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Short Communication

Novel missense polymorphism in the regulator of G-protein signaling 10 gene: analysis of association with schizophrenia

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ABSTRACT Dysfunction of neuronal signal transduction via G-protein has previously been speculated to be involved in the pathophysiology of schizophrenia. Regulator of G-protein signaling (RGS) is a protein that acts as a GTPase-activator for Gα protein. A total of 33 Japanese patients with schizophrenia were screened for mutations in the coding region of the RGS10 gene, and a novel missense polymorphism (Val38Met) in the RGS domain was detected. A case-control study did not reveal a significant association between this polymorphism and schizophrenia. The results do not provide evidence that the RGS10 gene is involved in biological vulnerability to schizophrenia.

Key words G-protein-coupled receptor, GTPase-activating protein, polymorphism, regulator of G-protein signaling, RGS10, schizophrenia.

INTRODUCTION

Dysfunction of neuronal signal transduction via G-protein is thought to be involved in the pathophysiology of schizophrenia.[1] Regulator of G-protein signaling (RGS) is a protein that plays a role in modulating signaling through G-protein pathways. Over 20 mammalian subtypes of RGS proteins have been identified to date. All RGS proteins share a conserved 120-amino acid sequence termed the RGS domain. They function primarily as GTPase-activating proteins (GAP) for Gα proteins, accelerating the hydrolysis of GTP-bound-Gα proteins and shortening the duration of intracellular signaling of many G-protein-coupled receptors (GPCR), including dopamine, GABA, 5-HT, glutamate, and other neurotransmitter systems.[2] Recently, transcription of RGS4, a member of the RGS family, was found to be decreased in postmortem cerebral cortex of schizophrenia patients.[3] Among 26 single nucleotide polymorphisms (SNP) of the RGS4 gene, several in the upstream sequence and in the first intron were found to be associated with schizophrenia.[4] In addition, striatal gene expression of RGS2 and RGS4 were found to be specifically mediated by dopamine D1 and D2 receptors.[5] Thus, RGS proteins may have some potential as targets for drugs for neuropsychiatric disorders.[6]

RGS10, which belongs to the RGS family, consists of 173 amino acids and the RGS10 gene encompasses five exons spanning more than 36.5 kb of genomic DNA. RGS10 acts specifically on activated forms of the two G-proteins subunits, Gαi3 and Gαz.[7] RGS10 is highly expressed in brain regions that have been implicated in the pathophysiology of schizophrenia, such as prefrontal cortex and hippocampus.[8] In the prefrontal cortex and the hippocampus, levels of RGS10 mRNA were significantly reduced 24 h after acute and chronic electroconvulsive seizures.[8] Such seizures are known to improve psychotic symptoms. In addition, the RGS10 gene is located on chromosome 10q26.11,[9] which has been linked to schizophrenia and/or bipolar affective disorder.[10,11] Based on these findings, we hypothesized

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that some polymorphisms of the RGS10 gene which cause amino acid substitutions, if they exist, are involved in the biological susceptibility to schizophrenia through interference in the functions of this protein, such as its GAP activity. To test this hypothesis, we screened for functional polymorphisms in the coding region of the RGS10 gene and examined whether any of them were associated with schizophrenia. In addition, we assessed the frequency of another previously identified SNP in the coding region of the RGS10 gene that causes an amino acid substitution and that possibly alters the functions of this protein.

METHODS

This study was approved by the Ethics Committee of Kobe University Graduate School of Medicine. All subjects were given written informed consent to participate in this study. The study group comprised 311 unrelated Japanese patients (178 males and 133 females; mean age ± SD, 50.7 ± 17.2 years) who met the Diagnostic and Statistical Manual-IV diagnostic criteria for schizophrenia. The control subjects comprised 345 adults (205 males and 140 females; mean age ± SD, 52.0 ± 18.5 years). They were recruited from hospital staff documented to be free of psychoses, and company employees who did not manifest psychiatric problems in brief interviews by psychiatrists.

We selected 33 of the 311 schizophrenia patients at random and screened them for genetic variations in the coding region of the RGS10 gene by direct sequencing. The genomic structure of the RGS10 gene was determined from the National Center for Biotechnology Information database[12] (NT_030059 and AF045229). Genomic DNA was extracted from whole blood. All coding regions were amplified by the polymerase chain reaction (PCR). The following primer pairs for the amplification of the coding sequences were generated: pair 1, 5′-GGA AAC CAG TGG CCA TCT GT-3′ and 5′-CTC TGT GTC CTC ACA GCA CA-3′ (fragment size 285 bp, annealing temperature [Ta] 60°C); pair 2, 5′-GGC AGT GCT GAC CAT TGC AT-3′ and 5′-GAG TGC TAC GAC AGA CA-3′ (308 bp, Ta 60°C); pair 3, 5′-GGG GTT TTC GAA GAG TAA CT-3′ and 5′-GGC AAC TTC AGA ATC TAT TG-3′ (295 bp, Ta 56°C); pair 4, 5′-CCT TTC TCT TGC CAC AGC AG-3′ and 5′-GGA GTC TAA CAT CGG GTG TT-3′ (346 bp, Ta 58°C); and pair 5, 5′-GGG ATG ATT TAT GGC CTG GA-3′ and 5′-CCC ATT GAA GGG TTT TGT AC-3′ (329 bp, Ta 56°C). The sequencing reaction was performed with the ABI PRISM DNA sequencing kit (Applied Biosystems, Foster City, CA, USA). The labeled products were purified and were analyzed with an automated capillary sequencer.

In genotyping for the identified SNP Val38Met, a 308-bp fragment of the RGS10 gene was amplified by PCR with the primer pair 2. Subsequently, the PCR products were digested with the restriction enzyme *Nla*III, followed by agarose gel electrophoresis. The Met38 allele was cleaved into 168, 119 and 21 bp fragments, whereas, the Val38 allele was cleaved into 287 and 21 bp fragments.

We also genotyped another SNP, GCC to GTC (Ala88Val) in exon 4, corresponding to cDNA nucleotide position 395, which was obtained from the NCBI SNP database (ID no. rs1802228). A 346-bp fragment of the RGS10 gene was amplified using the primer pair 4. Genotyping for the Ala88Val polymorphism was performed by digestion with the restriction enzyme *Stu*I, followed by agarose gel electrophoresis.

