Relationships of Cerebrospinal Fluid Monoamine Metabolite Levels With Clinical Variables in Major Depressive Disorder

Hyung Shin Yoon, PhD[a,b]; Kotaro Hattori, MD, PhD[a,c]; Shintaro Ogawa, PhD[a]; Daimei Sasayama, MD, PhD[a,d]; Miho Ota, MD, PhD[a]; Toshiya Teraishi, MD, PhD[a]; and Hiroshi Kunugi, MD, PhD[a,*]

ABSTRACT

Objective: Many studies have investigated cerebrospinal fluid (CSF) monoamine metabolite levels in depressive disorders. However, their clinical significance is still unclear. We tried to determine whether CSF monoamine metabolite levels could be a state-dependent marker for major depressive disorder (MDD) based on analyses stratified by clinical variables in a relatively large sample.

Methods: Subjects were 75 patients with MDD according to *DSM-IV* criteria and 87 healthy controls, matched for age, sex, and ethnicity (Japanese). They were recruited between May 2010 and November 2013. We measured homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA), and 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG) in CSF samples by high-performance liquid chromatography. We analyzed the relationships of the metabolite levels with age, sex, diagnosis, psychotropic medication use, and depression severity.

Results: There was a weak positive correlation between age and 5-HIAA levels in controls ($\rho = 0.26$, $P < .016$) and a similar trend in patients, while sex was unrelated to any metabolite. All monoamine metabolites in moderately to severely depressed patients (17-item Hamilton Depression Rating Scale score > 12) were significantly lower than those in controls ($P < .0005$ for all 3 metabolites). We found that antidepressants decreased the levels of 5-HIAA ($\rho = -0.39$, $P < .001$) and MHPG ($\rho = -0.49$, $P < .0001$) and that antipsychotics increased levels of HVA ($\rho = 0.24$, $P < .05$). There was a strong correlation between HVA and 5-HIAA levels (controls: $\rho = 0.79$, $P = .000001$; MDD: $\rho = 0.66$, $P = .000001$). HVA levels ($\rho = -0.43$, $P < .001$) and 5-HIAA levels ($\rho = -0.23$, $P < .05$), but not MHPG levels ($\rho = -0.18$, $P > .1$), were related to depression severity.

Conclusions: CSF 5-HIAA and HVA levels could be state-dependent markers in MDD patients. Since 5-HIAA levels greatly decrease with the use of antidepressants, HVA levels might be more useful in the clinical setting.

J Clin Psychiatry 2017;78(8):e947–e956
https://doi.org/10.4088/JCP.16m11144

[a]Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Kodaira, Tokyo, Japan
[b]Department of Physiology, Brain Korea 21 Plus Project for Medical Science, Brain Research Institute, Yonsei University College of Medicine, Seoul, South Korea
[c]Translational Medical Center, National Center of Neurology and Psychiatry, Kodaira, Tokyo, Japan
[d]Department of Psychiatry, Shinshu University School of Medicine, Matsumoto, Nagano, Japan
**Corresponding author:* Hiroshi Kunugi, MD, PhD, Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashi, Kodaira, Tokyo 187-8502, Japan (hkunugi@ncnp.go.jp).

Since the classical monoamine hypothesis was proposed in the 1950s,[1] monoamines have been major targets of therapeutic interventions and drug development for depression. Alterations in the monoamine metabolites homovanillic acid (HVA, a dopamine metabolite), 5-hydroxy-3-indoleacetic acid (5-HIAA, a serotonin metabolite), and 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG, a norepinephrine metabolite) in human cerebrospinal fluid (CSF) have been investigated in depressive disorders. Studies comparing CSF HVA,[2–24] 5-HIAA,[5–27] and MHPG[4–19,27–29] between patients with depression and healthy controls have had inconsistent results. Some studies have reported significantly decreased CSF HVA levels in patients with depression,[2–4,12,20,21,23,24] while others have not.[5–11,13–19,22] Similarly, some have reported decreased CSF 5-HIAA,[20,23,25] while others have found no significant alteration.[5–7,9–11,13–19,21,22,24,26,27] Two studies even reported increased 5-HIAA levels[8,12] in patients with depression. Increases,[4,12,14] decreases,[29] and no change[5–11,13,15–19,27,28] in CSF MHPG have been reported.

Several studies have suggested that background variables influence CSF monoamine metabolite levels. Some studies have found a relationship of sex with CSF 5-HIAA[12,13,30,31] and HVA[12,31]; age with CSF 5-HIAA,[12,20,31,32] HVA,[20] and MHPG[12,13,32]; and body height with CSF 5-HIAA[20] and HVA,[20] suggesting that controlling for sex and age may be essential. The inconsistencies in the literature may have arisen, at least in part, by lack of control for such variables. Indeed, even sex and age were not controlled for in previous studies.[4,12,14,20,21,28] Moreover, many studies[2,12–14,18,20–23,27,28] included patients with bipolar disorder as well as patients with unipolar depression.

Medication use and depression severity are thought to affect CSF monoamine metabolite levels. According to Little et al,[17] 11 of 12 previous studies using selective serotonin reuptake inhibitors (SSRIs) or serotonin-norepinephrine reuptake inhibitors have shown significant decreases in CSF 5-HIAA and MHPG, while only 2 showed a significant decrease in HVA. Bäckman et al[33] reported that antidepressants, such as SSRIs, tricyclics, or monoamine oxidase inhibitors (MAOIs), lower CSF 5-HIAA and MHPG levels. However, CSF 5-HIAA, but not MHPG, levels returned to baseline with long-term (>30 weeks) administration. Depression severity and CSF 5-HIAA,[34,35] MHPG,[36] and HVA[35,36] levels are reported to be negatively correlated. One study,[37] however, reported a positive correlation between depression severity and HVA and 5-HIAA levels. Other studies[5,21,25,26,38–48] have reported no correlation between depression severity and CSF monoamine metabolite levels.

Clinical Points

- The data obtained in this study confirm effects of antidepressants on the momoamine metabolites 5-hydroxyindoleacetic acid (5-HIAA) and 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG), but not on homovanillic acid (HVA), and the effect of antipsychotics on HVA.
- HVA and 5-HIAA, but not MHPG, levels are related to depression severity.
- Since the majority of patients with depression receive antidepressants rather than antipsychotics, cerebrospinal fluid HVA might be the best state-dependent marker for major depressive disorder among the 3 monoamine metabolites.

To our knowledge, no study has simultaneously examined the effects of age, sex, medication use, and depression severity on CSF monoamine metabolites. We examined CSF monoamine levels in a relatively large sample of patients with major depressive disorder (MDD) and sex- and age-matched healthy controls. Patients with bipolar disorder were not enrolled in the study. We analyzed CSF monoamine levels stratified by medication and depression severity. Our goal was to determine which CSF monoamine metabolite is useful as a state-dependent marker reflecting depression severity in patients with MDD.

METHODS

Subjects

Subjects were 75 patients with MDD and 87 age- and sex-matched healthy volunteers. They were recruited between May 2010 and November 2013. They were biologically unrelated Japanese individuals undergoing treatment at the outpatient clinic of the National Center of Neurology and Psychiatry (NCNP) Hospital (Tokyo, Japan) or recruited through advertisements in a free local magazine and announcement via the homepage of the NCNP website. Subjects received a structured Mini-International Neuropsychiatric Interview (MINI), Japanese version.[49,50] At least 2 psychiatrists diagnosed each patient according to the *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition (*DSM-IV*) criteria[51] on the basis of the MINI, additional unstructured interviews, and information from medical records. Patients with any comorbid Axis I disorders and those individuals with a medical history of central nervous system disease, severe head injury, or substance abuse/dependence were excluded. An experienced research psychiatrist assessed depressive symptoms using the Japanese version of the GRID Hamilton Depression Rating Scale, 17-item version ($HDRS_{17}$).[52] Daily doses of antipsychotics and antidepressants were converted to chlorpromazine- and imipramine-equivalent doses, respectively, using published guidelines.[53] Our study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee at NCNP. After describing the study to every subject, we obtained written informed consent.

