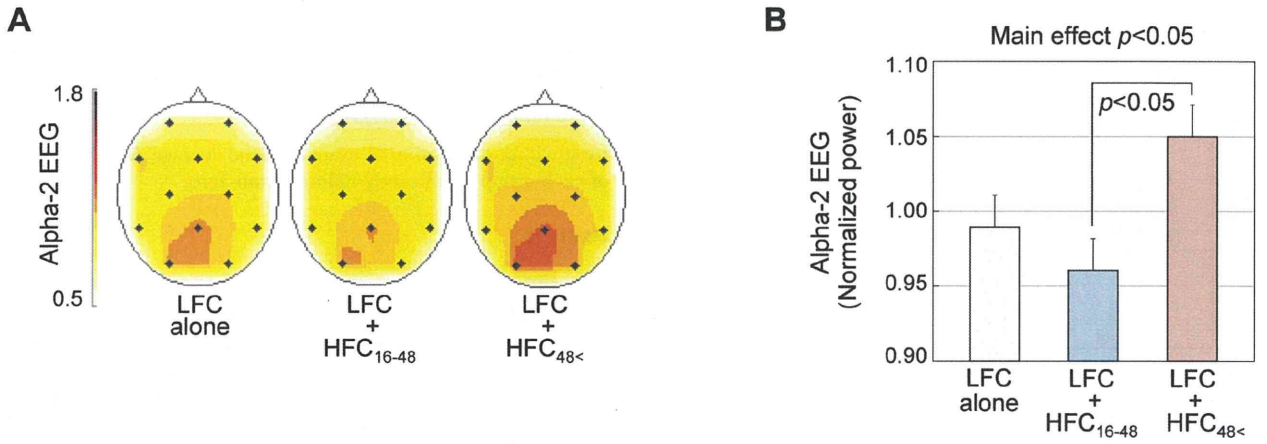


**Figure 4. Results of Experiment 1: broad examination of frequency dependency of the hypersonic effect.** A: Scalp distribution of electroencephalographic activity during application of different HFC frequencies. To overview the spatial distribution of the equivalent potential of alpha2 frequency band (10–13 Hz) of EEGs, colored contour line maps were constructed by using 2,565 scalp grid points computed by linear interpolation and extrapolation of alpha2 components from 12 electrodes [33,34]. Darker red indicates higher alpha2. Note that the alpha2 in the occipital region changed depending on the frequency of the HFC. B: Mean (+SE) value of Alpha-2 EEGs. Potential of alpha2 frequency band recorded from 7 electrodes in the centro-parieto-occipital region (C3, C4, T5, Pz, T6, O1 and O2) for the last 100 sec of sound application was averaged across all subjects. Analysis of variance (ANOVA) revealed the main effect of HFC to be significant, and Tukey's post-hoc test found significant difference between [LFC+$HFC_{16\text{-}48}$] and [LFC+$HFC_{48<}$].
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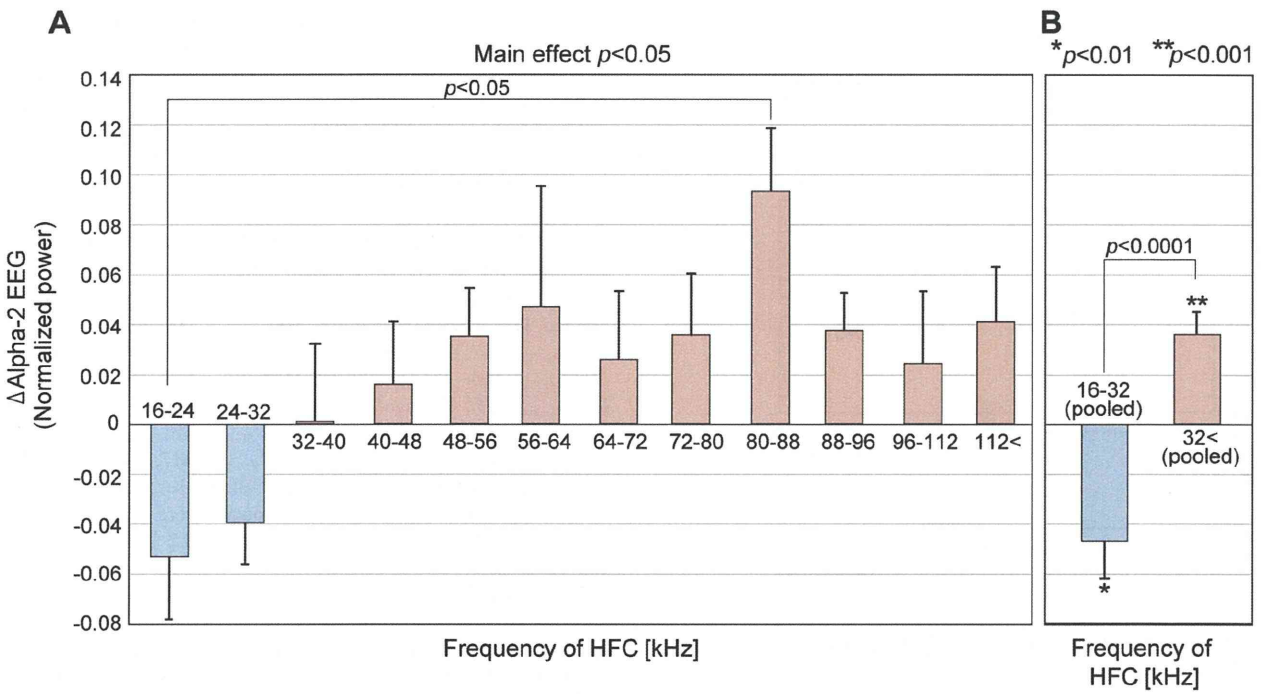


**Figure 5. Results of Experiment 2: detailed examinations of frequency dependency of the hypersonic effect.** A: Mean (+SE) values of ΔAlpha-2 EEG across the subjects in each of twelve sub-experiments. The frequencies indicate the frequency range of HFC that were applied together with LFC ([LFC+HFC]) in comparison with the control ([LFC] alone). ΔAlpha-2 EEG is calculated by subtracting Alpha-2 EEG obtained during [LFC] from those during [LFC+HFC] in each subject. Univariate ANOVA showed the main effect of the frequency of HFC ($p<0.05$). Tukey's post-hoc tests showed a significant difference between ΔAlpha-2 EEG obtained with [LFC+$HFC_{16\text{-}24}$] and that obtained with [LFC+$HFC_{80\text{-}88}$] ($p<0.05$). B: Comparison of ΔAlpha-2 EEG between two groups of pooled data obtained in the sub-experiments using HFC below 32 kHz and those obtained in the sub-experiments using HFC above 32 kHz. Unpaired t-tests showed significant difference between the two groups and 1-sampled t-tests showed significant difference from zero for each group, that is, ΔAlpha-2 EEG obtained by using HFC below 32 kHz was significantly negative ($p<0.01$), while those obtained by using HFC above 32 kHz was significantly positive ($p<0.001$).
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We carried out ANOVA using ΔAlpha-2 EEG as a variable and the frequency of the HFC as a factor. Results showed the main effect of the frequency of the applied HFC was significant ($F(11,92) = 1.980$, $p<0.05$). The Tukey's post-hoc test showed that ΔAlpha-2 EEG differed with statistical significance between the application of [LFC+$HFC_{16-24}$], which marked the lowest mean, and [LFC+$HFC_{80-88}$], the highest ($p<0.05$). The application of [LFC+$HFC_{24-32}$], the second lowest, and that of [LFC+$HFC_{80-88}$] did not show significant difference but the $p$-value marked close to the significance level ($p = 0.06$). In the experiment using $HFC_{80-88}$, 8 out of 9 subjects showed positive ΔAlpha-2 EEG. On the contrary, 8 out of 10 subjects in the experiment using $HFC_{16-24}$ and 8 out of 9 subjects in the sub-experiment using $HFC_{24-32}$ showed negative ΔAlpha-2 EEG.

Having observed that the mean value of ΔAlpha-2 EEG became higher as the frequency of the applied HFC increased and changed from negative to positive at around 32 kHz, we pooled and divided the data into two groups, one for HFC below 32 kHz (n = 19) and the other for HFC above 32 kHz (n = 85) (Figure 5B). Unpaired t-tests (two-sided test) showed that there was significant difference between the two groups ($t(103) = 4.06$, $p<0.0001$). We also performed 1-sampled t-tests (two-sided test) for each group. The ΔAlpha-2 EEG below 32 kHz was significantly negative ($t(18) = -3.082$, $p<0.01$), while that above 32 kHz was significantly positive ($t(84) = 4.014$, $p<0.001$).

## Discussion

Our two experiments suggest that the appearance of the hypersonic effect markedly changes depending on the frequency of the HFCs when applied along with lower audible components. Experiment 2, in particular, revealed that Alpha-2 EEG, used as an index of the emergence of the hypersonic effect, indicates contrary behaviors between higher and lower frequencies of applied HFC, at around 32 kHz; it decreases with lower frequency HFCs and increases with higher frequency HFCs. Furthermore, the magnitude of Alpha-2 EEG gradually changes according to the frequency of the HFCs. This study is the first to show that the emergence of the hypersonic effect depends on the frequency of the HFCs and that its effect may be either positive or negative.

In Experiment 1, we broadly examined the frequency dependency of the hypersonic effect. We found significant difference in Alpha-2 EEG between LFC associated with HFC below 48 kHz (i.e., LFC+$HFC_{16-48}$) and that above 48 kHz (i.e., LFC+$HFC_{48<}$). However, the difference was not significant when Alpha 2-EEG in LFC+$HFC_{16-48}$ and LFC+$HFC_{48<}$ were compared to the control, LFC alone, although there was a tendency for Alpha2-EEG to decrease in $HFC_{16-48}$ while it increased in $HFC_{48<}$. One possible reason could be that $HFC_{16-48}$ includes contrary components, one decreasing and the other increasing Alpha 2-EEG and thereby cancelling each other out.

