Table 1
Comparison of the Temperament and Character Inventory (TCI) subitem score among 3/3 vs. 3/4 and 4/4 repeats of MAOA gene polymorphism

TCI subitem	N[a]		Score[b]		P value
	3 × 3	3 × 4 and 4 × 4	3 × 3	3 × 4 and 4 × 4	
Novelty Seeking	79	129	22.3 ± 6.5	21.3 ± 5.6	0.23
Harm Avoidance	74	119	17.8 ± 6.5	17.9 ± 6.6	0.93
Reward Dependence	80	133	16.6 ± 3.6	17.4 ± 3.4	0.13
Persistence	79	133	4.8 ± 2.0	4.8 ± 2.1	0.90
Self-directedness	79	125	26.1 ± 6.2	26.6 ± 7.2	0.61
Cooperativeness	71	117	29.5 ± 5.5	30.3 ± 5.3	0.34
Self-transcendence	78	124	14.9 ± 5.8	14.8 ± 5.2	0.84

[a] Of a total of 219 subjects examined, 5 were excluded because of not submitting the TCI answer sheet. Since several blanks are found on each dimension in the TCI answer sheet of the remaining 214 subjects, the total number of subjects is different depending on each dimension.
[b] Values are expressed as mean ± standard deviation.

transcriptional activity of the MAO-A gene promoter. This mutation consists of a 30-bp repeated sequence present in 3, 3.5, 4, or 5 copies, each associated with different transcriptional efficiency; alleles with 3.5 or 4 copies of the repeat sequence are transcribed 2–10 times more efficiently than those with 3 copies of the repeat [3,14].

An association between this gene polymorphism (30-bp VNTR) and behavioral traits has been found in several studies. For example, Manuck et al. [11,12] reported that male subjects with 3.5 and 4 repeats scored significantly higher on scales of aggression than those with 3 and 5 repeats. On the other hand, Eley et al. [4] reported that males with high Neuroticism scores were significantly more likely to have 3.5 or more repeats than low scorers, while Garpenstrand et al. [5] and Jorm et al. [7] reported no such significant association. So far, the association between MAO-A gene polymorphism and behavioral traits in females has not been investigated.

The aim of the present study was to investigate the possible relationship between genotypes of MAO-A promoter polymorphism and behavioral traits in Japanese females.

This study was carried out after obtaining approval from the Ethics Committee of Nagoya University School of Medicine and Keio University School of Medicine. To minimize the effects of confounding variables such as race, age, and gender, the subjects enrolled were all Japanese female students in their first year at a nursing school ($n = 219$ females). Their mean age was 20 years (range: 18–32 years). After giving the subjects a full description of the study, written informed consent to participate was obtained from each of them.

The subjects were asked to complete the 240 items of the Japanese version of the TCI [2], whose reliability and validity had been established by Kijima et al. [8].

DNA was extracted from peripheral lymphocytes according to the standard method. Genotyping was carried out according to the standard protocol, with a slight modification from the published methods [4,11]. Each target segment was amplified by the polymerase chain reaction method and subjected to electrophoresis.

For statistical analysis, the 'SPSS for Windows ver11.0' (SPSS Japan Inc., Tokyo, Japan) software package was used. Genotype deviation from the Hardy–Weinberg equilibrium was evaluated by chi-squared test. Since MAO-A promoter activity is known to be decreased in subjects with 3/3 genotypes, and to be increased in subjects with 3/4 and 4/4 genotypes [3,14], the TCI scores were compared between 3/3 genotypes and others (3/4 and 4/4). The mean scores for the seven factors (Novelty Seeking, Harm Avoidance, Reward Dependence, Persistence, Self-directedness, Cooperativeness, Self-transcendence) of the TCI were compared among the genotypes (3/3 versus 3/4 and 4/4) using Student's t-test. The result was defined as being significant at $P < 0.05$.

The numbers of the genotypes 3/3, 3/4, and 4/4 were 83, 108, and 28, respectively. The 3.5 repeat allele was not detected at all in our samples. The genotype distribution was not significantly different from that expected according to the Hardy–Weinberg equilibrium. There were no significant differences in the mean scores for the seven TCI factors between the genotypes (3/3 and 3/4, 4/4).

The purpose of the present study was to examine the association between MAO-A gene polymorphism and personality traits. We detected no significant association between MAO-A genotype and TCI score, indicating that MAO-A gene promoter polymorphism is unlikely to have affected the personality traits in our sample. These results are consistent with other previous reports [5,7]. MAO-A is a crucial enzyme in the central nervous system, and Sabol et al. [14] reported that 3 repeats reflected low MAO-A activity, whereas 3.5 and 4 repeats reflected high MAO-A activity in vitro. However, on the basis of the present results, it seems unlikely that the MAO-A function reflected by this polymorphism is associated with the formation of personality traits.

Although the 3.5 repeat allele was not detected among our samples, this finding is in accordance with previous studies carried out in Japan. In fact, the distribution of the allele frequency in these studies was very similar to that observed in the present study [6,9].

The differences between our results and the previous studies showing a positive association between MAO-A and personality traits [4,11,12] may have been derived from differences in the demographic background of the

subjects and of the questionnaire used for personality evaluation.

It can be considered that the present study had a few limitations. First, our subjects were a fairly specifically defined population with only a narrow range of demographic factors, since they were all female students attending a nursing school, and thus their personality traits may have shown a similar trend. Second, there was a possibility of type II error in detecting significant differences, due to an insufficient sample size. Based on the results of power analysis [10], if the type I error is set at 0.05 and the power at 0.80, at least 285 samples are theoretically required to detect a significant difference of Reward Dependence, which showed the smallest *P* value observed in the present study.

Considering the relationship between MAO-A gene deficiency and violent personality, the possibility of some involvement of MAO-A polymorphism in personality traits cannot be ruled out. In addition, since other mechanisms regulating neurotransmitters such as COMT, MAO-B, and TPH2 may also be involved in compensation for altered MAO-A activity, relationships with these polymorphisms may also need to be considered. Further research with additional genotyping of other dopamine-related gene polymorphisms is needed using additional subjects with various demographic characteristics, in order to demonstrate more clearly any potential role of MAO-A polymorphism in the formation of personality traits.

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ORIGINAL ARTICLE

Haplotype association between $GABA_A$ receptor γ2 subunit gene (GABRG2) and methamphetamine use disorder

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ABSTRACT

Psychostimulant use disorder and schizophrenia have a substantial genetic basis. Evidence from human and animal studies on the involvement of the γ-aminobutyric acid (GABA) system in methamphetamine (METH) use disorder and schizophrenia is mounting. As we tested for the association of the human $GABA_A$ receptor gamma 2 subunit gene (GABRG2) with each diagnostic group, we used a case–control design with a set of 178 subjects with METH use disorder, 288 schizophrenics and 288 controls. First, we screened 96 controls and identified six SNPs in GABRG2, three of whom we newly reported. Next, we selected two SNPs, 315C>T and 1128+99C>A, as representatives of the linkage disequilibrium blocks for further case–control association analysis. Although no associations were found in either allelic or genotypic frequencies, we detected a haplotypic association in GABRG2 with METH use disorder, but not with schizophrenia. This finding partly replicates a recent case–control study of GABRG2 in METH use disorder, and thus indicates that GABRG2 may be one of the susceptibility genes of METH use disorder.

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Keywords: $GABA_A$ γ2 subunit gene; methamphetamine; substance use disorder; polymorphism; haplotype; schizophrenia

INTRODUCTION

In recent years there has been a pronounced increase in use of psychostimulants involving methamphetamine (METH).[1] Lifetime prevalence of psychostimulant use in some developed countries is found in 1–3% of the adult population,[2] and psychostimulant use in any form may lead to abuse or dependence with physiological, psychological and behavioral component.[3] Findings from family and twin studies suggest that the genetic contribution is important for the development of psychostimulant use disorders. Heritability estimates from a population-based twin study for METH use disorder are substantial,[4,5] for example, 66% for psychostimulant abuse.[6]

The dopamine system is a prime candidate for genetic influence on drug abuse, particularly METH abuse, because it is thought to be involved in the reward and reinforcing mechanism in the meso-cortico-limbic system in the nucleus accumbens.[7] Moreover, the primary site of biological activity of METH is the dopamine transporter in this system.