CSF Sampling and Measurement of Monoamine Metabolites

CSF was collected and extracted as described previously.[54,55] Briefly, CSF was drawn between 10:00 and 16:00 h from the L4–L5 or L3–L4 interspace with the subject in the left decubitus position, immediately transferred on ice and centrifuged ($4{,}000 \times g$ for 10 minutes, 4°C), aliquoted in low-protein adsorption tubes (Sumitomo Bakelite Co, Japan), and stored in a deep freezer (−80°C) until use. CSF monoamine metabolite levels were determined using high-performance liquid chromatography at SRL Inc (Tokyo, Japan).

Statistics

Clinical characteristics were analyzed by analysis of covariance controlling for sex and age. Relationships between monoamine metabolite levels and clinical measures were assessed using Spearman rank correlation coefficients. A P value of .05 (2-tailed) was considered statistically significant. Bonferroni correction was applied for multiple comparisons. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 11.0 (SPSS Japan, Japan).

RESULTS

Comparisons of Monoamine Metabolite Levels in All Subjects

Demographic and clinical characteristics of the participants are shown in Table 1. There were no differences in mean age ($t_{153.47} = -1.22$, $P = .22$), sex distribution ($\chi^2_1 = 0.55$, $P = .53$), or education years ($t_{126} = 1.80$, $P = .074$) between the patients and controls. HVA levels were not significantly different between the 2 groups, while 5-HIAA and MHPG levels were significantly reduced in the patients compared to those in the controls (HVA: $F_{1,157} = 1.2$, $P = .28$; 5-HIAA: $F_{1,157} = 12.9$, $P = .00043$; MHPG: $F_{1,157} = 17.4$, $P = .000050$) (Figure 1A–1C). HVA/5-HIAA ($F_{1,157} = 14.1$, $P = .00024$) and MHPG/5-HIAA ($F_{1,157} = 6.2$, $P = .014$) ratios were significantly increased in the patients compared to those in the controls, while HVA/MHPG was not significantly different ($F_{1,157} = 0.31$, $P = .58$) (Figure 1D–1F). There was a strong positive correlation between HVA and 5-HIAA levels in both groups (controls: $\rho = 0.79$, $P = .000001$; MDD: $\rho = 0.66$, $P = .000001$) (Figure 1G and 1H). MHPG and 5-HIAA levels were modestly but significantly correlated in both groups (controls: $\rho = 0.24$, $P = .027$; MDD: $\rho = 0.39$, $P = .00058$) (data not shown).

Clinical Variables and CSF Monoamine Metabolite Levels

There was no significant difference for any metabolite between men and women in either the patient or the control group (Supplementary eTable 1). Table 2 shows correlations between monoamine metabolite levels and age and medication doses. We observed a significant, albeit weak, positive correlation between age and 5-HIAA levels

Table 1. Demographic and Clinical Characteristics of Patients With MDD and Controls[a]

Clinical Factor	Healthy Controls	Patients With MDD		
			$HDRS_{17}$ Score	
		Total	Low (≤ 12)	High (> 12)
Total patients (male/female), n	87 (48/39)	75 (37/38)	41 (22/19)	34 (15/19)
Age, y	42.0 (15.6)	44.6 (10.8)	43.6 (9.8)	45.8 (12.1)
Education, y	15.6 (2.6)	14.7 (2.7)	15.0 (2.2)	14.4 (3.2)
Age at onset, y	…	36.2 (12.8)	33.8 (9.6)	39.2 (15.6)
Single episode/recurrent, n	…	35/38[b]	18/22	17/16
History of suicide attempts, yes/no, n	…	18/55[b]	9/32	9/23
History of ECT, yes/no, n	…	15/56[b]	8/33	7/23
Drug-naive subjects, n	…	9	5	4
Chlorpromazine-equivalent dose, mg/d	…	87.3 (175.1)	83.2 (184.8)	81.2 (156.5)
Imipramine-equivalent dose, mg/d	…	136.4 (138.7)	103.7 (127.2)	160.5 (141.9)
$HDRS_{17}$ score	…	12.8 (9.4)	5.9 (4.3)	21.3 (6.5)
Time of day of sampling, hours	13:21 (1:54)	13:27 (1:32)	13:34 (1:29)	13:17 (1:21)

[a]Values are shown as mean (SD) unless otherwise noted.
[b]Ambiguous data were excluded.
Abbreviations: ECT = electroconvulsive therapy, $HDRS_{17}$ = 17-item Hamilton Depression Rating Scale, MDD = major depressive disorder.
Symbol: … = not applicable.

in the controls ($\rho = 0.26$, $P = .016$; Table 2) and a similar trend in the patients ($\rho = 0.18$, $P = .065$), but no association of age with HVA or MHPG levels for in either group (Table 2). Antipsychotic use and HVA levels were positively correlated ($\rho = 0.238$, $P = .047$). Antidepressant use and 5-HIAA ($\rho = -0.388$, $P = .00090$) and MHPG ($\rho = -0.489$, $P = .000017$) levels were negatively correlated. Depression severity ($HDRS_{17}$ scores) was negatively correlated with HVA ($\rho = -0.43$, $P = .00013$) and 5-HIAA ($\rho = -0.23$, $P = .044$), but not with MHPG, levels.

Stratified Analyses by Medication and Depression Severity

Homovanillic acid. Figure 2A is a scatter plot of the significant negative correlation between CSF HVA levels and $HDRS_{17}$ scores ($\rho = -0.43$, $P = .00013$). When the patient group was divided into 2 groups based on depression severity using median $HDRS_{17}$ scores (Low ≤ 12 < High), HVA levels in the High group were significantly reduced compared to those of the controls ($F_{2,155} = 13.1$, $P = .00025$) and the Low group ($F_{2,155} = 13.1$, $P = .0000048$; Figure 2B). HVA levels stratified by depression severity and drug treatment (antidepressant-free vs antidepressant-treated groups) are shown in Figure 3A. Five patients were excluded due to ambiguous information on medication use. Reduced HVA levels were observed in the High group versus the Low group irrespective of antidepressant use (Figure 3A, center). Antipsychotics increased HVA levels ($F_{1,64} = 4.8$, $P = .032$, drug-free vs antipsychotics-only), which were attenuated with AD cotreatment (antidepressant + antipsychotic; $F_{1,64} = 4.5$, $P = .039$; Figure 3A, right).

