III. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
今村明、橋田あおい、中根允文	自閉症—幼児期精神病から発達障害へ—第10章遺伝研究	高木隆郎編	自閉症—幼児期精神病から発達障害へ—	星和書店	東京	2009	121-137
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宮田雄吾	こころの病気が分かる絵本　第2巻　てあらいがとまらないアライグマ[強迫性障害]			情報センター出版局	東京	平成22年	48
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IV. 研究成果の刊行物・別刷一覧

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ACTA PSYCHIATRICA SCANDINAVICA

Psychotic-like experiences are associated with suicidal feelings and deliberate self-harm behaviors in adolescents aged 12–15 years

Nishida A, Sasaki T, Nishimura Y, Tanii H, Hara N, Inoue K, Yamada T, Takami T, Shimodera S, Itokawa M, Asukai N, Okazaki Y. Psychotic-like experiences are associated with suicidal feelings and deliberate self-harm behaviors in adolescents aged 12–15 years.

Objective: Psychotic disorders are a significant risk factor for suicide, especially among young people. Psychotic-like experiences (PLEs) in the general population may share an etiological background with psychotic disorders. Therefore, the present study examined the association between PLEs and risk of suicide in a community sample of adolescents.
Method: Psychotic-like experiences, suicidal feelings, and self-harm behaviors were studied using a self-report questionnaire administered to 5073 Japanese adolescents. Depression and anxiety were evaluated using the 12-item General Health Questionnaire (GHQ).
Results: The presence of PLEs was significantly associated with suicidal feelings (OR = 3.1, 95% CI = 2.2–4.5) and deliberate self-harm behaviors (OR = 3.1, 95% CI = 2.0–4.8) after controlling for the effects of age, gender, GHQ-12 score, victimization, and substance use. Suicidal feelings and behaviors were more prevalent in subjects with a greater number of PLEs.
Conclusion: Psychotic-like experiences may increase the risk of suicidal problems among adolescents.

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Key words: psychotic-like experiences; adolescents; community sample; suicide; self-harm behaviors

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Significant outcome

- Psychotic-like experiences are associated with suicidal feelings and deliberate self-harm behaviors among adolescents aged 12–15 years.
- Suicidal feelings and deliberate self-harm behaviors are more prevalent in subjects with a greater number of psychotic-like experiences.

Limitations

- This is a cross-sectional study and subjects were recruited from public schools.
- A self-report questionnaire was used to assess the psychotic-like experiences, suicidal feelings, and self-harm behaviors.
- There was a lack of data about confounding factors, such as individual personality and family circumstances.

Introduction

Risk of suicide is significantly higher for subjects with psychotic disorders when compared to the general population (1, 2). The risk is even more pronounced during the early phase of the disorders (2, 3), with two-thirds of suicides occurring during the first 5 years after diagnosis (4, 5). As the onset of first episode of psychosis usually occurs in young people (6), the risk of suicide may be especially remarkable in young people with the disorders (7, 8).

Psychotic-like experiences (PLEs) are subclinical hallucinatory and delusional experiences. PLEs occur not only in persons with psychotic disorders but also in people in the community who may not have been clinically diagnosed with psychoses (9–11). It may be reasonable to suspect that PLEs might share an etiological background with psychotic disorders (9, 10, 12, 13). Previous epidemiologic studies have reported that PLEs were observed in more than 10% of the general population of adults (10, 11, 13). Although PLEs were originally studied in adult populations (10, 11), recent investigations suggest that PLEs may also frequently occur in children and adolescents (14–17). In longitudinal studies, PLEs in childhood and adolescence were identified as a risk factor for later psychiatric disorders and poor psychosocial outcomes (18, 19). There are few studies that have investigated the relationship of PLEs and the risk for suicide among young people in the community.

Aims of the study

The present study thus aimed to examine the associations of psychotic-like experiences (PLEs) with suicidal feelings and deliberate self-harm behaviors in a large community sample of adolescents.

Material and methods

Sample and survey procedures

In 2006, we recruited subjects (ages 12–15 years) from public junior high schools (7th–9th grade) and conducted a cross-sectional survey of psychopathologies among younger adolescents in Tsu-city, Mie Prefecture, Japan (16). Mie Prefecture is located in the central region of Japan, and Tsu-city is the prefectural capital. The total population of Tsu-city is approximately 280 000. There are 20 public junior high schools (with a total of 7127 students at the time of the survey) and attendance is compulsory, in accordance with Japanese law.

After the study was approved by the ethics committee of Mie University School of Medicine, the principal investigators (A.N. & Y.O.) approached the school principals about participation in the study. The principals then consulted with teachers and parents.

In the participating schools, the teachers were instructed on the guidelines for distribution and collection of questionnaires; then the teachers distributed the questionnaires and the envelopes to the students. The teachers also explained: i) that participation in the study was anonymous and voluntary, and ii) that strict confidentiality would be maintained. In addition, the students were asked to seal the completed questionnaire in the provided envelope. Each teacher also reported the total number of present and absent students (including those students who had been absent for more than a month) on the day of the survey. Research staff collected the sealed questionnaires at each school.

Measures

The questionnaires included items regarding the following: i) psychopathological and behavioral problems including PLEs, suicidal feelings and deliberate self-harm behaviors; ii) the Japanese version of the 12-item General Health Questionnaire (GHQ-12); and iii) other variables, including demographic characteristics. An expert in child and adolescent psychologist (N.K) and three schoolteachers (including a Japanese language teacher) from the participating schools examined the questions for age appropriate language and reading comprehension.

Psychotic-like experiences

Psychotic-like experiences were assessed using four items adopted from the schizophrenia section of the Diagnostic Interview Schedule for Children (DISC-C) (20). These items were previously used in a birth cohort study and were good predictors of schizophreniform disorder in adulthood (18). The items were as follows: i) 'Some people believe that their thoughts can be read. Have other people ever read your thoughts?' (thoughts read); ii) 'Have you ever had messages sent especially to you through the television or radio?' (special messages); iii) 'Have you ever thought that people are following you or spying on you?' (spied-upon); and iv) 'Have you ever heard voices that other people cannot hear?' (heard voices). Possible responses included: 'no', 'yes, likely' and 'yes, definitely'. We defined 'yes, definitely' as the presence of a hallucinatory and delusional experience and 'no' or 'yes, likely' as

no experience. The number of present experiences was designated as the 'total PLEs score', with a range of 0–4.

