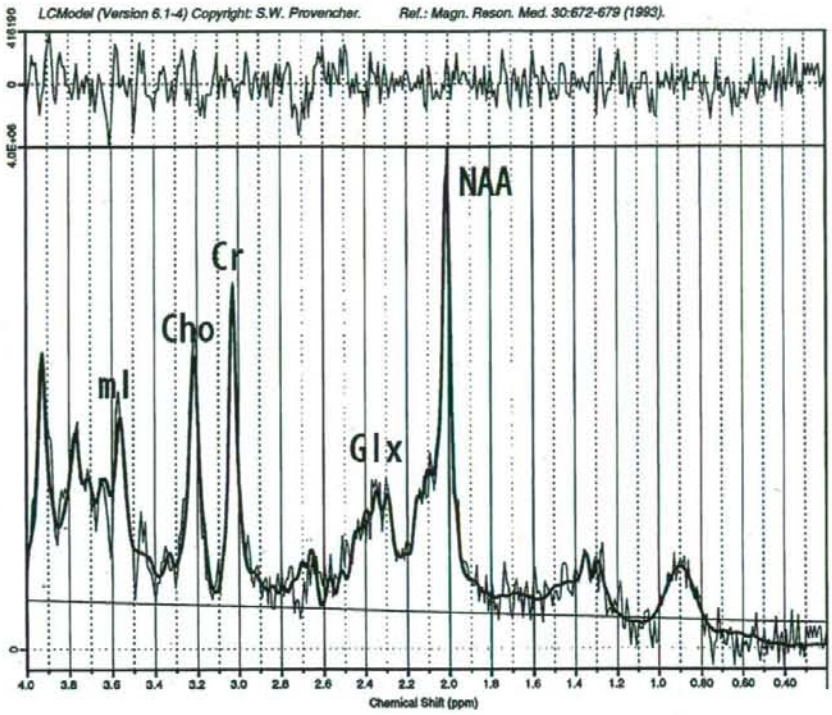
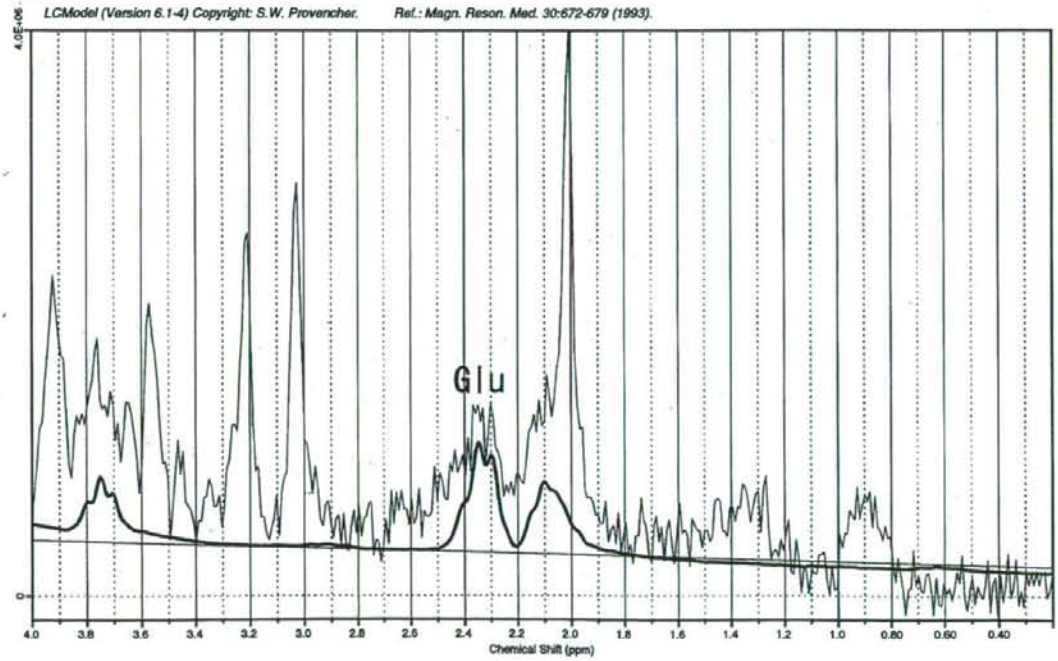
a


LCModel (Version 6.1-4) Copyright: S.W. Provencher.
Ref.: Magn. Reson. Med. 30:672-679 (1993).
4.0E+06
0
NAA
Cr
Cho
mI
Glx
4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.80 0.60 0.40
Chemical Shift (ppm)

b


LCModel (Version 6.1-4) Copyright: S.W. Provencher.
Ref.: Magn. Reson. Med. 30:672-679 (1993).
4.0E+06
0
Glu
4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.80 0.60 0.40
Chemical Shift (ppm)

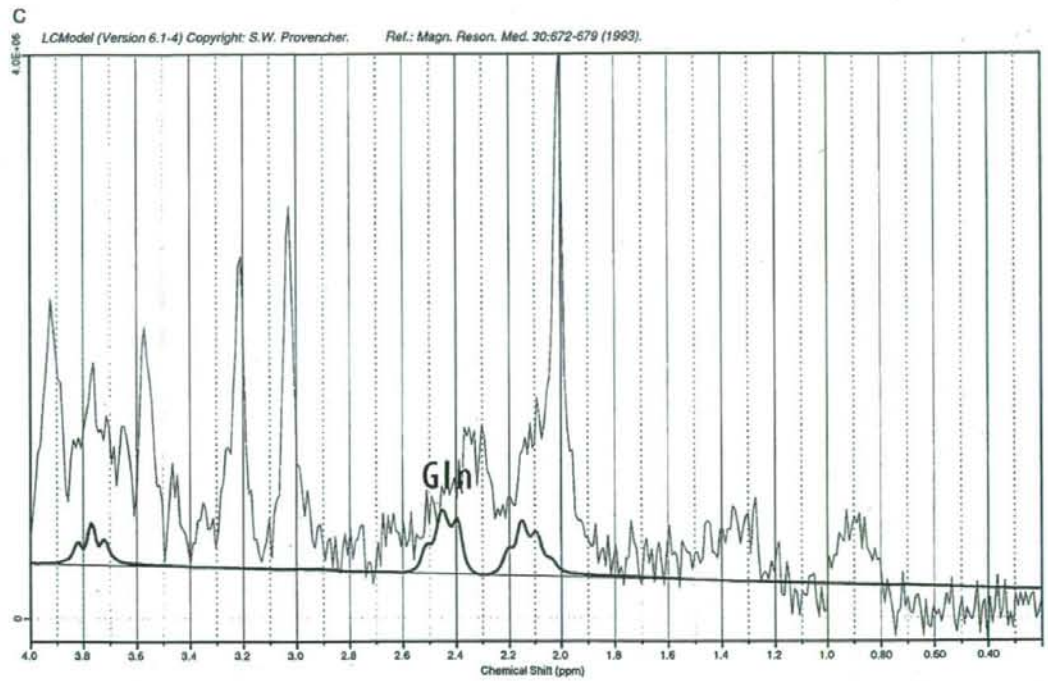


Fig. 2. Representative ^{1}H-MRS spectra of the anterior cingulate cortex (ACC) obtained from a subject in STEAM sequence. (a) shows the peaks that represent each compound. (b) and (c) show the separate peaks for Glu and Gln.

providing written informed consent in accordance with the guidelines of the ethical committee at Tokushima University. The epidemiological data from the subjects are shown in Tables 1a and 1b. All patients were assessed with the DSM-IV TR (American Psychiatric Association, 2000). All schizophrenic patients were outpatients or inpatients of the Tokushima University Hospital. Twenty-eight of the patients (thirteen males and fifteen females) were classified as having paranoid schizophrenia, one female patient was classified as having undifferentiated schizophrenia, and one male patient was classified as having disorganized schizophrenia. All patients were assessed using the Positive and Negative Syndrome Scale (PANSS, Kay et al., 1987). Eleven patients (five male and six female) received benzodiazepines (the 12.0±6.3 mg diazepam equivalent) and three patients (one male and two female) received paroxetine. All healthy volunteers were recruited from the same region and had no history of an Axis I psychiatric illness as determined by the DSM-IV TR. Both the schizophrenic patients and the healthy volunteers were Japanese and spoke Japanese as their mother tongue. None of the patients or healthy volunteers had a serious medical illness, or history of head injury or drug or alcohol abuse before the scan. All subjects were right-handed.

