Characterization of brain blood flow and the amplitude of low-frequency fluctuations in major depressive disorder: A multimodal meta-analysis

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Abstract

Background. No published meta-analysis has investigated changes in regional amplitude of low-frequency fluctuations (ALFF) in medication-free depressed patients. In healthy subjects, an association between ALFF and regional cerebral blood flow (rCBF) has been demonstrated in some brain regions. We sought to explore to what extent resting-state rCBF and ALFF changes co-occur in the depressed brain without the potential confound of medication.

Methods. Using the anisotropic effect-size version of signed differential mapping (AES-SDM), we conducted two meta-analyses, of rCBF and of ALFF studies of medication-free patients with major depressive disorder (MDD). We also conducted a multimodal meta-analysis to identify the brain regions showing abnormalities in both rCBF and ALFF. The potential effect of several relevant sociodemographic and clinical variables was examined by linear regression.

Results. After systematic literature searches, 14 rCBF studies and 8 ALFF studies met the inclusion criteria. We identified conjoint abnormalities in resting-state rCBF and ALFF in the cerebellum in medication-free MDD compared to healthy controls. Other changes include altered resting-state rCBF in the limbic-cortical pathways and precuneus as well as altered ALFF in the frontal–limbic circuits and task-positive networks/task-negative networks. Meta-regression found mean patient age to be negatively associated with resting-state rCBF in the left inferior frontal gyrus.

Conclusions. The conjoint alterations of ALFF and rCBF in the cerebellum may represent core neuropathological changes in medication-free MDD. Future studies could use this region as a region of interest for more in-depth analysis.

1. Introduction

MDD is a disorder characterized by the recurrence of discrete depressive episodes usually featuring symptoms such as low mood, anhedonia, poor motivation, impaired psychomotor activity, and reduced sleep, appetite, energy and libido (Fitzgerald *et al.*, 2008). Lifetime prevalence is high: in the United States 17% of people reportedly meet criteria for MDD at least once in their lifetime (Kessler RC *et al.*, 2005). Despite the development of new treatments for MDD, symptoms have considerable impact at the individual and societal level. However, understanding of the pathophysiology and aetiology of this important problem still remains relatively incomplete and unintegrated.

To elucidate brain processes involved in MDD, many functional imaging studies have been conducted during the past two decades. Recently resting state study, where subjects are asked to rest quietly with eyes closed during data acquisition, has become a popular tool to quantify intrinsic brain activity. Independent of particular tasks, resting-state studies provides a task-free approach that removes some performance-related confounders, and provides a reliable measure of 'baseline' brain activity and connectivity (Gusnard DA *et al.*, 2001 Oct). These resting studies have commonly used positron emission tomography (PET), single photon emission computed tomography (SPECT), and the magnetic resonance imaging-based techniques of arterial spin labeling (ASL) and amplitude of low-frequency fluctuations (ALFF) or fractional amplitude of low-frequency fluctuations (fALFF). PET, SPECT and ASL all measure regional cerebral blood flow (rCBF), which reflects brain energy demand-supply dynamics (Wintermark M *et al.*, 2005). ALFF can be used to detect regional neural synchronous activity using the power spectrum of low-frequency (0.01Hz–0.08Hz) fluctuations in the blood oxygen

level-dependent (BOLD) signal (Zang YF *et al.*, 2006). Since BOLD signals are modulated by rCBF (Kannurpatti SS *et al.*, 2008), it is assumed that ALFF and rCBF abnormalities are linked although there is no simple causal relation between the two measurements.

Resting state brain studies in depressed patients generally report abnormalities involving networks of cortical and subcortical limbic regions. However, inter-study differences in sample size, sociodemographic and clinical characteristics of the patients and in the technique of image acquisition and analysis have led to heterogeneity in the reported results. As the number of published resting-state rCBF and ALFF studies in MDD has grown, attention has turned to meta-analysis as a way to identify common abnormalities. Two previous meta-analyses of PET and SPECT studies found increased rCBF in medial frontal gyrus and thalamus, and decreased rCBF in pregenual anterior and posterior cingulate, pulvinar nucleus and superior temporal gyrus in MDD compared to controls (Fitzgerald PB *et al.*, 2008, Hamilton JP *et al.*, 2012). However, these studies included both medication-free patients and patients receiving antidepressant drugs at the time of scanning. There is accumulating evidence that antidepressant drugs can affect rCBF, although there are no prospective randomized controlled studies of the long-term effects of antidepressants. A serial PET study of MDD patients found that fluoxetine treatment altered the brain glucose metabolism in subcortical, limbic-paralimbic regions and neocortex by 1 week and in the anterior cingulate, dorsolateral prefrontal, and ventral frontal cortices and subgenual cingulate cortex by 6 weeks (Mayberg HS *et al.*, 2000). Moreover, medication-related differences in regional brain volumes have been reported in patients with mood disorders (Brambilla P *et al.*, 2002, Savitz J *et al.*, 2010, Smith R *et*

al., 2013). As a result, mixing medicated and medication-free patients may lead to biased results. To our knowledge, there has been no published meta-analysis of ALFF studies in medication-free MDD. With a large number of resting-state rCBF and ALFF studies of medication-free MDD patients being reported, it is timely to conduct a specific meta-analysis to isolate the intrinsic abnormalities in MDD, independent of confounding effects of antidepressant treatment.