In these experiments, the total power of sound was always larger in LFC+HFC than in LFC alone. Thus, it is possible to assume that changes in Alpha-2 EEG are related to the total power of applied sound rather than to frequency. In fact, it has been reported that increasing the intensity of inaudible HFCs enhances the hypersonic effect with a significant increase in the occipital alpha EEG and in the CLL of subjects [3,4]. To examine that possibility, we plotted power of sound stimuli and ΔAlpha-2 EEG against frequency in Figure S1. The total power was largest when HFCs of 24–32 kHz, which reduced ΔAlpha-2 EEG, were applied. On the other hand, it was much smaller when HFCs of 80–88 kHz, which showed the greatest increase of ΔAlpha-2 EEG, were applied. Thus it is difficult to explain the behavior of ΔAlpha-2 EEG in terms of the total power of sound.

Based on these findings, we have called the decrease in Alpha-2 EEG and other related phenomena observed when HFCs of 16–32 kHz are applied *the negative hypersonic effect*, to distinguish it from what we previously called the hypersonic effect, and what now we call *the positive hypersonic effect*, as appropriate.

Other studies have indicated a positive correlation between the occipital alpha EEG component and the rCBF of deep-lying brain regions [1,12–17], including the midbrain and the thalamus, which are reportedly activated in the hypersonic effect [1]. We also reported that the rCBF of these regions showed strong correlation with the occipital alpha2 EEG component, which is the faster component of the alpha rhythm (10–13 Hz) [10]. Since many factors contribute to the generation of alpha oscillations (e.g., [18]), we cannot determine a causal relationship between the changes in the Alpha-2 EEG observed in this study and the neuronal activity of the deep-lying brain structure. Omata et al. [17] reported, based on a simultaneous recording of EEG and fMRI, that the slow fluctuation component of alpha power time series with frequencies slower than 0.04 Hz was positively correlated with activity in the midbrain, the medial part of the thalamus and anterior cingulate cortex, which work as part of the reward-generating neuronal network, while the fast fluctuation component, such as waxing and waning [19], correlated with the lateral part of the thalamus. In this study, since we averaged Alpha-2 EEG over 100-sec analysis epoch, the observed changes are considered to correspond to the slow fluctuation component as examined in the Omata study. Of course, we do not argue that changes in EEG observed in this study uniquely reflect the activity of the reward-generating system since such structures likewise contribute to various cognitive functions other than reward generation. Nevertheless, considering the fact that the hypersonic effect involves the enhancement of the pleasure sensation of sound [1–3] and induces an approaching behavior [2–4], we envisage the possibility that the changes in EEG observed in this study may pertain to a certain relationship with the activity of the reward-generating neuronal network.

The mechanism explaining how a difference in the frequency of HFCs induces contrary behaviors of Alpha-2 EEG is not yet known, but we may look to rodent vocalization for reference, especially with regard to the relationship between the frequency of ultrasonic vocalization of rats and their affective reactions. The 22 kHz vocalization of rats relates negative affectation and activation of the punishment-relating neuronal network [20–23], inducing avoidance behavior [20,23–27], while the 50 kHz vocalization relates positive affectation and activation of the reward-generating neuronal network [21–23,28–30], inducing approach behavior [23,31]. As is widely known, the audible frequency of rats reaches a much higher frequency than that of humans; all of the frequencies used in these studies are within the audible range of rats. We cannot, therefore, conclude any common mechanism between rodent vocalization and what was observed in this study. However, these studies may provide a clue for subsequent investigation.

As for the positive hypersonic effect, HFCs at around 80−88 kHz induce the maximum activity of Alpha-2 EEG. Such frequencies are within the ultra-high frequency domain, which is far beyond and not contiguous to the 20 kHz upper limit of the human audible range. The authors had not anticipated that human brain activity would sharply respond to such ultra-high HFCs. Furthermore, the application of even higher HFCs, such as 96–112 kHz or even over 112 kHz, which are extremely faint in power, also increased Alpha-2 EEG no less than did HFCs of 40–

48 kHz and 48–56 kHz. Such data imply the existence of unknown human sensitivity to high frequency air vibrations, which may further contribute to discussion in the basic neuroscience field in light of the discovery of the hypersonic effect [32].

Such widely ranging sensitivity of humans to high frequency components causing a hypersonic effect calls for a re-examination of current audio formats such as CDs, SACDs, DVD-Audios, even of audio formats with higher resolution. The digital audio formats with a reproducible upper frequency below 32 kHz cannot induce a positive hypersonic effect and may not contribute to improved sound quality. If a negative hypersonic effect, observed when HFCs between 16 kHz and 32 kHz were applied, may pertain to a certain relationship with a decrease in the activity of the deep-lying brain structure, a biological assessment would be required in terms of the safe use of HFCs. Needless to say, not only audio formats but also the content itself should be examined to see if it contains a sufficient amount of higher HFCs. To utilize digital audio technologies effectively and safely, it is thus of utmost urgency to re-examine current audio formats in terms of the latest advances in life science.

## Conclusion

By observing Alpha-2 EEG, it became clear that the emergence of the hypersonic effect changes either positively or negatively depending on the frequency of the HFC applied along with the audible sound. We showed that Alpha-2 EEG increases when HFCs above approximately 32 kHz are applied, which indicates that a positive hypersonic effect has emerged, as shown in our earlier studies. Our present study reports, for the first time, that Alpha-2 EEG decreases when HFCs below approximately 32 kHz are applied, which indicates the emergence of a negative hypersonic effect.

## Supporting Information

**Figure S1 The powers of the applied sound stimuli and the change of Alpha-2 EEG.** To examine whether the change of Alpha-2 EEG was dependent on the total power of sound stimuli, the power of each frequency component of each sound stimulus was calculated as partial over all (POA) and plotted with ΔAlpha-2 EEG in Experiment 2. Each bar represents POA of LFC and HFC in each sound stimulus. POA of LFC (gray) is constant across all sub-experiments since identical LFC was always used, while POA of HFC (green) varies across sub-experiments. ΔAlpha-2 EEG was plotted in a red line. POA is a power between specific frequencies and calculated as follows. $\mathrm{POA}[\mathrm{fa}-\mathrm{fbkHz}]=\sum_{f=\mathrm{fa}}^{\mathrm{fb}}\{\mathrm{P}(f)-\mathrm{P}_{\mathrm{BL}}(f)\}$ Here, fa and fb is the lower and upper limit of the frequency range to be analyzed, respectively. $P(f)$ and $P_{BL}(f)$ is the averaged power spectrum for 200 sec of the gamelan music used in the present study and that of the background noise at frequency $f$, respectively. $P(f)$ and $P_{BL}(f)$ were calculated from 0 to 150 kHz using FFT analyzer. Since a slope of power spectrum existed outside the analysis frequency range according to the filter characteristics, the power of such components were also included. Therefore, POA corresponds to an area of power spectrum between specific frequencies.
(TIF)

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## Author Contributions

Conceived and designed the experiments: AF RY NK MH EN TO. Performed the experiments: AF RY NK EN TO. Analyzed the data: AF RY MH EN. Contributed reagents/materials/analysis tools: NK EN TO. Wrote the paper: AF MH EN TO.

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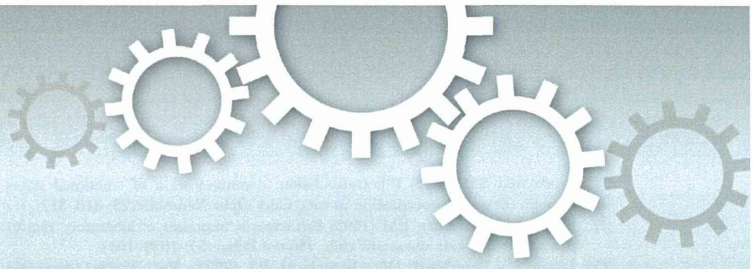

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# Reduced cerebrospinal fluid ethanolamine concentration in major depressive disorder

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**Amino acids play key roles in the function of the central nervous system, and their alterations are implicated in psychiatric disorders. In the search for a biomarker for major depressive disorder (MDD), we used high-performance liquid chromatography to measure amino acids and related molecules in the cerebrospinal fluid (CSF) of 52 patients with MDD (42 depressed and 10 remitted; DSM-IV) and 54 matched controls. Significant differences were found in four amino acid concentrations between the depressed patients and controls. After Bonferroni correction, only ethanolamine (EA) levels remained significantly reduced in depressed patients (nominal $P = 0.0000011$). A substantial proportion of the depressed patients (40.5%) showed abnormally low CSF EA levels (<12.1 μM) ($P = 0.000033$; OR = 11.6, 95% CI: 3.1–43.2). When patients with low EA and those with high EA levels were compared, the former had higher scores for overall depression severity ($P = 0.0033$) and 'Somatic Anxiety' symptoms ($P = 0.00026$). In unmedicated subjects, CSF EA levels showed a significant positive correlation with levels of homovanillic acid ($P = 0.0030$) and 5-hydroxyindoleacetic acid ($P = 0.019$). To our knowledge, this is the first study showing that patients with MDD have significantly lower CSF EA concentrations compared with control subjects. CSF EA could be a state-dependent biomarker for a subtype of MDD.**

Major depressive disorder (MDD) is a common disease with a prevalence rate estimated at 4.4% worldwide[1]. Since the pathophysiology of MDD remains elusive, no established biochemical marker is available for everyday use in the clinical setting and the diagnosis of MDD largely depends on the clinical interview[2]. Although many candidate molecules are present in peripheral blood[3], no study has successfully found a biomarker that is of practical use in the diagnosis, subtyping, or symptomatic assessment of MDD.

Since cerebrospinal fluid (CSF) contacts the interstitial fluid in the central nervous system (CNS)[4] and is mostly segregated from the peripheral circulation by the blood-brain barrier, CSF reflects molecular dynamics in the brain. The composition of CSF (electrolytes[5], amino acids[6], and proteins[7]) differs substantially from that of peripheral blood. Total tau and phosphorylated tau protein in CSF have been established as biomarkers for Alzheimer's disease[8], but are not detectable in peripheral blood. It is therefore feasible to search for a biomarker for MDD in the CSF. The proteomics approach to CSF samples seems to be promising[9].