2.2. ^{1}H-MRS procedure

Employing a 3 Tesla clinical magnetic resonance imaging (MRI) instrument (Signa 3T Excite, GE Healthcare, Milwaukee, WI, USA), ^{1}H-MRS was performed using the STEAM sequence with water suppression by CHESS pulses (TE = 18 ms, TR = 5000 ms, acquisition = 64 times) to minimize longitudinal and transverse relaxation efforts. Neurochemical metabolites that can be identified in short-echo ^{1}H-MRS include Cre, Gln, Glu, mI, NAA, and Cho. The area under the peak for each magnetic resonance is proportional to the concentration of that particular compound. Metabolite levels were estimated using linear combination model software (Provencher, 1993). Our basis-set was constructed from original in vitro data for each metabolite. On the basis of previous reports of functional anomalies, the region of interest (ROI) for ^{1}H-MRS was set for the ACC and the ltBG (the ROI size = 1.7 cm × 1.7 cm × 1.5 cm) using three oriented images. For a reference slice of the ACC, an axial cut approximately one cm above the upper end of the body of the lateral ventricle was chosen. The center of the ROI was centered on the frontal interhemispheric fissure, 3 cm in front of the central fissure and 2 cm above the corpus callosum. A reference slice of the ltBG was placed between the Sylvian fissure and the lateral ventricles to encompass the lenticular nucleus (Fig. 1a and b). Representative ^{1}H-MRS spectra of the ACC obtained from a subject with the peaks that represent each compound are shown in Fig. 2a, b, and c.

T1-weighted images (3D-SPGR) were acquired using the following parameters: TE = 4.2 ms, TR = 10 ms, slice thickness = 0.8 mm, matrix 512 × 512, FOV = 24 × 24 cm, and Flip angle = 15°. Thus, the brain images were composed of voxels (voxel size = 0.47 mm × 0.47 mm × 0.8 mm). Based on

Table 2a
Effect of illness in ACC

		Control (mmol)	Schizophrenia (mmol)		p
Cre		10.5±3.1	8.9±3.0	$F=3.82, df=1$	n.s.
	Male	11.1±3.0	8.1±3.2	$t=2.48$	.021
	Female	9.8±3.1	9.6.9±2.8	$t=0.22$	n.s.
Gln		5.6±2.3	4.9±1.8	$F=1.38, df=1$	n.s.
	Male	6.6±2.3	5.1±2.0	$t=1.74$	n.s.
	Female	4.5±1.7	4.7±1.6	$t=-0.3.6$	n.s.
Glu		11.5.±3.6	9.8±2.7	$F=4.07, df=1$	.049*
	Male	12.3±3.6	9.8±2.8	$t=2.43$	.022
	Female	10.7.±3.5	10.3±2.5	$t=-0.03$	n.s.
mI		8.2±2.3	6.8±2.2	$F=5.71, df=1$	.021*
	Male	8.6±2.2	6.3±2.2	$t=2.61$	.015
	Female	7.8±2.5	7.1±2.2	$t=0.79$	n.s.
NAA		11.7±3.3	100±3.3	$F=3.82$, $df=1$	n.s.
	Male	12.3±3.3	9.4±3.4	$t=2.23$	.035
	Female	11.1±3.4	10.5±3.1	$t=0.487$	n.s.
Cho		3.1±1.0	2.7±1.0	$F=2.27, df=1$	n.s.
	Male	3.4±0.9	2.5±1.1	$t=2.36$	0.27
	Female	2.8±0.9	2.8±1.0	$t=-0.25$	n.s.

The concentration of metabolite are shown as mean±S.D.
Abbreviations: Cre, creatine+phosphocreatine; Gln, glutamine; Glu, glutamate; mI, myo-inistol; NAA, N-acetylaspartate; Cho, choline containing compounds; n.s., no significant difference.
*Significant main effect of illness in two way ANOVA ($p<.05$).
†Significant in two sample t-test ($p<.05$).

Table 2b
Effect of illness in two way ANOVA and two sample t-test between schizrophrenic subject and healthy controls in each gender group in ltBG

		Control (mmol)	Schizophrenia (mmol)		p
Cre		5.4±0.6	5.4±0.6	$F=0.33, df=1$	n.s.
	Male	5.2±0.5	5.2.±0.6	$t=0.03$	n.s.
	Female	5.7.±0.6	5.5.±0.6	$t=0.76$	n.s.
Gln		2.7±0.6	2.7±0.7	$F=0.01, df=1$	n.s.
	Male	2.6.±0.7	2.9.±0.7	$t=-1.20$	n.s.
	Female	2.7±0.5	2.4.±0.7	$t=1.23$	n.s.
Glu		4.8.±0.8	4.8±0.8	$F=0.13, df=1$	n.s.
	Male	4.7±1.0	5.0±0.8	$t=-0.88$	n.s.
	Female	4.9.±0.4	4.7±0.7	$t=-0.72$	n.s.
mI		3.3±0.4	4.7±0.7	$F=0.13, df=1$	n.s.
	Male	3.0±0.4	3.1±0.5	$t=-0.62$	n.s.
	Female	3.5±0.3	3.0±0.9	$t=1.99$	n.s.
NAA		5.8±0.9	5.8±0.6	$F=0.08, df=1$	n.s.
	Male	5.3.±0.9	5.8.±0.6	$t=-0.98$	n.s.
	Female	6.3±0.4	5.9±0.6	$t=2.06$	n.s.
Cho		1.5±0.1	1.5±0.2	$F=0.00, df=1$	n.s.
	Male	1.5±0.1	1.5±0.2	$t=-0.58$	n.s.
	Female	1.5±0.2	1.5.±0.2	$t=-0.51$	n.s.

Abbreviation: n.s., no significant difference.
*Significant main effect of illness in two way ANOVA ($p<.05$). †Significant in two sample t-test ($p<.05$).

the histogram of voxel intensity, each voxel in the 3D-SPGR brain images was classified into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) using the ImageJ Ver.1.38 software package (http://rsb.info.nih.gov/ij). The voxels that were regarded as GM, WM and CSF in each ROI were counted using the 3D-Slicer Ver.2.6 software package (http://www.slicer.org) to obtain the desired ratio of these tissues. Metabolite concentrations were corrected for CSF in the ROI by dividing by the percentage of brain tissue in each ROI, assuming that metabolite concentrations in the CSF were equal to zero (Bustillo et al., 2001).

2.3. Statistical procedures

Statistical tests were performed using the SPSS version 11.5J software package (SPSS Japan Inc., Tokyo, Japan). Absolute metabolite levels measured with ^{1}H-MRS were analyzed. In ROIs for both the ACC and the ltBG, the mixed models approach for repeated two-way analysis of variance (ANOVA), including group (i.e., schizophrenia patients versus healthy volunteers) and gender (i.e., male versus female), was used to reveal the effects of these factors on each metabolite.

Since the partial volume effects of the GM and the WM may influence the metabolite levels in ^{1}H-MRS (Hetherrington et al., 1994; Kim et al., 2005; Pan et al., 2006), the ratios of the GM and the WM for each ROI were compared for either the schizophrenic subjects and the healthy controls or the male subjects and the female subjects using two sample t-tests.

Among the schizophrenic patients, correlations between clinical indices and each metabolite level were evaluated using Spearman's rank correlation test in both ROIs. In the eleven patients who received benzodiazepine, the correlations between the benzodiazepine dose and the metabolite levels were evaluated in both ROIs.

3. Results

Among the metabolites, ANOVA revealed that the illness significantly affected the Glu concentration ($F=4.07$, $df=1$, $p=.049$) and the mI concentration ($F=5.70$, $df=1$, $p=.021$) in the ACC; both metabolites were lower in schizophrenia patients than in control subjects (Tables 2a and 2b). Gender was shown to significantly affect the Gln concentration in the ACC ($F=5.88$, $df=1$, $p=.019$) and the concentrations of Cre

Table 2c
Effect of gender in ACC

		Control (mmol)	Schizophrenia (mmol)		p
Cre		9.5±3.4	9.7±2.9	$F=0.03, df=1$	n.s.
	Ctrl	11.1±3.0	9.8.±3.1	$t=1.00$	n.s.
	Sc	8.1.±3.2	9.6±2.8	$t=-1.33$	n.s.
Gln		5.8±2.2	4.6±1.6	$F=5.88, df=1$	.019*
	Ctrl	6.6.±2.3	4.5.±1.7	$t=2.62$	.016†
	Sc	5.1±2.0	4.7.±1.6	$t=0.62$	n.s.
Glu		10.7.±3.5	10.5±3.0	$F=0.09, df=1$	n.s.
	Ctrl	12.3±3.6	10.7±3.5	$t=1.13$	n.s.
	Sc	9.2.±2.9	10.3±2.5	$t=-1.09$	n.s.
mI		7.4±2.4	7.4±2.3	$F=0.00, df=1$	n.s.
	Ctrl	8.6±2.2	7.8±2.5	$t=0.82$	n.s.
	Sc	6.4±2.2	7.1±2.2	$t=-0.92$	n.s.
NAA		10.8±3.6	10.8±3.2	$F=0.00, df=1$	n.s.
	Ctrl	12.3.±3.3	11.1.±3.4	$t=0.89$	n.s.
	Sc	9.4±3.4	10.5±3.1	$t=-0.91$	n.s.
Cho		2.9±1.1	2.8±0.9	$F=0.25, df=1$	n.s.
	Ctrl	3.4±0.9	2.8±0.9	$t=1.66$	n.s.
	Sc	2.5±1.0	2.8.±1.0	$t=-0.97$	n.s.