Instead, a role for the γ-aminobutyric acid (GABA) system in drug abuse is also suggested in accumulating evidence. First, the irreversible GABA-transaminase inhibitor, γ-vinyl GABA, attenuates such increase of the dopamine release in the nucleus accumbens following acute administration of METH.[8] Second, QTL mapping for acute alcohol withdrawal severity suggests that a polymorphism in the $GABA_A$ receptor γ2 subunit gene in mice is genetically correlated with this phenotype.[9] A third line of evidence involves several case–control association studies, suggesting that the human $GABA_A$ receptor γ2 subunit gene (GABRG2) is marginally associated with METH use disorder,[10] and is also associated with alcoholism comorbid with antisocial personality disorder,[11] although there are conflicting results.[12,13] Therefore, it is possible that GABRG2 affects vulnerability to substance use disorder, including METH use disorder.

On the other hand, a number of post-mortem studies have reported an altered GABA neurotransmission in schizophrenia. These studies reported that release and uptake of GABA at synaptic terminals were reduced in schizophrenic cortex[14–16] and that the activity of glutamic acid decarboxylase (GAD), the synthesizing enzyme for GABA, GAD mRNA expression, and the density of GABAergic interneurons, were reduced in the prefrontal cortex (PFC) of schizophrenics.[17–20] Although there was reportedly no significant change in overall mRNA levels for $GABA_A$ receptor subunits,[17] expression of the alternately spliced short isoform of $GABA_A$ receptor γ2 subunit, γ2S, was markedly reduced in the PFC of schizophrenics.[21] The relative over-representation of the γ2L subunit, which possesses an additional phosphorylation site within the eight amino acids inserted, should result in a functionally less active form of the receptor,[22,23] and this defective GABAergic system may be involved in the development of schizophrenia. The evidence of linkage analysis from multiple genome scans of schizophrenia within 5q31–34 where GABRG2 locates also support the involvement of this gene in the development of schizophrenia.[24–27]

Here, we explored the possible contributions of GABRG2 in both METH use disorder and schizophrenia. We systemically searched all exons and the intronic branch sites of GABRG2 for polymorphisms, and examined haplotype-based case–control association analysis with both METH use disorder and schizophrenia.

RESULTS

Our screening of 96 controls in all exons and the flanking intronic splice sites of GABRG2 revealed six SNPs, which were designated 'Asn79Ser', '315C>T', '588T>C', '922+20G>A', '1129−1482A>C', and '1230C>T'. Minor allele frequencies and a schematic graph of these SNPs are presented in Table 1 and Figure 1, respectively. Of all identified SNPs, 315C>T, 588T>C (rs211037) and 922+20G>A have been reported elsewhere.

To evaluate the linkage disequilibrium (LD) in the 96 screened samples using several widely used measures (D', Δ_2 and *P*-value), we genotyped five SNPs in GABRG2 (two SNPs (315C>T, 588T>C) of identified SNPs, two SNPs (rs2268583, rs2284780) from the dbSNP database, and one SNP (1128+99C>A) reported as *Bam*HI RFLP previously[11]). These SNPs were selected because they showed sufficient heterozygosity (a frequency of minor allele>0.1) to detect a small effect of a susceptibility gene presumed to underlie complex disorders, and they were distributed almost evenly on the entire exonic regions of the gene (Figure 1).

Table 1 SNPs in GABRG2 and minor allele frequencies

SNP	*SNP position*	*Minor allele frequency*	*Reference*
107+740C>T	Intron 1	0.302	rs2268583
Asn79Ser	Exon 2	0.005	
315C>T	Exon 3	0.300	
588T>C	Exon 5	0.480	rs211037
922+20G>A	Intron 7	0.020	
923−466C>T	Intron 7	0.480	rs2284780
1128+99C>A	Intron 8	0.480	*Bam*HI C>A
1129−1482A>T	Intron 8	0.236	
1230C>T	Exon 9	0.005	

Estimation of LD between each pairwise SNP is presented in Table 2. These results show that the first three and the last two consecutive SNPs were in complete or nearly complete LD with each other. Therefore, we selected two SNPs (315C>T and 1128+99C>A) as representatives of these nearly complete LD regions for further case–control association analysis.

In addition to screened 96 samples, we genotyped 178 subjects with METH use disorder, 288 schizophrenics, and 288 controls in all. Two representative SNPs were in moderate LD with each other in METH use disorder ($D'=0.72$), schizophrenia ($D'=0.51$) and control subjects ($D'=0.61$). Genotypic and allelic frequencies of two SNPs in each population are summarized in Table 3. The genotypic distributions of each SNP did not significantly deviate from the Hardy–Weinberg equilibrium in either METH use disorder, schizophrenia or control subjects ($P=0.98$, 0.84 and 0.70 at 315C>T and $P=0.15$, 0.62 and 0.06 at 1128+99C>A, respectively). The distributions of each SNP did not differ significantly between each diagnostic group and controls in both allele and genotype frequencies (Table 3).

The distributions of haplotypic frequencies estimated using the expectation-maximization algorithm implemented in the Arlequin 2.0 significantly differed between METH use disorder and control subjects ($P=0.044$). In contrast, there was no significant difference in haplotypic distributions between schizophrenic and control subjects ($P=0.356$, Table 4). From examining at-risk haplotypes predisposed to METH use disorder, only two haplotypes, T–C and T–A (defined by 315C>T–1128+99C>A), were found to confer the significant susceptibility to this disorder. By applying the Bonferroni correction, this finding becomes nonsignificant for haplotype T–A (corrected $P=0.120$) and remains

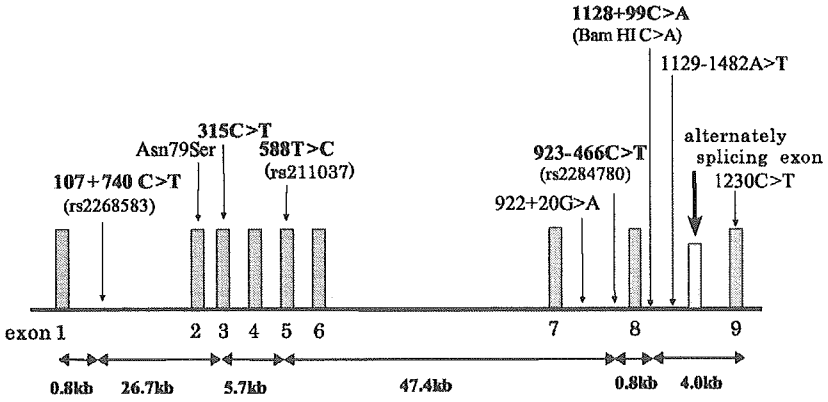

Figure 1 Schematic presentation of identified and reported GABRG2 SNPs. Solid box represents exons. The SNPs in bold type were used to evaluate LD structure.

Table 2 Pairwise linkage disequilibrium in controls

				D' Δ^2		
		rs2268583	*315C>T*	*588T>C*	*rs2284780*	*1128+99C>A*
P-value	rs2268583		1.000 0.976	0.926 0.457	0.376 0.058	0.376 0.058
	315C>T	$<1.0\times10^{-5}$		0.962 0.482	0.608 0.141	0.608 0.141
	588T>C	$<1.0\times10^{-5}$	$<1.0\times10^{-5}$		0.643 0.315	0.643 0.315
	rs2284780	0.002	0.0002	$<1.0\times10^{-5}$		1.000 1.000
	1128+99C>A	0.002	0.0002	$<1.0\times10^{-5}$	$<1.0\times10^{-5}$	

Table 3 Genotypic and allelic distributions of the GABRG2 SNPs in patients with METH use disorder and schizophrenia *vs* controls

SNP	*Sample*	*n*	*Genotype*			*Rarer allele*	*P-value*	
			CC	*CT*	*TT*	*T*	*Genotype*	*Allele*
315C>T	METH	178	87 (49%)	75 (42%)	16 (9%)	107 (30%)	0.374	0.174
	SCZ	288	151 (52%)	116 (40%)	21 (7%)	158 (27%)	0.818	0.594
	Control	288	157 (55%)	113 (39%)	18 (6%)	149 (26%)		
			CC	*CA*	*AA*	*A*	*Genotype*	*Allele*
1128+99C>A	METH	178	56 (31%)	79 (44%)	43 (24%)	165 (46%)	0.603	0.281
	SCZ	288	64 (22%)	139 (48%)	85 (30%)	309 (54%)	0.317	0.238
	Control	288	80 (28%)	128 (44%)	80 (28%)	288 (50%)		

Table 4 Haplotypic distributions of the GABRG2 gene in patients with METH use disorder and schizophrenia *vs* controls

Sample	*Haplotypes (315C>T–1128+99C>A)*				*P-value*
	C–C	*C–A*	*T–C*	*T–A*	
METH	0.275	0.425	0.262	0.039	0.044
SCZ	0.261	0.464	0.202	0.072	0.356
Control	0.314	0.428	0.186	0.072	

significant for haplotype T–C (corrected $P=0.028$). The presumed at-risk haplotype T–C has an estimated frequency of 18.6% among controls and 26.2% among METH use disorder subjects. The estimated odds ratio of haplotype T–C was 1.55 (95% CI (1.13–2.13)).