Differences in the genotype and allele frequencies of the polymorphisms between the patients with schizophrenia and the control subjects were tested for significance using the two-sided Fisher's exacts test. Probability differences of $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

One missense mutation GTG to ATG (Val38Met) was detected in exon 2 of the RGS10 gene, corresponding to cDNA nucleotide position 244. We failed to detect other missense SNP or synonymous SNP in the coding region. Table 1 shows the genotype distribution and allele frequencies of the Val38Met polymorphism in the patients with schizophrenia and the control subjects. The genotype distributions of the Val38Met polymorphism in both groups were in Hardy–Weinberg equilibrium. However, the genotype distribution and allele frequencies in the patients with schizophrenia did not differ from those in the control subjects (genotype: $\chi^2 = 1.371$, d.f. = 2, $P = 0.574$; allele: $\chi^2 = 0.060$, d.f. = 1, $P = 0.833$). The Ala88Val mutation was not found in our Japanese samples.

In this study, we detected a novel missense polymorphism in the RGS10 gene. Although it is not clear whether the Val38Met polymorphism affects the RGS10 functions, this polymorphism is located in the RGS domain which mediates the GAP activity of the RGS10 protein. Therefore, this polymorphism may change the duration of intracellular signaling of the G-protein-coupled receptors. Functional studies of this polymorphism are necessary to evaluate the enzymatic activity of RGS10.

We found no association between the Val38Met polymorphism of the RGS10 gene and schizophrenia. Our study did not provide evidence that the RGS10 gene plays a significant role in the genetic predisposi-

Table 1. Genotype distributions and allele frequencies for the Val38Met polymorphism of the RGS10 gene in patients with schizophrenia and the controls

Group	Genotype n (%)			Allele n (%)	
	Val/Val	Val/Met	Met/Met	Val	Met
Schizophrenia	300 (96.5)	11 (3.5)	0 (0.0)	611 (98.2)	11 (1.8)
Controls	335 (97.1)	9 (2.6)	1 (0.3)	679 (98.4)	11 (1.6)

tion to schizophrenia. However, the power of the association analysis was calculated at approximately 0.12.[13] We cannot exclude the possibility that our failure to find an association between the Val38Met polymorphism of the RGS10 gene and schizophrenia is due to a type II error. So, it is necessary to conduct association studies of this polymorphism with schizophrenia in large samples. Moreover, we should investigate other SNP at other sites in the RGS10 gene, such as the promoter region or intronic sequences or other flanking sequences, and make association studies with such polymorphisms to clarify whether the RGS10 gene is involved in biological vulnerability to schizophrenia. In addition, it is necessary to examine whether the Val38Met polymorphism is associated with other psychiatric disorders because the RGS10 gene is located on chromosome 10q26.11, which was confirmed to be one of the bipolar affective disorder susceptibility loci.[10]

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Association between Catechol-O-Methyltransferase Functional Polymorphism and Male Suicide Completers

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Suicide has been suggested to involve catecholaminergic dysfunction and to be related to genetics. Catechol-O-methyltransferase (COMT) 158Val/Met polymorphism (GenBank Accession No. Z26491) is a polymorphism of the gene encoding COMT, a major enzyme in catecholamine inactivation. The COMT 158Val/Met polymorphism affects COMT activity, that is, the alleles encoding Val and Met are associated with relatively high and relatively low COMT activity, respectively. In this study, we hypothesized that the COMT 158Val/Met polymorphism is associated with suicide. The study population consisted of 163 suicide completers (112 males and 51 females). We found that the genotype distribution of the COMT 158Val/Met polymorphism was significantly different between male suicide completers and male controls ($p = 0.036$), while the frequency of the Val/Val genotype, a high-activity COMT genotype, was significantly less in male suicide completers than in male controls (OR: 0.52; 95% CL: 0.31–0.89; $p = 0.016$). However, this was not the case in females. Our results suggest that the Val/Val genotype is a protective factor against suicide in males.

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Keywords: suicide; catecholaminergic dysfunction; COMT 158Val/Met polymorphism; association study; gender differences

INTRODUCTION

Suicide has been suggested to involve catecholaminergic dysfunction and to be related to genetics. Catecholaminergic dysfunction has been observed in suicide. For example, low concentrations of 3-methoxy-4-hydroxyphenylglycol, a metabolite of norepinephrine, and homovanillic acid, a metabolite of dopamine, were observed in the cerebrospinal fluid of suicide attempters (Lester, 1995; Roy *et al*, 1986; Jones *et al*, 1990), and high concentrations of norepinephrine and decreased α2-adrenergic binding were observed in the prefrontal cortex of suicide victims (Arango *et al*, 1993). Genetic factors in suicide have been observed in family, twin, and adoption studies, and were found to be independent of psychiatric disorders (Roy *et al*, 1997).

Catecholaminergic dysfunction in suicide appears to be related to gene polymorphisms in catecholaminergic systems. Catechol-O-methyltransferase (COMT) is a major enzyme in catecholamine inactivation. COMT has a polymorphism named COMT 158Val/Met polymorphism (GenBank Accession No. Z26491), in which Val at codon 158 is replaced with Met. The alleles encoding Val and Met are associated with relatively high and relatively low COMT activity, respectively. The Val/Val genotype leads to a level of enzymatic activity three to four times that generated by the Met/Met genotype, while the Val/Met genotype leads to an intermediate activity (Lachman *et al*, 1996).

The COMT 158Val/Met polymorphism has been shown to be associated with suicide-related disorders. For example, the Met allele (low COMT activity) was associated with aggressive behaviors or violent suicide attempts in schizophrenic patients (Strous *et al*, 1997; Lachman *et al*, 1998; Kotler *et al*, 1999; Nolan *et al*, 2000). The Met allele was also associated with depressive disorders (Ohara *et al*, 1998), although other studies did not find such an association (Frisch *et al*, 1999; Kunugi *et al*, 1997b).

We hypothesized, therefore, that the COMT 158Val/Met polymorphism is associated with suicide, independently of psychiatric disorders. To test this hypothesis, we conducted a study of the association between the COMT 158Val/Met polymorphism and suicide completers in a Japanese population.

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Table 1 Genotype and Allele Frequencies of COMT 158Val/Met Polymorphism in Suicide Completers and Controls

	Genotype frequency			Allele frequency	
	Val/Val (%)	**Val/Met (%)**	**Met/Met (%)**	**Val (%)**	**Met (%)**
Suicide completers ($n = 163$)	68 (42%)	79 (48%)	16 (10%)	215 (66%)	111 (34%)
Controls ($n = 169$)	90 (53%)	61 (36%)	18 (11%)	241 (71%)	97 (29%)
Male suicide completers ($n = 112$)	43 (38%)	60 (54%)	9 (8%)	146 (65%)	78 (35%)
Male controls ($n = 114$)	62 (54%)	42 (37%)	10 (9%)	166 (73%)	62 (27%)
Female suicide completers ($n = 51$)	25 (49%)	19 (37%)	7 (14%)	69 (68%)	33 (32%)
Female controls ($n = 55$)	28 (50%)	19 (35%)	8 (15%)	75 (68%)	35 (32%)

METHODS

Subjects

The study population consisted of 163 suicide completers (112 males: mean age±SD, 48.68±16.9 years; 51 females: 47.12±19.8 years), who were autopsied at the Division of Legal Medicine, Kobe University Graduate School of Medicine. All subjects were ethnically Japanese. The methods of suicide were hanging (80), jumping from heights (49), drug overdose (8), drowning (8), several deep cuts (5), jumping in front of a vehicle (4), burning (3), gas poisoning (2), and other methods (4). Most (155) of the cases were classified as violent suicides according to the criteria proposed by Asberg *et al* (1976). Accurate information about the clinical backgrounds of the suicide completers could not be obtained under our ethical code for genetic studies.