Abbreviation: Ctrl, Healthy Controls; Sc, Schizophrenic subjects; n.s., no significant difference.
*Significant main effect of gender in two way ANOVA ($p<.05$). †Significant in two sample t-test ($p<.05$).

Table 2d
Effect of gender in ltBG

		Male (mmol/l)	Female (mmol/l)		p
Cre		5.2±0.6	5.5±0.6	$F=4.58$, $df=1$	.037*
	Ctrl	5.2±0.5	5.7±0.6	$t=-1.89$	n.s.
	Sc	5.2±0.6	5.5±0.6	$t=-1.16$	n.s.
Gln		2.8±0.7	2.5±0.6	$F=1.75$, $df=1$	n.s.
	Ctrl	2.6±0.7	2.7±0.5	$t=-0.29$	n.s.
	Sc	3.0±0.7	2.4±0.7	$t=2.17$	.039†
Glu		4.8±0.9	4.8±0.6	$F=0.00$, $df=1$	n.s.
	Ctrl	4.7±1.0	4.9±0.4	$t=-0.77$	n.s.
	Sc	5.0±0.8	4.7±0.7	$t=0.81$	n.s.
mI		3.1±0.4	3.2±0.7	$F=1.10$, $df=1$	n.s.
	Ctrl	3.0±0.4	3.5±0.3	$t=-3.01$	.007†
	Sc	3.1±0.5	3.0±0.9	$t=0.48$	n.s.
NAA		5.5±0.7	6.1±0.5	$F=12.69$, $df=1$	.001*
	Ctrl	5.3±0.9	6.3±0.4	$t=-3.42$	.003†
	Sc	5.7±0.6	6.0±0.6	$t=-1.36$	n.s.
Cho		1.5±0.2	1.5±0.2	$F=0.02$, $df=1$	n.s.
	Ctrl	1.5±0.1	1.5±0.2	$t=-0.71$	n.s.
	Sc	1.5±0.2	1.5±0.2	$t=0.42$	n.s.

Abbreviation: n.s., no significant difference.
*Significant main effect of gender in two way ANOVA ($p<.05$). †Significant in two sample t-test ($p<.05$).

($F=4.58$, $df=1$, $p=.037$) and NAA ($F=12.7$, $df=1$, $p=.001$) in the ltBG. Gln levels in the ACC were significantly higher in male subjects as compared to female subjects (Table 2c and 2d). Among male subjects, Cre, Glu, mI, NAA, and Cho were significantly lower in the schizophrenic subjects than in the control subjects (Table 2a). Among the control subjects, Gln levels were significantly higher in the ACC of the male subjects as compared to the female subjects (Table 2c), and mI and NAA levels were significantly lower in the ltBG of the male subjects as compared to the female subjects (Table 2d). There was no significant illness (i.e., schizophrenic patients vs. control subjects)×gender (i.e., male subjects vs. female subjects) interaction in any metabolite level in either ROI (Table 3). The ratio of GM and WM for each ROI did not significantly differ either between the schizophrenic patients and the healthy controls or between the male subjects and the female subjects (Table 4).

Among the schizophrenic patients, the clinical indices and PANSS positive, negative, general, and total scores did not significantly correlate with the level of any specific metabolite. in addition, treatment with antipsychotics did not correlate with the level of any of the metabolites. The result

Table 3
The F values for illness × gender interaction in each metabolite level in both ROI's

	ACC		ltBG	
	F value (illness illness×gender)	p	F value (illness illness×gender)	p
Cre	2.71	n.s.	0.29	n.s.
Gln	2.61	n.s.	3.00	n.s.
Glu	2.54	n.s.	1.23	n.s.
mI	1.52	n.s.	3.29	n.s.
NAA	1.63	n.s.	3.73	n.s.
Cho	3.44	n.s.	0.57	n.s.

Abbreviations: ACC, anterior cingulate cortex; ltBG, left basal ganglia; n.s., no significant difference.

Table 4
The ratio of GM, WM in the ROIs of ACC and the ltBG

	Control	Schizophrenia	p
GM ratio in the ACC	0.36±0.11	0.42±0.13	n.s.
WM ratio in the ACC	0.17±0.09	0.16±0.09	n.s.
GM ratio in the ltBG	0.49±0.16	0.48±0.17	n.s.
WM ratio in the ltBG	0.49±0.18	0.50±0.18	n.s.
	Male	Female	p
GM ratio in the ACC	0.41±0.14	0.38±0.11	n.s.
WM ratio in the ACC	0.14±0.09	0.18±0.09	n.s.
GM ratio in the ltBG	0.52±0.17	0.45±0.16	n.s.
WM ratio in the ltBG	0.45±0.18	0.53±0.17	n.s.

Abbreviations: GM, gray matter; WM, white matter; n.s., no significant difference.

was similar when analyzed for each gender group. Treatment with benzodiazepine correlated with mI levels ($r=0.66$, $p=.027$) in the ACC and Cre ($r=0.73$, $p=0.11$), Gln ($r=.73$, $p=.011$) and Glu ($r=.66$, $p=.029$) levels in the ltBG.

4. Discussion

4.1. Glutamate change

In the ACC, Glu levels were significantly decreased in schizophrenic patients. Because of the difficulty in isolating the Glu signal from Gln and other signals using a low magnetic field MR device, most previous MRS studies reported the combined signals of these compounds (Glx: Glu plus Gln). In previous studies that used a high-magnetic MR device, Glu levels in the left ACC were significantly increased in first-episode schizophrenia patients (Theberge et al., 2002), whereas these levels were significantly decreased in medicated patients with chronic schizophrenia (Theberge et al., 2003). Wood et al. (2007) reported that Glx levels in the ACC did not change in relation to schizophrenia. Zavitsanou et al. (2002) reported that ionotropic glutamate receptors were observed in increased levels in the postmortem ACC of patients with chronic schizophrenia. This result suggests a postsynaptic compensation for the impaired glutamatergic neurotransmission. Oni-Orisan et al. (2008) reported that in the postmortem ACC of chronic schizophrenia patients, transcription of the vesicular glutamate transporter (VGLUT) – which is known to package Glu into vesicles in the presynaptic terminal for subsequent release into the synaptic cleft – increased, and the expression of VGLUT protein decreased. This study suggested that the Glu release in the ACC decreased in chronic schizophrenic subjects. The result of our study is compatible with this postmortem study. Since Glu concentration showed no significant correlation with age, duration of illness, duration of therapy, or dosage of antipsychotics, these factors are not the likely cause for the reduction of Glu.

4.2. Myo-inositol change

The mI concentration was significantly lower in schizophrenic patients as compared to the healthy subjects. Previous ^{1}H-MRS studies with schizophrenic patients did not show consistent results regarding mI concentration. Block

et al. (2000), a study of the left frontal lobe using 1.5-Tesla imaging; Delamillieure et al. (2000), a study of the bilateral thalamus using 1.5-Tesla imaging; and Theberge et al. (2003), a study of the left ACC and the left thalamus using 4.0-Tesla imaging, reported no significant change in mI levels. Bluml et al. (1999) reported that the mI level did not change in the parietal cortex in either drug-naïve patients or medicated patients, while Szulc et al. (2005) reported that the mI concentration in the thalamus of drug-naïve patients increased after they received risperidone. Since researchers have suggested that antipsychotics may be effective via a dampening action on an overactive phosphatidylinositol second messenger system, where mI plays an important role (Kim et al., 2005), the influence of medication might contribute to the inconsistency among these studies. In a post-mortem study, Shimon et al. (1998) reported that the mI concentration decreased in chronic schizophrenia patients in the frontal and occipital cortex. The result of our study is consistent with this postmortem study.

4.3. N-acetylaspartate stability

Several studies using low magnetic field devices reported a change in NAA levels in chronic schizophrenia patients (Deicken et al., 2000; Auer et al., 2001; Ende et al., 2000), while studies using high magnetic MR devices did not have results consistent with these findings. Theberge et al. (2003) reported that NAA levels in chronic schizophrenia patients did not significantly differ in the left ACC and the left thalamus, whereas Chang et al. (2007) reported a significant NAA decrease in the bilateral, frontal, and temporal white matter of elderly schizophrenia patients. In our study, the NAA level was not significantly decreased in schizophrenic subjects. However, a trend of decrease ($F=3.82$, $p=.056$) was observed, and if we had assembled a larger sample, we may have observed a statistically significant decrease.