It is also timely to consider the relationship between the two kinds of measurement. Several studies have shown abnormal ALFF of the cerebellum in depressed patients (Liu *et al.*, 2013, Wang *et al.*, 2012, Wang *et al.*, 2014, Yan R. *et al.*, 2014), and several rCBF studies have reported abnormal cerebellar blood flow in MDD patients (Germain *et al.*, 2007, Kimbrell *et al.*, 2002, Monkul *et al.*, 2012). A study of the relationship between BOLD fMRI-derived resting brain activity and CBF showed that ALFF were reliably correlated with rCBF in most of brain cortex in healthy people (Li *et al.*, 2012). We therefore hypothesized that there would be correlations between these two kinds of measurements in MDD.

Therefore, we conducted a novel voxel-based meta-analytical method to *multimodally* examine the relationship between rCBF and ALFF brain abnormalities in MDD without confounding effects of antidepressant treatment. The medication-free MDD patients included had either never taken antidepressants, or underwent a medication washout period before scanning. We also evaluated the impacts of clinical characteristics of patients on rCBF or ALFF by meta-regression analysis. We used the Anisotropic effect-size version of signed differential mapping (AES-SDM) (www.sdmproject.com) (Radua J *et al.*, 2013, Radua J *et al.*, 2014 Feb 10, Radua and Mataix-Cols, 2009, Radua

et al., 2011, Radua J. and Mataix-Cols D., 2012), which has been previously applied in neuropsychiatric populations (Bora *et al.*, 2011, Cooper *et al.*, 2014). In addition to standard meta-analyses, we summarize rCBF and ALFF findings in a single meta-analytic map, by assessing which brain regions showed both rCBF and ALFF abnormalities in medication-free MDD.

2. Method

2.1. Search strategies

Meta-analysis was conducted according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses guidelines (PRISMA) (Moher D *et al.*, 2009). Systematic search strategy was used to identify relevant studies published in PubMed, Embase and Knowledge of Science up to March 2015. Keyword searches used the following terms: (1)'depression' or 'unipolar depression' or 'depressive disorder' or 'major depression' or 'major depressive disorder' or 'depressed' (2) 'CBF' or 'cerebral flow' or 'PET' or 'positron emission tomography' or 'SPECT' or 'single photon emission computed tomography' or 'arterial spin labeling' or 'ASL' or 'neuroimaging' or 'ALFF' or 'amplitude of low-frequency fluctuations' or 'fractional ALFF' or 'FALFF' or 'LFF' or 'low frequency fluctuation' or 'low frequency oscillation' or 'LFO', and (3) 'rest' or 'resting state'. The reference lists of included studies were manually checked to identify further studies for inclusion.

2.2. Inclusion criteria

Studies were included if they met the following criteria: (1) published in an original paper in a peer-reviewed journal; (2) compared MDD patients with healthy control (HC) subjects; (3) employed whole brain imaging in both patients and controls; (4) the MDD patients either had never taken antidepressants or underwent a medication washout period before scanning (for details see Table 1) (5) reported either Talairach or Montreal Neurologic Institute (MNI) coordinates of altered brain regions. We excluded studies that only reported region of interest (ROI) or use seed-voxel-based analysis. We also excluded theoretical papers and reviews, as well as studies that reported adolescent or late-life depression and patients with psychiatric comorbid disorders including bipolar disorder, schizophrenia and substance abuse or dependence. For studies where multiple independent patient samples were compared with controls, the appropriate coordinates were included as separate data sets. For studies using overlapping samples, the study with the most subjects was included. To minimize the possibility of a biased sample set, we contacted the authors of studies in which the Talairach or MNI coordinates (necessary for the voxel-level quantitative meta-analysis) were not explicitly reported. Two authors (W.B.L. and Z.Q.C.) independently conducted the literature search. The results were compared, any inconsistent result was discussed, and a consensus decision was obtained.

2.3. Quality assessment

The quality of all the studies included was assessed using a 10-point checklist, focused

on both the clinical and demographic aspects of individual study samples and the imaging-specific methodology (see Supplementary Table S1), based on similar lists used in previous meta-analytic studies (Brambilla P *et al.*, 2003, Du M *et al.*, 2014, Shepherd AM *et al.*, 2012). At least two authors reviewed every paper and determined a completeness rating independently. These rating results were compared, and if different a consensus score was obtained by discussion. The 'Quality Score' for each study is presented in Table 1.

