The time of discharge was thoroughly discussed with the patients and their families and was determined according to whether the patient had improved sufficiently to be treated on an outpatient basis. Forty-seven healthy controls (table 1) were also included in the study. The healthy controls did not meet current or past criteria for any Axis I disorder of DSM-IV. All participants met the following criteria: (1) no systemic or neurologic disease; (2) no past head trauma with loss of consciousness; and (3) no lifetime history of alcohol or substance dependence. No healthy controls had diabetes mellitus and/or chronic renal disease.

Evaluation of Clinical Symptoms

Clinical symptoms were assessed using the Brief Psychiatric Rating Scale (BPRS) (each item was rated on a scale of 1–7).[8] The BPRS scores included direct interviews that were independently evaluated by well-trained experienced psychiatrists. The overall total rating and scores dealing with positive and negative symptom clusters were used.[9] The presence of a family history of psychiatric disease was defined as having a first- or second-degree relative with any neuropychiatric disorder.

A top priority of the Juntendo University Schizophrenia Projects (JUSP)[10–12] is to improve patients' symptoms in the most effective manner. Accordingly, the use of drug therapy was not controlled due to ethical considerations. The Ethics Committee of the Juntendo University School of Medicine approved the present study (2012083). All participants gave their written informed consent prior to participating in the study.

Measurements of Carbonyl Stress Markers

Measurements for pentosidine using a competitive enzyme-linked immunosorbent assay kit (FSK Pentosidine; Fushimi Pharmaceutical Co, Ltd),[13,14] and for vitamin B6 (pyridoxine, pyridoxal, and pyridoxamine) using high-performance liquid chromatography were described in detail at elsewhere (supplementary methods). Serum levels of pyridoxine and pyridoxamine were expected to be low in vivo, and indeed, serum levels of pyridoxine and pyridoxamine were below the lower limit of detection (3.0 ng/ml and 0.6 ng/ml, respectively). Thus, we used serum pyridoxal levels to represent serum vitamin B6 levels.

Genotyping of Functional Polymorphisms in GLO1

A relatively common missense mutation (*rs4746*, *Glu111Ala*, minor allele frequency; schizophrenia = .28, controls = .13) in exon 4 of *GLO1* causes a decrease in enzyme activity, resulting in the accumulation of pentosidine.[6] The influence of this mutation on levels of pentosidine was also investigated by TaqMan genotyping methods (see supplemental methods).[11]

Statistical Analysis

Chi-square tests were used to assess differences in the distribution of frequencies (eg, gender). The differences in the serum pentosidine and pyridoxal levels between the unpaired groups were examined using the 2-tailed Mann-Whitney *U* test for 2-group comparisons and the Kruskal-Wallis test for comparison of 3 or more groups. The differences in the pentosidine and pyridoxal levels in paired samples of patient serum between the time of

Table 1. Clinical Variables and Serum Pentosidine and Pyridoxal Levels in Healthy Controls and Patients With Schizophrenia at Admission

	Controls	Patients With Schizophrenia	Statistical Test and *P* Value	
			Mann-Whitney *U*	
Variables	(*n* = 47)	(*n* = 137)	χ^2	*P*
Sex, M/F	17/30	68/69	2.55	.11
Age, mean (y)	31.0 ± 5.0 (22–48)	38.9 ± 13.9 (16–76)	12.8	**<.001**
Onset (y)	NA	23.8 ± 9.0 (12–53)		
Duration of education (y)	NA	12.4 ± 2.5 (9–20)		
Family history (y/n)	NA	46/91		
Duration of illness (y)	NA	17.9 ± 14.2 (0–56)		
DUP (mo)	NA	16.7 ± 32.2 (0–300)		
Number of admissions	NA	3.1 ± 2.3 (1–10)		
CP dose (mg/day)	NA	735.7 ± 577.9 (0-2625)		
BPRS (Total)	NA	61.5 ± 14.1 (34–96)		
(Positive)	NA	16.6 ± 4.6 (7–25)		
(Negative)	NA	10.8 ± 3.5 (3–19)		
Pentosidine, ng/ml	36.2 ± 10.2 (10.7–60.0)	36.2 ± 17.4 (11.6–135.6)	−1.44	.151
Pyridoxal, ng/ml	11.7 ± 6.4 (3.7–29.8)	8.8 ± 6.9 (0.1–40.7)	−3.71	**<.001**

Note: Data are the mean ± SD (range).
P values with statistical significance are in bold.
BPRS, Brief Psychiatric Rating Scale; CP dose, chlorpromazine equivalent dose; DUP, duration of untreated psychosis; NA, not applicable.

admission and the time of discharge were examined using the Wilcoxon matched-pairs signed-rank test. The correlations between clinical features, such as duration of hospitalization, and measured serum substance levels were analyzed using Pearson's correlation test. The correlations between the BPRS scores and measured serum substances were analyzed using Spearman's correlation test.

Results

Carbonyl Stress Markers in Patients With Schizophrenia at the Acute Stage

The characteristics of all participants are given in table 1. No significant differences were found in the gender distribution between healthy controls and schizophrenic patients (table 1). Significant differences were noted in the age between patients with schizophrenia and controls (table 1). Thus, the correlation between age and the main measurements, levels of serum pentosidine and pyridoxal, were first analyzed. Serum levels of pentosidine and pyridoxal did not show significant correlations with age in either patients (r = .04 and −.13, P > .05) or controls (r = .02 and −.20, P > .05).