DISCUSSION

Our results provide supportive evidence for a haplotypic association in GABRG2 with METH use disorder, but not with schizophrenia. This association suggests that the susceptibility variant for METH use disorder may lie within the region in positive LD with the at-risk haplotype reconstructed in this study. The patterns of LD were shown to be two block like, the first block represented by 315C>T (covering rs2268583 at intron 1 to rs211037 at exon 5), and the second block represented by 1128+99C>A (covering rs2284780 at intron 7 to 1128+99C>A at intron 8). Since we found no association between each representative SNP and METH use disorder in either allelic or genotypic frequencies, the possibility arises that susceptibility variant can be located outside of these block-like regions. The second block includes the splicing regulatory elements surrounding the spliced exon, which bind to the polypyrimidine tract binding protein, the splicing regulator.[28–30] Actually, we screened this regulatory region thoroughly through direct sequencing of the 96 samples, however, could not find any variant in these elements. Other splicing regulatory elements that bind to another splicing regulator Nova-1 were located in intron 8, about 3.5 kb downstream of 1128+99C>A.[31,32] If the second block does not cover the latter splicing regulatory elements, these regions can be a susceptible candidate. Recently, a significant association was reported between rs4480617 at the 5′-UTR of GABRG2 and METH use disorder in females.[10] Therefore, this SNP or other variants in the promoter region also can be another candidate. Given that the sample size of 96 used to identify SNPs in this study provides more than 80% power to detect SNPs with about 1% minor allele frequency,[33] we are almost unlikely to overlook common nonsynonymous SNPs predisposed to METH use disorder.

As has been widely discussed, a spurious association can arise because of confounding such as population stratification and clinical heterogeneity, given the problems of reliability due to no use of structured interviews. However, our data are partly in agreement with a recent report[10] that found the significant association between GABRG2 and METH use disorder in females. This provides further corroboration that our haplotypic association with METH uses disorder is not spurious, although potential sources of bias such as ascertainment bias still remain possible. For example, subjects suffering from not only METH use disorder but also METH-induced psychosis are more likely to seek medical care and thus to be ascertained. Such 'spurious comorbidity'[34] of psychosis may account for the apparent association in this study. In the present study, we did not stratify the METH use disorder sample according to the comorbidity of METH-induced psychosis because the sample size was too small for reliable analysis. Although the precise prevalence of the comorbid METH-induced psychosis remains unknown, the data in the late 1940s and early 1950s in Japan indicating that about 10% of METH users had METH-induced psychosis[35] would suggest that comorbid METH-induced psychosis is over-represented in our clinically ascertained sample with METH use disorder.

As no association exists between GABRG2 and schizophrenia in our sample, association between GABRG2 and METH use disorder would not likely be attributable to spurious comorbid METH induced-psychosis, which may share the pathophysiology of susceptibility with schizophrenia, the so-called sensitization phenomena.[35] On the contrary, the comorbid polysubstance-related disorder over-represented in our sample with METH use disorder can account for the apparent association in this study. Indeed, previous findings suggesting nonspecific substance dependence vulnerability[5] supported the existence of such a 'misattributed' association in our study. In addition to concurrent comorbidity, we also cannot deny the possibility of spurious comorbid bias caused by the past comorbid diseases because of not examining the past history of any mental diseases systematically. METH use subjects in our study included a large number of patients who experienced first psychotic symptoms after METH use for a relatively short duration and participants in the special program designed for drug use disorder, in which they could not participate if they suffered from other psychiatric problems. The low levels of comorbidity in METH use subjects may reflect such biased ascertainment.

There is indeed a neuroscientific framework to link GABRG2 and METH use disorder. First, several lines of investigation[7] implicate the mesolimbic dopamine system in psychostimulant-induced motor activity. Furthermore, it was shown in a pharmacological study[36] that a GABAergic system in PFC modulated the motor response to psychostimulants by inhibiting PFC pyramidal neurons. Second, a tentative association was found for a GABRG2 SNP and the frontally located event-related potential (ERP) complex N100/P200 after auditory stimuli.[37] Thus, the prefrontal activation difference may reflect the differential GABRG2 activities derived from variants of the gene. Accordingly, GABRG2 activities in PFC could affect the modulation of mesolimbic reward circuitries, which might be associated with vulnerability of METH use disorder.

Overall our results indicate that GABRG2 may play a role in the risk of METH use disorder development in this population. Analysis of the promoter region or the splicing regulatory elements in intron 8 in a future study would be a logical next step in searching for a susceptible variant of GABRG2 in METH use disorder. However, it remains uncertain whether the associated phenotype may reflect the vulnerability of METH-specific abuse or nonspecific substance abuse.

METHODS

Subjects

All patients in this study were unrelated and recruited from three medical institutes participating the Japanese Genetics Initiative for Drug Abuse (JGAIDA).[38] They were diagnosed according to DSM-IV criteria by the consensus of at least two experienced psychiatrists on the basis of unstructured interviews and review of the medical records prior to genotyping.

The number of the patients with METH uses disorder, comprised of 164 METH-dependent subjects, and 14 METH abuse subjects, and schizophrenia were 178 (144 males and 34 females) and 288 (140 males and 148 females), respectively. The ages of each patient group were 18–69 years old (mean±SD; 36.7±12.0) and 15–75 (39.6±14.0), respectively. No patient with schizophrenia had severe physical complications or other Axis-I disorders according to DSM-IV when enrolled in this study, because seven schizophrenic subjects with METH use disorder were excluded based on the criteria that restricted a comorbid diagnosis of any psychotic disorder other than METH-induced psychosis. Among the subjects with METH use disorder, 150 (124 males and 25 females) have a comorbid diagnosis of METH-induced psychosis, three of anorexia nervosa, one of obsessive-compulsive disorder, and one of major depressive disorder. Additionally, 119 subjects with METH use disorder have abuse or dependence on drugs other than METH. The past history of any mental illness was not examined. The ages of METH-induced psychotic subgroup were 19–69 years old (37.7+12.3). No patient with METH use disorder had any severe physical complications when enrolled in this study. The 288 unrelated healthy volunteers (152 males and 136 females), aged 19–65 years (33.6±13.0), were comprised of hospital staff members and medical students at Fujita Health University. All healthy controls were also psychiatrically screened based on unstructured interviews. After complete description of the study to each subject, written informed consent was obtained. This study was approved by the ethics committee of each JGAIDA institute.

SNP Identification

Genomic DNA was isolated from whole blood using PUREGNER (Gentra system, Minneapolis, MN 55447, USA). For denaturing high-performance liquid chromatography (DHPLC) analysis, we designed specific primer sets amplifying all GABRG2 exons and the flanking intronic splice sites, based on GenBank sequence (NM000816 and NT023133) (primer sequences are available on request).

Polymerase chain reaction (PCR) was performed in a 10-μl volume containing 10 ng sample DNA, 0.4 M of each primer, 200 μM each dNTP, 1 × PCR Gold Buffer, 1.5 mM $MgCl_2$ and 0.25 U of Amplitaq Gold™ (Applied Biosystems Japan Ltd, Tokyo, Japan), using GeneAmp™ PCR system 9700 (Applied Biosystems Japan Ltd). PCR cycling conditions consisted of an initial denaturation step at 95°C for 9 min, followed by 45 cycles of 95°C for 15 s, 60°C for 20 s, 72°C for 30 s, and ending with a final extension step at 72°C for 7 min.

To screen for nucleotide variants, the obtained PCR products from all screened samples were analyzed by DHPLC with the WAVE™ system (Transgenomics Japan Ltd, Tokyo, Japan). The PCR products showing variant chromatograms were amplified again and then sequenced with an ABI PRISM™ 3100 Genetic Analyzer (Applied Biosystems Japan Ltd). Furthermore, to screen for any kinds of nucleotide variants in the splicing regulatory elements surrounding the spliced exon, we performed direct sequencing of the 96 controls. The conditions for DHPLC analysis and direct sequencing were reported previously.[39]

SNP Genotyping

To confirm the sequencing result and to genotype the variants in additional samples, the DHPLC analysis using the primer extension methods were developed for genotyping 588T>C by modifying the method of Hoogendoorn *et al*,[40] as reported previously.[39] All the remaining SNPs examined were genotyped using PCR-restriction fragment length polymorphism (PCR-RFLP) methods. Of four RFLP sites selected, the *Bam*HI restriction site in the eighth exon was genotyped as described by Loh *et al*,[12] while for the rest of the three SNPs, PCR-RFLP methods were developed (detailed information on experimental procedures is available upon request).