2.4. Meta-analyses of rCBF and ALFF abnormalities

Regional difference was separately analyzed using the anisotropic effect-size version of signed differential mapping (Anisotropic ES-SDM) (www.sdmproject.com) (Radua J *et al.*, 2014 Feb 10, Radua and Mataix-Cols, 2009, Radua *et al.*, 2011, Radua J. and Mataix-Cols D., 2012). AES-SDM is based on the previous versions of ES-SDM, which has been applied in neuropsychiatric populations (Bora *et al.*, 2011, Cooper *et al.*, 2014, Radua *et al.*, 2012). These used isotropic kernels (so the value of a voxel close to a peak depends only on the Euclidean distance between them). AES-SDM introduces a novel improvement: it adopts anisotropic kernels to assign different values to the different neighboring voxels based on the spatial correlation between them, so highly correlated voxels are estimated to have larger effect sizes whereas uncorrelated voxels are estimated to have smaller or null effect sizes (Radua J *et al.*, 2014 Feb 10). Isotropic kernels may underestimate the effect size in voxels strongly correlated with the peak (e.g. more likely to be from the same brain region), and overestimate the effect size in voxels weakly

correlated (e.g. less likely to be from the same brain region) (Radua J *et al.*, 2014 Feb 10). In this respect anisotropic kernels are more accurate.

The AES-SDM methods have been described in detail elsewhere (Radua and Mataix-Cols, 2009, Radua *et al.*, 2011, Radua J. and Mataix-Cols D., 2010, Radua J. *et al.*, 2011), and only the main points will be summarized here. First, the reported peak coordinates of all functional differences that were statistically significant at the whole-brain level were selected. We checked that the same statistical threshold was used throughout the brain in all included studies, to avoid potential bias to liberally-thresholded regions. Second, a map of the effect size of the differences between patients and controls for each study was recreated using the peak coordinates, converting the peak t value to Hedges' effect size and then applying an anisotropic Gaussian kernel which assigns higher effect sizes to the voxels more correlated with peaks (Radua J *et al.*, 2014 Feb 10). A relatively wide full-width at half-maximum (FWHM, 20mm) was used to control false positive results (Radua *et al.*, 2011). Both positive (i.e. increased rCBF or ALFF) and negative (i.e. decreased rCBF or ALFF) differences are reconstructed in the same map, which prevents a particular voxel appearing to be significant in opposite directions (Radua and Mataix-Cols, 2009). Findings from studies reporting no group differences were recreated with effect size and variance maps as in any other study, the only difference being that all voxels in the effect size group are estimated to have a null effect size, which was included in the meta-analysis like any other effect size. Third, as in standard meta-analyses, studies were combined with a random-effects model taking into account sample size, intrastudy variability and between-study heterogeneity. The null hypothesis consists in assuming that effect sizes (rather than only peaks) are randomly

distributed throughout the brain (Radua *et al.*, 2011). To balance optimally the sensitivity and specificity, uncorrected for false discovery rate (FDR) p=0.005 was used as the main threshold with additional peak height z=1 and cluster extent=10 voxels (Radua *et al.*, 2011). We used leave-one-out jackknife sensitivity analysis to test the replicability of findings of ALFF and rCBF studies; this consists in repeating the mean analysis as many times as the studies included, discarding one different study each one time, i.e. removing one study and repeating the analysis, then putting that study back and removing another study and repeating the analysis. If significant brain regions remain significant in all or most of the studies，it can be concluded that this finding is highly replicable.

2.5. Analysis of heterogeneity and publication bias

The statistical (between-studies) heterogeneity of individual clusters was examined using a random-effects model with Q statistics (chi-square distribution converted to z values and tested with a permutation approach (uncorrected for FDR $p<0.005$, peak height z=1, cluster extent=10 voxels). The possibility of publication bias for brain regions showing conjoint alteration of rCBF and ALFF was examined using the Egger test (Shepherd AM *et al.*, 2012 Nov).

2.6. Multimodal analysis of ALFF and rCBF

We summarized these two kinds of functional findings in a single meta-analytic map, showing brain regions with both ALFF and rCBF abnormalities. By computing the union

of the two functional p-values，the multimodal analysis could detect both ALFF and rCBF abnormalities, and signal the region as multi-modally-affected (Radua *et al.*, 2012). This method has already been used to meta-analyze structural and functional brain abnormalities in first episode psychosis (Radua *et al.*, 2012) and those at elevated genetic risk of developing schizophrenia (Cooper *et al.*, 2014).

2.7. Linear model analyses: meta-regression

The potential effect of several relevant sociodemographic and clinical variables is examined by means of linear regression (Radua *et al.*, 2011). To minimize the detection of spurious relationships we decreased the probability threshold to 0.0005, only admitted the findings detected both in the slope and in one of the extremes of the regressor, and discarded findings in regions not detected in the main analyses (Radua and Mataix-Cols, 2009). Finally, we inspected the regression plots to discard fits obviously driven by too few studies (Radua *et al.*, 2012).