No significant differences were found between serum pentosidine levels in patients at admission and healthy controls (table 1), but as previously reported,[6] extremely high pentosidine levels (>2 SD higher than the mean in controls, >57.6 ng/ml) were more frequently found in patients with schizophrenia (14 cases, 10.2%) than in controls (1 case, 2.1%) (table 1, figure 2A). Pyridoxal levels were significantly lower in patients with schizophrenia than in controls (table 1, figure 2B). Serum pentosidine and pyridoxal levels were not correlated at the time of admission in schizophrenia (r = −.153, P = .074) and well as in normal controls (r = .204, P = .169). With respect to clinical symptoms, the severity of symptoms was not significantly correlated with levels of pentosidine or pyridoxal. Interestingly, serum pentosidine levels from patients with schizophrenia at admission showed a significant positive association with daily chlorpromazine (CP) dose amount (r = .361, P < .001), whereas pyridoxal levels did not (r = −.028, P = .748). We established a speculative "total accumulation of dose amounts of antipsychotics" as "[(duration of illness)−(duration of untreated psychosis)]×(daily CP dose amount at admission)." The "total accumulation of dose amounts of antipsychotics" also showed a strong positive association with serum pentosidine levels (r = .490, P < .001) but not with pyridoxal levels (r = −.05, P = .569).

The schizophrenic patients in this study included 8 medication-free patients at admission. The levels of pentosidine in these 8 patients were lower than those in the 129 medicated patients, but the difference was not significant (unmedicated, 27.0 ± 9.3 ng/ml; medicated, 36.6 ± 17.7 ng/ml; χ^2 = 1.62; P = .10). Pyridoxal levels were also not significantly different (unmedicated, 8.1 ± 4.0 ng/ml; medicated, 8.8 ± 7.1 ng/ml; χ^2 = −0.32; P = .75). All clinical variables, such as age, duration of illness, duration of untreated psychosis, and numbers of admissions (table 1), did not show any significant correlations with levels of any carbonyl stress markers. Comparison of carbonyl stress markers between patients with and without a family history did not show a significant difference. Additionally, nutrition variables (body mass index, hemoglobin A1C, creatinine, glucose, total

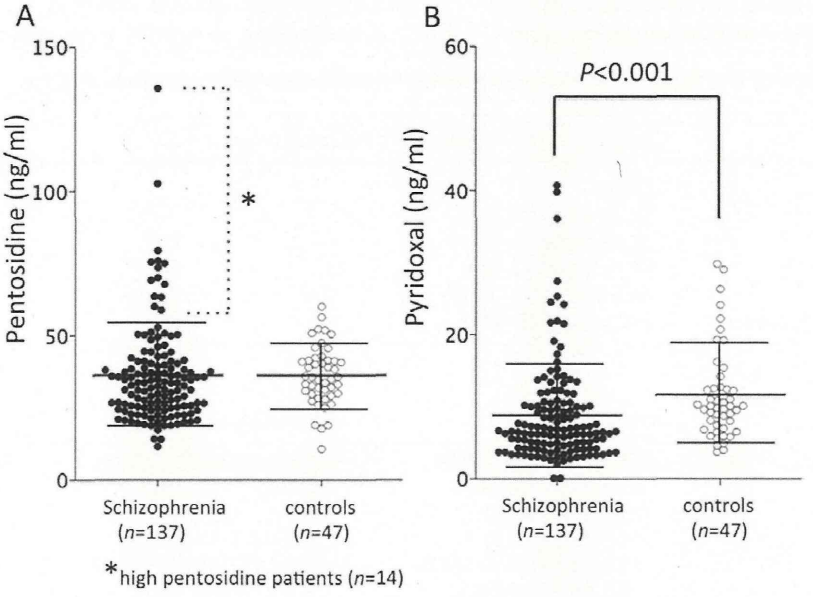

Fig. 2. Serum levels of carbonyl stress markers in normal controls and patients with schizophrenia at admission. A. Pentosidine. B. Pyridoxal. Fourteen patients with high pentosidine (>2 SD higher than the mean in controls, >57.6 ng/ml) are indicated with an asterisk. Values were compared with the 2-tailed Mann-Whitney U test. Error bars indicate mean and standard deviations.

cholesterol, triacylglycerol and total protein) and lifestyle factors (smoking and alcohol habit) were not associated with carbonyl stress markers (supplementary table 1).

Clinical Features of Patients With High Pentosidine Levels at the Acute Stage

Fourteen cases showed quite high pentosidine levels that were over 2 SD of control levels. A previous study suggested that these patients may be associated with development of a certain subtype of schizophrenia, carbonyl stress schizophrenia.[6] Thus, clinical variables were compared between patients with high pentosidine levels (>57.6ng/ml) and patients with normal levels (<57.6ng/ml) (in figure 2A, patients with high pentosidine levels are indicated with an asterisk). No significant differences in clinical symptoms or variables were found between patients with high pentosidine levels compared with those with normal pentosidine levels, except for the duration of illness and the daily CP dose amount (supplementary table 2). The duration of illness was significantly longer in high pentosidine patients (22.4±9.4 y vs 15.3±11.4 y, $\chi^2 = 2.221$, $P = .026$). Furthermore, the daily CP dose amount was approximately 2-fold higher in patients with elevated pentosidine (1291.8±636.2mg/day) than in patients with normal pentosidine (671.4±537.5mg/day, $\chi^2 = 3.598$, $P < .001$, supplementary table 2).