Statistical Analysis

Tests for Hardy–Weinberg equilibrium, the calculation of LD measures such as D′, Δ_2 and *P*-value and the estimation of haplotypic frequencies were carried out using Arlequin software 2.0.[41] The haplotypic frequencies between each patient group and controls were also compared using Arlequin software 2.0. The genotypic and allelic frequencies among each patient group and control group were compared with an exact test, using SPSS (version 10). A two-tailed level of 5% was chosen for the type I error rate. We have not corrected for multiple testing so as to avoid false negative findings.

Following Ohashi and Tokunaga,[40] we estimated the power of association analysis for our sample size of 178 subjects with METH use disorder, 288 schizophrenics and 288 controls under multiplicative model of inheritance, assuming a population susceptibility allele frequency of 0.30 at 315C>T and 0.48 at 1128+99C>A, the value in our screened samples. Setting the type I error rate at 5% and Genotype relative risk at more than 1.4 and 1.5, we obtained more than 80% power for direct association analysis of METH use disorder and schizophrenia, respectively.

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DUALITY OF INTEREST

None declared.

ABBREVIATIONS

METH	*methamphetamine*
GABA	*γ-aminobutyric acid*
GABRG2	*The human $GABA_A$ receptor gamma 2 subunit gene*
GAD	*glutamic acid decarboxylase*
PFC	*prefrontal cortex*
LD	*linkage disequilibrium*
DHPLC	*denaturing high-performance liquid chromatography*
PCR-RFLP	*polymerase chain reaction-restriction fragment length polymorphism*

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Letter to the Editors

A missense polymorphism (H204R) of a Rho GTPase-activating protein, the chimerin 2 gene, is associated with schizophrenia in men

Dear Editors:

Schizophrenia is a complex genetic disorder characterized by profound disturbances of cognition, emotion and social functioning. This disease is believed to involve genetic abnormalities in developmental/plasticity related processes (DeLisi, 2000). The pathophysiology of schizophrenia is still unclear; however, the high incidence developing schizophrenia was observed in mental retardation, suggesting common pathophysiological basis of these two diseases including genetic basis. As several X-linked mental retardation genes are involved in Rho signaling pathways (Ramakers, 2002), Rho GTPase related genes could be strong candidate genes for schizophrenia. The Rho family of GTP binding proteins act as a key regulator for developing neuronal network, e.g. neurite and growth cone formation (Negishi and Katoh, 2002). Chimerin 2 gene, CHN2, is one of the GTPase-activating proteins expressed in a variety of human tissues with the highest expression levels in brain (Yuan et al., 1995). Therefore, genetic variability of the chimerin 2 gene is of considerable interest in the evaluation of risk of schizophrenia. To our knowledge, however, there is no study examining the possible association between the CHN2 gene and schizophrenia.

The CHN2 maps to chromosome 7p15.3 and consisted of 13 exons and 12 introns, spanning 318 Kb (Yuan et al., 1995). We searched for polymorphisms in the CHN2 gene in silico and detected a common single nucleotide substitution (A611G; NCBI SNP ID: rs3750103) (Haga et al., 2002) in exon7 giving rise to an amino acid change of histidine to arginine at codon 204 (H204R) (amino acid numbering is according to NCBI protein data base accession NP_004058). In our search there was no other missense polymorphism reported in the CHN2 gene. Since this polymorphism may alter functions of the CHN2, we performed an association analysis between this polymorphism and schizophrenia in a Japanese sample of 293 patients (162 males and 131 females with mean age of 43.7 years [S.D. 14.2]) with schizophrenia and 450 healthy controls (222 males and 228 females with mean age of 36.5 years [S.D. 12.6]). Consensus diagnosis was made for each patient by at least two psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition(DSM-IV) criteria. After description of the study, written informed consent was obtained from every subject. The study protocol was approved by institutional ethical committees.

The H204R polymorphism genotypes were determined using the TaqMan 5′-exonuclease allelic discrimination assay, described previously (Hashimoto et al., 2004). Briefly, probes and primers for detection of the SNP are: forward primer, 5′-CAGATCTCCTC-CCTGGTTCGA-3′, reverse primer, 5′-TGCTTACCT-TAAAGTTGTGTGTCTTCT-3′, probe 1, 5′-VIC-CCCTCACACACAACGA-MGB-3′, and probe 2, 5′-FAM-CCTCACACGCAACGA-MGB-3′.

Genotype distributions and allele frequencies of the H204R missense polymorphism of the chimerin 2 gene among the patients and controls are shown in Table 1. The genotype distributions for the two groups and those of male and female patients as well as controls were in Hardy–Weinberg equilibrium (data not shown). There was a trend towards an increased frequency of the R204 allele in the patients than in the controls ($\chi^2=3.74$, $df=1$, $p=0.053$, odds ratio = 1.29, 95%CI 1.00–1.66). The individuals homozygous for the R204 allele were significantly

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Table 1
Genotype and allele distributions for the H204R polymorphism of the chimerin 2 gene between the patients with schizophrenia and controls

Group	*N*	Genotype distribution				Mantel Haenszel	Allele frequency			odds ratio (95%CI)
		His/His	His/Arg	Arg/Arg	*P* value	*P* value	His	Arg	*P* value	
Male										
Patients	162	95 (58.6%)	57 (35.2%)	10 (6.2%)	0.047	0.018	247 (76.2%)	77 (23.8%)	0.018	1.53 (1.07–2.19)
Controls	222	152 (68.4%)	65 (29.3%)	5 (2.3%)			369 (83.1%)	75 (16.9%)		
Female										
Patients	131	80 (61.2%)	44 (33.6%)	7 (5.3%)	0.703	0.678	204 (77.9%)	58 (22.1%)	0.681	1.08 (0.75–1.56)
Controls	228	141 (61.9%)	79 (34.6%)	8 (3.5%)			361 (23.8%)	95 (20.8%)		
Total										
Patients	293	175 (59.7%)	101 (34.5%)	17 (5.8%)	0.087	0.052	451 (77.0%)	135 (23.0%)	0.053	1.29 (1.00–1.66)
Controls	450	293 (65.1%)	144 (32.0%)	13 (2.9%)			730 (81.1%)	170 (18.9%)		

more common in the cases than in the controls ($\chi^2 = 3.94$, $df = 1$, $p = 0.049$, odds ratio = 2.07, 95%CI 0.99–4.33). The observed frequency for the minor allele (R204) in our control group (19%) was quite similar to that reported by Haga et al. (2002) (18%) estimated from 48 Japanese chromosomes. Thus, the observed significant difference in the allele frequency between the cases and controls cannot be ascribed to an unusually lower frequency of the R204 allele in our control subjects.

As gender differences occur in various aspects of the disease, including earlier age of onset, poorer course and medication response in men, we examined males and females separately. The R204 allele was excess in our cases when compared to controls among males ($\chi^2 = 5.57$, $df = 1$, $p = 0.018$, odds ratio = 1.53, 95%CI 1.07–2.19). Genotype distributions also revealed significant difference between male controls and male patients with schizophrenia ($\chi^2 = 6.12$, $df = 2$, $p = 0.047$; $\chi^2 = 5.56$, $df = 1$, $p = 0.018$ by Mantel Haenszel test). However, there was significant difference in neither allele frequency nor genotype distribution between the schizophrenics and controls in females.

CHN2 protein acts as a receptor of diacylglycerol/phorbol esters and regulates the activity of the Rac GTPase, one of the Rho GTPase family proteins (Caloca et al., 2003). The CHN2 inhibits Rac-GTP activation by the stimulation of epidermal growth factor (EGF). EGF protein levels were decreased in the prefrontal cortex of schizophrenic patients, and conversely, EGF receptor expression was elevated in the prefrontal cortex (Futamura et al., 2002). Serum EGF levels were markedly reduced in schizophrenic patients, even in young, drug-free patients (Futamura et al., 2002). Neonatal perturbation of EGF in rats resulted in abnormal sensorimotor gating and social interaction in adults (Futamura et al., 2003). In addition, neuregulin-1, one of the EGF family proteins, was reported as a schizophrenia susceptibility gene (Harrison and Owen, 2003) and the abnormal expression of neuregulin-1 has been observed in schizophrenic brain (Hashimoto et al., 2003). Therefore, the CHN2 H204R polymorphism might lead to the abnormality of neuregulin signaling pathways. As the location of H204R is close to diacylglycerol/phorbol ester binding domain (214–264 amino acid), this polymorphism could alter the protein structure of the region, which may change the second messenger signaling. H204R polymorphism, next to a casein kinase II phosphorylation site, might also play a potential role in the CHN2 phosphorylation state, although the physiological phosphorylation status is unclear.