4.4. Gender differences

Gender differences in the clinical features of schizophrenia are widely known (Seeman, 1997), but the biological differences have not been fully confirmed. Although MRS is a useful method to investigate these gender differences in schizophrenia, only a few MRS studies have referred to these issues. Buckley et al. (1994) reported that male schizophrenic patients showed significant decreases in NAA levels and increases in Cho levels in the frontal cortex as compared to both male controls and female patients, but these differences were concealed within overall patient-control comparisons. In recent ^{1}H-MRS studies that used high magnetic MR devices on chronic schizophrenia patients, the ratios of male subjects to female subjects were quite large, but gender differences were not examined (Theberge et al., 2003; Tang et al., 2007; Matsuzawa et al., 2008). In our study, ANOVA revealed that gender significantly affected the Gln level in the ACC and the Cre and NAA levels in the ltBG. In male subjects, the levels of Cre, Glu, mI, and NAA in the ACC were significantly decreased in the ACC of schizophrenic patients as compared to the control subjects, and no compound level significantly differed in the ltBG of schizophrenic patients versus control subjects. However, in female subjects, no compound level significantly differed in the ACC, while the NAA concentration significantly decreased in the ltBG of schizophrenic patients as compared to the control subjects (Tables 2a and 2b). Part of these gender differences might be caused by the menstrual cycle (Rasgon et al., 2001; Batra et al., 2008). Since the morphological changes in schizophrenic patients are more prominent in male subjects (Waddington, 1993; Moreno et al., 2005) and the gender difference in the morphology might be attributable to a greater vulnerability among males to neurodevelopmental forms of schizophrenia (Waddington, 1993), the more prominent metabolite changes in male subjects may relate to these morphological findings. The gender differences in our study suggest that the male/female ratio may influence the results of MRS studies and that previous findings in studies with predominantly male subjects may not be generalized to female patients.

4.5. PANSS scores and metabolite level

Within the patient groups, the PANSS positive, negative, general, and total scores did not significantly correlate with the levels of any of the metabolites in either ROI. Some previous studies reported significant correlations between the NAA concentration and the PANSS negative scores (Sigmundsson et al., 2003; Tanaka et al., 2006), but Wood et al. (2007) reported that a PANSS negative syndrome did not correlate with the NAA level in the ACC. No previous study reported a correlation between the NAA level in the ltBG and the PANSS scores. The functional difference in the region where the ROI was placed may cause the inconsistent result.

4.6. Effects of benzodiazepines

The dose of benzodiazepine positively correlated with the mI level in the ACC and with Cre, Gln and Glu levels in the ltBG. A few MRS studies regarding acute benzodiazepine administration have been conducted. After acute benzodiazepine administration, Brambilla et al. (2002) found no significant change in the levels of Cre, Glx, mI, NAA, and Cho, whereas Goddard et al. (2004) found significant GABA reduction and speculated that benzodiazepines may have an inhibitory effect on glutamic acid decarboxylase (GAD), which is involved in the synthesis of γ-aminobutyric acid (GABA) from Gln and Glu. In preclinical observations, Izzo et al. (2001) reported that a withdrawal from chronic diazepam administration is associated with a marked increase in cortical GAD_{65} mRNA expression, indicating that benzodiazepine exposure may tend to suppress GAD gene expression. Raol et al. (2005) reported that long-term treatment with benzodiazepine suppresses the level of mRNA expression of both GAD_{65} and GAD_{67}. These results suggest that benzodiazepine administration may produce an increase in Gln and Glu levels and a decrease in GABA levels. The positive correlation of benzodiazepine dosage with Gln and Glu concentrations may be partly accounted for by these mechanisms. However, no MRS study has reported on chronic benzodiazepine use to our knowledge. Additionally, the mechanisms for the change in Cre and mI levels by chronic benzodiazepine use are not clear. Since the dosage of benzodiazepine did not significantly correlate with the PANSS scores, which did not significantly correlate with the level of any compound, this result may not

simply be caused by the severity of the illness. Although the mechanisms are unknown, our result suggests that long-term benzodiazepine use may increase mI levels in the ACC and Cre, Glu and Gln levels in the ltBG.. In addition, benzodiazepines are often used in various psychiatric illnesses for different purposes. Future MRS studies should consider that long-term benzodiazepine use may become a confounding factor in the interpretation of results.

4.7. Limitations

Several limitations can be identified in our study. Some confounding factors are present and might influence the results. In MRS studies, the results might be influenced by the region or the size of the ROI, which is not necessarily composed of uniform tissue. The participants include different clinical types of schizophrenic patients. The therapies with which they were treated were not equal.

5. Conclusion

Using a high-magnetic field MR device, our study revealed significant decreases in the levels of Glu and mI in the ACC of schizophrenic patients. It also demonstrated the existence of gender differences in some brain metabolites and dose-dependent benzodiazepine effects on the levels of certain metabolites.

Role of the funding source

This work was supported in part by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science, and Technology (No.19790819) and a Grants-in-Aid for Scientific Research from the 21st Century COE Program, Human Nutritional Science on Stress Control Tokushima, Japan. The ministry had no further role in the study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Contributors

The author Tayoshi designed the study, wrote the protocol, managed the literature search, and undertook the statistical analysis. Authors Sumitani, Taniguchi, Shibuya-Tayoshi, Numata, Iga, and Nakataki recruited the subjects. Author Harada operated the MRS. Author Ueno also managed the literature searches and recruited subjects. Author Ohmori managed the progress of the entire study.

Conflict of interest

All authors declare that they have no conflicts of interest.

Acknowledgement

This work was supported in part by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science, and Technology (No.19790819) and a Grants-in-Aid for Scientific Research from the 21st Century COE Program, Human Nutritional Science on Stress Control Tokushima, Japan.

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Annals of Medicine. 2008; 40: 336–342

REVIEW ARTICLE

Molecular assessment of depression from mRNAs in the peripheral leukocytes

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Abstract

Depression is a disorder not only in the central nervous system (CNS), but also in the systemic neuroendocrine, autonomic nervous, and immune systems. The changes in these systems have been widely studied in depression by using serum proteins because they are easily and repetitively studied before, during, and after treatment. Recently, gene expressions in the peripheral blood leukocytes have been used to assess the depressive changes in the CNS by DNA microarrays and/or real-time polymerase chain reaction (PCR) methods. These studies will give us clues to assess depression because circulating peripheral leukocytes are influenced by systems that underlie depression, and the quantification of mRNAs in them is methodologically precise and easier than that of protein. In this paper, we review the studies on the leukocyte gene expression, including our own, and discuss the limitations and strengths of the current gene expression-based molecular assessment of depression by the leukocyte mRNA expression.

Key words: *Depression, DNA microarray, leukocytes, mRNA, RT-PCR*

Introduction

Depression affects about 10% of the population at some point in their lives and is the leading cause of disability in nations with developed economies (1). The disease is potentially fatal because 15% of patients with severe depression eventually die by suicide (2). If treated properly, most patients recover from the disease. However, studies have shown that depression, which lacks specific objective findings, is often misdiagnosed or undiagnosed (3). Establishing convenient and reliable biological markers would greatly improve the precise diagnosis and, consequently, the welfare of depressed patients.

While many studies utilize subjective questionnaires for assessing depression, a few objective methods have been introduced. Psychological changes in depression are known to stimulate the hypothalamus-pituitary-adrenal (HPA) axis (neuroendocrine system), autonomic nervous system, and immune system. These systems interact with each other, leading to the complex stress response (4,5). In addition to the HPA axis, the production of cytokines and inflammatory and immune responses

Key messages

- Depression is a disorder not only in the central nervous system (CNS), but also in the systemic neuroendocrine, autonomic nervous, and immune systems.
- Then, the changes of mRNA expressions in the peripheral leukocytes will give us clues to assess the changes in depression.
- Although mRNA expressions in the leukocytes may be useful tools for depression, further studies are needed to clarify the gene expression-based molecular assessment.

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ISSN 0785-3890 print/ISSN 1365-2060 online
DOI: 10.1080/07853890802082088

are changed in the depressive state (6). The measurement of these hormones or cytokines has been used to objectively assess levels of mental conditions. However, their usefulness as biological markers is limited because of the unsatisfactory sensitivity and/or specificity.

One of the emerging approaches for assessing mental conditions is measuring the leukocyte mRNA expressions by using new technologies, such as DNA microarrays and quantitative real-time polymerase chain reaction (RT-PCR) methods (Figure 1). DNA microarray allows us to measure the thousands of mRNA transcripts simultaneously, while RT-PCR allows us to measure the mRNA transcripts of candidate gene promptly and precisely. Both methods are now recognized as useful clinical tools for making diagnostic, therapeutic, or prognostic decisions for patients with physical illness (7–13).