Change in Pentosidine and Pyridoxal Levels According to the Clinical Course of Schizophrenia

To account for potential bias, the clinical variables in table 1 were compared between the 53 discharged patients with paired samples and 81 discharged patients with admission data only. There were no significant differences in the clinical variables, including BPRS scores at discharge (data were not shown). Fifty-three patients with schizophrenia could be followed from the time of admission to discharge, enabling paired comparisons of their serum biomarkers (table 2, figure 3A and 3B). As expected, total BPRS and positive and negative symptoms were significantly improved from the time of admission to the time of discharge. The daily CP dose amount did not significantly change in these patients. The paired samples showed a marginally significant increase in pyridoxal levels from the time of admission to discharge, but this was not seen for pentosidine. Although the levels increased, the pyridoxal in patients with schizophrenia at discharge (9.3±4.9ng/ml) were still lower than those in controls (11.7±6.4ng/ml), and the difference was close to statistical significance (Mann-Whitney U, $\chi^2 = -1.96$, $P = .050$). Among the 53 cases with paired samples, the pentosidine were decreased in approximately half of the patients [26 cases (49%); 27 cases (51%) were increased] from the time of admission to discharge (figure 3A). Pyridoxal were increased in more than half of patients (34 cases [63%]; 18 cases [37%] were decreased) (figure 3B). Interestingly, only patients with decreased pyridoxal levels (18 cases, figure 3B) from the time of admission to discharge showed a significant correlation between their change in pyridoxal and change in total BPRS scores ($r = -.542$, $P = .025$) and positive symptom scores ($r = -.528$, $P = .029$). The greater the decrease in pyridoxal levels, the lesser the improvement in symptoms (supplementary figure 1). The remaining 3 subgroups (patients with an increase and/or decrease in pentosidine, and an increase in pyridoxal levels) did not show any correlations between changes in markers and changes in clinical symptoms (all $P > .05$). Among the 14 patients with high pentosidine (figure 2A and supplementary table 2), 7 patients provided paired samples. Of these, 5 patients showed a decrease in serum pentosidine levels, and the

Table 2. Changes in Characteristics and Test Scores in the 53 Patients With Schizophrenia That Were Followed Up

Variables	Paired-sample Patients With Schizophrenia (n = 53)			
Sex, M/F	27/26			
Age, mean ± SD, y	38.4±14.5 (17–76)			
Onset (range), y	25.6±10.5 (12–53)			
Duration of illness (range), y	14.3±12.6 (0.1–48)			
DUP (range), mo	20.3±28.1 (0.1–120)			
Duration of hospitalization (range), days	108.5±86.5 (2–411)			
			Wilcoxon test	
	At Admission	At Discharge	Z	P
CP dose, mg/day	836.7±677.4 (0-2625)	937.4±453.9 (150–2475)	1.60	.110
BPRS scores (Total)	60.7±14.0 (41–96)	38.9±9.4 (18–63)	−5.91	**<.001**
(Positive)	16.5±4.6 (7–24)	9.9±3.2 (3–18)	−5.82	**<.001**
(Negative)	10.5±3.2 (3–18)	8.5±3.2 (3–18)	−4.66	**<.001**
Pentosidine, ng/ml	37.1±20.6 (14.1–135.6)	38.2±18.2 (14.1–128.4)	0.85	.397
Pyridoxal, ng/ml	8.2±6.3 (2.5–36.1)	9.3±4.9 (0.1–27.4)	2.00	**.046**

P values with statistical significance are in bold.
Abbreviations are explained in the first footnote to table 1.

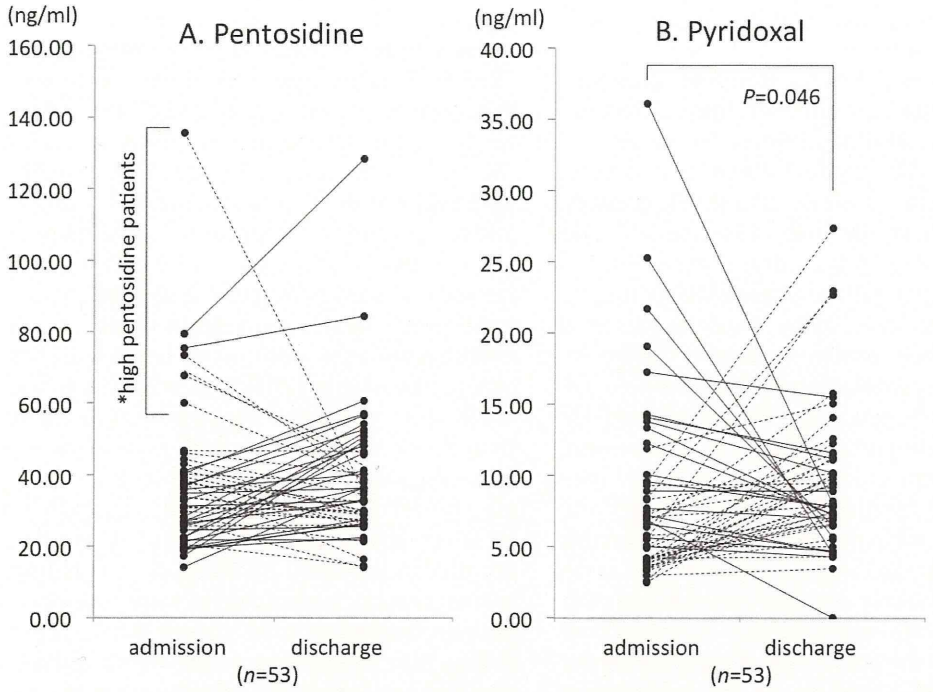

Fig. 3. Changes in serum carbonyl stress markers in paired-sample patients with schizophrenia (n = 53) who were followed from the time of admission to discharge. Dotted lines indicate a decrease in carbonyl stress (decrease in pentosidine and increase in pyridoxal), and solid lines indicate an increase in carbonyl stress (increase in pentosidine and decrease in pyridoxal) over time.

other 2 patients showed an increase (figure 3A, asterisk). One patient with a decrease in serum pentosidine levels showed no change in clinical symptoms, but the other 6 patients, including 2 with increased pentosidine levels, showed improvement in total, positive, and negative symptom scores from BPRS.

