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Cerebrospinal Fluid Metabolome in Mood Disorders-Remission State has a Unique Metabolic Profile

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Targeted metabolomics provides an approach to quantify metabolites involved in specific molecular pathways. We applied an electrochemistry-based, targeted metabolomics platform to define changes in tryptophan, tyrosine, purine and related pathways in the depressed and remitted phases of major depressive disorder (MDD). Biochemical profiles in the cerebrospinal fluid of unmedicated depressed (n=14; dMDD) or remitted MDD subjects (n=14; rMDD) were compared against those in healthy controls (n=18; HC). The rMDD group showed differences in tryptophan and tyrosine metabolism relative to the other groups. The rMDD group also had higher methionine levels and larger methionine-to-glutathione ratios than the other groups, implicating methylation and oxidative stress pathways. The dMDD sample showed nonsignificant differences in the same direction in several of the metabolic branches assessed. The reductions in metabolites associated with tryptophan and tyrosine pathways in rMDD may relate to the vulnerability this population shows for developing depressive symptoms under tryptophan or catecholamine depletion.

The biochemical underpinnings of major depressive disorder (MDD) and of this condition's phenotypically distinct illness phases, namely depression versus remission, have only been partly characterized. While multiple neurotransmitter systems appear dysregulated in MDD, the greatest amount of empirical research has focused upon the role of impairments in central monoaminergic function[1–4]. A major limitation of previous studies, however, is that they have not examined multiple compounds within a pathway or multiple interconnected pathways simultaneously.

The application of novel metabolomics technology enables mapping of biochemical pathways implicated in disease[5–10]. Through metabolomic profiling researchers recently have identified initial metabolic signatures for a variety of central nervous system (CNS) disorders, including depression, schizophrenia, and Parkinson's disease[11–18]. For example, preliminary metabolomic studies using plasma of MDD[11–12] patients revealed that the depressed state is associated with changes in amino acids, neurotransmitters including gamma-aminobutyric acid (GABA), dicarboxylic fatty acids and lipids. Nevertheless, no previous metabolite profiling study has been performed on the cerebrospinal fluid (CSF), the biofluid believed to be most closely linked to brain function[19].

Previous studies examined CSF concentrations of the primary metabolites of serotonin, dopamine and norepinephrine (i.e., 5-hydroxyindoleacetic acid [5-HIAA], homovanillic acid [HVA] and 4-hydroxy-3-methoxyphenyl glycol [MHPG], respectively) in currently depressed subjects. These studies found lower concentrations of HVA, and no significant difference in MHPG, in depressed subjects with *melancholic features* (i.e., a subgroup characterized by more prominent abnormalities of weight/appetite, thought, movement, and incapacity to experience pleasure) relative to controls[20], although variable results were obtained in samples selected more generally according to the MDD criteria alone[21]. Similarly, abnormal reductions in the CSF 5-HIAA concentrations were identified

in some but not most currently-depressed samples[22–23], although in the positive studies this abnormality sometimes was associated more specifically with clinical manifestations of aggression or suicidal behavior[24–25]. These previous studies did not, however, assess the expanded metabolomic profile where many monoaminergic neurotransmitter metabolites are measured quantitatively and simultaneously.

Moreover, few studies assessed monoaminergic neurotransmitter metabolites in the remitted phase, and the extant data from these studies remain inconclusive. Preliminary observations suggested that 5-HIAA levels may be abnormally reduced in a subgroup of individuals in the remitted phase of MDD[21,26–27]. In contrast, a previous study that assessed CSF HVA levels in remitted MDD subjects[27] did not detect a significant difference relative to currently depressed subjects or healthy controls .

Here we applied a targeted electrochemistry-based metabolomics platform to interrogate perturbations in the neurotransmitter pathways involving serotonin, dopamine and norepinephrine, and in related pathways for methionine and purine in both the symptomatic and recovered phases of MDD. A total of 26 metabolites were measured quantitatively and simultanously that cover the conversion of tryptophan to 5-HIAA and kynurenine (KYN), and the conversion of tyrosine (TYR) to catecholamines and HVA. Our aim was to characterize a possible altered biochemical profile in the CSF of participants with MDD studied while either depressed or remitted.

Results

Participant demographics. The baseline demographic characteristics of the groups are shown in Table 1. The subject groups did not differ significantly in gender, age or body mass index. There were a greater proportion of African-American individuals in the dMDD group.

Metabolic signatures for dMDD and rMDD - Analysis within pathways. Using an electrochemistry based metabolomics platform we quantified 26 metabolites within the tryptophan, tyrosine, purine, and methionine pathway (Supplemental Tables 1,2 and Figures 1a–d). We compared levels and ratios of metabolites within pathways and across pathways between the dMDD, rMDD and HC groups using univariate and multivariate approaches.

Tryptophan pathway. Analyses of the tryptophan pathway metabolites (N = 4, Table 2) and ratios (N = 4) (Supplemental Tables 1, 2 for names of metabolites and abbreviations) showed significant main effects of group for the 5-HIAA concentration ($p = .01$, Figure 2a) and the 5-HIAA/TRYP ($p = .008$, Figure 2b.) and 5-HIAA/KYN ($p = .0057$) ratios. Follow-up tests (Bonferroni-adjusted alpha=.017 for significance) revealed that the rMDD group had significantly lower levels of 5-HIAA than both the dMDD group ($p = .007$) and the HC group ($p = .014$) and significantly smaller 5-HIAA/TRYP and 5-HIAA/KYN ratios than both the dMDD group (ps = .006 and .002, respectively) and the HC group (ps = .006 and .007, respectively).

Tyrosine pathway. Analyses of the tyrosine pathway metabolites (N = 8, Table 2) and ratios (N = 10) (Supplemental Tables 1, 2) showed significant main effects of group for HVA concentrations ($p = 0.02$, See Figure 2c.) and the TYR/4HPLA ratio ($p<0.02$, See Figure 2d.). The test for HVA/MHPG ($p = 0.01$, Bonferroni alpha=0.008) was not considered significant using the Bonferroni adjustment, but was considered significant when correction for pFDR was applied. Follow-up tests (Bonferroni alpha = .017 for significance) revealed that the rMDD group had significantly lower levels of HVA than the HC group ($p = 0.007$). Examination of the ratios revealed that the rMDD group had a significantly larger TYR/4HPLA ratio than the HC group ($p = 0.008$). The rMDD group also had a significantly smaller HVA/MHPG ratio ($p = 0.003$) than the HC group. There was no significant difference between the HC and dMDD groups.