3. Results

3.1. Numbers of studies found

A total of 7 ALFF studies (Guo *et al.*, 2014, Liu *et al.*, 2013, Wang *et al.*, 2012, Wang *et al.*, 2014, Yan R. *et al.*, 2014, Zhang *et al.*, 2014, Zhao B. *et al.*, 2014), 1 fALFF study (Lai and Wu, 2015) as well as 14 rCBF studies, including 1 ASL study (Lui S *et al.*,

2009), 5 SPECT studies (Kohn *et al.*, 2007, Krausz *et al.*, 2007, Perico *et al.*, 2005, Skaf C.R. *et al.*, 2002, Vardi *et al.*, 2011) and 8 PET studies (Aihara *et al.*, 2007, Brody *et al.*, 2001, Drevets *et al.*, 1992, Germain *et al.*, 2007, Kennedy SH *et al.*, 2001, Kimbrell *et al.*, 2002, Monkul *et al.*, 2012, Saxena S *et al.*, 2001) met our inclusion criteria (Table 1). For 2 of these studies (Lui S *et al.*, 2009, Wang *et al.*, 2014) using subgroup patients with significant different clinical characteristics compared with HC separately, each subgroup comparison was included in the meta-analysis as a dataset.

Since PET, SPECT and ASL all provide measurements of rCBF, a total of 14 rCBF (PET, SPECT and ASL) studies (15 datasets) comparing 326 medication-free MDD patients with 367 HC were ultimately included in the rCBF meta-analysis. We also conducted subgroup meta-analyses of rCBF PET studies, given the different modalities applied in rCBF studies. A subgroup meta-analysis of SPECT or ASL studies was precluded by their small number. A total of 7 ALFF studies and 1 fALFF study (9 datasets) comparing 284 medication-free MDD patients with 277 HC were ultimately included in the ALFF meta-analysis. All MDD patients were currently depressed and medication-free, having either never taken antidepressants or undergone a ‘washout’ period before scanning. Fig. 1 details the process of the literature search and article inclusion.

3.2. ALFF meta-analysis

A total of 8 ALFF studies including 9 datasets were analyzed. Compared with HC, in MDD resting state ALFF was <u>increased</u> in left medial cingulate gyrus and right supplementary motor area and <u>decreased</u> in left cerebellum, right middle frontal gyrus

and left middle temporal gyrus (Fig.2A, Table 2). Whole-brain jackknife sensitivity found these results highly replicable, preserved in all 9 combinations of datasets (Table S2A).

3.3. rCBF meta-analysis

We analyzed 14 rCBF studies including 15 datasets. Compared with HC, in MDD resting state rCBF was increased in right caudate nucleus, right precuneus, right corpus callosum, and right hippocampus and decreased in bilateral inferior frontal gyrus and left anterior cingulate (Fig. 2B, Table 2). Whole-brain jackknife sensitivity found these results highly replicable, preserved in all 15 combinations of datasets (Table S2B).

Subgroup meta-analysis of rCBF PET studies includes 8 datasets comparing 161 medication-free MDD patients with 194 HC. Compared with HC, in MDD resting state rCBF was increased in right caudate nucleus and right lingual gyrus and decreased in bilateral middle frontal gyrus and left superior temporal gyrus. These results were all supported by more than 5 datasets; outcomes supported by fewer than 5 datasets were discarded through whole-brain jackknife sensitivity analysis (Table S2C).

3.4. Multimodal meta-analysis results

To demonstrate regions that showed both ALFF and rCBF abnormalities, the results were summarized by putting rCBF and ALFF findings into a single meta-analytic map. Compared to HC, in MDD this multimodal analysis reported that the only conjoint abnormality showing robust decreased ALFF together with increased rCBF was in the left

cerebellum, vermic lobule IV / V.

3.5. Analysis of heterogeneity and publication bias

Analysis of heterogeneity revealed that some regions with altered rCBF (caudate nucleus, corpus callosum, precuneus, inferior and middle frontal gyrus) or ALFF (cerebellum, middle temporal gyrus, inferior and middle frontal gyrus, corpus callosum, postcentral gyrus) showed significant statistical heterogeneity between studies ($p<0.005$) (see Table S3). Analysis of publication bias found that the Egger test was insignificant for the cerebellum in the ALFF meta-analysis ($p=0.627$) and the rCBF meta-analysis ($p=0.269$).

3.6. Meta-regression

Variables explored by meta-regression were mean age of patients, percentage of female patients and duration of illness.

Meta-regression analyses of ALFF studies showed no effect of percentage of female patients and age (available in all ALFF and fALFF studies) on brain ALFF in medication-free MDD patients; the 17-item HRSD score and illness duration in ALFF studies could not be studied because the data were only available in 9 studies (Radua and Mataix-Cols, 2009).

Meta-regression analyses of rCBF studies showed that the age of MDD patients (available in all rCBF studies) was negatively associated with resting state rCBF in the

left inferior frontal gyrus (Fig. 3), with predicted rCBF decrease in studies with more older patients; no effect of percentage of female patients (available in all rCBF studies) and illness duration (available in 8 studies (Aihara *et al.*, 2007, Brody *et al.*, 2001, Germain *et al.*, 2007, Kennedy SH *et al.*, 2001, Kimbrell *et al.*, 2002, Lui S *et al.*, 2009, Perico *et al.*, 2005, Skaf C.R. *et al.*, 2002)） was detected. The 17-item HRSD score could not be studied due to insufficient data.