We demonstrated, for the first time, the possible association between a missense polymorphism (H204R) of the CHN2 gene and schizophrenia in a Japanese population. A false-positive association due to population stratification could not be excluded in our case control designed study, despite the precaution of ethnic matching of this study. Therefore, it is necessary to carry out further investigations to confirm our findings in other samples. If our results are replicated, functional analysis of the CHN2 H204R polymorphism might contribute to understanding the molecular mechanisms of schizophrenia.

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Thioridazine Inhibits Risperidone Metabolism

A Clinically Relevant Drug Interaction

To the Editors:

Risperidone mainly undergoes 9-hydroxylation, yielding an active metabolite, 9-hydroxyrisperidone.[1] The plasma concentration of risperidone is predominantly influenced by CYP2D6 activity.[2,3] Thioridazine, a low-potency typical antipsychotic agent, has been widely used in schizophrenic patients with mild excitement and/or moderate to severe anxiety symptoms compared to other typical antipsychotics because of its sedative effects and lower risk of extrapyramidal symptoms.[4] Based on these characteristics, thioridazine is not uncommonly prescribed together with risperidone in some patients with mild excitement and/or moderate to severe anxiety symptoms despite receiving risperidone, although another combination between risperidone and benzodiazepine has been recently recommended in the treatment of anxiety symptoms. As an in vivo study has suggested that repeated administration of 50 mg/d thioridazine dramatically altered the metabolic ratio of debrisoquine,[5] it is most likely that thioridazine inhibits risperidone metabolism. Thus, we studied the effects of 2-week additional treatment with thioridazine on plasma concentrations of risperidone and 9-hydroxyrisperidone in schizophrenic patients with prevailingly anxiety symptoms.

The subjects were 12 schizophrenic inpatients (3 males and 9 females) with prevailingly anxiety symptoms despite treatment with risperidone 6 mg/d. All patients fulfilled the criteria for schizophrenia according to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*. The mean ± SD (range) of age was 52 ± 13 (30 to 65) years, body weight was 58 ± 11 (40 to 87) kg, and duration of illness was 165 ± 122 (12 to 388) months. The study was approved by the Ethics Committee of Hirosaki University Hospital, and written informed consent to participate in this study was obtained from the patients and their families.

The subjects had received risperidone 3 mg twice a day (8 AM and 8 PM) for 4 to 54 weeks. The drugs coadministered were flunitrazepam 1 to 4 mg/d in 8 cases, biperiden 4 to 6 mg/d in 6 cases, and sennoside 12 to 48 mg/d in 4 cases. The doses of these coadministered drugs were fixed throughout the study period. Thioridazine 25 mg twice a day (8 AM and 8 PM) was coadministered to all subjects for 2 weeks. Blood samplings were performed before and during thioridazine coadministration just before the morning dose (8 AM). On the same days as the blood samplings, severity of illness and side effects were evaluated by the Brief Psychiatric Rating Scale[6] and the Udvalg for Kliniske Underssgelser side effects rating scale,[7] respectively. Raters were blind to the study protocol and regimens of patients. The 18 items of the Brief Psychiatric Rating Scale were divided into 5 clusters: positive, excitement, cognitive, negative, and anxiety-depression symptoms.[8] Nineteen items selected from the Udvalg for Kliniske Underssgelser side effect rating scale were further divided into 3 subgroups: psychic, neurological, and autonomic side effects.

Plasma concentrations of risperidone and 9-hydroxyrisperidone were measured using a liquid chromatography–mass spectrometry–mass spectrometric method.[3] The *CYP2D6*1* (**1*), **2*, **3*, **4*, **5*, **10*, and **14* alleles and gene duplication were identified by polymerase chain reaction analysis.[3] Statistical analyses were performed using the Student *t* test and the Spearman rank test. A *P* value of 0.05 or less was regarded as significant.

Mean (±SD) plasma concentration of risperidone during thioridazine coadministration was significantly higher than the corresponding value before the coadministration (34.4 ± 15.4 vs. 6.9 ± 5.8 ng/mL, $P < 0.001$) (Fig. 1). Plasma concentration of 9-hydroxyrisperidone significantly decreased (28.7 ± 58.9 vs. 39.9 ± 11.4 ng/mL, $P < 0.01$), while active moiety (risperidone plus 9-hydroxyrisperidone) concentration significantly increased (65.7 ± 20.7 vs. 45.5 ± 512.4 ng/mL, $P < 0.01$) (Fig. 1). Ratio of risperidone/9-hydroxyrisperidone during thioridazine coadministration was significantly higher than the corresponding value before the coadministration (1.11 ± 0.47 vs. 0.18 ± 0.15, $P < 0.001$).

A significant correlation was found between the percentage of control in risperidone concentrations during thioridazine coadministration and the baseline ratios of risperidone/9-hydroxyrisperidone ($r_s = -0.825$, $P < 0.01$).

Three *CYP2D6* genotypes were identified in the patients: 2 homozygotes of the **1* allele, 5 heterozygotes of the **1* and **10* alleles, 2 heterozygotes of the **1* and **5* alleles, and 3 of homozygotes of **10* allele. These patients were divided into 3 groups according to the number of mutated alleles: 0 mutated allele in 2 cases, 1 mutated allele in 7 cases, and 2 mutated alleles in 3 cases. Large differences in the percentage of control in risperidone concentration and baseline ratios with thioridazine coadministration were observed between *CYP2D6* genotype groups. Percentages of control in patients with 0, 1, and 2 mutated alleles for *CYP2D6* were 1331% ± 704%, 936% ± 606%, and 267% ± 73% for risperidone concentration and 1914% ± 713%, 1382% ± 889%, and 260% ± 42% for ratio of risperidone/9-hydroxyrisperidone, respectively.

Brief Psychiatric Rating Scale total scores significantly decreased ($P < 0.05$), and Udvalg for Kliniske Underssgelser total scores significantly increased ($P < 0.05$) with thioridazine coadministration. However, there were no significant differences in clinical symptoms or side effects in any of subgroups.

The results of this study showed a pronounced elevation of risperidone and a slight but significant drop in 9-hydroxyrisperidone during coadministration of low-dose thioridazine. As a result, the active moiety concentration was significantly increased by thioridazine coadministration. These findings clearly indicate that thioridazine inhibits 9-hydroxylation of risperidone, catalyzed by CYP2D6, which are consistent with previous interaction studies using other CYP2D6 inhibitors such as fluoxetine[9] and paroxetine.[10] Thus, it is likely that steady-state plasma concentrations of risperidone are influenced during coadministration of other psychotropic agents that have CYP2D6 inhibitory effects, including perphenazine (Ki, 0.8 μM), chlorpromazine (Ki, 6.4 μM), and haloperidol (Ki, 7.2 μM).[11]

As no differences have been reported in 9-hydroxyrisperidone concentrations after concomitant administration of fluoxetine[9] and paroxetine,[10] we assumed that steady-state plasma concentration of 9-hydroxyrisperidone might not be significantly affected even when CYP2D6 activity is strongly inhibited by thioridazine. Contrary to our expectation, the steady-state plasma concentration of 9-hydroxyrisperidone was decreased. We have a plausible explanation for this discrepancy. Coadministration of low-dose thioridazine might be enough to inhibit formation of 9-hydroxyrisperidone from risperidone but not be enough to inhibit further metabolism of 9-hydroxyrisperidone.

The percentages of control in risperidone concentration during thioridazine coadministration were correlated to the concentration ratios of risperidone/9-hydroxyrisperidone, which were also closely associated with *CYP2D6* genotypes. Since the ratio of risperidone/9-hydroxyrisperidone is an index of CYP2D6 activity,[3] these findings imply that thioridazine coadministration has a stronger impact on risperidone metabolism in subjects with more activity of CYP2D6.