Examination of the unadjusted p-values associated with the Kruskal-Wallis tests revealed a non significant trend toward a group difference in the HVA/TYR ratio ($p = .024$). The rMDD group had a smaller HVA/TYR ratio than the HC group.

Purine pathway. None of the tests of the purine pathway metabolites (N = 7, Table 2) or the within pathway ratios (N = 10, Supplemental Tables 1, 2) identified significant differences. The hypoxanthine and

Table 1 | Clinical and Demographic Characteristics of currently depressed subjects with major depressive disorder (MDD), MDD subjects in full remission, and healthy controls

Characteristic	Healthy Control	Remitted MDD	Depressed MDD
Sample size (n)	18	14	14
Age (yrs): mean ± SD	40 ± 11	45 ± 9.5	38 ± 13
Gender (% male)	44	47	57
Body Mass Index: mean ± SD	28.5 ± 5.54	29.0 ± 7.58	26.5 ± 4.23
Race (% Caucasian)	78	89	36[a]
Current cigarette smoker: n (% of cases)		4 (29)	6 (43)
Past suicide attempts: n (% of cases)	0 (0)	2 (14)	3 (21)
Age at illness-onset (yrs): mean ± SD	n.a.	25 ± 11	18 ± 6.1
Illness duration[b] (yrs): mean ± SD (range)	n.a.	21 ± 12 (2 – 37)	21 ± 14 (4 – 43)
Time in remission (mos): mean ± SD (range)	n.a.	58 ± 52 (9 – 156)	n.a.
Naïve to antidepressant treatment: n (%)	18 (100)	4 (29)	2 (14)
Time unmedicated[c] (mos): mean ± SD (range	n.a.	44 ± 34 (3 – 103)	49 ± 59 (3 – 204)
Number of previous depressive episodes	n.a.	9	11
≥ 3 episodes (n)			
2 episodes (n)	n.a.	4	2
1 episode (n)	n.a.	1	1
MADRS	0 ± 0	1.3 ± 3.3	23 ± 10
HAM-D (17 item)	0 ± 0	1.6 ± 2.9	17 ± 5.9
HAM-D (A1-8 atypical feature items)	0 ± 0	0.5 ± 1.2	7.7 ± 3.5
HAM-A	0.06 ± 0.24	1.1 ± 1.9	11 ± 5.9

a. includes one Hispanic individual.
b. defined as the difference between current age and age-at illness-onset (years).
c. limited to subjects who were not naïve to antidepressant drug treatment.
Abbreviations: HAM-D – Hamilton Rating Scale for Depression; HAM-A – Hamilton Rating Scale for Anxiety; MADRS – Montgomery-Asberg Depression Rating Scale; SD – standard deviation.

Table 2 | Kruskal-Wallis One-Way ANOVA comparing remitted MDD, depressed MDD, and Healthy Control samples

Tryptophan Pathway					
	Median,HC (frstqrt,HC, thrdqrt,HC)	Median dMDD (frstqrt, dMDD, thrdqrt,dMDD)	Median rMDD (frstqrt,R, thrdqrt,R)	P value[1]	Significant Pair Test Results
5HIAA[3]	11.083 (5.73, 19.85)	11.887 (6.85, 15.03)	3.6 (2.03, 6.19)	0.014	rMDD<dMDD rMDD< HC
TRYP[3]	503.81 (440.65, 580.55)	504.09 (431.82, 599.93)	570.78 470.41, 624.10)	0.48[2]	
TPOL	1.498 (0.184,4.479)	0.897 (0.401, 1.674)	0.93 (0.24, 5.065)	0.9968	
KYN[3]	5.41 (4.7949, 6.2069)	5.14 (3.6376, 6.8153)	6.23 (5.5571, 7.0594)	0.81[2]	
KYN/TRYP	0.0113 (0.0092,0.0147)	0.0096 (0.0074, 0.0141)	0.0141 (0.0092, 0.0133)	0.38	
5HIAA/TRYP[3]	0.026 (0.012, 0.036)	0.025 (0.012, 0.033)	0.006 (0.004, 0.016)	0.0081	rMDD<dMDD rMDD< HC
TPOL/TRYP	0.004 (0.005, 0.008)	0.002 (0.001, 0.004)	0.002 (0, 0.011)	0.9713	
5HIAA/KYN	2.253 (0.7819,2.852)	2.2641 (1.7162, 2.9213)	0.6699 (0.3292, 1.3622)	0.0057[2]	rMDD<dMDD rMDD< HC
Tyrosine Pathway					
	Median,HC (frstqrt,HC, thrdqrt,HC)	Median, dMDD (frstqrt dMDD,thrdqrt,dMDD)	Median,rMDD (frstqrt,rMDD,thrdqrt,rMDD)	P value[1]	
HVA[3]	30.81 (23.38, 33.38)	23.35 (14.22, 33.8)	12.98 (9.995, 23.13)	0.02	rMDD < HC
LD[3]	0.463 (.25, .67)	0.61 (.35, .75)	0.447 (.14, .58)	0.66	
TYR[3]	1257 (1117, 1356)	1235 (1177, 1444)	1328 (1040, 1687)	0.91	
MHPG[3]	11.94 (10.29, 12.56)	11.79 (10.42, 13.12)	11.73 (9.31, 12.98)	0.81	
TYRA	2.356 (1.381, 4.347)	3.416 (1.44, 6.847)	0.911 (0.515, 1.398)	0.103[2]	
4HPAC	3.448 (2.956, 3.909)	3.074 (2.694, 5.016)	2.777 (2.472, 3.854)	0.4705	
4HPLA	34.36 (30.685, 39.504)	28.72 (22.888, 43.936)	25.811 (20.242, 28.066)	0.0697	
4HBAC	237.7 (127.3,403.7)	100 (71.7,261.3)	118 (73.4,240.9)	0.32[2]	
TYR/4HPLA[3]	35.05 (32.92, 38.92)	40.49 (36.18, 54.04)	58.00 (43.87, 69.66)	0.025	rMDD> HC
TYRA/TYR	0.002 (0.001, 0.003)	0.003 (0.001, 0.005)	0.001 (0, 0.001)	0.0714	
4HPAC/TYR	0.003 (0.002, 0.003)	0.003 (0.002, 0.004)	0.002 (0.002, 0.003)	0.2142	
4HPAC/TYRA	1.42 (0.767, 2.231)	1.375 (0.543, 2.655)	2.779 (1.443, 6.944)	0.276[2]	
HVA/MHPG[3]	2.522 (1.873, 2.84)	1.824 (1.446, 2.548)	1.126 (0.905, 1.769)	0.0105	rMDD< HC
HVA/LD	57.687 (42.925, 132.133)	53.734 (25.99, 85.884)	41.277 (18.846, 104.257)	0.3155	
HVA/TYR	0.024 (0.019, 0.026)	0.017 (0.012, 0.023)	0.009 (0.006, 0.022)	0.0242	
MHPG/LD	26.152 (16.553, 46.581)	21.062 (14.33, 34.464)	23.953 (15.133, 75.821)	0.8907	
MHPG/TYR	0.009 (0.008, 0.011)	0.009 (0.008, 0.011)	0.009 (0.007, 0.009)	0.7526	
LD/TYR	0.0004 (0.0002, 0.001)	0.0004 (0.0002, 0.001)	0.0003 (0.0001, 0.001)	0.746	
Purine Pathway					
	Median HC (frstqrt,HC, thrdqrt,HC)	Median MDD (frstqrt,MDD, thrdqrt,MDD)	Median rMDD (frstqrt,R, thrdqrt,R)	P value[1]	
HX	407.44 (324.60, 426.79)	345.27 (225.23, 490.56)	272.63 (224.08, 340.71)	0.055	