4. Discussion

To our knowledge this is the first whole-brain voxel-wise meta-analysis to evaluate resting state rCBF and ALFF in medication-free MDD patients compared to HC. The main finding was overlapping rCBF and ALFF changes in cerebellum. Other differences include altered resting-state rCBF in the right caudate nucleus, right precuneus, right corpus callosum, and right hippocampus, bilateral inferior frontal gyrus and left anterior cingulate, as well as altered ALFF in left median cingulate, right supplementary motor area, left cerebellum, right middle frontal gyrus and left middle temporal gyrus. In addition, the age of patients was negatively associated with rCBF in the left inferior frontal gyrus.

4.1. Findings in the ALFF studies

The meta-analysis of ALFF studies revealed increased ALFF in left medial cingulate

gyrus and right supplementary motor area and decreased ALFF in left cerebellum, right middle frontal gyrus and left middle temporal gyrus. The supplementary motor area belongs to the task-positive networks (TPN) which also include the dorsolateral prefrontal cortex, middle temporal gyrus, parietal lobe, and insula (Dutta *et al.*, 2014). This network typically responds with increased activation to attention-demanding tasks during wakeful rest, while the opposite pattern emerges during rest, with the task-negative network (TNN) becoming more active and the TPN less active (Hamilton *et al.*, 2011). During rest, the TPN has been claimed to subserve intermittent 'extrinsic awareness', defined as the conscious perception through different sensory modalities of one's surrounding environment (Vanhaudenhuyse *et al.*, 2011). The increase of ALFF in supplementary motor area as well as decrease in the middle temporal gyrus may indicate dysfunction of the TPN in MDD.

Conversely, our study showed increased ALFF in the cingulate gyrus which belongs to the TNN. The TNN has been termed the default mode network (DMN) subserving 'intrinsic awareness' (Vanhaudenhuyse *et al.*, 2011). Abnormalities of the TPN have been reported in MDD, attention-deficit/hyperactivity disorder (ADHD) and drug addiction (Rive *et al.*, 2013, Sripada CS, 2014, Wang L *et al.*, 2015 Feb 26), while abnormalities of the TNN have been reported in depression, anxiety, dementia, schizophrenia, epilepsy, autism and ADHD (Broyd SJ *et al.*, 2009). Marchetti et al. proposed TNN-TPN imbalance as the overarching neural mechanism involved in crucial cognitive risk factors for recurrent depression, namely rumination, impaired attentional control, and cognitive reactivity (Marchetti *et al.*, 2012).

Thus our study showed ALFF abnormalities in both the TPN and TNN in MDD

patients compared to HC, supporting the idea that disruption of the ‘extrinsic/intrinsic system’ may contribute to the development of MDD (Fox MD *et al.*, 2005). Whether these abnormalities of TPN-TNN are specific manifestations of MDD is worthy of further study.

The middle frontal gyrus is one of the most important regions in the fronto–limbic dysregulation model in MDD (Mayberg HS, 2003 , Seminowicz DA *et al.*, 2004 May, WC, 2001 Apr). The ventral components of the fronto-limbic network (including the subcortical, limbic and paralimbic regions) are thought to regulate vegetative and somatic features of depression (i.e. appetite, sleep and endocrine alterations), and the dorsal components (including the neocortical and superior limbic regions) are hypothesized to mediate attention and cognitive function (i.e. apathy and attention impairment) (Guo *et al.*, 2015). A breakdown of this network may underlie the evolution of depressive symptoms such as somatic complaints and negative bias to interpersonal feedback (Cullen KR *et al.*, 2009 Sep 4). Alterations in the fronto–limbic circuits are implicated in the mechanisms underlying emotional dysfunction (S and JL, 2002, WC, 2000). Fronto-limbic connectivity is reportedly decreased in depressed people both in task (GJ *et al.*, 2002) and resting-state (An *et al.*, 2009) conditions. Indeed, many studies have reported abnormal functional connectivity between the frontal and limbic regions (Anand A *et al.*, 2005 Jul, 2005 May 15, Cullen KR *et al.*, 2009 Sep 4, Horn DI *et al.*, 2010 Jul 15, Liu Z *et al.*, 2010 Jun 30, Su Q *et al.*, 2015 Jan). Consistent with the fronto-limbic model, in which MDD shows decreased frontal cortex function and increased limbic system function (Wang *et al.*, 2014), we found reduced regional activity in the right middle frontal gyrus in MDD. We suggest that decreased ALFF in middle frontal gyrus

may reflect a disconnection syndrome, which contributes to emotional dysregulation in MDD patients.

4.2. Findings in the rCBF studies

The pooled meta-analyses of rCBF studies revealed increased rCBF in right caudate nucleus, right precuneus, right corpus callosum, and right hippocampus，and decreased rCBF in bilateral inferior frontal gyrus and left anterior cingulate cortex.