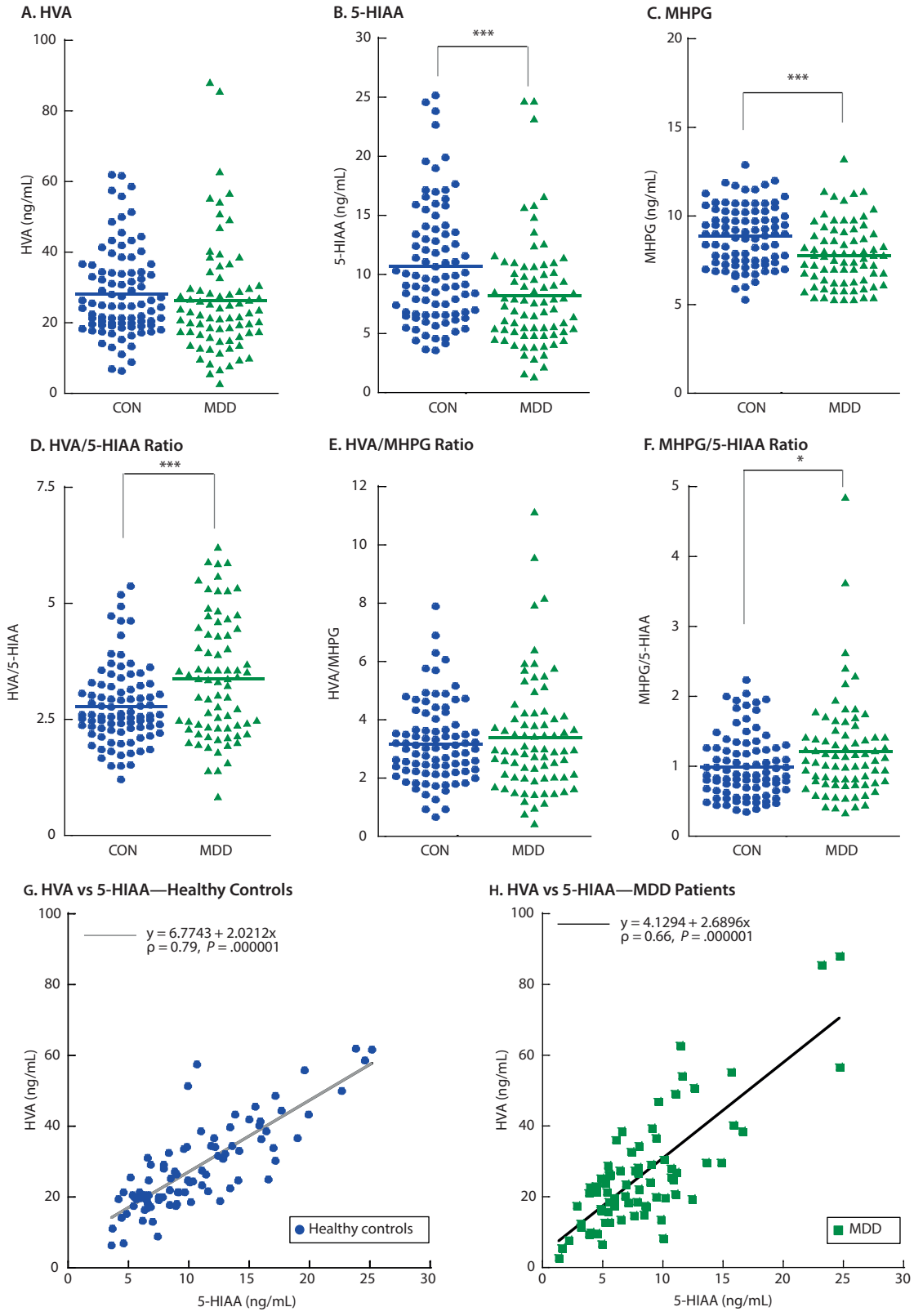
5-hydroxy-3-indoleacetic acid. When the patients were stratified using the same criteria used in the HVA analysis, 5-HIAA levels were significantly reduced in the High group versus the Low group ($F_{2,155} = 11.4$, $P = .0095$) and the controls ($P = .000013$, Figure 2C). 5-HIAA levels were stratified by depression severity and drug treatment (Figure 3B). The antidepressant-treated group had significantly lower 5-HIAA levels versus the antidepressant-free group ($F_{2,150} = 12.5$, $P = .0022$) and the controls ($P = .0000094$; Figure 3B, left). Mean 5-HIAA level was lower in the High group versus the Low group within the antidepressant-free group ($F_{1,64} = 4.6$, $P = .037$; Figure 3B, center). The mean 5-HIAA level in the antidepressant-treated Low group was significantly lower versus the antidepressant-free Low group ($F_{1,64} = 8.0$, $P = .0063$; Figure 3B, center). The antidepressant-treated High group had the lowest mean 5-HIAA level (mean = 5.65 ng/mL; Figure 3B, center). Unlike HVA levels, 5-HIAA levels did not increase with antipsychotic-only treatment ($F_{1,64} = 2.6$, $P = .11$), but patients receiving both antidepressants and antipsychotics had significantly lower mean 5-HIAA levels than those who received only antipsychotics ($F_{1,64} = 7.7$, $P = .0073$; Figure 3B, right).

3-methoxy-4-hydroxyphenylethyleneglycol. MHPG levels were negatively correlated only with imipramine-equivalent dose ($\rho = -0.489$, $P = .000017$; Table 2) in the MDD group. MHPG levels were significantly decreased in the Low group ($F_{2,155} = 9.7$, $P = .039$) and the High group ($F_{2,155} = 9.7$, $P = .00013$; Figure 2D) versus the controls. MHPG levels were significantly lower in antidepressant-treated patients but not in those receiving antipsychotic medication (Table 2, Figure 3C). When antidepressant-treated and antidepressant-free patients were examined separately, there was no difference in MHPG levels between the High and Low groups in antidepressant-treated or antidepressant-free cases (Figure 3C, center).

DISCUSSION

The findings of this study are as follows. (1) Overall, patients with MDD had significantly lower 5-HIAA and MHPG levels than controls, while there was no difference in HVA levels between patients and controls. (2) Although substantial inter-individual differences were noted in CSF HVA and 5-HIAA levels, the correlation between the two was strong and the HVA/5-HIAA ratio was relatively

Figure 1. Total Monoamine Metabolite Levels in CSF of Patients With MDD and Healthy Controls[a]



[a]As shown in parts A through C, absolute concentrations of HVA, 5-HIAA, and MHPG were compared by analysis of covariance controlling for sex and age. Ratios of respective monoamine metabolites are shown in parts D through F. The correlation between HVA and 5-HIAA is shown in parts G and H.
*P < .05. ***P < .001.