The peripheral blood leukocytes produce various cytokines, as well as proinflammatory cytokines, particularly gp130 family members which directly stimulate the HPA axis (14). At the same time, leukocytes express receptors for stress mediators, such as neurotransmitters, hormones, growth factors, and cytokines (15). Many studies showed similarities between receptor expression and mechanisms of transduction processes of cells in the central nervous system and lymphocytes (for review see (16)). Thus, investigating gene expression in the leukocytes may be a potential tool for evaluating psychological distress and depression. We review recent studies on the leukocyte gene expression to assess depression.

Molecular assessment of psychological distress with microarray

The neuroendocrine response, activated by psychological stress, makes stress into changes in mononuclear cell functions (17) and stimulates the production of tumor necrosis factor (TNF)-alpha, interferon (IFN)-gamma, interleukin (IL)-6, IL-10, and IL-1 receptor antagonists (18). It is reported that the mRNA levels of several genes, including receptors for cytokines and associated molecules, were significantly upregulated in graduate school students in the defense of their Ph.D. degree, with DNA microarrays (19). The altered genes included the IL-1 receptor (*IL1R1* and *IL1R2*), the TNF receptor homologue (*TNFRSF10C*), the TNF-alpha-induced protein (*TNFAIP6*), the IFN-receptor 2 (*IFNGR2*), the IFN-induced cellular resistance mediator protein (*MX2*), the IFN-regulatory factor-2 (*IRF2*), and IFN inducible proteins (*IFITM1* and *IFITM3*). However, in posttraumatic stress disorder (PTSD), it is reported that the production of those metabolic parameters (TNF-alpha, IL-1beta, IL-6, etc.) was not changed but that the mRNA expressions of *TXR1* (thioredoxin reductase 1), *IL-16*, *IL-18*, *SOD1* (superoxide reductase 1), and *EDG1*

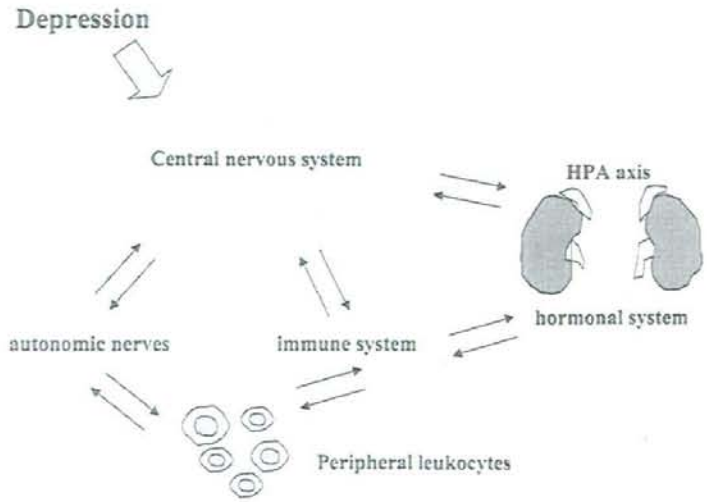

Figure 1. Depression is a mental disorder that affects the central nervous system. However, its dysfunction includes autonomic nerves, immune, and hormonal systems. Gene expressions in the peripheral leukocytes are under influence of these systemic dysfunctions and can be biological markers for depression. HPA = hypothalamus-pituitary-adrenal.

(endothelial differentiation sphingolipid G-protein-coupled receptor 1) were significantly reduced in the peripheral blood leukocytes using DNA microarray (20). It is suggested that reactive oxygen species (ROS) and/or different mechanisms are included in long-term stress reactions (21,22).

Molecular assessment of depression with quantitative real-time PCR

A large number of studies with laboratory examinations have been conducted to establish diagnostic markers for depression. Dexamethasone suppression test (DST) and its modified test, a dexamethasone-corticotrophin releasing hormone (DEX/CRH) test, have been extensively studied to detect hyperactivity of the HPA axis (23). Measurement of neurotransmitter receptors and transporters located in blood cells has also been vigorously studied with the assumption that they may reflect their counterparts in the CNS. For example, decreased serotonin transporter binding has been reported in the platelets of depressed patients (24,25), although some studies have reported no change (26–28). More recently, with the progress of experimental procedures, altered mRNA levels in leukocytes of major depression have been reported, such as dopamine D4 receptor mRNA levels (29) and cyclic adenosine monophosphate (AMP) response element-binding protein 1 (CREB) mRNA levels (30). We briefly summarize mRNA expression studies for depression using RT-PCR methods (Table I). Among them, we focused on serotonin transporter, calcium signaling, and trophic factors.

Table I. Molecular markers reported from peripheral blood leukocyte research.

Genes	Change	Authors (references)
Serotonin transporter	Increase	Iga 2005 (31), Tsao 2006 (32)
	Decrease	Lima 2005 (33)
cAMP response element-binding protein 1	Increase	Iga 2007 (34)
	No change	Lai 2003 (30)
Glucocorticoid receptor	Decrease	Matsubara 2006 (35)
Histone deacetylase 5	Increase	Iga 2007 (34)
Noradrenaline transporter	Decrease	Mata 2005 (36)
Dopamine receptor D4	Decrease	Rocc 2002 (29)
Vascular endothelial growth factor	Increase	Iga 2007 (37)
PDLIM5	Decrease	Iga 2006 (46)
Beta-arrestin1	Decrease	Matuzany-Ruban 2005 (38), Avissar 2006 (39)

cAMP, cyclic AMP or 3′-5′-cyclic adenosine monophosphate; ENH, Enigma homolog

Serotonin transporter

A serotonin transporter (5HTT) is the initial target for many classes of antidepressants, especially selective serotonin reuptake inhibitors (SSRI). 5HTT plays a key role in the regulation of serotonergic neurotransmission (40) and is one of the potential loci affecting the vulnerability to depression (41). The measurement of *5HTT* gene products in the peripheral leukocytes has been vigorously studied on the assumption that they reflect, to some extent, their counterparts in the CNS. We established the procedure for a precise measurement of *5HTT* mRNA levels in the leukocytes and measured the levels in the leukocytes of major depression before and after treatment with antidepressants (31). Baseline *5HTT* mRNA levels (before medication) were significantly higher in depressed patients than those in control subjects, and *5HTT* mRNA levels after 8 weeks of antidepressant treatment decreased significantly. Although some studies reported controversial results, our results have been reconfirmed by another group (32). Further studies will be needed to investigate the mechanism of these changes in depression.

Calcium signaling

LIM (PDLIM5) is a small protein that interacts with the protein kinase C-epsilon and the N-type calcium channel alpha-1B subunit and modulates neuronal calcium signaling (42,43). Recently, Iwamoto et al. reported that *PDLIM5* mRNA expression in postmortem brains and immortalized lymphoblastoid cells from mood disorder patients was different from that of healthy controls and seemed to be involved in the pathophysiology of mood disorder (44,45). Thus, we hypothesized that the *PDLIM5* mRNA level in the peripheral blood leukocytes might be a good candidate as a biological marker for mood disorders (46). The *PDLIM5* mRNA levels in the leukocytes of drug-free depressed patients were significantly lower than those of the controls and increased to almost the same level as the controls after recovery. These results indicate that the expression levels of *PDLIM5* mRNA in leukocytes are associated with the depressive state.

Recently, chromatin remodeling has attracted attention as an important factor in the treatment of depression because modifying histone acetylation alters depression-related behaviors in animal models of depression (47,48). One particularly interesting

target is histone deacetylase 5 (HDAC5) which is decreased by chronic antidepressant treatments (49). HDAC5 is known to be involved in calcium/calcium-dependent protein kinase signaling in the lymphocytes (49). On the other hand, hyperacetylation of histones catalyzed by histone acetyltransferases (HATs) is believed to facilitate gene transcription; this action is opposed by HDACs. CREB, a type of HAT, is one of the most important targets for antidepressants (50) and is related to calcium signaling (51). Because CREB is located downstream of HDAC5 in the lymphocyte calcium signaling (52), we have determined the expression levels of *HDAC5* and *CREB* mRNA in the leukocytes of depressed patients. Both *HDAC5* and *CREB* mRNA levels in the leukocytes of the untreated depressed patients are significantly higher than those of the control subjects and decreased to almost normal levels after antidepressant treatments (34). There is a positive correlation between *HDAC5* and *CREB* mRNA levels. Our results suggest that the alteration of *HDAC5* and *CREB* gene expression may represent the abnormal calcium signaling in major depression.

Trophic factors

A neurotrophic hypothesis of depression has been intensively studied. In particular, brain-derived neurotrophic factor (BDNF) has been demonstrated in the pathogenesis of major depressive disorder (MDD) (for review see (53,54)). Serum BDNF is consistently decreased not only in depressive patients (55,56) but also in other neuropsychiatic patients such as those with eating disorder (57), autism (58), and Huntington's disease (59). However, there is no report on the *BDNF* mRNA levels in the leukocytes of major depression because of its low revels in the leukocytes.