Table 2 | Continued

Purine Pathway

	Median HC (frstqrt,HC, thrdqrt,HC)	Median MDD (frstqrt,MDD, thrdqrt,MDD)	Median rMDD (frstqrt,R, thrdqrt,R)	P value[1]	
XAN	249.28 (220.85, 273.39)	210.71 (171.15, 271.57)	182.58 (173.04, 231.87)	0.048[2]	
XANTH	4.00 (2.40, 5.10)	3.07 (2.04, 4.89)	2.40 (1.84, 3.80)	0.344	
GR	3.95 (3.26, 4.66)	3.38 (3.10, 3.87)	3.48 (3.07, 3.90)	0.32[2]	
7MXAN	0.82 (0.34, 1.39)	0.82 (0.61, 1.34)	0.54 (0.39, 0.89)	0.436	
URIC	6831.23 (5514.46, 9164.00)	7436.60 (5635.26, 8792.94)	8324.69 (7422.20, 10799.59)	0.50[2]	
X7MG	2.65 (2.31, 2.82)	2.55 (2.20, 3.09)	2.56 (2.38, 2.74)	0.60[2]	
URIC/HX	18.06 (13.37, 23.30)	22.31 (12.57, 30.99)	30.55 (19.90, 47.70)	0.156	
URIC/GR	1705.57 (1508.64, 2040.76)	2298.52 (1625.76, 2702.63)	2264.40 (1935.46, 3329.48)	0.157	
XAN/HX	0.67 (0.57, 0.73)	0.62 (0.50, 0.77)	0.69 (0.67, 0.87)	0.339[2]	
URIC/XAN	28.70 (22.97, 35.08)	34.06 (23.52, 40.86)	41.39 (29.49, 54.10)	0.193	
URIC/XANTH	2045.64 (1394.97, 3052.20)	2081.13 (1537.92, 4238.69)	3109.14 (1869.48, 4372.19)	0.484	
XANTH/HX	0.011 (0.008, 0.013)	0.007 (0.005, 0.015)	0.009 (0.007, 0.015)	0.994[2]	
XANTH/GR	0.89 (0.57, 1.35)	0.82 (0.62, 1.13)	0.69 (0.55, 1.10)	0.684	
XAN/XANTH	71.82 (48.77, 93.91)	65.78 (52.28, 103.94)	69.74 (59.31, 117.03)	0.837	
XAN/GR	60.65 (54.39, 70.47)	59.94 (51.50, 69.99)	61.49 (48.47, 75.42)	0.861	

Methionine and Other Pathways

	Median HC (frstqrt, HC, thrdqrt,HC)	Median MDD (frstqrt, MDD, thrdqrt,MDD)	Median rMDD (frstqrt,HC, thrdqrt,R)	P value[1]	
MET[3]	350.003 (307.024, 377.479)	346.042 (322.215, 369.386)	445.028 (390.317, 469.8)	.02	rMDD > HC rMDD > dMDD
GLNTRP	2.638 (0.839, 3.295)	1.869 (1.082, 3.127)	1.911 (1.110, 3.373)	0.52[2]	
GSH	9.174 (8.586, 9.714)	9.574 (9.191, 10.091)	8.699 (8.450, 8.972)	0.0790	
ASC	11770.484 (8355.891, 13224.860)	10454.866 (8718.010, 12391.660)	11159.427 (7317.788,12974.98)	0.7202	
HHASC	233.306 (195.669, 303.334)	297.748 (212.466, 323.615)	219.603 (177.721, 276.052)	0.2159	
GLNTRP/GSH	0.296 (0.091, 0.369)	0.219 (0.111, 0.329)	0.267 (0.137, 0.453)	0.796[2]	
GSH/MET	0.028 (0.023, 0.032)	0.029 (0.027, 0.032)	0.021 (0.016, 0.022)	0.0026	rMDD < dMDD rMDD < HC

Ratios Across Pathways

XAN/HVA[3]	8.310 (7.486, 9.641)	9.249 (8.179, 12.794)	13.879 (10.951, 19.848)	0.028	rMDD > HC
HVA/5HIAA[3]	2.658 (1.654, 3.768)	2.160 (1.455, 2.649)	3.613 (2.128, 6.840)	0.039	rMDD >dMDD
5HIAA/HX[3]	.0316 (.0161, .0445)	.0264 (.0236, .0404)	.0178 (.0085, .0269)	0.06	
5HIAA/XAN[3]	.0472 (.0231, .0800	.0501 (.0372, .0671)	.0219 (.0121, .0355)	.007	rMDD < HC rMDD <dMDD
TYR/TRYP[3]	2.568 (2.301, 2.640)	2.445 (2.157, 2.923)	2.428 (2.243, 2.588)	.90	

[1]P-values are from the Kruskal Wallis 3-group test, or, the 3-group McSweeney rank ANCOVA test, if analyte and Race were correlated with Kendall's tau p<.05.
[2]McSweeney p-value used.
[3]Planned Comparison.
Note: For planned tests, significance was set at p<0.05. Bonferroni corrected alpha for the remaining Tryptophan pathway analytes, was 0.0125; for the remaining Tyrosine pathway analytes, 0.0042; for all Purine pathway analytes, 0.0031; and for the remaining Methionine cluster of analytes, 0.0083. When a result was significant, the last column displays the individual pair test results that were significant at p<.017, and direction of difference.