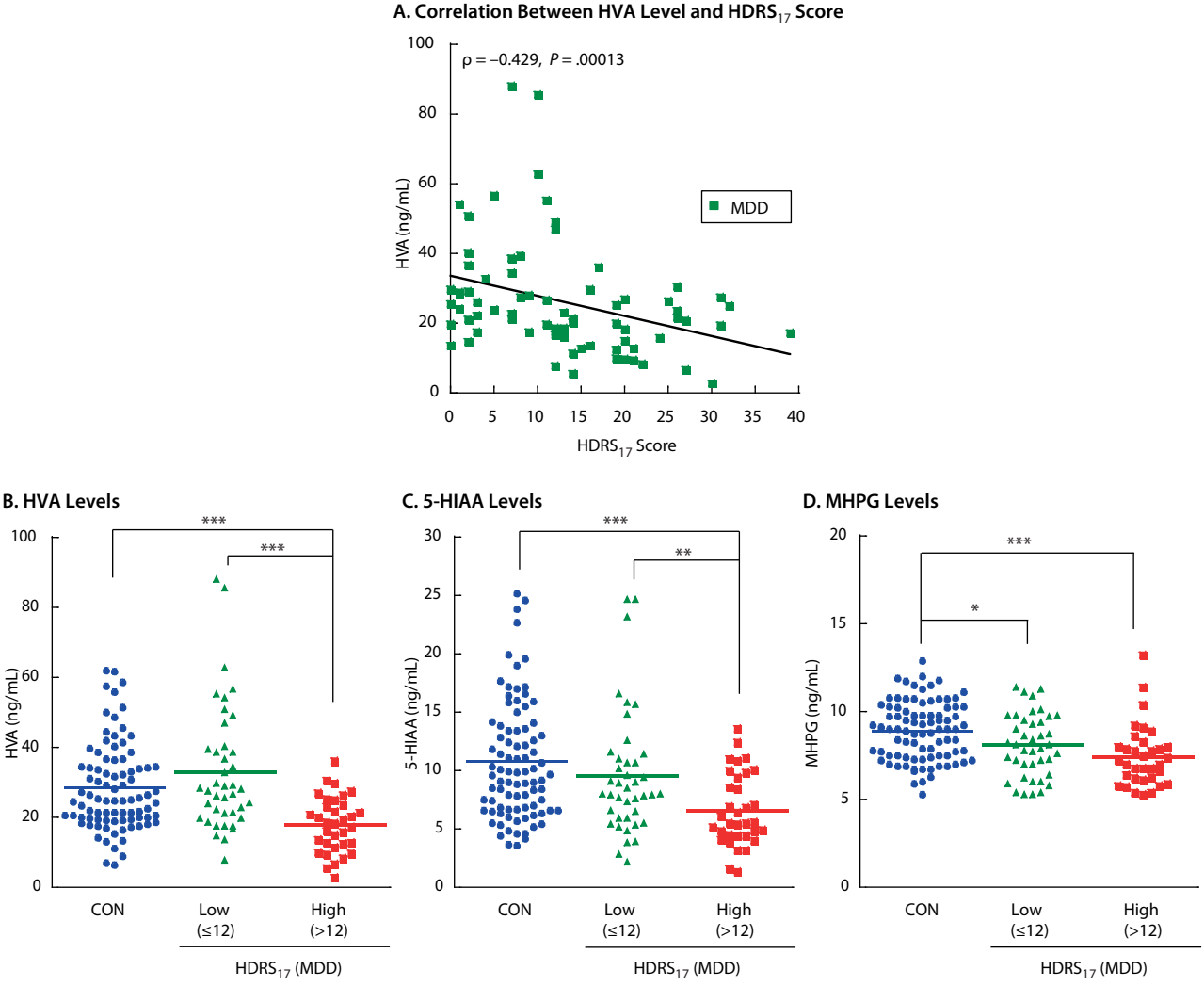
Abbreviations: CON = healthy controls, CSF = cerebrospinal fluid, 5-HIAA = 5-hydroxy-3-indoleacetic acid, HVA = homovanillic acid, MDD = major depressive disorder, MHPG = 3-methoxy-4-hydroxyphenylethyleneglycol.

Table 2. Correlations Between Respective Monoamine Metabolites and Clinical Factors[a]

	Healthy Controls						Patients With MDD					
	HVA		5-HIAA		MHPG		HVA		5-HIAA		MHPG	
Clinical Factor	ρ	*P* Value	ρ	*P* Value	ρ	*P* Value	ρ	*P* Value	ρ	*P* Value	ρ	*P* Value
Age (y)	0.11	.291	**0.259**	**.016**	0.1	.23	0.028	.406	0.177	.065	0.087	.228
CP (mg/d)	…	…	…	…	…	…	**0.238**	**.047**	0.099	.415	−0.126	.299
IMI (mg/d)	…	…	…	…	…	…	−0.1	.412	**−0.388**	**<.001**	**−0.489**	**<.0001**
$HDRS_{17}$ score	…	…	…	…	…	…	**−0.429**	**<.001**	**−0.233**	**.044**	−0.184	.115

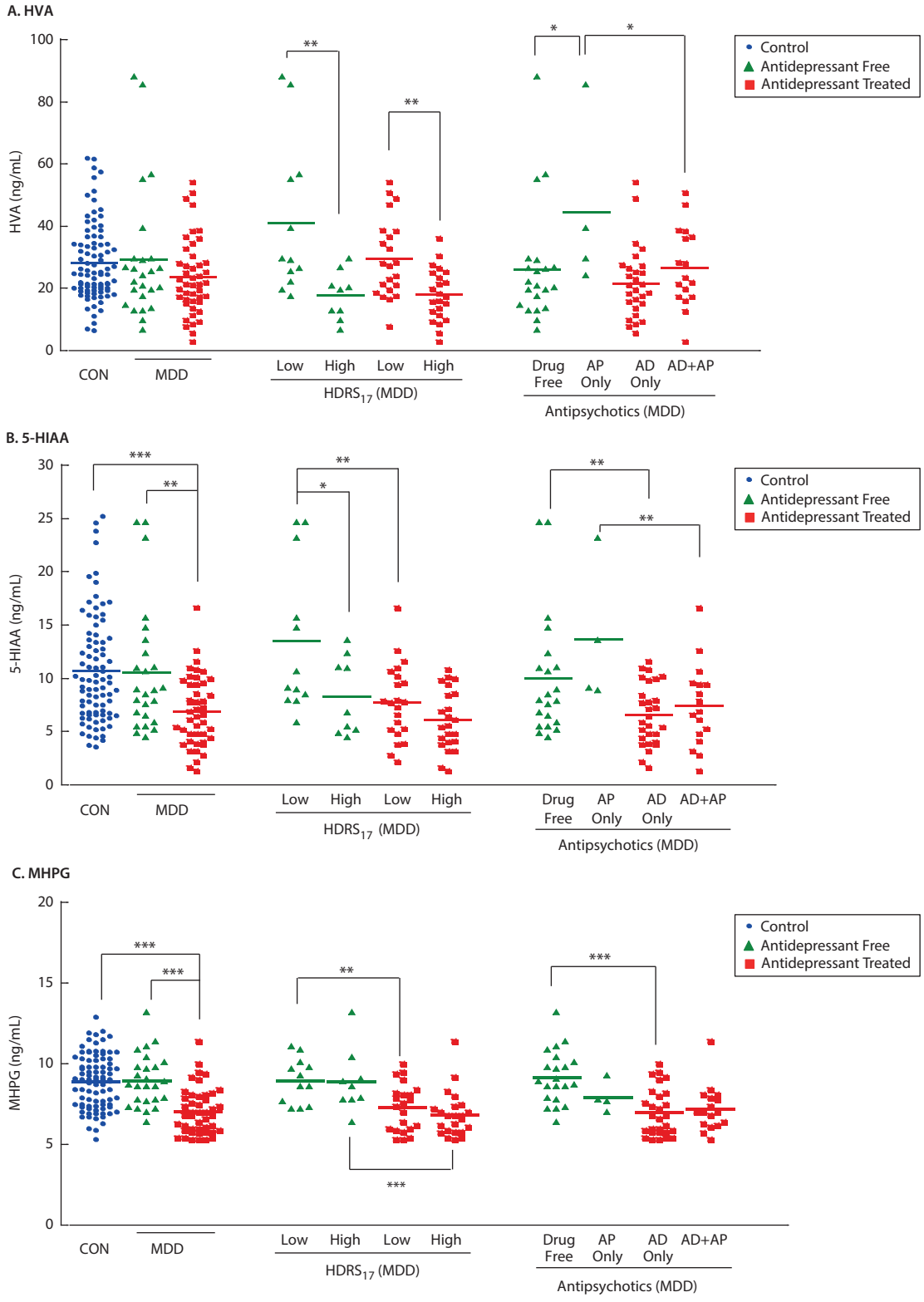
[a]Significant results are in bold type.
Abbreviations: CP = chlorpromazine-equivalent dose of antipsychotics, $HDRS_{17}$ = 17-item Hamilton Depression Rating Scale, 5-HIAA = 5-hydroxy-3-indoleacetic acid, HVA = homovanillic acid, IMI = imipramine-equivalent dose of antidepressants, MDD = major depressive disorder, ρ = Spearman rank correlation coefficient, MHPG = 3-methoxy-4-hydroxyphenylethyleneglycol.
Symbol: … = not applicable.