The changes [(value at "discharge" − value on "admission") / value at "admission"] in pentosidine and pyridoxal levels were not significantly correlated with changes in clinical symptoms (total, positive, and negative symptom cluster scores of BPRS: $r = -.092$ to .016, all $P > .05$). Δpentosidine and Δpyridoxal levels also did not show any significant correlation with Δdaily CP dose amount ($r = .105$ and $-.131$, respectively, all $P > .05$). In addition, Δpentosidine and Δpyridoxal levels did not show any correlation with each other ($r = .032$, $P = 0{,}821$).

At the time of discharge, nutrition variables did not show associations with carbonyl stress markers (supplementary table 1). In addition, at the time of discharge when accurate compliance was confirmed, there was no influence among the types of antipsychotics used (first-generation antipsychotics, FGA; second-generation antipsychotics, SGA; and concomitant use of both types) on carbonyl stress markers (supplementary table 3).

Classification of Patients With Schizophrenia by the Comprehensive Status of Carbonyl Stress

To investigate differences in clinical severity and improvement in symptoms, patients at admission and paired-sample patients were classified according to their comprehensive carbonyl stress status at the time of admission, discharge and during hospitalization (detailed categories, see supplementary table 4). Again, the degree of clinical symptoms and their improvements showed no significant differences among the groups categorized according to carbonyl stress status (supplementary table 5).

Genetic Influence of Functional Polymorphisms in GLO1 on Pentosidine and Pyridoxal Levels

The *rs4746* was genotyped in 74 patients with schizophrenia at admission, and 47 of these provided a sample at discharge and were considered as providing usable paired samples for comparison of a change in carbonyl stress markers. No patients had the homozygous minor allele (Ala/Ala). No significant differences in carbonyl stress marker levels were found between patients with Glu/Glu genotypes and Glu/Ala genotypes. In addition, Δcarbonyl stress markers were not different between these genotyped patients (supplementary table 6).

Discussion

This study reproduced a previous study by Arai et al[6] of schizophrenia in the chronic state. We also investigated whether the levels of serum carbonyl stress markers were altered in patients with schizophrenia even at the acute

stage, and if they changed according to the clinical course with multifarious parameters.

The pentosidine levels were not significantly altered in patients with schizophrenia and did not change according to the clinical course. The pentosidine levels did not show any correlation with physical factors, including nutrition status and lifestyle. However, as a previous study suggested,[6] some patients at admission (14 cases, 10.2%) indeed showed extremely high pentosidine (figure 2A). We expected that these patients can be referred to as having "carbonyl stress schizophrenia" with severe symptoms, resistance to treatment, and genetic features.[6,7] However, these 14 cases with high pentosidine showed no association with symptom severity, except for the duration of illness and daily CP dose amounts. Interestingly, the patient with the highest pentosidine (135 ng/ml) showed the highest daily CP dose amount (2625 mg/day). Furthermore, pentosidine levels from all patients with schizophrenia (n = 137) at admission showed a significant positive association with the daily CP dose amount, but not with the duration of illness. Thus, we speculate that accumulation of antipsychotics (dose × duration) may elevate the serum pentosidine levels. Indeed, analysis of the correlation between accumulation of antipsychotics before admission and pentosidine at admission showed a strong association. In addition, although the number of medication-free patients was small (n = 8), they showed a tendency for lower pentosidine levels than medicated patients. For patients with paired samples (n = 53) from admission to discharge, because their daily CP dose amount did not significantly increase (table 2), we found no significant association between Δpentosidine and Δdaily CP dose amounts. It may require only about 3 months (108.5 days) without an increase in the daily CP dose amount to increase pentosidine levels. However, these findings should be interpreted carefully, because drug-naive patients with an at-risk mental state show high pentosidine levels,[15] and the patients in this study with the highest pentosidine levels (135 ng/ml) showed a drastic decrease in pentosidine (35.3 ng/ml) that was accompanied by accumulation of the total CP dose amount (2475 mg/day and 245 days) and improvement in symptoms (Δtotal BPRS; −48.8%). Although the antipsychotic dose amount is likely a major factor in increasing pentosidine levels, other factors may also contribute.

The findings for pyridoxal were as follows: (1) levels were lower in schizophrenia compared with normal controls; (2) levels increased according to the clinical course, although the increased levels were still lower than those of normal controls (not statistically significant); and (3) 18 patients who showed a decrease in pyridoxal levels according to the clinical course showed that the greater the decrease in pyridoxal, the less improvement in symptoms. These findings were consistent with a previous study that also showed significantly lower serum pyridoxal in schizophrenia.[6] The present study reproduced these previous data, and we also speculate that these lower levels increased according to the clinical course from a worse state to a better state. Carbonyl stress may be an aspect of the pathophysiology of schizophrenia, because pyridoxal levels increased as symptoms improved. A major limitation of the present study is that we could not infer what the lower serum pyridoxal and the increase in clinical course directly reflected, because we could not show either a correlation between pyridoxal and the severity of symptoms at admission, or between a change in pyridoxal levels and the degree of improvement in symptoms according to the clinical course. It is difficult to presume that lower pyridoxal levels were caused by poor nutrition status at admission, because at the time of discharge, the nutrition status of all patients was good due to the hospital diets, and the pyridoxal levels were still lower than those in controls, as with previous study.[6] Of importance regarding the lower pyridoxal levels in schizophrenia, the details of the mechanism (eg, higher consumption or lower absorption) are unknown, but the lower levels are almost certainly involved in the pathophysiology of schizophrenia. Interestingly, some patients with schizophrenia showed that the greater the decrease in pyridoxal during their clinical course, the worse carbonyl stress likely was and the lowest the improvement in symptoms, especially positive symptoms, was observed. Even in chronic schizophrenia, lower pyridoxal levels show correlation with more severe symptoms, especially positive symptoms.[6] Thus, our present findings for pyridoxal in the acute stage could be interrelated with pyridoxal in the chronic stage in patients with treatment-resistant schizophrenia.[6] Pyridoxal is a candidate for augmentation therapy, perhaps not for all patients, but for treatment-resistant patients with lower pyridoxal levels and/or in cases where levels decrease during the clinical course. Taking supplemental vitamin B6 from the early stage of the disease, not from the late stage, may also be beneficial.[16]