Suicidal feelings and deliberate self-harm behaviors

Questions about lifetime experiences of suicidal feelings and deliberate self-harm behaviors in the previous year were included in the questionnaire. The item I – 'Have you ever had thoughts that your life is no longer worth living?', included four possible responses ('no', 'probably no', 'possibly yes' and 'yes') (21), and the item II – 'Have you intentionally hurt yourself within the past year?', included two possible responses ('yes' or 'no').

The GHQ-12

The GHQ-12 is one of the most widely used self-report screening tools for non-psychotic psychiatric symptoms, particularly symptoms of anxiety or depression (22). The validity and reliability of the Japanese version of the GHQ-12 have been confirmed (23, 24). The GHQ was originally applied to adult populations and subsequently used and validated for younger populations (25–28). A 4-point scale with binary scoring (0011), which is known as the GHQ method, was used for each of the questions. Responses of '1' were then added together to form the total score, with a range from 0 (best possible) to 12 (worst possible).

Other variables

Suicidal problems among young populations might be affected by other confounding factors, such as victimization and substance use, as reported in previous studies (29–32). In our questionnaire, we asked the participants about the experiences of being bullied (within the past year), violence from adults in the home (within the past month), alcohol use (within the past month), and use of recreational drugs (lifetime). The items on victimization ('being bullied' and 'violence from adults in the home') were answered as 'yes' or 'no'. The items on alcohol use and use of recreational drugs were answered as 'not at all' or 'once or more than once'.

Statistical analysis

Associations between PLEs and the lifetime experience of suicidal feelings or deliberate self-harm behaviors in the previous year were analyzed using logistic regression analysis adjusted for age, sex, GHQ-12 total score, victimization ('being bullied' and 'violence from adults in the home') and substance use (alcohol use and use of recreational drugs). 'Suicidal feelings' or 'deliberate self-harm behaviors' were the dependent variables.

Associations between each of the four PLEs (thoughts read, special messages, spied-upon, heard voices), and suicidal feelings or self-harm behaviors were tested by comparing individuals with each PLE to those without that type of PLEs. In addition, the effect of the total PLEs score was tested. Regarding the total PLEs score, scores of 3 and 4 were merged, and the subjects were classified into three subgroups according to Lataster et al. (33): 'no PLEs', '1 PLE' and '2 or more PLEs' groups. For the item about suicidal feelings, the responses on a 4-point scale, were converted into binary scoring (0001) when employed as a dependent variable in logistic regression.

All statistical analyzes were conducted using the Statistical Package for Social Sciences (SPSS), version 15.0 for Windows (SPSS Japan Inc., Tokyo, Japan). A P-value < 0.05 was considered statistically significant.

Results

Descriptive statistics

Fourteen out of the 20 public junior high schools in Tsu-city, with a total of 5335 students, agreed to participate in the survey. On the day of the survey, 205 students (3.8%) were absent (57/205 were long-term absentees); 18 (0.3%) refused to participate; 16 (0.3%) submitted blank questionnaires; and 23 (0.4%) gave all 'yes' answers (apparently frivolous). There were 179 (out of 5073) questionnaires missing data for critical items (PLEs, suicidal feelings, deliberate self-harm behaviors, sex, and age). Finally, we analyzed 4894 questionnaires which represented 92.1% of all junior high school students in the 14 participating schools. The demographics included: students aged 12–15 years [2523 boys (51.6%) and 2371 girls (48.4%), age: 13.3 ± 0.9 years (mean ± SD)]. Table 1 summarizes the GHQ-12 scores for the students by grade. The mean GHQ-12 scores were higher in the upper grades (Table 1).

Prevalence of PLEs, suicidal feelings, and deliberate self-harm behaviors

The prevalence of the four PLEs was as follows: 'thoughts read' was observed in 76 subjects (1.6%), 'special messages' in 33 (0.7%), 'spied-upon' in 363 (7.4%), and 'heard voices' in 487 (10.0%). The experience of at least one type of PLE was reported by 746 (15.2%); 182 students (3.7%) experienced

two or more symptoms of PLEs. The experience of lifetime suicidal feelings was observed in 908 (18.6%; 337 boys and 571 girls), while the experience of deliberate self-harm behaviors in the previous year was reported by 250 (5.1%; 75 boys and 175 girls).

Associations between PLEs and suicidal feelings/deliberate self-harm behaviors

The effect of each of the four PLEs was analyzed by logistic regression. After controlling for age, sex, non-psychotic psychiatric symptoms (the GHQ-12 score), victimization, and substance use; suicidal feelings were significantly associated with 'thoughts read', 'spied-upon', and 'heard voices' (Table 2). Deliberate self-harm behaviors were significantly associated with 'spied-upon' and 'heard voices' (Table 3).

The total PLEs score was significantly associated with suicidal feelings and deliberate self-harm behaviors, indicating that suicidal feelings and behaviors were more prevalent in subjects with a greater number of PLEs (Tables 4 and 5).

Discussion

The current study is the first to investigate and clearly show that PLEs are significantly associated with suicide-related problems in a community sample of younger adolescents. The risk of suicidal feelings and deliberate self-harm behaviors increases when more types of PLEs are experienced. The subjects experienced two or more types

Table 1. Demographic variables, mean General Health Questionnaire (GHQ-12) scores by school grade (n = 4894: males, 2523; females, 2371)

	No. subjects			Age	GHQ-12 score	
School grade	All	Male (%)	Female (%)	Mean	Mean	SD
Grade 7	1580	831 (52.6)	749 (47.4)	12.30	2.72	2.61
Grade 8	1645	842 (51.2)	803 (48.8)	13.29	3.15	2.79
Grade 9	1669	850 (50.9)	819 (49.1)	14.31	3.70	2.93
Overall	4894	2523 (51.6)	2371 (48.4)	13.32	3.20	2.81

Table 2. Associations between suicidal feelings and psychotic-like experiences in Japanese adolescents aged 12–15 years (n = 4894)