Figure 2. Alteration Pattern of CSF Monoamine Metabolite Levels in Patients With MDD When Stratified by Severity of MDD Symptoms[a]



[a]Correlation between HVA level and $HDRS_{17}$ score is shown in part A. Parts B through D depict levels of monoamine metabolites when the patient group was divided into 2 groups according to $HDRS_{17}$ score (1-way analysis of covariance controlling for sex and age).
*P < .05. **P < .01. ***P < .001.
Abbreviations: CON = healthy controls, CSF = cerebrospinal fluid, $HDRS_{17}$ = 17-item Hamilton Depression Rating Scale (score: Low ≤ 12 < High), 5-HIAA = 5-hydroxy-3-indoleacetic acid, HVA = homovanillic acid, MDD = major depressive disorder, MHPG = 3-methoxy-4-hydroxyphenylethyleneglycol.

Figure 3. Monoamine Metabolite Levels Stratified by Medication and Depression Severity[a]



[a]One-way analysis of covariance controlling for sex and age.
*P < .05. **P < .01. ***P < .001.

Abbreviations: AD = antidepressant, AP = antipsychotic, CON = healthy controls, CSF = cerebrospinal fluid, $HDRS_{17}$ = 17-item Hamilton Depression Rating Scale (score: Low ≤ 12 < High), 5-HIAA = 5-hydroxy-3-indoleacetic acid, HVA = homovanillic acid, MDD = major depressive disorder, MHPG = 3-methoxy-4-hydroxyphenylethyleneglycol.

constant. Significant, albeit weak, correlations were also observed between 5-HIAA and MHPG and between MHPG and HVA levels. (3) We found no significant sex differences for any metabolite. (4) Age was modestly correlated with 5-HIAA, but not HVA or MHPG, levels. (5) Patients taking antidepressants had significantly lower 5-HIAA and MHPG, but not HVA, levels than antidepressant-free patients. (6) Although the number of patients using antipsychotic medication was small, these patients had higher HVA levels than those not using antipsychotic medication. (7) In antidepressant-free patients, the High group ($HDRS_{17}$ score >12) had significantly lower HVA and 5-HIAA, but not MHPG, levels than the Low group. Furthermore, $HDRS_{17}$ scores and HVA levels were negatively correlated in the total patients.

Effects of Sex and Age

Some previous studies reported that sex is related to CSF levels of 5-HIAA[12,13,30,31] and HVA,[12,31] but our data, obtained using a large sample size, do not support sexual dimorphism in CSF monoamine metabolite levels. Some studies have reported that female patients have significantly higher CSF 5-HIAA[12,13,30,31] and HVA[12,31] levels than male patients, while other studies[11–13,22,29,40] have not found such a sex difference. Thus, there is very likely no major sex difference for any monoamine metabolite.

Relationships between CSF 5-HIAA,[12,13,20,32] HVA,[20] and MHPG[12,13,32] levels and age have been studied in the past. Age and CSF 5-HIAA and MHPG,[12,32] or only MHPG,[13] levels in patients have been shown to be correlated. CSF 5-HIAA and HVA also have significantly positive regression coefficients in multiple regression analysis.[20] Exceptionally, one[38] study reported a significantly negative correlation between age and CSF 5-HIAA. Other studies[7,8,16,17,24,39,41,45] have reported no relationship between age and monoamine metabolites. We detected a weak positive correlation between age and 5-HIAA levels in the controls and a similar trend in the patients. Age may thus have a positive, albeit weak, correlation with CSF 5-HIAA levels.

Correlations Between CSF Monoamine Metabolite Levels

We found that CSF HVA levels correlated strongly with 5-HIAA levels and that 5-HIAA levels correlated modestly with MHPG levels, which is consistent with previous reports.[22,36–38,42,44,47,48,56–61] The 3 monoamine metabolites are thus in balance with each other, which may be important for maintaining normal monoaminergic functions.[62] In an animal study,[63] dopamine input was shown to exert tonic excitatory effects on serotonin neurons in the intact brain, and lesions of dopaminergic neurons decreased serotonergic neuronal firing in the dorsal raphe. This may partially explain the balance between CSF HVA and 5-HIAA levels.

Effects of Medication

We found that patients taking antidepressant medication had significantly lower 5-HIAA and MHPG, but not HVA, levels, which is consistent with the majority of the previous studies[17] we described. A limitation to this point is that MAOI antidepressants, which may reduce CSF HVA,[17] were not commercially available in Japan. Although the number of patients using antipsychotic medication was small, we found a significant increase in HVA levels in patients using antipsychotic medications compared to those not using antipsychotics, indicating that antipsychotic medication increases CSF HVA levels. This is in line with previous findings.[64–66]

Depression Severity and CSF Monoamine Metabolites

We found a highly significantly negative correlation between CSF HVA levels and $HDRS_{17}$ scores. We found that CSF HVA levels in the High MDD group were significantly lower than those of the Low group and the controls, which is in line with previous reports.[2–4,12,20,21,23,24] That the Low group (ie, patients with less severe or remitted depression) did not have lower CSF HVA levels compared to the controls may explain, at least in part, the inconsistencies in previous studies. We also found the lowest 5-HIAA levels among the antidepressant recipients in the High MDD group (Figure 3B). This finding is most likely due to the decreasing effect of antidepressants on 5-HIAA levels, while it is also possible that nonresponders to antidepressants had a more profound disruption in serotonin neurotransmission than responders. Decreased CSF 5-HIAA in MDD is partially in line with previous studies.[20,23,25] The observed influences of antidepressant treatment or depression severity on CSF 5-HIAA levels may also have led to the inconsistent results of previous studies. CSF MHPG levels were decreased by antidepressants, consistent with a previous report,[33] but were not associated with severity (Figure 3C).

CSF HVA as a State-Dependent Marker for MDD

As we described earlier in this report, CSF HVA levels reflect depression severity and were not influenced by antidepressants, indicating that among the 3 CSF monoamine metabolites, HVA might be the best state-dependent marker for MDD in the clinical setting. A drawback is the effect of antipsychotics on HVA and the possible effect of MAOIs. Therefore, the use of CSF HVA as a state-dependent marker should be restricted to individuals who are not using antipsychotics or MAOIs. Although CSF 5-HIAA levels seem to correlate negatively with depression severity, they were subject to the strong effects of antidepressants. Given that the majority of MDD patients receive antidepressant treatment, the use of CSF 5-HIAA would be limited. CSF MHPG levels were not state-dependent and were strongly influenced by antidepressants, indicating that CSF MHPG levels cannot be a useful state-dependent marker for MDD.

Possible Mechanisms

Clinically, dopaminergic dysfunction, which includes decreased HVA levels, has been implicated in the pathophysiology of depression.[67,68] For example, pramipexole, a selective dopamine D_2/D_3 agonist, has

been suggested to be effective for treatment-resistant depression.[67, 69] Electroconvulsive therapy for treatment-resistant depression enhances the mesolimbic dopaminergic system and HVA levels in rats and humans, respectively, and has rapid and consistent effects on dopamine[70,71] (reviewed in 2 articles[72,73]).