We determined that there were no clinical features in relation to an assumed severe carbonyl stress status using a combination of pentosidine and pyridoxal levels in a cross-sectional and longitudinal study (supplementary tables 4 and 5). We found no important features, including related major genetic factors (supplementary table 6). Although some patients experienced carbonyl stress, this was not reflected in the severity of symptoms or other clinical features at the acute stage. The mechanism responsible for high pentosidine levels could be partly due to an accumulation of the daily dose of antipsychotics until the long-term clinical course, but other unknown factors are likely involved in schizophrenia. Only a decrease in pyridoxal during the clinical course reflected the low improvement of symptoms relatively early in the disease. The observation that altered pyridoxal and pentosidine levels in schizophrenia indicate only the existence of carbonyl stress in these patients and these levels did not directly contribute to the development of the disease. If anything, the identification of RCO-modified proteins might reveal more direct associations with the disease or its severity.

The limitations of the methodology of the present study were (1) healthy controls were younger; (2) the interval of the 2 measurements of biological markers and the duration of hospitalization were not controlled; and (3) the type of drug therapy was not controlled. Factors (1) and (2) are not likely to be major factors associated with carbonyl stress marker levels because correlations with these variables were not observed. For factor (3), there were no differences in the levels of carbonyl stress markers among the types of antipsychotics (FGA and SGA). However, these factors, in particular the duration of illness and individual antipsychotics should be controlled to properly assess the potential of carbonyl stress markers as "therapeutic" biological markers for schizophrenia. From a genetic point of view, we only investigated one major functional polymorphism in *GLO1*. Other gene-gene interactions, such as 22q11.2 deletion, paired-like homeobox 2b, and *GLO1*, may cause an increase in carbonyl stress.[17]

Conclusion

From the point of view of the carbonyl stress status, we can classify patients with schizophrenia as patients (1) with extremely high pentosidine levels that may be caused by higher antipsychotic dose amounts; (2) with lower pyridoxal levels that increased according to the clinical course; and (3) with pyridoxal levels that decreased according to the clinical course and that were accompanied by less improvement in symptoms. The role of carbonyl stress in schizophrenia is gradually being elucidated as a diagnostic and therapeutic biological marker.

Supplementary Material

Supplementary material is available at http://schizophreniabulletin.oxfordjournals.org.

Funding

Juntendo Institute of Mental Health (201109, 201209).

Acknowledgments

All authors contributed to the conceptualization, design, and writing of this manuscript. No authors have any conflicts of interest to disclose pertaining to this article.

References

1. Marchbanks RM, Ryan M, Day IN, Owen M, McGuffin P, Whatley SA. A mitochondrial DNA sequence variant associated with schizophrenia and oxidative stress. *Schizophr Res*. 2003;65:33–38.
2. Prabakaran S, Swatton JE, Ryan MM, et al. Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress. *Mol Psychiatry*. 2004;9:684–97, 643.
3. Yao JK, Reddy RD, van Kammen DP. Oxidative damage and schizophrenia: an overview of the evidence and its therapeutic implications. *CNS Drugs*. 2001;15:287–310.
4. Flatow J, Buckley P, Miller BJ. Meta-analysis of oxidative stress in schizophrenia. *Biol Psychiatry*. 2013;74:400–409.
5. Jaisson S, Gillery P. Evaluation of nonenzymatic posttranslational modification-derived products as biomarkers of molecular aging of proteins. *Clin Chem*. 2010;56:1401–1412.
6. Arai M, Yuzawa H, Nohara I, et al. Enhanced carbonyl stress in a subpopulation of schizophrenia. *Arch Gen Psychiatry*. 2010;67:589–597.
7. Miyashita M, Arai M, Kobori A, et al. Clinical features of schizophrenia with enhanced carbonyl stress. *Schizophr Bull*. September 23, 2013. doi:10.1093/schbul/sbt129.
8. Overall JE,Gorham DR. The Brief Psychiatry Rating Scale. *Psychol Rep*. 1962;10:799–812.
9. Bech P, Kastrup M, Rafaelsen OJ. Mini-compendium of rating scales for states of anxiety depression mania schizophrenia with corresponding DSM-III syndromes. *Acta Psychiatr Scand Suppl*. 1986;326:1–37.
10. Hatano T, Ohnuma T, Sakai Y, et al. Plasma alanine levels increase in patients with schizophrenia as their clinical symptoms improve-Results from the Juntendo University Schizophrenia Projects (JUSP). *Psychiatry Res*. 2010;177:27–31.
11. Maeshima H, Ohnuma T, Sakai Y, et al. Increased plasma glutamate by antipsychotic medication and its relationship to glutaminase 1 and 2 genotypes in schizophrenia – Juntendo University Schizophrenia Projects (JUSP). *Prog Neuropsychopharmacol Biol Psychiatry*. 2007;31:1410–1418.
12. Ohnuma T, Sakai Y, Maeshima H, et al. Changes in plasma glycine, L-serine, and D-serine levels in patients with schizophrenia as their clinical symptoms improve: results from the Juntendo University Schizophrenia Projects (JUSP). *Prog Neuropsychopharmacol Biol Psychiatry*. 2008;32:1905–1912.
13. Jinno M, Takeuchi M, Watanabe A, et al. Advanced glycation end-products accumulation compromises embryonic development and achievement of pregnancy by assisted reproductive technology. *Hum Reprod*. 2011;26:604–610.
14. Sanaka T, Funaki T, Tanaka T, et al. Plasma pentosidine levels measured by a newly developed method using ELISA in patients with chronic renal failure. *Nephron*. 2002;91:64–73.
15. Arai M, Koike S, Oshima N, et al. Idiopathic carbonyl stress in a drug-naive case of at-risk mental state. *Psychiatry Clin Neurosci*. 2011;65:606–607.
16. Lerner V, Miodownik C, Kaptsan A, Cohen H, Loewenthal U, Kotler M. Vitamin B6 as add-on treatment in chronic schizophrenic and schizoaffective patients: a double-blind, placebo-controlled study. *J Clin Psychiatry*. 2002;63:54–58.
17. Toyosima M, Maekawa M, Toyota T, et al. Schizophrenia with the 22q11.2 deletion and additional genetic defects: case history. *Br J Psychiatry*. 2011;199:245–246.
18. Monnier VM, Sell DR, Saxena A, et al. Measurement of oxidative stress: Technology, biomarkers and applications. Glycoxidative and carbonyl stress in aging and age-related diseases. In: Cutler RG, Rodriguez H, eds. *Critical Reviews of Oxidative Stress and Aging.Advances in Basic Science, Diagnostics and Intervention*, Vol 2. Singapore: World Scientific; 2003:414–426.