Increased reductive or oxidative stress to the cell or activation of numerous protein kinase pathways are thought to induce growth factor expression, among which the most important is vascular endothelial growth factor (VEGF). Elevated VEGF production in the serum has been detected in myocardial infarction (60,61), diabetic retinopathy (62), hyperlipidemia (63), and hypertension (64). Since VEGF has also been implicated in neuronal survival, neuroprotection, regeneration, growth, differentiation, and axonal outgrowth (65), we hypothesized that the expression of the *VEGF* mRNA in the leukocytes might be a good candidate as a biological marker for major depressive disorder (MDD) (37). The *VEGF* mRNA levels in the leukocytes of untreated depressive patients were significantly higher and decreased after antidepressant treatments. Its reduction is significantly correlated with clinical improvement. Our result may be related to previous reports showing an increased expression level of *VEGF* mRNA in the peripheral monocytes from diabetic patients with coronary artery disease (66). The relationship between major depression and cardiovascular disease is well known (67). Although there was no patient afflicted by cardiovascular disease in our study, the elevated *VEGF* mRNA expression in the leukocytes of depressed patients may reflect systemic oxidative stress, and the risk of cardiovascular events and the reduction of systemic stresses decrease the *VEGF* mRNA expression.

Molecular assessment of depression with microarray

An altered expression of one gene may be a useful biomarker of depression, but it may be more useful to use some of the altered expressions of genes in combination to create a more sensitive and reliable biomarker. For this purpose, DNA microarray or DNA tip seems suitable and intriguing. A preliminary study has been conducted to establish new biological markers for depression by a microarray specifically designed to measure the mRNA levels of stress-related genes in the leukocytes (68). The microarray analyses reveal that the expression of a dozen genes shows significant changes in the total group of depressed patients, compared to the controls (data not shown). These preliminary results reveal sets of gene expressions that could distinguish depressed patients from healthy controls, volunteers after psychological and physical stress, and from those in preliminary samples of patients with schizophrenia. Although mechanisms of alteration remain unclear, neurotransmitter, endocrinological, and immunological abnormalities are thought to have contributed to the alteration of expression to some extent. Some alteration may directly reflect intracellular abnormalities of depression that might be present in the leukocytes.

Limitations and future perspectives

According to the current hypotheses of major depression, most reports were focused on the mRNA expression of neurotransmitter transporter, second messengers, and trophic factors (Table I). Some findings were replicated; however, contradictory results were also reported. Possible reasons for these contradictions may come from the heterogeneous

etiology and pathophysiology of depression. Most studies use Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria for the diagnosis of depression. However, the major depressive disorder described symptomatologically by DSM-IV may include depressive episodes of different pathophysiology. Genetic polymorphisms which affect the gene expression may also contribute to these contradictions. For example, it is known that serotonin transporter has two major allelic variants (5HTTLPR at promoter and 5HTTVNTR at intron 2) which affect gene expression, and there are racial differences in the distribution of these gene polymorphisms (41). In the interpretation of the results of gene expression levels, careful consideration should be given to genetic and racial differences of samples.

With new technologies, such as microarray and RT-PCR, we must pay attention to technical confounding factors. The ability for intra- and inter-laboratory reproduction of results must be determined, and the standardization of methodology must be established. The results may be influenced by tissue acquisition methods as well as sample handling. Particularly, the method for RNA isolation has a significant impact on gene expression profiles obtained from human whole blood or circulating blood leukocytes and needs to be considered as a critical variable in the design of the experiment (69). The relative amount of each gene mRNA should be standardized with at least two housekeeping mRNAs (70).

Another important factor that might affect the result of studies is whether one extracts mRNAs from whole blood cells or from particular cell populations of blood cells. mRNAs from a particular cell population such as lymphocyte may have the advantage to detect more sensitively the systemic dysfunction of depression, but the complicated extraction procedures may change gene expression patterns significantly. With a kit, such as a PAXgene Blood RNA kit (Qiagen), one can extract mRNAs directly from a small amount of whole blood without complicated procedures which might influence gene expressions (71). However, with this method, mRNAs come from total leukocytes that consist of neutrophils, lymphocytes, monocytes, etc., and which cell compartment contributes to the results remains unknown. Differences in the cell populations used for mRNA extraction might contribute to the discrepancy of the results. The gene expression profiles among different cell populations should be studied further.

Studying gene expression in leukocytes is an interesting tool for assessing the role of target genes in stress-related disorders. In clinical research for psychiatric diseases, the peripheral leukocyte is a very useful tissue because of its accessibility. We can compare gene expressions at several points of the clinical course. When gene expressions in the leukocytes are used as a marker for the changes in the CNS, the assumption is that gene expressions in the leukocyte and the CNS are correlated with each other. This assumption may not be always true, since preclinical studies show tissue-specific differences in glucocorticoid receptors among cells and tissues of the immune systems (6). One can examine the expression levels of genes in the leukocytes which have important roles in psychiatric diseases; however, the function of these genes in leukocytes is not yet well known, and the interpretation of changes should be treated with caution. Although molecular assessment of depression with peripheral leukocytes is still in the early stages, it is worthwhile studying further. It is necessary to examine if these markers discriminate major depression from bipolar depression, euthymic from depressed, drug-naive from drug-treated.

Molecular assessment with peripheral leukocytes may lead us to a paradigm shift in the discovery process of the pathophysiology of depression. The initial targets can be discovered from the microarray and real-time PCR analysis of clinical samples. This new approach is intriguing because it will likely lead us to possible and even unexpected targets that are relevant for depression.

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Psychiatry and Clinical Neurosciences 2008; 62: 404–411 doi:10.1111/j.1440-1819.2008.01818.x

Regular Article

Predictors of subjective and objective quality of life in outpatients with schizophrenia

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Aim: In recent years, greater attention has been given to quality of life (QOL) in schizophrenia and several studies reported that negative and depressive symptoms and cognitive dysfunction are related to patient QOL. But because a variety of QOL measures have been used in the previous studies, there seems to be no unanimous predictors for subjective and objective QOL. The purpose of the present study was to elucidate the relationship between clinical variables and subjective and objective QOL in outpatients with schizophrenia, using schizophrenia disease-specific QOL measures. Particular attention was paid to cognitive function as a predictor of QOL.

Methods: Schizophrenia symptoms of the Positive and Negative Syndrome Scale (PANSS) were divided into five factors: positive factor, negative factor, cognitive factor, emotional discomfort, and hostility. The study sample consisted of 84 schizophrenia outpatients. Subjective and objective QOL were assessed with Schizophrenia Quality of Life Scale (SQLS) and the Quality of Life Scale (QLS), respectively.

Results: Subjective QOL correlated significantly with emotional discomfort, positive factor, negative factor, extrapyramidal symptoms and cognitive factor, while objective QOL correlated with negative factor, cognitive factor, emotional discomfort, extrapyramidal symptoms, and dose of antipsychotics. Total score and three of four subscales in the QLS correlated significantly with cognitive factor, while cognitive factor had a significant correlation with only one of three scales of SQLS. Stepwise regression showed that subjective QOL was significantly predicted by emotional discomfort and extrapyramidal symptoms, while negative factor was the most important predictor of objective QOL.

Conclusion: Cognitive dysfunction had a greater influence on objective QOL than subjective QOL. Treating depressive and negative symptoms and extrapyramidal symptoms might contribute to enhanced subjective and objective QOL.

Key words: cognitive dysfunction, depressive and negative symptoms, objective quality of life, schizophrenia, subjective quality of life.

OVER THE PAST two decades, the concept of quality of life (QOL) has become an important attribute in patient care and clinical research.[1,2] Although there seems to be no unanimous definition of QOL, QOL is generally thought to include life satisfaction, social functioning, daily living activities, and physical health, and it has been recognized as an important indicator of how well patients with schizophrenia can function.[2–4] QOL has been measured from two different viewpoints. One is subjective

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Received 9 May 2007; revised 6 March 2008; accepted 1 April 2008.

QOL, rated by patients themselves, and the other is objective QOL, rated by observers. Objective measures of QOL include indicators of health and living conditions, sociodemographic items and role functioning, whereas subjective indicators of QOL measure life satisfaction in general and within different life domains. Because patients with schizophrenia were thought to be unable to assess their QOL themselves because of their cognitive deficit function, objective QOL have been frequently used in many studies and the evaluation of treatments for schizophrenia was mainly based on objective assessment of the psychotic symptoms. But now there is general agreement that symptomatically stabilized patients are able to evaluate their QOL themselves.[5]

The clinical factors related to levels of QOL have been variously reported. Several studies including our own have suggested that depressive mood may be the most important determinant for subjective QOL.[6–11] Other studies reported that positive symptoms[12] or akathisia symptoms as well as the total severity of psychopathology[1] predicted subjective QOL.