One possible mechanism underlying the pathogenesis of MDD is stress-induced dopaminergic alteration. In an animal study, acute stress potently attenuated dopaminergic activity and reduced dopamine response to stimuli such as psychostimulants.[74] In an animal model using chronic mild stress, dopaminergic activity in the ventral tegmental area was significantly decreased,[75] which may lead to decreased dopamine response to external stimuli.[76] Similarly, in patients with posttraumatic stress disorder, a significant decrease in HVA levels and a tendency for decreased 5-HIAA levels in the CSF were observed after exposure to anxiety-inducing cues,[77] indicating that decreased monoamine levels may be induced by stress and result in anxiety.

Limitations

Although our sample size was relatively large, the number of drug-free subjects was limited and the numbers of subjects in the subgroups were not very large after stratification. This may have resulted in our overlooking subtle effects of clinical variables on CSF monoamine levels. When we examined the effect of medication use, all classes of antidepressants or antipsychotics were combined together. Thus, we could not see the effects of each drug on CSF monoamine metabolites. Moreover, MAOIs and dopamine and norepinephrine reuptake inhibitors such as bupropion were not commercially available in Japan. Therefore, our data do not reflect potential effects of such antidepressant classes. Previous studies suggested that MAOIs have a decreasing effect on CSF HVA, while bupropion does not (reviewed in Little et al[17]). Finally, our study was cross-sectional and did not confirm the state-dependency of monoamine levels and effects of medication use in a longitudinal manner.

Clinical Significance and Future Directions

We demonstrated that HVA and 5-HIAA levels are both state-dependent, although the latter are greatly influenced by the effect of antidepressants. The CSF HVA level can be used as an effective state-dependent marker (ie, an objective marker reflecting depression severity) even when the subjects take antidepressants, while CSF 5-HIAA level may be a useful state-dependent marker for drug-naive subjects or those who receive non-pharmacologic treatment such as cognitive-behavioral therapy without medication. According to our data, CSF MHPG level is not useful as a marker for depression severity.

As shown in our data, there are huge interindividual differences in CSF monoamine metabolite levels, while their levels highly correlate within an individual, particularly the correlation between 5-HIAA and HVA levels (Figure 1G and 1H). However, mechanisms underlying these phenomena are largely unknown. Future basic and clinical research exploring the causes of and mechanisms for the interindividual differences and correlation between the metabolites is definitely required and will provide important insights into the pathophysiology of MDD and lead to the development of novel treatment for the illness. "Omics" approaches, such as proteomics and metabolomics, to CSF samples will be particularly useful to reveal the molecular basis of monoamine metabolite dynamics, which is currently underway in our laboratory.

CONCLUSIONS

We examined CSF monoamine levels stratified by medication and depression severity in a relatively large sample of patients with MDD and healthy controls. We clearly showed that CSF 5-HIAA and HVA levels could be a state-dependent marker in MDD patients. Since 5-HIAA levels greatly decrease by antidepressants, HVA levels might be more useful in the clinical setting.

Submitted: August 12, 2016; accepted December 13, 2016.

Published online: July 25, 2017.

Potential conflicts of interest: All authors declare no conflicts of interest.

Funding/support: This study was supported by a Grant-in-Aid for Scientific Research (A) from the Japan Society for the Promotion of Science (JSPS, No. 25253075 for H.K. and K.H.); a JSPS fellowship (24-02218 for H.S.Y.); an Intramural Research Grant for Neurological and Psychiatric Disorders from the National Center of Neurology and Psychiatry, Japan (24-11 and 27-1 for H.K.); Health and Labor Sciences Research Grants for Comprehensive Research on Persons with Disabilities (24-ippan-001 and 27260301 for H.K. and K.H) from the Ministry of Health and Welfare, Japan; and from Japan Agency for Medical Research and Development (AMED; 15dk0310061h0001 and 16dk0307062h0001 for H.K. and K.H.).

Role of the sponsors: The funding agencies had no role in study design, acquisition and interpretation of data, or writing of this article.

Previous presentation: Presented at the 30th World Congress of the International College of Neuropsychopharmacology (CINP); July 3, 2016; Seoul, South Korea.

Acknowledgments: The authors thank all volunteers who took part in the study.

Supplementary material: See accompanying pages.

REFERENCES

1. Freis ED. Mental depression in hypertensive patients treated for long periods with large doses of reserpine. *N Engl J Med*. 1954;251(25):1006–1008.
2. Kasa K, Otsuki S, Yamamoto M, et al. Cerebrospinal fluid gamma-aminobutyric acid and homovanillic acid in depressive disorders. *Biol Psychiatry*. 1982;17(8):877–883.
3. Sher L, Mann JJ, Traskman-Bendz L, et al. Lower cerebrospinal fluid homovanillic acid levels in depressed suicide attempters. *J Affect Disord*. 2006;90(1):83–89.
4. Swann AC, Katz MM, Bowden CL, et al. Psychomotor performance and monoamine function in bipolar and unipolar affective disorders. *Biol Psychiatry*. 1999;45(8):979–988.
5. Sher L, Oquendo MA, Li S, et al. Lower CSF homovanillic acid levels in depressed patients with a history of alcoholism. *Neuropsychopharmacology*. 2003;28(9):1712–1719.
6. Sher L, Oquendo MA, Li S, et al. Higher cerebrospinal fluid homovanillic acid levels in depressed patients with comorbid posttraumatic stress disorder. *Eur Neuropsychopharmacol*. 2005;15(2):203–209.
7. Widerlöv E, Bissette G, Nemeroff CB. Monoamine metabolites, corticotropin releasing factor and somatostatin as CSF markers in depressed patients. *J Affect Disord*. 1988;14(2):99–107.
8. Sullivan GM, Oquendo MA, Huang YY, et al. Elevated cerebrospinal fluid 5-hydroxyindoleacetic acid levels in women with comorbid depression and panic disorder. *Int J Neuropsychopharmacol*. 2006;9(5):547–556.
9. Sullivan GM, Mann JJ, Oquendo MA, et al. Low cerebrospinal fluid transthyretin levels in

