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Gene expression and association analysis of the epithelial membrane protein 1 gene in major depressive disorder in the Japanese population

Masahito Nakataki[a,*], Jun-ichi Iga[a], Shusuke Numata[a], Eriko Yoshimoto[a], Kanami Kodera[a], Shin-ya Watanabe[a], Hongwei Song[a], Shu-ichi Ueno[b], Tetsuro Ohmori[a]

[a] Department of Psychiatry, Course of Integrated Brain Sciences, Medical Informatics, Institute of Health Biosciences, The University of Tokushima Graduate School, 18-15 Kuramoto-cho 3, Tokushima 770-8509, Japan

[b] Department of Neuropsychiatry, Neuroscience, Ehime University Graduate School of Medicine, Japan

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ABSTRACT

The epithelial membrane protein 1 (*EMP1*) plays a role in neuronal differentiation and neurite outgrowth, which are involved in the pathogenesis of major depressive disorder (MDD). We sought to determine whether the *EMP1* gene is implicated in MDD. We determined the mRNA expression levels of the *EMP1* gene in peripheral-blood leukocytes of patients and control subjects ($n = 27$ each). Next, we performed case–control association analyses (MDD, $n = 182$; controls, $n = 350$) in the Japanese population. The level of expression of the *EMP1* mRNA was significantly lower in medication-free patients compared with control subjects ($P < 0.001$). The association analysis revealed an absence of association between the polymorphisms studied and MDD, whereas a gender-specific association was observed between male controls and male patients for marker rs7315725 (permutation $P = 0.039$). Our results suggest that the *EMP1* gene may be implicated in the pathophysiology of MDD in the Japanese population.

The lifetime population prevalence of major depressive disorder (MDD) is 5–10%. The heritability of MDD, as assessed based on twin studies, is 40–50%, and adoption studies provide some support for a role for genetic factors in this disease [20]. Recently, it has been proposed that a deficit in neurogenesis is involved in MDD and that the mechanism of action of antidepressant medications may involve the promotion of neurogenesis [10]. The epithelial membrane protein 1 (*EMP1*) is a tetraspan transmembrane protein that plays a role in cell–cell adhesion and in interactions with the extracellular membrane [6], and is a biomarker of tumor resistance [15]. Earlier research found that *EMP1* is expressed in the immature mouse brain, but is lost in adult animals [32]. In human tissues, the *EMP1* mRNA was not detected in the adult brain or peripheral leukocytes, as assessed by Northern blot analysis [8]. However, recent reports using microarray or quantitative reverse transcriptase polymerase chain reaction (RT-PCR) analyses revealed that the *EMP1* gene is expressed in the adult brain [3,21] and peripheral leukocytes [25]. Thus, *EMP1* may be an important molecule for neuronal migration and neurite outgrowth [21,32].

Previously, we reported an association between the phosphodiesterase 4B (*PDE4B*) gene and MDD [24]. *PDE4B* plays an important role in the regulation of cyclic adenosine monophosphate (cAMP) signaling, which is a second messenger that is implicated in learning, memory, and mood [5,9,17]. Millar et al. [23] reported that disrupted-in-schizophrenia 1 (DISC1), which is an important genetic risk factor for MDD [14], interacts with *PDE4B* and that elevation of cellular cAMP levels leads to dissociation of *PDE4B* from DISC1 and an increase in *PDE4B* activity [23]. A recent report showed that the level of expression of the *PDE4B* mRNA was significantly elevated in the monocytes of bipolar depression patients compared with healthy controls and correlated with the level of the *EMP1* mRNA [25].

Based on the above-described findings, the *EMP1* gene appears to be a good candidate for a genetic study of MDD. To our knowledge, however, there are no reports on the association between the *EMP1* gene and MDD.

In this study, we determined the mRNA expression levels of the *EMP1* gene in the peripheral blood leukocytes of patients with MDD and in control subjects ($n = 27$ each). Next, we performed case–control association analyses (MDD, $n = 182$; controls, $n = 350$) in the Japanese population to determine whether the *EMP1* gene was implicated in MDD.

For the gene expression study, we enrolled 27 medication-free MDD patients (eight males (mean age, 40.9 ± 11.5 years) and 19 females (mean age, 42.4 ± 15.2 years)) from four psychiatric hospitals in the Tokushima Prefecture of Japan. Twenty-four patients were in their first depressive episode and were drug-naïve, and another three patients were having a recurrent episode

* Corresponding author. Tel.: +81 88 633 7130; fax: +81 88 633 7131.
E-mail address: nktk@clin.med.tokushima-u.ac.jp (M. Nakataki).

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and had been without antidepressant treatment for at least two months. The diagnosis of MDD was established according to Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria [1] by at least two experienced psychiatrists. We excluded patients who had organic disorders or alcohol or substance abuse. Blood samples were obtained from MDD patients before and 8 weeks after antidepressant treatment. Physicians were allowed to choose the antidepressants and to assign doses of medication according to clinical assessment. Clinical symptoms were evaluated using the 17-item Hamilton depressive rating scale (HAM-D) at the time blood samples were collected. Twenty-seven sex- and age-matched healthy controls were selected from a pool of volunteers (eight males (mean age, 41.9 ± 12.7 years) and 19 females (mean age, 40.2 ± 11.4 years)) who had no history of either psychiatric or serious somatic disease, and were not taking any medication. Clinical assessment was performed by an experienced psychiatrist before blood collection. Almost all samples are collected in the morning. Besides, we found no apparent fluctuation in the expression levels in blood samples from the same healthy person taken at 9:00, 14:00 and 19:00 in a day.

Total RNA was extracted from the peripheral leukocytes of whole blood samples using the PAXgene Blood RNA kit (Qiagen, Tokyo, Japan) according to the protocol recommended by the manufacturer. Two micrograms of total RNA was used for cDNA synthesis using random (N6) primers and Quantiscript Reverse Transcriptase (Qiagen, Tokyo, Japan) after assessing RNA quality and quantity using NanoDrop (NanoDrop Technologies, Wilmington, DE). Real-time quantitative RT-PCR analysis was performed on an ABI 7500 Fast Real Time PCR System (Applied Biosystems, Foster City, CA). Taqman primer/probes for the *EMP1* (Hs00608055_m1) gene were purchased from Applied Biosystems. Two housekeeping genes were used for normalization (beta-actin gene (ACTB) and glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH)). All reactions were performed in triplicate. A validation experiment using the comparative threshold cycle (C_t) method was performed as described previously [24]. The amounts of *EMP1* mRNA were normalized to the endogenous reference and were expressed relative to the calibrator as $2^{-\Delta\Delta C_t}$ (comparative C_t method).

For the genetic association study, we enrolled 182 MDD patients (76 males, median age, 46.5 years (interquartile range, 17.5 years); 106 females, median age, 44.5 years (interquartile range, 29.3 years)) from four psychiatric hospitals in the neighboring area of the Tokushima Prefecture and from the Ehime University Hospital in Japan. We also selected 350 controls from a pool of volunteers (147 males, median age, 47 years (interquartile range, 16 years); 203 females, median age, 45 years (interquartile range, 19 years)). All subjects were genotyped. The diagnosis of MDD was established according to DSM-IV criteria [1] by at least two experienced psychiatrists. Control subjects were healthy volunteers who had no current or past contact with psychiatric services.

Genotyping was performed using commercially available TaqMan probes for the *EMP1* gene on an ABI 7500 Fast Real Time PCR System, according to the protocol recommended by the manufacturer. We selected four single-nucleotide polymorphic (SNP) markers at an average density of 5 kb across the *EMP1* gene, based on information from the International HapMap Project (Fig. 2). The reference SNP ID numbers and the location of these four SNPs were as follows: SNP1 (rs7315725, 5′ untranslated region (UTR)), SNP2 (rs4763327, intron 1), SNP3 (rs2291060, intron 2), and SNP4 (rs8885, 3′ UTR).

All subjects were biologically unrelated Japanese. All subjects signed written informed consent, which was approved by the Ethical Committee of the University of Tokushima or the Graduate School of Ehime University.

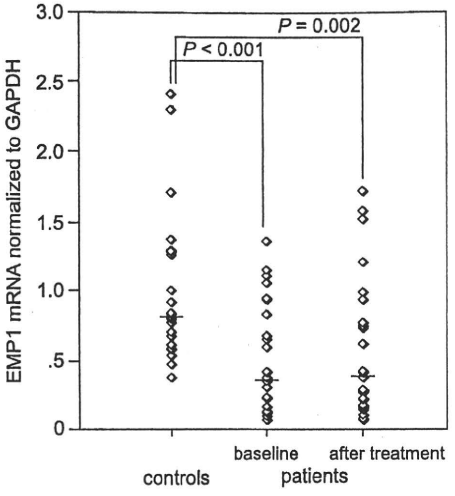

Fig. 1. Epithelial membrane protein 1 (*EMP1*) mRNA expression levels. Bars indicate the median of the values for each subject ($n = 27$). The *EMP1* mRNA levels of patients were significantly lower than those of controls, regardless of the absence (controls, 0.81 (0.70); patients at baseline, 0.36 (0.66); Mann–Whitney *U*-test, $P < 0.001$) or presence (after treatment, 0.39 (0.59); Mann–Whitney *U*-test, $P = 0.002$) of treatment. The *EMP1* mRNA levels did not vary significantly during the treatment period (Wilcoxon signed-rank test, $P = 0.068$).

Statistical calculations were performed using SPSS 17 (SPSS Inc., Chicago, USA). Data were expressed as mean ± standard deviation or as median (interquartile range) values for normally or not normally distributed continuous variables, respectively. The Fisher exact test was used for categorical data. The Student's *t*-test was used for normally distributed data, and the Mann–Whitney *U*-test and the Wilcoxon signed-rank test were used for data that was not normally distributed. Spearman's rho or Pearson's correlation coefficients were calculated to assess the correlations between parameters, depending on the distribution of the data. A multiple linear regression analysis was performed to evaluate the independent relationship of the variables under study.