Mayberg have proposed a working model of depression with three main compartments (cortical, subcortical and limbic) implicating failure of the coordinated interactions of a distributed of network of limbic-cortical pathways (Mayberg HS, 2003). In this model, abnormal chronic activity of limbic-subcortical structures tends to produce a persistent negative mood, with partial compensation by frontal hyperactivity (Monkul *et al.*, 2012); failure to initiate or maintain such a frontal compensatory state causes frontal hypometabolism, with resultant apathy, psychomotor slowness, and impaired executive functioning (Mayberg HS, 2003). This limbic-cortical dysregulation model in MDD is consistent with our findings of decreased rCBF in inferior frontal gyrus and anterior cingulate (cortical) and increased rCBF in caudate nucleus and hippocampus (limbic-subcortical part).

We found increased rCBF in right precuneus in medication-free MDD patients compared to controls. In normal subjects, the precuneus is activated in self-related mental representations, at rest and during tasks about reflection on one's own personality traits and physical appearance (Kjaer TW *et al.*, 2002 Oct). Our finding of increased

precuneus rCBF could be related to exaggerated self-referential processing (Lemogne C *et al.*, 2011 Sep), which distorts interpretations of social cues and maintains social fears because of maladaptive cognitions regarding self (i.e. as socially incompetent) and others (i.e. as critical judges) (Bögels SM and W, 2004 Nov) (Mor N and J, 2002 Jul)). Dumas et al. have reported a correlation between decreased perfusion in the precuneus and improvement of the quality of life in MDD patients (Dumas R *et al.*, 2012 Jul).

4.3. Conjoint findings between the rCBF and ALFF meta-analyses

The multimodal analysis of ALFF and rCBF showed decreased ALFF and increased rCBF in the cerebellum, vermic lobule IV / V.

The cerebellum contributes to cognitive processing and emotional control in addition to its role in motor coordination. It has connection with nonmotor cortical and subcortical areas, including the limbic system (Schmahmann JD and DN, 1997) and the prefrontal cortex (N, 2006 Jul); it also connects with brain stem nuclei, which supply the limbic system and cerebrum with serotonin, norepinephrine, and dopamine (Konarski JZ *et al.*, 2005 May). There is evidence that regions in the cerebellum are functionally connected with the dorsal executive, default-mode, affective, and motor networks of the cerebrum: the lateral cerebellar hemisphere with the dorsolateral prefrontal cortex, suggesting its potential involvement in executive function (Alalade *et al.*, 2011, Krienen and Buckner, 2009), and crus I of the cerebellum with the medial prefrontal cortex and anterior cingulate cortex, indicating its involvement in default-mode activity and emotional processing (Krienen and Buckner, 2009). The cognitive and affective symptoms

following cerebellar dysfunction have been termed a ‘dysmetria of thought’ (Schmahmann JD and JC, 1998 Apr); clinically, patients with cerebellar lesions are more likely to have depression, deficits in the ability to experience emotions, and behavioral difficulties (Wolf *et al.*, 2009). Using both task-related and resting-state fMRI, previous studies showed abnormal cerebellar activity and connectivity in MDD (Frodl *et al.*, 2010, Guo *et al.*, 2013). In a recent morphometric study, MDD patients showed reduced grey matter in right cerebellum (Crus I) and cerebellar vermis than healthy subjects (Machino A *et al.*, 2014 Oct 15). In a study of healthy volunteers, cerebellar volume was inversely related to depression scores (Schutter DJ *et al.*, 2012). In the present study, we identified coincident abnormalities of cerebellum in both ALFF and rCBF studies, consistent with an important role for the cerebellum in the neuropathology of MDD.

4.4. Influence of age: rCBF in the inferior frontal gyrus

Our meta-regression analysis found that rCBF in the left inferior frontal gyrus was negatively associated with the age of the patients: older MDD patients tend to have decreased rCBF.

In healthy subjects aging is associated with a decline in frontal lobe executive functioning (Jurado MB and M., 2007 Sep) and higher-order cognition (Bugg JM *et al.*, 2006 Oct)). MDD is associated with dysfunctions in similar cognitive domains that impact the functioning and quality of life (Gualtieri CT *et al.*, 2006, Nakano Y *et al.*, 2008 Nov). Cognitively stable healthy older individuals also show decreases in inferior frontal gyrus rCBF with age (Beason-Held LL *et al.*, 2008). Moreover, a longitudinal

study of healthy older adults found significant grey matter loss in the left inferior frontal gyrus with age (Bartzokis G *et al.*, 2001 May, Resnick S *et al.*, 2003). A longitudinal study in depression which directly investigates the relation between age and rCBF in the inferior frontal gyrus would clearly be of interest.

5. Limitations

This study has several limitations. First, the meta-analyses use summarized maps (i.e. coordinates from published studies) rather than raw statistical brain maps, which may reduce accuracy (Salimi-Khorshidi G *et al.*, 2009). Second, patients in the included studies were studied after a medication washout period, so we cannot exclude the long-term influences of the medication. However, a recent study detected no difference between 'drug-naive' and 'drug-washout' in acutely depressed patients (Fountoulakis KN *et al.*, 2013). Third, the rCBF studies included in our meta-analysis are only of western people while most of the ALFF studies include eastern people. Fourth, several regions with altered rCBF or ALFF had significant statistical heterogeneity between included studies. Meta-regression analyses and subgroup analyses were conducted in order to examine the moderator variables which may contribute to this heterogeneity (Radua and Mataix-Cols, 2009). Fifth, our use of data acquired and analyzed using different functional neuroimaging modalities (i.e. ALFF, PET, SPECT and ALS) may have decreased the sensitivity of the analysis. When more suitable studies have been published a modality-specific meta-analysis will be able to address the pathophysiological questions more directly.

