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Introduction

Additional Exploratory Hypothesis

The main goal of the present study was to use a pattern recognition technique to classify depressed individuals, case by case, as either bipolar or unipolar depressed based on measures of resting blood flow using arterial spin labeling in different ACC subdivisions. In more exploratory analyses, we examined in clinical follow up which, if any, of those depressed individuals with a current unipolar depression diagnosis whom the pattern recognition algorithm classified as having bipolar depression, actually went on to develop a hypo/manic episode.

Method

Exclusion Criteria

Exclusion criteria included history of head injury (from medical records and participant report), systemic medical illness, cognitive impairment (score<24 in the Mini-Mental State Examination, premorbid IQ estimate<85 using the National Adult Reading Test), and general exclusion criteria for MRI (presence/questionable history of metallic objects in the body, positive pregnancy test/self-reporting of pregnancy, and proneness to panicking in enclosed spaces). Left-handed participants were also excluded. All females performed a saliva and urine screen on the day of the scan, and were free of current abuse of alcohol and illicit substance. For healthy females, previous history of alcohol and illicit substance abuse (determined by SCID-I, saliva and urine screen) were further exclusion criteria. All depressed females had a minimum duration of two months' free from alcohol and illicit substance abuse.

Medication Load

A problem for all neuroimaging studies of unipolar and bipolar depression is the potential confounding effect of psychotropic medication, as it is difficult to recruit medication-free subjects into such studies. We therefore computed medication load, an index that reflects the number and dose of different medications for each female (the greater the number and dose of the medication, the greater the medication load). This strategy has been employed in our previous neuroimaging studies (1-4).

Arterial Spin Labelling

Arterial spin labelling is a non-invasive perfusion magnetic resonance imaging technique to quantify cerebral blood flow. ASL is based upon the subtraction of two consecutively acquired images: one image with, and another image without, magnetically-labelled water in arterial blood(5-7). ASL uses an endogenous perfusion tracer (water) to measure resting cerebral blood flow, and is therefore a safe neuroimaging technique. In comparison, cerebral blood flow measured using positron emission tomography (PET) poses many disadvantages; such as: greater expense, the necessity for PET studies to be centered in academic institutions, and the invasive nature of PET due the use of radioligands. These features of PET studies seriously limit the application of the technique to widespread clinical practice. Published comparison between ASL and $H_2{}^{15}O$ positron emission tomography PET in healthy individuals demonstrate significant positive correlations between measures of resting cerebral blood flow in gray matter derived from the two-neuroimaging techniques (8).

Data Acquisition

Neuroimaging data were collected using a 3Tesla Siemens Trio MRI scanner at the Magnetic Resonance Research Center in UPMC. Structural 3D axial MPRAGE image (TE:3.29ms, TR:2200ms, Flip angle9°, FOV=256x192mm, slice thickness:1mm, Matrix:256x256, 192 continuous slices), and 40 pairs of pulsed ASL (pASL) functional images (5min22sec of duration, whole brain coverage with 21 axial slices, voxel size:3.8x3.8x5mm^3, TR/TE=4000/18msec, FOV=240x240mm, matrix=64x64; Flip angle90°; post-inversion delay of 700msec; IR slab thickness: 142mm) were acquired in the same session.

Imaging Data Analysis

For each participant, functional images were first realigned to correct for head motion. Subjects with a range of movement larger than 4mm were excluded. None of our participants moved more than 4mm, and, therefore, we did not exclude any subject from the analysis due head movement. Next, the average of the images was coregistered with the anatomical image and segmented. Perfusion weighted image series were then generated by pairwise subtraction of the label and control functional images, followed by conversion to absolute cerebral blood flow (CBF) image series based on a single compartment pASL perfusion moSdel (5-7, 9, 10). One mean CBF image was generated for each participant, normalised to the Montreal Neurological Institute (MNI) template using the parameters derived from the segmentation, resampled to 2x2x2 mm and smoothed with full-width at half maximum at 8mm kernel.

