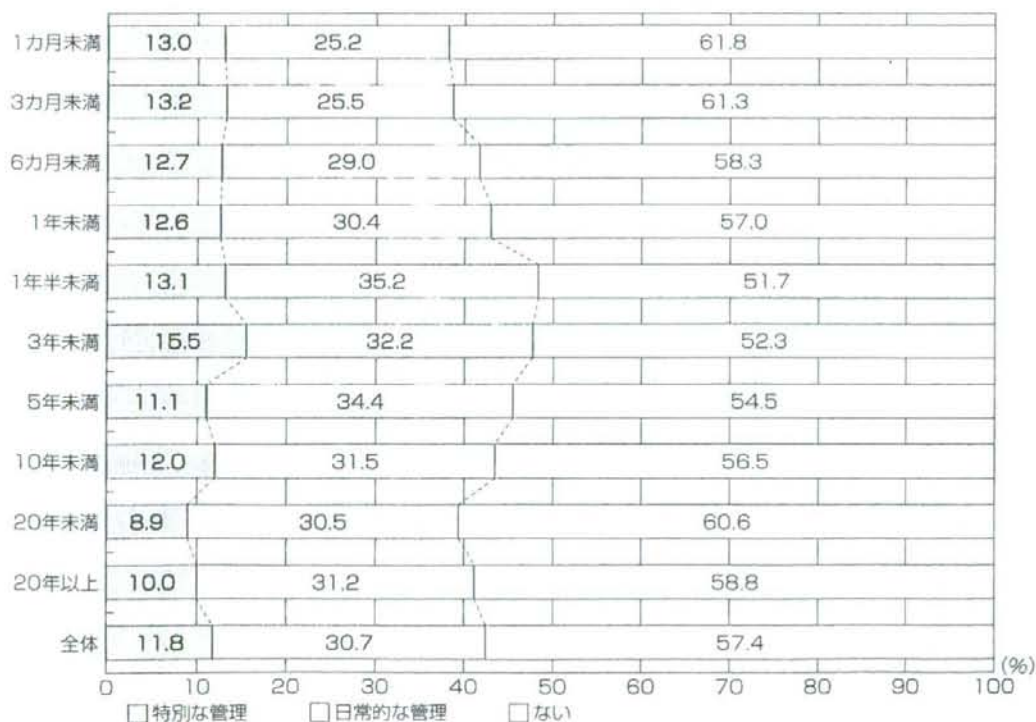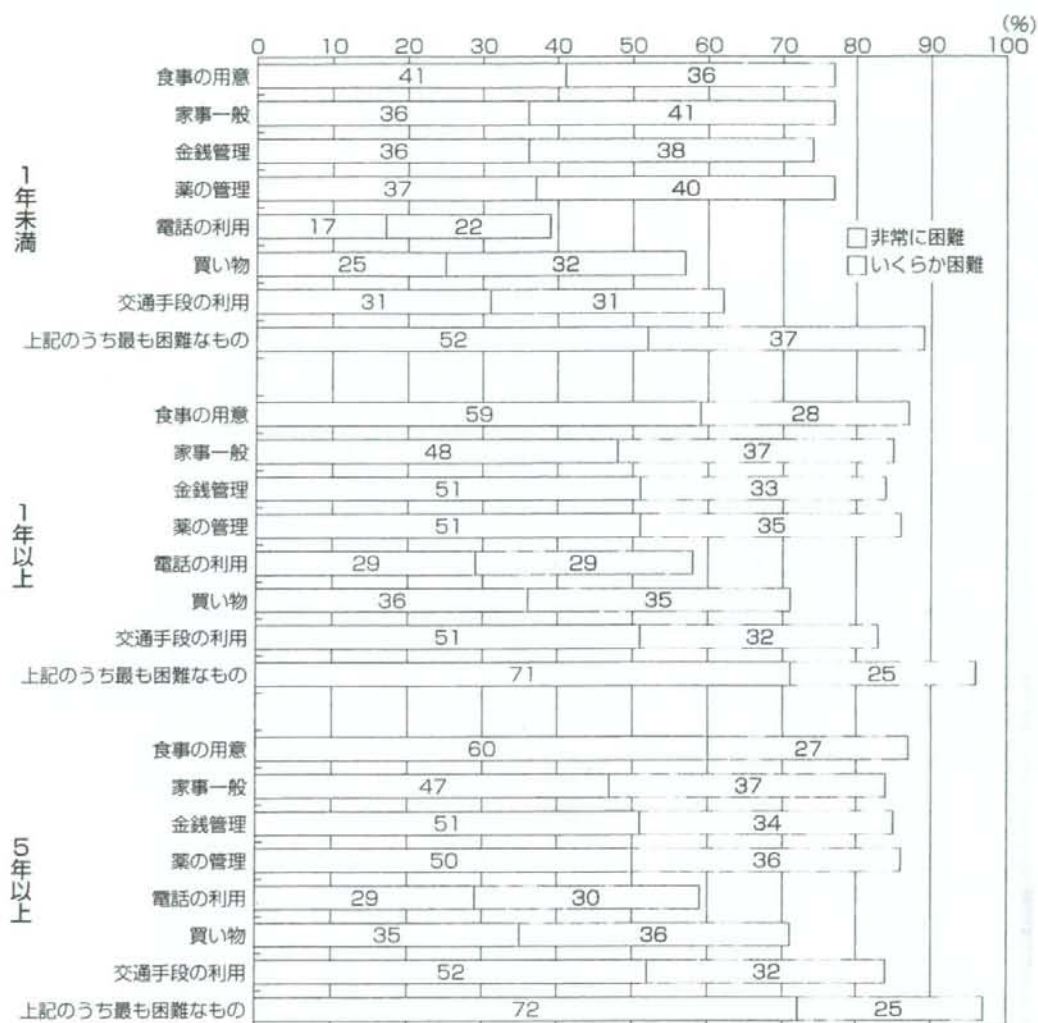図 7　入院期間別 ADL の支援のレベル（F20 のみ抽出）

図 8　身体合併症×入院期間（F0 を除く）

図9　入院期間別 IADL の困難度（F20 のみ抽出）

らかにする必要がある。その1つとして ADL の
結果を代表的な統合失調症（F20）に限定して検
索を行った。この結果，図7に示すように，
ADL の障害の程度は1年未満と1年以上群との
間には大きな差はなかった。最も支援を必要とす
る項目だけを抽出しても，それは25％を超える
ことはなく，いずれの項目（食事・排泄等）も20
％を超えることはほとんどなかった。さらに，
ADL に大きな影響を与える身体合併症の併発状

況をみると，特別な管理を要するものは15％以
下にとどまり，また，入院期間に大きく左右され
ることはなかった（F0を除いたデータ）（図8）。
　IADL について統合失調症（F20）のみについ
て分析すると（図9），1年以上入院群と5年以
上入院群とでは障害の程度に大きな相違はないが，
1年未満群と1年以上群を比較すると，1年以上
群は明らかに IADL については低下していること
がわかる。その内容は，食事の用意，家事一般

表 3

(A) 入院の状況（患者調査と同じ）

	全　体		F0		F20	
	患者数	割　合	患者数	割　合	患者数	割　合
生命の危険は少ないが入院治療を要する	10,822	62.6	1,901	55.9	6,712	67.1
生命の危険がある	500	2.9	173	5.1	228	2.3
受け入れ条件が整えば退院可能	5,810	33.6	1,294	38.1	2,989	29.9
検査入院	10	0.1	3	0.1	3	0.0
その他	146	0.8	27	0.8	66	0.7
計	17,288	100.0	3,398	100.0	9,996	100.0

(B) 入院の状況×支援が整った場合の退院の可能性（割合）

問 27 ＼ 問 13	生命の危険は少ないが入院治療を要する	生命の危険がある	受け入れ条件が整えば退院可能	検査入院	その他	計
現在の状態でも，居住先・支援が整えば退院可能	3.4	0.1	5.2	0.0	0.2	9.0
状態の改善が見込まれるので，居住先・支援などを新たに用意しなくても近い将来退院可能	3.5	0.1	2.0	0.0	0.1	5.8
状態の改善が見込まれるので，居住先・支援が整えば近い将来退院可能	20.3	0.5	24.6	0.0	0.1	45.6
状態の改善は見込まれず，居住先・支援を整えても近い将来退院の可能性なし	35.3	2.1	1.8	0.0	0.4	39.5
計	62.5	2.9	33.7	0.1	0.9	100.0
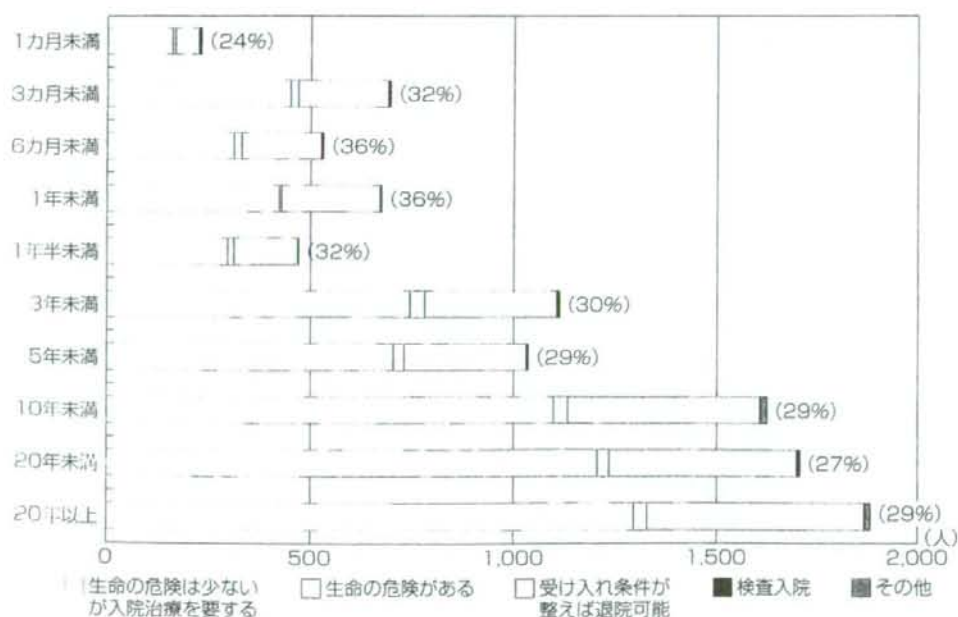
図 10　入院の状況×入院期間（F20）

図 11　退院の可能性×入院期間（F0 を除く）

入院期間	現在の状態でも居住先・支援が整えば可能	条件を整えなくても近い将来可能	条件が整えば近い将来可能	近い将来退院の可能性はない
1カ月未満	30.3	28.5	33.8	7.4
3カ月未満	27.3	27.3	35.5	9.9
6カ月未満	20.4	15.7	46.6	17.3
1年未満	11.9	8.9	53.7	25.5
1年半未満	6.4	4.0	51.9	37.7
3年未満	4.8	2.1	51.1	42.0
5年未満	3.6	1.0	47.9	47.5
10年未満	5.1	0.7	46.1	48.1
20年未満	3.3	0.5	41.0	55.2
20年以上	4.1	0.4	39.9	55.6
全体	9.4	6.5	44.4	39.7
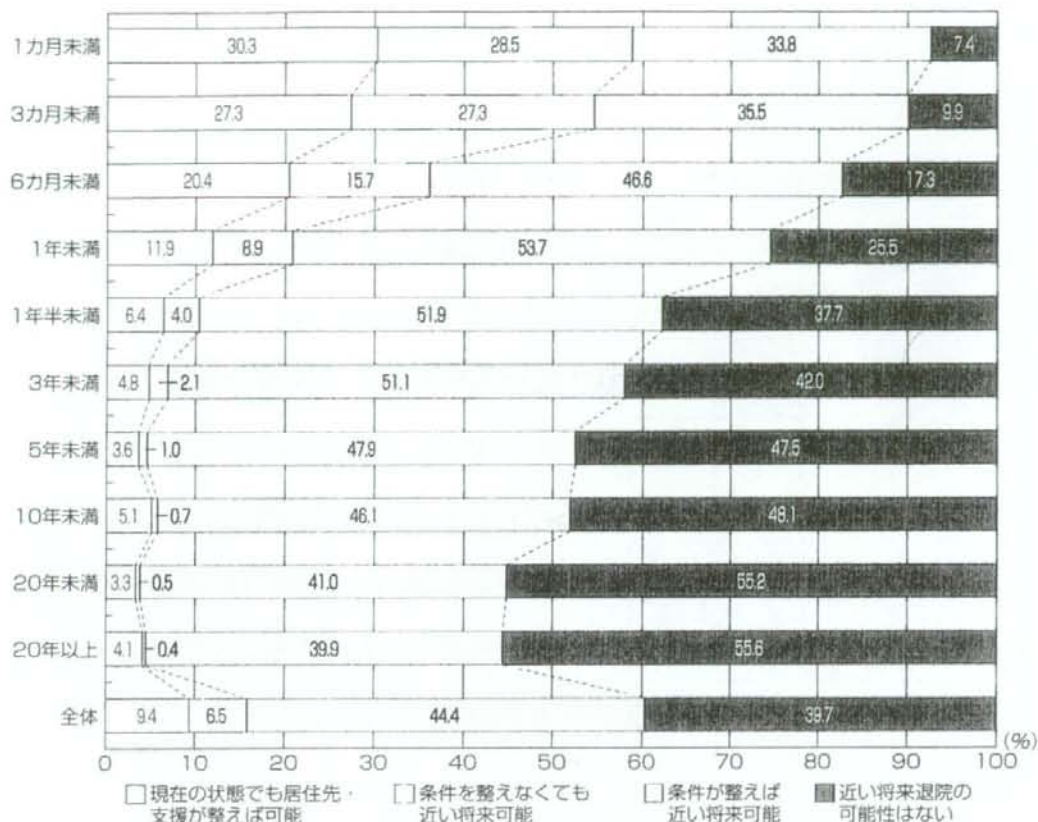
（保清），金銭管理，薬の管理などでは，「いくらか困難」が 75％ ～ 80％ と高率を示しており，「非常に困難」はほぼ50％に達している。最も困難な項目のみを抽出した場合には，90％以上においてIADLは「いくらか困難」であり，「非常に困難」な状態は70％に達している。このことは，2軸評価・GAFの分析で示された1年以上の長期入院患者における退院困難性の大部分は，IADLの低下が大きく影響しているためであると言うことができる。