In some studies, the severity of negative symptoms[7,10,11,13] or the presence of tardive dyskinesia[14] was reported to be associated with a poor objective QOL. Levels of insight into the illness showed no significant relationship with QOL levels.[15] In addition to clinical symptoms, sociodemographic factors also influence objective QOL of patients with schizophrenia.[16]

In recent years, greater attention has been given to the cognitive dimension in schizophrenia. One of the reasons is that atypical antipsychotics improve cognitive function while conventional antipsychotics produce minimal cognitive improvement.[17,18] Several studies strongly indicate that cognitive function has a greater impact on QOL in patients with schizophrenia than do positive symptoms.[19–21] Executive functioning and verbal learning appear to be especially valid predictors of work status.[19,22–24] Other studies reported that unemployed patients with schizophrenia were impaired on measures of memory and problem solving, even when IQ was within an average range.[25,26]

The purpose of the present study was to further elucidate clinical determinants of both subjective and objective QOL. In the present study, subjective and objective QOL were assessed on the Schizophrenia Quality of Life Scale (SQLS)[27,28] and the Quality of Life Scale (QLS),[29,30] respectively. Schizophrenia symptoms were assessed with the Positive and Negative Syndrome Scale (PANSS).[31] Evidence from recent factor analysis studies conducted with PANSS has suggested that a five-dimensional structure appears to be a better representation of the psychopathological data. Analysis for the present study is based on five orthogonal dimensions according to Bell *et al.*:[32] positive factor, negative factor, cognitive factor, emotional discomfort, and hostility. Contribution of these five symptom factors as well as duration of illness, number of hospitalizations, dose of antipsychotics and extrapyramidal symptoms to the levels of QOL was investigated. Few studies have investigated the relationship between subjective and objective QOL and the five PANSS factors. Particular interest was paid to the PANSS cognitive factor as a predictor of subjective or objective QOL.

METHODS

Subjects

Clinical data were collected at Department of Psychiatry, Tokushima University Hospital from 18 May to 5 August 2005. After obtaining written consent from all subjects, we investigated a sample of 105 outpatients whose diagnosis was confirmed by at least two psychiatrists according to the DSM-IV.[33]

Subjects were excluded if they presented with any organic central nervous system disorder, epilepsy, mental retardation, severe somatic disorder, drug dependence, or alcohol dependence. Of 105 patients, 84 completed the questionnaire.

The present subjects were all clinically stable and received outpatient treatment regularly. Seventy-two had never been hospitalized during the previous 1 year including 28 who had never had inpatient treatment, while 12 had inpatient treatment during the previous 1 year. The antipsychotic regimen of 75 subjects had been unchanged for at least 6 months before the recruitment, and only nine subjects had a little change in regimen during the previous 6 months, but the nine were judged as clinically stabilized by the treating psychiatrists.

Procedure

To assess subjective QOL, we used the SQLS.[27,28] Objective QOL was evaluated using the QLS.[29,30] Psychotic symptoms were evaluated using the PANSS.[31] Drug-induced extrapyramidal symptoms were

assessed using the Drug-Induced Extrapyramidal Symptoms Scale (DIEPSS).[34]

The SQLS is a self-reported, 30-item questionnaire for measuring QOL specific to patients with schizophrenia with good reliability and validity.[27,28] It is composed of three scales: psychosocial, motivation/energy and symptoms/side-effects. Lower scores indicate higher levels of subjective QOL.

The QLS assesses objective QOL by means of a semistructured interview. The reliability and validity of the scale has been verified.[29,30] The ratings are based upon patient self-report and observer judgment about patient functioning and life circumstances. This instrument has four subscales: interpersonal relations, instrumental role, intrapsychic foundation, and common objects and activities. Higher scores indicate higher levels of objective QOL. Some of the authors, who are all experienced psychiatrists, conducted the interviews according to the Evaluation Manual for the QLS.[30]

The PANSS was originally designed as a rating scale that represents positive, negative and general psychopathology.[31] The score ranges are 30–210 for the global score; 7–49 for the positive score; 7–49 for the negative score; and 16–112 for the general psychopathology score. A breakdown into five factors was used according to Bell *et al.*:[32] positive factor (score range 6–42; items: delusions, hallucinations, grandiosity, suspiciousness, somatic concern, unusual thought content); negative factor (score range 8–56; items: blunted affect, emotional withdrawal, poor rapport, passive social withdrawal, lack of spontaneity, motor retardation, disturbance of volition, preoccupation); cognitive factor (score range 7–49; items: difficulty in abstract thinking, stereotyped thinking, conceptual disorganization, lack of judgment and insight, poor attention, tension, mannerisms and posturing); emotional discomfort factor (score range 4–28; items: depression, anxiety, guilt feeling, active social avoidance); and hostility factor (score range 4–28; items: excitement, hostility, poor impulse control, uncooperativeness).

The DIEPSS is composed of eight individual parameters (gait, bradykinesia, sialorrhea, muscle rigidity, tremor, akathisia, dystonia, and dyskinesia) and one global assessment constructed to assess extrapyramidal adverse effects, using a 5-point scale that ranges from 0 to 4 (0, none; 4, severe). The reliability and validity of the scale have been verified.[34]

All the scales except the SQLS were applied by the authors, who were experienced psychiatrists. Inter-rater consistencies of all the scales in our group have been shown to be satisfactory.[35]

Statistical analysis

Pearson's correlation coefficients were calculated to study the relationship between subjective and objective QOL and clinical variables (duration of illness, number of hospitalizations, dose of antipsychotics, PANSS positive symptoms score, PANSS negative symptoms score, PANSS cognitive score, PANSS emotional discomfort score, PANSS hostility score and the DIEPSS score). Because data were normal continuous variables except for the PANSS positive symptoms score, duration of illness and dose of antipsychotics, and because the sample size was large, we used parametric test. Then, using clinical variables that showed significant correlation, stepwise regression was done to determine which clinical variables were the best predictors for each dependent variable. The SQLS score and the QLS total score and subscales were chosen as dependent variables. Statistical analysis was done using SPSS version 11.5J (SPSS, Chicago, IL, USA).

RESULTS

Demographic characteristics and means and standard deviations of the clinical indices are presented in Table 1. All subjects were Japanese, and 42 were male and 42 were female. We used the chlorpromazine conversion chart[36] to determine the dosage of antipsychotic medication.

The correlations between the SQLS scores and clinical variables are shown in Table 2. Only positive factor and emotional discomfort were correlated significantly with the score of psychosocial scale. The score of the motivation and energy scale correlated significantly with positive factor, negative factor and emotional discomfort. Positive factor, cognitive factor, and extrapyramidal symptoms were correlated significantly with the score of the symptoms and side-effects scale.

The correlations between the scores of the QLS total and subscales and clinical variables are shown in Table 3. Negative factor was correlated with total and all subscales. Total score and three subscales of four correlated with cognitive factor.

Table 4 shows the results of stepwise regression on the SQLS and the QLS.

Table 1. Subject characteristics (mean ± SD)

n (M/F)		84 (42/42)
Age (years)		40.7 ± 12.6
Duration of illness (years)		14.6 ± 10.4
No. hospitalizations		1.4 ± 1.6
Dose of antipsychotics (mg/day)†		534 ± 542
Type of schizophrenia (*n*)	Paranoid	65
	Residual	13
	Disorganized	1
	Catatonic	4
	Undifferentiated	1
Marital state (*n*)	Married	19
	Never married	63
	Divorced	2
PANSS	Total	61.7 ± 11.7
	Positive factor	12.1 ± 4.9
	Negative factor	21.1 ± 6.9
	Cognitive factor	14.4 ± 5.5
	Emotional discomfort	5.6 ± 2.1
	Hostility	8.3 ± 2.5
DIEPSS (Overall)		1.3 ± 2.3
SQLS	Psychosocial	24.3 ± 10.7
	Motivation/energy	14.3 ± 4.6
	Symptoms/side-effects	7.9 ± 4.7
QLS	Total	65.4 ± 22.9
	Interpersonal Relations	21.2 ± 10.6
	Instrumental Role	13.2 ± 5.0
	Intrapsychic Foundations	23.6 ± 8.0
	Common Objects and Activities	7.3 ± 2.0

†Chlorpromazine equivalent.
DIEPSS, Drug-Induced Extrapyramidal Symptoms Scale; PANSS, Positive and Negative Syndrome Scale; QLS, Quality of Life Scale; SQLS, Schizophrenia Quality of Life Scale.

The psychosocial scale score and the motivation/energy scale score were significantly predicted only by emotional discomfort. The symptoms/side-effects scale score was significantly predicted only by extrapyramidal symptoms.