Grey matter volume

As in our previous study (11), structural images were segmented into grey and white matter (GM and WM, respectively), normalised and resampled (2x2x2mm) using a unified model (12). Voxel values were modulated. Finally, images were smoothed with a 12-mm Gaussian kernel.

Pattern Recognition Analysis Using ACC Blood Flow and Grey Matter Volume

The PROBID toolbox (www.kcl.ac.uk/iop/depts/neuroimaging/research/imaginganalysis/Software/PROBID.aspx) was used to perform the pattern classification analysis. PROBID is a Matlab toolbox for pattern recognition analysis of brain imaging data. In PROBID each brain image defines a spatial pattern and it can include whole brain voxels or voxels within a region of interest. The support vector machine (SVM) option in PROBID uses a linear kernel, which helps prevent overfitting and allows direct extraction of the classifier's weights as an image(i.e. discriminating pattern). The parameter C, which controls the trade-off between having zero training errors and allowing misclassification, was set to 1 for all cases. To classify a new example, the classifier computes a weighted sum of the voxel activity values within the region used to define the spatial pattern and passes it through a decision function. This value corresponds to the distance to the decision boundary or test margin. In the case of SVM, if the test margin is above zero, the individual subject is classified as group 1; if the test margin is below zero, the individual subject is classified as group 2.

For pattern recognition based classification, each brain image is treated as a point in a high dimensional space (space dimension=number of voxels in the image). The task of classifying a set of images into two classes (e.g., unipolar vs. bipolar depression) can thus be viewed as a task of finding a separating hyperplane or decision boundary between the two groups. The

classification procedure consists of two phases: training and testing. During the training phase, the algorithm finds a hyperplane that separates the training examples in the input space according to their class labels. The classifier is trained by providing examples of the form <**x**,c>, where **x** represents a spatial pattern (e.g., the pattern of cerebral blood flow or greymatter volume across voxels within a region of interest) and c is the group membership(e.g., unipolar depression or bipolar depression). Once the decision function is learned from the training data, it can be used to predict the group membership of test individuals. The SVM algorithm(13) finds the largest margin hyperplane. The margin is the distance from the separating hyperplane to the closest training examples. It has been demonstrated that the optimal hyperplane is the one with maximal margin(i.e., more separation between the classes). A larger margin corresponds to better generalisation performance. A more detailed description of the SVM can be found in (14, 15). The PROBID methods are discussed in detail in previous studies by Mourao-Miranda et al, and Ecker et al(16, 17). We evaluated the performance of SVM to discriminate between groups using a leave-one-pair-out cross validation test. Here, we first used data from all but one individual in each group to train the classifier. Then, we predicted group membership using the brain scans of the remaining individual (one per each group).

Permutation testing was then used to derive a p-value for the SVM accuracies. Here, we permuted each group's labels 1000 times (i.e., each time randomly assigning group 1and group 2 labels to each pattern of brain activation) and repeated the cross-validation procedure. We then counted the number of times the accuracy was higher than the one obtained for the real labels. Dividing this number by 1000 we derived a p-value for the classification accuracies.

Univariate Analysis of Anterior Cingulate Cortical Blood Flow and GreyMatter Volume

For completeness, we also report parallel analyses of the same data set using standard neuroimaging approaches: specifically, with pairwise comparisons with standard analysis with general linear model based on random field theory for each neuroimaging modality (cerebral blood flow or greymatter volume; Table DS5).

Region of Interest Masks

Region-of-interest (ROI) analyses were performed on a single mask comprising bilateral anterior cingulate cortices, derived from TD_Labels_Anterior_Cingulate added with bilateral TD_brodmann_areas_25 from the toolbox WFUpickatlas (correction for multiple comparison based on a cluster-level false positive detection rate at $p<0.05$ using a voxel threshold of $p<0.05$ with a cluster (k) extent empirically determined by Monte Carlo simulations implemented in AlphaSim). Total cerebral blood flow and age were entered as covariates in between-group cerebral blood flow comparisons; and total wholebrain greymatter volume and age were entered as covariates in between-group greymatter comparisons.