3．「受け入れ条件が整えば退院可能」な患者の分析

1）患者数とその割合

　表3では，まず，（A）では，従来の患者調査と同様に「受け入れ条件が整えば退院可能」とされる患者数とその割合を示した。全体で 33.6％，認知症（F0）では 38.1％，統合失調症（F20）では 29.9％ である。この数値は，21.6％（7万2千人）とされた平成11年調査に比較して明らかに高い数値を示している。このことでは，認知症患者の増加が影響していると思われるが，それだけではなく，回答を行った主治医そのものの意識の変化も影響していることも否定できない。

　さらに，（B）では「患者調査の項目」と「居住施設・支援が整ったと仮定した場合の退院の可能性」とのクロス集計を行ったものである。これをみると「受け入れ条件が整えば退院可能」とされた患者のうち，「現在の状態でも，居住先・支援が整えば退院可能」となっているのはわずかに
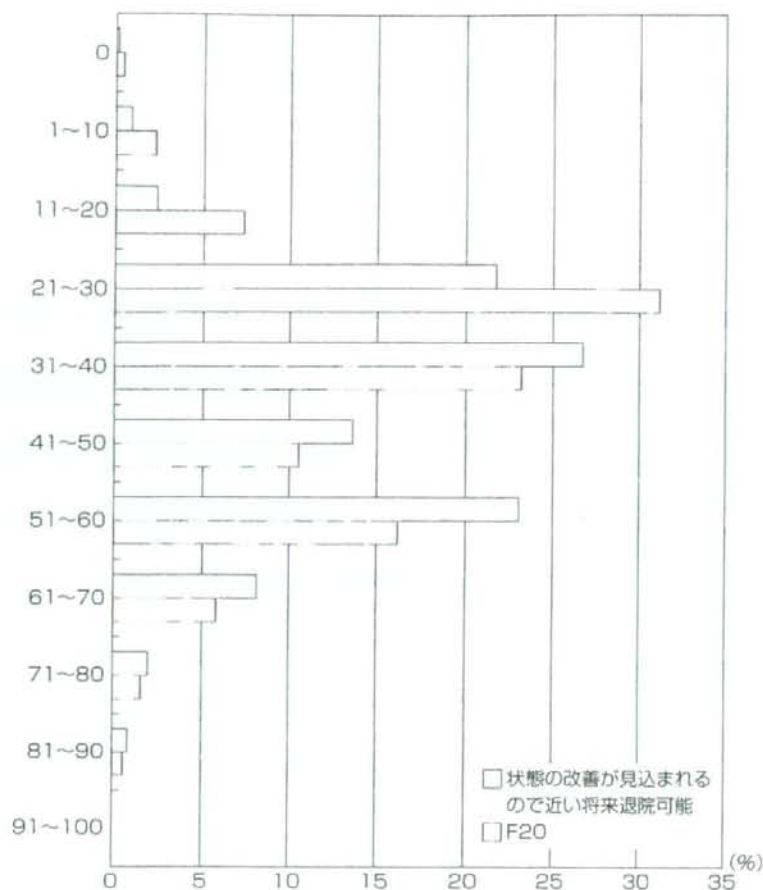
図 12　GAF の分布（F020 全体と近い将来退院可能群との比較）

5.2%（受け入れ条件が整えば退院可能とされた
患者の 15.5%）にすぎない。26.6%（受け入れ条
件が整えば退院可能とされた患者の 72.0%）につ
いては，「状態の改善が見込まれるので」と状態
改善を希望的に予測している例が多いことがわか
る。換言すれば，「受け入れ条件が整えば退院が
可能のようにみえる患者」と言ってもよい群では
ないか。

2）患者数と入院期間

　前述のように全体の 33.6%が「受け入れ条件が
整えば退院可能」とされた。このうち，1 年未満
群では 36.5%で 1 年以上では 32.5%で，入院期間

表 4

		全　体	F0 を除く全体	退院可能なF20 群
1	群	10.3%	12.0%	14.5%
2	群	7.8%	8.9%	19.7%
3	群	13.1%	14.3%	25.0%
4	群	3.5%	2.8%	2.7%
5	群	16.9%	19.2%	20.1%
6	群	48.3%	42.8%	23.1%

が長期になるほど次第に減少する傾向がある。と
くに統合失調症（F20）だけを抽出すると 29.9%
であるが，1 年未満群では 32%であり，1 年以
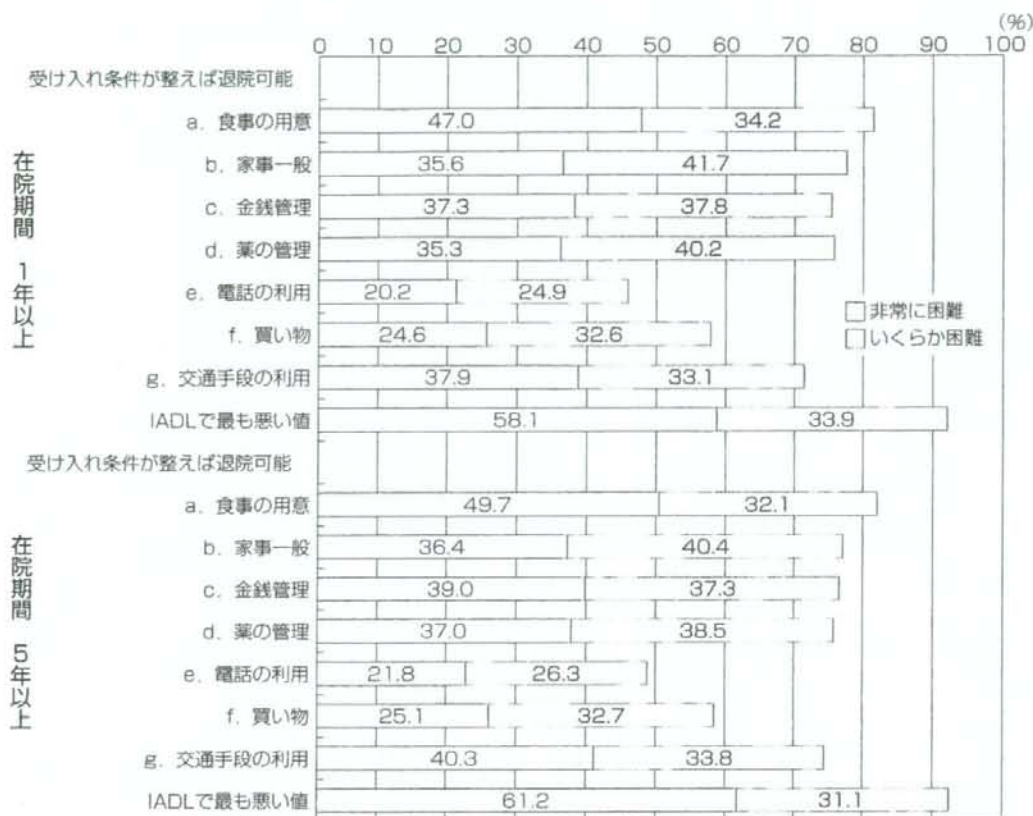上では 29.3%とわずかに低い。図 10 では，入院
期間ごとに「受け入れ条件が整えば退院可能」の

図13 入院期間別 IADL の困難度（F0 を除く退院可能な者）

占める割合を示しているが，入院期間が長期になるに従って減少はするものの，その差は大きなものではない。

3）退院可能性と入院期間

図11は，「条件が整えば近い将来退院可能」とされた人たちの入院期間による変化を示したものである。前述したように「受け入れ条件が整えば退院可能」の73.0％において近い将来退院可能とされたが，この割合は図に示すように，入院6カ月後から急速に増加している。同時に，「近い将来退院の可能性がない」とされた人たちは，入院1年の時点では37.7％であるが，10年未満では48.1％，さらに10年以上で55％を超えている。いずれにせよ，長期入院患者（1年以上）であっ

ても，「受け入れ条件が整えば退院可能」群は著しく増加することはないが，その中身をみると，「条件が整えば近い将来退院可能」の割合が増加し，「現在の状態でも退院可能」は5％以下にまで減少している。

4）精神症状と生活障害の程度

図12では，GAFスコアをF20全体の分布と「近い将来退院可能群」との比較をしたものである。グラフでみる限り両者の間には大きな相違は認められない。表4では，2軸評価表から，各群の分布を調査対象ごとに比較したものである。「近い将来退院可能なF20群」では，他の集団に比較して，2群と3群が明らかに増加し，6群が著しく減少していることが示されている。GAF

では，退院可能性の中身は明らかではないが，「退院可能群」では精神症状が比較的安定していることが示唆されているが，能力障害の面では著しい6群のものを除けば，大きな差異がないことがわかる。すなわち，精神症状は一定程度改善はしているが，能力障害（生活障害）の改善はいまだ不十分なままであることが示されている。図13で示されているとおり，IADLを中心とする生活障害は図9に比較して一定の改善は認められるが，いまだ生活障害は高度に残遺しており，手厚い生活援助が必要な状態であることは明らかである。

今後の検討

新たな調査では，「受け入れ条件が整えば退院可能」，さらに，「近い将来退院可能」とされた人たちについて，より詳しい分析が行われた。そのなかでは，これらの人たちが一見退院可能であるかにみえるが，比較的重度の生活障害を持っており，このために，「退院可能」にみえて，実際には退院に移行することはたやすくないことが示された。退院当初には夜間を含めて援助が可能な「居住施設」と「手厚い生活支援」が必要であることが示された。

これを実現するために何が必要であるか，あるいは，利用者自身が何を必要としているかなど，さらに，詳細な分析が必要である。紙面の関係から，さらなる分析は他の機会に報告したい。

文　献

1）大島　巌，他：長期入院精神障害者の退院可能性と，退院に必要な社会資源およびその数の推計－全国の精神科医療施設4万床を対象とした調査から－．精神経誌　93（7）：582 - 602，1991.
2）高橋正和：在院患者調査中間報告－精神科医療マスタープランのための入院患者調査報告－．日精協誌　12（10）：971 - 1015，1993.
3）黒田研二，他：長期入院患者の退院可能性とリハビリテーションニーズに関する調査：調査結果の速報．精神経誌　101（9）：762 - 776，1999.
4）日本精神科病院協会：平成14年マスタープラン調査データ集．2003.
5）日本精神科病院協会：精神障害者社会復帰サービスニーズ等調査事業報告書．2003.
6）厚生労働省科学研究費補助金こころの健康科学研究事業：精神医療の質的実態把握と最適化に関する総合研究．2008.

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Negative Correlation between Brain Glutathione Level and Negative Symptoms in Schizophrenia: A 3T 1H-MRS Study

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Abstract

Background: Glutathione (GSH), a major intracellular antioxidant, plays a role in NMDA receptor-mediated neurotransmission, which is involved in the pathophysiology of schizophrenia. In the present study, we aimed to investigate whether GSH levels are altered in the posterior medial frontal cortex of schizophrenic patients. Furthermore, we examined correlations between GSH levels and clinical variables in patients.

Methods and Findings: Twenty schizophrenia patients and 16 age- and gender-matched normal controls were enrolled to examine the levels of GSH in the posterior medial frontal cortex by using 3T SIGNA EXCITE 1H-MRS with the spectral editing technique, MEGA-PRESS. Clinical variables of patients were assessed by the Global Assessment of Functioning (GAF), Scale for the Assessment of Negative Symptoms (SANS), Brief Psychiatric Rating Scale (BPRS), Drug-Induced Extra-Pyramidal Symptoms Scale (DIEPSS), and five cognitive performance tests (Word Fluency Test, Stroop Test, Trail Making Test, Wisconsin Card Sorting Test and Digit Span Distractibility Test). Levels of GSH in the posterior medial frontal cortex of schizophrenic patients were not different from those of normal controls. However, we found a significant negative correlation between GSH levels and the severity of negative symptoms (SANS total score and negative symptom subscore on BPRS) in patients. There were no correlations between brain GSH levels and scores on any cognitive performance test except Trail Making Test part A.

Conclusion: These results suggest that GSH levels in the posterior medial frontal cortex may be related to negative symptoms in schizophrenic patients. Therefore, agents that increase GSH levels in the brain could be potential therapeutic drugs for negative symptoms in schizophrenia.