Significant changes in clinical symptoms or side effects were observed during thioridazine coadministration, These findings imply 2 possibilities. First, an approximately 40% elevation in active moiety concentration might enhance drug efficacy in some patients and/or it might reach toxic ranges in other patients. Second, some pharmacological characteristics of thioridazine itself might contribute to the pharmacodynamic changes in the present study. Because this combination therapy was generally well tolerated under the conditions of this study, no dosage adjustment of risperidone is likely to be necessary. From a pharmacokinetic point of view, however, benzodiazepine in addition to risperidone is preferable to control anxiety symptoms in schizophrenia.

In conclusion, this study showed that thioridazine coadministration significantly increases the steady-state plasma concentrations of risperidone

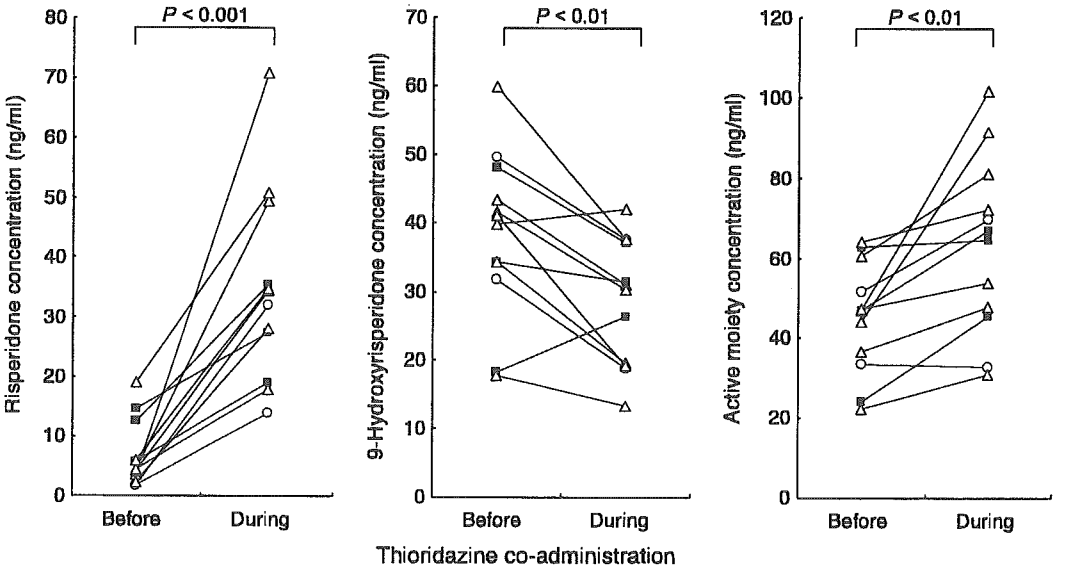

FIGURE 1. Individual changes in steady-state plasma concentrations of risperidone, 9-hydroxyrisperidone, and active moiety before and during thioridazine coadministration. Open circles indicate patients without mutated alleles for CYP2D6. Open triangles indicate those with 1 mutated allele. Solid squares indicate those with 2 mutated alleles.

and decreases those of 9-hydroxyrisperidone, probably by inhibiting the metabolism of risperidone. A greater elevation in risperidone concentrations was observed in subjects with more capacity for CYP2D6-dependent 9-hydroxylation of risperidone.

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Cannabis-Induced Extrapyramidalism in a Patient on Neuroleptic Treatment

To the Editors:

Although cannabis has been long considered a cause of psychotic episodes[1] and remains a controversial risk factor for schizophrenia,[2] no reports of its involvement in extrapyramidal side effects have insofar been made. Central cannabinoid receptors are located in the postsynaptic terminal of neurons in both the lateral globus pallidus and substantia nigra, thus suggesting a role in motor activity regulation.[3] There is also some evidence of the role played by endogenous cannabinoid system within basal ganglia transmission circuitry, mediated through an increase in γ-aminobutyric acid activity, inhibition of glutamate release, and interference with dopamine reuptake. Several movement disorders are caused by dysfunctions of basal thalamus–cortical circuits and again a role is ascribed to the central cannabinoid system in either pathophysiology or therapeutics. Current literature remains contradictory as far as its possible therapeutic use in movement disorders is concerned. While some research suggests efficacy in treating tics in Tourette syndrome,[4] or dopa-induced tardive dyskinesia in Parkinson disease,[3] the only double-blind, randomized, placebo-controlled study known to us[5] showed no significant reduction of dystonia in patients on treatment with the synthetic cannabinoid agonist nabilone.

Mr R is a 20-years-old Caucasian male with no previous history of movement disorders. He is a cannabis abuser since the age of 16. One year ago, he was diagnosed of paranoid schizophrenia and successfully treated during hospitalization with risperidone 9 mg/d and clorazepate 10 to 20 mg/d, with no noteworthy side effects [Udvalg for Kliniske Underssgelser side effect rating scale, neurologic subescale: 3/27; global assessment of interference with daily performance, both patient and doctor: 1/3 (no interference)][6] during the 4 weeks spent in our clinic. On discharge, he resumed cannabis misuse. He then suffered from a series of cervical and jaw dystonia episodes, as well as oculogyric crises, which were immediately relieved in the emergency room by intramuscular biperiden and showed a clear-cut connection in time with the use of cannabis. Every admission was preceded by high-dose consumption of cannabis, as a chart that correlated cannabis intake and extrapyramidal side effects retrospectively ascertained it. Prophylactic biperiden (2 to 4 mg/d), although used after the first episode, failed to prevent the crisis, and intramuscular extra doses were always needed.

Following treatment discontinuation and a consequent new episode of psychosis, he was admitted again in our unit. Due to the side effects already

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Short Communication

Influence of duration of untreated psychosis on auditory P300 in drug-naive and first-episode schizophrenia

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Abstract P300 amplitude reduction in schizophrenia is, according to previous studies, partially recovered by treatment with neuroleptics. However, whether this medication-induced P300 recovery is associated with duration of untreated psychosis (DUP) remains unreported; the present study is a preliminary examination of this question. Auditory P300 was recorded from 18 drug-naive and first-episode schizophrenia patients, among whom 10 were identified as short DUP, and eight as long DUP. Follow-up event-related potential tests were carried out after treatment with haloperidol or bromperidol for approximately 2 months. Recovery of P300 amplitude was replicated after neuroleptic medication was administered. A significant interaction was found between DUP and the medication effect in P300 amplitude over the left temporo-parietal area; a significant P300 recovery was seen in short DUP but not in long DUP. These results suggest that first-episode schizophrenia patients with long DUP might have severe impairments in the left temporal structures, supporting DUP as a key variable in future neurobiological studies of first-episode schizophrenia.

Key words duration of untreated psychosis (DUP), first-episode schizophrenia, neuroleptic medication, P300.

INTRODUCTION

Duration of untreated psychosis (DUP) is the period of time from the first appearance of psychotic symptoms to the time adequate treatment is sought and secured. In schizophrenia, longer DUP is often associated with an unfavorable outcome in multiple ways, including the time or level of recovery from the first episode, the time to or likelihood of relapse after the first episode, and long-term outcome measured globally for up to 5 years after treatment is begun for first-episode patients.[1] The DUP may be important in the sense that first-episode patients with long DUP may be well beyond the period of formation of active deficit processes in their brains, as suggested by McGlashan in 1999. He postulated the following.

Active treatment in long DUP cases will not have any possible impact upon underlying deficit processes, because they are no longer active. On the other hand, the brains of some patients with short DUP may still be undergoing actively destructive neurobiological changes, with changing patterns of the manifest illness being a signal of such activity. In such short DUP cases, successful treatment of the psychosis may have the potential to do more than treat active symptoms; it may also impact upon these still active deficit processes, with the possibility of truncating the development of further chronicity.[2]

Cognitive impairment is an important clinical feature in schizophrenia patients. It has been extensively investigated using the P300 component of auditory event-related potentials (ERP), with P300 amplitude reduction being the most replicated electrophysiological finding.[3] This abnormality reflects the high-level attention-dependent cognitive deficits in schizo-

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phrenia. While it remains controversial whether conventional neuroleptic medications bring about improvement in cognitive deficits in schizophrenia, these medications have been found to induce a partial recovery of P300 amplitude reduction in unmedicated schizophrenia patients in several studies.[4,5] However, whether the neuroleptic medication-induced P300 recovery would be influenced by DUP remains unclear. Based on the finding of some neuropsychological studies that worse cognitive deterioration could be predicted by longer DUP,[6] a poorer P300 recovery might be expected in longer DUP patients after the administration of neuroleptic medication. The intention of the present study was to examine the relationship between DUP and neuroleptic medication-induced P300 recovery in first-episode patients. In order to avoid the effect on P300 of a past treatment history,[3] only drug-naive patients were recruited.