We then examined between group differences in anterior cingulate cortical blood flow after covarying additionally for mean greymatter volume extracted from the above bilateral anterior cingulate cortical anatomical ROI masks.

Exploratory Analyses

In exploratory analyses, cerebral blood flow and greymatter volume measures showing significant abnormalities in univariate and multivariate analysis were also explored for possible relationships with: age, age of illness onset, illness duration, depression severity (measured using

the HRSD25), mania severity (measured using the Young Mania Rating Scale), medication load, taking versus not taking individual psychotropic medication classes, and lifetime history of comorbid anxiety and/or substance disorder. Given the exploratory nature of these analyses, we used a statistical threshold of p=0.05.

Assessing Manic/hypomanic Episode in Clinical Follow-up

By liaison with the clinical team responsible for management of depressed study participants, and with approval from UPMC Institutional Review Board, we were able to follow up as far as three years and a six months(range 11 months–3 years and 6 months) following the scanning assessment all unipolar depressed females receiving a ”false positive” bipolar disorder diagnosis in pattern classification analyses. This allowed us to determine if any of these individuals did in fact go on to develop a hypo/manic episode, and thereby examine the extent to which our pattern recognition approach was able to detect bipolar disorder at earlier stages than clinical assessment with DSM-IV criteria.

Results

Pattern Recognition Analysis Using ACC GreyMatter Volume

None of the between-group pairwise comparisons using pattern recognition analysis based on SVM using the pattern of regional greymatter volume across voxels was significant for any of the three ACC subdivisions (Table DS4).

Univariate Analysis of Anterior Cingulate Cortical Blood Flow and GreyMatter Volume

Resting Anterior Cingulate Cortical Blood Flow

There was a significant increase in cerebral blood flow in a large area of the rostral/perigenual anterior cingulate cortex (BA24 and 32) in females with unipolar depression relative to females with bipolar depression (t=3.1, cluster size=402 voxels, p=0.002 corrected; Table DS5).

Females with unipolar depression also showed a significant increase in cerebral blood flow in rostral anterior cingulate cortex (BA32) relative to healthy females (t=3, cluster size=248 voxels, p=0.003 corrected; Table DS5).

Females with bipolar depression showed a significant decrease in cerebral blood flow in all subdivisions of the anterior cingulate cortex (BA25, 24 and 32) relative to healthy females (cluster size=262, BA25: t=2.85, p=0.004; BA24: t=2.45, p=0.01; BA32: t=2.24, p=0.016 corrected; Table DS5)

Anterior Cingulate Cortical GreyMatter Volume

None of the three pairwise comparisons was significant. Females with bipolar depression showed increases in greymatter volume in rostral/perigenual anterior cingulate cortex relative to healthy females (BA24/32), and unipolar depressed females (BA 32), but these comparisons failed to meet significance (Table DS5).

Resting State Anterior Cingulate Cortical Blood Flow covaried for GreyMatter Volume

Including regional anterior cingulate cortical greymatter volume as a covariate in analysis of between group differences in anterior cingulate cortical cerebral blood flow revealed an even larger cluster of increased blood flow in the anterior cingulate cortex, now including the subgenual division, in females with unipolar depression (BA25, 32 and 24, t=3.14, cluster=445 voxels, p=0.002 corrected, Table DS5) relative to females with bipolar depression.

After covarying for greymatter volume, the between-group differences in cerebral blood flow for comparisons of depressed relative to healthy females were reduced, both in cluster size and in magnitude of effect size (Table DS5).