Introduction

Accumulating evidence suggests that oxidative stress associated with impaired metabolism of the antioxidant glutathione (GSH) plays a key role in the pathogenesis of schizophrenia [1,2]. First, activity of glutathione peroxidase (GSH-Px), a key antioxidant enzyme, was found to be decreased in red blood cells [3,4] and plasma [5] of some, but not all schizophrenic patients [6,7]. Furthermore, plasma GSH-Px levels were significantly and positively correlated with psychosis rating scores in schizophrenic patients [8]. Second, it has been reported that the activity of glutamate cysteine ligase (GCL), the rate-limiting enzyme for GSH synthesis, as well as expression of the catalytic GCL subunit (GCLL) protein in cultured skin fibroblasts from schizophrenic patients were significantly decreased compared to those in

comparison subjects, and that decreased GCL activity was correlated with decreased GCLL protein expression [9]. Third, Do et al. [10] reported that levels of GSH in the cerebrospinal fluid of drug-free patients of schizophrenia were significantly decreased compared to those in normal comparisons. Furthermore, a study using postmortem brain samples demonstrated decreased levels of GSH, oxidized GSH (GSSG), GSH-Px, and GSH reductase in the caudate region of brains from schizophrenic patients, suggesting impaired GSH metabolism in schizophrenic brains [11]. Moreover, a 1.5T 1H-magnetic resonance spectroscopy (MRS) study with double quantum coherence technique demonstrated significant reduction (52 %) in GSH levels in the medial frontal cortex of schizophrenic patients compared to comparisons [10]. However, Terpstra et al. [12] reported that levels of GSH in the anterior cingulated cortex, measured by 4T

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^{1}H-MRS with MEGA-PRESS (MEscher-GArwood-Point RE-Solved Spectroscopy) sequence, did not differ in schizophrenic patients and comparisons. Fourth, several genes involved in GSH metabolism have been shown as potential candidate genes for schizophrenia. Association of the glutathione-S-transferase (GST) M1 gene was shown in schizophrenic subgroups in Japanese [13] and Korean populations [14]. Recently, Tosic et al. [15] reported that the levels of mRNA for GCLM and glutathione synthetase, which are responsible for GSH synthesis, were significantly decreased in the fibroblasts of schizophrenic patients in a Swiss population. Subsequently, they reported the GCLM gene as a susceptibility gene for schizophrenia in Swiss and Danish populations [9,15]. Taken together, these findings provide genetic and functional evidence that an impaired capacity to synthesize GSH under conditions of oxidative stress is a vulnerability factor for schizophrenia.

GSH plays a major role in the modulation of redox-sensitive sites on the N-methyl-D-aspartate (NMDA) receptors [16–18], which are implicated in the pathophysiology of schizophrenia [19–23]. Considering the NMDA receptor hypofunction hypothesis for schizophrenia [19–23], it is of great interest to study whether levels of GSH are altered in the brains of schizophrenic patients. In the present study, we aimed to investigate whether GSH levels are altered in the posterior medial frontal cortex of schizophrenic patients. Furthermore, we examined the correlations between GSH levels and clinical features including the severity of clinical symptoms (positive symptoms, negative symptoms and cognitive deficits). In addition, we performed genetic analysis for the genes involved in GSH metabolism: namely, GCLM, glutathione peroxidase 1 (GPX1), and several classes (GSTM1, GSTO1, GSTP1, GSTT1 and GSTT2) of glutathione-S-transferase (GST).

Materials and Methods

Subjects

This research was performed under approval of the ethics committee of Chiba University Graduate School of Medicine and National Institute of Radiological Science. The experiments were thoroughly explained to the subjects, and written informed consent was obtained from all. Twenty schizophrenic patients and 16 age- and gender- matched normal controls with no past history of psychotic disorders or drug dependence were enrolled in the study. Characteristics of subjects are shown in **Table 1**. Due to a few highly educated comparisons, the extent of education and estimated IQ were significantly different between the two groups, but the estimated IQ of all patients was within the normal range. All patients were outpatients meeting the DSM-IV criteria for schizophrenia [24] and having no other psychiatric disorders. All patients were taking second-generation neuroleptics: i.e., risperidone (2–12 mg/day, n = 9), olanzapine (5–20 mg/day, n = 5), aripiprazole (6–12 mg/day, n = 4), quetiapine (500 mg/day, n = 1) or perospirone (48 mg/day, n = 1), with no change in their medication for the past month. Of the patients, twelve were diagnosed as residual type and eight were as paranoid type.

^{1}H-MRS measurement and data analysis

All data were acquired using the 3T SIGNA EXCITE (GE) with a standard quadrature coil. GSH spectra were acquired by the MEGA-PRESS sequence [25]. A GSH peak at chemical shift 2.95 ppm originating from cysteinyl β-CH_2 was observed by editing pulse at 4.95 ppm α-CH resonance line J-coupled to the observed spins. Acquisition parameters for the measurement were as follows: echo time (TE) = 94 ms, repetition time (TR) = 1500 ms, number of

Table 1. Characteristics and clinical variables of subjects enrolled in this study

Variable	Controls (n = 16)	Schizophrenia (n = 20)	P values
Sex, Male/Female	12/4	12/8	0.481 [a]
Age (year)	30.0±7.2 (21–41)	30.7±5.8 (20–39)	0.581 [b]
Education (year)	15.2±2.9 (12–21)	13.5±1.7 (12–16)	0.04 [b]
Estimated IQ[*1]	107.4±17.3 (90–128)	98.6±10.9 (80–114)	0.03 [b]
age at onset of illness (year)		23.6±5.5 (11–31)	
Duration of illness (year)		7.30±5.2 (1–21)	
GAF scale		51.5±11.5 (29–71)	
Amount of medication[*2]		283.1±216(80–667)	
BPRS score		26.2±8.6 (13–43)	
BPRS positive score		12.2±5.7 (4–24)	
BPRS negative score		6.1±2.9 (2–12)	
SANS score		76.9±12.9 (60–103)	
DIEPSS score		0.41±0.15 (0.11–0.78)	
GSH (mM)	0.928±0.24 (0.608–1.465)	0.808±0.26 (0.432–1.250)	0.166 [b]

All values are shown as mean±SD (range).
[a]: Chi-squire test.
[b]: Student t-test.
[*1]: Short form version of Wechsler Adult Intelligence Scale, Revised (WAIS-R)
[*2]: Chlorpromazine equivalent (mg)
GAF: Global Assessment of Functioning, BPRS: Brief Psychiatric Rating Scale, SANS: Scale for the Assessment of Negative Symptoms, DIEPSS: Drug Induced Extra-Pyramidal Symptoms Scale
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excitations (NEX) = 512, band width 2.5 kHz, data point 4096. TE and TR were set experimentally as optimum for our system after confirming the GSH signal changes to be within a certain range (TE: 62–101 ms, TR: 1077 ms–12000 ms) with both phantom solutions and human subjects. The short TR enabled us to increase NEX and obtain a satisfying signal/noise (S/N) ratio in the human brains. For the quantification of GSH, we prepared eight phantom solutions containing different concentration of GSH (0.3–30.0 mM) with N-acetyl aspartate (NAA, 10 mM) and creatine (8 mM) to get the reference spectra. During the phantom data acquisition, the solutions were kept at 37±0.6°C.

For the acquisition of human spectra, an 18.6-ml (28×30×22 mm) volume of interest (VOI) was placed on the posterior medial frontal cortex under the guidance of T_2-weighed images (**Figure 1A**). The posterior medial frontal cortex was selected since reduction in the GSH levels in this region of schizophrenic patients has been reported previously [10]. To minimize variation in the positioning of the head, subjects were positioned by the same investigator. The overall examination time was 1 hour or less.

For all data acquisition, high-order shim followed by automatic local shim adjustment was used and repeated until the half linewidth was accomplished under 3 Hz (phantom) or 8 Hz (human). The raw data of both phantom solutions and human subjects were processed on GE analysis software (GE Medical Systems, Milwaukee, WI). Fourier transform was done with an exponential weighting function of 2 Hz. The area of the GSH signal was measured on Image J (http://rsb.info.nih.gov/ij/) software.
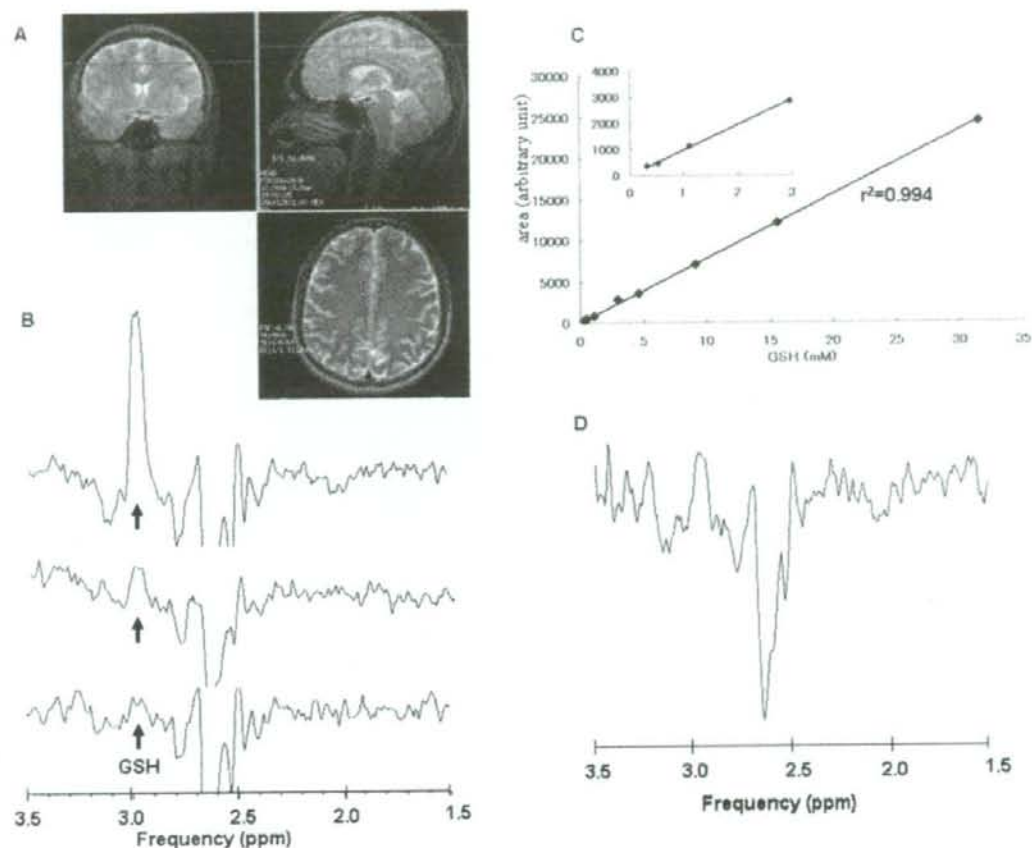
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Figure 1. Proton MRS of GSH. (A): T2-weighed magnetic resonance imaging of the targeted region. The blue boxes show the voxel size (28 × 22 × 30 mm) in the posterior medial frontal cortex of a human brain. (B): representative data of reference phantom spectra of GSH (0.5, 1.0, 3.0 mM). Note that the GSH signal increases according to the phantom concentration. (C): Quantification of GSH. Plots showing a linear correlation (r2 = 0.994) between the GSH signal area at 2.95 ppm and the concentration of GSH. (D): Representative data of GSH signals of the posterior medial frontal cortex of a human subject. The GSH level was calculated as 0.735 mM by applying the linear concentration curve on (C).
doi:10.1371/journal.pone.0001944.g001

Evaluation of clinical variables

The Scale for the Assessment of Negative Symptoms (SANS) and Brief Psychiatry Rating Scale (BPRS) were used to evaluate the severity of negative symptoms and psychotic symptoms (positive and negative symptoms), respectively. The Drug-Induced Extrapyramidal Symptoms Scale (DIEPSS) was used to evaluate and exclude the effects of drug-induced extrapyramidal symptoms which could affect the severity of symptoms in schizophrenic patients. Functional disability was assessed using the Global Assessment of Functioning (GAF) scale.

Cognitive function tests

Several cognitive function tests were used. In the Word Fluency Task (letter, category), subjects were given an initial letter (letter fluency task) or a certain category (category fluency task) as a cue [26]. Both tasks consisted of three trials, and the number of words produced in one minute for each trial was recorded for evaluation.

In the Stroop Test, a list of twenty four colored dots (D), a baseline test, and 24 colored words incongruent with the color (C) were used. The difference between the reaction time (C-D) was assessed [27]. In the Wisconsin Card Sorting Test (WCST), subjects were instructed to sort cards according to a rule (color, shape, or number). The numbers of achieved categories and perseverative errors were assessed [28]. In the Trail-Making Test part (TMT) A, subjects drew lines as quickly as possible to connect 25 consecutively numbered circles. In the TMT part B, subjects connected 25 consecutively numbered and lettered circles by alternating between the two sequences. The time taken to complete each part of the test was recorded in seconds [29]. In the Digit Span Distractibility Test (DSDT), subjects were asked to remember a tape-recorded string of digits read by a female voice while ignoring the digits read by a male voice (distracter) [30].The percentages of digits correctly recalled in conditions with and without distracters were assessed separately.

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Genotyping

Genetic analysis for the genes involved in GSH metabolism-GCLM, glutathione peroxidase 1 (GPX1), and several classes of glutathione-S-transferase (GSTM1, GSTO1, GSTP1, GSTT1 and GSTT2)-as performed by the methods described previously [15,31–33].

Statistical analysis

All calculations were performed with SPSS software (SPSS version 12.0J, Tokyo, Japan). Student's t-test (unpaired) was employed for the comparison of GSH levels between schizophrenic patients and normal control subjects and of the scores of the cognitive function tests between the two groups. For the genotyping results, the differences between patients and controls were evaluated by Fisher's exact test. Pearson's correlation coefficients were examined to identify any correlations of GSH levels with the clinical severity (BPRS, SANS, and DIEPSS) of schizophrenic patients and with the scores of cognitive function tests of all subjects. A value of p<0.05 was used as the standard for statistical significance in all analyses.