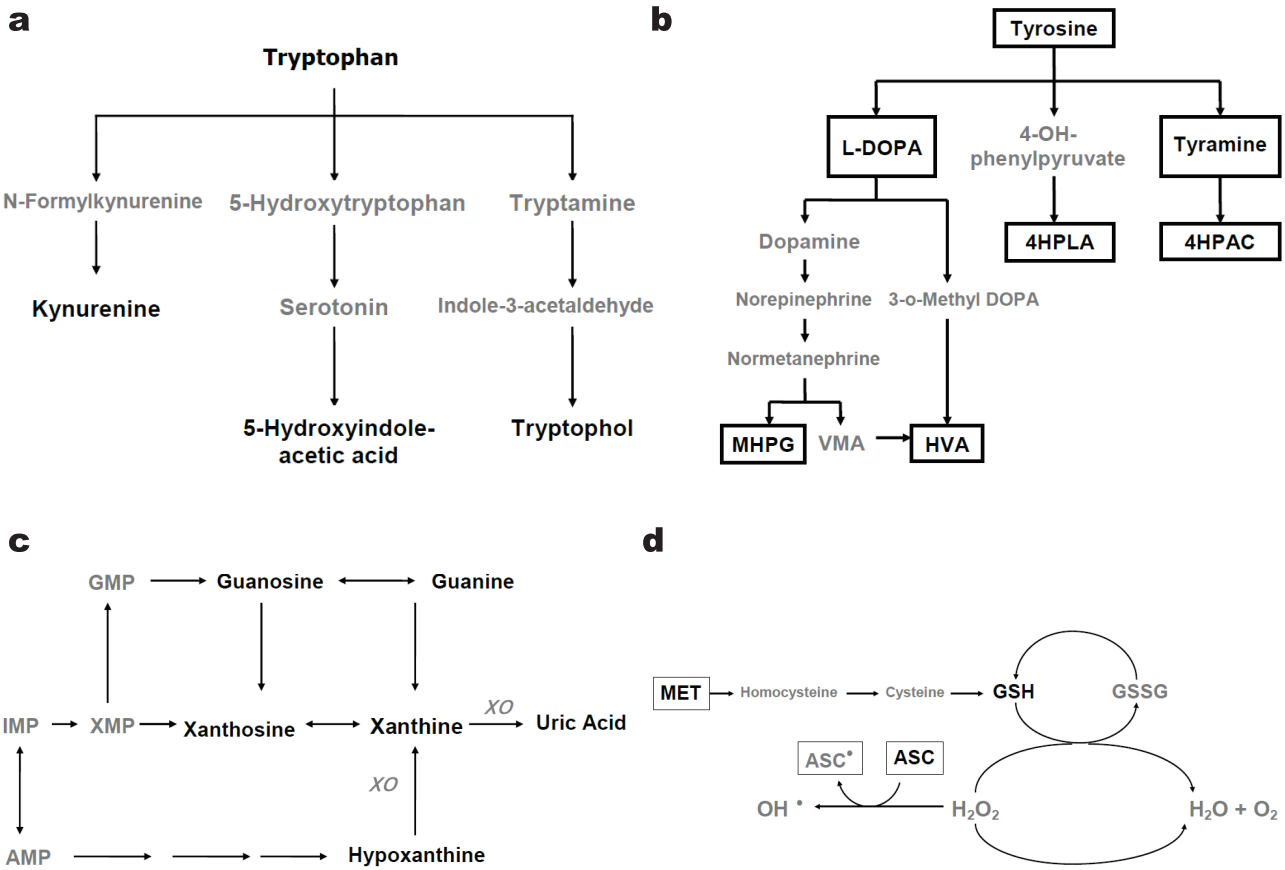

Figure 1 | (a) Tryptophan pathway metabolites quantitated by the LCECA platform. (b)Tyrosine pathway metabolites quantitated by the LCECA platform. (c) Purine pathway metabolites quantitated by the LCECA platform. (d) Methionine pathway metabolites quantitated by the LCECA platform.

xanthine concentrations showed a non significant trend toward being lower in the rMDD group than the HC group.

Methionine and related pathways. Analyses of the metabolites (N = 5, Table 2) and ratios (N = 2) of these pathways (Supplemental Tables 1, 2) revealed significant main effects of group for the methionine concentration (MET; p = 0.02, Figure 3a.) and the glutathione (GSH)/MET ratio (p = 0.003, Bonferroni alpha = 0.008, Figure 3b.). Follow-up tests (Bonferroni alpha = 0.017 for significance) revealed that the rMDD group had significantly higher levels of MET than the HC group (p = 0.007) and a trend toward having higher levels of MET (p = 0.03) than the dMDD group. The rMDD group also had significantly smaller GSH/MET ratios than the dMDD (p = 0.001) and HC (p = 0.002) groups. The GSH/MET effect was due to the

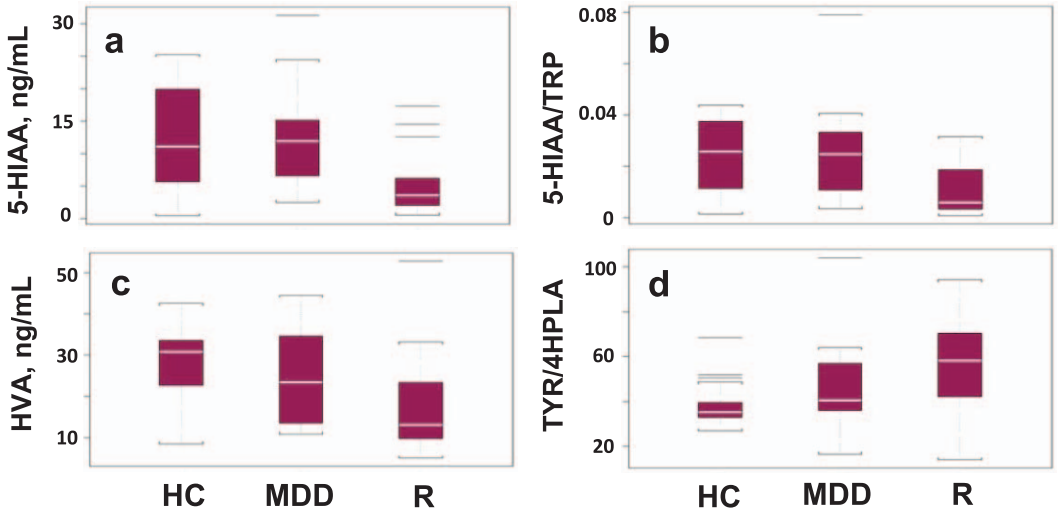

Figure 2 | (a) 5HIAA levels in healthy control, depressed MDD, and remitted MDD samples. (b) 5-HIAA/TRP ratios in healthy control, depressed MDD, and remitted MDD samples. (c) HVA levels in healthy control, depressed MDD, and remitted MDD samples. (d) TYR/4HPLA ratios in healthy control, depressed MDD, and remitted MDD samples.