Control subjects (159 males, 223 females) were recruited from the general population of Kobe city area, Japan. All were healthy and of Japanese descent and none manifested psychiatric problems in brief interviews by psychiatrists. To match the age and gender of the suicide subjects, 169 subjects (114 males: 45.33±15.5 years; 55 females: 48.9±18.7 years; all unrelated) were randomly selected from the above group. Informed consent was obtained from each control subject. This study was approved by the Ethical Committee for Genetic Studies of Kobe University Graduate School of Medicine.

Genotyping

Peripheral blood was drawn from suicide completers and controls, and leukocyte DNA was extracted for genotype determination. The genotypes of the COMT 158Val/Met polymorphism were determined by the method of Daniels *et al* (1996).

Statistical Analyses

The genotype distribution and Hardy-Weinberg equilibrium were tested with the χ^2 test for quality of fit. Comparisons of the genotype or allele frequencies between groups were performed with a χ^2 test. The level of significance was set at $p = 0.05$.

RESULTS

Table 1 shows the genotype and allele frequencies of the COMT 158Val/Met polymorphism in suicide completers and control subjects. There is a strong trend towards deviation from Hardy–Weinberg equilibrium in the male completers ($\chi^2 = 3.6$; df = 1, $p = 0.057$), while the genotype distributions in male/female controls and female suicide completers are in Hardy–Weinberg equilibrium. The allele frequencies in the controls were similar to those previously established for a Japanese population (Kunugi *et al*, 1997a; Ohmori *et al*, 1998; Ohara *et al*, 1998).

When the results for both genders were combined, the genotype distribution tended to be different between suicide completers and controls, although the difference was not significant ($\chi^2 = 5.4$; df = 2, $p = 0.068$). The allele frequencies were not different between suicide completers and controls ($\chi^2 = 2.2$; df = 1, $p = 0.14$). Similar results were obtained when only the violent suicide completers (155 of 163 subjects) were considered (data not shown).

In males, the genotype distribution was significantly different between suicide completers and controls ($\chi^2 = 6.7$; df = 2, $p = 0.036$). The genotype distribution of male suicide completers is also significantly different from controls of both genders combined ($\chi^2 = 8.40$; df = 2, $p = 0.015$, statistically significant tests are those having p values less than 0.017, alpha = 0.05/3). The Val/Val genotype appeared less frequently in male suicide completers than in male controls. The Odds ratio for the Val/Val genotype *vs* the other genotypes was 0.52 (95% CL: 0.31–0.89; $p = 0.016$) in male suicide completers. The Val/Met genotype appeared more frequently in male suicide completers than in male controls. The Odds ratio for the Val/Met genotype *vs* the other genotypes was 1.98 (95% CL: 1.16–3.37; $p = 0.012$) in male suicide completers. In allele frequencies, the Val allele tended to appear less frequently in male suicide completers than in male controls ($\chi^2 = 3.08$; df = 1, $p = 0.080$).

Among females, no significant differences were found between suicide completers and controls in either genotype distribution ($\chi^2 = 0.086$; df = 2, $p = 0.96$), or in allele frequencies ($\chi^2 = 0.007$; df = 1, $p = 0.93$).

DISCUSSION

This is the first study to examine the association between the COMT 158Val/Met polymorphism and suicide completers. We found that the Val/Val genotype, a high-activity COMT genotype, appeared less frequently in male suicide completers than in male controls. Among males, the risk for suicide in the Val/Val genotype carriers was only about half that in other genotype carriers (Odds ratio 0.52). However, this was not the case in females. Consequently, our results suggest that (1) the Val/Val genotype is a factor that protects against suicide, and (2) the COMT 158Val/Met polymorphism is associated with suicide, specifically in males.

Our finding that the Val/Val genotype is a factor that protects against suicide implies that the other genotypes including the Met allele, a low-activity COMT allele, increase suicide risk. This generally supports the results of previous reports that the Met allele is associated with suicide-related disorders (Ohara *et al*, 1998; Strous *et al*, 1997; Lachman *et al*, 1998; Kotler *et al*, 1999; Nolan *et al*, 2000). In our study, the Met allele tended to appear more common in suicide completers, although it did not reach statistical significance. The higher frequency of the Met allele was mainly due to the increased Val/Met genotype rather than to an increased Met/Met genotype. Why the Met/Met genotype did not increase is not clear. One possibility is that the frequency of the Met/Met genotype in our Japanese subjects is so low that the sample size is insufficient to detect a difference of the Met/Met genotype frequencies between suicide completers and controls. The frequency of the Met allele in Japanese subjects (approximately 0.3) is lower than that in Caucasian subjects (approximately 0.5) (Palmatier *et al*, 1999). Furthermore, in another Japanese study, the Val/Met genotype appeared more frequently in schizophrenics than in the controls, while the frequency of the Met/Met genotype was not significantly different between the two groups (Ohmori *et al*, 1998).

Our finding that the COMT 158Val/Met polymorphism is associated with suicide specifically in males implies that this polymorphism affects catecholaminergic systems differently in males and females. One possible explanation for the gender-specific association is that estrogen in females modulates neurotransmission and neuronal excitability of catecholaminergic systems (Balthazart *et al*, 1996). In previous studies, the COMT 158Val/Met polymorphism has been gender-specifically associated with several neuropsychiatric disorders: obsessive–compulsive disorders (Karayiorgou *et al*, 1999), narcolepsy (Dauvilliers *et al*, 2001), and attention deficit hyperactivity disorder (Qian *et al*, 2003). Moreover, the Met allele has been associated with violent suicide attempts specifically in male schizophrenic patients (Nolan *et al*, 2000).