In the case–control association study, the HAPLOVIEW program [4] was used to estimate haplotype frequencies, linkage disequilibrium (LD), permutation *P* values (50,000 permutations), and deviation from the Hardy–Weinberg distribution of alleles. Pairwise LD indices (D') were calculated for the control subjects. Power calculations for sample size were performed using the G*Power program [11].

All tests were two sided and $P < 0.05$ were considered significant. To account for multiple testing, Bonferroni criterion ($\alpha = 0.05$ divided by the number of tests) was used to interpret findings and determine significance.

HAM-D scores improved significantly after eight weeks of antidepressant treatment ($n = 27$; at baseline median, 21 (interquartile range, 8); after treatment, 6 (12); Wilcoxon signed-rank test, $P < 0.0001$). The following antidepressant drugs were administered: paroxetine ($n = 18$), sertraline ($n = 4$), fluvoxamine ($n = 3$), milnacipran ($n = 2$), amitriptyline ($n = 1$), and maprotiline ($n = 1$). The dose of antidepressants was 37.5–300 mg/day of the imipramine equivalent.

The relative amount of the *EMP1* mRNA in peripheral leukocytes was standardized to GAPDH mRNA, which was used as an internal standard. At baseline, patients showed significantly lower *EMP1* mRNA levels than did control subjects (controls, 0.81 (0.70); patients at baseline, 0.36 (0.66); Mann–Whitney *U*-test, $P < 0.001$). After antidepressant treatment, there remained a significant difference in the levels of *EMP1* mRNA normalized by the GAPDH between controls and patients (patients after treatment, 0.39 (0.59); Mann–Whitney *U*-test, $P = 0.002$) (Fig. 1). This difference

Table 1
Allele frequencies of the four *EMP1* SNPs in patients with MDD and in controls.

SNP	Marker	Position	Diagnosis	*n*	Genotype (%)			*P* value	HWE	Allele		*P* value	MAF
All subjects													
					A/A	A/G	G/G			A	G		
SNP1	rs7315725	13239012	MDD	182	16 (8.8)	77 (42.3)	89 (48.9)	0.261	0.910	109	255	0.146	0.299
		5′ upstream	control	346	23 (6.6)	132 (38.2)	191 (55.2)		0.976	178	514		0.257
					C/C	C/T	T/T			C	T		
SNP2	rs4763327	13246467	MDD	182	8 (4.4)	66 (36.3)	108 (59.3)	0.248	0.600	82	282	0.205	0.225
		intron1	control	350	29 (8.3)	125 (35.7)	196 (56.0)		0.160	183	517		0.261
					G/G	A/G	A/A			G	A		
SNP3	rs2291060	13256321	MDD	182	6 (3.3)	52 (28.6)	124 (68.1)	0.736	0.848	64	300	0.801	0.176
		intron2	control	347	10 (2.9)	108 (31.1)	229 (66.0)		0.520	128	566		0.184
					C/C	C/T	T/T			C	T		
SNP4	rs8885	13260463	MDD	182	35 (19.2)	101 (55.5)	46 (25.3)	0.500	0.124	171	193	1.000	0.470
		3′ UTR	control	350	77 (22.0)	175 (50.0)	98 (28.0)		0.946	329	371		0.470
Male subjects													
					A/A	A/G	G/G			A	G		
SNP1	rs7315725	13239012	MDD	76	10 (13.2)	37 (48.7)	29 (38.2)	**0.031**	0.737	57	95	**0.012**	0.375
		5′ upstream	control	145	9 (6.2)	56 (38.6)	80 (55.2)		0.847	74	216		0.255
					C/C	C/T	T/T			T	C		
SNP2	rs4763327	13246467	MDD	76	0 (0)	28 (36.8)	48 (63.2)	**0.008**	0.049	28	124	**0.028**	0.184
		intron1	control	147	14 (9.5)	54 (36.7)	79 (53.7)		0.293	82	212		0.279
					G/G	A/G	A/A			G	A		
SNP3	rs2291060	13256321	MDD	76	0 (0)	21 (27.6)	55 (72.4)	0.190	0.162	21	131	0.149	0.138
		intron2	control	145	5 (3.4)	47 (32.4)	93 (64.1)		0.752	57	233		0.197
					C/C	C/T	T/T			T	C		
SNP4	rs8885	13260463	MDD	76	14 (18.4)	46 (60.5)	16 (21.1)	0.233	0.065	74	78	0.842	0.487
		3′ UTR	control	147	34 (23.1)	71 (48.3)	42 (28.6)		0.706	139	155		0.473
Female subjects													
					A/A	A/G	G/G			A	G		
SNP1	rs7315725	13239012	MDD	106	6 (5.7)	40 (37.7)	60 (56.6)	0.944	0.843	72	140	0.929	0.340
		5′ upstream	control	201	14 (7.0)	76 (37.8)	111 (55.2)		0.840	139	263		0.346
					C/C	C/T	T/T			C	T		
SNP2	rs4763327	13246467	MDD	106	8 (7.5)	38 (35.8)	60 (56.6)	0.982	0.566	54	158	0.922	0.255
		intron1	control	203	15 (7.4)	71 (35.0)	117 (57.6)		0.360	101	305		0.249
					G/G	A/G	A/A			G	A		
SNP3	rs2291060	13256321	MDD	106	6 (5.7)	31 (29.2)	69 (65.1)	0.371	0.325	43	169	0.445	0.203
		intron2	control	202	5 (2.5)	61 (30.2)	136 (67.3)		0.547	71	333		0.176
					C/C	C/T	T/T			C	T		
SNP4	rs8885	13260463	MDD	106	21 (19.8)	55 (51.9)	30 (28.3)	0.974	0.641	97	115	0.865	0.458
		3′ UTR	control	203	43 (21.2)	104 (51.2)	56 (27.6)		0.681	190	216		0.468

Statistical differences in genotypic and allelic distributions were evaluated using the Fisher exact test. Values of $P < 0.05$ are shown in bold.

was also confirmed using normalization by the ACTB gene (controls, 1.10 (0.60); patients at baseline, 0.76 (0.82); Mann–Whitney *U*-test, $P = 0.018$: patients after treatment, 0.69 (0.94); Mann–Whitney *U*-test, $P < 0.001$). After eight weeks of treatment, the *EMP1* mRNA levels of patients showed a trend toward upregulation; however, this result was not significant (Wilcoxon signed-rank test, $P = 0.068$). There were no significant differences in the levels of the *EMP1* mRNA either before or after treatment according to gender, age and other demographic characteristics (number of episodes, age of onset, hereditary load, dose of antidepressants, and HAM-D scores before and after treatment).

The genotypic and allelic frequencies of the four SNPs located in the *EMP1* gene are shown in Table 1. There were no significant deviations from the Hardy–Weinberg equilibrium for all four SNPs, in either patients or control subjects.

There was no significant association between either the allelic frequencies or the genotypic distributions of these SNPs and MDD. To assess the presence of gender differences, we examined males and females separately. Significant differences were observed between male patients and male controls for the genotypes of two SNPs (rs7315725, $P = 0.031$; rs4763327, $P = 0.008$) and for their allelic frequencies (rs7315725, $P = 0.012$; rs4763327, $P = 0.028$). After application of the permutation test (50,000 permutations) to correct for multiple testing, rs7315725 retained significant allelic associations with MDD (permutation $P = 0.039$). Among females, there were no significant differences in either allele frequency or genotype distribution between patients and controls.

Next, we performed haplotype analyses. The values of absolute D' for the control subjects are presented in Fig. 2. There was one LD block in the *EMP1* gene (rs2291060 and rs8885), which resided in block 1. The permutation test of the two markers revealed no significant differences (permutation $P = 0.99$).

No correlations were detected between *EMP1* genotypes and clinical subtype (age, sex, age of onset, psychotic features, suicidal behavior, and family history).

Twenty four patients participated in both mRNA expression and genetic analysis. The expression level of the *EMP1* mRNA before and after treatment was investigated via multiple linear regression analysis with adjustment for cofounders (age, sex, number of episodes, age of onset, hereditary load, dose of antidepressants, HAM-D scores before and after treatment, and single SNP genotype). However, we did not observe any significant correlations between the level of the *EMP1* mRNA and these parameters.

In the present study, we performed an mRNA expression analysis of the *EMP1* gene in the peripheral blood leukocytes of MDD and control subjects. We also performed a case–control association analysis of the *EMP1* gene to clarify its implication in MDD.