Results

GSH concentration between schizophrenic patients and healthy comparisons

We used eight phantom solutions of different GSH concentrations (0.3–30 mM) to acquire reference spectra for quantification. As shown in **Figure 1B**, acquired GSH phantom spectra clearly increased their areas at chemical shift 2.95 ppm in a concentration-dependent manner. In **Figure 1C**, plots show a linear correlation ($r^2 = 0.994$) between the GSH signal area and the GSH concentration. The areas of GSH spectra acquired from human subjects *in vivo* were applied to the linear concentration curve for
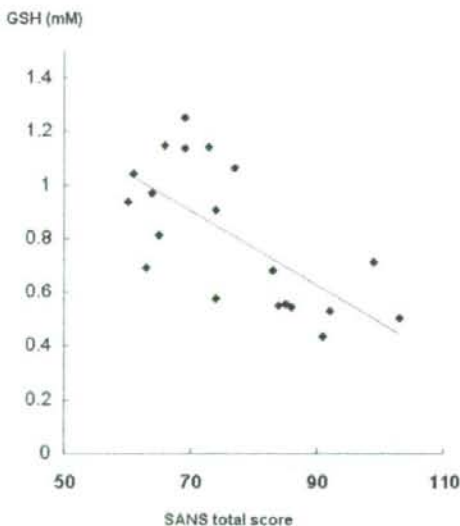
GSH (mM)

Figure 2. Correlation between GSH levels and the severity of negative symptoms in schizophrenia. There was a significant negative correlation ($r = -068$, p<0.001) between GSH levels and SANS total scores of schizophrenic patients (n = 20).
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quantification (**Figure 1D**). As shown in **Table 1**, GSH concentration (0.808 ± 0.26 mM (mean $\pm$ SD)) in the posterior medial frontal cortex of schizophrenic patients (n = 20) did not differ (t = 1.416, df = 34, p = 0.166) from that (0.928 ± 0.24 mM (mean $\pm$ SD)) of age- and gender-matched normal healthy controls (n = 16)(**Table 1**). Furthermore, there were no correlations between GSH levels and clinical variables (age, education, estimated IQ, age at onset of illness, duration of illness, GAF, and amount of medication) in the subjects.

Correlation between GSH concentration and clinical variables

We examined the correlation between GSH level and the severity of clinical symptoms (scores of SANS, BPRS and DIEPSS) in the schizophrenic patients (n = 20). Interestingly, there was a significant negative correlation ($r = -0.68$, p<0.001) between GSH level and SANS total score in schizophrenic patients (**Figure 2**). Of five subscale-symptom groups in SANS, significant negative correlations with GSH level were detected in four subscales (S1: affective flattering-blunting ($r = -0.57$, p = 0.009), S2: alogia ($r = -0.67$, p = 0.001), S3: avolition-apathy ($r = -0.52$, p = 0.02), S4: anhedonia-asociality ($r = -0.62$, p = 0.004)), but not in attention impairment ($r = -0.27$, p = 0.252). Furthermore, we also found a significant correlation ($r = -0.60$, p = 0.005) between GSH levels and the negative symptom subscore on BPRS. However, there were no significant correlations between GSH level and BPRS total score ($r = -0.41$, p = 0.076), BPRS positive symptom score ($r = -0.43$, p = 0.059) and DIEPSS score ($r = -0.32$, p = 0.167). Because these correlations might have been affected by medication, we controlled for the doses of antipsychotics using partial correlation coefficients. Even when the administered antipsychotics (chlorpromazine equivalents) were adjusted for using partial correlation coefficients, the relationships between GSH level and SANS score (partial correlation coefficient = -0.60, p = 0.007) or BPRS negative score (partial correlation coefficient = -0.52, p = 0.02) remained significant.

Correlation between GSH concentration and cognitive functions

As shown in **Table 2**, significant differences were observed between schizophrenia patients and normal controls in all cognitive function tests: Word Fluency (letters: t = 4.67, df = 34, p<0.001; category: t = 3.57, df = 34, p<0.01), Stroop Task (t = -3.47, df = 34, p<0.01), WCST (category: t = 3.95, df = 34, p<0.001; perseverative error : t = -4.61, df = 34, p<0.001), Trail Making Test (TMT-A: t = -3.21, df = 34, p<0.001; TMT-B: t = -3.43, df = 34, p<0.01; TMT-B-A; t = -2.17, df = 34, p = 0.03), and DSDT (without distracter: t = 1.35, df = 34, p = 0.18; with distracter; t = 3.23, df = 34, p<0.01).

Then, we examined the correlations between GSH levels and the scores of cognitive function tests. We found a significant negative correlation ($r = -0.36$, p = 0.03) between GSH level and TMT-A scores in all subjects (n = 36)(**Table 2**). There were no correlations between GSH levels and the scores of other cognitive function tests (**Table 2**).

Correlations between GSH concentration and the genotypes of enzymes related with GSH metabolism

There was a significantly (p = 0.017) different genotype distribution for the GSTT2 gene between schizophrenic patients and healthy controls. No different distribution was observed in other genes (**Table S1**). Then, we investigated whether or not these genotypes affected GSH levels in the posterior medial frontal

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Table 2. Performance on cognitive function tests and their correlations with GSH level

Cognitive function test	mean scores ± SD		Coefficients with GSH level (r)*[a]
	Control subjects (n = 16)	Schizophrenia (n = 20)	
Word Fluency (letter)	41.3 ± 8.8	28.4 ± 7.8**	0.15
Word Fluency (category)	48.9 ± 8.4	39.8 ± 6.9**	0.21
Stroop test (C-D, sec)	5.8 ± 3.9	12.1 ± 6.4**	−0.05
WCST (category)	5.1 ± 1.9	2.7 ± 1.8**	0.01
WCST (perserverative error)	2.1 ± 2.5	11.5 ± 8.4***	−0.23
TMT-A (sec)	21.8 ± 6.7	32.0 ± 8.4**	−0.36*
TMT-B (sec)	48.4 ± 18.2	80.5 ± 32.1**	−0.14
TMT B-A (sec)	26.6 ± 13.5	53.6 ± 51.3*	0.06
DSDT (without distractor)	87.9 ± 12.8	80.3 ± 20.8	0.18
DSDT (with distractor)	93.0 ± 7.2	74.4 ± 22.1**	0.31

*$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$ (vs.Control)

[a]Pearson's coefficients between GSH level in all subjects (n = 36). *$P < 0.05$

WCST : Wisconsin Card Sorting Test, TMT : Trail Making Test, DSDT : Digit Span Distractibility Test

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cortex. There were no significant differences in GSH levels relevant to those genotypes. However, we found a difference in GSH levels between patients (n = 15) and normal controls (n = 5) in subjects with the G/G genotype of the GSTT2 (Met139Ile) gene, although the difference only showed a trend toward statistical significance (p = 0.058) (**Figure S1**). We also found a difference in GSH levels between patients (n = 13) and normal controls (n = 11) in subjects with the C/C genotype of the GCLM (ss60297536) gene; again, the differences only showed a trend toward statistical significance (p = 0.099) (**Figure S1**).

Discussion

The major finding of this study was that GSH levels in the posterior medial frontal cortex of schizophrenic patients were significantly correlated with the severity of their negative symptoms. To the best of our knowledge, this is the first report demonstrating the negative correlation between brain levels of GSH and the severity of negative symptoms in schizophrenia.

The measurement of brain GSH levels by ^{1}H-MRS has been elusive up until now because GSH exists at a relatively low concentration and the cysteinyl β-CH$_2$ signal of GSH at 2.95ppm overlaps with other resonances such as those of aspartate, γ-aminobutylic acid (GABA), and especially creatine, with its high concentration in human brain. The MEGA-PRESS sequence is able to highlight the GSH signal by adding two editing pulses with a normal PRESS sequence. Sufficient GSH signal was obtained by setting an optimum condition with repeated preliminary measurements using both phantom solutions and human subjects, and the shorter TR than in previous studies [12,25,34] enabled us to increase the number of scans within the short examination time.

In this study, we found no alteration in GSH concentrations in the brains of schizophrenic patients, which was consistent with a previous report using the MEGA-PRESS sequence [12], but not a previous report using a double quantum coherence filter technique [10]. The reasons underlying this discrepancy are currently

unclear. One possibility may be due to the difference of technique (MEGA-PRESS sequence vs. a double quantum coherence filter) for GSH measurement. Another possibility may be due to medication. The patients enrolled in the study of Do et al. [10] were first-episode patients whereas those in the present study and that of Terpstra et al. [12] were medicated. However, in this study, we found no effect of medication on GSH levels in schizophrenic patients. Therefore, it is unlikely that medication contributes to this discrepancy, although further study is necessary.

The present finding suggests that increasing the brain levels of GSH should be considered a potential therapeutic approach for negative symptoms in schizophrenia. It is well known that oral administration of GSH does not result in its effective increase in the brain because of its poor penetration through the blood-brain barrier, indicating that GSH is not a suitable agent for treating neuropsychiatric diseases such as schizophrenia. The antioxidant N-acetyl-L-cysteine (NAC) has been widely used as a donor of cysteine, the limiting precursor in the synthesis of GSH, and NAC has a good penetration through the blood-brain barrier. Recently, Lavoie et al. [35] reported that treatment of schizophrenic patients with NAC significantly improved impaired mismatch negativity, which is an auditory evoked potential component related to NMDA receptor function [36]. Furthermore, a multi-center double-blinded trial of NAC showed improvement of negative symptoms on the Positive and Negative Symptoms Scale after 6 months of treatment with NAC ([36], Berk et al, unpublished work). Interestingly, it has been reported that GSH-deficient mice showed enhanced dopamine neurotransmission, altered serotonin function, and augmented locomotor responses to low doses of the NMDA receptor antagonist phencyclidine, suggesting that the GSH deficiency produced alterations in monoaminergic function and behavior in mice relevant to schizophrenia [37]. Moreover, we reported that NAC could attenuate behavioral changes and neurotoxicity in rodents and non-human primates after repeated administration of the psychostimulant methamphetamine [38,39]. Taken together, the findings suggest that NAC has potential as a therapeutic drug for negative symptoms in schizophrenia.

In this study, we found a weak negative correlation between GSH levels in the posterior medial frontal cortex and TMT-A scores. There was also a positive correlation (r = 0.47, p = 0.024) between TMT-A scores and SANS total scores in schizophrenic patients. The posterior medial frontal cortex can be divided functionally into two parts: an upper half including Brodman areas 8 and 9 and a lower half including part of the anterior cingulate cortex, Brodman areas 24 and 32 [40]. Both parts are shown to play a role in self monitoring and control of action demanded in the context of social cognitive processes [40]. The relation between GSH level and cognitive symptoms might be assessed in more detail by setting smaller and more specific VOI in the brain, although it is currently difficult to get sufficient GSH signal with smaller VOI. Nonetheless, it seems that GSH levels in the posterior medial frontal cortex may be associated with cognitive impairment as well as negative symptoms in schizophrenia. Therefore, GSH levels in the posterior medial frontal cortex may be a predictive biological factor for the severity of cognitive impairment and negative symptoms in schizophrenia.

In this study, GSH levels were not affected by the genotypes of several genes related to GSH metabolism. The genotype distribution of GSTT2 was significantly (p = 0.017) different between patients (n = 20) and normal controls (n = 16), but this was considered to be a type I error due to the small sample size, as our study using a larger sample size (over 200 of both groups) revealed no significant difference (Matsuzawa et al, submitted). Interestingly, we found that brain GSH levels in patients with the C/C genotype of the GCLM

(ss60297536) gene were lower than those of controls with the C/C genotype of the GCLM (ss60297536) gene although the differences only showed a trend toward statistical significance (p = 0.099). Further study using a large sample will be necessary to study the relationship between GCLM gene polymorphism and GSH levels in schizophrenic patients.

In conclusion, the present study suggests a negative correlation between GSH levels in the posterior medial frontal cortex and the severity of negative symptoms in schizophrenia. Therefore, agents (e.g., NAC) that can increase brain GSH levels should be considered potential therapeutic drugs for negative symptoms in schizophrenia.

