Discovery and replication of cerebral blood flow differences in major depressive disorder

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Abstract

Major depressive disorder (MDD) is a serious, heterogeneous disorder accompanied by brain-related changes, many of which are still to be discovered or refined. Arterial spin labeling (ASL) is a neuroimaging technique used to measure cerebral blood flow (CBF; perfusion) to understand brain function and detect differences among groups. CBF differences have been detected in MDD, and may reveal biosignatures of disease-state. The current work aimed to *discover* and *replicate* differences in CBF between MDD participants and healthy controls (HC) as part of the EMBARC study. Participants underwent neuroimaging at baseline, prior to starting study medication, to investigate biosignatures in MDD. Relative CBF (rCBF) was calculated and compared between 106 MDD and 36 HC EMBARC participants (whole-brain *Discovery*); and 58 MDD EMBARC participants and 58 HC from the DLBS study (region-of-interest *Replication*). Both analyses revealed reduced rCBF in the right parahippocampus, thalamus, fusiform and middle temporal gyri, as well as the left and right insula, for those with MDD relative to HC. Both samples also revealed increased rCBF in MDD relative to HC in both the left and right inferior parietal lobule, including the supramarginal and angular gyri. Cingulate and prefrontal regions did not fully replicate. Lastly, significant associations were detected between rCBF in replicated regions and clinical measures of MDD chronicity. These results (1) provide reliable evidence for ASL in detecting differences in perfusion for multiple brain regions thought to be important in MDD, and (2) highlight the potential role of using perfusion as a biosignature of MDD.

Introduction

Major depressive disorder (MDD) is a common, serious, and heterogeneous mood disorder. Despite the conception that it is associated with brain-related changes, brain-based biomarkers have not been established for either use in

diagnosis or treatment decision-making. Neuroimaging is continuing to be an increasingly important tool to identify biomarkers for several neurological conditions, including stroke, multiple sclerosis, and Alzheimer's disease, but such biomarkers still require more investigation for use in mood disorders. Magnetic resonance imaging (MRI) provides a

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noninvasive medical test that may identify the key brain biosignatures useful for MDD diagnostics, treatment matching, and tracking treatment response [1]. However, there remains a need to replicate and validate the reliability of these tests for MDD before investigating such biosignatures as biomarkers to use clinically.

Arterial spin labeling (ASL) is a noninvasive neuroimaging technique used to measure brain function, similar to blood-oxygen-level-dependent (BOLD) and positron emission tomography (PET). However, opposed to using a radioligand like PET, ASL uses a magnetic pulse to label blood as it perfuses through the brain to provide oxygen and nutrients to tissue. As opposed to measuring connectivity between regions at rest, ASL at rest provides a quantifiable measure of blood perfusion within a given region. Therefore, ASL-derived cerebral blood flow (CBF; perfusion) may be useful to understand brain function in MDD [2]. Yet, only one small study ($n = 44$; 22 MDD and 22 healthy controls; HC) has reported absolute CBF differences in the default-mode (i.e., resting/idle state) network before controlling for whole-brain gray matter and age [3]. Another small ASL study was able to accurately distinguish unipolar ($n = 18$) from bipolar depression ($n = 18$) based on differences in CBF in the anterior cingulate cortex with 81% accuracy, but did not accurately distinguish MDD from HC [4]. A third study observed lower CBF in MDD ($n = 27$) participants relative to HC ($n = 43$) within prefrontal and cingulate regions [5]. However, MDD participants were currently being treated for their depression, some of which were classified as non-remitters, which may limit both replicability and generalizability of the findings. Further studies using larger samples are needed to determine if ASL can serve as a clinical tool for MDD diagnosis and treatment decision-making.

The current study, Establishing Moderators/Biosignatures of Antidepressant Response in Clinical Care (EMBARC [6]) is a double-blind, randomized, placebo-controlled trial aiming to detect biosignatures and a treatment response index for MDD [6]. A crucial step toward establishing reliable biomarkers for clinical care is to identify and replicate features (biosignatures) that are altered in the disease-state for the measure of interest. CBF was evaluated as a potential biosignature of MDD using a *Discovery-Replication* approach. We predicted that participants with MDD would show CBF differences in brain regions involved in major functional networks, including default-mode, emotion, reward, and salience processing (e.g., prefrontal, insula, and cingulate cortices), and they would replicate. Other neuroimaging modalities have observed these networks to be disrupted in MDD (for a review on multi-modal imaging and these pathophysiologic processes in MDD, see [1]). Moreover, we predicted MDD participants would have lower CBF in the cingulate cortex, inferior and middle frontal

gyri, prefrontal cortex, and thalamus, relative to HC, replicating prior ASL work in MDD [5, 7, 8]. The addition of CBF-based findings may aid in identifying brain-based biomarkers of MDD.