6. Conclusions

We used the AES-SDM method to identify conjoint abnormalities of rCBF and ALFF in the cerebellum at resting state in medication-free patients, avoiding the potential confounding influence of antidepressant medication. These may be an important neuropathological change in MDD and future studies could use the cerebellum as a region of interest (ROI) for more in-depth researches. We also identified altered resting-state rCBF in precuneus and limbic-cortical pathways, and altered resting-state ALFF in the fronto-limbic network and widely distributed abnormalities in the TPN/TNN networks. Abnormalities in these regions are likely to reflect damage to cognitive, affective, self-referential processing function in depression. Our meta-regression analysis identified that the age of patients was negatively associated with rCBF in the inferior frontal gyrus, which merits further study.

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Declaration of interest:

None.

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Table1: Demographic and clinical characteristics of subjects in the functional neuroimaging datasets included in the meta-analyses

Studies	Modality / Analysis	Number(%female)		Mean age(y)		Mean illness duration (months)	HRSD score (17 Items)	Clinical details	Drug status	Quality score (out of 10)
		MDD	HC	MDD	HC					
ALFF Studies										
Guo et al 2014	ALFF	44(50%)	44(50%)	27.52	29.39	19.61	25.18		Drug-naive	9.5
Lai et al 2014	fALFF	47(49%)	27(56%)	36.9	38.29	NA	22.07		Drug-naive	9.5
Liu et al 2013	ALFF	22(45%)	19(47%)	28.09	24.37	2.95	25.89		Drug-naive	10
Wang et al 2012	ALFF	18(50%)	18(50%)	34	35	5	25		Drug-naive	10
Wang et al 2014	ALFF	30(57%)	33(58%)	35.7	31.45	5.48	24.97	Treatment-nonresponsive MDD	Drug-naive	10
Wang et al 2014	ALFF	26(62%)	33(58%)	32.54	31.45	4	27.5	Treatment-responsive MDD	Drug-naive	10
Yan et al 2014	ALFF	14(100%)	18(100%)	36	33	4	25.7		Drug-naive or >2 w washout	10
Zhang et al 2014	ALFF	32(56%)	35(49%)	20.53	20.97	NA	NA		Drug-naive	9.5
Zhao et al 2014	ALFF	51(53%)	50(56%)	28	29	NA	NA		Drug-naive	8.5
rCBF Studies										
Kohn et al 2007	^{99}mTc -HMPAO SPECT	33(58%)	25(52%)	53	49	NA	NA		>2 w washout	9.5
Krausz et al 2007	^{99}mTc -HMPAO SPECT	10(90%)	10(90%)	49.1	49.7	NA	NA	Treatment-sensitive MDD	>3 w washout	8
Perico et al 2005	^{99}mTc-ECD SPECT	15(80%)	15(60%)	34.5	33.27	30.6	26.9		>4 w washout	9.5
Skaf et al 2002	^{99}mTc-ECD SPECT	9(44%)	12(50%)	41	34	127	35.22		>4 w washout	8
Vardi et al 2011	^{99}mTc -HMPAO SPECT	37(57%)	27(58%)	55	50	NA	NA		>2 w washout	9.5
Aihara et al 2007	^{18}F-FDG PET	24(62.5%)	23(65.2%)	52.4	54.8	2.5	NA		Drug naive	10
Brody et al 2001	^{18}F-FDG PET	24(54%)	16(50%)	38.9	35.6	223.2	19.4		>2 w washout	9.5
Drevets et al 1992	^{15}O-H_2O PET	13(54%)	33(61%)	36	30	NA	NA	MDD with MDD first-degree relatives	>3 w washout	9.5
Germain et al 2007	^{18}F-FDG PET	12(83%)	13(77%)	38.1	37.3	12.7	NA		>2 w washout	10
Kennedy et al 2001	^{18}F-FDG PET	13(0%)	24(0%)	36	31.7	3.6	22.42		>4 w washout	10
Kimbrell et al 2002	^{18}F-FDG PET	38(66%)	37(65%)	43.4	43.4	321.6	NA		>4 w washout	9.5
Monkul et al 2012	^{15}O-H_2O PET	20(75%)	21(65%)	37.2	34.8	NA	NA		>2 w washout	9.5
Saxena et al 2001	^{18}F-FDG PET	17(50%)	27(50%)	32.5	38.2	NA	20.8		>4 w washout	9.5
Lui et al 2009	ASL	24(33%)	42(36%)	35	37	192	22	Refractory MDD	Drug naive	9.5