Supporting Information

Figure S1 GSH levels and the relevance with polymorphisms of GCLM and GSTT2 gene. The plots show GSH levels of controls

References

1. Mahadik SP, Mukherjee S (1996) Free radical pathology and antioxidant defense in schizophrenia: A review. Schizophr Res 19: 1–17.
2. Yao JK, Reddy RD, van Kammen DP (2001) Oxidative damage and schizophrenia: An overview of the evidence and its therapeutic implications. CNS Drugs 15: 287–310.
3. Abdalla DS, Monteiro HP, Oliveira JA, Bechara EJ (1986) Activities of superoxide dismutase and glutathione peroxidase in schizophrenic and manic-depressive patients. Clin Chem 32: 805–807.
4. Ben Othmen L, Mechri A, Fendri C, Bost M, Chazot G, et al. (2008) Altered antioxidant defense system in clinically stable patients with schizophrenia and their unaffected siblings. Progress in Neuro-Psychopharmacology & Biological Psychiatry 32: 155–159.
5. Zhang XY, Tan YL, Zhou DF, Cao LY, Wu GY, et al. (2007) Disrupted antioxidant enzyme activity and elevated lipid peroxidation products in schizophrenic patients with tardive dyskinesia. J Clin Psychiatry 68: 754–760.
6. Reddy R, Sahebarao MP, Mukherjee S, Murthy JN (1991) Enzymes of the antioxidant defense system in chronic schizophrenic patients. Biol Psychiatry 30: 409–412.
7. Akyol O, Herken H, Uz E, Fadillioglu E, Unal S, et al. (2002) The indices of endogenous oxidative and antioxidative processes in plasma from schizophrenic patients. The possible role of oxidant/antioxidant imbalance. Prog Neuropsychopharmacol Biol Psychiatry 26: 995–1005.
8. Yao JK, Reddy RD, van Kammen DP (1999) Human plasma glutathione peroxidase and symptom severity in schizophrenia. Biol Psychiatry 45: 1512–1515.
9. Gysin R, Kraftsik R, Sandell J, Bovet P, Chappuis C, et al. (2007) Impaired glutathione synthesis in schizophrenia: Convergent genetic and functional evidence. Proc Natl Acad Sci USA 104: 16621–16626.
10. Do KQ, Trabesinger AH, Kirsten-Kruger M, Lauer CJ, Dydak U, et al. (2000) Schizophrenia: Glutathione deficit in cerebrospinal fluid and prefrontal cortex in vivo. Eur J Neurosci 12: 3721–3728.
11. Yao JK, Leonard S, Reddy R (2006) Altered glutathione redox state in schizophrenia. Dis Markers 22: 83–93.
12. Terpstra M, Vaughan TJ, Ugurbil K, Lim KO, Schulz SC, et al. (2005) Validation of glutathione quantitation from STEAM spectra against edited 1H NMR spectroscopy at 4T: Application to schizophrenia. MAGMA 18: 276–282.
13. Harada S, Tachikawa H, Kawanishi Y (2001) Glutathione S-transferase M1 gene deletion may be associated with susceptibility to certain forms of schizophrenia. Biochem Biophys Res Commun 281: 267–271.
14. Pae CU, Yu HS, Kim JJ, Kim W, Lee CU, et al. (2004) Glutathione S-transferase M1 polymorphism may contribute to schizophrenia in the korean population. Psychiatr Genet 14: 147–150.
15. Tosic M, Ott J, Barral S, Bovet P, Deppen P, et al. (2006) Schizophrenia and oxidative stress: Glutamate cysteine ligase modifier as a susceptibility gene. Am J Hum Genet 79: 586–592.
16. Sucher NJ, Lipton SA (1991) Redox modulatory site of the NMDA receptor-channel complex: Regulation by oxidized glutathione. J Neurosci Res 30: 582–591.
17. Kohr G, Eckardt S, Luddens H, Monyer H, Seeburg PH (1994) NMDA receptor channels: Subunit-specific potentiation by reducing agents. Neuron 12: 1031–1040.
18. Varga V, Jenei Z, Janaky R, Saransaari P, Oja SS (1997) Glutathione is an endogenous ligand of rat brain N-methyl-D-aspartate (NMDA) and 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors. Neurochem Res 22: 1165–1171.
19. Javitt DC, Zukin SR (1991) Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatry 148: 1301–1308.
20. Tamminga CA (1998) Schizophrenia and glutamatergic transmission. Crit Rev Neurobiol 12: 21–36.
21. Goff DC, Coyle JT (2001) The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. Am J Psychiatry 158: 1367–1377.
22. Hashimoto K, Fukushima T, Shimizu E, Komatsu N, Watanabe H, et al. (2003) Decreased serum levels of D-serine in patients with schizophrenia: Evidence in support of the N-methyl-D-aspartate receptor hypofunction hypothesis of schizophrenia. Arch Gen Psychiatry 60: 572–576.
23. Hashimoto K, Okamura N, Shimizu E, Iyo M (2003) Glutamate hypothesis of schizophrenia and approach for possible therapeutic drugs. Curr Med Chem-CNS Agents 60: 572–576.
24. American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders (4th Ed). Washington, D.C.: American Psychiatric Press.
25. Terpstra M, Henry PG, Gruetter R (2003) Measurement of reduced glutathione (GSH) in human brain using LCModel analysis of difference-edited spectra. Magn Reson Med 50: 19–23.
26. Sumiyoshi C, Sumiyoshi T, Nohara S, Yamashita I, Matsui M, et al. (2005) Disorganization of semantic memory underlies alogia in schizophrenia: An analysis of verbal fluency performance in japanese subjects. Schizophr Res 74: 91–100.
27. Carter CS, Mintun M, Cohen JD (1995) Interference and facilitation effects during selective attention: An H215O PET study of stroop task performance. Neuroimage 2: 264–272.
28. Shad MU, Tamminga CA, Cullum M, Haas GL, Keshavan MS (2006) Insight and frontal cortical function in schizophrenia: A review. Schizophr Res 86: 54–70.
29. Reitan R, Wolfson D (1985) The halstead-reitan neuropsychological test battery. Tuscon: Neuropsychology Press.
30. Wilder-Willis KE, Sax KW, Rosenberg HL, Fleck DE, Shear PK, et al. (2001) Persistent attentional dysfunction in remitted bipolar disorder. Bipolar Disord 3: 58–62.
31. Matsuzawa D, Hashimoto K, Shimizu E, Fujisaki M, Iyo M (2005) Functional polymorphism of the glutathione peroxidase 1 gene is associated with personality traits in healthy subjects. Neuropsychobiology 52: 68–70.
32. Hashimoto T, Hashimoto K, Matsuzawa D, Shimizu E, Sekine Y, et al. (2005) A functional glutathione S-transferase P1 gene polymorphism is associated with methamphetamine-induced psychosis in japanese population. Am J Med Genet B Neuropsychiatr Genet 135: 5–9.
33. Hashimoto T, Hashimoto K, Miyatake R, Matsuzawa D, Sekine Y, et al. (2008) Association study between polymorphisms in glutathione-related genes and methamphetamine use disorder in a japanese population. Am J Med Genet B Neuropsychiatr Genet in press.
34. Satoh T, Yoshioka Y (2006) Contribution of reduced and oxidized glutathione to signals detected by magnetic resonance spectroscopy as indicators of local brain redox state. Neurosci Res 55: 34–39.
35. Lavoie S, Murray MM, Deppen P, Knyazeva MG, Berk M, et al. (2007) Glutathione precursor, N-acetyl-cysteine, improves mismatch negativity in schizophrenia patients. Neuropsychopharmacology in press.
36. Javitt DC, Steinschneider M, Schroeder CE, Arezzo JC (1996) Role of cortical N-methyl-D-aspartate receptors in auditory sensory memory and mismatch negativity generation: Implications for schizophrenia. Proc Natl Acad Sci USA 93: 11962–11967.
37. Jacobsen JP, Rodriguiz RM, Mork A, Wetsel WC (2005) Monoaminergic dysregulation in glutathione-deficient mice: Possible relevance to schizophrenia? Neuroscience 132: 1055–1072.
38. Fukami G, Hashimoto K, Koike K, Okamura N, Shimizu E, et al. (2004) Effect of antioxidant N-acetyl-L-cysteine on behavioral changes and neurotoxicity in rats after administration of methamphetamine. Brain Res 1016: 90–95.
39. Hashimoto K, Tsukada H, Nishiyama S, Fukumoto D, Kakiuchi T, et al. (2004) Protective effects of N-acetyl-L-cysteine on the reduction of dopamine transporters in the striatum of monkeys treated with methamphetamine. Neuropsychopharmacology 29: 2018–2023.
40. Amodio DM, Frith CD (2006) Meeting of minds: The medial frontal cortex and social cognition. Nat Rev Neurosci 7: 268–277.

and patients with each genotype of GCLM-588 (left) and GSTT2 (right). The bars represent mean GSH level ± standard deviation (mM).
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Table S1
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Author Contributions

Conceived and designed the experiments: KH DM. Performed the experiments: ES DM TO YS HN EY HI MI YK. Analyzed the data: KH DM YS. Contributed reagents/materials/analysis tools: TO YS HN EY JT TM HI MI YK. Wrote the paper: KH DM.

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Does hypofrontality expand to global brain area in progression of schizophrenia?: A cross-sectional study between first-episode and chronic schizophrenia

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ABSTRACT

Although to date there have been no conclusive pathophysiological findings in support of the degenerative theory of the etiology of schizophrenia, the results of neuroimaging studies have suggested that progressive changes in the brain do occur during the clinical course of schizophrenia. However, there has been no report on alterations in regional cerebral blood flow (rCBF) under resting condition, which was compared between the first-episode and the chronic patients of schizophrenia and healthy controls. Therefore, in this study, we applied three-dimensional stereotactic surface projection analysis of resting SPECT (3D-SSP SPECT) in patients with first-episode ($n=18$) and chronic schizophrenia ($n=23$) and age-/sex-matched healthy controls ($n=40$). The rCBFs in the middle/inferior/medial frontal gyrus and the anterior cingulate gyrus were significantly decreased in both patient groups, relative to the respective controls ($Z > 3.0$, $P<0.001$, uncorrected). The chronic group showed significant hypoperfused region in the left inferior parietal lobule and middle/inferior temporal gyrus. Furthermore, within-cases comparison between the first-episode and chronic schizophrenia, revealed that the significant hypoperfused regions in the chronic group, compared to the first-episode group, were not only the lateral and medial prefrontal cortex, but also the inferior parietal cortex, posterior part of the temporal lobe, and the cuneus. The present study suggested that the reduction in rCBF occurs in the posterior brain area in addition to the frontal lobe across all clinical stages of schizophrenia.

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1. Introduction

Schizophrenia has been considered a progressive disorder in view of clinical symptoms and functional disability ever since the report of Kraepelin (1919/1971). The progressive disability has been considered to be associated with progressive biological changes in the brain; hence, it is important to clarify these changes in brain morphology and function for our understanding of the disease pathophysiology and the development of therapeutic strategies. Following the reports on hypofrontality in schizophrenia under the resting state by Ingvar and Franzen (1974), various neuroimaging studies using SPECT or PET have documented hypofrontality in patients with schizophrenia (Ashton et al., 2000; Gonus et al., 2003; Malaspina et al., 2004; Molina et al., 2005; Sachdev et al., 1997; Siegel et al., 1993). However, recent functional imaging studies under resting state (Andreasen et al., 1997; Kim et al., 2000), as well as studies conducted while subjects performed some cognitive tasks (Fletcher et al., 1999; Meyer-Lindenberg et al., 2005), have suggested that multiple regions including the lateral prefrontal cortex (LPFC), the medial prefrontal cortex (MPFC), and the temporal lobe cortices, rather than simple hypofrontality, are involved in the pathophysiology of schizophrenia.

On the other hand, recent MRI studies also indicate morphological changes with progression of the disease. The changes following the disease onset have been demonstrated as ventricular enlargement (Cahn et al., 2002; DeLisi et al., 1995, 1997) and degeneration in several

regions such as the prefrontal cortex (Gur et al., 1998; Ho et al., 2003; Mathalon et al., 2001) and temporal lobe cortices (Mathalon et al., 2001; Kasai et al., 2003a,b), although several studies have denied progressive volume reductions (Degreef et al., 1991; DeLisi et al., 2004; Gur et al., 1998; Whitworth et al., 2005). In addition, there is some evidence of these morphological changes at the initiation of disease, i.e., already before disease onset (Borgwardt et al., 2007; Job et al., 2005; Pantelis et al., 2003; Sun et al., in press). Progressive volume reduction in structural neuroimaging suggests that hypoperfusion in the brain, not only in functional neuroimaging but also under a resting state, becomes exacerbated over the clinical stages in schizophrenia. However, surprisingly, there have been few studies on this topic (Desco et al., 2003; Gur et al., 1995).

The three-dimensional stereotactic surface projection (3D-SSP) method used in this study has been known to minimize the effects of brain atrophy more than statistic parametric mapping (SPM), and thus is a method suitable for investigating regional cerebral blood flow (rCBF) and regional glucose metabolism ratio (rGMR) in diseases with brain degeneration, such as Alzheimer's disease and Parkinson's disease (Ishii et al., 2001; Matsui et al., 2005; Minoshima et al., 1995, 1997). The 3D-SSP method is considered a suitable tool for assessment of rCBF in schizophrenia as well, since volume reductions in the brain have been indicated. In this study, therefore, we used 3D-SSP SPECT to observe rCBF in patients with first-episode and chronic schizophrenia, in order to clarify whether or not brain hypoperfusion progresses with clinical stage in schizophrenia. In this study, each patient group had age-matched control groups, since several studies on normal subjects have suggested age-related alterations in rCBF, even if in relatively younger adults (Inoue et al., 2003; Kuji et al., 1999; Pagini et al., 2002; Van Laere and Dierckx, 2001).