To better characterize differences identified in MDD using brain imaging, clinical and translational research often investigate how clinical features relate to these differences. For CBF, one study showed MDD severity was associated with lower perfusion in the cingulate cortex and inferior parietal lobule [3]. So, tertiary to the *Discovery-Replication* predictions, we hypothesized that clinical features related to MDD chronicity and severity would also associate with perfusion levels, although the directions were exploratory. Characterizing biosignature-clinical relationships may augment diagnostic and treatment response indices.

Materials and methods

Design and overview

EMBARC MDD participants were scanned using MRI, including an ASL sequence, pretreatment and one week after starting treatment. All participants' data were collected at one of four sites. Baseline CBF was compared between HC and MDD groups split into *Discovery-Replication*, 2/3–1/3 subgroups. The *Discovery* model was exploratory using a whole-brain approach with a less stringent p -value threshold of standard cluster size for ASL data. This would allow both corrected and uncorrected significant *Discovery*-derived regions to be investigated as replicable or spurious upon *Replication*. The *Replication* model was conservative using a region of interest (ROI) approach with a more stringent threshold correcting for multiple comparisons. Work following similar designs can be found in Genome-Wide Association Study literature [9].

Participants

EMBARC included 200 MDD participants and 40 HC. Additional HC ($n = 58$) came from the Dallas Lifespan Brain Study (DLBS) and were used to augment the EMBARC HC sample and provide independent validation for *Discovery-Replication* data analysis sets. Participants were 18–65 years old and fluent in English. MDD participants were diagnosed using the Structural Clinical Interview for DSM-IV [10], had early-onset (before age 30), chronic (two or more episodes) MDD, were not undergoing treatment for MDD, antidepressant medication-free for 21 days (5 weeks for fluoxetine) with no failed antidepressant trials of adequate dose and duration (as defined by the MGH-ATRQ), in the current episode or inadequate response or poor tolerability to study medication. EMBARC HC

Table 1 Demographic and clinical characteristics for the *Discovery* and *Replication* datasets

	Discovery dataset		Replication dataset	
	EMBARC healthy controls	EMBARC MDD sample	DLBS healthy controls	EMBARC MDD replication sample
No. of participants	36	106	58	58
Age, mean years (SE)	37.49 (2.62)	37.65 (1.32)	37.59 (1.67)	37.71 (1.71)
Degree of right-handedness/ambidextrous 1–100, Mean (SE)	69.56 (9.08)	68.53 (4.82)	Not collected	73.75 (6.31)
Female (%)	24 (68.6)	67 (63.2)	36 (62.1)	36 (62.1)
Race/ethnicity				
Caucasian (%)	22 (62.9)	74 (69.8)	49 (84.5)	37 (63.8)
Afr. Amer. (%)	8 (22.9)	22 (20.8)	4 (6.9)	12 (20.7)
Asian (%)	3 (8.6)	5 (4.7)	2 (3.5)	5 (8.6)
Amer. Indian/Alask. Nat. (%)	0 (0)	1 (0.9)	0 (0)	0 (0)
Nat. Haw./Pacif. Islander (%)	0 (0)	0 (0)	0 (0)	0 (0)
Other (%)	2 (5.7)	4 (3.8)	3 (5.2)	4 (6.9)
Years of education, Mean (SE)	15.76 (.79)	14.71 (.25)	16.72 (.37)	14.72 (.35)
QIDS-SR mean score (SE)	1.43 (.21)	18.56 (.29)	Not collected	18.12 (.33)
HAM-D-17 Mean score (SE)	.54 (.13)	18.92 (.46)	Not collected	18.74 (.57)
Age of onset (SE)	0 (0)	16.00 (.58)	Not collected	17.12 (.86)
Number of MDD episodes (SE)	0 (0)	11.98 (2.24)	Not collected	8.72 (1.93)

MDD Major Depressive Disorder, *SE* standard error, *QIDS-SR* Quick Inventory of Depressive Symptomatology–Self-Report, *HAM-D* Hamilton Scale for Depression

participants had no history of mental disorders and scored ≤ 8 on the Quick Inventory of Depressive Symptomatology–Self-Report (QIDS-SR), while MDD participants scored ≥ 14 [11]. For DLBS inclusion, HC participants had to be predominately right handed, of good medical condition, and cognitively healthy. MDD participants were excluded if any other mental disorder was primary to MDD, if they had a lifetime history of psychotic depressive, schizophrenic, bipolar (I, II, or NOS), schizoaffective, other Axis I psychotic disorders, eating disorder, or had the presence of a medical condition that would compromise MDD-specific findings¹. Both studies implemented general MRI exclusion criteria. See Table 1 for demographics. The study was approved by the Institutional Review Board at each site. All participants signed informed consent.