	Lifetime prevalence of suicidal feelings*		Unadjusted odds ratio			Adjusted odds ratio‡§		
Psychotic-like experiences (PLEs)	n	%	OR†	95% CI	P-value	OR†	95% CI	P-value
Thoughts read (n = 76)	33	43.42	3.45	2.18–5.46	<0.001	2.47	1.40–4.34	0.002
Special messages (n = 33)	12	36.36	2.52	1.24–5.14	0.011	1.93	0.83–4.47	0.127
Spied-upon (n = 363)	173	47.66	4.69	3.76–5.84	<0.001	2.44	1.87–3.18	<0.001
Hearing voices (n = 487)	203	41.68	3.74	3.07–4.56	<0.001	2.26	1.79–2.87	<0.001

*The number of individuals with the lifetime experience of suicidal feelings among those with each PLE.
†Odds ratio comparing the groups with and without PLE.
‡Odds ratio adjusted forage, sex, the total score of the GHQ-12, being bullied, violence from adults in the home, alcohol use, and use of recreational drugs.
§In each section, the missing data have been excluded from the statistical analyzes.

Table 3. Associations between deliberate self-harm behaviors and psychotic-like experiences in Japanese adolescents aged 12–15 years (n = 4694)

	Prevalence of self-harm behaviors*		Unadjusted odds ratio			Adjusted odds ratio‡§		
Psychotic-like experiences (PLEs)	n	%	OR†	95% CI	P-value	OR†	95% CI	P-value
Thoughts read (n = 76)	10	13.15	2.88	1.47–5.69	0.002	1.56	0.71–3.43	0.267
Special messages (n = 33)	4	12.12	2.59	0.90–7.42	0.077	1.62	0.48–5.50	0.439
Spied-upon (n = 363)	60	16.53	4.52	3.31–6.18	<0.001	1.93	1.34–2,77	<0.001
Hearing voices (n = 487)	74	15.26	4.33	3.24 –5.78	<0.001	2.32	1.67–3.22	<0.001

*The number of individuals with the experience of deliberate self-harm behaviors in the previous year among those with each PLE.
†Odds ratio comparing the groups with and without PLE.
‡Odds ratio adjusted for age, sex, the total score of the GHQ-12, being bullied, violence from adults in the home, alcohol use, and use of recreational drugs.
§In each section, the missing data have been excluded from the statistical analyzes.

Table 4. Association between the lifetime experience of suicidal feelings and severity of psychotic-like experiences in Japanese adolescents aged 12–15 years (N = 4894)

	Lifetime prevalence of suicidal [feelings]†		Unadjusted odds ratio			Adjusted odds ratio‡§		
Psychotic-like experiences (PLEs)*	*n*	%	OR	95%	*P*-value	OR	95% CI	*P*-value
No symptom group (n = 4148)	602	14.51	1.00		<0.001	1.00		<0.001
1 symptom group (n = 564)	209	37.06	3.46	2.85–4.18		2.18	1.74–2.74	
2 or more –symptoms group (n = 18)	97	53.30	6.70	4.94–9.07		3.14	2.17–4.53	

*No symptom group = individuals with no PLE; 1 symptom group = individuals with one PLE; 2 or more symptoms group = individuals with two or more PLEs.
†The number of individuals with the lifetime experience of suicidal feelings.
‡Odds ratio adjusted forage, sex, the total score of the GHQ-12, being bullied,
violence from adults in the home, alcohol use, and use of recreational drugs.
§In each section, the missing data have been excluded from the statistical analyzes.

Table 5. Association between deliberate self-harm behaviors and severity of psychotic-like experiences in Japanese adolescents aged 12–15 years (N = 4894)

	Prevalence of self-harm behaviors†		Unadjusted odds ratio			Adjusted odds ratio‡§		
Psychotic-like experiences (PLEs)*	*n*	%	OR	95% CI	*P*-value	OR	95% CI	*P*-value
No symptom group (n = 4150)	150	3.62	1.00		<0.001	1.00		<0.001
1 symptom group (n = 562)	59	10.50	3.12	2.23–4.29		1.68	1.18–2.40	
2 or more symptoms group (n = 182)	41	22.53	7.75	5.28–11.38		3.06	1.96–4.78	

*No symptom group = individuals with no PLE; 1 symptom group = individuals with one PLE; 2 or more symptom group = individuals with two or more PLEs.
†The number of individuals with the experience of deliberate self-harm behaviors in the previous year.
‡Odds ratio adjusted for age, sex, the total score of the GHQ-12, being bullied, violence (torn adults in the home, alcohol use, and use of recreational drugs.
§In each section, the missing data have been excluded from the statistical analyzes.

of PLEs were approximately three times more likely to experience suicidal feelings and self-harm behaviors than those who did not experience PLEs.

When each PLE was analyzed, the prevalence of 'heard voices' was the most frequent (10.0%), followed by 'spied-upon' (7.4%). Compared with these two PLEs, 'thoughts read' and 'special messages' were much less common (1.6% and 0.7%). 'Spied-upon' and 'heard voices' were significantly associated with both suicidal feelings and deliberate self-harm behaviors, after controlling for confounding factors. 'Thoughts read' was significantly associated with suicidal feelings but not with deliberate self-harm behaviors, whereas 'special messages' was not significantly associated with either the feelings or the behaviors. These results suggest that 'heard voices' and 'spied-upon' could be useful markers to identify younger adolescents who are at risk for suicide-related feelings and behaviors.

The risk of suicide behaviors is significantly higher in subjects with psychosis, including schizophrenia, during the early phase of the disease (2, 8). The risk for suicide during the early stage of psychosis may be higher for those who have greater insight about their disease (3, 34). A recent study reported that such insight might begin to significantly elevate the risk of self-harm behaviors in subjects with the first episode of psychosis in the pretreatment phase (3). Insight about the unusual nature of the PLEs could increase the risk of the suicidal feelings/behaviors even for younger teenagers, because of the fear and distress associated with PLEs. Future studies about the relationship of insight and distress are needed to understand the effects.