First, we observed significantly decreased levels of expression of the *EMP1* mRNA in the peripheral blood leukocytes of medication-free MDD patients compared with control subjects. Recent reports revealed that the *EMP1* gene is expressed in the brain [3,21] and peripheral leukocytes [25]. *EMP1* has the single N-linked carbohydrate chain attached to the protein backbone, which carries the HNK-1/L2 carbohydrate epitope [22,29]. This special structure may

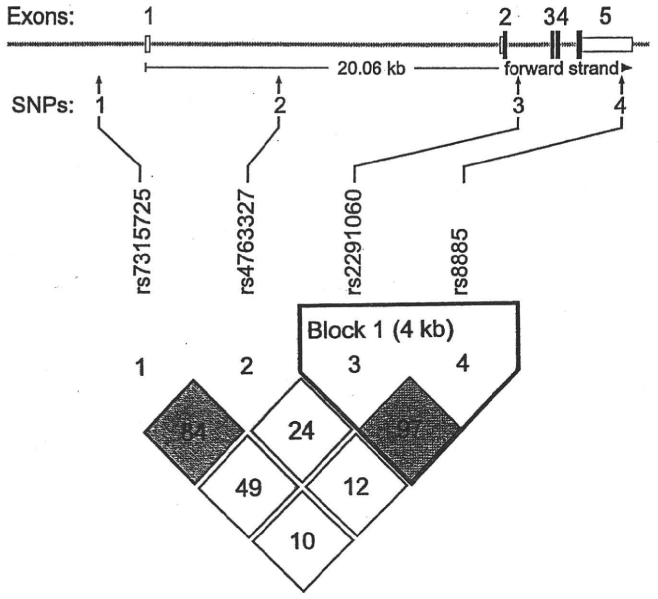

Fig. 2. Schematic representation of the *EMP1* gene. SNP locations and haplotype-block structure of *EMP1* gene. The horizontal line represents the genomic sequence; filled or empty boxes represent exons; and connected lines represent introns. Coding-region exons are shown as filled boxes; 5′ and 3′ UTRs are shown as empty boxes; and vertical arrows indicate the SNPs that were investigated in this study. Haplotype-block structure was determined using the Haploview program [4]. There was one linkage-disequilibrium block in the *EMP1* gene. Each box represents the *D′* values that correspond to each pairwise SNP.

not only contribute to cell–cell or cell–extracellular matrix recognition functions, but also affect differentiation, proliferation, and cell growth [27]. Thus, *EMP1* may be required during neurogenesis and the establishment of neural connectivity [32]. The observation that the *EMP1* mRNA level was decreased in leukocytes may provide a clue on the pathophysiology of MDD, as lymphocytes may reflect the metabolism of brain cells, and may be exploited as a neural and possible genetic probe in studies of psychiatric disorders [13,30]. Padmos et al. reported that the level of *EMP1* mRNA in monocytes was significantly elevated in bipolar depression patients compared with healthy controls [25]. Our results seem to contradict their findings. However, our findings were in line with recent reports that propose the neurotrophic hypothesis in which decreased expression of growth factors, notably brain-derived neurotrophic factor (BDNF) [7,18,26], contributes to depression, and upregulation of neurotrophic factors plays a role in the action of antidepressant treatment [10]. At the present time, we can only speculate on the reasons for this inconsistency, which may reflect differences such as cell type (monocytes vs. whole leukocytes) and disease subtype (bipolar depression vs. MDD). Though there is an overlap of core clinical features between bipolar depression and MDD, these diseases exhibit opposite differences regarding specific biological markers, e.g., BDNF [12,28].

Another possibility is that the *EMP1* mRNA change in leukocytes may reflect an activated inflammatory response system which is associated with MDD. Several studies have reported abnormalities in the immune system of patients with MDD [13,19]. Although the function of the *EMP1* gene is not entirely known, it is reported that *EMP1* mRNA in monocytes was correlated with the expression of the genes for proinflammatory cytokines mutually and strongly [25]. This suggests that *EMP1* may be involved in inflammation-related processes.

Second, although the *EMP1* mRNA levels of MDD patients after eight weeks of antidepressant treatment did not show significant changes from baseline levels, 70% of patients (19 out of 27) exhibited *EMP1* mRNA upregulation after treatment. Whether the upregulation of the *EMP1* mRNA after treatment is caused by pharmacological effects of the antidepressants or by clinical improvement remains to be elucidated. It is possible to assume that upregulation of the *EMP1* mRNA levels is more likely to be detectable after a period longer than a few weeks of treatment. Regarding the results of our study, though we did not examine whether the *EMP1* mRNA levels of patients were normal at the time of recovery, further studies (in particular a long-term study of a larger cohort of patients) are required to determine the clinical significance of *EMP1* mRNA expression in peripheral leukocytes.

Third, we investigated the genetic association between the *EMP1* gene and MDD in the Japanese population. We did not find any association of the four *EMP1* SNPs tested with the disease. However, rs7315725 exhibited significant allelic association with male MDD patients after subdivision of the subjects according to gender. To our knowledge, this is the first study of *EMP1* genetic variants in MDD. A potential explanation for this gender-specific effect is that MDD may involve different genetic factors in men and women. Several studies provide evidence of gender differences in the genotypic risk to develop MDD, such as the serotonin transporter [2], monoamine oxidase [16], and catechol-O-methyltransferase [31] genes. Our results suggest the existence of a gender-specific association between the *EMP1* genotype and MDD. Power calculations using the G*Power program [11] revealed that the size of our sample required an effect size of 0.16 (i.e., relatively weak) to archive a power of 80% (which is usually considered as sufficient power) at the 0.05 significance level (two tailed). Considering that the effect size of rs7315725 observed among male subjects was 0.177, our sample size may have been insufficient to reliably detect weak gene effects. Future studies using larger sample sizes may uncover additional associations between other *EMP1* polymorphisms and MDD.

Our study had several limitations. The size of the sample used in the expression analysis was small and the follow-up period was relatively short. It is possible that the MDD diagnosis will change into bipolar disorder with time in some MDD patients who participated in this study. The prognostic significance of *EMP1* mRNA levels warrants further investigation using additional studies with a larger number of patients and a longer follow-up period. The size of the sample used in the genetic association analysis was relatively small. Larger studies including participants of different ethnic backgrounds and meta-analyses are warranted to confirm the associations found here. Finally, we did not address how SNPs in the *EMP1* gene, which were significantly associated with MDD in our study, alter its function. Further investigations including intermediate phenotype approaches are needed to determine the effect of genetic variations in the *EMP1* gene on the etiology of MDD.

A significantly lower expression of the *EMP1* mRNA was observed in the leukocytes of MDD patients compared with control subjects. The *EMP1* genotype exhibited a significant association between male MDD patients and male controls. Our results suggest that *EMP1* may be implicated in the pathophysiology of MDD in the Japanese population.

Conflicts of interest

The authors declare that they have no conflict of interest.

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GABA concentration in schizophrenia patients and the effects of antipsychotic medication: A proton magnetic resonance spectroscopy study

Shin'Ya Tayoshi [a], Masahito Nakataki [a,*], Satsuki Sumitani [a], Kyoko Taniguchi [a], Sumiko Shibuya-Tayoshi [a], Shusuke Numata [a], Jun-ichi Iga [a], Shu-ichi Ueno [b], Masafumi Harada [c], Tetsuro Ohmori [a]

[a] Department of Psychiatry, Course of Integrated Brain Sciences, Medical Informatics, Institute of Health Biosciences, The University of Tokushima Graduate School, Japan
[b] Department of Neuropsychiatry, Neuroscience, Ehime University Graduate School of Medicine, Japan
[c] Department of Radiology, Major in Radiology, Institute of Health Biosciences, The University of Tokushima Graduate School, Japan

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ABSTRACT

Gamma-amino butyric acid (GABA) is thought to play a role in the pathophysiology of schizophrenia. High magnetic field proton magnetic resonance spectroscopy (^{1}H-MRS) provides a reliable measurement of GABA in specific regions of the brain. This study measured GABA concentration in the anterior cingulate cortex (ACC) and in the left basal ganglia (ltBG) in 38 patients with chronic schizophrenia and 29 healthy control subjects.
There was no significant difference in GABA concentration between the schizophrenia patients and the healthy controls in either the ACC (1.36 ± 0.45 mmol/l in schizophrenia patients and 1.52 ± 0.54 mmol/l in control subjects) or the ltBG (1.13 ± 0.26 mmol/l in schizophrenia patients and 1.18 ± 0.20 mmol/l in control subjects). Among the right handed schizophrenia patients, the GABA concentration in the ltBG was significantly higher in patients taking typical antipsychotics (1.25 ± 0.24 mmol/l) than in those taking atypical antipsychotics (1.03 ± 0.24 mmol/l, $p = 0.026$). In the ACC, the GABA concentration was negatively correlated with the dose of the antipsychotics ($rs = -0.347$, $p = 0.035$). In the ltBG, the GABA concentration was positively correlated with the dose of the anticholinergics ($rs = 0.403$, $p = 0.015$).
To the best of our knowledge, this is the first study to have directly measured GABA concentrations in schizophrenia patients using ^{1}H-MRS. Our results suggest that there are no differences in GABA concentrations in the ACC or the ltBG of schizophrenia patients compared to healthy controls. Antipsychotic medication may cause changes in GABA concentration, and atypical and typical antipsychotics may have differing effects. It is possible that medication effects conceal inherent differences in GABA concentrations between schizophrenia patients and healthy controls.

1. Introduction

1.1. The Gamma-amino butyric acid (GABA) system in schizophrenia

Gamma-amino butyric acid (GABA) is thought to play a role in the pathophysiology of schizophrenia (Guidotti et al., 2005; Wassef et al., 2003).

1.1.1. Postmortem studies

Postmortem studies of $GABA_A$ receptors in chronic schizophrenia have reported inconsistent findings. Some

* Corresponding author. Department of Psychiatry, Course of Integrated Brain Sciences, Medical Informatics, Institute of Health Biosciences, The University of Tokushima Graduate School, 3-8-15 Kuramoto-cho Tokushima 770-8503, Japan. Tel.: +81 88 633 7130; fax: +81 88 633 7131.
E-mail address: nktk@clin.med.tokushima-u.ac.jp (M. Nakataki).

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case–control studies have reported increased $GABA_A$ receptor binding in the cingulate cortex (Hanada et al., 1987; Benes et al., 1992), whereas others have found it to be decreased (Squires et al., 1993) or unchanged (Pandey et al., 1997). The $GABA_A$ receptor is composed of various subunits. Ishikawa et al. (2004) found a higher density of alpha 1 and beta 2/3 subunits in the prefrontal cortex (PFC) of schizophrenia patients compared to control subjects.

The 65 and 67 kDa isoforms of glutamic acid decarboxylase (GAD) are key enzymes in GABA synthesis, and a number of studies have investigated their significance in schizophrenia. Bird et al. (1977) found that GAD levels were decreased in the nucleus accumbuns, amygdala, hippocampus, and putamen of schizophrenia patients. Benes et al. (2000) observed no change in GAD density in the anterior cingulate cortex (ACC) of schizophrenia patients. Woo et al. (2004) found a decrease in the number of GAD67 mRNA-containing neurons in the ACC of schizophrenia patients compared to control subjects. Dracheva et al. (2004) reported an increased expression of GAD65 and GAD67 mRNA in the dorsolateral PFC and in the occipital cortex of schizophrenia patients compared to control subjects.