Data acquisition and imaging parameters

EMBARC MRI data were collected at four sites on 3T scanners: Columbia University (CU-General Electric), Massachusetts General Hospital (MG-Siemens), University of Michigan (UM-Philips), and the University of Texas Southwestern Medical Center (TX-Philips). DLBS was

collected at TX. ASL was performed at the end of a seven-sequence protocol². ASL was acquired last for two reasons: (1) it was the more exploratory scan of the protocol, and (2) the completion of two non-task scans prior to the ASL sequence (>20 min), would allow return of resting-state function less confounded by residual task function.

All ASL sequences implemented a resting-state, pseudo-continuous technique with a 1516 ms labeling duration and 1500 post-labeling delay. Other ASL parameters were: TR/TE = 4460 ms/17 ms for EMBARC, 4150 ms/13 ms for DLBS, in-plane resolution $3.4 \times 3.4 \text{ mm}^2$, with 5 mm thickness covering the whole brain, field of view (FOV) = $220 \times 220 \times 145 \text{ mm}$ for EMBARC, $240 \times 240 \times 135 \text{ mm}$ for DLBS, matrix = 64×64 ; flip angle = 90° , multi-slice acquisition in ascending order, 29 slices for EMBARC and 27 slices for DLBS, 70 dynamics, no background suppression, with scan duration of approximately 5 min. These imaging parameters are generally compatible with recommendations from the recent consensus paper [2]. T1-weighted, high-resolution, structural-3D sagittal images were acquired in the same session.

¹ Exclusionary medical conditions included the presence of a neurological condition requiring an anticonvulsant; a medical condition not stable with medication, required hospitalization or deemed clinically relevant by the investigators; or any abnormal laboratory results the site principal investigator considered clinically significant.

² Order of sequences (total scan time ≈ 1 h): high-resolution structural MRI (MPRAGE ≈ 5 min), pre-task resting-state BOLD (≈ 6 min; B0 ≈ 2 min), event-related BOLD (emotion conflict task ≈ 13 min), event-related BOLD (reward task ≈ 8 min), post-task resting-state BOLD (≈ 6 min), diffusion tensor imaging (DTI ≈ 15 min with B0), and arterial spin labeling (≈ 5 min).

Data processing and analysis

Of the 40 HC and 200 MDD EMBARC participants, 36 HC and 164 MDD baseline ASL scans were used. Others were not collected or excluded due to poor quality (i.e., incomplete sequence, artifact, signal loss, missing brain due to poor FOV placement, and/or excessive motion). All 58 DLBS HC scans were usable. We implemented relative CBF (rCBF) to detect and replicate differences between HC and MDD. Each participant's rCBF is the normalization of their absolute CBF (aCBF), which divides the aCBF of each voxel by the whole-brain averaged aCBF. CBF is reportedly reliable in HC populations across scanning sessions (for EMABRC HC data see [12]) [13]. However, rCBF reportedly has greater sensitivity and reliability in detecting differences in perfusion between groups when compared to aCBF [14]. rCBF assists in controlling for individual differences that are not disease-specific (e.g., gender). rCBF's normalization process is also useful to decrease cross-site differences introduced across scanners. Figure 1 depicts aCBF and rCBF for this dataset.

The baseline ASL data were preprocessed and analyzed with SPM8 (Wellcome Department of Cognitive Neurology, UK) in Matlab (MathWorks, Natick, MA). Processing followed: (1) realignment of the ASL time-series to correct for head motion, (2) generation of perfusion-weighted image series by pairwise subtraction of the label and control functional images, (3) conversion to aCBF image series based on a single-compartment ASL perfusion model (a center, thalamic-level slice of the control image was used as a global index representing equilibrium magnetization, i.e. M0), (4)

generation of a mean CBF image for each participant, (5) co-registration of the mean image with the anatomical image, (6) normalization to the Montreal Neurological Institute (MNI) template, (7) resampling of CBF image to $2 \times 2 \times 2$ mm and smoothed with full-width at half-maximum 8 mm kernel within gray and white matter, and (8) extracting the rCBF image by dividing out the aCBF global individual mean from each aCBF voxel³.

Data for rCBF analyses were divided into *Discovery* and *Replication* sets modeling precision medicine approaches (i.e., detection in larger to smaller samples). EMBARC HC were used for *Discovery*, and DLBS age- and gender-matched HC were used for *Replication* to serve as independent and external validation set (see Fig. 2). MDD participants used for the *Discovery* and *Replication* datasets were pseudo-randomly assigned, controlling for important characteristics: site, age, gender, race, handedness, education level, MDD severity, age of MDD onset, and number of episodes (see Table 1).