In our study, the genotype distribution in the male suicide completers tended to deviate from Hardy–Weinberg equilibrium. There is little possibility of genotyping error only in the male suicide completers because the genotype distributions in other groups (male/female controls and female suicide completers) are in Hardy–Weinberg equilibrium. There is also little possibility of false-positive results due to population stratification in our study because the Japanese population is considered ethnically homogeneous due to its geographical and historical isolation (Katoh *et al*, 2002).

Our research contains some limitations. First, psychiatric diagnoses were not available in this study under our ethical code for genetic studies. We cannot completely exclude the possibility that the genotype differences are secondary to the different frequencies of psychiatric disorders and not directly related to risk for suicide. Second, we did not test several SNPs and haplotypes in the COMT gene. A haplotype analysis (Shifman *et al*, 2002) could have detected a smaller effect of the COMT 158Val/Met polymorphism on suicide completers. Third, the sample size of the subjects enrolled may be insufficient. Especially, in the comparison between female suicide completers and female controls, the power of the analysis was calculated to be 0.07. Considering that the COMT 158Val/Met polymorphism might have a very small effect on female suicide completers, we cannot completely exclude the possibility that our failure to find an association between the COMT 158Val/Met polymorphism and female suicide completers is due to a type II error. A more conclusive study with a substantially larger sample size may be required. Despite these limitations, our study provides new evidence regarding a protective factor for suicide.

In conclusion, we propose that the Val/Val genotype of the COMT 158Val/Met polymorphism, a high-activity COMT genotype, is a factor that protects against suicide specifically in males in the Japanese population.

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Cytochrome P450 II D6 gene polymorphisms and the neuroleptic-induced extrapyramidal symptoms in Japanese schizophrenic patients

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Objective The purpose of this study was to examine whether the neuroleptic-induced extrapyramidal symptoms are associated with the CYP2D6 activity.

Methods The CYP2D6 gene polymorphisms (CYP2D6*2, CYP2D6*3, CYP2D6*4, CYP2D6*10, and CYP2D6*12) were genotyped in 196 normal controls and 320 schizophrenic patients receiving neuroleptics. The relationships with susceptibility to extrapyramidal symptoms (EPS) and tardive dyskinesia, and with steady-state serum haloperidol levels in maintenance therapy, were investigated.

Results The allele frequency of CYP2D6*2 was significantly higher, while that of CYP2D6*10 tended to be higher in the schizophrenic patients susceptible to acute EPS. The steady-state serum haloperidol levels per daily dosage were observed to be significantly higher in schizophrenic patients with the mutant-type homozygote of CYP2D6*2, while this difference was trend level in those of CYP2D6*10. However, no significant difference was observed in the distribution of both CYP2D6*2 (C2938T) and CYP2D6*10 (C188T) polymorphisms between schizophrenic patients with or without tardive dyskinesia.

Conclusion The present results suggest that the homozygotes of CYP2D6*2 and CYP2D6*10 appear to be a susceptibility factor for developing acute EPS in schizophrenic patients and for impaired neuroleptic metabolism in Japanese schizophrenic patients. *Psychiatr Genet* 13:163–168 © 2003 Lippincott Williams & Wilkins.

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Keywords: CYP2D6, gene, polymorphism, schizophrenia, extrapyramidal symptom, tardive dyskinesia, haloperidol

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Introduction

Extrapyramidal symptoms (EPS) associated with typical antipsychotic drugs have been one of the major problems in the pharmacological treatment of schizophrenic patients. These symptoms, especially in severe cases, often affect patients' daily living and induce non-compliance with continued medication, resulting in the relapse of primary psychosis. Furthermore, the appearance of EPS in the initial phase of antipsychotic drug treatment (Kane *et al.*, 1986; Saltz *et al.*, 1991) and high cumulative neuroleptic exposure (Morgenstern and Glazer, 1993; Chakos *et al.*, 1996) have been reported to be risk factors for the troublesome late-onset involuntary movement disorders, tardive dyskinesia.

It is known that the development of acute EPS is substantially influenced by the serum neuroleptic level, which is considered to be regulated by the daily neuroleptic dose administered and the individual enzyme activity of hepatic cytochrome P450IID6 (CYP2D6). Most neuroleptic agents, including haloperidol (HPD), are eliminated through oxidative metabolism by CYP2D6. Therefore, its enzyme activity has been considered to form, at least in part, characteristics of the antipsychotic drug treatment, such as therapeutic response and vulnerability to side effects, by way of affecting the metabolism of antipsychotic drugs in individual patients. In the recent several years, mutations or polymorphisms that could alter the function of enzyme activity in the CYP2D6 gene have been found and most of their functional consequences have been established (Daly *et al.*, 1996; Sachse *et al.*, 1997).

Several reports have suggested that genetic factors are involved in the development of EPS (Myrianthopoulos *et al.*, 1962) and tardive dyskinesia (Weinhold *et al.*, 1981;

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Yassa and Ananth, 1981). In the 1990s, findings showing the positive association between CYP2D6 mutations and susceptibility to Parkinson's disease (Armstrong *et al.*, 1992; Smith *et al.*, 1992; Tsuneoka *et al.*, 1993) or subgroups of this disease (Agundez *et al.*, 1995; Sandy *et al.*, 1996) have been reported. These findings, although they are still controversial and have not been established, raise the hypothesis as to whether the genetic association between the CYP2D6 mutations remains eligible not only for idiopathic Parkinson's disease, but could also apply to neuroleptic-induced parkinsonism.

In the present study, the status of EPS and tardive dyskinesia, steady-state serum HPD levels and some other clinical variables concerning maintenance neuroleptic therapy were examined in the Japanese schizophrenic patients receiving antipsychotic agents, and the relationships with the CYP2D6 gene polymorphisms were investigated. The main purposes of this study were: (1) to clarify the effects of CYP2D6 gene polymorphisms that are known to decrease the enzyme activity in the Japanese schizophrenic patients; and (2) to discuss the possibility of a clinical application in psychiatry, whereby these polymorphisms help the prediction of the development of acute EPS in neuroleptic-naive schizophrenic patients and of late-onset movement disorders in patients receiving long-term neuroleptic treatment, that can be predicted by the knowledge of CYP2D6 genotypes. Such individuals could be specially monitored for side effects, and dosages of antipsychotics could be better controlled to avoid long-term and short-term side effects.

Subjects and methods

Ethical considerations

The present study was initiated after approval by the ethical committee of the National Center of Neurology and Psychiatry in the Kohnodai area. Written informed consent was obtained from all subjects, after adequate explanation of the study.

Subjects

Schizophrenic subjects were recruited from inpatients of a mental hospital in the suburbs of metropolitan Tokyo. Patients all satisfied the diagnostic criteria for DSM-III-R (American Psychiatric Association, 1987) of schizophrenia and had been receiving neuroleptic therapy for longer than 6 months. Control subjects, who volunteered to participate in the study, were mostly medical staff with no history of psychosis or substance abuse or of receiving antipsychotic medication. All subjects and their parents were Japanese.