1.1.2. In vivo neuroimaging studies

Neuroimaging studies using radio active ligands have also reported inconsistent findings. Some studies using single photon emission computed tomography (SPECT) have failed to find any evidence of $GABA_A$ receptor binding abnormalities in the brains of schizophrenia patients compared to healthy controls (Busatto et al., 1997; Verhoeff et al., 1999; Abi-Dargham et al., 1999). One study, however, found a significant correlation between task performance and $GABA_A$/benzodiazepine receptor binding in the frontal and occipital cortices of schizophrenia patients (Ball et al., 1998). Using positron emission tomography (PET), Asai et al. (2008) reported no differences in [11C] Rol5-4513 binding (which represents the density of the alpha 5 subunit of the $GABA_A$/benzodiazepine receptor) in the PFC and the hippocampus of schizophrenia patients compared to control subjects; among the schizophrenia patients, the degree of binding was found to be negatively correlated with negative symptom scores.

1.1.3. The effects of antipsychotic medication on the GABA system in the basal ganglia and cingulate cortex

Gunne et al. (1984) reported an inhibition of GAD activity in monkeys following treatment with antipsychotics. Studies in rats have reported that treatment with typical antipsychotic drugs such as haloperidol (Jolkkonen et al., 1994; Delfs et al., 1995a,b; Laprade and Soghomonian, 1995; Sakai et al., 2001), fluphenazine (Chen and Weiss, 1993; Johnson et al., 1994), and sulpiride (Laprade and Soghomonian, 1995) increased the expression of GAD67 and GAD67 mRNA in the basal ganglia, whereas atypical antipsychotic drugs such as clozapine (Delfs et al., 1995a) and olanzapine (Sakai et al., 2001) did not. These changes may be reflected in the dyskinetic and antipsychotic actions of typical antipsychotics (Delfs et al., 1995b; Sakai et al., 2001). Zink et al. (2004) reported that both haloperidol and clozapine increased [^{3}H]-muscimol binding to $GABA_A$ receptors in the ACC, whereas increased $GABA_A$ receptor binding in the basal ganglia was only induced by haloperidol. Although the underlying mechanism is unclear, these results suggest that antipsychotics may affect the GABA system, and that typical and atypical antipsychotics may have differing effects.

1.2. The role of the ACC and the basal ganglia in schizophrenia

Several changes in the ACC of schizophrenia patients have been reported: (1) alterations in GAD levels (Woo et al., 2004), (2) morphological change (Baiano et al., 2007; Fujiwara et al., 2007; Zetzsche et al., 2007), and (3) activation deficits during cognitive tasks (Liddle et al., 2006; Yücel et al., 2007; Brüne et al., 2008; Koch et al., 2008). Menzies et al. (2007) found that GABA-modulating drugs affected working memory performance and induced activation changes in the ACC of schizophrenia patients. The basal ganglia contain the striatum, the globus pallidus, and other structures. The striatum is thought to receive GABAergic interneurons from other regions of the brain, in particular the globus pallidus and the cerebral cortex (Bolam et al., 2000). The PFC is thought to be involved in the pathophysiology of schizophrenia on three levels: morphologically (Meda et al., 2008), functionally (Lee et al., 2006), and histologically (Woo et al., 2008). The PFC tonically inhibits striatal dopamine projections, and it is thought that this is mediated by GABA interneurons (Carlsson, 2001; Akil et al., 2003; Perlman et al., 2004). The globus pallidus is also thought to be involved in the pathophysiology of schizophrenia (Galeno et al., 2004; Spinks et al., 2005). An increase in $GABA_A$ receptor binding in the basal ganglia following the administration of antipsychotics has been reported (Zink et al., 2004).

1.3. Magnetic resonance spectroscopy

Proton magnetic resonance spectroscopy (^{1}H-MRS) provides an in vivo measurement of brain metabolites such as myo-inositol, N-acetylaspartate, choline-containing compounds, Glx (glutamate plus glutamine), creatine, and phosphocreatine in the human brain. The recent introduction of high magnetic field MRS has enabled the reliable measurement of GABA in specific brain regions. Reduced concentrations of GABA in depressed patients (Hasler et al., 2007) and unchanged concentrations of GABA in panic disorder patients (Hasler et al., 2008) have been reported in areas of the frontal lobe. To the best of our knowledge, no previous ^{1}H-MRS study has examined GABA concentrations in schizophrenia patients. In the present study, ^{1}H-MRS was used to compare GABA concentrations in medicated chronic schizophrenia patients with those of healthy controls using a high magnetic field device. The regions of interest (ROIs) were located in the ACC and in the left basal ganglia (ltBG); the ltBG contain the striatum, globus pallidus, and other structures. The effects of typical and atypical antipsychotic medication on GABA concentrations in the basal ganglia and cingulate cortex were also examined.

2. Method

2.1. Subjects

Thirty-eight patients with chronic schizophrenia and twenty-nine healthy control subjects participated in this

study. All study participants gave written informed consent in accordance with the guidelines of the ethics committee of the University of Tokushima. All diagnoses were assigned according to DSM-IV TR criteria. The schizophrenia patients were classified into the following two groups in order to compare the effects of the type of antipsychotic regime: (1) the TYP group, who were taking typical antipsychotics with or without concomitant atypical antipsychotics, and (2) the ATY group, who were taking atypical antipsychotics without concomitant typical antipsychotics. The patients were also classified according to schizophrenia subtype: paranoid schizophrenia ($n=36$), undifferentiated schizophrenia ($n=1$), and disorganized schizophrenia ($n=1$). Epidemiological data, including age, duration of illness, handedness, Positive and Negative Syndrome Scale scores (PANSS, Kay et al., 1987), and dose of antipsychotics at the time of the scan, are shown in Table 1. The healthy control subjects had no history of any Axis I psychiatric disorder according to DSM-IV TR criteria. None of the patients or healthy control subjects had a serious medical illness, history of head injury, or history of drug or alcohol abuse prior to the scan. All of the schizophrenia patients and healthy control subjects were Japanese and came from the same region, and their native language was Japanese. Handedness was assessed using the Edinburgh Handedness Inventory (Oldfield, 1971). With the exception of two schizophrenia patients, all study participants were right handed.

2.2. The ^{1}H-MRI/MRS procedures

^{1}H-MRS was performed using MEGA-PRESS according to previously reported methods (Mescher et al., 1998). A 3 T clinical magnetic resonance imaging (MRI) scanner was used (Sigma 3T Excite, GE, Milwaukee, WI, USA). Gradients were employed surrounding the frequency selective pulses at 1.9 ppm to diphase transverse magnetization. Water suppression involved three conventional CHESS pulses after manual optimization. The sequence parameters were as follows: (1) TR = 1500 ms, (2) TE = 68 ms, (3) size of ROI = $3.0\times3.0\times3.0$ cm^3 (27 ml), (4) summation = 256 signals for each spectrum, and (5) total acquisition time = 13 min. Alternate measurements with and without frequency selective pulses were taken; J evolution for GABA was refocused during odd-numbered acquisitions but not during even-numbered acquisitions. The difference in the acquired spectra provided an edited spectrum of GABA. The in vitro data for GABA were acquired from MEGA-PRESS, and were used as a basis for the Linear combination model (LC Model) (Provencher, 1993). The quantification of signals from the different MEGA-PRESS spectra was performed with the LC Model. An axial cut approximately 1 cm above the upper end of the body of the lateral ventricles was chosen as a reference slice of the ROI in the ACC. The center of the interhemispheric fissure was 3 cm in front of the central fissure and 2 cm above the corpus callosum (Fig. 1a). An axial cut approximately 1 cm above the genu of the corpus callosum, which provided a continuous view of the anterior and posterior horns of the lateral ventricles, was chosen as a reference slice of the ROI in the ltBG. The ROI of the ltBG was located between the sylvian fissure and the lateral ventricles in order to incorporate the lenticular nucleus (Fig. 1b). The representative ^{1}H-MRS spectra from the MEGA-PRESS sequence are shown in Fig. 2.

T1-weighted three dimensional images were acquired using the following parameters: (1) TE = 4.2 ms, (2) TR = 10 ms, (3) slice thickness = 0.8 mm, (4) matrix 512×512, (5) FOV = 24×24 cm, and (6) Flip angle = 15°. The brain images were thus composed of voxels that were $0.47\text{mm}\times0.47\text{mm}\times0.8\text{mm}$ in size. On the basis of the histogram of voxel intensity, each voxel in the 3D-SPGR brain images was classified as gray matter (GM), white matter (WM), or cerebrospinal fluid (CSF) using the "ImageJ Ver. 1.38" software package (http://rsb.info.nih.gov/ij). The voxels that were considered GM, WM, and CSF in each ROI were counted using the "3D-Slicer Ver.2.6" software package (http://www.slicer.org) in order to obtain the ratio of these tissues. Metabolite concentrations in the ROI were corrected for CSF by dividing the percentage of brain tissues in each ROI, under the assumption that the metabolite concentrations in CSF were equal to zero (Bustillo et al., 2001).

The criteria for selecting the reliable metabolite concentrations were based on the %SD of the fit for each metabolite, reflecting the Cramer–Rao lower bounds (CRLB) for LC Model analysis. The data included in the present study showed %SD of less than 20%. In our previous study, the intraclass correlation of two measurements in the same study participant was greater than 0.7, indicating acceptable reliability as a clinical instrument.

Table 1
Epidemiologic data.