METHODS

Subjects

Eighteen schizophrenia patients were included in the initial ERP testing at their first visit to the Neuropsychiatry Department of University of the Ryukyus, Okinawa, Japan. Background data are shown in Table 1. The diagnosis of schizophrenia was made based on *Diagnostic and Statistical Manual of Mental Disorders* (4th edn; DSM-IV) criteria. The DUP was determined retrospectively and was defined as the period from the onset of the first non-specific psychotic symptoms, as reported by patients, family or family doctors, to the time that neuroleptic medication was initiated. The first psychotic symptoms included both positive symptoms (delusions, hallucinations, disorganized/bizarre behaviors, formal thought disorder and catatonic motor behaviors) as well as prodromal symptoms that were mild variants of either positive or negative symptoms (e.g. social isolation, impairment in role functioning, peculiar behaviors, impaired hygiene and grooming, blunted affect, digressive or vague speech, odd or magical thinking, and unusual perceptual experiences). The onset was determined to be at the time when a symptom had lasted throughout the day for several days, or appeared several times a week. The final determination of DUP involved consensus of two of the investigators (HH and ST). According to the bimodal distributions of DUP with highest frequencies at approximately 3 months and 2 years,[2] eight patients with a DUP of ≥2 years were identified as long-DUP subjects. The other 10 were identified as short-DUP subjects. After the patients were placed on neuroleptic medication (haloperidol in 14 patients, at a dosage of 3.75 ± 1.93 mg/day; bromperidol in four patients, at a dosage of 6.75 ± 1.50 mg/day) for approximately 2 months, a follow-up ERP test was performed. Clinical symptoms were evaluated using the 18-item Brief Psychiatric Rating Scale (BPRS).[7] Nineteen age- and gender-matched healthy subjects were included as the normal controls. All subjects were right-handed. The present study was approved by the Ethics Committee of the Faculty of Medicine, University of the Ryukyus, Okinawa, Japan. Informed con-

Table 1. Patient variables

	Short DUP	Long DUP	Normal controls
No. patients	10	8	19
Gender (M/F)	7/3	4/4	12/7
Age (years)	30.0 ± 8.6	29.6 ± 6.2	27.8 ± 6.2
Onset age (years)	29.7 ± 8.7	24.6 ± 3.9	
DUP (months)			
Mean	5.5 ± 4.0	61.1 ± 43.5	
Range	1–12	24–132	
Subtypes (cases)			
Paranoid	5	2	
Non-paranoid	5	6	
Medication period (days)	68.4 ± 43.4	68.8 ± 42.6	
Medication dosage (mg/day)	4.8 ± 2.1	3.9 ± 2.4	
BPRS			
Baseline	47.0 ± 7.8	48.5 ± 9.8	
Follow up	30.5 ± 9.9	33.1 ± 6.0	

BPRS, Brief Pyschiatric Rating Scale; DUP, duration of untreated psychosis.

sent was obtained from each subject before the ERP test was performed.

Event-related potentials recording

The electroencephalogram (EEG) data were collected from 16 Ag-AgCl disc electrodes placed at Fp1, Fp2, F7, F3, Fz, F4, F8, C3, C4, T5, P3, Pz, P4, T6, O1 and O2, and referred to linked earlobes using a laboratory computer (DP 1200, Japan). P300 from auditory stimuli was elicited using an oddball paradigm. Stimuli were infrequent target tones [2000 Hz, 75 dB sound pressure level (SPL), $P = 0.20$] and frequent standard tones (1000 Hz, 75 dB SPL, $P = 0.80$). The subjects were instructed to silently count the number of target tones. Recording was terminated when 40 artifact-free responses to infrequent stimuli were collected. The ERP were averaged online separately for target and standard tones. Trials were automatically rejected if at any point during the averaging epoch the voltages exceeded ±100 μV in the electro-oculogram (EOG) lead. The averaged potentials were baselined to the mean potential of the 200-ms period before stimulus onset. Details of the recording procedure have been published previously.[8]

Data analysis

The P300 component was defined as the most positive voltage in the target ERP waveform sampled within 260–450 ms after the stimulus onset. Both peak P300 amplitude and P300 latency were measured at all electrode sites. Data analyses concerned baseline comparisons between schizophrenia patients and the normal controls, and the interaction of medication effect × DUP in patients, using repeated measures ANOVA. The baseline comparisons were performed among three groups (controls, short DUP and long DUP) and with one within-subject factor, the recording site. Examination of the interaction of DUP × medication was performed only between the two patient groups (short DUP and long DUP), but with two within-subject factors: medication (drug-naive and medicated) and recording site. Because the inclusion of all 16 electrodes as being level in one factor of the recording site might reduce the power of statistical analysis, electrode-based regions of interest (ROI) were defined. The ROI were defined as frontal (F3, Fz, F4), parietal (P3, Pz, P4), left temporo-parietal (C3, P3, T5) and right temporo-parietal (C4, P4, T6). When the Mauchly sphericity assumption about the repeated measure factor was violated, Greenhouse–Geisser correction of degrees of freedom was applied, with only the corrected probability values reported. Post-hoc assessment of multiple comparisons employed Tukey's test. Results were considered significant for $P \leq 0.05$.

RESULTS

First symptom and psychopathological assessments

The first psychotic symptoms indicating onset of the illness course were as following: unusual perceptual experience in one short and one long DUP, second- or third-person auditory hallucinations in four short and one long DUP, delusions in two short and two long DUP, odd or magical thinking in two short DUP, digressive or vague speech in two long DUP, bizarre behavior in one long DUP, catatonic motor behavior in one short DUP, and impaired hygiene and grooming in one long DUP. The mean DUP of all 18 patient subjects was 30.0 ± 40.0 months.

Results of the BPRS and its subscales are shown in Table 2. Total BPRS scores and its five factor scores were reduced significantly by neuroleptic medication in these patients (total BPRS score, $F_{1,16} = 60.1$, $P < 0.001$; anxiety–depression factor score, $F_{1,16} = 21.9$, $P < 0.001$; anergia factor score, $F_{1,16} = 13.5$, $P = 0.002$;

Table 2. BPRS total scores and five subscale scores of schizophrenia patients

Items	Short DUP		Long DUP	
	Baseline	Follow up	Baseline	Follow up
Total	47.0 ± 7.8	30.5 ± 9.9	48.5 ± 9.8	33.1 ± 6.0
Anxiety–depression	9.0 ± 7.3	6.6 ± 7.0	9.6 ± 8.0	6.5 ± 6.0
Anergia	9.5 ± 7.5	6.9 ± 7.4	12.1 ± 6.9	9.0 ± 7.5
Thought disturbance	11.8 ± 6.3	7.4 ± 6.5	10.8 ± 7.2	7.4 ± 5.9
Activation	7.9 ± 5.6	4.9 ± 4.5	7.5 ± 5.7	4.8 ± 3.9
Hostile–suspiciousness	8.4 ± 4.3	4.9 ± 4.6	8.5 ± 7.3	5.5 ± 5.1

BPRS, Brief Pyschiatric Rating Scale; DUP, duration of untreated psychosis.

thought disturbance factor score, $F_{1,16} = 56.4$, $P < 0.001$; activation factor score, $F_{1,16} = 21.1$, $P < 0.001$; hostile–suspiciousness factor score, $F_{1,16} = 34.7$, $P < 0.001$). However, no difference was shown between the two DUP groups either prior to or after treatment with neuroleptic medication, and no interaction between DUP and medication was found.

Baseline comparisons between patients and controls

P300 amplitude had a significant group difference for all ROI (frontal, $F_{2,34} = 20.4$, $P < 0.001$; parietal, $F_{2,34} = 15.1$, $P < 0.001$; left temporo-parietal, $F_{2,34} = 16.7$, $P < 0.001$; right temporo-parietal, $F_{2,34} = 15.8$, $P < 0.001$). Post-hoc tests revealed a reduction in P300 amplitude over all ROI in both short DUP and long DUP, as compared to the unaffected controls. P300 amplitude did not differ between the two patient groups. P300 latency demonstrated a group difference only over the parietal ROI ($F_{2,34} = 3.7$, $P = 0.035$). Post-hoc tests further detected a prolonged P300 latency in the long DUP patients ($t_s = 3.6$, $P < 0.05$) but not in the short DUP patients ($t_s = 2.0$, $P > 0.05$) compared to the unaffected controls.