For *Discovery*, a whole-brain, analysis of variance (ANOVA) on Group (HC, MDD), controlling for site as a fixed effect (CU, MG, TX, UM), was conducted on rCBF at $p < 0.05$ (cluster-level; and 0.01 corrected cluster-level) and extent threshold of 100 continuous voxels using SPM8. The ANOVA compared the 36 EMBARC HC to 106 EMBARC MDD participants. Gray matter coordinates for peak voxels in each significant *Discovery* cluster, reaching greater than $p = 0.001$ voxel significance, were used to build 10 mm ROI-spheres in MarsBar. ROI-sphere extractions were used for *Replication* analysis. Fit and significance of ROI-spheres were confirmed for the *Discovery* sample prior to using in *Replication* sample (all $f > 4.10$; $p < 0.04$). For *Replication*, Group (HC, MDD) ROI ANOVAs were conducted on rCBF means at $p < 0.05$ (Bonferroni adjustment), also controlling for site as a fixed effect using SPSS (IBM, New York, US). *Replication* ANOVAs compared the 58 DLBS HC to the remaining 58 EMBARC MDD participants. Effect sizes (partial eta-squared; η_p^2) for each *Replication* ROI are reported alongside p -values to provide additional information as to the relevance, or promise, of a given region being a biosignature applied to individual cases. Brain regions were defined by the AAL atlas and Brodmann areas using MNI coordinates [15].

In addition to observing disease-state differences in rCBF between HC and MDD groups, correlations were conducted on those regions that replicated, using the *Replication* MDD sample, to explore relationships between rCBF and MDD clinical features. Uncorrected Spearman's

Fig. 1 Site effect for **a** absolute cerebral blood flow (CBF) and **b** relative CBF. Regions for which site had a positive effect are in Red. Regions for which site had a negative effect are in Blue

³ Prior to rCBF analyses, equivalency of aCBF was confirmed between HC and MDD groups, $f = 0.06$; $p = 0.80$, and between TX DLBS HC and TX EMBARC HC as to code them under the TX site, $t = -0.03$; $p = 0.98$.

Fig. 2 Study sample and analytic flow diagram. EMBARC participants with major depressive disorder (MDD) were pseudo-randomly divided into 2/3–1/3 *Discovery-Replication* analyses. EMBARC healthy

controls were used for *Discovery* whole-brain analysis. DLBS healthy controls were used for *Replication* region of interest (ROI) analyses derived from the *Discovery* clusters

rho correlations were conducted on rCBF and clinical measures of MDD chronicity, using duration of current episode (months), length of illness (years), age of onset (year of age), and number of major depressive episodes (MDEs), as well as MDD severity, using both the QIDS-SR, self-reported inventory of major symptoms, and the 17-item Hamilton Depression Rating Scale (HAM-D₁₇) [16], a standard measure of symptom severity.

Results

Lower rCBF in MDD than HC

Discovery sample

The MDD group showed significantly *lower perfusion* than HC during *Discovery* analyses in five clusters: Cluster 1—a large cluster encompassing bilateral cerebellar and midbrain regions, extending anteriorly to the right thalamus, parahippocampal gyrus, fusiform gyrus, and insula; Cluster 2—the right posterior cingulate cortex; Cluster 3—the left inferior temporal gyrus; Cluster 4—the right middle temporal gyrus and temporal pole; and Cluster 5—the left insula (marginally significant). See Table 2 for detailed cluster information and Fig. 3 for cluster depictions.

Replication sample

Discovery-derived ROIs that had significantly *lower perfusion* in the MDD group compared to HC upon *Replication* included: Cluster 1 ROIs—the cerebellum, parahippocampal gyrus, fusiform gyrus, and insula, with the thalamus being marginally significant; Cluster 4 ROI—the right middle temporal gyrus and pole (marginally significant); and Cluster 5 ROI—the left insula. See Table 2 for detailed cluster information and effect sizes, and Fig. 3 for cluster depictions.

Greater rCBF in MDD than HC

Discovery sample

The MDD group showed significantly *greater perfusion* than HC during *Discovery* analyses in five clusters: Cluster 1—the left ventromedial prefrontal cortex extending dorsally to the ventral and dorsal anterior cingulate cortex; Cluster 2—the dorsolateral prefrontal cortex and anterior prefrontal cortices; Cluster 3—the right inferior parietal lobule containing the angular and supramarginal gyri; Cluster 4—left inferior parietal lobule containing the angular and supramarginal gyri; and Cluster 5—the right precuneus. See Table 2 for detailed cluster information and Fig. 3 for cluster depictions.