We have focused on amino acids and related molecules in the CSF to identify a biomarker for MDD, because alterations in the serotonin, noradrenaline, dopamine, glutamate, and γ-amino-butyric acid (GABA) systems are implicated in MDD[10]. These neurotransmitters are themselves amino acids or are synthesized from amino acids. Previous studies examined amino acid levels in the peripheral blood of MDD patients, although their results are equivocal[11–14]. We have recently reported a meta-analysis demonstrating that the plasma L-tryptophan concentration is significantly lower in MDD patients than healthy controls ($P = 0.000059$)[15]. In this context, we chose to examine amino acids and related molecules in the CSF of MDD patients.

Although many studies compared CSF amino acid concentrations between MDD patients and healthy controls[16–28], the majority examined a single amino acid or a few amino acids. Some researchers found reduced CSF GABA levels in depressed patients compared with controls[18,19,21,28], while others reported contradictive negative results[17,20,22]. Increased glutamine levels in depressed patients[24], and reduced glutamate and glycine concentrations in refractory patients were reported[25], however, no differences in CSF glutamate and glutamine levels between 2 groups were inconsistently found[27]. No significant difference between patients and controls was found for tryptophan[21,23], tyrosine[21] or alanine[24]. To our knowledge, only two studies examined comprehensive amino acid profiles in CSF. An early study examined 32 amino acids and related molecules including ethanolamine (EA); however, that sample included only 8 subjects with unipolar depression and 2 controls[16]. A metabolomics-based approach in 14 currently-depressed patients, 14 remitted patients, and 18 controls found that CSF methionine levels were significantly increased in remitted patients compared with controls[26]. Thus, previous information on CSF amino acid levels in MDD patients is surprisingly limited.

We measured CSF amino acids and related molecules in a relatively large sample to search for a biomarker for MDD. We also analyzed the correlations between CSF amino acid levels and depression severity, psychotropic medication, and monoamine metabolites.

## Subjects and methods

**Subjects.** Subjects were 52 patients with MDD and 54 healthy controls matched for age and sex. All participants were biologically-unrelated Japanese. Patients were recruited at the National Center of Neurology and Psychiatry (NCNP) Hospital (Tokyo, Japan), or through advertisements in free local magazines, and by our website. Healthy controls were from the same geographical area (i.e., western Tokyo metropolitan) via advertisements in magazines and website. Trained psychologists or psychiatrists conducted a structured Mini-International Neuropsychiatric Interview (M.I.N.I.)[29], Japanese version with all participants. A consensus diagnosis was made according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria[30] based on the M.I.N.I., an additional unstructured interview, and information from medical records if available. Patients with any comorbid axis I disorder were excluded, as were individuals with a prior medical history of CNS disease, severe head injury, or substance abuse/dependence. After the nature of the study procedures had been fully explained, written informed consent was obtained from all subjects. MDD symptoms were assessed using the Japanese version of the 17-item Hamilton Depression Rating Scale (HAMD-17)[31], and the cut-off score for remission was ≤7[32]. Ten of the 52 patients were remitted. Among all MDD patients (depressed + remitted), 39 patients were medicated and the remaining 13 (11 depressed and 2 remitted) were not on psychotropic medication. Daily doses of benzodiazepine derivatives, antidepressants, and antipsychotics were converted to equivalent doses of diazepam, imipramine, and chlorpromazine respectively, using published guidelines[33] When each class of drugs was not medicated, the dose was considered to be zero. The present experiments on our participants were conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the ethics committee at the NCNP (No. 305).

**Sample collection.** Between 10:00 h and 16:00 h, CSF samples were obtained by lumbar puncture from the L4–5 or L3–4 interspace of participants, in the left decubitus position. CSF samples were immediately placed on ice, and centrifuged at 4000 × *g*. Supernatants were aliquoted and stored at −80°C until assays were performed. All samples were collected from August 2010 to August 2013. Initial 2 mL of CSF was used for measuring glucose, chloride, total protein levels and monoamine metabolites. Homovanillic acid (HVA), 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG), and 5-hydroxyindoleacetic acid (5-HIAA) were measured by high-performance liquid chromatography (HPLC) at SRL Co., Inc. (Tokyo, Japan).

**Determination of CSF levels of amino acids and related molecules by HPLC.** CSF sample was mixed with 4% 5-sulfosalicylic acid dihydrate (WAKO, Tokyo, Japan), and centrifuged for 10 min at 12,000 × *g* and 4°C. Each supernatant was transferred to a micro-tube, filtered using a 0.22-μm pore-diameter syringe-filter (AS ONE, Osaka, Japan), and subjected to HPLC (JASCO, Tokyo, Japan). Acquired data were processed and quantified on the chromatography data station ChromNAV (JASCO). A detailed description of the HPLC protocol is available in the Supplementary Methods.

**Validation by capillary electrophoresis time-of-flight mass spectrometry (CE-TOF-MS).** Amino acids and related molecules were also measured using CE-TOF-MS for a subset of the subjects (24 depressed, 3 remitted patients, and 27 controls). The CE-TOF-MS measurements were performed at Human Metabolome Technologies, Inc. (Yamagata, Japan), by coauthors who were blinded to the HPLC

Table 1 | Demographic and Clinical data on subjects

| | dMDD | rMDD | HC | Statistics |
|---|---|---|---|---|
| **N (Male/Female)** | 42 (19/23) | 10 (6/4) | 54 (28/26) | $\chi^2 = 0.86$, $df = 2$, $P = 0.63$ [1] |
| **Age, year** | 45.5 ± 12.2 | 42.9 ± 14.9 | 43.6 ± 15.3 | $F = 0.26$, $df = 2$, $P = 0.77$ [2] |
| **BZD, mg/day (ratio)** | 14.1 ± 14.4 (29/42) | 12.1 ± 17.9 (7/10) | N.A. | $t = 0.38$, $df = 50$, $P = 0.71$, 95% CI: −8.66 to 12.63 [3] |
| **AD, mg/day (ratio)** | 136.9 ± 131.1 (27/42) | 149.4 ± 170.4 (7/10) | N.A. | $t = -0.26$, $df = 50$, $P = 0.80$, 95% CI: −110.72 to 85.78 [3] |
| **AP, mg/day (ratio)** | 83.7 ± 176.2 (13/42) | 56.5 ± 125.5 (3/10) | N.A. | $t = 0.46$, $df = 50$, $P = 0.65$, 95% CI: −91.67 to 146.14 [3] |
| **HAMD-17, score** | 18.0 ± 7.2 | 4.3 ± 2.4 | N.A. | $t = 10.20$, $df = 44.93$, $P < 5 \times 10^{-13}$, 95% CI: 9.00 to 18.36 [3] |
| **Time of CSF sampling, min [4]** | 185.2 ± 95.4 | 221.5 ± 111.9 | 172.2 ± 118.6 | $F = 0.89$, $df = 2$, $P = 0.42$ [2] |
| **Days from CSF sampling, day [5]** | 554.9 ± 313.5 | 721.5 ± 276.9 | 479.7 ± 286.4 | $F = 3.01$, $df = 2$, $P = 0.054$ [2] |

[1]Based on the chi-squared test with exact probability.
[2]Based on the analysis of variance.
[3]Based on the *t*test.
[4]Sampling time is expressed as minutes from 10:00 AM.
[5]Number of days from the lumbar puncture day to Sep 1, 2013.
Abbreviations: dMDD, depressed (non-remitted) patients with major depressive disorder; rMDD, remitted patients; HC, healthy controls; HAMD-17, 17-item Hamilton Depression Rating Scale; CSF, cerebrospinal fluid; BZD, daily diazepam equivalent dose of benzodiazepine derivatives; AD, daily imipramine equivalent dose of antidepressants; AP, daily chrolpromazine equivalent dose of antipsychotics; CI, confidence interval for the difference between two means.

Table 2 | Comparisons between dMDD and HC for amino acids and related molecule profiles in cerebrospinal fluid