2. Methods

2.1. Subjects and study design

We distinguished between the first-episode and the chronic patients, based on the duration of illness following the onset of positive symptoms. The first-episode group included those who had a continuous episode that lasted for up to 2 years, whereas the chronic group included those who had a duration of illness of more than 10 years prior to SPECT scanning. Both groups also met the DSM-IV criteria for schizophrenia (American Psychiatric Association, 1994). From July 2005 to October 2008, we recruited inpatients and outpatients at Chiba University Hospital or Chiba Psychiatry Medical Center (CPMC), who met the above criteria in serial order. We then divided the patients who agreed to participate in the study into first-episode (n = 18) and chronic (n = 23) groups. All patients were also evaluated using the Structured Clinical Interview for DSM-IV (SCID; American Psychiatric Association, 1994). Furthermore, reevaluation of the diagnosis of schizophrenia at 6 months after SPECT scanning, based on a semi-structured clinical interview and medical records, was performed for each subject by two experienced psychiatrists (N.K. and E.S.). As regards pharmacotherapy, the first-episode group had either no history of taking antipsychotic medication (neuroleptic-naive; n = 7) or had been treated with atypical antipsychotics such as risperidone (RIS), olanzapine (OLZ), quetiapine (QTP), or perospirone (PER) prior to enrollment in the study (mean duration of treatment: 3.0 months; n = 11; RIS: n = 8, OLZ: n = 1, QTP: n = 1, PER: n = 1), whereas patients in the chronic group were drug-free (n = 6) due to discontinuation of therapy, or they had been treated with atypical antipsychotics (n = 17; RIS: n = 9, OLZ: n = 8) for at least one year. Patients' clinical symptoms were assessed with the 18-item Brief Psychiatric Rating Scale (BPRS; Overall and Gorham, 1964) and concurrently, the shortened version of the Wechsler Adult Intelligence Scale-Revised (WAIS-R; Wechsler 1981), consisting of knowledge, picture completion and digit span forward/backward to evaluate the estimated IQ. On the other hand, the control group consisted of 40 healthy volunteers, who are free from any Axis I or II psychiatric disorder, based on the Structured Clinical Interview for DSM-IV Non-patient Edition (SCID-NP), and was divided into two age- and sex-matched groups (control-1 corresponds to the first-episode patient group and control-2 corresponds to the chronic patient group) (Table 1). Exclusion criteria for the both patients and healthy groups were a history of loss of consciousness, organic brain disorder, alcohol/drug abuse for previous life time, pregnancy or any physical disease on the basis of medical interview, physical examination, brain MRI, and laboratory data. The study protocol was approved by the ethics committee of both Chiba University and CPMC. The patients gave their written informed consent.

2.2. SPECT scanning procedure

The SPECT images were obtained using ^{123}I-IMP and acquired on a PRISM3000XP (Picker International, Cleveland, OH), 3-headed SPECT camera with ultrahigh-resolution fan-beam collimators. All subjects were examined in the supine resting position with closed eyes in a silent room. They were injected with 111 MBq of ^{123}I-IMP in the antecubital vein, and scanning was begun after 30 min. The data acquisition parameters were 128 × 128 matrices, 3 per step, 120 views,

Table 1
Demographic characteristics of the groups

Clinical variables	First-episode schizophrenia	Normal control-1	Chronic schizophrenia	Normal control-2	Statistical values (df = 39) first-episode vs chronic
Male/female	M10/F8[a]	M10/F8	M19/F4[b]	M18/F4	N.S. (Fisher's exact)
Age	27.0±4.5[c]	26.4±4.7	38.0±7.0[d]	36.1±7.8	P<0.001
(Range)	19–36	19–35	27–53	22–51	
Duration of illness (years)	1.3±0.3		15.5±6.2		P<0.001
Age at onset	25.5±4.7		22.0±5.2		P<0.05
Years of education	14.7±2.5[c]	15.0±1.9	14.0±2.3[d]	14.6±3.1	N.S.
Dose of antipsychotics[e]	284.9±319.5		447.8±405.6		N.S.
Duration of treatment (years)	0.25±0.34		6.57±6.79		P<0.001
BPRS (total)	34.6±11.7		36.8±10.5		N.S.
Positive symptoms[f]	11.8±5.6		10.4±4.1		N.S.
Negative symptoms[g]	5.8±4.3		7.7±3.5		N.S.
Estimated IQ	86.4±20.0		89.9±23.6		N.S.

[a] n.s. in comparisons between the first-episode and normal control-1 (Fisher's exact test).
[b] n.s. in comparisons between the chronic patient and normal control-2 (Fisher's exact test).
[c] n.s. in comparisons between the first-episode and normal control-1 (t-test).
[d] n.s. in comparisons between the chronic patient and normal control-2 (t-test).
[e] Corresponding to daily chlorpromazine dose (mg).
[f] BPRS positive scores consist of those of conceptual disorganization, suspiciousness, hallucinations and unusual thought content.
[g] BPRS negative scores consist of those of emotional withdrawal, motor retardation and blunted affect.
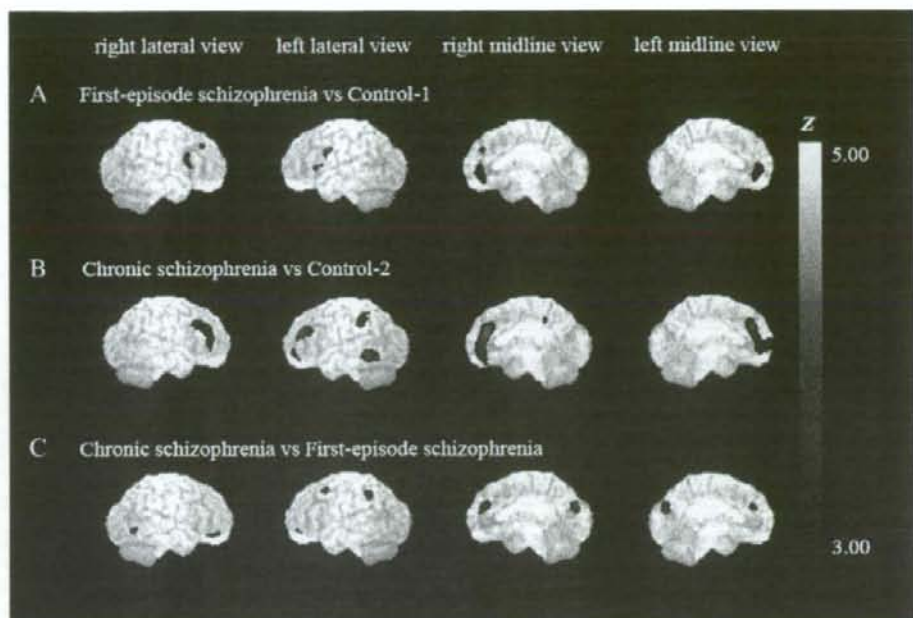
Fig. 1. 3D-SSP maps of the rCBF of patients with schizophrenia derived using ^{123}I-IMP, and compared with those of normal controls. (a) Significantly decreased regions in the first-episode patients compared with the controls; (b) Significantly decreased regions in the chronic patients compared with the controls; (c) Significantly decreased regions in the chronic patients compared with the first-episode patients ($Z>3.00$, corresponding to $P<0.001$, uncorrected).

7.5 s per view, and a 159-keV (±10%) energy window. Reconstruction was performed by filtered back projection using a Butterworth filter (cut-off frequency 2.5/order 4), and attenuation correction of ramp filters was performed using the Chang 8-order method. To confirm that there was no head movement or opening of the eyes, each subject was monitored by video camera.

2.3. Statistical analysis

Analysis with the interface software iSSP (version 3.5; Nihon Medi-Physics Corporation, Nishinomiya, Japan) followed the 3D-SSP method established by Minoshima (Bartenstein et al., 1997; Minoshima et al., 1994). We used the cerebellum as the reference region for normalization, as this was the approach taken in a number of similar studies (Sabri et al., 1997; Sachdev et al., 1997; Vita et al., 1995).

To demonstrate differences in the rCBF distributions, 2-sample t-test values were calculated on a pixel-by-pixel basis and then transformed to Z values by a probability integral transformation (Worsley et al., 1996). And we set the level of significant thresholds of $Z>3.00$ (corresponding to uncorrected $P<0.001$) revised on the basis of random Gaussian field theory (Hanyu et al., 2001). Regarding cluster size, we defined a region having more than 50 contiguous

Table 2
Regional cerebral blood flow assesses in first-episode and chronic schizophrenia groups, compared to normal healthy subjects ($Z>3.00$, $P<0.001$, uncorrected)

Region with decreased rCBF	First-episode schizophrenia				Chronic schizophrenia			
	Brodmann's area	Cluster size (ml)	Highest Z value	Coordinates (x, y, z)	Brodmann's area	Cluster size (ml)	Highest Z value	Coordinates (x, y, z)
Frontal lobe								
Middle frontal gyrus/	L6/9	1.50	3.90	−55, 5, 32	L9/10/46	6.15	3.86	−44, 44, 25
inferior frontal gyrus	L44/45	0.82	3.35	−53, 23, 5	L10/47	3.96	3.41	−30, 32, −13
	R8/9	1.09	3.25	39, 39, 38	R9/45/46/47	8.95	3.47	46, 41, 27
	R44/45/46	2.87	3.20	57, 17, 16				
Medial frontal gyrus/	L10/11/32	4.03	3.40	−1, 44, −2	L9/10/32	12.02	3.73	−1, 39, 27
anterior cingulate gyrus	R10/11/32	4.72	3.58	1, 46, −2	R6/9/10/11/32	12.51	4.21	1, 39, 23
	R6/9	1.30	3.24	1, 44, 34				
Parietal lobe								
Inferior parietal lobule					L40	4.17	3.48	−60, −40, 43
Temporal lobe								
Middle temporal gyrus/ inferior temporal gyrus/ fusiform gyrus					L19/21/37	5.26	3.71	−60, −49, −9
Limbic regions								
Cingulate gyrus					L31	0.22	3.40	1, −37, 38

significant pixels as a hypoperfused region and converted them to volume. These procedures were performed with stereotactic extraction estimation (SEE ver. 2; Nihon Medi-Physics Corporation).

3. Results

Table 1 shows the characteristics of the participants. Age and sex were matched between the two patient groups and the corresponding normal healthy subjects, respectively. Furthermore, BPRS positive and negative symptoms were not significantly different between the first-episode and chronic schizophrenia groups.

3.1. Comparisons of rCBF ratios between the first-episode schizophrenia and control-1, and between the chronic schizophrenia and control-2

The pixel-by-pixel based comparison with the significant threshold of $Z>3.00$ (uncorrected $P<0.001$) revealed that the LPFC (middle/inferior frontal gyrus) and MPFC (medial frontal gyrus and ACG [anterior cingulate gyrus]) were the significant hypoperfused regions common to both schizophrenia groups (Fig. 1a,b, Table 2). On the other hand, the regions that were significantly hypoperfused only in chronic schizophrenia, and not in the first-episode group, were the left inferior parietal lobule, the left middle/inferior temporal/fusiform gyrus, and cigulate gyrus (Fig. 1b, Table 2). The stricter significance threshold ($Z>4.53$, corrected $P<0.05$) revealed that neither the first-episode group, nor the chronic group have the significantly hypoperfused region.

3.2. Comparison of rCBF ratios between first-episode and chronic schizophrenia

In order to explore rCBF alteration depending on progressive clinical change of the disease, we performed a within-cases comparison (i.e., first-episode schizophrenia vs chronic schizophrenia). The analysis showed that rCBF ratios in the bilateral LPFC (middle frontal gyrus) and the MPFC (medial frontal gyrus and ACG) in the chronic group revealed the significant hypoperfused regions, compared to the ratios in the first-episode group ($Z>3.0$, uncorrected $P<0.001$; Fig. 1c and Table 3). Furthermore, rCBF ratios in the left parietal lobe (inferior parietal lobule and the supramarginal gyrus), the right posterior part of the temporal lobe (middle/inferior temporal gyrus), and the bilateral medial phase of the occipital gyrus (cuneus) in the chronic group were significantly lower than those in the first-episode group (Fig. 1c and Table 3). In the analysis with a stricter significance threshold ($Z>4.53$, corrected $P<0.05$), we did not find any significant region that indicated

Table 3
Significant hypoperfused regions in chronic schizophrenia compared to first-episode schizophrenia ($Z>3.00$, $P<0.001$, uncorrected)

Region with decreased rCBF	Brodmann's area	Cluster size (ml)	Highest Z value	Coordinates (x, y, z)
Frontal lobe				
Middle frontal gyrus	L6/8	1.23	3.38	−46, 17, 47
	L10/11	0.75	3.17	−39, 53, −7
	R11/47	2.94	3.70	39, 39, −11
Medial frontal gyrus/	L9/32	1.64	3.42	−1, 39, 27
anterior cingulate gyrus	R6/9/32	3.35	3.60	1, 41, 27
Parietal lobe				
Inferior parietal lobule/	L40	2.12	3.48	−60, −46, 41
supramarginal gyrus				
Temporal lobe				
Middle temporal gyrus/	R19/37	1.37	3.16	53, −62, −7
inferior temporal gyrus				
Occipital lobe				
Cuneus	L18/19/31	3.55	3.57	−1, −78, 27
	R18/19/31	3.52	3.52	1, −78, 23

a greater decrease of rCBF ratios in the chronic group than in the first-episode group. On the other hand, the analysis indicated that there is no region, where is the significantly decreased in the first-episode group compared to the chronic group. In addition, the within-controls comparison (control-1 vs control-2) revealed no significant difference in rCBF ratios between the two groups.

4. Discussion

In this study we found, first, that there was hypoperfusion in the LPFC and MPFC in both the first-episode and chronic schizophrenia groups compared with the respective control groups, which suggest the hypoperfusion in these regions is consistent throughout the course of the disease. Second, hypoperfusion in the frontal lobe tended to be more severe in chronic than in first-episode schizophrenia (Fig. 1a,b). Third, the results of this study were suggestive of a greater reduction in rCBF in the posterior brain regions (the parietal cortex, the posterior part in temporal lobe and the cuneus) in the chronic schizophrenia than in the first-episode schizophrenia. Taken together, these results suggest that hypoperfusion in the brain of the chronic schizophrenia may progress from the frontal regions to other regions, especially the posterior regions, over the course of the disease.