		Schizophrenia	Controls	p
Age	(all)	34.0 ± 10.0	34.0 ± 10.2	n.s.[a]
(mean ± S.D. years old)	(rt handed)	34.7 ± 10.0	34.0 ± 10.2	n.s.[a]
Number (male/female)	(all)	38 (20/18)	29 (17/12)	n.s.[b]
	(rt handed)	36(19/17)	29 (17/12)	n.s.[b]
Duration of illness (years)		11.1 ± 9.4		
PANSS total score (mean ± S.D.)		54.0 ± 14.4		
PANSS positive score (mean ± S.D.)		13.2 ± 6.0		
PANSS negative score (mean ± S.D.)		15.1 ± 5.3		
PANSS general score (mean ± S.D.)		26.7 ± 6.9		
Dose of antipsychotics[c] (mean ± S.D.)		423.7 ± 362.3		

Abbreviation: rt handed, right handed; PANSS, Positive and Negative Syndrome Scale; n.s., not significant.
[a] Two sample Student's *t*-test.
[b] χ square test.
[c] Chlorpromazine equivalent.

2.3. Statistical procedures

Statistical tests were performed using the "SPSS Version 11.5" software package (SPSS Japan Inc., Tokyo, Japan). The absolute GABA levels, as measured with ^{1}H-MRS, were analyzed.

For both ROIs, two sample *t*-tests were used to compare the mean values of the GABA concentrations in schizophrenia patients and healthy control subjects. The GABA concentration of the TYP group was compared to that of the ATY group using analysis of covariance (ANCOVA), with covariance for age.

For both ROIs, the correlation between GABA concentration and each of the clinical measures (e.g., age, dose of benzodiazepine, dose of antipsychotics, dose of anticholinergics, and the positive, negative, and general PANSS scores) was

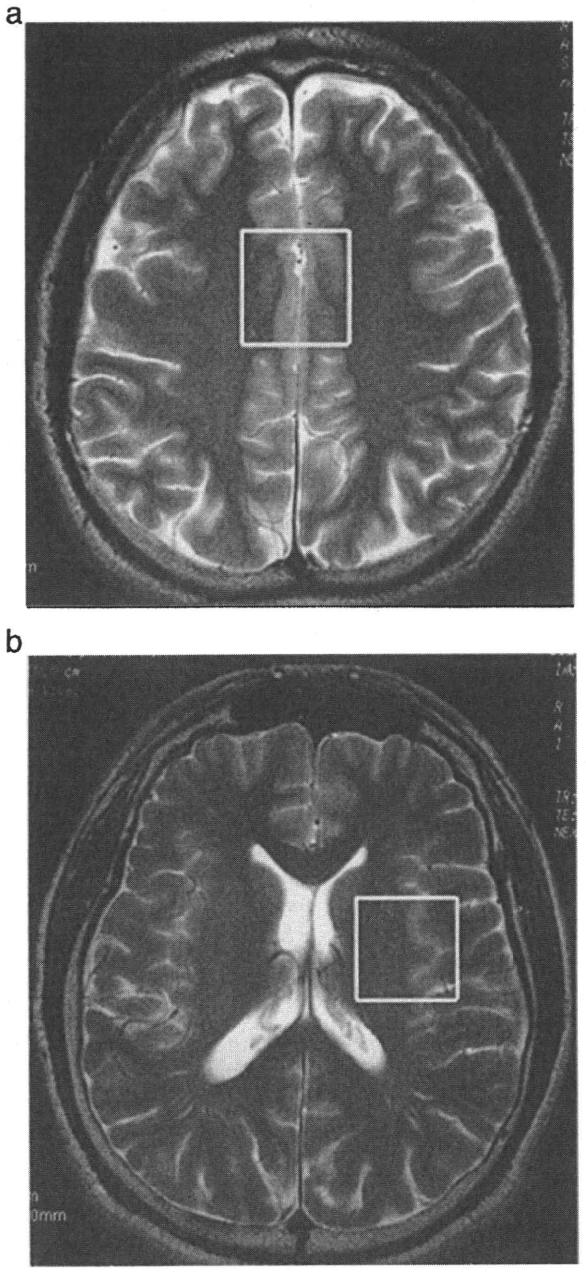

Fig. 1. The region of interest (ROI) positions in the anterior cingulate cortex (ACC) (a) and left basal ganglia (ltBG) (b) for spectroscopic measurement by MEGA-PRESS sequence. The white box represents the location of the ROI (3 cm×3 cm×3 cm) that was used in the MEGA-PRESS sequence in the horizontal image.

evaluated using Spearman's rank correlation test. Since handedness could have influenced the results, the analysis in the ltBG was only performed for right handed patients i.e. the two left handed schizophrenia patients were excluded.

Statistical significance was set at $p<0.05$.

Since the partial volume effects of the GM and the WM may influence the GABA concentration in ^{1}H-MRS (Choi et al., 2006), a two sample *t*-test was used to make the following comparisons of the GM:WM ratio for each ROI: (1) schizophrenia patients and healthy control subjects, and (2) male study participants and female study participants.

3. Results

There were no significant differences between the schizophrenia patients and the healthy control subjects in age range or male:female ratio. No significant difference in GABA concentration was observed between schizophrenia patients and healthy control subjects in either the ACC (1.36 ± 0.45 mmol/l in schizophrenia patients and 1.52 ± 0.54 mmol/l in healthy control subjects, $p=0.18$) or the ltBG (1.13 ± 0.26 mmol/l in schizophrenia patients and 1.18 ± 0.20 mmol/l in healthy control subjects, $p=0.36$).

For the schizophrenia patients, the clinical data of the TYP group and the ATY group are shown in Table 2. The TYP group consisted of 16 patients and the ATY group consisted of 22 patients. For each patient, the name and dose of the antipsychotic medication prescribed at the time of the scan are provided in Tables 3a and b. Eight of the TYP group and 8 of the ATY group were prescribed benzodiazepines, and 13 of the TYP group and 9 of the ATY group were prescribed anticholinergic medication (Tables 3a and b). Three patients were prescribed concomitant mood stabilizers, and a further three patients were prescribed antidepressants (Tables 3a and b). There were no significant differences between the TYP group and the ATY group for the following factors: (1) PANSS positive, negative, and general scores, (2) dose of benzodiazepine (diazepam equivalent dose), and (3) dose of antipsychotics (chlorpromazine equivalent dose). However, age and duration of illness were significantly higher in the TYP group than the ATY group. The TYP group were prescribed anticholinergic medication significantly more often than the ATY group ($X^2=6.18$, $p=0.013$). The mean dose of anticholinergic medication (biperiden equivalent dose) did not differ between the TYP group and the ATY group.

ANCOVA was performed using the type of antipsychotic (i.e. TYP versus ATY) and the use of anticholinergic medication (i.e. patients with versus patients without anticholinergic medication) as two independent factors and with age as a covariate. In the ltBG, ANCOVA revealed a significant effect for the type of antipsychotic ($F=5.48$, $p=0.026$) but not for the use of anticholinergic medication; no interaction between the type of antipsychotic and the use of anticholinergic medication was observed. In the ACC, no significant effect was observed for the type of antipsychotic or the use of anticholinergic medication, and no interaction between these two factors was observed. In the ltBG, the mean ± SD of the GABA concentrations for the TYP group and the ATY group were 1.25 ± 0.24 mmol/l and 1.03 ± 0.24 mmol/l, respectively. In the ACC, the mean ± SD of the GABA concentrations for the TYP group and the ATY group were 1.40 ± 0.48 mmol/l and 1.27 ± 0.51 mmol/l, respectively.

A significant negative correlation was found between the dose of the antipsychotics and the GABA concentration in the ACC ($rs=-0.387$, $p=0.016$) but not in the ltBG. A significant positive correlation was found between the dose of the anticholinergic medication and the GABA concentration in the ltBG ($rs=0.399$, $p=0.016$) but not in the ACC. There was no correlation between the remaining clinical measures (i.e.

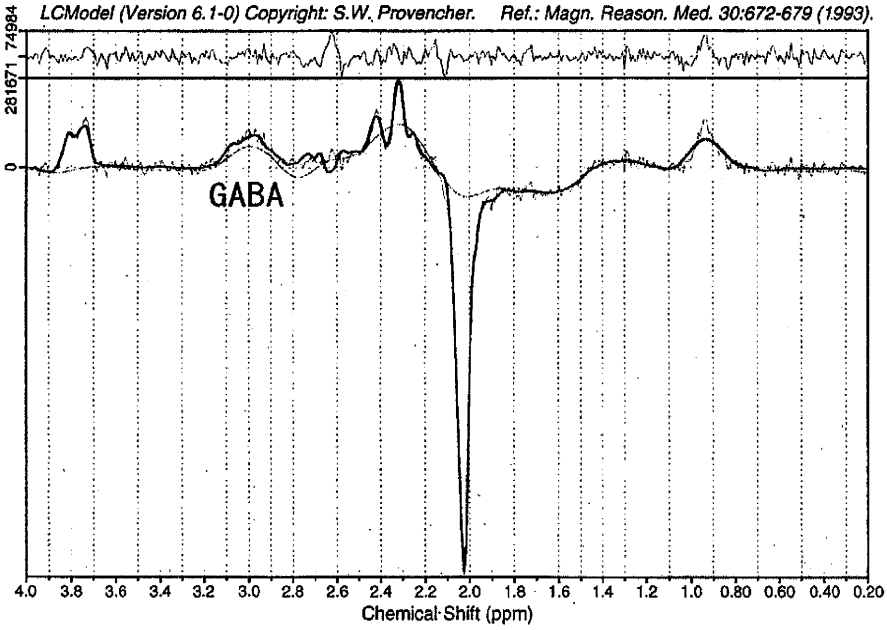

Fig. 2. Representative ^{1}H-MRS spectra from a study participant before the MEGA-PRESS sequence. The peaks that represent GABA are shown.

age, PANSS scores, and dose of benzodiazepine) and the GABA concentrations in the ACC and the ltBG.