Replication sample

Discovery-derived ROIs that had significantly *greater perfusion* in the MDD group compared to HC upon *Replication* included: Cluster 2 ROIs—the left anterior prefrontal and dorsolateral prefrontal cortex; Cluster 3 ROI—the right inferior parietal lobule cluster, including the angular and supramarginal gyri; and Cluster 4 ROI—the left inferior parietal lobule cluster, including the angular and supramarginal gyri. However, the Cluster 2 ROIs were not replicated in the expected direction. HC participants had greater perfusion in these regions than MDD participants. See Table 2 for detailed cluster information and effect sizes, and Fig. 3 for cluster depictions.

Exploratory correlations between rCBF and clinical features

For replicated ROIs that had significantly *lower* rCBF in the MDD group relative to HC, right parahippocampal gyrus rCBF was positively correlated with duration of current episode, such that longer duration was associated with higher perfusion ($r_s = 0.26$; $p < 0.05$). However, the right

Table 2 Clusters, i.e., brain regions, in which the healthy control (HC) and major depressive disorder (MDD) groups showed differences in relative cerebral blood flow (rCBF; perfusion) for *Discovery* and *Replication* analyses separated by contrast, regions in which those with MDD had lower perfusion and regions in which they had greater perfusion relative to HC

Discovery cluster Inset replication region of interest	MNI coordinates (x, y, z)	BA	No. of voxels	<i>t</i> -value <i>f</i> -value	<i>p</i> -value	η_p^2	<i>M</i>	<i>SD</i>
MDD < HC								
Cluster 1-Red	20, -26, -22		9617	5.59	0.001^b			
<i>Right cerebellum</i>	20, -26, -22			6.02	0.02	0.05	1.23	0.24
<i>Right cerebellum</i>	14, -30, -16			2.00	0.16	0.02	1.33	0.28
<i>Right thalamus</i>	20, -16, 2			3.48	0.06	0.03	0.89	0.16
<i>Right parahippocampus</i>	28, -12, -28	27		32.63	0.001	0.23	0.90	0.20
<i>Right fusiform gyrus</i>	32, -42, -14	37		12.92	0.001	0.10	1.14	0.17
<i>Right insula, anterior</i>	40, -4, -4	13		18.46	0.001	0.14	1.30	0.24
Cluster 2-Blue	4, -34, 26		1078	3.90	0.04^b			
<i>Right posterior cingulate, ventral</i>	5, -42, 27	23		2.26	0.14	0.02	1.54	0.25
Cluster 3-Green	-62, -46, -22		1037	4.45	0.05^b			
<i>Left inferior temporal gyrus</i>	-62, -46, -22	20		0.47	0.50	0.01	0.58	0.15
<i>Left inferior temporal gyrus</i>	-56, -52, -20	20		2.93	0.09	0.03	0.84	0.18
Cluster 4-Yellow	58, 16, -30		304	3.33	0.05^a			
<i>Right middle temporal gyus, pole</i>	58, 16, -30	38		3.50	0.06	0.03	0.55	0.17
<i>Right middle temporal gyus</i>	68, -4, -22	21		2.38	0.13	0.02	0.56	0.13
Cluster 5-Violet	-42, 10, -10		243	3.71	0.07^a			
<i>Left insula</i>	-42, 10, -10	13		6.71	0.01	0.06	1.38	0.27
MDD > HC								
Cluster 1-Red	-8, 28, -16		445	3.85	0.02^a			
<i>Left ventromedial prefrontal cortex</i>	-8, 28, -16	11		0.46	0.50	0.004	0.61	0.18
<i>Left anterior cingulate, dorsal</i>	-8, 38, 12	32		0.01	0.96	0.001	0.91	0.19
Cluster 2-Blue	-10, 56, 12		318	3.34	0.05^a			
<i>Left superior medial frontal gyrus, anterior prefrontal</i>	-10, 56, 12	10		6.62	0.01	0.06	0.98	0.15
<i>Left middle frontal gyrus, dorsolateral prefrontal</i>	-30, 44, 20	46		7.14	0.01	0.06	1.12	0.17
Cluster 3-Green	58, -46, 44		638	3.81	0.01^a			
<i>Right inferior parietal lobule, supramarginal gyrus</i>	58, -46, 44	40		47.48	0.05	0.30	0.97	0.18
<i>Right inferior parietal lobule, angular gyrus</i>	46, -68, 40	39		36.28	0.001	0.25	1.01	0.18
Cluster 4-Yellow	-60, -42, 42		372	2.84	0.03^a			
<i>Left inferior parietal lobule, supramarginal gyrus</i>	-60, -42, 42	40		37.73	0.001	0.25	0.86	0.20
<i>Left inferior parietal lobule, angular gyrus</i>	-44, -68, 44	39		58.87	0.001	0.35	0.94	0.21
Cluster 5-Violet	4, -60, 38		284	3.70	0.05^a			
<i>Right precuneus</i>	4, -60, 38			0.007	0.93	0.001	1.50	0.21