| | dMDD ($N = 42$) | | HC ($N = 54$) | | Statistics for ANCOVA | | | |
|---|---|---|---|---|---|---|---|---|
| Name | μM ± *SD* | NMISS [1] | μM ± *SD* | NMISS | *F* | *df* | *P* | 95% CI |
| **Phosphoethanolamine** | 4.7 ± 1.2 | 0 | 5.1 ± 1.1 | 0 | 2.12 | 1 | 0.15 | −0.75 to 0.11 |
| **Threonine** | 33.1 ± 9.3 | 0 | 29.8 ± 5.3 | 0 | 4.30 | 1 | 0.041 | 0.13 to 6.030 |
| **Serine** | 29.3 ± 7.5 | 0 | 27.9 ± 6.1 | 0 | 0.75 | 1 | 0.39 | −1.51 to 3.86 |
| **Asparagine** | 7.2 ± 1.4 | 0 | 6.6 ± 1.3 | 0 | 3.85 | 1 | 0.053 | −0.0065 to 1.11 |
| **Glutamine** | 711.3 ± 142.5 | 0 | 644.3 ± 108.8 | 0 | 6.70 | 1 | 0.011 | 15.16 to 115.15 |
| **Glycine** | 6.4 ± 2.0 | 0 | 6.0 ± 1.8 | 0 | 0.95 | 1 | 0.33 | −0.38 to 1.12 |
| **Alanine** | 37.5 ± 10.3 | 0 | 33.7 ± 9.0 | 0 | 3.34 | 1 | 0.071 | −0.31 to 7.47 |
| **α-Amino-n-butyric acid** | 2.7 ± 1.0 | 0 | 2.6 ± 0.9 | 0 | 0.30 | 1 | 0.59 | −0.29 to 0.51 |
| **Valine** | 16.6 ± 4.8 | 0 | 15.2 ± 4.6 | 0 | 2.41 | 1 | 0.12 | −0.39 to 3.23 |
| **Methionine** | 3.3 ± 0.9 | 0 | 3.5 ± 0.8 | 0 | 1.68 | 1 | 0.20 | −0.56 to 0.12 |
| **Isoleucine** | 5.2 ± 1.5 | 0 | 4.8 ± 1.4 | 0 | 2.88 | 1 | 0.093 | −0.080 to 1.017 |
| **Leucine** | 12.5 ± 3.4 | 0 | 11.5 ± 2.9 | 0 | 3.42 | 1 | 0.068 | −0.082 to 2.27 |
| **Tyrosine** | 9.0 ± 1.8 | 0 | 9.4 ± 2.3 | 0 | 1.50 | 1 | 0.22 | −1.34 to 0.32 |
| **Phenylalanine** | 10.1 ± 2.1 | 0 | 10.0 ± 2.3 | 0 | 0.038 | 1 | 0.85 | −0.79 to 0.96 |
| **Ethanolamine** | 12.3 ± 2.3 | 0 | 14.8 ± 2.2 | 0 | **27.36** | **1** | **0.0000011** [2] | **−3.29 to −1.48** |
| **Lysine** | 24.3 ± 7.1 | 0 | 22.2 ± 3.9 | 0 | 3.11 | 1 | 0.081 | −0.25 to 4.17 |
| **Histidine + 1-Methylhistidine** | 8.4 ± 2.7 | 0 | 8.0 ± 1.4 | 0 | 0.69 | 1 | 0.41 | −0.50 to 1.21 |
| **Arginine** | 21.8 ± 5.1 | 0 | 22.6 ± 4.7 | 0 | 0.58 | 1 | 0.45 | −2.79 to 1.25 |
| **Aspartate** | 0.7 ± 0.3 | 17 | 0.7 ± 0.2 | 21 | 1.091 | 1 | 0.30 | −0.062 to 0.20 |
| **Glutamate** | 9.1 ± 5.4 | 2 | 8.3 ± 5.2 | 1 | 0.57 | 1 | 0.45 | −1.39 to 3.088 |
| **Cystine** | 2.9 ± 0.7 | 6 | 2.8 ± 0.8 | 8 | 0.20 | 1 | 0.66 | −0.25 to 0.40 |
| **Tryptophan** | 1.8 ± 0.7 | 19 | 2.0 ± 0.4 | 18 | 2.64 | 1 | 0.11 | −0.55 to 0.058 |
| **Ornithine** | 2.9 ± 1.4 | 6 | 3.2 ± 1.5 | 7 | 1.52 | 1 | 0.22 | −1.034 to 0.24 |
| **Carnosine** | 2.4 ± 1.5 | 7 | 3.3 ± 2.1 | 10 | 5.30 | 1 | 0.024 | −1.58 to −0.11 |
| **γ-Aminobutyric acid** | 0.3 ± 0.1 | 24 | 0.3 ± 0.1 | 30 | 1.061 | 1 | 0.31 | −0.023 to 0.072 |

[1]Missing values are replaced with blanks in the ANCOVA analysis.
[2]Adjusted significance was set at $P < 0.002$, and significant *P*-values after Bonferroni correction are in bold type.
Abbreviations: dMDD, depressed (non-remitted) patients with major depressive disorder; HC, healthy controls; SD, standard deviation; NMISS, number of missing values; ANCOVA, analysis of covariance; CI, confidence interval for the difference between two means.

data obtained at the NCNP. Compounds were identified by their peaks using annotated tables with *m/z* values and normalized by migration times. Detailed CE-TOF-MS procedures are described elsewhere[34].

**Statistical analysis.** Data are reported as means ± standard deviation (*SD*). Means were compared using *t*-tests or analysis of variance. Categorical variables were compared using the $\chi^2$ test with exact probability. Analysis of covariance (ANCOVA), controlling for age and sex, was performed to compare CSF levels of amino acids and related molecules in the patients and controls and between subgroups of the patients. Partial correlation analysis, controlling for age and sex, was performed to examine the correlations between equivalent doses of psychotropic drugs and concentrations of amino acids and related molecules, and between EA levels and other CSF substances including biogenic amine metabolites. The Mann-Whitney *U* test with exact probability was used to compare clinical symptoms (HAMD-17 scores) between MDD subgroups (Low-EA vs. High-EA). We obtained 95% CI for the difference between two medians for Mann-Whitney *U* using Hodges-Lehmann estimate. HAMD-17 items were assigned to the following subscales: 'Core' (items 1, 2, 7, 8, 10, 13), 'Sleep' (items 4, 5, 6), 'Activity' (items 7, 8), 'Psychic Anxiety' (items 9, 10), and 'Somatic Anxiety' (items 11, 12, 13), according to Serretti *et al.*[35]. We used non-parametric estimate of receiver operating characteristic (ROC) curves for the assessment of specificity and sensitivity of EA to discriminate between MDD patients and controls, and between depressed and remitted MDD groups. Statistical significance for a two-tailed *P*-value was <0.05, and was corrected for multiple comparisons using the Bonferroni method. All analyses were performed using IBM SPSS Statistics 22.0 Japanese version (IBM Japan, Tokyo, Japan).

## Results

Demographic and clinical characteristics of the subjects are shown in Table 1. There were no significant differences in sex ratio, age distribution, time of CSF sampling, or number or days from sample collection among the depressed, remitted, and control subjects. No significant difference was observed in psychotropic drug doses between depressed and remitted MDD groups. CSF concentrations of 25 of the 41 amino acids and related molecules were successfully determined in the majority of subjects (Table 2). There was nominally a significant difference in the concentrations of threonine, glutamine, EA, and carnosine between the depressed MDD and control groups. When the critical $P = 0.002$ (0.05/25) was conservatively applied after the Bonferroni correction, only EA remained significant (nominal $P = 0.0000011$).

We then performed detailed analyses on the possible relationships between CSF EA levels and clinical variables. In controls, there was no significant difference in CSF EA levels between men and women controlled for age ($F = 0.045$, $df = 1$, $P = 0.83$; 95% CI: −1.10 to 1.36), and no significant correlation between EA and age controlled for sex ($r = -0.063$, $df = 51$, $P = 0.66$; 95% CI: −0.33 to 0.21), between EA and time of CSF sampling controlled for sex and age ($r = 0.061$, $df = 50$, $P = 0.67$: 95% CI: −0.22 to 0.33), or between EA and number of days from sample collection controlled for sex and age ($r = 0.0062$, $df = 50$, $P = 0.97$; 95% CI: −0.27 to 0.28), in a partial correlation analysis. Figure 1a shows dot plots of CSF EA concentrations in depressed MDD patients and controls. Among all MDD patients, no significant difference in EA levels was observed between the medicated and unmedicated patients using ANCOVA, controlling for sex and age ($F = 0.0030$, $df = 1$, $P = 0.96$; 95%CI: −1.57 to 1.66) (Figure 1b), and there were no significant differences in age, sex, or total HAMD-17 score. When unmedicated MDD patients and controls were compared, there was a significant difference in CSF EA based on ANCOVA ($F = 7.0$, $df = 1$, $P = 0.010$; 95% CI: −3.28 to −0.46). Remitted MDD patients had significantly higher EA levels than depressed MDD patients (ANCOVA; $F = 8.1$, $df = 1$, $P = 0.0066$; 95%CI: 0.67 to 3.90) (Figure 1c), and they showed no significant differences in age or sex. There was no significant difference in CSF EA levels between remitted patients and controls ($F = 0.0092$, $df = 1$, $P = 0.92$; 95%CI: −1.68 to 1.53). These comparisons for CSF EA concentrations between subgroups are summarized in Table 3. Area under curve (AUC) by the ROC curve to discriminate between depressed (non-remitted) MDD patients and controls was 0.77 (Supplementary Fig. S1a), that between depressed and remitted MDD groups was 0.75 (Supplementary Fig. S1b), indicating 'fair test' for these discriminations. When "abnormally low CSF EA levels"