As far as we know, there has been no cross-sectional study or longitudinal study on rCBF distribution at different stages of schizophrenia. Our study employed strict age/sex-matched control groups (control-1 and control-2) for the corresponding patient groups, first-episode and chronic schizophrenia, respectively. Therefore, the significantly decreased regions in rCBF ratios in both first-episode and chronic schizophrenia, compared to their respective controls, indicate disease-related alterations at each clinical stage of schizophrenia (Fig. 1a, b, Table 2). Importantly, we found that the rCBFs in the posterior brain regions, in addition to the prefrontal cortex in the chronic group, were significantly lower than those in the first-episode group. This finding cannot be explained only by aging (first-episode group, 27.0 years; chronic group, 38.0 years), since the rCBF distributions in control-1 and control-2 were very similar. Moreover, the regions with different rCBF ratios between the two clinical stages (Fig. 1c), can be predicted from the two results showing the disease-related changes at each clinical stage (Fig. 1a, b).

Hypoperfusion in the frontal lobe, particularly the MPFC, showed the greatest reduction from healthy levels among all regions in the brain in both the first-episode and chronic stage patients. This result suggests that rCBF in the frontal lobe already begin to fall within 2 years of disease onset. Several recent MRI studies on the early stage in schizophrenia have shown that morphological alterations occur dynamically over the first 2–3 years following onset, which could be differentiated from subsequent phases of the disease (Ho et al., 2003; Whitford et al., 2006); they also support our results for the first-episode group.

The current finding of hypofrontality in subjects in a resting state starting at the early stage appears to be inconsistent with previous fMRI studies in which increased activity was observed in the frontal regions (Manoach et al., 1999, 2000). However, Manoach (2003) suggested that the level of activity may be influenced by the kind of cognitive tasks loaded, and that observations of increased activity may be reflective of hypofrontality in patients with schizophrenia. Therefore, results of fMRI studies showing increased activity (Manoach et al., 1999, 2000) may indeed be consistent with our results.

Contrary to our hypothesis, hypofrontality in the LPFC in the chronic group failed to show obvious exacerbation relative to the first-episode group (Fig. 1c, Table 3). Desco et al. (2003) conducted a comparative study on FDG-PET between recent-onset schizophrenia, with illness duration up to 3 years, and chronic schizophrenia, and found that the LPFC in the chronic group showed significantly lower rGMR than the recent-onset group. In addition to differences in rGMR

by PET and rCBF by SPECT, one of the possible explanations for the lack of significant progressive frontal rCBF reduction in our study may be the patients' profiles. Particularly in the chronic group, as shown in Table 1, negative symptoms and estimated IQ in this group were similar to those of first-episode, and there were no long-term institutionalized patients in the present study, suggesting that the chronic patients who participated in this study had better prognoses than more general chronic patients in clinical practice. Nevertheless, severe rCBF reduction in the ACG throughout clinical stages may be associated with abnormalities directly demonstrated by MRI studies showing volume reduction (Hanznedar et al., 2004; Mitelman et al., 2005; Wang et al., 2007) or postmortem studies showing histopathological changes in this region (Benes et al., 1991; Broadbelt et al., 2006; Jone et al., 2002).

On the other hand, results for the parietal, temporal, and occipital lobes, which indicated significant decreases in rCBF in the chronic group relative to the normal comparison, also showed a significant difference by within-cases comparison. These results suggest a greater alteration of rCBF in the posterior region than in the frontal region during a timeframe ranging from the early to the chronic phase of illness. Although little attention has been paid to the parietal lobe, the posterior temporal lobe, and the cuneus in schizophrenia, rCBF/rGMR in chronic schizophrenia was found to be significantly reduced in these lobes (Kim et al., 2000; Potkin et al., 2002; Wolkin et al., 1985). However, these studies did not show relationships between these abnormalities and illness duration. In recent fMRI researches on chronic schizophrenia, function in the inferior parietal lobule has been suggested to differ from that in healthy subjects in concept of self (Spence et al., 1997) and decision-making (Collete et al., 2005; Paulus et al., 2002); furthermore, the posterior part of the middle temporal gyrus involving semantic priming has been reported to be functionally disturbed in schizophrenia (Han et al., 2007; Kuperberg et al., 2007). Although there are far fewer reports on the medial phase of the occipital lobe than on the parietal/temporal lobe in schizophrenia, several studies found dysfunction of visual processing (Butler and Javitt, 2005; O'Donnell et al., 1996; Tek et al., 2002). In addition, structural MRI studies suggested that reduced brain volume in widespread regions including the posterior brain area are related with the duration of illness (Meisenzahl et al., 2008) or poorer outcomes of the disease (Mitelman et al., 2003). These cortices have strong connectivity with the prefrontal cortex, and disconnectivities among the prefrontal and other cortices are presumed to contribute to the core pathophysiology of schizophrenia (Fletcher et al., 1999; Frith et al., 1995; Quintana et al., 2003). These regions in the posterior brain area showing hypoperfusion in chronic schizophrenia in the current study may be responsible for the pathophysiology underlying the disease, but in combination with other regions, including the prefrontal cortex, rather than alone.

The results of the present study are preliminary, and it would therefore be inappropriate to conclude that they are reflective of a general pattern of progression of hypoperfusion across clinical stages of this disease, since this study has following some limitations related to study design. First, the present study was not conceived as a longitudinal study, i.e., there were no rigorous comparisons of changes in rCBF ratio at different stages of illness within the same patient group. Therefore, although the use of two age-matched control groups did enable us to observe a clear difference in rCBF ratio in some posterior brain regions between our two patient groups (Fig. 1c, Table 3), these results will require further examination in future studies. Second, antipsychotics administered to both patient groups may have had some effect on rCBF distribution. Different patterns of effects on perfusion have been reported for haloperidol and risperidone (Miller et al., 2001). Metabolic and perfusion changes have also been reported for clozapine and risperidone (Molina et al., 2003, 2005). However, our samples consisted of patients who had taken neither haloperidol nor clozapine. Furthermore, the chlorpromazine-equivalent dosages of antipsychotic

drugs did not statistically differ between groups (Table 1). Thus, the effects caused by medication differences may have been low in this study. Finally, our sample size was relatively small. Despite these limitations, this study suggests that SPECT is a useful means to explore the alterations of rCBF over the stages of schizophrenia, beyond its relatively poor spatial resolution. In order to compare two patient groups with different clinical stages more strictly and reach conclusive evidence on this issue, a further longitudinal study with both larger and homogeneous samples should be conducted.

5. Conclusions

In conclusion, the present study demonstrated that hypofrontality in the LPFC and MPFC was unchanged between first-episode and chronic schizophrenia. In the chronic stage, however, the rCBF reductions in the frontal lobe tended to extend to posterior brain regions such as the parietal lobe, the posterior temporal lobe and the occipital lobe.

References

American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 4th ed. Washington, DC.: American Psychiatric Press; 1994
Andreasen NC, O'Leary DS, Flaum M, Nopoulos P, Watkins GL, Ponto LLB. Hypofrontality in schizophrenia: distributed dysfunction circuits in neuroleptic-naïve patients. Lancet 1997;349:1730-4.
Ashton L, Barnes A, Livingston M, Wyper D. The Scottish schizophrenia research group. Cingulate abnormalities associated with PANSS negative scores in first episode schizophrenia. Behav Neurol 2000;12:93-101.
Bartenstein P, Minoshima S, Hirsch C, Buch K, Willoch F, Mösch D, et al. Quantitative assessment of cerebral blood flow in patients with Alzheimer's disease by SPECT. J Nucl Med 1997;38:1095-101.
Benes FM, McSprren J, Bird ED, SanGiovanni JP, Vincent SL. Deficits in small interneurons in prefrontal and cingulate cortices of schizophrenics and schizoaffective patients. Arch Gen Psychiatry 1991;48:996-1001.
Borgwardt SJ, Riecher-Rössler A, Dazzan P, Chitnis X, Aston J, Drewe M, et al. Regional gray matter volume abnormalities in the at risk mental state. Biol Psychiatry 2007;161:1148-56.
Broadbelt K, Ramprasaud A, Jones LB. Evidence of alterd neurogranin immunoreactivity in areas 9 and 32 of schizophrenic prefrontal cortex. Schizophr Res 2006;87:6-14.
Butler PD, Javitt DC. Early-stage visual processing deficits in schizophrenia. Curr Opin Psychiatry 2005;18:151-7.
Cahn W, Hulshoff Pol HE, Lems EB, van Haren NE, Schnack HG, van der Linden JA, et al. Brain volume changes in first-episode schizophrenia: a 1-year follow-up study. Arch Gen Psychiatry 2002;59:1002-10.
Collete F, Olivier L, Van der Linden M, Laureys S, Delfiore G, Luxen A, et al. Involvement of both prefrontal and inferior parietal cortex in duel-task performance. Brain Res Cogn 2005;24:237-51.
Degreef G, Ashtari M, Wu H, Borestein M, Geisler S, Lieberman JA. Follow up MRI study in first episode schizophrenia. Schizophr Res 1991;5:204-6.
DeLisi LE, Tew W, Xie S, Hoff AL, Sakuma M, Kushner M, et al. A prospective follow-up study of brain morphology and cognition in first-episode schizophrenic patients: preliminary findings. Biol Psychiatry 1995;38:349-60.
DeLisi LE, Sakuma M, Tew W, Kushner M, Hoff AL, Grimson R. Schizophrenia as a chronic active brain process: a study of progressive brain structural change subsequent to the onset of schizophrenia. Psychiatry Res Neuroimaging 1997;74:129-40.
DeLisi LE, Sakuma M, Maurizio AM, Relja M, Hoff AL. Cerebral ventricular change over the first 10 years after the onset of schizophrenia. Psychiatry Res 2004:130:57-70.
Desco M, Gispert JD, Reig S, Sanz J, Pascau J, et al. Cerebral metabolic patterns in chronic and recent-onset schizophrenia. Psychiatry Res Neuroimaging 2003;122:125-35.
Fletcher P, McKenna PJ, Friston KJ, Frith CD, Dolan RJ. Abnormal cingulated modulation of fronto-temporal connectivity in schizophrenia. Neuroimage 1999;9:337-42.
Frith CD, Friston KJ, Herold S, Silbersweig D, Fletcher P, Cahill C, et al. Regional brain activity in chronic schizophrenic patients during the performance of a verbal fluency task. Br J Psychiatry 1995;167:343-9.
Gonus AS, Kula M, Esel E, Tutus A, Sofuoglu S. A Tc-99m HMPAO SPECT study of regional cerebral blood flow in drug-free schizophrenia patients with deficit and non-deficit syndrome. Psychiatry Res Neuroimaging 2003;123:199-205.
Gur RE, Mozley PD, Resnick SM, Mozley LH, Shtasel DL, Gallacher F, et al. Resting cerebral glucose metabolism in first-episode and previously treated patients with schizophrenia relates to clinical features. Arch Gen Psychiatry 1995;52:657-67.
Gur RE, Cowell P, Turetsky BI, Gallacher F, Cannon T, Bilker W, et al. A follow-up magnetic resonance imaging study of schizophrenia: relationship of neuroanatomical

changes to clinical and neurobehavioural measures. Arch Gen Psychiatry 1998;55:145–52.

Han SD, Nestor PG, Hale-Spencer M, Cohen A, Niznikiewicz M, McCarley RW, et al. Functional neuroimaging of word priming in males with chronic schizophrenia. Neuroimage 2007;35:273–82.

Hanyu H, Asano T, Kogure D, Abe S, Iwamoto T, Takahashi M. Diagnosis of Alzheimer's disease using brain SPECT with three-dimentional stereotactic surface projections. Rinsho Shinkeigaku 2001;41:582–7 Article in Japanese.

Hanznedar MM, Buchsbaum MS, Hanzlett EA, Shihabuddin L, New A, Siever LJ. Cingulate gyrus volume and metabolism in the schizophrenia spectrum. Schizophr Res 2004;71:249–62.

Ho BC, Andreasen NC, Npoulos P, Arndt S, Magnotta V, Flaum M. Progressive structural brain abnormalities and their relationship to clinical outcome: a longitudinal magnetic resonance imaging study early in schizophrenia. Arch Gen Psychiatry 2003;60:585–94.

Ingvar DH, Franzen G. Abnormalities of cerebral blood flow distribution in patients with chronic schizophrenia. Acta Psychiatr Scand 1974;50:425–62.

Inoue K, Nakagawa M, Goto R, Kinomura S, Sato T, Sato K, et al. Regional differences between 99mTc-ECD and 99mTC-HMPAO SPET in perfusion changes with age and gender in healthy adults. Eur J Nucl Med Mol Imaging 2003;30:1489–97.

Ishii K, Willoch F, Minoshima S, Drzezga A, Ficaro EP, Cross DJ, et al. Statistical brain mapping of 18F-FDG PET in Alzheimer's disease: validation of anatomic standardization for atrophied brains. J Nucl Med 2001;42:548–57.

Job DE, Whalley HC, Johnstone EC, Lawrie SM. Grey matter changes over time in high risk subjects developing schizophrenia. Neuroimage 2005;25:1023–30.

Jone LB, Johnson N, Byne W. Alterations in MAP2 immunocytochemistry in areas 9 and 32 of schizophrenic prefrontal cortex. Psychiatry Res 2002;114:137–48.

Kasai K, Shenton ME, Salisbury DF, Hirayasu Y, Onitsuka T, Spencer MH, et al. Progressive decrease of left Heschl gyrus and planum temporal gray matter volume in first-episode schizophrenia. Arch Gen Psychiatry 2003a;60:766–75.