Whole-brain clusters from the Discovery analysis are represented in bold

Regions of interest used in the Replication analysis are presented below their corresponding Discovery cluster in italics

MNI Montreal Neurological Institute, BA Brodmann Area, *M* means, *SD* standard deviations for replication cluster data

^aUncorrected cluster-level

^bCorrected cluster-level

anterior insula negatively correlated with age of onset ($r_s = -0.26$; $p = 0.05$), such that younger age of onset was associated with higher perfusion, while the left insula negatively correlated with length of illness ($r_s = -0.29$; $p < 0.03$), such that longer length of illness was associated with lower perfusion. Despite only reaching marginal significance upon replication, perfusion in the thalamus positively correlated with

length of illness ($r_s = 0.46$; $p < 0.001$) and number of MDEs ($r_s = 0.32$; $p < 0.02$), such that longer length of illness and higher number of MDEs was associated with higher perfusion. Only perfusion in the middle temporal pole positively correlated with depression severity (QIDS-SR), such that higher depression severity was associated with higher perfusion ($r_s = 0.28$; $p = 0.03$). For regions that had significantly higher rCBF

Fig. 3 Brain regions in which groups showed differences in relative cerebral blood flow (rCBF; perfusion). **a** Overall effect of Group controlling for Site where participants with major depressive disorder (MDD) had lower rCBF relative to healthy controls (HC). **b** Regions for which rCBF was lower for those with MDD relative to HC. Discovery clusters are represented in color based on Table 1 definitions (Cluster 1-Red; Cluster 2-Blue; Cluster 3-Green; Cluster 4-Yellow; and Cluster 5-Violet) and the Replication region of interest-spheres (ROIs), derived from the Discovery clusters, imposed in cyan. **c** Overall effect of Group controlling for Site where MDD participants had greater rCBF relative to HC. **d** Regions for which rCBF for was greater in those with MDD than HC. Discovery clusters are represented in color based on Table 1 definitions (Cluster 1-Red; Cluster 2-Blue; Cluster 3-Green; Cluster 4-Yellow; and Cluster 5-Violet) and replication ROIs imposed in cyan

in the MDD group relative to HC, only rCBF in the supramarginal gyrus was negatively correlated with duration of current episode, such that longer episode duration was associated with lower perfusion ($r_s = -0.26$; $p < 0.05$).

Discussion

The present study is the first to discover and replicate rCBF differences in MDD using a large, well characterized population. The study examined 164 MDD participants and divided them into 2/3–1/3 *Discovery-Replication* samples.

The *Discovery* sample provided further evidence for the role of ASL in detecting CBF differences in brain regions implicated in the phenotype of MDD [17–19]. The *Replication* sample provided independent, unbiased confirmation for these rCBF differences. Both samples revealed that HC have consistently increased perfusion relative to MDD participants, whose dysfunction in these regions has been characterized using other MRI modalities examining brain networks. Specifically, the midbrain cluster encompassed regions involved in multiple cognitive functions, sleep, salience, and reward processing, the insula in salience, interoceptive and emotion processing, and regions of the temporal lobe in higher order sensory processing [20–27]. HC participants had a majority of their perfusion differences in the frontal lobe (executive function), while MDD participants' were in the inferior parietal lobule (angular gyrus; default-mode network and attention) highlighting shifts in cognitive networks [28, 29]. Disruptions in connectivity within, and between, these large networks arguably play a significant role in the pathophysiology of MDD such as persistent negative mood, emotion processing, self-focus, and ruminating thoughts [30–32].

In addition to observing replicable lower perfusion, this study's rCBF findings also revealed MDD participants to have replicable greater perfusion than HC in other brain regions. Nearly all ASL research in MDD has only observed CBF to be lower in those with MDD relative to HC, and not greater rCBF or both [5, 7, 8]. However, one other study that scanned treatment-responders, prior to starting their antidepressant treatment, observed greater CBF in MDD than HC in regions of the occipital lobe and limbic system [8]. The MDD samples used for the EMBARC *Discovery-Replication* analyses likely include responders and non-responders to antidepressant treatment, limiting replication. The heterogeneity of the disease within this population may also limit consistency across all findings. Other considerations are their complicated symptom profile and the sensitivity of the CBF measure implemented (aCBF vs. rCBF).