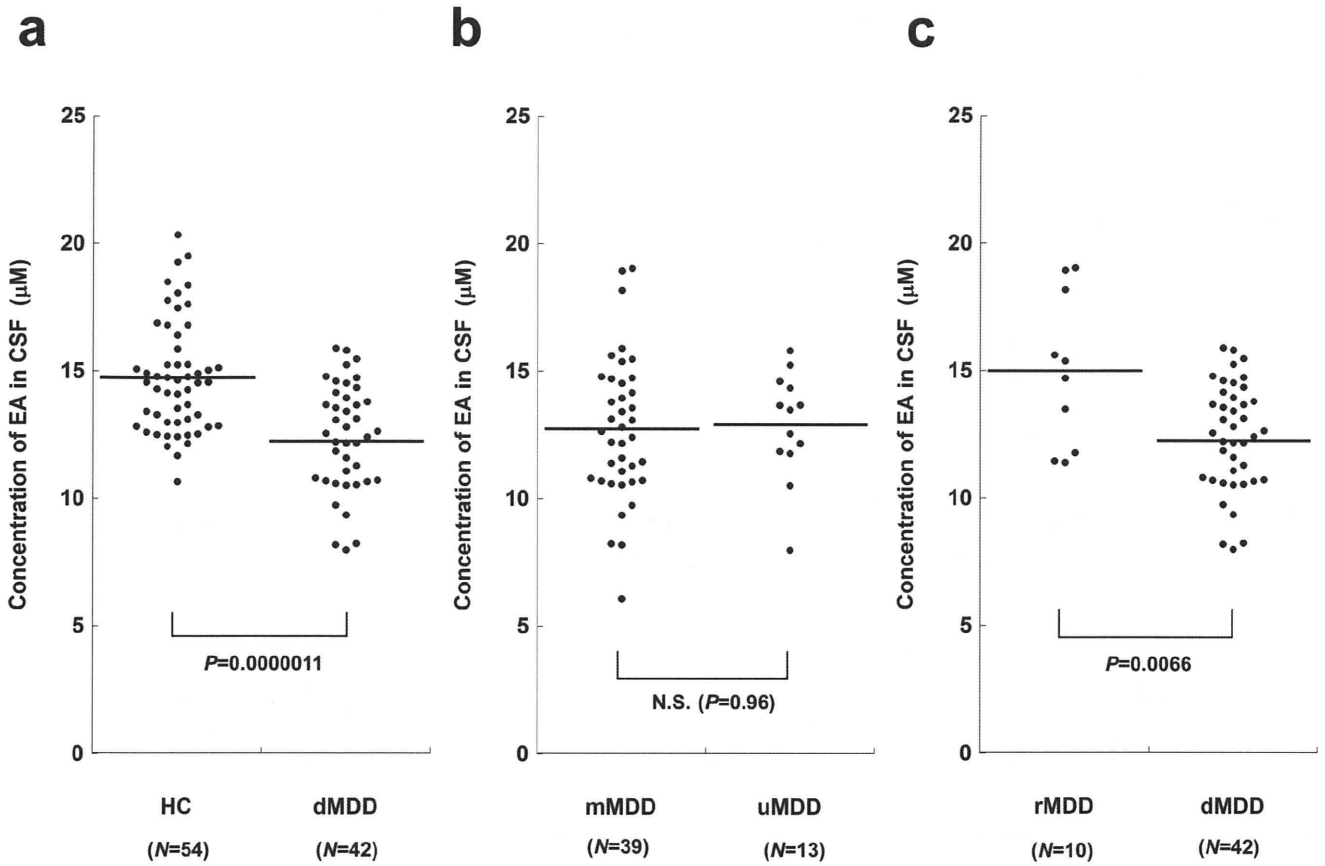


**Figure 1 | Dot plots of CSF EA concentrations in patients with MDD and controls.** Horizontal bars represent mean values of the groups. Statistical analyses were performed by ANCOVA, controlling for age and sex. (a) Comparison between dMDD patients and HC, (b) that between mMDD and uMDD, and (c) that between rMDD and dMDD. Abbreviations: CSF, cerebrospinal fluid; EA, ethanolamine; HC, healthy controls; dMDD, depressed (non-remitted) patients with major depressive disorder; rMDD, remitted patients; mMDD, medicated patients; uMDD, unmedicated patients; ANCOVA, analysis of covariance.

were defined as the 5th percentile value of the controls (<12.1 μM), 17 depressed patients (40.5%) and 3 controls fell within this range ($\chi^2$ = 17.5, $df$ = 1, $P$ = 0.000033; OR = 11.6, 95% CI: 3.1 to 43.2).

To investigate the relationship between patient's symptoms and CSF EA levels, we defined MDD patients (depressed + remitted) whose levels fell below the 1st quartile of CSF EA values as 'Low-EA' ($N$ = 13) and above the 3rd quartile as 'High-EA' ($N$ = 13). The Low-EA group had higher HAMD-17 total scores ($P$ = 0.0033, Mann-Whitney $U$ test), and 'Core' ($P$ = 0.034) and 'Somatic Anxiety' ($P$ = 0.00026) subscale scores (Figure 2). Other HAMD subscales ('Sleep', 'Activity', and 'Psychic Anxiety') showed no significant differences between Low-EA and High-EA groups.

Although there was no significant difference in CSF EA levels between medicated and unmedicated MDD patients, we examined the possible correlation between the equivalent dose of psychotropic drugs and CSF amino acid levels in the depressed MDD group (Table S1). CSF EA levels did not significantly correlate with benzodiazepine derivatives ($P$ = 0.29), antidepressants ($P$ = 0.34), or antipsychotics ($P$ = 0.21). With respect to other molecules, several amino acid concentrations nominally showed significant correlations with medications (Supplementary Table S1). The correlations between isoleucine and antidepressants ($P$ = 0.00056) (Supplementary Fig. S2), remained significant even after correcting for multiple comparisons [critical $P$-value of 0.05/(25 × 2) = 0.001].

We further investigated the correlations between CSF EA levels and other CSF substances in unmedicated patients with MDD and controls (total $N$ = 67), based on reports that levels of CSF biogenic amine metabolites are affected by psychotropic drugs[36]. HVA, a catabolite of dopamine ($P$ = 0.0030), and 5-HIAA, a catabolite of serotonin ($P$ = 0.019), showed significant correlations, while other substances (total protein, glucose, chloride, and MHPG) did not (Figure 3 and Table 4).

Table 3 | Summarized comparisons in CSF EA concentrations between subgroups

| Comparisons | Differences | Statistics for ANCOVA [1] | 95% CI |
|---|---|---|---|
| **dMDD ($N$ = 42) vs HC ($N$ = 54)** | 12.3 ± 2.3 vs 14.8 ± 2.2 | **$F$ = 27.36, $df$ = 1, $P$ = 0.0000011** | **−3.29 to −1.48** |
| **mMDD ($N$ = 39) vs uMDD ($N$ = 13)** | 12.8 ± 2.8 vs 12.9 ± 2.1 | $F$ = 0.0030, $df$ = 1, $P$ = 0.96 | −1.57 to 1.66 |
| **dMDD ($N$ = 42) vs rMDD ($N$ = 10)** | 12.3 ± 2.3 vs 15.0 ± 3.0 | **$F$ = 8.073, $df$ = 1, $P$ = 0.0066** | **−3.90 to −0.67** |

[1]Statistical values derived from ANCOVA controlling for sex and age.
Significant $P$-values are shown in bold type.
Abbreviations: CSF, cerebrospinal fluid; EA, ethanolamine; ANCOVA, analysis of covariance; dMDD, depressed (non-remitted) patients with major depressive disorder; rMDD, remitted patients; HC, healthy controls; uMDD, unmedicated patients; mMDD, medicated patients; CI, confidence interval for the difference between two means.

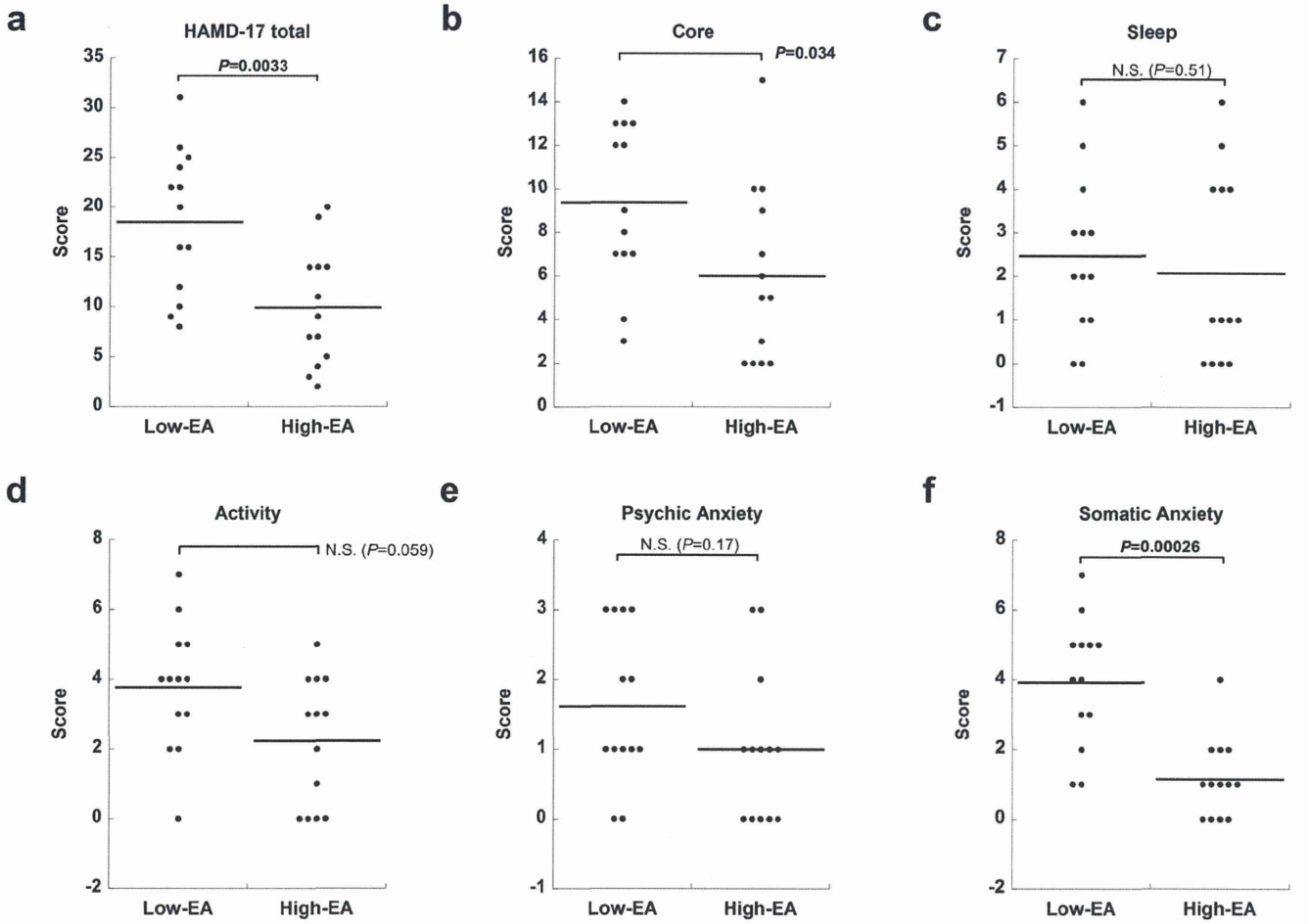


**Figure 2 | Dot plots of HAMD-17 total score and subscale scores in High and Low-EA patients.** We defined MDD patients with CSF EA values below the 1st quartile as 'Low-EA' ($N = 13$), and MDD patients with CSF EA values above the 3rd quartile as 'High-EA' ($N = 13$) to compare the CSF EA levels with symptoms. Horizontal bars in dot clusters represent mean values of the groups. *P*-values were obtained by the Mann-Whitney *U* test. (a) Low-EA showed a significantly higher total score for HAMD-17 than High-EA ($U = 29.0$, $P = 0.0033$; 95% CI: 3.0 to 15.0). (b) Low-EA showed a significantly higher score for the 'Core' subscale than High-EA ($U = 43.5$, $P = 0.034$; 95% CI: 1.0 to 7.0). There were no significant differences between the two groups for (c) 'Sleep' ($U = 71.5$, $P = 0.51$; 95% CI: −1.0 to 2.0), (d) 'Activity' ($U = 48.0$, $P = 0.059$; 95% CI: 0.0 to 3.0), and (e) 'Psychic Anxiety' ($U = 57.5$, $P = 0.17$; 95% CI: 0.0 to 2.0). (f) Low-EA showed a significantly higher score for 'Somatic Anxiety' than High-EA ($U = 18.5$, $P = 0.00026$; 95% CI: 1.0 to 4.0). Abbreviations: HAMD-17, 17-item Hamilton Depression Rating Scale; CSF, cerebrospinal fluid; EA, ethanolamine; Low-EA, patients with CSF EA values below the 1st quartile; High-EA, patients with CSF EA values above the 3rd quartile; CI, confidence interval for the difference between two medians.