depression: correlations with suicidal ideation and low serotonin function. *Biol Psychiatry*. 2006;60(5):500–506.
10. Berger PA, Faull KF, Kilkowski J, et al. CSF monoamine metabolites in depression and schizophrenia. *Am J Psychiatry*. 1980;137(2):174–180.
11. Oreland L, Wiberg A, Asberg M, et al. Platelet MAO activity and monoamine metabolites in cerebrospinal fluid in depressed and suicidal patients and in healthy controls. *Psychiatry Res*. 1981;4(1):21–29.
12. Koslow SH, Maas JW, Bowden CL, et al. CSF and urinary biogenic amines and metabolites in depression and mania: a controlled, univariate analysis. *Arch Gen Psychiatry*. 1983;40(9):999–1010.
13. Gerner RH, Fairbanks L, Anderson GM, et al. CSF neurochemistry in depressed, manic, and schizophrenic patients compared with that of normal controls. *Am J Psychiatry*. 1984;141(12):1533–1540.
14. Roy A, Pickar D, De Jong J, et al. Norepinephrine and its metabolites in cerebrospinal fluid, plasma, and urine: relationship to hypothalamic-pituitary-adrenal axis function in depression. *Arch Gen Psychiatry*. 1988;45(9):849–857.
15. Molchan SE, Lawlor BA, Hill JL, et al. CSF monoamine metabolites and somatostatin in Alzheimer's disease and major depression. *Biol Psychiatry*. 1991;29(11):1110–1118.
16. De Bellis MD, Geracioti TD Jr, Altemus M, et al. Cerebrospinal fluid monoamine metabolites in fluoxetine-treated patients with major depression and in healthy volunteers. *Biol Psychiatry*. 1993;33(8–9):636–641.
17. Little JT, Ketter TA, Mathé AA, et al. Venlafaxine but not bupropion decreases cerebrospinal fluid 5-hydroxyindoleacetic acid in unipolar depression. *Biol Psychiatry*. 1999;45(3):285–289.
18. Ehnvall A, Sjögren M, Zachrisson OC, et al. Lifetime burden of mood swings and activation of brain norepinephrine turnover in patients with treatment-refractory depressive illness. *J Affect Disord*. 2003;74(2):185–189.
19. Kaddurah-Daouk R, Yuan P, Boyle SH, et al. Cerebrospinal fluid metabolome in mood disorders-remission state has a unique metabolic profile. *Sci Rep*. 2012;2:667.
20. Asberg M, Bertilsson L, Mårtensson B, et al. CSF monoamine metabolites in melancholia. *Acta Psychiatr Scand*. 1984;69(3):201–219.
21. Roy A, Agren H, Pickar D, et al. Reduced CSF concentrations of homovanillic acid and homovanillic acid to 5-hydroxyindoleacetic acid ratios in depressed patients: relationship to suicidal behavior and dexamethasone nonsuppression. *Am J Psychiatry*. 1986;143(12):1539–1545.
22. Westenberg HG, Verhoeven WM. CSF monoamine metabolites in patients and controls: support for a bimodal distribution in major affective disorders. *Acta Psychiatr Scand*. 1988;78(5):541–549.
23. Jones JS, Stanley B, Mann JJ, et al. CSF 5-HIAA and HVA concentrations in elderly depressed patients who attempted suicide. *Am J Psychiatry*. 1990;147(9):1225–1227.
24. Reddy PL, Khanna S, Subhash MN, et al. CSF amine metabolites in depression. *Biol Psychiatry*. 1992;31(2):112–118.
25. Palaniappun V, Ramachandran V, Somasundaram O. Norepinephrine and serotonin metabolism and clinical response to combined imipramine and amitriptyline therapy in depression. *Indian J Psychiatry*. 1991;33(3):193–199.
26. Hou C, Jia F, Liu Y, et al. CSF serotonin, 5-hydroxyindolacetic acid and neuropeptide Y levels in severe major depressive disorder. *Brain Res*. 2006;1095(1):154–158.
27. Geracioti TD Jr, Loosen PT, Ekhator NN, et al. Uncoupling of serotonergic and noradrenergic systems in depression: preliminary evidence from continuous cerebrospinal fluid sampling. *Depress Anxiety*. 1997;6(3):89–94.
28. Roy A, Pickar D, De Jong J, et al. Suicidal behavior in depression: relationship to noradrenergic function. *Biol Psychiatry*. 1989;25(3):341–350.
29. Post RM, Gordon EK, Goodwin FK, et al. Central norepinephrine metabolism in affective illness: MHPG in the cerebrospinal fluid. *Science*. 1973;179(4077):1002–1003.
30. Gjerris A, Sørensen AS, Rafaelsen OJ, et al. 5-HT and 5-HIAA in cerebrospinal fluid in depression. *J Affect Disord*. 1987;12(1):13–22.
31. Kishida I, Aklillu E, Kawanishi C, et al. Monoamine metabolites level in CSF is related to the 5-HTT gene polymorphism in treatment-resistant depression. *Neuropsychopharmacology*. 2007;32(10):2143–2151.
32. Gjerris A, Werdelin L, Gjerris F, et al. CSF-amine metabolites in depression, dementia and in controls. *Acta Psychiatr Scand*. 1987;75(6):619–628.
33. Bäckman J, Alling C, Alsén M, et al. Changes of cerebrospinal fluid monoamine metabolites during long-term antidepressant treatment. *Eur Neuropsychopharmacol*. 2000;10(5):341–349.
34. Asberg M, Thorén P, Träskman L, et al. "Serotonin depression"—a biochemical subgroup within the affective disorders? *Science*. 1976;191(4226):478–480.
35. Peabody CA, Faull KF, King RJ, et al. CSF amine metabolites and depression. *Psychiatry Res*. 1987;21(1):1–7.
36. Timmerman L, de Beurs P, Tan BK, et al. A double-blind comparative clinical trial of citalopram vs maprotiline in hospitalized depressed patients. *Int Clin Psychopharmacol*. 1987;2(3):239–253.
37. Roy A, Pickar D, Linnoila M, et al. Cerebrospinal fluid monoamine and monoamine metabolite concentrations in melancholia. *Psychiatry Res*. 1985;15(4):281–292.
38. Mann JJ, Malone KM. Cerebrospinal fluid amines and higher-lethality suicide attempts in depressed inpatients. *Biol Psychiatry*. 1997;41(2):162–171.
39. Papeschi R, McClure DJ. Homovanillic and 5-hydroxyindoleacetic acid in cerebrospinal fluid of depressed patients. *Arch Gen Psychiatry*. 1971;25(4):354–358.
40. Coppen A, Prange AJ Jr, Whybrow PC, et al. Abnormalities of indoleamines in affective disorders. *Arch Gen Psychiatry*. 1972;26(5):474–478.
41. Goodwin FK, Post RM, Dunner DL, et al. Cerebrospinal fluid amine metabolites in affective illness: the probenecid technique. *Am J Psychiatry*. 1973;130(1):73–79.
42. Placidi GP, Oquendo MA, Malone KM, et al. Aggressivity, suicide attempts, and depression: relationship to cerebrospinal fluid monoamine metabolite levels. *Biol Psychiatry*. 2001;50(10):783–791.
43. Galfalvy H, Currier D, Oquendo MA, et al. Lower CSF MHPG predicts short-term risk for suicide attempt. *Int J Neuropsychopharmacol*. 2009;12(10):1327–1335.
44. Mann JJ, Oquendo MA, Watson KT, et al. Anxiety in major depression and cerebrospinal fluid free gamma-aminobutyric acid. *Depress Anxiety*. 2014;31(10):814–821.
45. Banki CM, Molnar G, Fekete I. Correlation of individual symptoms and other clinical variables with cerebrospinal fluid amine metabolites and tryptophan in depression. *Arch Psychiatr Nervenkr (1970)*. 1981;229(4):345–353.
46. Banki CM, Molnar G, Vojnik M. Cerebrospinal fluid amine metabolites, tryptophan and clinical parameters in depression, part 2: psychopathological symptoms. *J Affect Disord*. 1981;3(2):91–99.
47. Banki CM, Arató M. Amine metabolites and neuroendocrine responses related to depression and suicide. *J Affect Disord*. 1983;5(3):223–232.
48. Banki CM, Vojnik M, Papp Z, et al. Cerebrospinal fluid magnesium and calcium related to amine metabolites, diagnosis, and suicide attempts. *Biol Psychiatry*. 1985;20(2):163–171.
49. Sheehan DV, Lecrubier Y, Sheehan KH, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for *DSM-IV* and *ICD-10*. *J Clin Psychiatry*. 1998;59(suppl 20):22–33, quiz 34–57.
50. Otsubo T, Tanaka K, Koda R, et al. Reliability and validity of Japanese version of the Mini-International Neuropsychiatric Interview. *Psychiatry Clin Neurosci*. 2005;59(5):517–526.
51. American Psychiatric Association. *Diagnostic and Statistical Manual for Mental Disorders*. Fourth Edition. Washington, DC: American Psychiatric Association; 1994.
52. Williams JB, Kobak KA, Bech P, et al. The GRID-HAMD: standardization of the Hamilton Depression Rating Scale. *Int Clin Psychopharmacol*. 2008;23(3):120–129.
53. Inagaki A, Inada T, Fujiii Y, et al, eds. *Equivalent Dose of Psychotropics*. Tokyo, Japan: Seiwa Shoten; 1999.
54. Sasayama D, Hattori K, Wakabayashi C, et al. Increased cerebrospinal fluid interleukin-6 levels in patients with schizophrenia and those with major depressive disorder. *J Psychiatr Res*. 2013;47(3):401–406.
55. Ogawa S, Hattori K, Sasayama D, et al. Reduced cerebrospinal fluid ethanolamine concentration in major depressive disorder. *Sci Rep*. 2015;5:7796.
56. Soderstrom H, Blennow K, Sjodin AK, et al. New evidence for an association between the CSF HVA:5-HIAA ratio and psychopathic traits. *J Neurol Neurosurg Psychiatry*. 2003;74(7):918–921.
57. Hsiao JK, Colison J, Bartko JJ, et al. Monoamine neurotransmitter interactions in drug-free and neuroleptic-treated schizophrenics. *Arch Gen Psychiatry*. 1993;50(8):606–614.
58. Martinez M, Arnalich F, Vazquez JJ, et al. Altered cerebrospinal fluid amino acid pattern in the anorexia of aging: relationship with biogenic amine metabolism. *Life Sci*. 1993;53(21):1643–1650.
59. Hsiao JK, Potter WZ, Agren H, et al. Clinical investigation of monoamine neurotransmitter interactions. *Psychopharmacology (Berl)*. 1993;112(suppl):S76–S84.
60. Nilsson LK, Nordin C, Jönsson EG, et al. Cerebrospinal fluid kynurenic acid in male and female controls—correlation with monoamine metabolites and influences of confounding factors. *J Psychiatr Res*. 2007;41(1–2):144–151.
61. Jokinen J, Nordström AL, Nordström P. Cerebrospinal fluid monoamine metabolites and suicide. *Nord J Psychiatry*. 2009;63(4):276–279.
62. Geracioti TD Jr, Keck PE Jr, Ekhator NN, et al. Continuous covariability of dopamine and serotonin metabolites in human cerebrospinal fluid. *Biol Psychiatry*. 1998;44(3):228–233.