Kasai K, Shenton ME, Salisbury DF, Hirayasu Y, Lee CU, Ciszewski AA, et al. Progressive decrease of left superior temporal gyrus gray matter volume in patients with first-episode schizophrenia. Am J Psychiatry 2003b;160:156–64.

Kim JJ, Mohamed S, Andreasen NC, O'Leary DS, Watkins GL, et al. Regional neural dysfunction in chronic schizophrenia. Studied with positron emission tomography. Am J Psychiatry 2000;157:542–8.

Kraepelin E. Dementia Praecox (trans. R. Barclay). Edinburgh: Livingstone; 1919/1971.

Kuji I, Sumiya H, Niida Y, Takizawa N, Ikeda E, Tsuji S, et al. Age-related changes in the cerebral distribution of 99mTc-ECD from infancy to adulthood. J Nucl Med 1999;40:1818–23.

Kuperberg GR, Deckersbach T, Holt DJ, Goff D, West C. Increased temporal and prefrontal activity in response to semantic association in schizophrenia. Arch Gen Psychiatry 2007;64:138–51.

Malaspina D, Harkavy-Friedman J, Corcoran C, Mujica-Parodi L, Printz D, et al. Resting neural activity distinguishes subgroup of schizophrenia patients. Biol Psychiatry 2004;56:931–7.

Manoach DS. Prefrontal cortex dysfunction during working memory performance in schizophrenia: reconciling discrepant findings. Schizophr Res 2003;60:285–98.

Manoach DS, Press DZ, Thangaraj V, Searl MM, Goff DC, Halpern E, et al. Schizophrenic subjects activate dorsolateral cortex during a working memory task, as measured by fMRI. Biol Psychiatry 1999;45:1128–37.

Manoach DS, Gollub RL, Benson ES, Searl MM, Goff DC, Halpern E, et al. Schizophrenic subjects show aberrant fMRI activation of dorsolateral prefrontal cortex and basal ganglia during working memory performance. Biol Psychiatry 2000;48:99–109.

Mathalon DH, Sullivan EV, Lim KO, Pfefferbaum A. Progressive brain volume changes and the clinical course of schizophrenia in men: a longitudinal magnetic resonance imaging study. Arch Gen Psychiatry 2001;58:148–57.

Matsui H, Udaka F, Miyoshi T, Hara N, Tamura A, Oda M, et al. Brain perfusion differences in Parkinson's disease and multiple system atrophy with predominant Parkinsonian features. Pakinson Relat Disorders 2005;11:227–32.

Meisenzahl EM, Koutsouleris N, Bottlender R, Scheuerecker J, Jäger M, Teipel SJ, et al. Structural brain alterations at different stages of schizophrenia: a voxel-based morphometric study. Schizophr Res 2008;104:44–60.

Meyer-Lindenberg AS, Olsen RK, Kohn PD, Brown T, Eagan MF, Weinberger DR, et al. Regionally specific disturbance of dorsolateral prefrontal-hippocampal function connectivity in schizophrenia. Arch Gen Psychiatry 2005;62:379–86.

Miller DD, Andreasen NC, O'Leary DS, Watkins GL, Ponto LLB, Hichwa RD. Comparison of the effects of risperidone and haloperidol on regional cerebral blood flow in schizophrenia. Biol Psychiatry 2001;49:704–15.

Minoshima S, Koeppe RA, Frey KA, Kuhl DE. Anatomic standardization: Linear scaling and nonlinear warping of functional brain imagings. J Nucl Med 1994;35:1528–37.

Minoshima S, Frey KA, Koeppe RA. A diagnostic approach in Alzheimer's disease using three-stereotactic surface projections of fluorine-18-FDG PET. J Nucl Med 1995;36:1238–48.

Minoshima S, Giordani B, Berent S, Frey KA, Foster NL, Kuhl DE. Metabolic reduction in the posterior cingulate cortex in very early Alzheimer's disease. Ann Neurol 1997;42:85–94.

Mitelman SA, Shihabuddin L, Brickman AM, Hanzlett EA, Buchsbaum MS. MRI assessment of grey and white matter distribution in Brodmann's areas of the cortex in patients with schizophrenia with good and poor outcomes. Am J Psychiatry 2003;160:2154–68.

Mitelman SA, Shihabuddin L, Brickman AM, Hanzlett EA, Buchsbaum MS. Volume of the cingulated and outcome in schizophrenia. Schizophr Res 2005;72:91–108.

Molina V, Gispert S, Reig S, Sanz J, Pascau J, Santos A, et al. Cerebral metabolism and risperidone treatment in schizophrenia. Schizophr Res 2003;60:1–7.

Molina V, Gispert JD, Reig S, Sanz J, Pascau J, Santos A, et al. Cerebral metabolic changes induced by clozapine in schizophrenia and related to clinical improvement. Psychopharmacology 2005;17–26.

O'Donnell BF, Swearer JM, Smith LT, Nestor PG, Shenton ME, McCarley RW. Selective deficits in visual perception and recognition in schizophrenia. Am J Psychiatry 1996;153:687–92.

Overall JE, Gorham DR. The brief psychiatric rating scale. Psychological Rep 1964;10:799–812.

Pagini M, Salmaso D, Jonsson C, Hatherly R, Jacobsson H, Larsson SA, et al. Regional cerebral blood flow as assessed by principal component analysis and 99mTc-HMPAO SPET in healthy subjects at rest: normal distribution and effect of age and gender. Eur J Nucl Med 2002;29:67–75.

Pantelis C, Velakoulis D, McGorry PD, Wood SJ, Suckling J, Phillips LJ, et al. Neuroanatomical abnormalities before and after onset of psychosis: a cross-sectional and longitudinal MRI comparison. Lancet 2003;361:281–8.

Paulus MP, Hozack NE, Zauscher BE, Frank L, Brown GG, McDowell J, et al. Parietal dysfunction is associated with increased outcome-related decision-making in schizophrenia patients. Biol Psychiatry 2002;51:995–1004.

Potkin SG, Alva G, Fleming K, Anand R, Keator D, Carreon D, et al. A PET study of the pathophysiology of negative symptoms in schizophrenia. Am J Psychiatry 2002;159:227–37.

Quintana J, Wong T, Oriz-Portillo E, Kovalik E, Davidson T, Marder SR, et al. Prefrontal-posterior parietal networks in schizophrenia: primary dysfunctions and secondary compensations. Biol Psychiatry 2003;53:12–24.

Sabri O, Erkwoh R, Schreckenberger M, Owega A, Sass H, Buell U. Correlation of positive symptoms exclusively to hyperperfusion or hypoperfusion of cerebral cortex in never-treated schizophrenics. Lancet 1997;349:1735–9.

Sachdev P, Brodaty H, Rose N, Haindl W. Regional cerebral blood flow in late-onset schizophrenia: a SPECT study using 99mTc-HMPAO. Schizophr Res 1997;27:105–17.

Siegel BV, Buchsbaum MS, Bunney WE, Gottschalk LA, Haier RJ, Lohr JB, et al. Cortical-striatal-thalamic circuits and brain glucose metabolic activity in 70 unmedicated male schizophrenic patients. Am J Psychiatry 1993;150:1325–36.

Spence SA, Brooks DJ, Hirsch SR, Liddle PF, Meehan J, Grasby PM. A pet study of voluntary movement in schizophrenic patients experiencing passivity phenomena (delusion of alien control). Brain 1997;120:1997–2011.

Sun, D, Phillips, L, Velakoulis, D, Yung, A, McGorry, PD, Wood SJ, et al. Progressive brain structural changes mapped as psychosis develops in 'at risk' individuals. Schizophr Res in press.

Tek C, Gold J, Blaxton T, Wilk C, McMahon RP, Buchanan RW. Visual perceptual and working memory impairments in schizophrenia. Arch Gen Psychiatry 2002;59:146–53.

Van Laere KJ, Dierckx RA. Brain perfusion SPECT: age- and sex-related effects correlated with voxel-based morphometric findings in healthy adults. Nucl Med 2001;221:810–7.

Vita A, Bressi S, Perani D, Invernizzi G, Giobbio GM, Dieci M, et al. High-resolution SPECT study of regional cerebral blood flow in drug-free and drug-naive schizophrenic patients. Am J Psychiatry 1995;152:876–82.

Wang L, Hosakere M, Trein JC, Miller A, Ratnanather JT. Abnormalities of cingulated gyrus neuroanatomy in schizophrenia. Schizophr Res 2007;93:66–78.

Wechsler D. WAIS-R manual. New York: The Psychological Corporation; 1981.

Whitford TJ, Grieve SM, Farrow TFD, Gomes L, Brennan J, Harris AW, et al. Progressive grey matter atrophy over the first 2–3 years of illness in first-episode schizophrenia: a tensor-based morphometry study. Neuroimage 2006;32:511–9.

Whitworth AB, Kemmler G, Honeder M, Kremser C, Felber S, Hausmann A, et al. Longitudinal volumetric MRI study in first- and multiple-episode male schizophrenia patients. Psychiatry Res Neuroimaging 2005;140:225–37.

Wolkin A, Jaeger J, Brodie JD, Wolf AP, Fowler J, Rotrosen J, et al. Persistence of cerebral metabolic abnormalities in chronic schizophrenia as determined by positron emission tomography. Am J Psychiatry 1985;142:564–71.

Worsley KJ, Marret S, Neelin P, Evans AC. A unified statistical approach for determining significant signals in location and scale space images of cerebral activation. In: Myers R, Cunningham V, Baily D, et al, editors. Quantification of brain function using PET. San Diego: Academic press; 1996. p. 327–33.

Letter to the Editor (Case report)

Fluvoxamine as a sigma-1 receptor agonist improved cognitive impairments in a patient with schizophrenia

1. Introduction

Patients with schizophrenia exhibit positive and negative symptoms, and these symptoms have been targets for the development of new antipsychotics. In recent years, cognitive impairments in these patients, which may persist across cycles in other aspects of the illness and may be strongly related to the functional outcome, have drawn considerable attention (Hughes et al., 2003; Harvey et al., 2001). It has been reported that the patients themselves are aware of and experience distress due to their cognitive impairments (Ginsberg et al., 2005). Some of the new atypical antipsychotics can improve the impairments, but such amelioration generally remains unsatisfying, and the development of more effective drugs is still necessary (Lehman et al., 1995). Recently, we reported on the efficacy of fluvoxamine, a potent sigma-1 receptor agonist, in improving cognitive impairments in an animal model of schizophrenia (Hashimoto et al., 2007). In the present report, cognitive impairments in a female patient with schizophrenia were dramatically improved by adjunctive treatment of fluvoxamine added to risperidone.

2. Case report

A 19-year-old female college student began to experience auditory hallucinations, including a voice admonishing her to kill herself, and delusions of persecution and reference. She responded by withdrawing socially and engaging in various impulsive behaviors, including wrist-cutting. Her mother took her to a mental clinic, where she was diagnosed with borderline personality disorder. She was prescribed zotepine 50 mg and haloperidol 1.25 mg for approximately 6 months, but her withdrawal and impulsive behaviors did not improve, and she was referred to our hospital at the age of 20. No abnormalities were found in her general laboratory examinations or brain CT. She reported having consistent pathological experiences, such as hallucination and delusions, and was diagnosed with schizophrenia according to the DSM-IV criteria. We discontinued her previous medications and started her on risperidone 4 mg. She quickly stopped hearing voices and cutting her wrists. However, she continued to suffer from impairments of concentration, attention and memory, which she reported having experienced for several years, and felt distress as these impairments often disturbed her motivation to watch TV, read a book or help her mother with housework.

A ten-month treatment with risperidone at a dose ranging from 4 to 8 mg almost completely eliminated her psychotic symptoms, and she was again able to go shopping with her mother several times a week. However, she still reported distressing cognitive impairments. Therefore, we added 20-mg paroxetine in order to reduce her anxiety over the impairments. A five-month treatment with paroxetine did not improve the distress or the cognitive impairments, but rather worsened her anxiety. We therefore stopped the paroxetine and, 3 months later, added 50 mg fluvoxamine. One month after the start of fluvoxamine, she reported that her ability to follow the plots of television dramas had improved, along with her quickness of mind and concentration. Two weeks later, she began to help her mother with housework, including cooking and cleaning rooms, and she stated that these changes were due to her improved concentration. She is now 25 years old and has been treated with the two drugs for 18 months. Her cognitive function remains improved and she continues to help her mother with the housework and to search for a suitable job.

3. Discussion

We have presented the case of a young patient with schizophrenia whose psychotic symptoms were improved by treatment with risperidone, one of the new antipsychotics. Her improvement was complicated by cognitive impairments and resulting stress. Her cognitive impairments were consistent with those often observed in schizophrenia, i.e., impairments in quickness of mind, concentration, memory and executive function, which generally appear early and persist regardless of the phase of the illness.

Paroxetine was administered in the hope of eliminating her anxiety and possibly her cognitive impairments, but it did not improve either even after 5 months of treatment. On the other hand, fluvoxamine prominently and quickly improved her cognitive impairments as measured both subjectively and objectively. It has been reported that the combination therapy of fluvoxamine, but not paroxetine, and antipsychotics improves negative symptoms (Silver, 2004). The improvement of the cognitive impairments in the present patient may have been partly correlated with an improvement in negative symptoms, although the patient felt anxiety rather than loss of affect at the impairments, which disturbed her motivation. Both fluvoxamine and paroxetine are serotonin reuptake inhibitors. However, only fluvoxamine exhibits sigma-1 receptor agonism, i.e., the affinity of fluvoxamine is more than 50 times higher than that of paroxetine (Narita et al., 1996), and we have previously shown that fluvoxamine, but not paroxetine, improved cognitive dysfunction in