The replication of 52% of our discovery-derived clusters is exciting given the lack of discovery-replication work in the field. This is augmented by the associations detected between these replicated clusters and MDD clinical features. The majority of the clinical-perfusion relationships were chronicity-based MDD features. Associations were detected for rCBF and duration of current episode (parahippocampal and supramarginal gyri), length of illness (thalamus and insula), age of onset (insula), and number of MDEs (thalamus). These chronicity-based relationships may suggest that levels of perfusion in MDD could be markers of trait or long-term effects of MDD (i.e., brain scarring). Paired with the observation that most of these relationships existed for regions that were also identified to have medium to large effect sizes between MDD and HC, these regions may not just provide

markers of disease, but may also provide mediators of treatment response.

Limitations

To reduce biologic heterogeneity, EMBARC participants had chronic, early-onset MDD. This may limit the generalizability of the findings to newly depressed or late-stage MDD populations. The DLBS study did not collect clinically relevant information like EMBARC. The inability to confirm that the DLBS HC sample clinically matched the EMBARC HC sample is a limitation and may play a role in some replication discrepancies. Other effects such as age or MDD severity may not have been completely controlled for in the pseudo-randomization of the MDD population into the *Discovery* and *Replication* samples. rCBF did not differ by gender in the MDD sample, but thalamic rCBF was positively correlated with age prior to multiple comparison correction. Residual effects of age on rCBF could be of interest with studies of a wider range and greater representation by age. EMBARC's protocols optimized quality and performance while maintaining similar parameters (in line with ASL recommendations [2, 33]) and revealed great HC test–retest reliability [12]. However, site variations could also still play a role in *Discovery-Replication* discrepancies despite use of rCBF. The inclusion of the DLBS HC likely impacted the ability to adequately control for site variations in the *Replication* analyses. Future methodological work on across- and within-site scanner reliability and between-group effects could still assist in understanding CBF data and its discrepancies in MDD. ASL data compared to other imaging modalities can have a lower signal-to-noise ratio and magnetic susceptibility signal voiding/artifact in regions like the frontal lobes. This could play an additional role in the inconsistent findings of the prefrontal regions. A recent study investigating CBF and glucose metabolism in MDD observed glucose metabolism to be more sensitive than CBF in identifying functional abnormalities in the frontal lobes [34]. Perfusion, neuronal activity, and metabolism of a given region may inform or influence each other. Recent findings on CBF and cerebral metabolic rate of oxygen identified a 2:1 ratio of perfusion to neuronal consumption that mediated the BOLD signature in aging populations, suggesting age-related changes in perfusion and neuronal activity are not equal, and mediate one another [35]. MDD-related changes in these brain physiology relationships might also exist to some degree. Modeling other imaging measures with rCBF may assist in better characterization of perfusion markers in MDD.

Future directions

ASL has become a more widely used research tool, but of the many MRI modalities, it has ideal prospects of being translated into a tool for clinical diagnostics and informing treatment decisions. The short scan-time, capacity for automated processing, and direct, quantifiable physiological measure are all desired qualities of a biomarker. In combination with clinical symptom profiles, CBF-defined regional disruptions could inform tailored antidepressant or therapy programs, or even provide anatomical targets for treatments (e.g., transcranial magnetic stimulation). Beyond its utility in diagnosing and choosing treatment, CBF can provide a noninvasive biomarker of disease progression and possibly predict relapse by tracking changes, or lack thereof, in perfusion over time.

The current study is the first to present independent internal and external replication of CBF in MDD for regions that lack and exceed perfusions levels of HC. The replication and overlap in brain regions detected in our perfusion data and those investigated using other imaging modalities of treatment response is promising for the future development of reliable MDD biosignatures (e.g., default-mode network connectivity and serotonin norepinephrine reuptake inhibitor treatment [36]; insula metabolism and cognitive behavioral therapy vs selective serotonin reuptake inhibitors [37]; superior temporal gyrus and insula gray matter volume and transcranial magnetic stimulation [38]; for a review of resting fMRI and various treatments, see [39]). These ASL findings have implications for future studies aimed to develop CBF as both a diagnostic and treatment biomarker in clinical populations. A follow-up study using the complete EMBARC outcomes dataset with 300 MDD participants may provide further information regarding the application of CBF in predicting treatment response in MDD.