Status of EPS

Clinical evaluation of neuroleptic-induced EPS was performed systematically in as many schizophrenic patients as possible who participated in this study. The EPS was assessed using the Drug-Induced Extrapyramidal Symptoms Scale (Inada *et al.*, 2002). Tardive dyskinesia was assessed using the Japanese version of the Abnormal Involuntary Movement Scale (Inada *et al.*, 1991). Subclassification in terms of acute EPS susceptibility and tardive dyskinesia vulnerability was carried out in as many patients as possible, using the criteria reported previously (Inada *et al.*, 1997, 1999). All clinical ratings were performed by trained raters, before the genotyping. Patients with scarce or incomplete information about acute EPS in the initial phase of neuroleptic therapy for their first episode of schizophrenia were excluded from the acute EPS susceptibility study, while those whose tardive dyskinesia status could not be obtained adequately were omitted from the tardive dyskinesia vulnerability study.

Neuroleptic dosage

Recent 1-year cumulative neuroleptic dosage was calculated in schizophrenic patients who had been receiving neuroleptics for longer than 1 year. Of these patients, steady-state daily neuroleptic dosage was calculated in only those who had been continuously receiving the identical neuroleptic medication as maintenance therapy for longer than 1 year. The chlorpromazine equivalent neuroleptic dosage administered to each patient was calculated using the conversion table specifically developed for the Japanese (Inagaki *et al.*, 1998).

HPD concentration

Serum HPD levels were routinely examined at approximately 2-month intervals in schizophrenic subjects who had been receiving HDL. Of these, only those who had been receiving a constant dose of HPD as maintenance therapy for longer than 3 months without any other butyrophenone derivatives (bromperidol, timiperone, pipamperone, moperone, and spiperone) were selected for the HPD concentration study. An average value was adopted when steady-state serum HPD concentrations were measured twice or more.

Determination of CYP2D6 gene polymorphism

The following CYP2D6 gene polymorphisms were examined: CYP2D6*2 (C2938T), CYP2D6*3, CYP2D6*4, CYP2D6*10 (C188T) and CYP2D6*12. Genotyping was carried out according to the standard protocol, slightly modified from the published methods. Briefly, genomic DNA was extracted from the leukocyte nuclei of peripheral blood. Each target segment was amplified by the polymerase chain reaction (PCR) method and the PCR products were digested with the restriction enzymes, and then electrophoresed.

Statistics

Comparisons of allele and genotype frequencies were performed using the chi-squared test for 2×2 and 2×3

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contingency tables. Comparisons of variables concerning neuroleptic therapy among three subgroups of CYP2D6 genotype were performed using either the Kruskal–Wallis test or one-way analysis of variance followed by the Scheffe test. The name of the statistical test used to analyze the data is described in each table. For statistical analyses, the computer package 'SPSS for Windows 98' (SPSS Japan Inc., Tokyo, Japan) was used. Significance was defined when $P < 0.05$.

Results

Profiles of the subjects

The demographic background of the subjects is presented in Table 1. The initial research protocol was designed for the following three polymorphisms: genotyping of the CYP2D6*2, the CYP2D6*3 and the CYP2D6*4. Additional experiments, genotyping of CYP2D6*10 and CYP2D6*12, were performed subsequently as an independent study.

CYP2D6*3, CYP2D6*4 and CYP2D6*12

Since these mutations are known to result in the lack of enzyme activity, no poor metabolizers were found in the subjects: no CYP2D6*3 and CYP2D6*12 alleles were detected, while a heterozygote of CYP2D6*1 and CYP2D6*4 was observed in one schizophrenic patient.

CYP2D6*2 (C2938T) and CYP2D6*10 (C188T)

The genotype and allele frequencies of CYP2D6*2 (C2938T) and CYP2D6*10 (C188T) polymorphisms are presented in Tables 2 and 3, respectively. The genotype distribution was not significantly different from that expected according to the Hardy–Weinberg equilibrium in any group of the subjects. No significant difference was observed either between schizophrenic patients and controls or between schizophrenic patients with or without tardive dyskinesia in both CYP2D6*2 (C2938T) and CYP2D6*10 (C188T) polymorphisms. The allele frequency of CYP2D6*2 was significantly higher [$\chi^2 = 5.6$, degrees of freedom (df) = 1, $P = 0.018$], while that of CYP2D6*10 ($\chi^2 = 2.7$, df = 1, $P = 0.099$) tended to be higher in the 'acute EPS present' group than in the 'acute EPS absent' group ($P = 0.018$). The genotype distribution of CYP2D6*2 also differed significantly between these two groups ($\chi^2 = 6.6$, df = 2, $P = 0.037$), while no significant difference was observed in the genotype distribution of CYP2D6*10 between these two groups.

Tables 4 and 5 indicate the results of the comparison of profiles of neuroleptic therapy among three subgroups of CYP2D6*2 and CYP2D6*10, respectively. Although the daily dosage of HPD, the steady-state daily neuroleptic dose and the recent 1-year cumulative neuroleptic dose

Table 1 Demographic background of the subjects

Group name	Age (years) [mean ± SD (minimum–maximum)]	Gender	
		Male	Female
CYP2D6*2 (C2938T), CYP2D6*3, CYP2D6*4			
Schizophrenia (n=309)	53 ± 14 (18–90)	191	118
Control (n=99)	42 ± 14 (21–87)	51	48
CYP2D6*10 (C188T), CYP2D6*12			
Schizophrenia (n=214)	53 ± 13 (19–81)	139	75
Control (n=196)	45 ± 13 (18–75)	56	140

Table 2 Genotype and allele frequencies of the CYP2D6*2 (C2938T)

Group	Genotype (GT)[a]			Allele frequency (AF) (%)		Statistics[b] (P value)
	WW	WM	MM	W ratio	M ratio	
Disease etiology						
Schizophrenia (n=309)	234 (232.4)	68 (71.1)	7 (5.4)	86.7	13.3	GT: 0.721
Control (n=99)	75 (75.6)	23 (21.8)	1 (1.6)	87.4	12.6	AF: 0.816
TD vulnerability						
Patients vulnerable to TD (n=41)	30 (30.7)	11 (9.5)	0 (0.7)	86.6	13.4	GT: 0.515
Patients not vulnerable to TD (n=142)	105 (104.0)	33 (35.1)	4 (3.0)	85.6	14.4	AF: 0.815
Acute EPS susceptibility						
Patients vulnerable to EPS (n=38)	20 (18.5)	13 (16.0)	5 (3.5)	69.7	30.3	GT: 0.037
Patients not vulnerable to EPS (n=41)	29 (29.9)	12 (10.2)	0 (0.9)	85.4	14.6	AF: 0.018

TD, tardive diskynesia; EPS, extrapyramidal symptoms.
[a]Expected genotype counts according to Hardy–Weinberg equilibrium are in parentheses. W, Wild type (CYP2D6*1); M, mutant type (CYP2D6*2).
[b]Chi-squared test was performed for 2X3 (GT: Genotype) and 2X2 (AF: Allele frequency).

