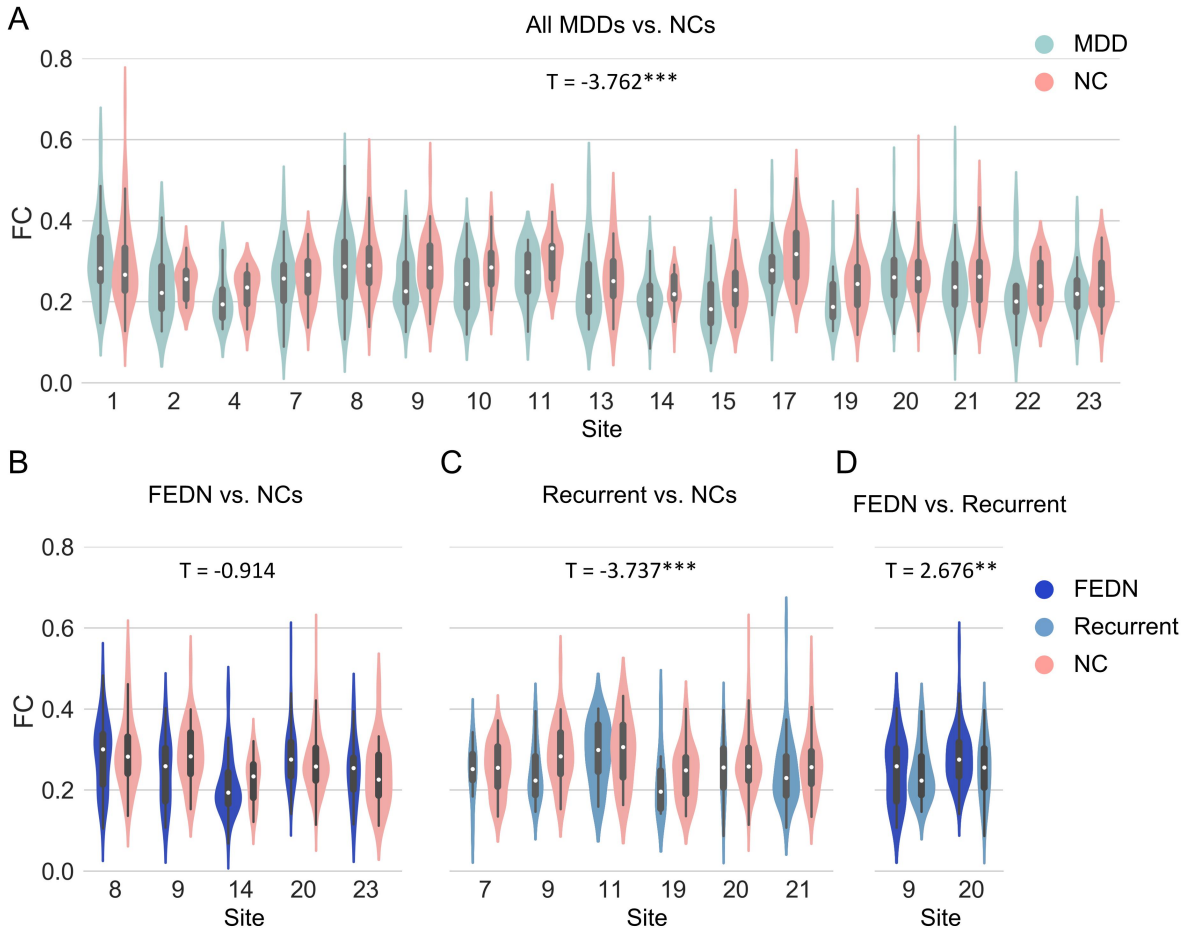

Figure 3. The effects of illness duration and medication on decreased DMN functional connectivity in MDD patients. The violin figures show the distribution of mean DMN within-network FC for each contributing site, for first episode drug naïve (FEDN) MDDs with long vs. short illness duration (A left), for pooled MDDs with long vs. short illness duration (A right), and for first episode MDDs with vs. without medication usage (B). The T values are the statistics for these comparisons in Linear Mixed Model analyses. **, p < 0.01.