Lui et al 2009	ASL	37(30%)	42(36%)	33	37	24	24	Nonrefractory MDD	Drug naive	9.5

^{15}O-H_2O PET = ^{15}O-H_2O positron emission tomography; 17-item HRSD = 17-item Hamilton Rating Scale for Depression; ^{18}F-FDG PET = ^{18}F-fluorodeoxyglucose positron emission tomography; ^{99}mTc-ECD SPECT = Technetium-^{99}m ethyl cysteinate dimer single photon emission computed tomography; ^{99}mTc-HMPAO SPECT = Technetium-^{99}m hexamethylpropyleneamine oxime single photon emission computed tomography; ALFF = amplitude of low-frequency fluctuations; ASL = arterial spin labeling; fALFF = fractional amplitude of low-frequency fluctuations; HC = healthy controls; MDD = major depressive disorder; NA = not available; rCBF = regional cerebral blood flow.

Table 2: Regional Differences between Medication-free MDD Patients and Healthy Controls in the rCBF and ALFF Meta-Analyses

Region	Talairach coordinate			SDM z-score	p-value	number of voxels	cluster breakdown (number of voxels)
	x	y	z				
Meta-analysis of ALFF studies							
MDD > HC							
L medial cingulate	-2	-32	40	1.301	0.0027	97	R median cingulate / paracingulate gyrus(64) L median cingulate / paracingulate gyrus(33)
R supplementary motor area	-2	-8	58	1.302	0.0027	171	R supplementary motor area(105) L supplementary motor area(66)
MDD < HC							
L cerebellum	-6	-44	-2	-2.338	~0	907	cerebellum, vermic lobule IV / V(125) corpus callosum(86) L cerebellum, hemispheric lobule IV / V(167) L median network(75) cerebellum, vermic lobule III(55) L lingual gyrus(172) L precuneus(97) L cerebellum, hemispheric lobule III(33) L calcarine fissure / surrounding cortex(66) L cerebellum, hemispheric lobule III(31)
R middle frontal gyrus	34	48	-12	-1.517	0.00074	397	R middle frontal gyrus(307) R inferior frontal gyrus(90)
L middle temporal gyrus	-44	-60	-4	-1.194	0.00389	15	L middle temporal gyrus(12) L middle occipital gyrus(3)
Meta-analysis of rCBF studies							
MDD > HC							
R caudate nucleus	12	-2	12	1.723	0.0000206	591	R caudate nucleus(252) R anterior thalamic projections(310) corpus callosum(29)

R precuneus	12	-42	4	1.154	0.001832	126	R precuneus(44) R lingual gyrus(30) cerebellum, vermic lobule IV / V(21) R posterior cingulate gyrus(17) L precuneus(14)
R corpus callosum	20	-28	6	1.290	0.000701845	66	corpus callosum(39) R thalamus(17)
R hippocampus	26	-10	-18	1.092	0.002585	8	R hippocampus(10) R hippocampus(8)
<u>MDD < HC</u>							
L inferior frontal gyrus	-48	28	-2	-2.297	0.0000103	2247	L inferior frontal gyrus(1069) L temporal pole(470) L insula(357) L superior temporal gyrus(220) L rolandic operculum(131)
R anterior cingulate	0	42	20	-1.946	0.000175	1008	R anterior cingulate(325) L anterior cingulate(351) R superior frontal gyrus(103)
R inferior frontal gyrus	58	20	12	-1.992	0.000108	578	L superior frontal gyrus(229) R inferior frontal gyrus(545) R insula(33)

*Regions identified by meta-analyses of coordinates from 15 rCBF datasets and 9 ALFF/fALFF datasets separately (voxel-wise $p < 0.005$ and FWHM 20 mm).

ALFF = amplitude of low-frequency fluctuations; fALFF = fractional amplitude of low-frequency fluctuations; L = left; MDD = major depressive disorder; R = right; rCBF = regional cerebral blood flow; SDM = signed differential mapping.

Figure Captions

Fig. 1: Meta-analysis of resting-state rCBF and ALFF studies in medication-free patients with major depressive disorder.

Abbreviations: ALFF = amplitude of low-frequency fluctuations; fALFF = fractional amplitude of low-frequency fluctuations; rCBF = regional cerebral blood flow

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Fig. 2: The areas of increased (red) and decreased (blue) resting state brain activity in medication-free MDD patients compared with the healthy controls in the meta-analyses of ALFF studies (**A**) and rCBF studies (**B**).

Abbreviations: ALFF = amplitude of low-frequency fluctuations; IFG = inferior frontal gyrus; L = left; MFG = middle frontal gyrus; MTG = middle temporal gyrus; R = right; rCBF = regional cerebral blood flow; SMA = supplementary motor area

Fig. 3: Results of the meta-regression analyses of rCBF studies for resting-state rCBF against age in medication-free depressed patients.

The mean age is negatively associated with resting-state rCBF in the left IFG. Each study is represented as a dot, with lager dots symbolizing lager sample size. The regression line (meta-regression signed differential mapping slope) is presented as a straight line.

Abbreviations: IFG = inferior frontal gyrus; L = left; rCBF = regional cerebral blood flow