Exploratory Analyses

In females with unipolar depression, there were negative correlations between cerebral blood flow and age at scan (BA32: r=-0.47, p=0.049, Table DS7a: older females had lower blood flow), depression severity (BA32: r=-0.485, p=0.041: more severe depression had lower blood flow), previous history of substance abuse (BA32: t(13.9)=2.23, p=0.043: those with positive history had lower blood flow), and comorbid anxiety disorder (BA24: t(16)=2.13, p=0.049: those with positive history had lower blood flow). In females with bipolar depression, there was a relationship with cerebral blood flow and benzodiazepine use (BA25: t(15.7)=2.58, p=0.02: those taking benzodiazepine had higher blood flow; Table DS7a. Healthy females relationships were not significant (Table DS7b).

Interestingly, older age, greater depression severity, history of substance abuse, and comorbid anxiety disorder were all associated with reduced blood flow in rostral/perigenual anterior cingulate cortex, a pattern of blood flow approximating to that observed in bipolar depressed females. Furthermore, females with bipolar depression taking benzodiazepines had higher subgenual anterior cingulate cortical blood flow, which might represent a normalising effect of this medication, given that this pattern of regional blood flow approximates to the pattern seen in healthy females

Manic/hypomanic Episode Onset in Clinical Follow-up

Two females out of the four females with a diagnosis of unipolar depression at study entry who were misclassified as having bipolar depression (i.e. “false positives”), based on pattern recognition analyses with subgenual cingulate cortical blood flow (Figure 1), went on to develop a manic/hypomanic episode requiring hospitalisation or treatment approximately one year and seven months after taking part in the neuroimaging study.

We currently have only partial follow-up related to the unipolar depressed sample from a relatively short period of time. Future studies can also aim to replicate our present findings in independent samples of bipolar I and unipolar depressed females; and with longer follow-up durations.

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Table DS1. Demographic and Clinical Variables

Group	HF (n=18)		UD (n=18)		BD (n=18)		stat.	p
Age at Scan (mean/SD)	32.9	6.2	32.5	8.5	32.1	8.8	F(2,53)=0.044	0.96
Age of Illness Onset (mean/SD)	-		18.1	9.0	17.0	5.7	t(34)=0.443	0.7
Illness Duration (mean/SD)	-		14.4	9.1	15.1	7.8	t(34)=0.264	0.8
Medication Load (mean/SD)	-		2.2	1.5	3.1	2.5	t(27.8)=1.31	0.2
HRSD-25 (mean/SD)	1.8	2.6	28.2	6.5	24.2	8.4	F(2,53)=91.28	**<0.001**[a]
YMRS (mean/SD)	0.8	1.4	4.4	2.4	4.0	2.8	F(2,53)=13.29	**<0.001**[b]
NART (mean/SD)	110.1	6.8	113.6	7.9	113.7	9.1	F(2,53)=1.2	0.31
Mean Wholebrain Cerebral Blood Flow	44.7	4.3	41.5	4.3	41.8	4.6	F(2,53)=2.773	0.07
Mood Stabilizators (ON/OFF - percent yes)	-		1/17	5.6	10/8	55.6	$x^2(1)=10.6$	**0.001**
Anti Psychotic Medications (ON/OFF - percent yes)	-		2/16	11.1	9/9	50	$x^2(1)=6.415$	**0.01**
Anti Depressants (ON/OFF - percent yes)	-		15/3	83.3	7/11	38.9	$x^2(1)=7.481$	**0.01**
Benzodiazepines (ON/OFF - percent yes)	-		5/13	27.8	3/15	16.7	$x^2(1)=0.643$	0.4
Lifetime Presence of Anxiety Disorders (Yes/No - percent yes)	-		13/5	72.2	12/6	66.7	$x^2(1)=0.131$	0.7
Lifetime Presence of Alcohol/Drug Abuse or Dependence (Yes/No - percent yes)	-		6/12	33.3	6/12	33.3	$x^2(1)=0$	>0.9