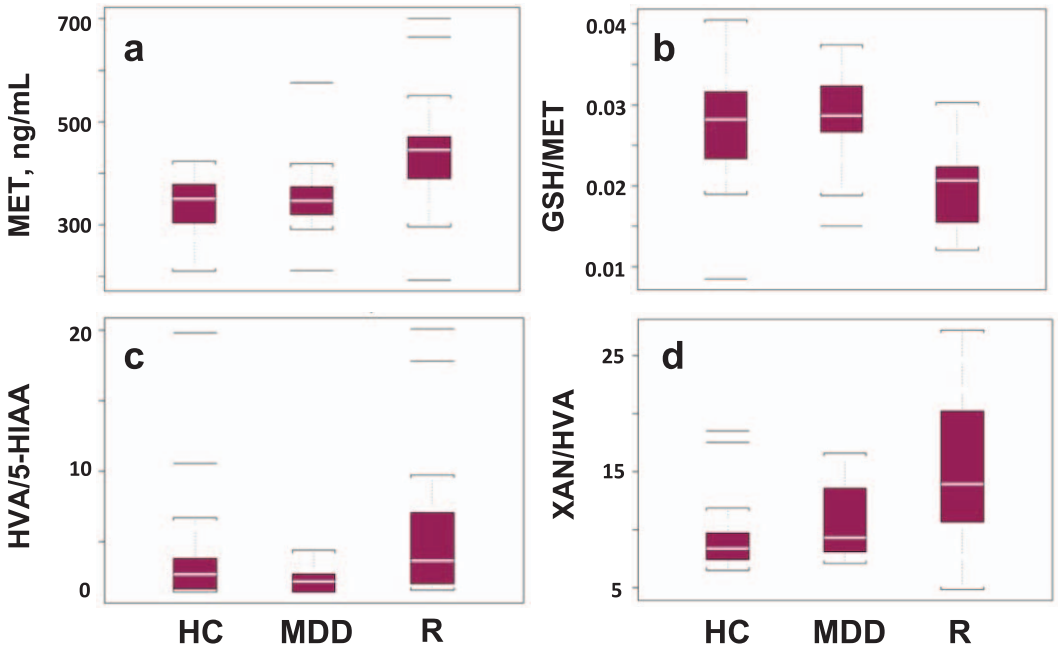

Figure 3 | (a) Methionine levels in healthy control, depressed MDD, and remitted MDD samples. (b) GSH/MET ratios in healthy control, depressed MDD, and remitted MDD samples. (c) HVA/5HIAA ratios in healthy control, depressed MDD, and remitted MDD samples. (d) XAN/HVA ratios in healthy control, depressed MDD, and remitted MDD samples.

rMDD group having both higher MET levels and lower GSH levels than the other two groups. There was no significant difference between the HC and dMDD groups.

Metabolic Signatures for dMDD and rMDD - *Ratios across pathways.* Kruskal-Wallis tests on the selected across pathway ratios (N = 5, Table 2) showed significant effects for HVA/5HIAA ($p = 0.04$, Figure 3c.), XAN/HVA ($p = 0.03$, Figure 3b.), and 5HIAA/XAN ($p = 0.007$) ratios. Follow-up tests (Bonferroni alpha = 0.017 for significance) revealed that the rMDD group had significantly larger XAN/HVA ratios than the HC group ($p = 0.008$) and a significantly larger HVA/5HIAA ratio than the dMDD group ($p = 0.014$). The rMDD group also had a significantly smaller 5HIAA/XAN ratio than both the HC ($p = 0.014$) and dMDD ($p = 0.003$) groups.

Associations of metabolites with disease severity indices. There were several significant correlations between levels of metabolites (N = 26) and indices of disease severity in the dMDD group. TPOL was negatively associated with HAM-D-17 scores ($r = -.61$, $p = .036$) indicating that lower levels of this compound are associated with higher levels of melancholic symptoms. TYRA was negatively associated with the HAM-D-A1-8 scores ($r = -.58$, $p = .049$) indicating that patients with lower levels of TYRA have more severe symptoms of atypical depression (a phenotype characterized by increases in appetite/weight and sleep, and preservation of mood reactivity in response to pleasurable events). HAM-A scores were positively associated with 5HIAA ($r = .60$, $p = .023$), HX ($r = .55$, $p = .04$), and MHPG ($r = .65$, $p = .01$) and negatively associated with TRYP ($r = -.59$, $p = .026$). This suggests that dMDD patients with more severe symptoms of anxiety have higher levels of 5-HIAA, HX, and MHPG and lower levels of TRYP.

Discussion

We compared metabolites from the tryptophan, tryrosine, purine and methionine pathways between rMDD and dMDD subjects and healthy controls using the LCECA platform. This approach detects a subset of the metabolome, namely compounds amenable to oxidation-reduction, with greater sensitivity for studying monoamine neurotransmitter pathways than can be achieved using other technologies. Since cellular metabolism reflects the integrated interconversion of metabolic substrates through enzyme-catalyzed biochemical reactions, an alteration in the enzyme function at one reaction can affect the fluxes of one or several subsequent reactions[28]. In this CSF metabolite pathway analysis we observed that the levels of several metabolites within the investigated pathways were altered in unmedicated-remitted individuals with MDD. In contrast, unmedicated-depressed individuals did not exhibit statistically significant perturbations within the same metabolic branches, consistent with the results of most previous studies on 5-HIAA, HVA, and purine metabolites in depressed individuals selected according to similar diagnostic criteria.