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Table 3 Genotype and allele frequencies of the CYP2D6*10 (C188T)

Group	Genotype[a,b]			Allele frequency (%)		Statistics[b] (*P* value)
	WW	WM	MM	W ratio	M ratio	
Disease etiology						
Schizophrenia (*n*=214)	78 (74.8)	97 (103.5)	39 (35.8)	59.1	40.9	GT: 0.910
Control (*n*=196)	69 (68.1)	93 (94.9)	34 (33.1)	58.9	41.1	AF: 0.957
TD vulnerability						
Patients vulnerable to TD (*n*=27)	10 (10.1)	13 (12.8)	4 (4.1)	61.1	38.9	GT: 0.950
Patients not vulnerable to TD (*n*=106)	40 (38.6)	48 (50.7)	18 (16.6)	60.4	39.6	AF: 0.922
Acute EPS susceptibility						
Patients vulnerable to EPS (*n*=32)	10 (10.1)	16 (15.8)	6 (6.1)	56.3	43.8	GT: 0.260
Patients not vulnerable to EPS (*n*=32)	16(15.8)	13 (13.4)	3 (2.8)	70.3	29.7	AF: 0.099

TD, tardive diskynesia; EPS, extrapyramidal symptoms.
[a]Expected genotype counts according to Hardy–Weinberg equilibrium are in parentheses. W, Wild type (CYP2D6*1); M, mutant type (CYP2D6*10).
[b]Chi-squared test was performed for 2X3 (GT, genotype) and 2X2 (AF, allele frequency).

Table 4 Comparison of profiles of neuroleptic therapy among three subgroups of CYP2D*2 (C2938T) genotype

Group	Genotype[a]			Statistics[b] (*P* value)
	WW	WM	MM	
HPD concentration study (*n*=184)				
Daily dosage of HPD (mg/day) (A)	16.0±12.3 (*n*=141)	15.9±10.3 (*n*=40)	9.0±7.9 (*n*=3)	0.495
Serum HPD levels (ng/ml) (B)	16.2±12.3 (*n*=141)	16.2±9.9 (*n*=40)	14.8±5.5 (*n*=3)	0.917
B/A (ng/ml per mg per day)	1.1±0.6 (*n*=141)	1.2±0.6 (*n*=40)	2.4±1.3 (*n*=3)	0.003
Steady-state daily neuroleptic dose[c] (C) (*n*=96)	1,449±2378 (*n*=63)	1,598±3097 (*n*=30)	1,240±1547 (*n*=3)	0.678
Recent 1-year cumulative neuroleptic dose[c] (D) (*n*=203)	410,153±694,590 (*n*=149)	480,910±721,473 (*n*=50)	385,474±480,128 (*n*=4)	0.685

Data are expressed as mean±standard deviation. Number of patients used to analyze the data are shown in parentheses. HPD, haloperidol.
[a]W, Wild type (CYP2D6*1); M, mutant type (CYP2D6*2).
[b]Kruskal–Wallis test was performed for A, B, C and D, while one-way analysis of variance for B/A.
[c]Neuroleptic dose are expressed as chlorpromazine equivalent mg/day calculated using the table of the TRS-RG version (Inagaki *et al*., 1998).

Table 5 Comparison of profiles of neuroleptic therapy among 3 subgroups of CYP2D*10 (C188T) genotype

Group	Genotype[a]			Statistics[b] (*P* value)
	WW	WM	MM	
HPD concentration study (*n*=190)				
Daily dosage of HPD (mg/day) (A)	14.2±10.2 (*n*=66)	15.8±12.1 (*n*=86)	12.4±10.9 (*n*=38)	0.268
Serum HPD levels (ng/ml) (B)	13.8±9.2 (*n*=66)	16.8±12.6 (*n*=86)	16.3±9.6 (*n*=38)	0.359
B/A (ng/ml per mg per day)	1.2±0.7 (*n*=66)	1.2±0.6 (*n*=86)	1.4±0.6 (*n*=38)	0.097
Steady state daily neuroleptic dose[c] (C) (*n*=102)	1,117±2027 (*n*=41)	1,427±2275 (*n*=53)	813±570 (*n*=8)	0.486
Recent 1-year cumulative neuroleptic dose[c] (D) (*n*=179)	460,598±658,350 (*n*=68)	459,260±695,196 (*n*=81)	373,564±359,405 (*n*=30)	0.153

Data are expressed as mean±standard deviation. Number of patients used to analyse the data are shown in parentheses. HPD, haloperidol.
[a]W, Wild type (CYP2D6*1); M, mutant type (CYP2D6*10).
[b]Kruskal–Wallis test was performed for A, B, C and D, while one-way analysis of variance for B/A.
[c]Neuroleptic dose are expressed as chlorpromazine equivalent mg/day calculated using the table of the TRS-RG version (Inagaki *et al*., 1998).

were observed to be lower in the homozygote groups of both CYP2D6*2 and CYP2D6*10 when compared with the remaining groups, their differences did not reach a statistically significant level. Steady-state serum HPD levels per daily dosage were significantly higher ($P < 0.05$) in the CYP2D6*2 homozygote group than that in the remaining groups. Likewise, it was observed to be higher in the CYP2D6*10 homozygote group, although this difference was trend level ($P < 0.1$).