The association between PLEs and suicidal feelings/behaviors was significant after controlling for anxiety/depression and use of substances. Thus far, psychotic disorders may have been considered less of a contributing factor in suicide in young people compared to mood disorders and substance use disorders (35, 36). In psychological autopsy studies, the information was mostly obtained from family members and other relevant people (7, 35, 36). Subclinical psychotic symptoms or signs which do not manifest in the behaviors of the subjects would be more likely to be overlooked than depression or substance misuse. In the present study, a substantial portion of the subjects with suicidal problems were suffering from PLEs; 306 out of 908 subjects with suicidal feelings (34%) and 100 out of 250 subjects with suicidal behaviors (40%) experienced one or more types of PLEs.

More attention should be focused on subclinical psychotic signs/symptoms in future studies of suicidal problems in young people.

This study had several limitations. First, we could not obtain answers from absent students. Poor mental health status and psychopathology may be more prevalent among frequent or long-term absentees. Second, because we used a self-report questionnaire, there may be more over-reporting and/or under-reporting on certain topics than there would be in an interview-based survey. For these reasons, the estimated prevalence of psychopathology might not be totally precise. Third, we did not ask the participants to give detailed descriptions about the PLEs which they experienced. Therefore, we could not identify possible discrepancies in what subjects perceived as real PLEs. Fourth, sufficient information about confounding factors such as personality (37, 38) and family circumstances (39, 40), which might be associated with suicidal problems, was not available in the present study. Finally, we used a cross-sectional survey, and therefore, we were not able to identify cause and effect relationships between PLEs and other factors. Hence, in the future, follow-up studies will be needed to investigate the nature of the chronological relationships between PLEs and other factors including suicide-related problems.

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Declaration of interest

None.

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ORIGINAL ARTICLE

Enhanced Carbonyl Stress in a Subpopulation of Schizophrenia

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Context: Various factors are involved in the pathogenesis of schizophrenia. Accumulation of advanced glycation end products, including pentosidine, results from carbonyl stress, a state featuring an increase in reactive carbonyl compounds (RCOs) and their attendant protein modifications. Vitamin B_6 is known to detoxify RCOs, including advanced glycation end products. Glyoxalase I (GLO1) is one of the enzymes required for the cellular detoxification of RCOs.

Objectives: To examine whether plasma levels of pentosidine and serum vitamin B_6 are altered in patients with schizophrenia and to evaluate the functionality of *GLO1* variations linked to concomitant carbonyl stress.

Design: An observational biochemical and genetic analysis study.

Setting: Multiple centers in Japan.

Participants: One hundred six individuals (45 schizophrenic patients and 61 control subjects) were recruited for biochemical measurements. Deep resequencing of *GLO1* derived from peripheral blood or postmortem brain tissue was performed in 1761 patients with schizophrenia and 1921 control subjects.

Main Outcome Measures: Pentosidine and vitamin B_6 concentrations were determined by high-performance liquid chromatographic assay. Protein expression and enzymatic activity were quantified in red blood cells and lymphoblastoid cells using Western blot and spectrophotometric techniques.

Results: We found that a subpopulation of individuals with schizophrenia exhibit high plasma pentosidine and low serum pyridoxal (vitamin B_6) levels. We also detected genetic and functional alterations in *GLO1*. Marked reductions in enzymatic activity were associated with pentosidine accumulation and vitamin B_6 depletion, except in some healthy subjects. Most patients with schizophrenia who carried the genetic defects exhibited high pentosidine and low vitamin B_6 levels in contrast with control subjects with the genetic defects, suggesting the existence of compensatory mechanisms.

Conclusions: Our findings suggest that GLO1 deficits and carbonyl stress are linked to the development of a certain subtype of schizophrenia. Elevated plasma pentosidine and concomitant low vitamin B_6 levels could be the most cogent and easily measurable biomarkers in schizophrenia and should be helpful for classifying heterogeneous types of schizophrenia on the basis of their biological causes.

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SCHIZOPHRENIA IS A DEBILITATING and complex mental disorder with a prevalence of approximately 1% worldwide. Its pathophysiology remains unclear, despite extensive research.[1,2] Biochemical and pharmacological studies using human samples and animal models suggest that oxidative/carbonyl stress contributes to the pathophysiology of schizophrenia.[3-6] Oxidative stress is a central mediator of advanced glycation end product (AGE) formation, and pyridoxamine (vitamin B_6, biosynthesized from pyridoxal in vivo) is known to detoxify reactive carbonyl compounds via carbonyl-amine chemistry. Toxic reactive carbonyl compounds such as α-oxoaldehydes (eg, methylglyoxal, glyoxal, and 3-deoxyglucosone) are formed from sugars, lipids, and amino acids.[7-9] Accumulation of such reactive carbonyl compounds, referred to as carbonyl stress,[10] results in the modification of proteins and the eventual formation of AGEs such as pentosidine. Cellular removal of AGEs hinges largely on the activity of the zinc metalloenzyme glyoxalase I (GLO1).[11] The glyoxalase detoxifi-

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Table 1. Genetic and Biochemical Analyses in Schizophrenic Patients and Control Subjects

Characteristic	No. (%) Schizophrenic Patients (n=1761)	No. (%) Control Subjects (n=1921)	Main Application
Institutions where DNA was collected, No.			
Tokyo Institute of Psychiatry	261	302	Resequence
Tokyo Metropolitan Matsuzawa Hospital (postmortem brain tissue)	70	1	Resequence plasmid construction
RIKEN Brain Science Institute	1156	1502	Resequence
Okayama University	274	116	Resequence
Pentosidine level[a]			
Very high, >130 ng/mL	3 (6.7)[b]	0	HPLC
High, >55.2 ng/mL	18 (40.0)	2 (3.3)	
Normal, <55.2 ng/mL	24 (53.3)	59 (96.7)	
Vitamin B_6, pyridoxal level[a]			
Normal: male, 6-27 ng/mL; female, 4-42 ng/mL	19 (42.2)	54 (88.5)	HPLC
Low: male, <6 ng/mL; female, <4 ng/mL	15 (33.3)	7 (11.5)	
Very low, <3 ng/mL	11 (24.4)[b]	0	

Abbreviation: HPLC, high-performance liquid chromatography.
[a] Forty-five schizophrenic patients; 61 healthy control subjects.
[b] For detailed information, see Table 3.