63. Guiard BP, El Mansari M, Merali Z, et al. Functional interactions between dopamine, serotonin and norepinephrine neurons: an in-vivo electrophysiological study in rats with monoaminergic lesions. *Int J Neuropsychopharmacol*. 2008;11(5):625–639.
64. Doran AR, Rubinow DR, Wolkowitz OM, et al. Fluphenazine treatment reduces CSF somatostatin in patients with schizophrenia: correlations with CSF HVA. *Biol Psychiatry*. 1989;25(4):431–439.
65. Nikisch G, Baumann P, Wiedemann G, et al. Quetiapine and norquetiapine in plasma and cerebrospinal fluid of schizophrenic patients treated with quetiapine: correlations to clinical outcome and HVA, 5-HIAA, and MHPG in CSF. *J Clin Psychopharmacol*. 2010;30(5):496–503.
66. Fyrö B, Wode-Helgodt B, Borg S, et al. The effect of chlorpromazine on homovanillic acid levels in cerebrospinal fluid of schizophrenic patients. *Psychopharmacologia*. 1974;35(4):287–294.
67. Hori H, Kunugi H. The efficacy of pramipexole, a dopamine receptor agonist, as an adjunctive treatment in treatment-resistant depression: an open-label trial. *ScientificWorldJournal*. 2012;2012:372474.
68. Dunlop BW, Nemeroff CB. The role of dopamine in the pathophysiology of depression. *Arch Gen Psychiatry*. 2007;64(3):327–337.
69. Cusin C, Iovieno N, Iosifescu DV, et al. A randomized, double-blind, placebo-controlled trial of pramipexole augmentation in treatment-resistant major depressive disorder. *J Clin Psychiatry*. 2013;74(7):e636–e641.
70. Lammers CH, Diaz J, Schwartz JC, et al. Selective increase of dopamine D3 receptor gene expression as a common effect of chronic antidepressant treatments. *Mol Psychiatry*. 2000;5(4):378–388.
71. Nikisch G, Mathé AA. CSF monoamine metabolites and neuropeptides in depressed patients before and after electroconvulsive therapy. *Eur Psychiatry*. 2008;23(5):356–359.
72. Baldinger P, Lotan A, Frey R, et al. Neurotransmitters and electroconvulsive therapy. *J ECT*. 2014;30(2):116–121.
73. Leggio GM, Salomone S, Bucolo C, et al. Dopamine D(3) receptor as a new pharmacological target for the treatment of depression. *Eur J Pharmacol*. 2013;719(1–3):25–33.
74. Chang CH, Grace AA. Amygdala β-noradrenergic receptors modulate delayed downregulation of dopamine activity following restraint. *J Neurosci*. 2013;33(4):1441–1450.
75. Chang CH, Grace AA. Amygdala-ventral pallidum pathway decreases dopamine activity after chronic mild stress in rats. *Biol Psychiatry*. 2014;76(3):223–230.
76. Lodge DJ, Grace AA. The hippocampus modulates dopamine neuron responsivity by regulating the intensity of phasic neuron activation. *Neuropsychopharmacology*. 2006;31(7):1356–1361.
77. Geracioti TD Jr, Jefferson-Wilson L, Strawn JR, et al. Effect of traumatic imagery on cerebrospinal fluid dopamine and serotonin metabolites in posttraumatic stress disorder. *J Psychiatr Res*. 2013;47(7):995–998.

Supplementary material follows this article.

THE JOURNAL OF CLINICAL PSYCHIATRY
THE OFFICIAL JOURNAL OF THE AMERICAN SOCIETY OF CLINICAL PSYCHOPHARMACOLOGY

Supplementary Material

Article Title: Relationships of Cerebrospinal Fluid Monoamine Metabolite Levels With Clinical Variables in Major Depressive Disorder

Author(s): Hyung Shin Yoon, PhDa,b; Kotaro Hattori, MD, PhDa,c; Shintaro Ogawa, PhDa; Daimei Sasayama, MD, PhDa,d; Miho Ota, MD, PhDa; Toshiya Teraishi, MD, PhDa; and Hiroshi Kunugi MD, PhDa,*

DOI Number: https://doi.org/10.4088/JCP.16m11144

List of Supplementary Material for the article

1. **eTable 1** There were no significant differences in any monoamine metabolite levels between males and females in either the patient or control group

Disclaimer

This Supplementary Material has been provided by the author(s) as an enhancement to the published article. It has been approved by peer review; however, it has undergone neither editing nor formatting by in-house editorial staff. The material is presented in the manner supplied by the author.

Supplementary eTable 1. There were no significant differences in any monoamine metabolite levels between males and females in either the patient or control group

Sex	Healthy controls				Patients with MDD			
	HVA	5-HIAA	MHPG	n	HVA	5-HIAA	MHPG	n
Male	27.2 (12.0)	10.1 (4.5)	9.0 (1.6)	48	25.6 (14.8)	7.9 (4.5)	7.7 (1.7)	37
Female	30.0 (13.0)	11.5 (5.5)	8.9 (1.8)	39	27.1 (17.4)	8.6 (4.9)	7.9 (1.8)	38
p -value	0.29	0.17	0.8		0.67	0.57	0.58	

Values are shown as mean (standard deviation).

MDD: major depressive disorder; HVA: homovanillic acid; 5-HIAA: 5-hydroxy-3-indoleacetic acid; MHPG: 4-hydroxy-3-methoxyphenylethyleneglycol