Discussion

In the present study, a positive association between CYP2D6 polymorphisms (CYP2D6*2 and CYP2D6*10)

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and an acute EPS episode within 3 months of the initial neuroleptic therapy was observed. A possible explanation is that a high risk for acute EPS in patients with a homozygote of CYP2D6*2 could be related to the high serum concentration of HPD resulting from impaired metabolism of neuroleptics due to genetic mutation. As presented in Tables 4 and 5, a significantly higher steady-state serum concentration-to-dose ratio for HPD was observed in patients with a homozygote of CYP2D6*2 than in the remaining patients, while a trend level in patients with a homozygote of CYP2D6*10. The present results suggests that both CYP2D6*2 and CYP2D6*10 are associated with the appearance of EPS in the initial phase of antipsychotic therapy in the Japanese schizophrenic subjects. This may be sharp contrast with the previous findings obtained from the Caucasian subjects that the CYP2D6*2 allele does not induce significant decrease in its enzyme activity (Kroemer and Eichelbaum, 1995; Sachse *et al.*, 1997). It is known that there are racial differences in the CYP2D6 enzyme activity. The rates of poor metabolizers who lack enzyme activity completely has been estimated as about 5–10% in Caucasians, while as less than 1% in Japanese. Therefore, CYP2D6 gene polymorphisms resulting in poor metabolizers are usually used to examine the role of CYP2D6 gene polymorphisms on EPS in the Caucasian study. Arthur *et al.* (1995) reported a positive relationship between the degree of impaired CYP2D6 activity and the severity of extrapyramidal side effects during neuroleptic treatment, although no over-representation of poor metabolizers was observed among neuroleptic-treated Swedish patients with tardive dyskinesia. Armstrong *et al.* (1997) examined the CYP2D6 genotypes in 76 schizophrenic patients of mainly European Caucasian origin and reported that the CYP2D6 genotype is not always a determinant of susceptibility to acute dystonic reactions, but may be a contributory factor in antipsychotic drug-induced movement disorders including tardive dyskinesia. As shown in this study, the frequencies of CYP2D6*3 and CYP2D6*4, which are representative poor metabolizers/extensive metabolizers markers in the Caucasian population, are quite low in the Japanese subjects so they cannot be suitable markers for this population. It is known that pharmacokinetic responses differ significantly among the various races. For example, Asian patients have been reported to have a higher serum HPD concentration than Caucasians for the same dosage of HPD (Lin and Poland, 1995). Ethnic difference is also considered to exist in respect of vulnerability to EPS (Binder and Levy, 1981) and tardive dyskinesia. A review of Japanese epidemiological studies of tardive dyskinesia suggests that its prevalence is significantly lower in Japanese than in Caucasian subjects (Inada and Yagi, 1995).

No significant difference was observed between schizophrenic patients with or without tardive dyskinesia in both CYP2D6*2 (C2938T) and CYP2D6*10 (C188T) polymorphisms. This suggests that CYP2D6*2 and CYP2D6*10 polymorphisms are unlikely to be of major aetiologic importance in the development of tardive dyskinesia. Ohmori *et al.* (1998, 1999) reported a significant association between tardive dyskinesia and CYP2D6*10 but no association was found with CYP2D6*2 in the Japanese schizophrenic patients. Differential results from the present study may come from the difference of the definition of tardive dyskinesia status. Subclassification of tardive dyskinesia/non-tardive dyskinesia in the present study is based on the longitudinal observation with multiple evaluation for at least 1 year, while that in the study by Ohmori *et al.* (1998, 1999) is based on the cross-sectional one.

In the present study, differential EPS response to neuroleptic medication was ascertained among the schizophrenic patients with the differential CYP2D6 polymorphism. Since polymorphisms that lack enzyme activity are quite rare in the Japanese population, genotyping of these polymorphisms that are known to decrease enzyme activity could be a useful marker in predicting the development of acute EPS in this population, especially for neuroleptic-naïve first-episode schizophrenic patients. It may help patients avoid neuroleptic non-compliance if the development of severe side effects can be screened during the initial phase of disease onset. In addition, our present HPD concentration study has indicated that this polymorphism could provide some information for estimating the required dose in maintenance therapy. However, the prediction of susceptibility to neuroleptic-induced acute EPS and tardive dyskinesia is difficult without considering ethnic and/or genetic factors. Further research is needed to clarify the exact roles of these factors on the susceptibility to neuroleptic-induced extrapyramidal side-effects for the purpose of accurate prediction of their risk in advance of initial neuroleptic therapy.

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Cytochrome P450 2D6 polymorphism and character traits

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Objective **It has been suggested that cytochrome P450 2D6 (CYP2D6) is involved in dopamine metabolism within the brain. The dopamine system is suggested to play a role in determining normal character. The purpose of this study was to examine whether character traits are dependent on cytochrome P450 2D6 activity.**

Methods **We investigated the association between temperament and CYP2D6 gene polymorphism. The subjects were all Japanese and the polymorphism genotyped in the present study was CYP2D6*10. Character traits were assessed using the Temperament and Character Inventory.**

Results **There was no overall or specific association between personality traits and the CYP2D6*10 allele and genotype frequencies.**

Conclusions **The present results do not support the hypothesis that CYP2D6 activity affects temperament and character. *Psychiatr Genet* 13:111–113 © 2003 Lippincott Williams & Wilkins.**

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Keywords: cytochrome P450 2D6, CYP2D6, polymorphism, character, TCI, dopamine

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Introduction

Dopamine is a neurotransmitter considered to be involved in the aetiologies of several mental disorders such as schizophrenia and mood disorder. Dopamine is formed from L-tyrosine by the action of tyrosine hydroxylase and aromatic L-amino acid decarboxylase. Some recent *in vivo* and *in vitro* studies have suggested that mixed function oxidases catalyse the oxidation of the aromatic ring of dopamine. Hiroi *et al.* (1998) showed that cytochrome P450 2D6 (CYP2D6) metabolizes tyramine to dopamine *in vivo*. Cytochrome P450 2D6 is also expressed in the brain, especially the midbrain (McFadyen *et al.*, 1997), where dopamine is produced. Furthermore, the value for the Michaelis constant is low enough to produce dopamine in the brain (Hiroi *et al.*, 1998).

Recently, it has been suggested that dopamine transmission in the central nervous system (CNS) is responsible for individual variations in personality. Ebstein *et al.* (1996) and Benjamin *et al.* (1996) reported that individuals with long alleles of polymorphic exon III, which has a repeat sequence of the dopamine D_4 receptor (D4DR) gene, are more likely to be novelty-seeking individuals than those with short alleles. Novelty seeking is characterized as impulsive, exploratory, fickle, excitable, quick-tempered and extravagant behaviour. This, defined by Cloninger *et al.* (1993), is one of the four mutually independent dimensions of normal temperament traits: Novelty Seeking, Harm Avoidance, Reward Dependence, and Persistence. Ohno *et al.* (1997) reported that there is an association between D4DR gene polymorphism and novelty seeking among the Japanese population too, suggesting that this character is determined mainly by genetic factors. Thus, the relationship between individual variation in novelty seeking and the receptor site of the dopamine system in the CNS has been well established. However, little is known about the relationship between personality and dopamine metabolism.