cation system is ubiquitous in human tissues, including the brain. The GLO1 detoxification system interacts with several metabolizing cascades, and some compounds in these cascades have been reported as candidates for involvement in the etiology of schizophrenia, such as glutathione, homocysteine, and folic acid metabolites (eFigure 1, available at http://www.prit.go.jp/En/PSchizo/TSchizo/archives.html).[12-19]

Recent studies have revealed that dysfunction of GLO1 is involved not only in systemic diseases such as diabetes mellitus[20] and vascular injury,[21] but also in neuropsychiatric disorders such as mood disorder,[22] autism,[23,24] anxiety disorders,[25] alcoholism,[26] and Alzheimer disease.[7] In mice, levels of *Glo1* expression have been associated with anxiety-like behavioral phenotypes.[27-29] *GLO1* has been mapped to chromosome 6p21, a linkage region for schizophrenia.[30-32] A missense polymorphism, Glu111/Ala111, has been reported in 2 multiplex Caucasian pedigrees with schizophrenia spectrum disorders.[33] However, the functional significance of this polymorphism has not been addressed.

The present study examined whether plasma levels of pentosidine and serum vitamin B_6 are altered in patients with schizophrenia. If so, *GLO1* polymorphisms associated with functional deficits could be an underlying substrate of schizophrenia. To the best of our knowledge, this is the first study to suggest enhanced carbonyl stress as an underlying mechanism of schizophrenia.

METHODS

SUBJECTS

Materials for resequencing of the *GLO1* gene were obtained from 1761 schizophrenic patients (mean age, 50.1 years [SD, 13.9 years]) and 1921 healthy control subjects (mean age, 42.5 years [SD, 14.4 years]) (**Table 1**). For genetic study, the affected individuals were randomly recruited from among both inpatients and outpatients. Cases were composed of 961 men (mean age, 49.0 years [SD, 13.4 years]) and 800 women (mean age, 51.4 years [SD, 14.3 years]). Control subjects were composed of 779 men (mean age, 41.2 years [SD, 13.6 years]) and 1142 women (mean age, 43.0 years [SD, 14.8 years]). DNA extracted from 71 postmortem brain tissue specimens was used for resequencing. We did not assess associations between common variants and schizophrenia, as the aim of this study was to focus on rare variations to reveal large biological effects, thus enabling clarification of pathophysiology in rare cases of schizophrenia. These samples were therefore not matched by age or sex. Schizophrenia was diagnosed according to the *DSM-IV* to obtain a best-estimate lifetime diagnosis, with consensus of at least 2 experienced psychiatrists. No structured interviews were performed. Ten percent of patients exhibited discordant subtypes. The available medical records and family informant reports were also taken into consideration. Control subjects were recruited from among hospital staff and company employees documented to be free from mental illness based on brief interviews by experienced psychiatrists. The companies that provided employees as control subjects for our study were biochemical, pharmaceutical, and medical device manufacturers. We personally announced recruitment of volunteers for our research at annual meetings such as those of the Japanese Society of Biological Psychiatry and the Japanese Society of Schizophrenia Research.

Fresh plasma and serum samples were obtained from 45 available schizophrenic patients and 61 healthy controls among the subjects included in the genetic study (Table 1). Diabetes mellitus and renal dysfunction were criteria for exclusion in selecting patients and healthy control subjects, as these diseases may potentially increase pentosidine levels.

All participants provided written informed consent, and the study protocols were approved by the ethics committees of all participating institutions (Tokyo Institute of Psychiatry,[34] Tokai University, RIKEN Brain Science Institute,[35-38] Okayama University,[39] Tokyo Metropolitan Matsuzawa Hospital, Hamamatsu University, Chiba University, and Tohoku University).

RESEQUENCING ANALYSIS OF *GLO1*

All the coding regions and exon-intron boundaries as well as the 5′ upstream region of *GLO1* were examined by direct sequencing of the polymerase chain reaction (PCR) products. Polymerase chain reaction amplification was performed using the sets of

primers listed in eTable 1 and Blend Taq polymerase (Toyobo, Osaka, Japan). Detailed information on the PCR amplification conditions is available from the authors upon request. Sequencing of PCR products was performed using a BigDye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems, Foster City, California) and an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). We read both strands when an inserted or deleted nucleotide yielded dual signals derived from wild-type and mutant-type strands. Moreover, to confirm a single base insertion or deletion, PCR fragments were subcloned into a pTA2 plasmid vector (Toyobo) and sequenced.

GLO1 ENZYMATIC ASSAY

Fresh blood samples were obtained from 45 schizophrenic patients and 61 healthy control subjects (Table 1). Red blood cells (RBC), plasma, and serum were separated by centrifugation and used in subsequent studies. Glyoxalase I enzymatic activity in RBC was determined using the spectrophotometric method described by McLellan and Thornalley.[40] Briefly, washed RBC were lysed with 4 volumes of ice-cold distilled water and kept on ice for more than 30 minutes to complete hemolysis. Debris was removed by centrifugation and the supernatant was assayed for enzymatic activity. Activity of the GLO1 enzyme is given in units/10^6 RBC, where 1 unit is the amount of enzyme required to catalyze the formation of 1 µmol of S-D-lactoylglutathione per minute from hemithioacetal. Hemithioacetal was prepared by preincubation of 2mM methylglyoxal with 2mM glutathione in a 50mM sodium phosphate buffer (pH 6.6) at 37°C for 10 minutes. The increase in absorbance at 240 nm owing to the formation of S-D-lactoylglutathione was measured by spectrophotometry. Prominently low enzymatic activities were confirmed by at least 3 measurements.