展望

統合失調症の遺伝子研究における課題と展望*

異種性と複雑系

糸川昌成[1,2]　新井　誠[1]　宮下光弘[1,2]　小堀晶子
畠山幸子　鳥海和也[1]　市川智恵　大島健一[1,2]
新里和弘　岡崎祐士　齋藤正彦

Key words

Heterogeneity, Complex system, Carbonyl stress, Association study

はじめに

統合失調症に遺伝要因が関連することから，原因解明には遺伝子研究が有望な手段と考えられてきた。しかしながら，遺伝子研究は当初考えられていたほど病態解明に結びつく成果を挙げていない。本稿では，統合失調症を対象とする遺伝子研究が難航してきた経緯を紹介し，近年盛んに取り組まれている大規模研究とは異なった手法を試みた自験例に触れ，臨床的視点の意義と重要性について考察する。

統合失調症の遺伝要因

統合失調症に罹患した人の親族では，一般人口より統合失調症の発症危険率が高いとされている。最初の報告は，1928年に発表されたWeinberg[20]の論文であり，以後1960年代までに25編に及ぶ報告がある。東京大学脳研究施設の井上は，これら25編に双生児研究11編と一般集団の発症率調査26編を加えて解析し，統合失調症の遺伝要因について検討した(図1)[7]。この結果をみると，統合失調症発端者の親族における発症危険率はいずれも一般集団の0.82～0.83より大きい。しかも，発端者と血縁が近い――同じ遺伝子型を共有する率が高い――ほど発症危険率が高くなっている。このデータは，統合失調症の発症に遺伝要因が関連している事実を示していると考えられた。しかし，井上は，この解釈に慎重であるべき点を指摘した。すなわち，血縁が近いほど環境要因も共有するものが多くなるはずであり，血縁の近さと発症危険率の相関をそのまま遺伝要因に関連付けることができないとした。

* Assignment and Perspective of Genetic Research on Schizophrenia：Heterogeneity and complex system

1) 東京都医学総合研究所統合失調症・うつ病プロジェクト(〒156-8506 東京都世田谷区上北沢2-1-6)，Itokawa Masanari, Arai Makoto, Miyashita Mitsuhiro, Kobori Akiko, Hatakeyama Sachiko, Toriumi Kazuya, Ichikawa Tomoe, Oshima Kenichi, Niizato Kazuhiro, Okazaki Yuji, Saito Masahiko：Project for Schizophrenia and Affective Disorders Research, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan
2) 東京都立松沢病院精神科，Department of Psychiatry, Tokyo Metropolitan Matsuzawa Hospital