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Compliance with ethical standards

Conflict of interest Crystal Cooper, Cherise Chin Fatt, Peiying Liu, Bruce Grannemann, Thomas Carmody, Jorge Almeida, Ashley Malchow, Melvin McInnis, Ramin Parsey, Elizabeth Bartlett, and Hanzhang Lu declare that they have no conflict of interest. Thilo Deckersbach's research has been funded by NIH, NIMH, NARSAD, TSA, IOCDF, Tufts University, DBDAT, Otsuka Pharmaceuticals, Cogito, Inc. and Sunovion. He has received honoraria, consultation fees and/or royalties from the MGH Psychiatry Academy, BrainCells Inc., Clintara, LLC., Systems Research and Applications Corporation, Boston University, the Catalan Agency for Health Technology Assessment and Research, the National Association of Social Workers Massachusetts, the Massachusetts Medical Society, Tufts University, NIDA, NIMH, and Oxford University Press. He has also participated in research funded by DARPA, NIH, NIMH, NIA, AHRQ, PCORI, Janssen Pharmaceuticals, The Forest Research Institute, Shire Development Inc., Medtronic, Cyberonics, Northstar, Takeda and AlphaSigma. Maurizio Fava has received research support from Abbott Laboratories; Acadia Pharmaceuticals; Alkermes, Inc.; American Cyanamid; Aspect Medical Systems; AstraZeneca; Avanir Pharmaceuticals; AXSOME Therapeutics; BioResearch; BrainCells Inc.; Bristol-Myers Squibb; CeNeRx BioPharma; Cephalon; Cerecor; Clintara, LLC; Covance; Covidien; Eli Lilly and Company; EnVivo Pharmaceuticals, Inc.; Euthymics Bioscience, Inc.; Forest Pharmaceuticals, Inc.; FORUM Pharmaceuticals; Ganeden Biotech, Inc.; GlaxoSmithKline; Harvard Clinical Research Institute; Hoffman-LaRoche; Icon Clinical Research; i3 Innovus/Ingenix; Janssen R&D, LLC; Jed Foundation; Johnson & Johnson Pharmaceutical Research & Development; Lichtwer Pharma GmbH; Lorex Pharmaceuticals; Lundbeck Inc.; MedAvante; Methylation Sciences Inc; National Alliance for Research on Schizophrenia & Depression (NARSAD); National Center for Complementary and Alternative Medicine (NCCAM); National Coordinating Center for Integrated Medicine (NiiCM); National Institute of Drug Abuse (NIDA); National Institute of Mental Health (NIMH); Neuralstem, Inc.; NeuroRx; Novartis AG; Organon Pharmaceuticals; PamLab, LLC.; Pfizer Inc.; Pharmacia-Upjohn; Pharmaceutical Research Associates, Inc.; Pharmavite® LLC; PharmoRx Therapeutics; Photothera; Reckitt Benckiser; Roche Pharmaceuticals; RCT Logic, LLC (formerly Clinical Trials Solutions, LLC); Sanofi-Aventis US LLC; Shire; Solvay Pharmaceuticals, Inc.; Stanley Medical Research Institute (SMRI); Synthelabo; Takeda Pharmaceuticals; Tal Medical; VistaGen); Wyeth-Ayerst Laboratories; he has served as advisor or consultant to Abbott Laboratories; Acadia; Affectis Pharmaceuticals AG; Alkermes, Inc.; Amarin Pharma Inc.; Aspect Medical Systems; AstraZeneca; Auspex Pharmaceuticals; Avanir Pharmaceuticals; AXSOME Therapeutics; Bayer AG; Best Practice Project Management, Inc.; Biogen; BioMarin Pharmaceuticals, Inc.; Biovail Corporation; BrainCells Inc; Bristol-Myers Squibb; CeNeRx BioPharma; Cephalon, Inc.; Cerecor; CNS Response, Inc.; Compellis Pharmaceuticals; Cypress Pharmaceutical, Inc.; DiagnoSearch Life Sciences (P) Ltd.; Dinippon Sumitomo Pharma Co. Inc.; Dov Pharmaceuticals, Inc.; Edgemont Pharmaceuticals, Inc.; Eisai Inc.; Eli Lilly and Company; EnVivo Pharmaceuticals, Inc.; ePharmaSolutions; EPIX Pharmaceuticals, Inc.; Euthymics Bioscience, Inc.; Fabre-Kramer Pharmaceuticals, Inc.; Forest Pharmaceuticals, Inc.; Forum Pharmaceuticals; GenOmind, LLC; GlaxoSmithKline; Grunenthal GmbH; Indivior; i3 Innovus/Ingenix; Intracellular; Janssen Pharmaceutica; Jazz Pharmaceuticals, Inc.; Johnson & Johnson Pharmaceutical Research & Development, LLC; Knoll Pharmaceuticals Corp.; Labopharm Inc.; Lorex Pharmaceuticals; Lundbeck Inc.; MedAvante, Inc.; Merck & Co., Inc.; MSI Methylation Sciences, Inc.; Naurex, Inc.; Nestle Health Sciences; Neuralstem, Inc.; Neuronetics, Inc.; NextWave Pharmaceuticals; Novartis AG; Nutrition 21; Orexigen Therapeutics, Inc.; Organon Pharmaceuticals; Osmotica; Otsuka Pharmaceuticals; PamLab,

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