The GM:WM ratio did not differ significantly between the schizophrenia patients and the healthy control subjects, or between the TYP group and the ATY group in either of the two ROIs (Table 4).

4. Discussion

4.1. Main findings

This study contributes four main findings. Firstly, no difference in GABA concentration was observed between the schizophrenia patients and the healthy control subjects. Secondly, among the schizophrenia patients, the GABA concentration in the ltBG was significantly higher in the TYP group than in the ATY group. Thirdly, there was a significant negative correlation between the GABA concentration in the ACC and the dose of antipsychotics. Finally, there was a positive correlation between the GABA concentration in the ltBG and the dose of anticholinergic medication.

Table 2
The clinical indices of the schizophrenic patients and types of medication.

		TYP	ATY	p
Number	(all)	16	22	
	(rt handed)	15	21	
Age	(all)	38.4±10.7	30.8±8.3	.025*
	(rt handed)	38.7±11.1	31.6±8.2	.046*
Duration of illness	(all)	16.2±11.0	7.1±6.2	.005*
	(rt handed)	16.2±11.0	7.2±6.5	.010*
PANSS positive		12.4±2.6	13.7±7.5	n.s.
PANSS negative		15.0±4.0	15.2±6.1	n.s.
PANSS general		27.0±6.3	27.0±7.3	n.s.
Dose of antipsychotics		435.9±432.9	414.8±311.7	n.s.
Dose of benzodiazepine		6.6±8.3	3.3±4.7	n.s.

Abbreviation: TYP, patients taking typical antipsychotics with or without concomitant atypical antipsychotics; ATY, patients taking atypical antipsychotics without concomitant typical antipsychotics; rt handed, right handed; PANSS, Positive and negative syndrome scale.
* Significantly different in two sample t-test ($p<0.05$).

4.2. No difference in the GABA concentration between schizophrenia patients and healthy control subjects

Analysis of GABA receptor binding sites and measurement of GAD have frequently been used to investigate the GABA

Table 3a
Typical antipsychotic group.

Age (years)	Sex	Antipsychotic drugs	BZD	Anticholin
40	F	HPD 0.75 mg	0	0
30	F	HPD 3 mg	0	3
50	F	HPD 1.5 mg	0	3
36	M	HPD 3 mg	0	3
26	F	HPD 2.25 mg	10	2
48	F	HPD 4 mg	10	2
54	M	HPD 2.25 mg	0	6
46	F	SPD 150 mg	10	0
24	F	OLZ 10 mg, HPD 4.5 mg	30	4
39	M	OLZ 30 mg, LP 100 mg	10	3
21	F	PER 16 mg, BPD 1.5 mg	0	2
33	M	QTP 400 mg, RIS 6 mg, LP 150mg	10	4
24	M	RIS 2 mg, LP 25 mg	0	1
36	M	RIS 3 mg, CP 100 mg, LP 100 mg	15	1
52	M	RIS 6 mg, BPD 6 mg	0	3
46	M	RIS 9 mg, LP 50 mg, ZTP 75 mg	10	0

Abbreviations: BZD, benzodiazepine dose expressed as diazepam equivalent dose; Anticholin, anticholinergic drugs expressed as biperiden equivalent dose; HPD, haloperidol; SPD, sulpiride; LP, levomepromadine; BPD, bromperidol; CP, chlorpromadine; ZTP, zotepin.

Table 3b
Atypical antipsychotic group.

Age (years)	Sex	Antipsychotic drugs	BZD	Anticholin
16	F	APZ 6 mg	0	0
25	M	APZ 12 mg	0	0
37	M	APZ 12 mg	10	1
30	F	APZ 6 mg, OLZ 2.5 mg	0	0
21	F	APZ 6 mg, RIS 1 mg	10	1
27	M	OLZ 5 mg	10	0
25	M	OLZ 5 mg	0	0
35	M	OLZ 15 mg	0	0
40	M	OLZ 20 mg	5	0
20	F	OLZ 20 mg	10	0
21	M	OLZ 25 mg	6.7	1
38	F	OLZ 25 mg	0	0
27	M	PER 2 mg	0	0
28	F	PER 16 mg	8.33	0
38	F	PER 20 mg	0	2
28	M	QTP 750 mg	0	2
25	F	RIS 2 mg	0	2
37	F	RIS 2 mg	0	0
36	M	RIS 2 mg	0	0
48	M	RIS 5 mg	0	1
25	F	RIS 8 mg	0	4
41	M	RIS 8mg	13	4

Abbreviations: BZD, benzodiazepine dose expressed as diazepam equivalent dose; Anticholin, anticholinergic drugs expressed as biperiden equivalent dose; HPD, haloperidol; SPD, sulpiride; LP, levomepromadine; BPD: bromperidol; CP, chlorpromadine; ZTP, zotepin.

system of the brain. Findings from postmortem studies of GABAA receptor binding have been inconsistent, and have included reports of an increase (Hanada et al., 1987; Benes et al., 1992), a decrease (Squires et al., 1993), and no change (Pandey et al., 1997). Studies that have used SPECT (Busatto et al., 1997; Verhoeff et al., 1999; Abi-Dargham et al., 1999) or PET (Asai et al., 2008) to measure GABA receptor binding have reported that it is unchanged. GAD is the key enzyme in GABA synthesis. Postmortem studies of schizophrenia patients have reported that GAD levels are decreased in the nucleus accumbuns, amygdala, hippocampus, and putamen (Bird et al., 1977), and unchanged in the ACC (Benes et al., 2000). It has been shown that GAD65 and GAD67 mRNA are increased in the dorsolateral PFC and in the occipital cortex of schizophrenia patients (Dracheva et al., 2004). A reduction in GAD67 mRNA-containing neurons in the ACC has been reported in schizophrenia patients (Woo et al., 2004).

Table 4
The ratio of gray matter and white matter in each ROI.

		Schizophrenia		Control	p
		TYP	ATY		
GM in ACC	(all)	0.38 ± 0.08		0.37 ± 0.05	n.s.
	(all)	0.39 ± 0.08	0.37 ± 0.07		n.s.
WM in ACC	(all)	0.22 ± 0.07		0.22 ± 0.07	n.s.
	(all)	0.22 ± 0.07	0.21 ± 0.08		n.s.
GM in ltBG	(rt handed)	0.34 ± 0.11		0.35 ± 0.10	n.s.
	(rt handed)	0.37 ± 0.13	0.32 ± 0.08		n.s.
WM in ltBG	(rt handed)	0.62 ± 0.12		0.63 ± 0.11	n.s.
	(rt handed)	0.59 ± 0.13	0.65 ± 0.10		n.s.

Abbreviations: GM, gray matter; WM, white matter; rt handed, right handed; ACC, anterior cingulate cortex; ltBG, left basal ganglia; TYP, the patients taking typical antipsychotics with or without concomitant atypical antipsychotics; ATY, the patients taking atypical antipsychotics without concomitant typical antipsychotics; n.s., not significantly different in two sample *t*-test.

Our study has demonstrated that the in vivo GABA concentrations of the ACC and the ltBG did not differ significantly between medicated chronic schizophrenia patients and healthy control subjects.

4.3. Higher ltBG GABA concentration in the TYP group than the ATY group

Animal studies have reported that typical antipsychotics increased the expression of GAD67 and GAD67 mRNA in the basal ganglia (Chen and Weiss, 1993; Johnson et al., 1994; Jolkkonen et al., 1994; Delfs et al., 1995a,b; Laprade and Soghomonian, 1995; Sakai et al., 2001), but that administration of atypical antipsychotic drugs did not (Delfs et al., 1995a; Sakai et al., 2001). Our observation of significantly higher GABA concentrations in the TYP group compared to the ATY group is therefore compatible with the findings of these animal studies. This difference in GABA concentration may be reflected in the dyskinetic effects of typical antipsychotics (Delfs et al., 1995b; Sakai et al., 2001), although the severity of dyskinesia did not differ significantly between the two groups of patients in the present study (data not shown). The true extent of the influence of typical and atypical antipsychotics on GABA concentration in the present patient sample remains unknown since we did not include unmedicated patients. Unmedicated schizophrenia patients might be expected to have lower GABA concentrations than patients treated with typical antipsychotics, but further studies would be necessary to test this hypothesis.

4.4. Relationship between GABA concentration and the dose of antipsychotics in the ACC, and the dose of anticholinergics in the ltBG

Results from animal studies have suggested that antipsychotic medication may influence the GABA system in the cingulate cortex (Sharp et al., 1994, 2001; Vincent et al., 1994; Squires and Saederup, 2000). Zink et al. (2004) reported that both clozapine and haloperidol increased $GABA_A$ receptor binding in the ACC, while only haloperidol increased $GABA_A$ receptor binding in the basal ganglia. These animal studies indicated that both atypical and typical antipsychotics have an effect on the GABA system in the cingulate cortex.

Previous MRS studies have examined the effect of antipsychotic treatment on the combined signals of glutamate, glutamine and GABA (Glx). Although one study reported a decrease in Glx levels following antipsychotic treatment (Choe et al., 1996), another reported no significant difference in the PFC following treatment with risperidone (Szulc et al., 2005). A further study reported no change in the cingulate cortex following a switch from typical antipsychotics to olanzapine (Goff et al., 2002). It is difficult to estimate the exact effect of antipsychotics on GABA concentrations from these studies. In a previous study, we found no significant correlation between glutamate or glutamine concentrations and the dose of antipsychotics (Tayoshi et al., 2009).

The present study found a significant negative correlation between the GABA concentration in the ACC and the dose of antipsychotics i.e. a higher dose of antipsychotics was associated with a lower concentration of GABA in the ACC.

All antipsychotic medications, including both typical and atypical antipsychotics, may decrease the GABA concentration in the ACC. Although no difference was found in the GABA concentration in the ACC of medicated patients compared to healthy control subjects, it would be reasonable to hypothesize that unmedicated patients might show a higher GABA concentration in the ACC. Studies of unmedicated patients would provide valuable insights into this issue.