0488-1281/13/￥500/論文/JCOPY

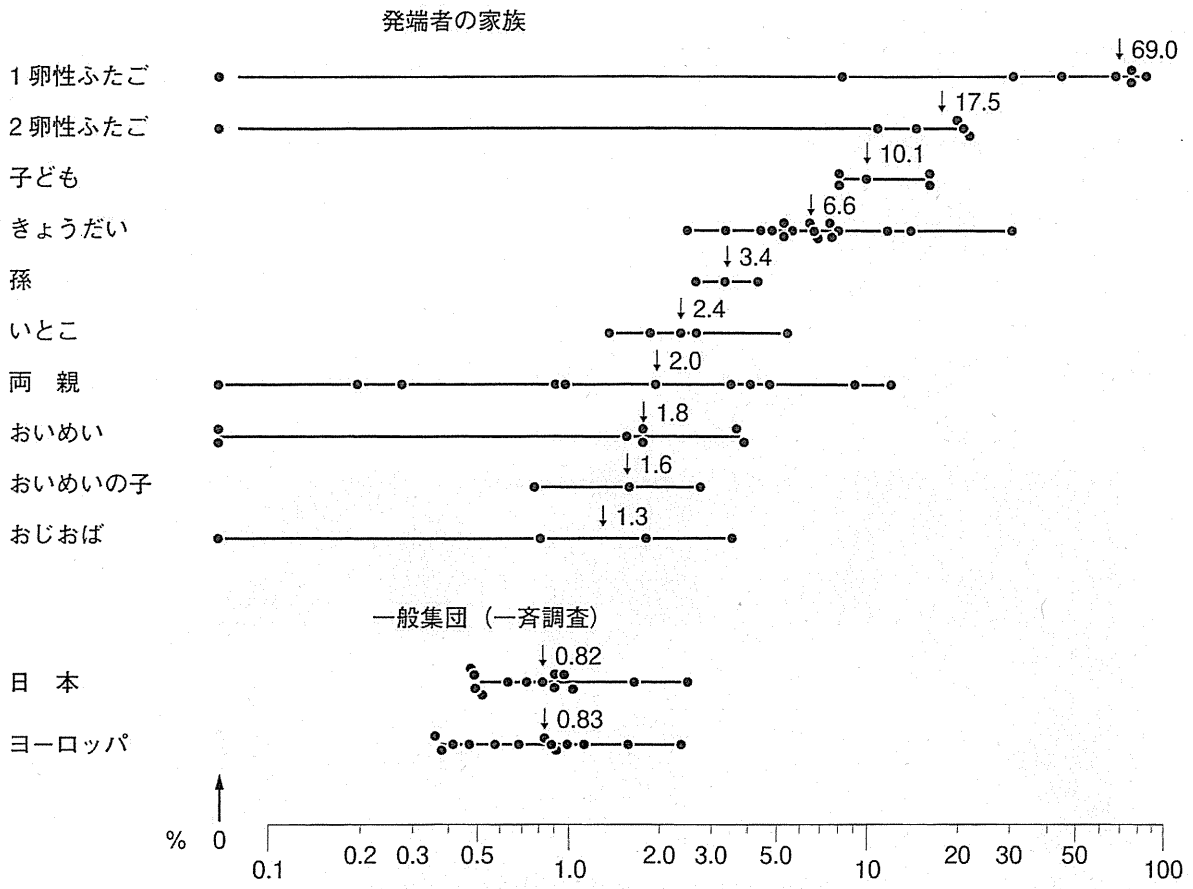

図1　統合失調症の発症危険率（↓は中位数）[7]
統合失調症発端者の親族における発症危険率はいずれも一般集団の0.82～0.83より大きく，発端者と血縁が近いほど発症危険率が高くなっている。

井上も指摘したような家族研究の課題を解決するために，多くの養子研究が行われた。すなわち，「血縁が近いほど環境も近い」親子を，養子縁組によって「血縁が近くても環境は近くない」組み合わせにして，発症危険率を検証した。代表的な養子研究にHarvard大学精神科教授のKetyらの研究がある[11]。Ketyらは米国NIMH（National Institutes of Mental Health）のRosentalと協力して，Denmark司法省の記録をもとに，1924年から1948年までに縁組された実父母と里親の間に血縁がない5,483組の養子を調査した。その結果，精神科受診歴を持った養子が507名見つかった。この中から英語圏の診断基準に照らして統合失調症と診断しうる33例の発端者を抽出した。対照には，年齢，性別，社会経済的水準，養子前生活状況が発端者らと等しい33例の精神科受診歴のない養子を選別した。この66例の養子（統合失調症33例，対照33例）について，Denmark人口台帳を用いて生物学的あるいは養子先の両親，同胞，半同胞（異母兄弟姉妹）など463名の親族を調べた。

33例の統合失調症発端者には150例の血縁者がいたが，その中から13例が統合失調症を発症していた。対照33例には156例の血縁者がいたが3例しか統合失調症を発症しなかった（$p=0.0072$）（表1上段）。一方，統合失調症発端者33例の養子先親族74例のうち統合失調症は2例で，対照33例の養子先親族83例中統合失調症3例と有意差がなかった。もし，統合失調症の発症に環境要因が遺伝要因より大きく作用するのであれば，生物学的親族の発症率は発端者（150例）・対照（156例）間で有意差がなく，養子先親族の発端者（74例）・対照（83例）間で有意差がつくはずである。Ketyのデータはそうならなかったこと

表1　養子における生物学的親族と里親の親族における統合失調症発症率[7]

被験者	生物学的親族(総数)	養子先の親族(総数)
総被験者		
発端者(n=33)	13(150)	2(74)
対照(n=33)	3(156)	3(83)
有意水準*	p=.0072	n.s.
生後1か月以内に養子に出された被験者		
発端者(n=19)	9(93)	2(45)
対照(n=20)	0(92)	1(51)
有意水準*	p=.0018	n.s.

文献7より改変して引用(原文は英語)。

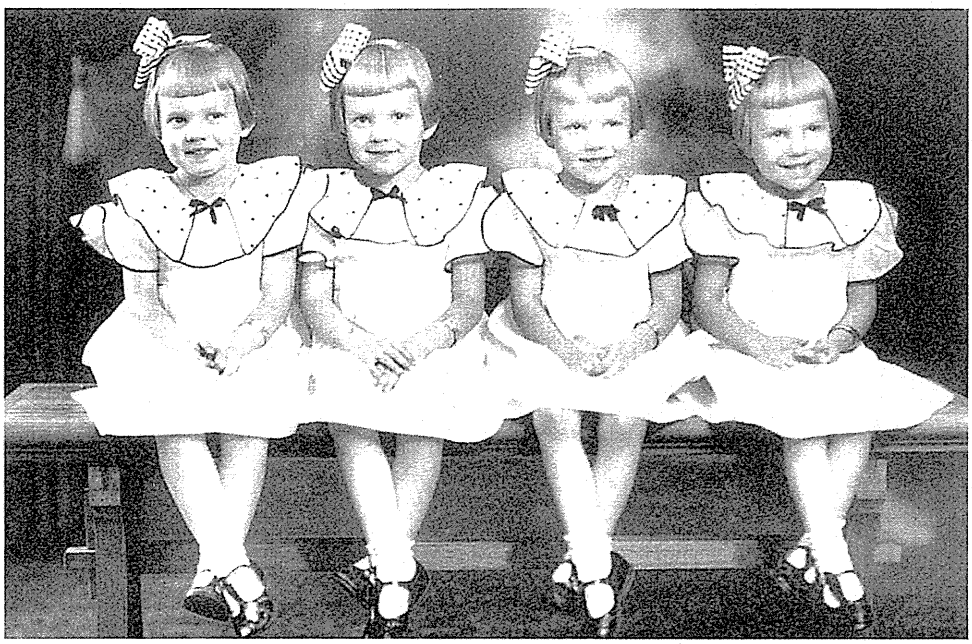

図2　Genain家の一卵性4つ子
1930年，Genain家にNora，Iris，Myra，Hesterの4人姉妹が生まれ，1951年から5年のあいだに相次いで4人とも統合失調症を発症した。Barondes S. H. Molecules and Mental Illness Scientific American Library 1993より引用。