MEASUREMENT OF PENTOSIDINE AND VITAMIN B_6

Pentosidine, an AGE, was determined by high-performance liquid chromatography assay as described previously.[41] In brief, the plasma sample was lyophilized, hydrolyzed in 100 µL of 6N of hydrochloric acid for 16 hours at 110°C under nitrogen, neutralized with 100 µL of 5N of sodium hydroxide and 200 µL of a 0.5M sodium phosphate buffer (pH 7.4), filtered through a 0.5-µm filter, and diluted with phosphate-buffered saline (PBS). A sample (corresponding to 25 µg of protein) was injected into a high-performance liquid chromatography system and fractionated on a C18 reverse-phase column. Effluent was monitored at excitation-emission wavelengths of 335/385 nm using a fluorescence detector (RF-10A; Shimadzu, Kyoto, Japan). Synthetic pentosidine was used to obtain a standard curve. We measured pentosidine at least twice, and additional measurements were performed 3 times to confirm 3 outliers. Three forms of vitamin B_6 (pyridoxine, pyridoxal, and pyridoxamine) were measured in serum samples by high-performance liquid chromatography according to a previously described method.[42] Other parameters (glucose, glycohemoglobin A_{1C}, total cholesterol, triglyceride, aspartate aminotransferase, alanine aminotransferase, creatinine, urea nitrogen, total protein, and albumin) were measured in blood samples. Glomerular filtration rate was estimated using the abbreviated Modification of Diet in Renal Diseases study equation.[43]

WESTERN BLOTTING

The GLO1 protein expression in RBC lysate was assessed by Western blotting analysis after sodium dodecyl sulfate–polyacrylamide gel electrophoresis using 5% to 20% polyacrylamide gradient gel. Polyclonal anti-GLO1 sera, designated NT2, were raised in rabbits by immunization with a human GLO1 peptide MAEPQPPSGGLTDEAALSC (corresponding to amino acids 1-19) conjugated to keyhole limpet hemocyanin. Equal volumes of RBC lysates were treated with Laemmli buffer, boiled at 100°C for 5 minutes, applied to the gel, and transferred to polyvinylidene fluoride membranes. Blots were treated with 100% BlockingOne (Nacalai, Kyoto, Japan) to block any nonspecific binding sites at 4°C overnight. The membrane was washed with PBS containing 0.05% Tween 20 (PBS-T) and then incubated with 1-µg/mL rabbit anti-GLO1 antibody (NT2) and mouse anti–glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody (1:200; Santa Cruz Biotechnology, Santa Cruz, California) as an internal control in PBS-T containing 5% BlockingOne for 1 hour at room temperature. Anti-GLO1 antibody was affinity-purified using beads coupled with the antigen peptide. The membrane was washed again 3 times with PBS-T and then incubated with peroxidase-conjugated anti-mouse Ig (1:1000) and peroxidase-conjugated anti-rabbit Ig (1:1000) (Vector, Burlingame, California) for 1 hour at room temperature, followed again by a wash and eventual development with 3,3′-diaminobenzidine tetrahydrochloride solution (Sigma, St Louis, Missouri). The GLO1 signals that were normalized to GAPDH were quantified using National Institutes of Health image software (http://rsb.info.nih.gov/nih-image/). Researchers were blind to *GLO1* genotypes during experiments with Western blotting. We performed at least 2 determinations for each sample.

CELL CULTURE

Epstein-Barr virus–transformed lymphoblastoid cell lines derived from patients and normal subjects were established at SRL Inc (Tokyo, Japan). Lymphoblastoid cell lines were grown in RPMI 1640 medium (Wako, Osaka, Japan) supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, California) and antibiotic liquid (Nacalai, Kyoto, Japan). Cell lines were cultured at 37°C in a humidified atmosphere incubator under 5% carbon dioxide.

STATISTICAL ANALYSIS

Data were analyzed using PRISM software (GraphPad Software, San Diego, California). Simple comparisons of means and standard errors of data were performed using an unpaired *t* test or the Mann-Whitney test (both 2-tailed). The χ^2 and Pearson correlation tests were used to assess the significance of association between the data. For comparison of more than 2 groups, 1-way analysis of variance was used. If the results of analysis of variance were significant, the Bonferroni procedure was used as a post hoc test. Significance was defined as $P < .05$.

RESULTS

PENTOSIDINE ACCUMULATION AND PYRIDOXAL DEPLETION

We measured plasma pentosidine and serum pyridoxal (vitamin B_6) levels using samples from 45 patients with schizophrenia and 61 mentally healthy subjects (**Figure 1**). Neither schizophrenic patients nor healthy subjects had diabetes mellitus or chronic kidney disease (estimated glomerular filtration rate >60 mL/min), which are 2 major causes of elevated AGEs. An increase in plasma pentosidine (to above the mean plus 2 SDs of control sub-

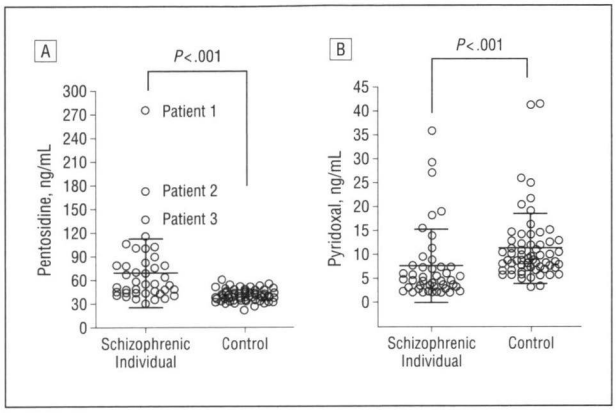

Figure 1. Plasma pentosidine accumulation and serum pyridoxal (vitamin B_6) depletion. Levels of plasma pentosidine (A) and serum pyridoxal (B) were analyzed using high-performance liquid chromatography techniques. Values were compared using the Mann-Whitney *U* test (2-tailed). Error bars indicate standard deviations.

jects, >55.2 ng/mL) was observed in 21 schizophrenic individuals (approximately 47%), as shown in Table 1. Three patients (patients 1, 2, and 3 in Figure 1A) exhibited extremely high pentosidine levels. The mean pentosidine level was 1.73-fold higher in schizophrenic individuals than in control subjects ($P<.001$) (Figure 1A and **Table 2**).

A concomitant marked decrease in pyridoxal levels was found in 11 schizophrenic patients (Table 1), most of whom were hospitalized and had been treated with well-controlled daily nutrition by a registered dietitian approved by the Japanese Ministry of Health, Labour, and Welfare based on the National Dietitian Law. Significant reduction of pyridoxal level was observed in schizophrenic patients compared with healthy control subjects ($P<.001$) (Figure 1B).