To validate the HPLC measurements, CSF EA levels were measured in a subset of the subjects using CE-TOF-MS. The EA values obtained using CE-TOF-MS and HPLC methods showed a near-perfect correlation ($r = 0.89$, $P < 5 \times 10^{-18}$) in partial correlation analysis, with age and sex as covariates (Supplementary Fig. S3a). Similar to Figure 1a, a significant difference in CSF EA values based on CE-TOF-MS data was observed between the depressed MDD patients and controls ($P = 0.0052$, ANCOVA) (Supplementary Fig. S3b).

## Discussion

Several of our findings are potentially of clinical significance. The levels of some CSF amino acids differed between MDD patients and controls. EA (also known as monoethanolamine or 2-aminoethanol), in particular, remained significantly lower in patients than controls after correcting for multiple comparisons ($P = 0.0000011$). Notably, as many as 40% of the depressed patients showed abnormally low levels, and EA levels were significantly lower in depressed patients than remitted patients. When relationships with clinical variables were examined in the patients, the Low-EA group showed higher HAMD-17 and subscale scores compared with the High-EA group. CSF EA did not correlate with the dose of psychotropic drugs, although isoleucine showed a correlation with antidepressants even after correcting for multiple testing. Lastly, CSF EA values were significantly correlated with CSF HVA and 5-HIAA levels in unmedicated subjects.

An early study by Goodnick *et al.*[16] reported that CSF tyrosine levels differed across diagnostic groups (bipolar, unipolar depression, and controls). The authors reported the CSF EA levels; however, there were only two controls in their sample and it is difficult to compare their results with ours. Frye *et al.*[25] reported that CSF glutamate and glycine levels were decreased in patients with mood disorder. By contrast, we found no significant difference in the levels of either amino acid between depressed patients and controls. One of the reasons for the inconsistency may be that the majority of the patients in Frye *et al.*'s study had bipolar disorder. Another reason may be that their subjects were all unmedicated; however, we could not confirm the results of Frye *et al.* even when our unmedicated patients were compared with controls (data not shown). Levine *et al.* reported that depressed patients (MDD and bipolar disorder) showed increased CSF glutamine levels than controls[24]. CSF glutamine levels in our MDD patients were also significantly increased

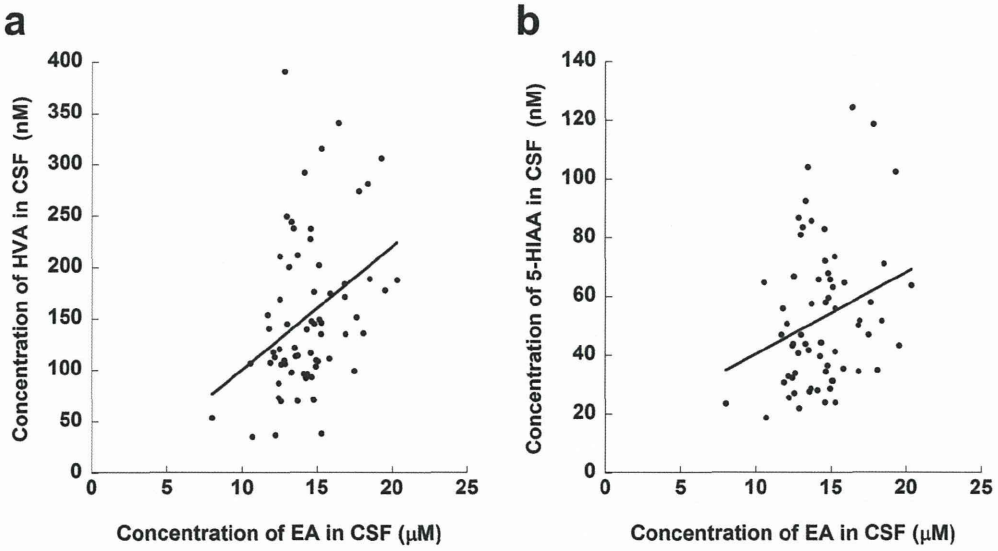


**Figure 3 | Scatter plots and regression lines for the relationships of EA with HVA and 5-HIAA in CSF.** Partial correlation test controlling for age and sex was performed in healthy controls ($N = 54$) and unmedicated patients with major depressive disorder ($N = 13$). Significant correlations of EA with (a) HVA and (b) 5-HIAA in CSF are shown. Abbreviations: EA, ethanolamine; CSF, cerebrospinal fluid; HVA, homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid.

than in controls. However, there was no significant difference between our unmedicated patients ($N = 11$) and controls (629.5 ± 72.9 vs. 644.3 ± 108.8 μM, $F = 0.046$, $df = 1$, $P = 0.83$, 95% CI: −69.33 to 55.89). Therefore, the observed increase in glutamine in our total subjects may be attributable to medication. Regarding CSF GABA, several previous reports[18,19,21,28] showed its decrease in depressed subjects; however, we observed no significant differences between patients and controls, which is in line with other studies[17,20,22]. Recently, Kaddurah-Daouk *et al.*, who employed a metabolomics-based approach, reported that methionine was increased in remitted MDD patients compared with depressed patients and healthy controls[26]. Our subjects showed similar results. Although CSF methionine levels did not differ significantly between the currently depressed patients and controls, our remitted patients had significantly higher CSF methionine levels than depressed patients (4.1 ± 1.2 vs. 3.3 ± 0.9 μM, $F = 9.1$, $df = 1$, $P = 0.0041$; 95% CI: 0.31 to 1.57, ANCOVA) and controls (vs. 3.5 ± 0.8, $F = 5.7$, $df = 1$, $P = 0.020$; 95% CI: 0.11 to 1.29). Methionine might be involved in the recovery processes of MDD and could be a biomarker for remission.

The significantly higher levels of CSF EA in remitted patients compared with depressed patients suggest that CSF EA levels might be state-dependent. In line, our Low-EA patients showed a higher HAMD-17 total score than the High-EA group. Vagus nerve stimulation (VNS), which is effective for MDD patients and alters the metabolites of neurotransmitters[37], was reported to elevate CSF EA levels in epileptic patients[38]. VNS may exert its effect through mechanisms that increase central EA. Longitudinal studies are warranted to examine whether antidepressant treatments increase CSF EA.

Table 4 | Partial correlations between levels of CSF substances and EA concentrations

| | Statistics | | | |
|---|---|---|---|---|
| Substances | *r* | *df* | *P* | 95% CI |
| **Total protein** | −0.055 | 63 | 0.66 | −0.30 to 0.19 |
| **Glucose** | −0.075 | 63 | 0.55 | −0.31 to 0.17 |
| **Chloride** | 0.077 | 63 | 0.54 | −0.17 to 0.31 |
| **HVA** | 0.36 | 63 | **0.0030** [1] | **0.13 to 0.56** |
| **MHPG** | 0.14 | 63 | 0.27 | −0.11 to 0.37 |
| **5-HIAA** | 0.29 | 63 | **0.019** | **0.051 to 0.50** |

[1]Significant *P*-values in bold type.
Abbreviations: CSF, cerebrospinal fluid; EA, ethanolamine; CI, confidence interval; HVA, homovanillic acid; MHPG, 3-methoxy-4-hydroxyphenylethyleneglycol; 5-HIAA, 5-hydroxyindoleacetic acid.

When we defined abnormally low EA levels based on the 5th percentile of the controls, approximately 40% of the depressed patients fell into this range, suggesting that a substantial proportion of subjects with MDD could be distinguished from normal subjects based on CSF EA levels, which would be useful for diagnosis. MDD patients with low CSF EA levels may constitute a subtype of MDD. Indeed, the Low-EA group was characterized by higher 'Core' and 'Somatic Anxiety' symptoms. In addition, we found a significant positive correlation between CSF EA and HVA and 5-HIAA levels, suggesting that MDD characterized by low CSF EA levels reflects impaired dopaminergic and serotonergic functions in the CNS, possibly due to synaptic dysregulation by an altered endocannabinoid system (see below).

Since most of our patients were medicated, the observed decrease in CSF EA may be attributable to medication. However, this possibility is unlikely because CSF EA was decreased in unmedicated patients compared with controls; there was no significant difference in CSF EA levels between medicated and unmedicated patients; and there was no significant correlation between CSF EA levels and the dose of any class of psychotropic drugs. Our results therefore suggest that CSF EA could be a useful biomarker even in medicated patients. With respect to the effect of antidepressants, we found a significant correlation with CSF isoleucine levels even after correcting for multiple comparisons. To our knowledge, no study has examined the effect of antidepressants on CSF amino acid levels; therefore, further studies are warranted.