The significant positive correlation found between the dose of anticholinergic medication and the GABA concentration in the ltBG may be of clinical significance. The striatum receives dense cholinergic innervations from local interneurons, and the main synaptic targets of these cholinergic interneurons are GABAergic projection neurons (Graybiel, 1990; Izzo and Bolam, 1988). Muscarinic receptors on the GABA neurons are known to reduce GABA-mediated synaptic potentials and GABA release (Calabresi et al., 2000). It has been proposed that blockade of muscarinic stimulation by anticholinergic medication may increase the GABA concentration in the basal ganglia of schizophrenia patients.

4.5. Limitations

The present study has certain limitations. Firstly, the volume of the ROIs was set relatively high in order to measure the GABA concentrations. The volume was 27 ml, and the ROIs might therefore have contained heterogeneous tissues. Different results might have been obtained if the GABA concentration had been measured with smaller ROIs containing more homogenous tissues. Secondly, schizophrenia is thought to be a heterogeneous disorder, and the inherent heterogeneity of the schizophrenia patients might have affected the results, although the fact that most of the schizophrenia patients in our study were diagnosed as paranoid type may have minimized this effect. However, had the schizophrenia patients been divided into subgroups on the basis of other factors (e.g., genetic factors), significant differences in the GABA concentration between some of the schizophrenia subgroups and healthy control subjects might have been revealed. Thirdly, since antipsychotic medication may influence GABA concentrations, measurements in patients not taking antipsychotic medication would have provided valuable insights. Finally, although around half of the participants in the present study were female, we did not control for the effect of the menstrual cycle, a factor which may have an effect on GABA concentrations (Epperson et al., 2005).

5. Conclusion

Using ^{1}H-MRS, we have been the first to measure GABA concentrations in schizophrenia patients, but have found no differences in either the ACC or the ltBG between patients and healthy control subjects. Among the schizophrenia patients, the GABA concentration in the ltBG was higher in those taking typical antipsychotics than in those taking atypical antipsychotics. The GABA concentration in the ACC was found to be negatively correlated with the dose of the antipsychotics. Although the underlying mechanism is unclear, our results suggest that antipsychotic medication may induce changes in GABA concentration, and that these changes are dependent upon the type of medication. Antipsychotic medication may conceal inherent differences in GABA concentrations between schizophrenia patients and healthy controls.

Role of funding source

The funding sources had no involvement in the study design; collection, analysis and interpretation of the data; or writing of the report and the decision to submit it for publication.

Contributors

Author Tayoshi designed the study, wrote the protocol, managed the literature search, and undertook the statistical analysis. Author Nakataki recruited study participants and undertook additional statistical analysis. Authors Sumitani, Taniguchi, Shibuya-Tayoshi, Numata, and Iga recruited study participants. Author Harada operated the ^{1}H-MRS. Author Ueno also managed the literature search and recruited study participants. Author Ohmori managed the progress of the entire study.

Conflict of interest

The authors have no conflict of interest to declare.

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ORIGINAL PAPER

Association Study Between the Pericentrin (*PCNT*) Gene and Schizophrenia

Shusuke Numata · Masahito Nakataki · Jun-ichi Iga · Toshihito Tanahashi · Yoshihiro Nakadoi · Kazutaka Ohi · Ryota Hashimoto · Masatoshi Takeda · Mitsuo Itakura · Shu-ichi Ueno · Tetsuro Ohmori

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Abstract Disrupted-in-schizophrenia 1 (*DISC1*), a known genetic risk factor for schizophrenia (SZ) and major depressive disorder (MDD), interacts with several proteins and some of them are reported to be genetically associated with SZ. Pericentrin (*PCNT*) also interacts with *DISC1* and recently single-nucleotide polymorphisms (SNPs) within the *PCNT* gene have been found to show significant associations with SZ and MDD. In this study, case-controlled association analysis was performed to determine if the *PCNT* gene is implicated in SZ. Nine SNPs were analyzed in 1,477 individuals (726 patients with SZ and 751 healthy controls). No significant difference was observed between the controls and the patients in allelic frequencies or genotypic distributions of eight SNPs. Although allelic distribution of rs11702684 was different between the two groups ($P = 0.042$), the difference did not reach statistical significance after permutation correction for multiple comparisons. In the haplotypic analysis, we could not find any significant association in our subjects, either. This gene may not play a major role independently in the etiology of SZ in the Japanese population.

Keywords Schizophrenia · PCNT · Kendrin · Case–control association study · DISC1

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S. Numata (✉) · M. Nakataki · J. Iga · Y. Nakadoi · T. Ohmori
Department of Psychiatry, Course of Integrated Brain Sciences, Medical Informatics, Institute of Health Biosciences, The University of Tokushima Graduate School, 3-8-15 Kuramoto-cho, Tokushima 770-8503, Japan
e-mail: shu-numata@umin.ac.jp

T. Tanahashi · M. Itakura
Division of Genetic Information, Institute for Genome Research, The University of Tokushima Graduate School, Tokushima, Japan

Y. Nakadoi
Kagawa Prefectural Marugame Hospital, Kagawa, Japan

K. Ohi · R. Hashimoto · M. Takeda
Department of Psychiatry, Osaka University Graduate School of Medicine, Osaka, Japan

R. Hashimoto · M. Takeda
The Osaka-Hamamatsu Joint Research Center for Child Mental Development, Osaka University Graduate School of Medicine, Osaka, Japan

S. Ueno
Department of Neuropsychiatry, Ehime University School of Medicine, Ehime, Japan

Introduction

Schizophrenia (SZ) is a complex psychiatric disorder that afflicts approximately 1% of the population throughout the world and has high heritability (Craddock et al. 2005). The pericentrin gene (the official symbol; *PCNT* and also called kendrin) is located at 21q22.3, which is one of chromosomal lesions prevalent in SZ by cytogenetic analysis (Demirhan and Tastemir 2003). *PCNT* is a coiled-coil protein localized specifically to the centrosome throughout the cell cycle (Flory et al. 2000) and an integral component of the pericentriolar material (Li et al. 2001). This protein provides sites for microtubule nucleation in the centrosome by anchoring gamma-tubulin complex (Takahashi et al. 2002), then it plays an important role in microtubule organization, spindle organization, and chromosome segregation (Doxsey et al. 1994; Purohit et al. 1999). Disrupted-in-schizophrenia 1

(*DISC1*), a known genetic risk factor for SZ and major depressive disorder (MDD) (Cannon et al. 2005; Chen et al. 2007; Hashimoto et al. 2006; Hennah et al. 2003; Millar et al. 2000; Thomson et al. 2005), localizes to the centrosome by binding to *PCNT* (Miyoshi et al. 2004). Shimizu et al. showed that overexpression of the *DISC1*-binding regions of *PCNT* or the *DISC1* deletion mutant lacking the *PCNT*-binding region impaired the microtubule organization and they suggested that the *DISC1–PCNT* interaction played a key role in the microtubule network formation (Shimizu et al. 2008). Recently, single-nucleotide polymorphisms (SNPs) within the *PCNT* gene have been found to show allelic associations with SZ and MDD (Anitha et al. 2009; Numata et al. 2009). In addition, Mitkus et al. reported a trend for an increase mRNA levels of the *PCNT* gene in the dorsolateral prefrontal cortex of patients with SZ, compared with the control groups (Mitkus et al. 2006). In this study, case-controlled association analysis was performed in the Japanese population to determine if the *PCNT* gene is implicated in SZ.

Materials and Methods

Subjects

We used genomic DNA samples from 726 SZ patients: 406 male (mean age 48.6 ± 13.8 years), 320 female (mean age 49.2 ± 14.5 years) from the Tokushima University Hospital, affiliated psychiatric hospitals of the University of Tokushima, the Ehime University Hospital and the Osaka University Hospital in Japan. The diagnosis of SZ was made by at least two experienced psychiatrists according to DSM-IV criteria on the basis of extensive clinical interviews and review of medical records. Seven hundred fifty-one controls, 422 male (mean age 45.5 ± 11.1 years) and 329 female (mean age 45.2 ± 10.5), were selected from volunteers who were recruited from hospital staff and students and company employees documented to be free from either psychiatric problems or past mental histories. All subjects were unrelated Japanese origin and signed written informed consent to participate in the genetic association studies approved by the institutional ethics committees.

Genotyping

We initially selected eight tagging SNPs by SNPBrowser 3.5 (De La Vega et al. 2006) (Applied Biosystems, Foster, CA, USA, Pair-wise $r^2 > 85\%$, MAF > 20%, Japanese population) (rs11702684, rs2249057, rs11701058, rs2839226, rs2839231, rs3788265, rs2073376, rs1010111) (Supplementary Table 1). After that, we selected rs2073380 additionally because eight tagging SNPs did not seem to cover the third block of the *PCNT* gene from HapMap data. Genotyping was performed using commercially available TaqMan probes for the *PCNT* gene with ABI Prism 7900 HT Sequence Detection System and ABI 7500 Real Time PCR System (Applied Biosystems). Haplotype block structure was determined using the HAPLOVIEW program (Barrett et al. 2005). Blocks were defined according to the criteria of Gabriel et al. (2002).

Statistical Analysis

Allelic and genotypic frequencies of patients and control subjects were compared using χ^2 test. The SNPAlyze 3.2Pro software (DYNACOM, Japan) was used to estimate haplotype frequencies, linkage disequilibrium (LD), permutation *P*-values (10,000 replications) and deviation from Hardy–Weinberg Equilibrium (HWE) distribution of alleles. Power calculations for our sample size performed using the G*Power program (Erdfelder et al. 1996). The criterion for significance was set at $P < 0.05$ for all tests.