Duration of untreated psychosis and effect of medication

Neither P300 amplitude nor P300 latency differed between the two patient groups. The effect of neuroleptic medication on P300 amplitude of schizophrenic patients was significant for all ROI (frontal, $F_{1,16} = 8.1$, $P = 0.012$; parietal, $F_{1,16} = 19.0$, $P < 0.001$; left temporo-parietal, $F_{1,16} = 15.4$, $P = 0.001$; right temporo-parietal, $F_{1,16} = 16.5$, $P = 0.001$;). This effect demonstrated an interaction with DUP only for the left temporo-parietal ROI ($F_{1,16} = 4.7$, $P = 0.045$) but not for any other ROI (frontal, $F_{1,16} = 1.4$, $P > 0.05$; parietal, $F_{1,16} = 2.3$, $P > 0.05$; right temporo-parietal, $F_{1,16} = 0.2$, $P > 0.05$). For the left temporo-parietal ROI, follow-up ANOVA separately examining the medication effect in each patient group revealed that the neuroleptic medication significantly increased P300 amplitude in the short-DUP patients ($F_{1,9} = 17.9$, $P = 0.002$) but not in the long-DUP patients ($F_{1,7} = 1.7$, $P > 0.05$; Fig. 1). The effect of neuroleptic medication on P300 latency was not significant, and also had no interaction with the DUP factor.

DISCUSSION

This is the first report to examine the relationship between DUP and auditory P300 in schizophrenia patients. The DUP is difficult to ascertain in some cases because the onset of psychosis is often subtle and insidious. Despite the retrospective definition of DUP used in the present study, the length was consistent with the generally reported DUP dating from the onset of first non-specific psychotic symptoms.[9]

In the present study, both patient groups compared to unaffected controls had P300 amplitude reduction for all ROI; the long-DUP group also had P300 latency prolongation for the parietal ROI prior to treatment with neuroleptic medication. This confirms findings in our previous studies of P300 abnormalities in drug-naive and first-episode schizophrenia.[3,8] However, the group difference of P300 between short DUP and long DUP was not significant, possibly due to the small sample size and/or the increased individual variability within the patients.

The present study replicated the previous finding that P300 amplitude reduction could be partially recovered by treatment with the conventional neuroleptics, haloperidol and bromperidol, in drug-naive and first-episode schizophrenia patients, suggesting that early treatment will do more than treat active symptoms. Although there are studies negating medication-induced P300 recovery in schizophrenia, schizophrenia subjects in those studies had not been limited to first-episode patients.[10,11] First-episode patients have a higher rate of therapeutic response and symptom remission with use of conventional neuroleptics compared to patients with multiple prior episodes.[12] For first-episode schizophrenia patients who remain in treatment even with typical neuroleptics, their cognitive deficits possibly do not deteriorate and may improve in the early stage of the illness course, as shown in some neuropsychological studies.[13] The positive effect of medication on P300 amplitude adds neurobiological evidence to this. Neuroleptic medication did not have a significant effect on P300 latency in the present study. The effect of neuroleptic medication on P300 latency is somewhat complicated; both prolongation and shortening of P300 latency could be induced, respectively, as a side-effect and as a therapeutic effect of the neuroleptics.[4,5]

The present study has demonstrated an interaction between DUP and the neuroleptic medication for P300 amplitude over the left temporo-parietal area, where P300 amplitude recovery was significant only in short DUP but not in long DUP. This finding further attests to the pathophysiological significance of left temporal impairment in schizophrenia. Smaller left temporal P300 amplitude in first-episode schizophrenia has been associated with smaller left posterior superior temporal gyrus volume in magnetic resonance imaging (MRI).[14] Because longitudinal MRI studies have dem-

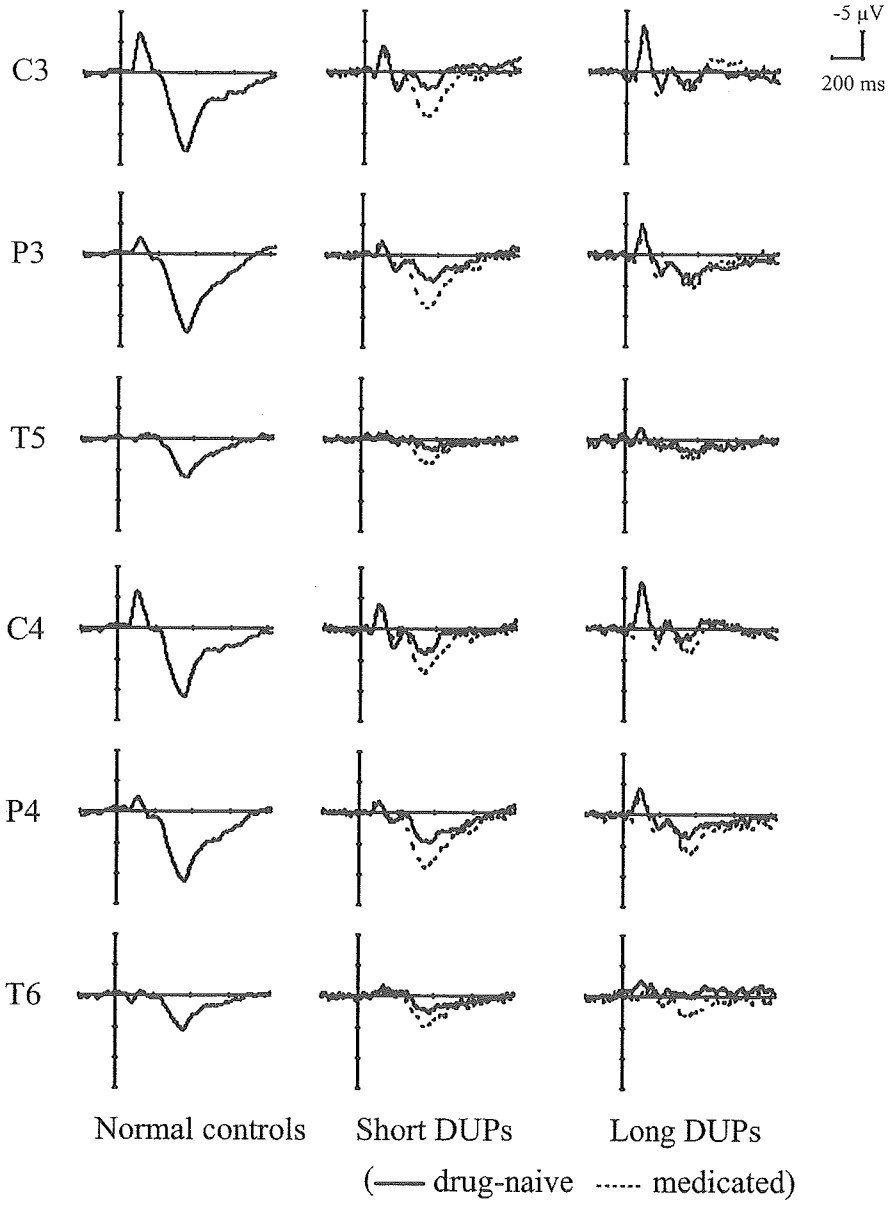

Figure 1. Grand average event-related potential (ERP) waveforms to target stimuli obtained at electrodes of left and right temporo-parietal areas. Prior to medication, both short-duration of untreated psychosis (DUP) and long-DUP patients showed a similar P300 amplitude reduction as compared to the unaffected controls. After the administration of neuroleptic medication, P300 recovery was symmetrical in the short-DUP patients, and asymmetrical in the long-DUP patients. Note that the P300 change over the left temporo-parietal area (C3, P3, T5) between the drug-naive state and the medicated state was not significant in the long-DUP patients.

onstrated a progressive volume reduction of the left posterior superior temporal gyrus gray matter, and a left-biased progressive volume reduction in the Heschl gyrus and planum temporale gray matter in patients with first-episode schizophrenia,[15,16] longer DUP is certainly associated with more pervasive gray matter loss in these aforementioned areas. Considering that the mean DUP length of the long-DUP group was around 5 years in the present study, impairments in the left temporal structures might be severe enough to be no longer active in some of these patients, which in turn leads to their poor P300 response to neuroleptic medication. Therefore, our finding could be linked to the postulation of McGlashan in the sense that first-episode patients with long DUP may be well beyond the period of active deficit processes in some brain regions, for instance, in the left temporo-parietal area.[2] In contrast, the deficit processes in other brain structures (in the frontal lobes etc.) might still be active in the long-DUP patients.[17,18]

We acknowledge several limitations in the present study. First, the retrospective definition of DUP was somewhat ambiguous and arbitrary. Second, the small sample size made our findings only suggestive, and confirmation with additional subjects is necessary. Because the effect of clozapine on P300 was more positive than