HF: healthy Females; UD: females with unipolar depression in depressed episode; BD: females with bipolar disorder type 1 in depressed episode, SD: standard deviation; HRSD-25: 25-item Hamilton Rating Scale for Depression; YMRS: Young Mania Rating Scale; stat.: statistical test value; p: p value; x^2=chi-square test; t= independent t-test; NART: National Adult Reading Test; a: Post hoc analysis revealed no differences UD vs. BD (t(34)=1.6, p=0.1; and increased HRSD-25 scores in UD (t(22.3)=16, p=0.001) and BD (t(20)=10, p=0.001)when compared to HF. b: Post hoc analysis revealed no differences UD vs. BD (t(34)=0.4, p=0.7; and increased YMRS scores in UD (t(28)=5.4, p=0.001) and BD (t(25)=4.3, p=0.001)when compared to HF.

Table DS2. Medication List of each Individual

Females with Unipolar Depression

Case	Medications
1	lithium (900mg), trazodone (100mg), clonazepam (0.5mg)
2	not taking
3	escitalopram (20mg)
4	venlafaxine (225mg), trazodone (200mg)
5	citalopram (10mg)
6	bupropion (450mg)
7	aripiprazole (10mg), venlafaxine (300mg), zolpidem (10mg)
8	fluvoxamine (400mg), clonazepan (0.5mg), risperidone (0.5mg)
9	citalopram (20mg)
10	bupropion (300mg)
11	citalopram (60mg)
12	fluvoxamine (400mg), clonazepan (1mg)
13	not taking
14	duloxetine (60mg)
15	citalopram (40mg), zolpidem (5mg)
16	not taking
17	venlafaxine (300mg), bupropion (450mg)
18	bupropion (300mg), escitalopram (10mg), trazodone (100mg)

Females with Bipolar Depression

1	not taking
2	valproic acid (500mg), quetiapine (150mg)
3	venlafaxine (150mg), clonazepam (1mg), quetiapine (100mg), aripiprazole (15mg)
4	lamotrigine (100mg), lithium (900mg)
5	quetiapine (50mg)
6	not taking
7	not taking
8	carbamazepine (400mg), risperidone (0.5mg), lithium (900mg)
9	venlafaxine (300mg), lamotrigine (175mg), quetiapine (100mg), ziprasidone (60mg), eszopiclone (2mg)
10	duloxetine (90mg), clonazepam (0.5mg), risperidone (4mg)
11	lamotrigine (300mg), carbamazepine (400mg), topiramato (200mg), lorazepam (1mg), buspirone (15mg)
12	lamotrigine (200mg), clomipramine (200mg), risperidone (3mg)
13	not taking
14	valproic acid (500mg), trazodone (100mg)
15	bupropione (400mg), lamotrigine (400mg), lithium (600mg)
16	ziprasidone (100mg), lamotrigine (200mg), bupropione (200mg)
17	valproic acid (500mg), atomoxetine (80mg)
18	quetiapine (150mg)

Table DS3: Pattern Recognition Analysis Discriminating Unipolar and Bipolar Depression and Healthy Females using Cerebral Blood Flow at Rest

	Sensitivity (%)	Specificity (%)	Accuracy (%)	p-value
BD (n=18) x UD (n=18)				
resting cerebral blood flow				
rostral ACC (BA32)	61.1	50.0	55.6	0.30
rostral/perigenual ACC (BA24)	61.1	66.7	63.9	0.07
subgenual ACC (BA25)	83.3	77.8	80.6	**0.001**
wholebrain	33.3	38.9	36.1	0.97
BD (n=18) x HF (n=18)				
resting cerebral blood flow				
rostral ACC (BA32)	50.0	44.4	47.2	0.69
rostral/perigenual ACC (BA24)	44.4	61.1	52.8	0.45
subgenual ACC (BA25)	38.9	44.4	41.7	0.88
wholebrain	44.4	50.0	47.2	0.73
UD (n=18) x HF (n=18)				
resting cerebral blood flow				
rostral ACC (BA32)	44.4	55.6	50.0	0.57
rostral/perigenual ACC (BA24)	50.0	83.3	66.7	**0.02**
subgenual ACC (BA25)	50.0	55.6	52.8	0.42
wholebrain	22.2	50.0	36.1	0.96