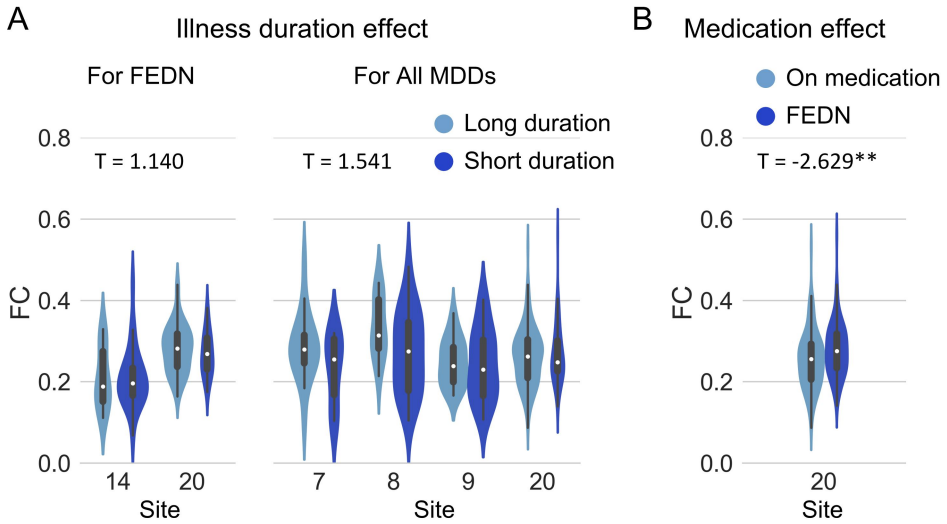
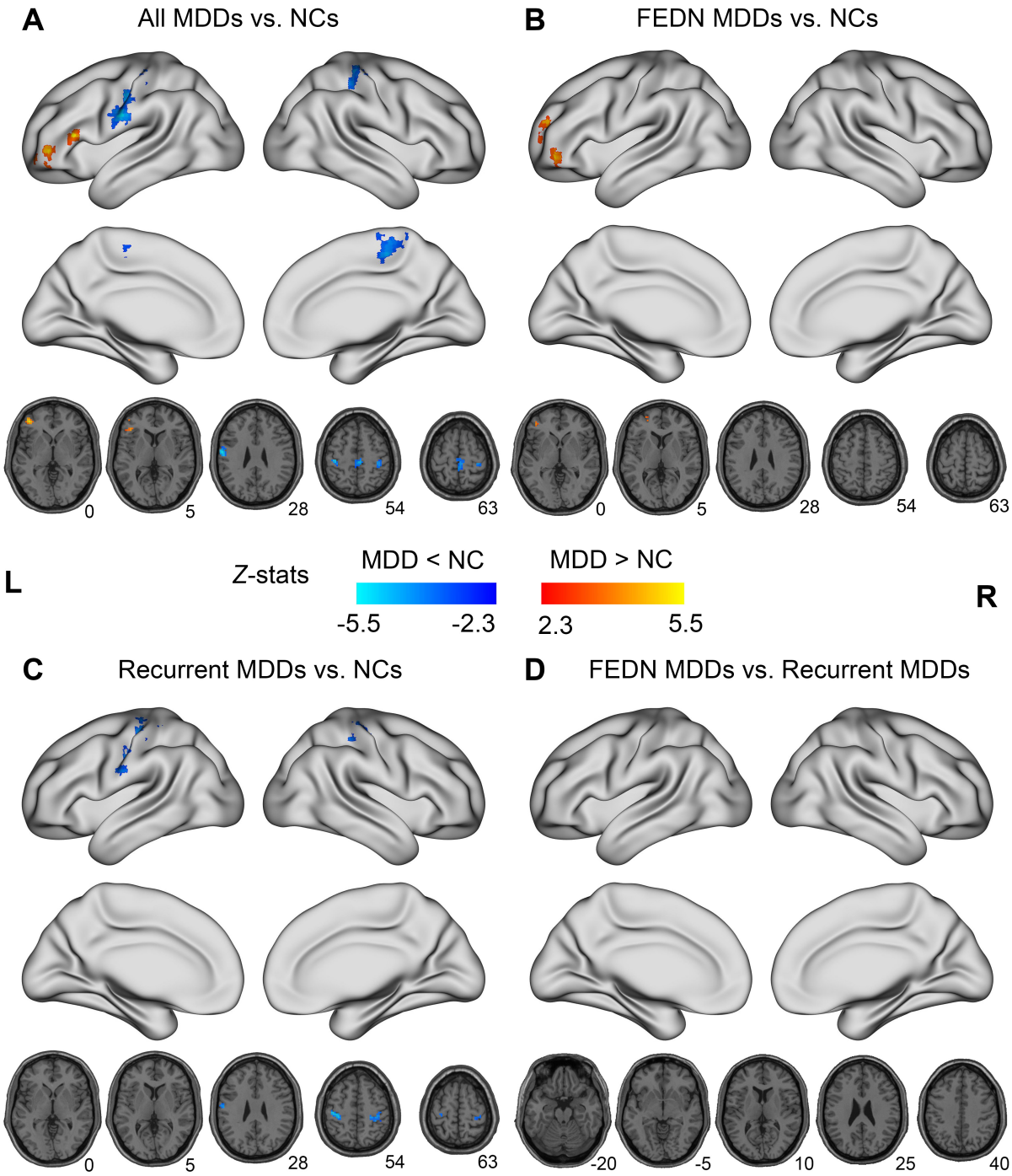
Figure 4. Regional homogeneity (ReHo) abnormalities in MDD patients. Significant group differences between (A) all individuals with MDD and NCs, (B) first episode drug naïve (FEDN) MDDs and NCs, (C) recurrent MDDs and NCs, (D) FEDN and recurrent MDDs were depicted. Gaussian random field (GRF) theory correction was employed to control family-wise error rates (voxel-level P < 0.0005; cluster-level P < 0.025 for each tail, two-tailed). L, Left hemisphere; R, right hemisphere.