Mean values of pentosidine and vitamin B_6 in control samples were 39.6 ng/mL (SD, 7.8 ng/mL) and 11.1 ng/mL (SD, 7.3 ng/mL), respectively. These values do not deviate markedly from the standard levels in adult subjects without diabetes mellitus or renal dysfunction reported in previous studies.[44-46]

GENETIC ANALYSES OF *GLO1*

We next attempted to determine the mechanism underlying the alterations in pentosidine/pyridoxal levels observed in schizophrenia by resequencing analysis (all exons and flanking introns) of *GLO1* using 1761 patients with schizophrenia and 1921 control subjects (Table 1). These subjects included not only those for whom pentosidine/pyridoxal levels were examined, but also many other schizophrenic individuals and controls to ensure thorough genetic scrutiny. This analysis detected 2 heterozygous frameshift mutations. The first was an adenine insertion at nt 79 in exon 1, causing a frameshift starting from codon 27 and introducing a premature termination codon after aberrant translation of 15 amino acid residues (T27NfsX15) in 1 patient with schizophrenia (**Figure 2**A and eTable 2). The second heterozygous frameshift mutation, c.365delC, generated a frameshift from codon 122 in exon 4 and a premature termination after an aberrant 27–amino acid addition (P122LfsX27) (Figure 2B). This mutation was detected in 4 schizophrenic individuals and 10 control subjects (eTable 2). No relatives of subjects exhibiting c.365delC were available for analysis.

Furthermore, we identified 36 nucleotide changes, including 8 common polymorphisms (minor allele frequency >0.03) and 28 rare variants (eTable 2 and eTable 3). We also identified 13 homozygous Ala111 carriers: 9 schizophrenic patients and 4 controls (9 of 1586 schizophrenic patients [0.6%]); 4 of 1685 control subjects [0.2%]) (Figure 2C and eTable 3).

Seven heterozygous frameshift carriers (3 schizophrenic individuals and 4 controls), 10 homozygotes for Ala111 (7 schizophrenic individuals and 3 controls), 22 subjects with Glu111/Ala111 genotype (12 schizophrenic individuals and 10 controls), and 67 subjects with Glu111/Glu111 genotype (23 schizophrenic individuals and 44 controls) were available for biochemical assays (Figure 1 and Table 2).

BIOCHEMICAL ANALYSES OF GLO1

We focused on the heterozygous frameshift mutations and Glu111/Ala111 variation of *GLO1* in an attempt to assess the functional significance of these changes. We first quantified the levels of expression of GLO1 protein in RBC by Western blotting in 45 schizophrenic patients and 61 control subjects. Marked reductions (40%-50%) to full-length GLO1 protein expression were found in 10 subjects carrying heterozygous frameshift mutations ($P<.001$) (Table 2 and eFigure 2A). Significantly reduced (approximately 15%) GLO1 expression was observed in 7 homozygous Ala111 carriers compared with homozygous Glu111 or heterozygous Glu111/Ala111carriers in the schizophrenia group (both $P<.05$) (Table 2). In control subjects, levels of GLO1 protein expression in 3 homozygous Ala111 carriers did not differ significantly from those carrying other genotypes (Table 2).

The GLO1 enzymatic activity in RBC was measured by spectrophotometric assay (Table 2). Marked reductions (40%-50%) in enzymatic activity were found in all individuals carrying heterozygous frameshift mutations ($P<.01$). The 7 homozygous Ala111 carriers also exhibited significantly decreased enzymatic activity (an approximately 20% reduction) compared with homozygous Glu111 carriers in the schizophrenic group ($P<.001$) but not in control subjects.

In addition, we established a cell line from lymphocytes of a heterozygous frameshift carrier and performed functional analysis of these cell lysates (eFigure 2B). They exhibited the same functional abnormalities as identified in RBC, ie, decrease in GLO1 activity and its protein expression.

CONFOUNDING FACTORS AND BIOCHEMICAL DATA

Three patients (patients 1, 2, and 3 in Figure 1A) exhibiting extremely high pentosidine levels had especially severe schizophrenia, though they were free of systemic disease. These 3 schizophrenic individuals had chronic and

Table 2. Samples Used in the Biochemical Analyses

Characteristic	Mean (SD)									
	Schizophrenic Patients					Control Subjects				
	All (n=45)	Glu/Glu (n=23)	Glu/Ala (n=12)	Ala/Ala (n=7)	Frameshift (n=3)	All (n=61)	Glu/Glu (n=44)	Glu/Ala (n=10)	Ala/Ala (n=3)	Frameshift (n=4)
Sex, No., M/F	29/16	13/10	9/3	5/2	2/1	23/38	17/27	3/7	0/3	3/1
Age, y	51.0 (12.2)[a]	47.6 (12.5)[a]	51.5 (12.7)	59.0 (8.6)[a]	57.3 (4.6)[a]	36.0 (9.4)	35.1 (9.4)	41.9 (8.2)	24.3 (1.5)	40.5 (5.7)
Age at onset, y	25.0 (8.7)	24.4 (5.8)	25.8 (11.9)	28.0 (12.7)	20.0 (2.6)					
Relative protein expression	0.95 (0.15)[b]	0.99 (0.11)	1.01 (0.08)	0.86 (0.06)[c]	0.55 (0.09)[d]	0.88 (0.12)	0.91 (0.10)	0.87 (0.09)	0.86 (0.05)	0.60 (0.06)[e]
Enzymatic activity, mU/10^6 RBC	5.43 (1.00)[f]	6.00 (0.75)	5.47 (0.35)	4.70 (0.65)[g]	3.00 (0.20)[h]	5.94 (1.00)	6.18 (0.61)	6.11 (0.69)	5.83 (0.29)	2.90 (0.08)[i]
Pentosidine, ng/mL	68.37 (43.42)[j]	64.73 (32.8)[k]	54.96 (17.83)[l]	97.95 (82.67)[m]	80.91 (53.26)	39.59 (7.82)	39.17 (8.41)	39.27 (6.25)	39.08 (3.24)	45.34 (6.12)
Pyridoxal, ng/mL[n]	7.46 (7.56)[o]	8.20 (8.70)[p]	7.36 (7.66)	6.82 (4.69)	3.60 (2.12)	11.14 (7.31)	11.91 (8.02)	8.45 (2.76)	14.63 (8.95)	6.88 (1.56)