ACC: anterior cingulate cortex; BA, Brodmann area; HF: healthy Females; UD: females with unipolar depression in depressed episode; BD: females with bipolar disorder type 1 in depressed episode

Table DS4: Pattern Recognition Analysis Discriminating Unipolar and Bipolar Depression and Healthy Females using Gray Matter Volume

UD (n=11) x BD (n=11)	Sensitivity (%)	Specificity (%)	Accuracy (%)	p-value
BD (n=18) x UD (n=18)				
Gray Matter Volume				
rostral ACC (BA32)	27.8	27.8	27.8	1.00
rostral/perigenual ACC (BA24)	33.3	44.4	38.9	0.98
subgenual ACC (BA25)	22.2	66.7	44.4	0.89
wholebrain	61.1	55.6	58.3	0.13
BD (n=18) x HF (n=18)				
Gray Matter Volume				
rostral ACC (BA32)	50.0	33.3	41.7	0.90
rostral/perigenual ACC (BA24)	27.8	44.4	36.1	0.98
subgenual ACC (BA25)	5.6	22.2	13.9	1.00
wholebrain	61.1	55.6	58.3	0.16
UD (n=18) x HF (n=18)				
Gray Matter Volume				
rostral ACC (BA32)	33.3	38.9	36.1	0.98
rostral/perigenual ACC (BA24)	33.3	50.0	41.7	0.92
subgenual ACC (BA25)	50.0	50.0	50.0	0.59
wholebrain	50.0	61.1	55.6	0.31

ACC: anterior cingulate cortex; BA, Brodmann area; HF: healthy Females; UD: females with unipolar depression in depressed episode; BD: females with bipolar disorder type 1 in depressed episode

Table DS5. Anterior Cingulate Cortical Gray Matter Volume and Blood Flow in Females with Unipolar and Bipolar Depression and in Healthy Females

		MNI coordinates						
Region	**Side**	**X**	**Y**	**Z**	**K**	**t**	**z**	**p**
resting ACC blood flow								
UD >HF								
rostral ACC (BA32)	L	-16	24	28	248	3	2.8	0.003
rostral ACC (BA32)	L	-14	34	14		2.74	2.57	0.005
rostral ACC (BA32)	L	-16	46	10		2.49	2.36	0.009
BD < HF								
subgenual ACC (BA25)	R	2	18	-2	262	2.85	2.67	0.004
rostral/perigenual ACC (BA24)	R	4	32	16		2.45	2.33	0.01
rostral ACC (BA32)	R	2	46	-4		2.24	2.15	0.016
UD > BD								
rostral/perigenual ACC (BA24)	R	4	24	-2	402	3.09	2.87	0.002
rostral/perigenual ACC (BA24)	R	4	32	16		2.99	2.79	0.003
rostral ACC (BA32)	R	2	46	-4		2.57	2.43	0.007
ACC gray matter volume								
BD > HF								
rostral ACC (BA32)	R	14	26	28	42	2.08	2	0.023 unc
rostral/perigenual ACC (BA24)	R	8	18	20		2.06	1.99	0.023 unc
BD > UD								
rostral ACC (BA32)	R	18	50	0	62	3.04	2.83	0.002 unc
rostral ACC (BA32)	R	18	24	28	126	2.62	2.48	0.007 unc
rostral ACC (BA32)	L	-12	36	6	78	2.54	2.41	0.008 unc
resting ACC blood flow covaring for local gray matter volume								
UD > HF								
rostral ACC (BA32)	L	-16	24	28	171	2.76	2.59	0.005
rostral ACC (BA32)	L	-14	34	14		2.47	2.34	0.01
rostral ACC (BA32)	L	-16	46	10		2.4	2.29	0.011
BD <HF								
subgenual ACC (BA25)	R	2	18	-2	218	2.8	2.63	0.004
rostral/perigenual ACC (BA24)	R	4	32	16		2.37	2.26	0.012
rostral ACC (BA32)	R	2	46	-4		2.21	2.12	0.017
UD > BD								
subgenual ACC (BA25)	R	2	18	-2	445	3.14	2.91	0.002
rostral/perigenual ACC (BA24)	R	4	32	14		2.97	2.76	0.009
rostral ACC (BA32)	R	2	46	-4		2.54	2.4	0.008