から，統合失調症への遺伝要因の関連が示唆された。1か月以内に養子に出された発端者19例と対照20例の比較では，生物学的親族の発症率の有意差が強まっている(p=0.0018)(表1下段)。これについて，Ketyらは精神科的に問題をかかえた親ほど早めに養子に出す傾向があったためだろうと考察している。

Genain家の4つ子

Rosentalらは，上述の養子研究を発表する10年前に，興味深い一卵性4つ子の統合失調症一致例を報告している(図2)[18]。Henrry Genainというドイツ系アメリカ人の農夫と，その妻Gertrude Hood Genainの間に，1930年，Nora，Iris，Myra，Hesterの4人姉妹が生まれた。1951年にNoraが21歳で統合失調症を発症し，Irisが22歳，Hesterが24歳，Myraも25歳で相次いで発症した。Genain家の一卵性4つ子は，発症一致という点で統合失調症と遺伝要因をシンボリックに関連付ける存在だった。一方で，4人は臨床的に多くの不一致点を呈しており，疾患に果たす遺伝要因の複雑さも示している[14]。

Noraは速記書記として2年間勤務したが，幻聴と妄想を訴えるようになりNIMHへ3年間に4回入院し電気痙攣療法を19回施された。州立病院へ転院後さらに18か月入院したのち自宅へ退院し母親と同居したが，その後も2回の入院歴がある。

Irisは，身体症状の訴え，不眠，注察妄想，幻聴，カタレプシーを呈してNIMHに3年入院したあと，州立病院へ転院し18年入院した。入院歴は6回であり，合計67回電気痙攣療法を受けた。退院後はケアホームに入所し，デイケアに通った。

Myraは，精神運動静止，ヒステリー様症状，反復性の抑うつ状態，不安を認め，中間施設に入所しながらNIMHで治療を受けた。退院後に抗精神病薬を中止して事務職に就き，1966年には結婚して2人の男児を出産した。1976年に上司が代わったのをきっかけとして妄想的となり2か月州立病院へ入院している。その後，自殺企図で3週間入院歴があるが，電気痙攣療法を受けたことはない。

Hesterは情緒不安定で高校を中退した。25歳のとき恐怖，昏迷，幻聴を認めNIMHへ3年入院した後，州立病院へ転院し12年入院した。

4姉妹は州立病院を含め複数回にわたり診断の記録があるが，NIMHが1955年と1981年に4姉妹の診断を行っている。1981年の診断はDSM-Ⅲを用いており，Noraがchronic undifferentiated affective component，Irisがundifferentiated (paranoid)＋affective features，Myraがschizoaffective (schizophrenia residual)，Hesterがchronic undifferentiatedである[14]。

4姉妹は一卵性にもかかわらず臨床症状は多彩であり，下位診断も不一致がみられた。経過と予後についても投薬を中止して結婚し出産，就労まで遂げたMyraから，電気痙攣療法を67回も受けたIrisまで大きな違いがみられた。ちなみに，GenainはRosentalらの作った仮名である(4姉妹の頭文字をとるとNIMHになる)。

候補遺伝子研究

1990年代に入ると候補遺伝子の多型頻度を症例・対照で比較する関連研究が盛んに行われるようになった。筆者らも，ドーパミン仮説に基づいてドーパミンD_2受容体を候補遺伝子とした多型解析を行った。D_2受容体は443アミノ酸からなるが，311番目のセリンがシステインに置換するミスセンス多型(S311C)を新規に同定した(図3)[8]。156例の統合失調症と300例の健常対照でS311Cの頻度を比較したところ，S311Cが有意に統合失調症で高い頻度で認められた($p<0.01$)[4]。アレル頻度は対照で1.8%，統合失調症156例で5.4%だったが，発症年齢が25歳以下の89例では9.0%，家族歴のある28例では13.5%と上昇した。また，Manchester scaleで評価した臨床症状の重症度を野生型(セリン/セリン)とシステインアレルを持つ症例(セリン/システイン，システイン/システイン)で比較すると，幻覚と妄想の項目では有意差がなかったが，思考滅裂($p=0.022$)，感情の平板化($p=0.006$)，精神運動静止($p=0.045$)では有意にシステインアレルを持つ症例の得点が低かった。

2011年までに31編の追試が発表されたが，オッズ比が1.0を超えた報告は18編にとどまり，関連を否定する研究が数多くみられた。2013年までに4件のメタ解析が行われ，いずれも有意にシステインが統合失調症で対照より頻度が高いとした(表2)[5,6,9,13]。

筆者らのS311Cのように，有意な関連が報告されると，それが再現されたと肯定する報告と有意差は認められないと否定する追試が蓄積されメタ解析によって決着をみるといったパターンが，その後の候補遺伝子研究全般で繰り返された。2011年までに統合失調症の候補遺伝子研究は1,727編発表され，遺伝子数は1,008(8,788多型)におよび，メタ解析は237件行われた[19]。しかし，ほとんどのメタ解析は1.5前後のオッズ比を示す弱い効果にとどまり，統合失調症の病態を明確に説明できるほど強い影響を与える遺伝子多型