The counterintuitive findings that the CSF 5-HIAA levels and the 5-HIAA/TRYP ratio were significantly lower in the rMDD versus the dMDD subjects conceivably may reflect greater biochemical heterogeneity within the dMDD group. One source of this heterogeneity may arise from variation in symptom severity, as suggested by the associations observed between the tryptophan, tyrosine, and purine pathway metabolites and depression or anxiety ratings. For example, Nordin[29] found that the relationship between 5-HIAA concentrations and depressive symptoms in MDD was curvilinear, such that individuals with the lowest and highest 5-HIAA levels had the lowest depression ratings. Our data appear compatible with this finding, since most of the currently depressed subjects we studied had depression ratings in the moderate severity range. Moreover, the observation that anxiety ratings correlated *positively* with 5-HIAA levels but *negatively* with TRYP levels suggests that the greater anxiety symptoms associated with active depressive illness may contribute to the higher 5-HIAA levels and the higher 5-HIAA/ TRYP ratio in the currently ill versus the currently remitted MDD samples. In preclinical studies of anxiety, rats exposed to stressors or threats show adaptive increases in serotonin turnover in several brain structures[30], which putatively would increase the CSF 5-HIAA levels. Thus the anxiety symptoms associated with depressive episodes may result in an elevation of 5-HT turnover, potentially obscuring a basal deficit in 5-HIAA levels that is evident during remission.

Another potential explanation for the more prominent difference in the remitted sample is that the entrance criteria used to select the rMDD subjects yielded a more homogenous subphenotype of MDD. The rMDD subjects were characterized by manifesting an episodic course (i.e., episodes of illness separated by epochs of remission) and the ability to remain in remission while unmedicated. In contrast, while the depressed sample included some subjects with an episodic course, most of the dMDD subjects manifested chronic illness. Thus, one interpretation of the marked reduction in 5-HIAA and HVA levels in the rMDD subjects is that the entrance criteria for this sample identifies a subphenotype that is more likely to maintain euthymia because of having a lower dopamine and serotonin turnover rate, potentially due to genetic variation that results in slower metabolism of these neurotransmitters (so that their availability within the synapse is prolonged). Compatible with such a hypothesis, drugs that achieve such a condition through monoamine oxidase inhibition exert potent antidepressant effects. Thus the persistently remitted group may manifest biological differences that confer resilience and prevent illness chronicity in the course of MDD. Nevertheless, the cross-sectional design of our study did not allow us to distinguish whether these CSF abnormalities reflect trait-like abnormalities or compensatory changes arising in response to past illness.

In either case the findings in remitted MDD subjects appear compatible with other evidence that, despite symptom remission, such individuals show behavioral and biochemical deficits relative to never-depressed controls. Clinically, unmedicated-remitted MDD subjects show a greater risk of depressive relapse, higher rates of suicide attempts and ideation, lower pleasure ratings and impaired socio-occupational functioning relative to controls[31]. Such individuals also show neurobiological abnormalities that in some cases resemble[32], but in other cases contrast with, those shown by their currently-depressed counterparts. For example, under acute tryptophan depletion or catecholamine depletion, unmedicated rMDD subjects redevelop depressive symptoms, neuropsychological deficits, and neurophysiological abnormalities characteristically seen in the depressed phase[33–34,35], while unmedicated-*depressed* MDD subjects show no exacerbation of depressive symptoms under these challenges[36,37]. These findings suggest that alterations in the tryptophan or tyrosine pathways limit the capacity to remain in remission in rMDD subjects, consistent with their abnormal reductions in CSF 5-HIAA and HVA levels shown herein.

The cross-sectional design also did not allow us to exclude the possibility that persistent effects of previous treatment influenced our findings, since only a minority of the subjects was treatment-naïve. Nevertheless, while several studies reported decreased CSF concentrations of 5-HIAA following acute or subacute administration of selective serotonin reuptake inhibitors (SSRIs)[38–40,41,42], after chronic SSRI treatment the CSF 5-HIAA levels return to the pre-treatment baseline[43]. Similarly, HVA levels are not changed significantly by chronic SSRI treatment[43]. Thus our findings more likely relate to the pathophysiology of MDD, and as such may underlie predisposing factors or adaptive responses to illness, as opposed to homeostatic mechanisms arising in response to past treatment[44]. For example, the abnormal reduction in CSF 5-HIAA levels in the rMDD sample is consistent with evidence that MDD is associated with neuropathological involvement of brainstem serotonergic nuclei[45], and that maintenance treatment with drugs that increase 5-HT transmission reduce the risk of illness recurrence in rMDD patients[46–47].

Our findings in the rMDD sample appear compatible with those of previous studies that measured CSF 5-HIAA in isolation from the remainder of the tryptophan pathway. van Praag and de Hann[48] reported that 33 of 54 depressed individuals with low *post* probenecid accumulation of CSF 5-HIAA also showed this low accumulation after recovery. Furthermore, Vestergaard et al.[27] reported that CSF 5-HIAA and HVA levels were significantly lower following recovery than during depression in 16 mood disordered subjects, although the differences in the 5-HIAA levels with respect to healthy controls did not reach significance in either the depressed or the recovered phases. Our data thus were the first to document that remitted MDD subjects show significantly reduced mean 5-HIAA levels relative to controls in the absence of probenicid challenge.

The significantly smaller 5HIAA/TRP and 5HIAA/KYN ratios in the rMDD group versus the dMDD and HC groups are noteworthy, in light of evidence that the pathophysiology of MDD involves activation of pro-inflammatory pathways[49]. Activation of the KYN pathway is initiated by pro-inflammatory cytokines via induction of the enzyme, indoleamine 2,3-dioxygenase (IDO), which metabolizes TRP into KYN. Activation of the KYN pathway is hypothesized to contribute to reductions in 5-HT synthesis in MDD and in individuals who develop depressive symptoms during cytokine administration[50,51]. Potentially compatible with this hypothesis, the reduced 5HIAA/KYN ratio in the rMDD sample suggests that more TRP is being shunted toward the KYN pathway than toward 5-HT synthesis. These findings thus appear compatible with evidence that a pro-inflammatory state exists in remitted MDD subjects, despite their recovery from depressive symptoms[52].

Within the tyrosine pathway, the rMDD group showed significantly higher TYR/4HPLA ratios, lower TYRA levels, and smaller HVA/MHPG and HVA/TYR ratios versus the HC group. This combination of findings suggests that of the TYR routed toward catecholamine synthesis, relatively more is being routed toward norepinephrine utilization than toward dopamine utilization (figure 1b). Given the role of dopamine in reward processing, these data together with the abnormal reduction in HVA levels in the rMDD group appear compatible with evidence that hypohedonia (diminished ability to enjoy pleasurable activities) is a trait-like marker of MDD[53]. In addition, the quartile data (table 2) suggested that a subset of the currently depressed subjects also manifested reduced HVA levels, consistent with previous reports that subjects with the melancholic subtype of MDD show reductions in the CSF HVA concentration (see Introduction).