ACC: anterior cingulate cortex; sgACC: subgenual anterior cingulate cortex;R, right; L, left; BA, Brodmann area; Coordinates correspond to the stereotaxic array of Montreal Neurologic Institute; k: cluster size; HF: healthy Females; UD: females with unipolar depression in depressed episode; BD: females with bipolar disorder type 1 in depressed episode

Table DS6. Relation Between Support Vector Machine Test Margin with Clinical and Demographic Variables in females with Unipolar Depression and Bipolar Depression

	Test Margin - ACC blood flow							
	subgenual ACC (BA25)				rostral/perigenual ACC (BA24)			
	UD (n=18)		BD (n=18)		UD (n=18)		HF (n=18)	
	r	p value	r	p value	r	p value	r	p value
Age at Scan	-0.4	0.1	-0.1	0.7	-0.3	0.3	0.5	0.05
Age of Illness Onset	-0.2	0.4	-0.2	0.5	-0.02	0.9	-	
Illness Duration	-0.2	0.5	0.02	0.9	-0.2	0.3	-	
Medication Load	-0.3	0.2	0.1	0.7	0.1	0.7	-	
HRSD-25	-0.3	0.2	-0.3	0.2	0.04	0.9	0.3	0.2
YMRS	-0.4	0.1	-0.3	0.3	-0.2	0.4	-0.1	0.7
	t-test	p value	t-test	p value	t-test	p value	t-test	p value
Mood Stabilizators (ON/OFF)	0.6	0.6	0.6	0.5	1.3	0.2	-	
Anti Psychotic Medications (ON/OFF)	0.4	0.7	2.0	0.1	0.3	0.8	-	
Anti Depressants (ON/OFF)	0.8	0.5	0.4	0.7	1.6	0.1	-	
Benzodiazepines (ON/OFF)	0.3	0.8	0.6	0.5	0.2	0.9	-	
Lifetime History of Substance Abuse or Dependence (YES/NO)	0.9	0.4	1.1	0.3	0.6	0.6	-	
Lifetime Presence of Anxiety Disorder (YES/NO)	1.1	0.3	1.0	0.3	0.2	0.8	-	

HF: healthy Females; UD: females with unipolar depression in depressed episode; BD: females with bipolar disorder type 1 in depressed episode; ACC: anterior cingulate cortex; HRSD-25: 25-item Hamilton Rating Scale for Depression; YMRS: Young Mania Rating Scale; t-test: independent t-test; r: Pearson correlation

Table DS7a. Univariate Relationship Between ACC Blood Flow and Clinical and Demographic Variables in Females with Unipolar Depression and Bipolar Depression