The QLS total score was predicted independently by negative factor and dose of antipsychotics. Four subscales were predicted independently by negative factor.

DISCUSSION

In recent years, greater attention has been given to QOL in schizophrenia and several symptoms have been reported to be related to patient QOL. But in the < previous studies, because a variety of QOL measures have been used, there seems to be no unanimous predictors for subjective and objective QOL. The purpose of the present study was to elucidate the relationship between clinical variables and subjective and objective QOL, using PANSS five-factor analysis and schizophrenia disease-specific QOL measures. Several recent studies strongly indicate that cognitive function has a greater impact on QOL in patients with schizophrenia than do positive symptoms.[19–21] Therefore we paid particular attention to the PANSS cognitive factor and explored the relationship between cognitive dysfunction and patient QOL.

The clinical factors related to levels of QOL have been variously reported. For the clinical factors associated with subjective QOL, Dickerson *et al.* found that patients' subjective QOL measured by the Quality of Life interview was related to the depression factor in PANSS.[6] Huppert *et al.* reported that more severe depression as rated on the brief Psychiatric Rating Scale (BPRS) was associated with lower subjective QOL measured by the Quality of Life interview.[8] Fitzgerald *et al.* reported that subjectively reported life satisfaction was more influenced by depressive symptom on the Montgomery–Asberg Depression Rating Scale (MADRS) than positive symptom or negative symptom.[7] Other similar studies including our own also support the association of depressive symptom with subjective QOL.[9–11] In the present study, emotional discomfort and positive factor were correlated with psychosocial scale scores of SQLS, and stepwise regression showed that emotional discomfort predicted the psychosocial score. Emotional discomfort, negative factor and positive factor were correlated with motivation/energy scores of SQLS, and stepwise regression showed that emotional discomfort predicted the motivation/energy scale score. The present results are consistent with those reported by Tomotake *et al.* and Aki *et al.*, who assessed subjective QOL and depressive symptoms with SQLS and the Calgary Depression Scale for Schizophrenia, respectively.[10,11]

In the current study, drug-induced extrapyramidal symptoms, cognitive factor, and positive factor were correlated with symptoms/side-effects scale scores of SQLS, and stepwise regression showed that drug-induced extrapyramidal symptoms predicted the symptoms/side-effects scale score. The present result suggests that the extrapyramidal symptom is a factor negatively influencing subjective QOL. The influence

Table 2. SQLS scores and clinical variables

	SQLS		
	Psychosocial	Motivation/energy	Symptoms/side-effects
PANSS			
Positive factor	0.274*	0.227*	0.260*
Negative factor	0.184	0.293**	0.056
Cognitive factor	0.19	0.176	0.266*
Emotional discomfort	0.449***	0.408***	0.195
Hostility	0.152	0.141	0.120
DIEPSS	0.204	0.179	0.279*
Duration of illness	0.170	−0.111	0.140
Number of hospitalization	0.149	−0.016	0.151
Dose of antipsychotics	0.210	0.011	0.047

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, Pearson correlations.
DIEPSS, Drug-Induced Extrapyramidal Symptoms Scale; PANSS, Positive and Negative Syndrome Scale; SQLS, Schizophrenia Quality of Life Scale.

of extrapyramidal adverse effects has already been documented. Ritsner *et al.*, using the MADRS, the Talbieh Brief Distress Inventory (TBDI), the Abnormal Involuntary Movement Scale (AIMS) and the Quality of Life Enjoyment and Satisfaction Questionnaire in schizophrenia patients, reported that the depression score on the TBDI and the score at the AIMS were predictors of poor QOL.[37] Awad *et al.* reported that, using PANSS, Hillside Akathisia scale and the Drug Attitude Inventory, subjective QOL is greatly influenced by psychopathology, akathisia and patients' subjective tolerance of medications, and concluded that effort should be directed towards effective control of psychotic symptoms and minimizing the side-effects of antipsychotic drugs in order to improve the QOL of schizophrenia patients.[1] These two studies, however, used subjective QOL measures, which, unlike SQLS, did not have a subscale focused on symptoms/side-effects. The current study suggests that patients with drug-induced extrapyramidal symptoms have subjective discomfort with respect to their symptoms and side-effects.

Table 3. QLS total and subscale scores and clinical variables

	QLS				
	Total	Interpersonal relations	Instrumental role	Intrapsychic foundation	Common objects and activities
PANSS					
Positive factor	−0.098	−0.059	−0.143	−0.110	−0.002
Negative factor	−0.535***	−0.487***	−0.340**	−0.584***	−0.337**
Cognitive factor	−0.303**	−0.228*	−0.267*	−0.350**	−0.177
Emotional discomfort	−0.272*	−0.263*	−0.208	−0.269*	−0.121
Hostility	−0.022	−0.039	−0.022	−0.025	0.108
DIEPSS	−0.252*	−0.241*	−0.206	−0.207	−0.248*
Duration of illness	−0.043	−0.053	0.137	−0.128	−0.038
Number of hospitalization	0.003	−0.046	0.013	0.023	0.149
Dose of antipsychotics	−0.272*	−0.258*	−0.253*	−0.222*	−0.210

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, Pearson correlations.
DIEPSS, Drug-Induced Extrapyramidal Symptoms Scale; PANSS, Positive and Negative Syndrome Scale; QLS, Quality of Life Scale.

Table 4. Stepwise regression for SQLS and QLS

	Dependent variable	Independent variable	Adjusted R^2	β
SQLS	Psychosocial	Emotional discomfort	0.191***	0.449***
	Motivation/energy	Emotional discomfort	0.156***	0.408***
	Symptoms/side-effects	DIEPSS	0.067**	0.279**
QLS	Total	Negative factor	0.334***	−0.504***
		Dose of antipsychotics		−0.190*
	Interpersonal Relations	Negative factor	0.228***	−0.487***
	Instrumental Role	Negative factor	0.105**	−0.340**
	Intrapsychic Foundations	Negative factor	0.333***	−0.584***
	Common Objects and Activities	Negative factor	0.180***	−0.337**

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
DIEPSS, Drug-Induced Extrapyramidal Symptoms Scale; QLS, Quality of Life Scale; SQLS, Schizophrenia Quality of Life Scale.

The present study suggests that negative symptoms predict objective QOL (QLS total and all the subscales). The present results are consistent with those reported by Tomotake *et al.* and Aki *et al.*, who assessed objective QOL and negative symptoms using QLS and BPRS, respectively.[10,11] Considering that QLS was originally designed to assess deficit symptoms and the dysfunctions related to them,[29] the correlation between negative symptoms and QLS scores seems to be reasonable.

The influence of negative symptoms on objective QOL has already been documented. Fitzgerald *et al.*, using QLS, PANSS and MADRAS, indicated a significant positive relationship between all of the four QLS subscales and PANSS negative scores, but none of the QLS subscales was related significantly to PANSS positive scores and MADRAS scores.[7] Norman *et al.*, using QLS, Scale for the Assessment of Positive Symptoms and Scale for the Assessment of Negative Symptoms (SANS), reported that negative symptom, level of functioning and positive symptom related to the scores on QLS and that QLS was most strongly related to negative symptom.[12] Browne *et al.* also investigated the relationship between objective QOL assessed with QLS and clinical variables, and reported that total QLS score correlated significantly with negative symptom rated with SANS.[14]

Greater attention has been given to the cognitive dimension in schizophrenia in recent years. Several studies strongly indicate that cognitive function has a greater impact on QOL in patients with schizophrenia than do positive symptoms.[19–21] Bell *et al.* found that higher scores on PANSS cognitive component were significantly correlated with poorer performance on neuropsychological tests.[32,38] Hofer *et al.* reported that, using the cognition subscale of the PANSS, poorer cognition scores reduced the competitive employment and that PANSS cognition subscale as well as negative symptom and positive symptom were found to contribute significantly to the Global Assessment of Functioning score.[39] Consistent with these previous studies, the QLS total score and all the subscale scores of QLS except common objects and activities subscale correlated significantly with PANSS cognitive score in the present study. Although stepwise regression showed that negative factor alone significantly predicted objective QOL, cognitive function also had an apparent influence on it. But Hofer *et al.* found that clinical assessment of cognitive deficits on PANSS is not a viable alternative to neuropsychological testing to obtain information about cognitive functioning in schizophrenia.[40] Their finding limits the interpretation of the present results. To elucidate influence of the cognitive dysfunction on QOL, further studies using neuropsychological tests such as Brief Assessment of Cognition in Schizophrenia[41] are necessary.

In contrast, in the present study, cognitive dysfunction did not predict subjective QOL, although the symptoms/side-effects scale score of SQLS was significantly correlated with cognitive dysfunction. Consistent with the present result, Reine *et al.* reported that only one of eight subscales in the short version