Our data further suggest that a balance between dopaminergic and serotonergic function may be relevant to the switch between depression and remission. Previously Roy et al.[54] reported that the CSF HVA-to-5-HIAA ratio was lower in depressed MDD subjects than in controls. In our study the significant difference in the HVA/5HIAA ratio across groups (table 2) was attributable to a lower ratio in the dMDD subjects versus the HC and rMDD subjects (consistent with Roy et al.[54]). These data imply that the ratio of dopamine turnover to serotonin turnover is lower in dMDD than in rMDD cases, raising the possibility that imbalances between 5-HT and DA neurotransmission contribute to the development of depressive *symptoms*. Notably the literature characterizing interactions between dopaminergic and serotonergic neurotransmission has emphasized functionally antagonistic relationships between these systems[55].

The MET concentration was higher and the GSH/MET ratio was lower in the rMDD group than in the HC and dMDD groups. These data conceivably reflect a reduction in the conversion rate of MET to GSH, possibly implicating a defect in 1-carbon metabolism in MDD. Methionine plays a critical role in protein synthesis, methylation, and polyamine biosynthesis, and its derivative S-adenosyl methionine (SAM) serves as a methyl donor for several processes including DNA methylation. A role for DNA methylation in mood regulation is suggested by the antidepressant effect of S-adenosyl methionine[56–58], the *post mortem* evidence that DNA methylation of genes involved in catecholamine metabolism are altered in mood disorders[59–61], and preclinical evidence that epigenetic processes underlie both the resilience against and the vulnerability toward the development of depressive behaviors under stress[62]. Our data raise the possibility that higher MET concentrations support the maintenance of symptom remission in MDD[58]. Conversely, the reduction in GSH levels observed in the remitted sample conceivably may confer the vulnerability of the rMDD population to illness recurrence. Glutathione is

the major antioxidant in the brain, and thus plays a key role in defending against oxidative damage. Glutathione is thought to play an important role in the neuroprotective effects of mood stabilizing drugs[63], given the accumulating evidence for oxidative stress mechanisms as common pathophysiological pathways in mood disorders[64–67]. We previously reported[10] that glycine levels differed significantly between remitters and non-remitters to SSRI treatment, and proposed that glycine's link to folate and methionine cycles and methylation processes might play a role in recovery from the depressed state.

Although none of the purine metabolites showed significant effects on their own, some ratios involving the purine metabolites and the metabolites within other pathways differed across groups (i.e., XAN/HVA and 5-HIAA/XAN). Previous studies reported strong positive correlations between CSF levels of xanthine and hypoxanthine with corresponding levels of HVA and 5HIAA, despite finding no significant difference in the mean concentrations of these purine metabolites between depressives and controls[68,69]. The strong correlation in the lumbar CSF pool between HVA and XAN across the purine and tyrosine pathways has been observed in previous studies[70]. This cross pathway correlation appears to extend the relationship between XAN and tyrosine/tryptophan evident in the brain and the ventricular CSF to the lumbar CSF, as the end product metabolites of tyrosine and tryptophan resulting from spinal cord metabolism. The purine and tyrosine pathways also share the pterin compounds as common cofactors, potentially contributing both to the similarities in the direction of correlation and to the differences in the ratio obtained across the rMDD group and the other two groups.

This study provides new insights about the metabolic state associated with remission from MDD, and distinguishes this state from the normative metabolic state. The relatively small sample size in our study limited the statistical sensitivity to detect other potentially meaningful differences between groups. The lack of difference between the dMDD and HC groups may reflect both the limited statistical power and the greater biochemical heterogeneity seen among chronically depressed subjects. Moreover, greater coverage of the metabolome using complementary platforms may reveal perturbations in dMDD that were not detected in our study. Finally, correlations between biochemical changes in the CSF with those observed in plasma might enable the connection of central and peripheral changes.

In conclusion, the correlation of metabolites within and across the tryptophan, tyrosine and methionine pathways revealed differences in remitted MDD subjects which suggest alterations in the regulatory feedback within these pathways. Our data thus demonstrate that metabolomics approaches hold the potential to elucidate failures in the regulation of specific biochemical pathways and networks in mood disorders. The differentiation of whether these abnormalities reflect consequences of prior depressive episodes or vulnerabilities toward the development of future mood episodes awaits longitudinal studies in at-risk samples that assess the function of these pathways before and following illness-onset. Furthermore, the biochemical heterogeneity extant within the depressed MDD population suggests that future studies should characterize sufficiently large samples that subphenotypes can be identified which manifest more homogenous metabolomic signatures (potentially analogous to those found in the rMDD sample).

Methods

Participants met DSM-IV-TR criteria[71] for MDD in full remission (rMDD; n= 14) or MDD in a current major depressive episode (dMDD; n=14). The control group was psychiatrically healthy (n=18). Diagnosis was established using the Structured Clinical Interview for DSM-IV[72] and an unstructured interview with a psychiatrist. To assess general health, physical examination, urine drug screening, and laboratory testing of electrolytes, blood count, thyroid function, hepatic and renal function, viral titers, and pregnancy status were performed. Volunteers were excluded if they had major medical or neurological disorders (including hypertension or endocrine disorders), past head injury, pregnancy, electrolyte disturbance, anemia, positive drug or viral (HIV, hepatitis) screen, current serious suicidal ideation or recent suicidal behavior, delusions or hallucinations, or met DSM-IV-TR criteria for substance abuse within one year or substance dependence within the lifetime. The rMDD sample additionally met the entrance criterion of having been in full remission (DSM-IV-TR) and free of exposure to psychotropic medications >3 months. The dMDD and HC subjects were excluded if they had been exposed to psychotropic or other medications likely to alter monoamine neurochemistry within 3 weeks. In no case was a medication discontinued for the purpose of this study; volunteers either were naïve to psychotropic medications or had discontinued medication for other reasons. The HC subjects met the additional exclusion criteria of having had no lifetime history of a psychiatric disorder and no first-degree relative with a mood or anxiety disorder, as established using the SCID and the Family Interview for Genetic Studies (FIGS)[73]. Females had not received hormonal treatments other than oral contraceptives. Subjects gave written informed consent, as approved by the NIH Combined Neuroscience IRB.

The 17 item Hamilton Rating Scale for Depression (HAM-D) and the Montgomery-Asberg Depression Rating Scale were administered to provide an overall depression severity index. The 8 item "atypical feature" component from the 25 item HAM-D reflecting reversed neurovegetative changes (i.e., increased sleep, appetite and weight) also was administered. The Hamilton Anxiety Rating Scale (HAM-A) was used to rate anxiety symptom severity.