	ACC blood flow															
	UD greater HF		BD lesser HF						UD greater BD							
	rostral ACC (BA32)		subgenual ACC (BA25)		rostral / perigenual ACC (BA24)		rostral ACC (BA32)		rostral/perigenual ACC (BA24)				rostral ACC (BA32)			
	UD (n=18)		BD (n=18)		BD (n=18)		BD (n=18)		UD (n=18)		BD (n=18)		UD (n=18)		BD (n=18)	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
Age at Scan	-0.5	**0.05**	-0.3	0.3	-0.1	0.7	0.0	0.9	-0.3	0.3	-0.4	0.1	-0.1	0.6	0.0	0.9
Age of Illness Onset	-0.1	0.8	0.1	0.6	-0.3	0.2	0.0	1.0	-0.1	0.8	0.0	1.0	-0.5	**0.04**	0.0	1.0
Illness Duration	0.0	0.9	0.1	0.8	-0.3	0.2	0.0	0.9	-0.2	0.4	0.1	0.8	-0.4	0.1	0.0	0.9
Medication Load	-0.2	0.5	-0.1	0.7	-0.1	0.7	-0.2	0.4	-0.2	0.4	-0.1	0.6	-0.1	0.8	-0.2	0.4
HRSD-25	-0.3	0.3	-0.2	0.4	-0.1	0.8	0.1	0.7	0.0	0.9	-0.3	0.2	-0.1	0.8	0.1	0.7
YMRS	0.2	0.4	0.2	0.5	0.1	0.8	0.1	0.7	0.1	0.6	0.2	0.4	-0.1	0.6	0.1	0.7
	t-test	p	t-test	p	t-test	p	t-test	p	t-test	p	t-test	p	t-test	p	t-test	p
Mood Stabilizators (ON/OFF)	1.4	0.2	-0.8	0.4	-0.8	0.5	-0.7	0.5	2.3	**0.04**	-0.8	0.4	2.1	**0.05**	-0.7	0.5
Anti Psychotic Medications (ON/OFF)	1.3	0.2	1.8	0.1	0.3	0.7	0.3	0.8	0.3	0.8	1.6	0.1	-0.2	0.8	0.3	0.8
Anti Depressants (ON/OFF)	1.4	0.2	0.9	0.4	0.4	0.7	1.2	0.2	1.1	0.3	1.5	0.2	0.1	0.9	1.2	0.2
Benzodiazepines (ON/OFF)	0.9	0.4	2.6	**0.02**	-0.2	0.9	-0.1	1.0	1.0	0.3	0.8	0.4	0.9	0.4	-0.1	1.0
Lifetime Presence of Alcohol/Drugs Abuse or Dependence (YES/NO)	0.6	0.6	-0.5	0.6	-0.4	0.7	0.1	0.9	0.6	0.6	0.0	1.0	1.6	0.1	0.1	0.9
Lifetime Presence of Anxiety Disorder (YES/NO)	1.7	0.1	-1.3	0.2	1.0	0.4	0.4	0.7	2.1	**0.05**	-0.9	0.4	1.8	0.1	0.4	0.7

HF: healthy Females; UD: females with unipolar depression in depressed episode; BD: females with bipolar disorder type 1 in depressed episode; ACC: anterior cingulate cortex; HRSD-25: 25-item Hamilton Rating Scale for Depression; YMRS: Young Mania Rating Scale; t-test: independent t-test; r: Pearson correlation

Table DS7b. Univariate Relationship Between ACC Blood Flow and Clinical and Demographic Variables in Healthy Females

	ACC blood flow							
	UD greater HF		BD lesser HF					
	rostral ACC (BA32)		subgenual ACC (BA25)		rostral / perigenual ACC (BA24)		rostral ACC (BA32)	
	HF (n=18)		HF (n=18)		HF (n=18)		HF (n=18)	
	r	p	r	p	r	p	r	p
Age at Scan	0.2	0.5	0.2	0.4	-0.2	0.4	-0.1	0.8
HRSD-25	0.4	0.1	0.4	0.1	-0.1	0.6	0.1	0.6
YMRS	0.2	0.4	-0.1	0.7	-0.1	0.6	-0.3	0.2

HF: healthy Females; UD: females with unipolar depression in depressed episode; BD: females with bipolar disorder type 1 in depressed episode; ACC: anterior cingulate cortex; HRSD-25: 25-item Hamilton Rating Scale for Depression; YMRS: Young Mania Rating Scale; t-test: independent t-test; r: Pearson correlation