Results

Genotypic and allelic frequencies of the *PCNT* gene are shown in Table 1. Genotypic distributions of these nine SNPs did not deviate significantly from HWE in either group ($P > 0.05$). No significant difference was observed in genotypic frequency between the controls and patients in eight SNPs. Although allelic distribution of rs11702684 was different between the two groups ($P = 0.042$), the difference did not reach statistical significance after permutation correction for multiple comparisons. In power calculations using the G*Power program, our sample size had >0.98 power for detecting a significant association (alpha < 0.05) when an effect size index of 0.2 was used.

Several papers reported that there were gender-specific genetic components involved in the pathology of SZ in the *DISC1* gene (Hennah et al. 2003; Chen et al. 2007) and the *DISC1*-related genes (Hennah et al. 2007; Pickard et al. 2007; Qu et al. 2008). In our study, when the data were subdivided on the basis of gender, allelic distribution of rs11702684 was different between the two groups in only male samples ($P = 0.033$). However, the difference did not survive statistical significance after permutation correction for multiple comparisons.

There were three LD blocks in the *PCNT* gene with rs2249057, rs11701058, rs2839226, and rs2839231 residing in block 1 and rs3788265 and rs2073376 residing in block 2, and rs2073380 and rs1010111 residing in block 3 (Gabriel et al. 2002, Fig. 1). These constructed marker haplotypes of blocks 1–3 were not associated with SZ (permutation $P = 0.184$, 0.137, and 0.601, respectively).

Table 1 Genotypes and allele frequencies of nine single SNPs in the PCNT gene in patients with SZ and controls

SNP	Diagnosis	Allele		*P*-value	Genotype			*P*-value	Frequency
rs11702684		C	T		C/C	C/T	T/T		
	SC	913	515	0.042	296	321	97	0.085	0.361
	CT	892	588		265	362	113		0.397
		C	A		C/C	C/A	A/A		
rs2249057	SC	862	590	0.504	255	352	119	0.691	0.406
	CT	870	626		247	376	125		0.418
		C	T		C/C	C/T	T/T		
rs11701058	SC	669	783	0.181	153	363	210	0.297	0.461
	CT	728	772		168	392	190		0.485
		C	T		C/C	C/T	T/T		
rs2839226	SC	378	1072	0.111	47	284	394	0.19	0.261
	CT	353	1147		34	285	431		0.235
		A	G		A/A	A/G	G/G		
rs2839231	SC	408	1042	0.562	63	282	380	0.52	0.281
	CT	405	1085		53	299	393		0.272
		G	T		G/G	G/T	T/T		
rs3788265	SC	821	627	0.998	234	353	137	0.506	0.433
	CT	846	646		230	386	130		0.433
		A	G		A/A	A/G	G/G		
rs2073376	SC	445	1001	0.403	75	295	353	0.51	0.308
	CT	478	1006		77	324	341		0.322
		C	A		C/C	C/A	A/A		
rs2073380	SC	642	796	0.839	144	354	221	0.552	0.446
	CT	669	817		141	387	215		0.45
		A	G		A/A	A/G	G/G		
rs1010111	SC	1079	363	0.298	402	275	44	0.343	0.252
	CT	1141	351		428	285	33		0.235

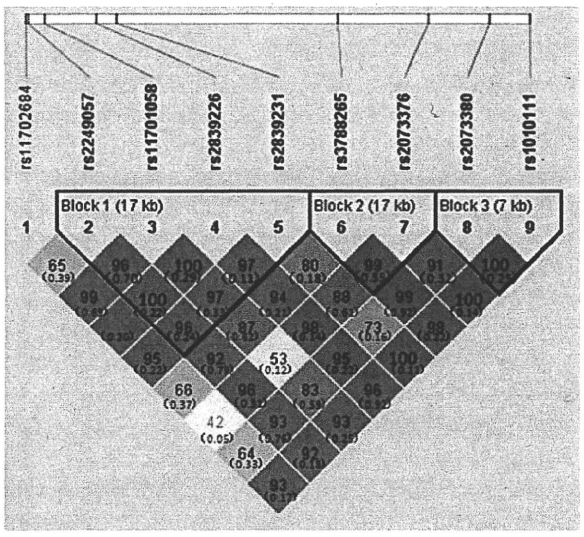

Fig. 1 LD and haplotype structure of the PCNT gene. Haplotype block structure was determined using the HAPLOVIEW program (Barrett et al. 2005). Blocks were defined according to the criteria of Gabriel et al. (2002). Each box represents the D' (r^2) values corresponding to each pair-wise SNP

Discussion

In this study, we examined the association of nine SNPs in the *PCNT* gene and SZ. No significant difference was observed between the controls and the patients in either allelic frequencies or genotypic distributions of nine SNPs after permutation correction for multiple comparisons. In the haplotypic analysis, we could not find any significant association in our subjects. This result was concordance with another study in a Caucasian population (Tomppo et al. 2009).

During the preparation of this article, Anitha et al. reported that rs2249057 of the *PCNT* gene and haplotypes involving this SNP were significantly associated with SZ after correction for multiple comparisons in the Japanese population (Anitha et al. 2009). Although SNPs examined in our study contained rs2249057, we could not find any significant associations in our subjects. The statistical power of our study was sufficient to detect an association between the variants and SZ (SZ $n = 726$; control $n = 751$). Surprisingly, the control minor allele frequency of rs2249057 in Anitha's study (0.48) was higher than

those of our study, HapMap data, and ABI data (0.42, 0.40, and 0.41, respectively). This differing allele frequency between these two studies may be caused by samples' recruited areas. Anitha et al. used subjects from further east compared to ours. However, it is reported that there is no significant population stratification in Japanese (Arinami et al. 2005; Yamaguchi-Kabata et al. 2008).

There are several limitations in our study. First, we applied MAF > 20% when we selected the tagging SNPs and it is difficult to evaluate the association of rare variants in our study. Second, we cannot rule out a possibility that *DISC1–PCNT* interaction may be involved in the etiology of SZ. Third, our findings only represented the Japanese population and studies in other populations would still be warranted due to differing allele frequencies between populations.

Conclusions

In conclusion, we did not find any significant association between the *PCNT* gene and the SZ in the Japanese population. This gene may not play a major role independently in the etiology of SZ.

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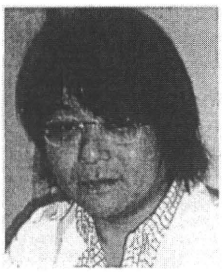
難治性うつ病への対応

Management of refractory depression in Japan in 2010



岡本長久　坂本広太
Nagahisa Okamoto and Kota Sakamoto
国立精神・神経医療研究センター病院精神科

◎“難治性うつ病”と一口にいっても難治性に関して厳密な定義があるわけではない．臨床現場において何種類もの薬物療法を試みても改善しない治療抵抗性うつ病や，容易に再発し頻回に病相を反復するうつ病などを指しており，うつ病全体の30%程度にみられる．アメリカで行われた大規模臨床試験 STAR*D 研究(Systematic Treatment Alternatives to Relieve Depression；4段階に分けたアルゴリズムに沿って各治療を比較した臨床試験)では最終段階までの累積寛解率は67%で，4段階の薬物治療戦略を試みても寛解に至らない症例が1/3存在することが示されている．難治性うつ病に対する治療法として，抗うつ薬にリチウム，甲状腺ホルモン，非定型抗精神病薬，ドパミンアゴニストなどを併用する増強療法や電気痙攣療法などが臨床使用されているが，その治療戦略に関して，エビデンスに基づき治療を標準化するための十分な数の臨床研究はまだない．加えて日本では市販されている抗うつ薬の種類が少ないこともあり，難治性うつ病の臨床研究やアルゴリズム作成は遅れをとっている．わが国でも標準化された治療戦略を確立するため，エビデンスを蓄積していくことが臨床における重要な課題である．本稿ではその現状および今後の展望について述べる．

Key word：難治性うつ病，治療抵抗性うつ病，増強療法，併用療法，修正型電気痙攣療法

薬物療法はうつ病治療の第一選択肢であり，わが国では1960年前後より第一世代三環系抗うつ薬(Tricyclic antidepressant：TCA)が，1980年代以降には第二世代のTCAや四環系抗うつ薬が発売された．またこの10年間は，選択的セロトニン再取込み阻害薬(Selective Serotonin Reuptake Inhibitor：SSRI)やセロトニン・ノルアドレナリン再取込み阻害薬(Serotonin Noradrenalin Reuptake Inhibitor：SNRI)が各国でつぎつぎと承認，発売され，わが国でもSSRIでは1999年マレイン酸フルボキサミン，2000年塩酸パロキセチン，2006年塩酸セルトラリンが，SNRIでは2000年塩酸ミルナシプランが発売され，抗うつ薬の選択の幅は広がっている．また，2009年には新しい作用機序をもつノルアドレナリン作動性・特異的セロトニン作動性抗うつ薬(Noradrenergic and Specific Serotonergic Antidepressant：NaSSA)であるミルタザピンが発売され，2010年にはSNRIである塩酸デュロキセチンが発売される予定である．

海外ではこれらの作用機序の抗うつ薬に加えてドパミン・ノルアドレナリン再取込み阻害薬であるブプロピオン，モノアミン酸化酵素A阻害薬などが発売されており，わが国でもモノアミン酸化酵素A阻害薬以外の抗うつ薬は治験が行われ，今後導入される可能性が高い．

しかし，初回治療の抗うつ薬で寛解しない患者が2/3程度存在し，最終的にうつ病患者の約1/3程度が適切な薬物治療戦略によって改善しない難治性うつ病であることが知られている[1]．

難治性うつ病の定義

一般的に難治性うつ病という言葉は，従来治療に反応しないうつ病に用いられてきた．しかし，すべての抗うつ薬とmECTや認知行動療法を試しすべて無効であった後に難治性うつ病と診断するのでは，各国により発売されている抗うつ薬に

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