Abbreviation: RBC, red blood cell.
[a] Unpaired *t* test, $P<.05$ (vs controls).
[b] Mann-Whitney test, $P<.01$ (vs controls).
[c] Analysis of variance, $F_{3,41}=21.76$, $P<.001$; Bonferroni multiple comparison test, $P<.05$ in schizophrenic patients (vs Glu/Glu and Glu/Ala).
[d] Analysis of variance, $F_{3,41}=21.76$, $P<001$; Bonferroni multiple comparison test, $P<.001$ in schizophrenic patients (vs Glu/Glu, Glu/Ala, and Ala/Ala).
[e] Analysis of variance, $F_{3,57}=13.71$, $P<.001$; Bonferroni multiple comparison test, $P<.01$ in controls (vs Glu/Glu, Glu/Ala, and Ala/Ala).
[f] Mann-Whitney test, $P<.001$ (vs controls).
[g] Analysis of variance, $F_{3,41}=23.44$, $P<.001$; Bonferroni multiple comparison test, $P<.001$ in schizophrenic patients (vs Glu/Glu).
[h] Analysis of variance, $F_{3,41}=23.44$, $P<.001$; Bonferroni multiple comparison test, $P<.01$ in schizophrenic patients (vs Glu/Glu, Glu/Ala, and Ala/Ala).
[i] Analysis of variance, $F_{3,57}=37.41$, $P<.001$; Bonferroni multiple comparison test, $P<.001$ in controls (vs Glu/Glu, Glu/Ala, and Ala/Ala).
[j] Mann-Whitney test, $P<.001$ (vs controls).
[k] Mann-Whitney test, $P<.001$ (vs controls).
[l] Mann-Whitney test, $P<.01$ (vs controls).
[m] Mann-Whitney test, $P<.05$ (vs controls).
[n] Pyridoxal levels less than 2.0 were calculated as 2.0.
[o] Mann-Whitney test, $P<.001$ (vs controls).
[p] Mann-Whitney test, $P<.001$ (vs controls).

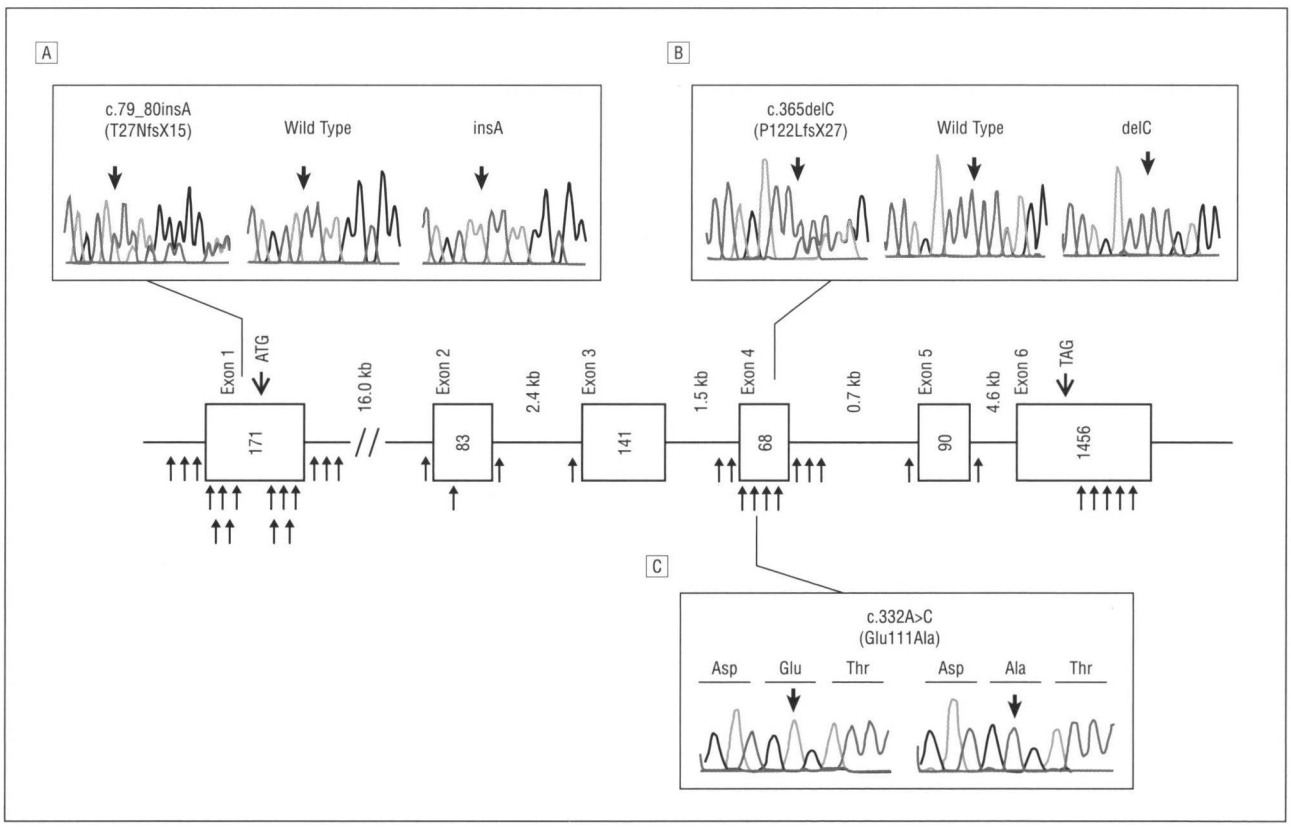

Figure 2. DNA sequence chromatograms showing frameshift and missense variants. Heterozygous sequence traces derived from individuals carrying an adenine insertion within exon 1 (A) and a cytosine deletion within exon 4 (B). TA cloning and subsequent sequencing analyses revealed normal (denoted "wild type") and mutant (denoted insA or delC) sequences. C, Chromatogram showing a Glu111/Ala111 missense variant located within exon 4. Positions of common and rare variants of *GLO1* are indicated by arrows (see also eTable 2 and eTable 3). kb indicates kilobase pairs.