EA is closely related to endocannabinoid signaling in the CNS (see Supplementary Fig. S4). EA is both a precursor to, and a metabolite of, anandamide (*N*-arachidonoylethanolamine), a ligand for cannabinoid receptors (CBs), and transient receptor potential vanilloid type 1 (TRPV1). The endocannabinoid system is implicated in depression, suicide, and stress-related affective disorders[39]. $CB_1$ receptor density is high at presynaptic axon terminals, where it functions to inhibit neurotransmitter release[40]. This may substantiate our

observation that CSF EA was correlated with HVA and 5-HIAA levels. In human studies, inconsistent results have been reported on serum endocannabinoid levels in MDD[41–43]. One study found no significant difference in the CSF anandamide level between MDD patients and controls, although it was elevated in unmedicated patients with schizophrenia[44]. Anandamide is synthesized on demand, binds with high affinity to extracellular $CB_1$ receptors, and is rapidly inactivated by active transport into neurons, followed by hydrolysis[45]. EA might be a stable surrogate marker for the anandamide system.

Decreased CSF EA may be due to inflammatory responses that have been implicated in MDD[46]. We previously reported elevated CSF IL-6 levels in MDD[47] suggesting the involvement of neuroinflammation. In the inflammatory process, activation of microglia and upregulation of cyclooxygenase-2 may facilitate conversion of EA to *N*-acylethanolamines or prostaglandin $H_2$ ethanolamide[48,49].

There are several limitations to this study. Firstly, the numbers of unmedicated patients with MDD ($N = 13$) and remitted individuals ($N = 10$) were small. However, we detected a significant difference in CSF EA levels between unmedicated patients and controls; and between depressed and remitted patients, which suggests large effect sizes. Secondly, the measurement of the CSF sample took place in a real-world setting; the majority of patients were medicated, and sampling was not performed after fasting or at a fixed time. However, we did not observe any correlation of EA with psychotropic medication or CSF sampling time. This makes CSF EA a feasible biomarker for everyday use in the clinical setting. Nevertheless, studies are necessary to elucidate the possible effects of fasting. Thirdly, there were missing values for several amino acids (see Table 2), which were likely due to small values below the detection limit and might have caused false negative results. Fourthly, small proportion of patients ($N = 13$) received antipsychotic medication, which may have an effect on CSF EA levels. However, there was no significant correlation between daily chlorpromazine equivalent doses of antipsychotics and CSF EA levels; therefore, the possible effect might be minimal. Finally, we obtained data only for MDD patients and controls. Further studies on other neuropsychiatric disorders are necessary to determine whether low EA is specific to MDD.

In conclusion, we found, for the first time, that CSF EA levels were reduced independently of medication in a substantial proportion (40%) of depressed MDD patients. Such patients had characteristic symptomatology (i.e., 'Somatic Anxiety') and CSF monoamine metabolite profiles (i.e., reduced HVA and 5-HIAA), and thus constitute a subtype of MDD.


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## Author contributions

S.O. designed the study, managed the literature searches, profiled the CSF samples by HPLC, undertook the statistical analyses, and wrote the draft of the manuscript. K.H. and Y.Y. recruited the participants. K.H. and D.S. diagnosed the participants, and collected the CSF samples. K.H. selected the sample set. Y.Y. and R.M. made psychological assessments. R.M. created and maintained database system. M.O., H.H. and T.T. screened the participants and diagnosed the patients. Y.O. and H.S. performed the CE-TOF-MS measuring for data validation. N.M. and J.M. reviewed the draft and gave critical comments on the manuscript. T.H., S.Y. and T.N. contributed to the recruitment of clinical volunteers. H.K. supervised the entire project and gave critical comments on the manuscript. All authors contributed to and have approved the final manuscript.

## Additional information

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

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動き

# 精神医学研究の新潮流
# Computational Psychiatry 2013

山下祐一

2013年10月22，23日，マイアミ(米国)で行われたComputational Psychiatry 2013に参加する機会を得た。本会議はComputational Psychiatry(計算論的精神医学)と銘打った世界最初の国際会議である。研究分野としてはまだ若いが，米国国立精神衛生研究所(NIMH)が提案するResearch Domain Criteria(RDoC)といった精神医学研究の新しい潮流とも密接に関係する重要なトピックであると考えられるため，現地で体感したその動向を報告したい。

計算論的精神医学という言葉は多くの精神科医にとって，耳慣れないと思われる。まず，計算論的精神医学とはどういう研究分野なのかについて簡単に説明したい。脳が外界といかに相互作用し情報を処理しているのか，という情報処理機構としての脳・神経システムの計算原理を探求する研究手法を計算論的アプローチという。計算論的精神医学とは，この手法を精神医学研究に積極的に応用しようという試みであり，近年その重要性の認識が非常に高まりつつある[2~4]。

会議の参加者は総勢100名(うち臨床経験を持つ研究者は約2割)ほどで，招待講演を中心とするコンパクトな会議であった。講演では，Read Montague博士，Peter Dayan博士(ロンドン大学)，Michel Frank博士(ブラウン大学)ら本会議の発起人たちに加えて，多くの計算論的神経科学の俊英たちによるチュートリアル的講演が行われた。強化学習という機械学習の計算理論に基づいた意思決定過程の理論，ベイズ推定という統計理論を用いた，脳の知覚体験様式の理論など，神経活動レベルの観察と行動レベルを橋渡しし，正常のみならず病態も説明しうる理論として紹介された(各理論の詳細は文献[1~4]を参照)。中でも，統合失調症の機能的断裂症候群仮説でも有名なKlaas Stephan博士(チューリッヒ大学)が提案した，仮説に基づく認知機能のモデル化と大規模データからの特徴抽出という計算論の強みを合わせたTranslational Neuromodelingというアイデアは，DSMに縛られない個別の症例に則した診断と治療の可能性まで視野に入れており，印象的であった。NIMHの元所長でRDoCの推進派であるSteve Hyman博士の基調講演でも，DSMを脱却した精神医学臨床研究に対して，計算論的アプローチが貢献することへの期待が熱く語られた。円卓会議と題されたディスカッションセッションでは，臨床家への理論的基礎教育の必要性について熱い議論が交わされた。総じて会議は熱気と興奮に満ちており，今後10年の精神医学研究の一つの方向性が示されたという印象を受けた。

(独立行政法人国立精神・神経医療研究センター神経研究所疾病研究第七部)

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学会告知板

# 千里ライフサイエンス技術講習会
# 次世代シークエンサーを用いた遺伝子発現解析の実際

**会期**　2014年5月16日(金)　10：00～16：30

**会場**　千里ライフセンタービル　5階501・502・503号会議室
(地下鉄御堂筋線千里中央駅北口すぐ)

**コーディネーター**　二村圭祐　大阪大学大学院医学系研究科 遺伝子治療学 助教

**プログラム**

技術解説(午前)
1.「遺伝子解析の歴史と現状」(二村圭祐)
2.「Ion Torrent™次世代シークエンサー～原理，ワークフロー～」(徳永裕子＊)
3.「Ion Torrent™次世代シークエンサーを用いた発現データ解析」(石倉　隆＊)
＊：ライフテクノロジーズジャパン株式会社

技術実習(午後)　次世代シークエンサーから得た遺伝子発現データの解析
(ライフテクノロジーズ社のクラウドサービスを含むインターネット環境を利用)

**参加資格**　分子生物学の研究者などで，今後遺伝子の解析に興味がある方
実習参加者はノート型PC(詳細は下記財団ホームページ参照)を持参できる方
なお，実習時のPCの故障・破損，動作不良，ウイルス感染などについて主催者は責任を負えません。予めご了解の上，お申込みください。

**定　員**　技術解説のみ30名，技術解説と実習参加20名

**参加費**　技術解説のみ：3,000円，技術解説と技術実習：5,000円

**申込み方法**　以下の要領にてお申込みください。
①氏名，勤務先，所属，役職名，〒，所在地，電話，FAX番号を明記の上，E-mailで(dsp@senri-life.or.jp)お申込みください。
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**主　催**　公益財団法人 千里ライフサイエンス振興財団(http://www.senri-life.or.jp)