CSF collection. Baseline cerebrospinal fluid (CSF) samples were collected via lumbar puncture (LP) into the subarachnoid space between the L3-4 or L4-5 interspaces. During the 3 days prior to LP, subjects were requested to observe a monoamine free diet. All subjects were admitted to the NIMH Inpatient Unit at 7 pm the day prior to LP, and LP was performed at 9 am after overnight stay.

Metabolomics analysis: liquid chromatography electrochemical array platform. Metabolic profiling of the samples was performed using LCECA coulometric array detection, which has been previously described in other studies[17–19,74–77] (See Supplement for a detailed description). This platform quantified 26 known compounds, primarily within the tyrosine, tryptophan, purine and methionine pathways. Levels of anthranilic acid and guanine were below the detectable limit of the platform in over 50% of the samples, so they were not included in the analysis. Of the remaining 24 compounds (See Supplementary Table 1), tryptophol (TPOL), L-DOPA (LD), and 7-methylxanthine (7MXAN) had fewer than 20% such samples and all other metabolites had no censored values.

Statistical analysis. Descriptive statistics were computed for each group. The raw data were viewed by quantile-quantile normal and chi-square plots, and by variable-pair scatterplots, to assess normality and nonlinear relationships. As most analytes were not approximately normally distributed, nonparametric Kruskal Wallis tests were used to test for differences across groups. A significant Kruskal-Wallis test was followed by post-hoc comparisons. When ties were present, and notably when values were censored below their minimum value, the normal approximations for the above Kruskal Wallis test statistics[78] were used in the tests. The first set of analyses focused on a series of compounds and ratios of compounds for which there was a clear basis from previous literature to be involved in the pathophysiology of MDD. These included the following compounds: tryptophan (TRYP), 5-HIAA, KYN, TYR, LD, HVA, MHPG, and methionine (MET). We also examined the following ratios: HVA/MHPG, 5HIAA/TRYP, xanthine(XAN)/HVA, HVA/5-HIAA, 5-HIAA/XAN, 5HIAA/HX, TYR/4HPLA, TYR/TRYP. Ratios of compounds inform us about the relative effectiveness of the enzymes in regulating the pathway or pathways involved. Thus, the analysis of ratios can help identify pathways that may play a role in MDD. For compounds and ratios reported to be abnormal in MDD in previous studies, we did not apply a correction for the number of Kruskal Wallis tests performed. When making post-hoc tests, a Bonferroni corrected p-value for three comparisons (.05/3=0.017) was used in judging significance. The imbalance in Race across the groups could affect the results for an analyte if that analyte were correlated with Race. We addressed this possibility by testing each analyte for a nonzero Kendall's tau correlation with Race, rejecting the null for $p<0.05$. Kendall's tau is known to handle ties well, even up to dichotomous variables. In such cases, we used McSweeney and Porter's rank ANCOVA[79] (e.g., see Barrett, 2011) with 3 groups and Race the covariate, to test for group differences, and the McSweeney p-value is presented in lieu of the Kruskal-Wallis p-value (Table 2).

The second set of analyses was exploratory and involved the 38 remaining compounds and ratios within pathways (see Table 2). The metabolites and ratios of metabolites were grouped according to their biochemical roles in four pathways (tryptophan, tyrosine, purine, methionine). Control of Type I error for this multiple testing process was effected by applying the Bonferroni correction to alpha=.05 for the (exploratory) remainder of analytes and ratios within each pathway, separately for each pathway. Here again, Kruskal Wallis or McSweeney tests of group differences were done.

Correlations of metabolites with disease severity. Spearman correlations were used to examine associations of metabolite levels to disease severity indicators in the dMDD group. Disease severity was measured with the HAM-D-17, HAM-D-A8, and HAM-A scales. The HAM-D-17 and HAM-D-A8 scales provide ratings that emphasize the melancholic and atypical features of depression, respectively. The HAM-A scale rates the severity of anxiety symptoms.

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Author contributions

R.K.D., was involved in study design, data interpretation and drafting of the manuscript. P.Y. was involved in the design of the study and data interpretation. S.H.B. was involved in data interpretation and drafting of the manuscript. W.M. profiled the CSF samples and contributed to the biochemical interpretation of the data. Z.W., Z.B.Z. and H.Z. played a role in data analysis and interpretation. G.G.D., conducted data analysis and provided input on interpretation of the data . J.Y. was involved in the biochemical interpretation of the data. G.C. was involved with research design, data acquisition, and data interpretation. X.G. was involved with research design, data acquisition, and data interpretation. P.C. oversaw the storage and organization of the cerebrospinal fluid samples and participated in preparing the data for publication. A.N. recruited the subject participants and obtained the cerebrospinal fluid samples and participated in the study design. C.Z. was involved in subject recruitment and evaluation. R.R.K. was involved in data interpretation. H.K.M. was involved with research design, data acquisition, and data interpretation. W.D. contributed to the study design, the subject clinical assessment, the interpretation of the results, and the drafting of the manuscript. He also oversaw the laboratory in which the research samples were collected and obtained funding for this part of the study. All authors reviewed the manuscript.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

Competing financial interests: Rima Kaddurah-Daouk is equity holder in Metabolon Inc., a biotechnology company in the metabolomics domain, and also an inventor on patents in the metabolomics field. She has received funding or consultancy fees for BMS, Pfizer Inc., AstraZeneca and Lundbeck. George G. Dougherty owns shares of Pfizer and Merck. Carlos Zarate is listed as a co-inventor on a patent for the use of ketamine in major depression. Dr. Zarate has assigned his patent rights on ketamine to the U.S. government. Ranga R Krishnan has received consultancy fees from Amgen, Bristol-Myers Squibb, CeNeRx, Corcept, GlaxoSmithKline, Johnson & Johnson, Lundbeck, Merck, Organon, Pfizer, Sepracor, and Wyeth. Wayne Drevets has received consultancy fees from Pfizer, Rules Based Medicine, Eisai, Abbott, and Johnson & Johnson, and has a use patent filed on scopolamine in the treatment of depression. Peixiong Yuan, Stephen H. Boyle, Wayne Matson, Zhi Wang, Zhao-bang Zeng, Hongjie Zhu, Jeffrey Yao, Guang Chen, Xavier Guitart, Paul J. Carlson, Alexander Neumeister, and Husseini K. Manji have no relevant financial interests to disclose.

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