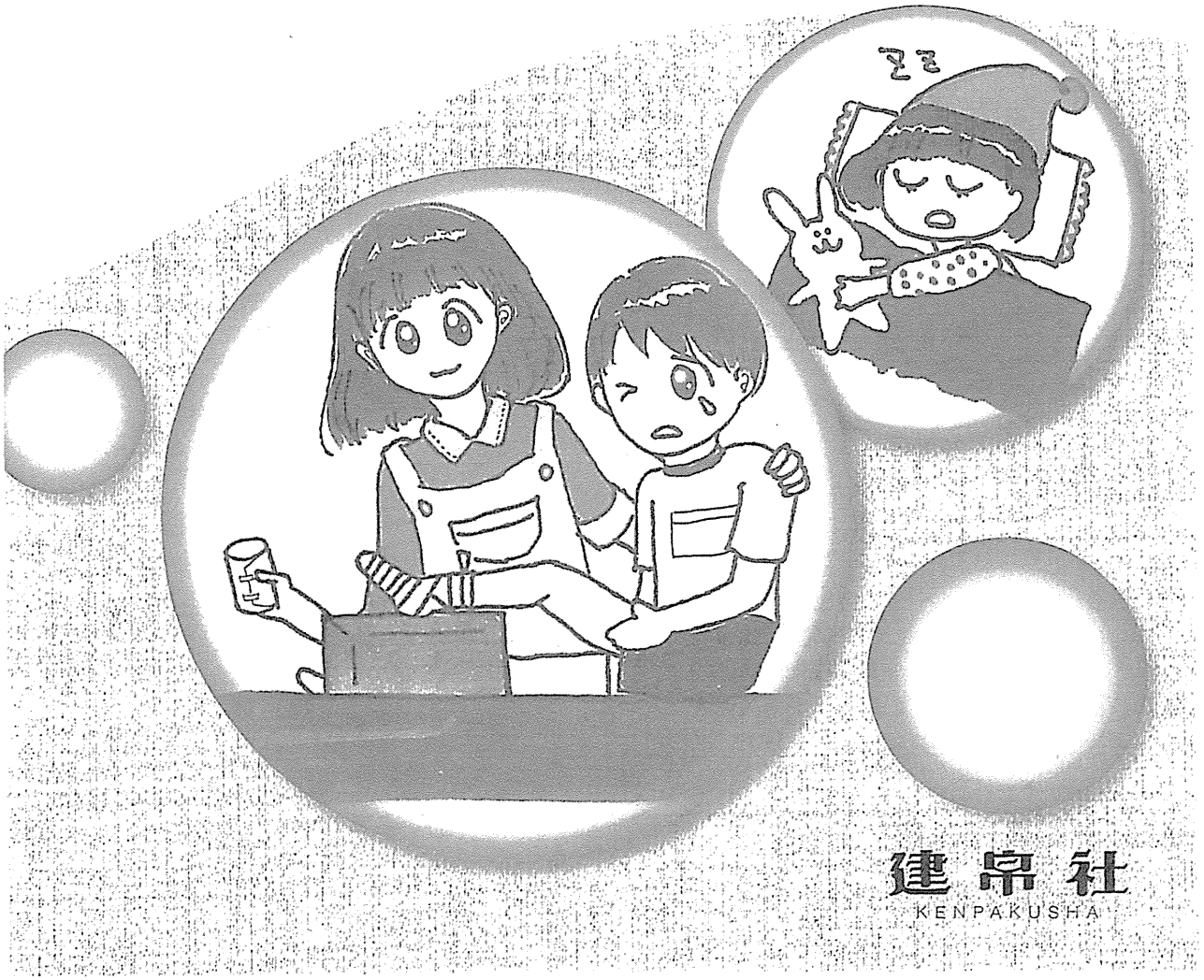
**協　力**　ライフテクノロジーズジャパン株式会社

新版

# 基礎から学ぶ 学校保健

瀧澤利行　編著

青柳直子・入澤裕樹・小浜　明・小松正子・後和美朝
宍戸洲美・柴若光昭・勝二博亮・高橋弘彦・中垣晴男
七木田文彦・花澤　寿・横田正義・吉田寿美子　共著



建帛社
KENPAKUSHA

### 6）起立性調節障害〔orthostatic disregulation：OD〕

起立性調節障害は，自律神経が失調し，起立時の循環器系の調節が不十分となることにより起こる。主な症状は立ちくらみ，脳貧血（脳の血液循環が悪くなり起こる）などである。診断は，従来表3-2の基準によって行われているが，2006（平成18）年に日本心身医学会からも診断・治療ガイドラインが出されている。

表3－2　OD診断基準

| | |
|---|---|
| 大症状 | A）立ちくらみあるいはめまいを起こしやすい<br>B）立っていると気持ちが悪くなる。ひどくなると倒れる<br>C）入浴時あるいはいやなことを見聞きすると気持ちが悪くなる<br>D）少し動くと動悸あるいは息切れがする<br>E）朝なかなか起きられず，午前中調子が悪い |
| 小症状 | a）顔色が青白い<br>b）食欲不振<br>c）強い腹痛をときどき訴える<br>d）倦怠あるいは疲れやすい<br>e）頭痛をしばしば訴える<br>f）乗り物に酔いやすい<br>g）起立試験で脈圧狭小16 mmHg以上<br>h）起立試験で収縮期血圧低下21 mmHg以上<br>i）起立試験で脈拍数増加21回/分以上<br>j）起立試験で立位心電図の$T_{II}$の0.2 mV以上の減高，その他の変化 |
| 判断 | 大1・小3，大2・小1，大3，以上で器質性疾患を除外できた場合をODとする |

### 7）貧　　血〔anemia〕

貧血は血色素（ヘモグロビン）濃度の低下した状態であり，血色素による酸素運搬が減少することにより，顔色が悪い，動悸，倦怠感などがみられる。学齢期には，血色素の成分である鉄分の不足による鉄欠乏性貧血が多い。身体発育に伴い鉄需要が増しているので，鉄を含めてバランスよい食事摂取をすることが重要である。

## 2．学校でみられるこころの健康問題

いじめ，不登校，家庭内暴力や自殺などこころの健康の重要性が高まっている。そこで，これらの理解を深めるために，学校現場でみられるこころの問題を簡単に概説する。

### 1）ひきこもり

いろいろな定義があるが，ここでは精神科医である斎藤環の定義を紹介する。それによると，ひきこもりとは「20代後半までに問題化し，6ヶ月以上，自宅に引きこもって社会参加しない状態が持続しており，他の精神疾患がその第一の原因とは考えにくいもの」としている[1]。10代後半から20代前半に起こり，男性に多い。失恋やケガなど周囲からみると些細な挫折体験をきっかけにひきこもることが多い。自宅の自室にひきこもり，家

族ともコミュニケーションを絶つ例も少なくない。精神的には不安や焦燥が強く，他者の評価に敏感で，家人に猜疑的となり家庭内暴力に発展する場合がある。このような状況から，精神疾患を併発する場合もあるので注意が必要である。

特筆すべきは，ひきこもりの60％以上が不登校経験者であり，いったんひきこもると10年以上も長期化することが少なくないので，不登校の段階での適切な対処が重要と考えられている。

2）心理的発達の障害〔ICD-10：F 8〕（第9章参照）

① 自閉症〔autism〕　カナー（L. Kanner）が「聡明な容貌・常同行動・高い記憶力・機械操作の愛好」などを特徴とする一群の幼児に対し，自閉症（オーティズム）と名づけた[2]。その後，親の育て方で自閉症になるなどの間違った認識があったが，現在は先天性の脳障害であることがわかり，全般性発達障害の小児自閉症（自閉症）に分類されている。この障害には自閉症状と知的障害の2つの障害がいろいろな割合で存在し，多くは3歳までに気づかれる。知的障害がないものはアスペルガー症候群（自閉症スペクトラム障害，ASD）として自閉症とは区別されている。

自閉症状で問題となるのはコミュニケーションの障害や興味や関心が限局していることで，具体的には「視線を合わせない」，「おうむ返しの返答」，「友達と遊ばない」，「変化を嫌い，儀式などに固執する」，「思いどおりにならないとひどい癇癪を起こしたり，手首を噛むなどの自傷行為を行う」ことである。今のところ治療法はなく，療育が中心となり，補助的に薬物治療を行うこともある。

② 学習障害〔learning disorders〕　学習障害は，精神科疾患として心身発達の障害に分類されている。しかし，診断を受けても医学的治療法がないことなどから，医療場面より教育現場で問題となる。

文部科学省は，「学習障害とは，基本的には全般的な知的発達に遅れはないが，聞く，話す，読む，書く，計算する又は推論する能力のうち特定のものの習得と使用に著しい困難を示す様々な状態を指すものである。学習障害は，その原因として，中枢神経系に何らかの機能障害があると推定されるが，視覚障害，聴覚障害，知的障害，情緒障害などの障害や，環境的な要因が直接の原因となるものではない」（文部科学省，1999年）と定義している。

実際の学校現場では，学習の障害よりもむしろ随伴する自己制御困難（授業中に私語が多い，ルールを守れない）や対人関係困難（人の嫌がることを言う，自分勝手な言動）が問題になることが多い。学力の向上だけにとらわれず，社会生活に適応できる援助が重要と思われる。

3）注意欠陥多動性障害〔attention-deficit hyperactivity disorder；ADHD，ICD-10：F 90〕（第9章参照）

ADHDはICD-10では多動性障害に分類され，いわば極端に落ち着きがなく注意散漫で衝動的な行動をとる傾向のある子どもである。生後5か月ごろに発症し，女子より男子に

多い。衝動的で，事故を起こしやすく，不注意から軽率な規則違反を起こし，親の躾(しつけ)の問題と誤解されることがある。しかし，これは脳の機能障害が原因と考えられ，親の躾が原因ではない。知的障害を伴わなければ，知能は普通である。不注意や衝動性などから学業の成績によい時と悪い時，波があることが多い。

薬物療法としては行動を鎮静するために覚醒剤の一種であるメチルフェニデートなどが効果を示す場合がある。いずれにせよ薬物療法は補助的であり，学校と家庭が連携して療育を行うことが肝要と思われる。むやみにしかったり，教師と親の対応がまったく異なるなど学校と家庭の療育の連携が悪いと，反抗挑戦性障害や行為障害，最悪の場合犯罪に発展する場合もあると考えられている（DBD マーチ，DBD：disruptive behavior disorder，破壊的行動障害）[1]。児童・思春期の精神疾患はいずれも学校や家庭の連携ある対応が重要であるが，ADHD においてはその重要性はさらに高いと思われる。

4）精神疾患（第 10 章参照）

厚生労働省の 2008（平成 20）年の調査では，精神疾患の患者は 323 万人にのぼり，237 万人の糖尿病，152 万人のがんなど他の 4 大疾病を大幅に上まわった。このような現状を受け，同省は 2013（平成 25）年 4 月から精神疾患をがん，脳卒中，急性心筋梗塞，糖尿病と並ぶ 5 大疾病と位置づけ，5 疾病 5 事業をスタートさせた。この事業は精神疾患の早期発見と共に地域での精神医療（病院・診療所・訪問看護ステーションの連携強化など）の向上を目指している。

## §2．学校でみられる生活習慣病

成人の生活習慣病と同様に学齢期においても，近代的生活による運動不足，過食などに起因する肥満や高血圧，糖尿病，脂質異常症は動脈硬化を促進し，心疾患および脳血管疾患のリスクとなっている。

### 1．糖 尿 病〔diabetes〕

糖尿病には，免疫異常等による膵臓のインスリン分泌不全である 1 型と，過食・運動不足等によりインスリンの作用・分泌が低下する 2 型がある。1 型は，インスリン注射が必要である。

2 型は，食事療法により食事を適量バランスよく食べて肥満を改善すること，運動により細胞のインスリン感受性を向上させることなどでコントロールする。

### 2．高 血 圧〔hypertension〕

高血圧は，体質に加え，肥満や食塩過剰摂取により起こるので，それらの改善が肝要である。