Previous studies have shown that when personalities are assessed by the Karolinska Scales of Personality, there are significant differences between extensive and poor metabolizers of CYP2D6 (Bertilsson *et al.*, 1989; Llerena *et al.*, 1993). Recently, it was also reported that there is a genetic variation in CYP2D6 that affects enzymatic activity. Taking these findings together, we hypothesized that CYP2D6 gene polymorphism affects dopamine metabolism in the CNS, thereby influencing personality. To assess the credibility of this hypothesis, we studied the association between personality and CYP2D6 gene polymorphism.

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Table 1 Comparison of the Temperament and Character Inventory (TCI) subitem score between CYP2D6*10/*10 and others

TCI subitem	*n*		Score		*P* value
	*1/*1 and *1/*10	*10/*10	*1/*1 and *1/*10	*10/*10	
Novelty Seeking	147	67	21.4±5.7	22.4±6.2	0.25
Harm Avoidance	140	60	17.8±7.3	17.8±5.6	0.97
Reward Dependence	150	66	16.7±3.7	17.1±3.7	0.40
Persistence	151	66	4.8±2.0	4.5±2.1	0.37
Self-directedness	144	65	26.1±7.6	26.3±6.6	0.87
Cooperativeness	136	58	29.3±5.6	29.4±5.9	0.97
Self-transcendence	142	65	14.0±5.5	14.9±5.6	0.33

Subjects and methods

The present study was conducted following approval from the Ethics Committee of the Department of Neuropsychiatry, Keio University, and the National Center of Neurology and Psychiatry in the Kohnodai area. To minimize the effects of confounding variables such as race, age, and gender, we examined a homogeneous group of Japanese students in their first year at Keio Nursing School ($n=255$; 222 females). The mean ± standard deviation age was 19.6 ± 2.1 years.

Upon receiving written informed consent, the character of each subject was assessed with the Temperament and Character Inventory (TCI). The TCI used in this study was a Japanese, 226-item version with established reliability and validity (Kijima *et al.*, 1996).

DNA was extracted from peripheral lymphocytes according to the standard method. Genotyping was carried out according to the standard protocol that had been slightly modified from the published methods. Briefly, genomic DNA was extracted from the leukocyte nuclei of peripheral blood samples. Each target segment was amplified by the polymerase chain reaction method. The polymerase chain reaction products were then digested with restriction enzymes and subjected to electrophoresis. The polymorphism genotyped in this study was CYP2D6*10. The laboratory analyses were performed by the second author (Y.K.).

For statistical analyses, the 'SPSS for Windows 98' (SPSS Japan Inc., Tokyo, Japan) software package was used. The mean scores of seven factors (Novelty Seeking, Harm avoidance, Reward dependence, Persistence, Self-directedness, Cooperativeness, Self-transcendence) in the TCI were compared among genotypes (*10/*10 versus others) using Student's t test. The result was defined as being significant when $P<0.05$.

Results

The number of each genotype, *1/*1, *1/*10, and *10/*10, was 65, 92, and 68, respectively. Since the CYP2D6 enzyme activity is known to be decreased in subjects with the genotype of homozygote *10/*10, the TCI subitem scores were compared between CYP2D6*10/*10 and others, and the results are presented in Table 1. There was no association with CYP2D6*10 genotype frequencies in any of the personality traits.

Discussion

As described earlier, some previous studies have suggested that CYP2D6 activity induces differences in personality (Bertilsson *et al.*, 1989; Llerena *et al.*, 1993). Since the *10/*10 genotype is known to decrease the CYP2D6 enzyme activity in comparison with the *1/*1 and *1/*10 genotypes (Daly *et al.*, 1996), the personalities of the subjects were compared between these two groups. However, there was no significant difference in any of the personalities observed. There are two possible reasons for the discrepancy between the previous findings and ours. First, there were some differences in the subjects and methodologies used between this and the previous studies. For example, the test used for estimation of personality and the race of the subjects in the present study were different from those of the previous study. Another possibility is that the degree of decrease in the CYP2D6 enzyme activity might not have been sufficient to differentiate the personality traits, although the homozygote of CYP2D6 is known to have decreased enzyme activity. To verify this possibility, it would be best to examine Caucasian subjects because 5–10% of them are poor metabolizers completely lacking CYP2D6 enzyme activity.

Irrespective of the aforementioned reasons, we found no evidence in the present study to support the hypothesis that differences in CYP2D6-related dopamine metabolism in the CNS are associated with variations of personality traits. However, many pathways that are related to dopamine metabolism in the CNS remain to be examined. Further research is needed to clarify the relationships between personality and dopamine metabolism in the CNS.

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The human frizzled-3 (*FZD3*) gene on chromosome 8p21, a receptor gene for Wnt ligands, is associated with the susceptibility to schizophrenia

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Abstract

Neurodevelopmental abnormalities have been reported in studies on the pathogenesis of schizophrenia. The Wnt-signaling pathway has been implicated in a variety of processes in neurodevelopment, and the frizzled proteins have been identified as receptors for Wnt ligands. Of the frizzled proteins, frizzled-3 (FZD3) is required for formation of the neural crest and for development of major fiber tracts in the CNS. The human *FZD3* gene is located on chromosome 8p21, a positive linkage locus for schizophrenia. We analyzed polymorphisms of the *FZD3* gene in patients with schizophrenia and control subjects in the Japanese population. We found a significant association between schizophrenia and the *FZD3* gene in single nucleotide polymorphisms and haplotype analyses. Our data suggest that dysregulation of the Wnt-signaling pathway may be involved in the susceptibility to schizophrenia.
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Keywords: FZD3; Wnt; Schizophrenia; Association study; Chromosome 8p21; Japanese

Schizophrenia is a common mental illness with a lifetime prevalence of approximately 1% of the world's population [15]. While the causes of schizophrenia remain unknown, it is now clearly established, on the basis of evidence from twin, family, and adoption studies, that genetic factors play a strong etiologic role in schizophrenia [4,13]. Recently, evidence has accumulated to suggest that this severe psychiatric illness is attributable to neurodevelopmental abnormalities that might occur early in life [5,17]. Therefore, it is likely that mutations in genes involved in neurogenesis play a role in the pathogenesis of this disorder.

Wnt proteins have established roles in a number of diverse processes including embryonic patterning and the regulation of cell proliferation, polarity and fate determination [3,19], and their importance in the regulation of patterning during cortical development has been documented [7]. Members of the frizzled family of seven pass transmembrane proteins have been identified as receptors for Wnt ligands [1,20]. It has been shown that one member of the frizzled family, frizzled-3, is required for Wnt signaling in the formation of the neural crest in the developing vertebrate embryo in Xenopus [6] and for the development of major fiber tracts in the rostral CNS [18]. Hence, dysfunction of frizzled-3 may result in neurodevelopmental abnormalities. In light of the neurodevelopmental hypotheses of schizophrenia, the gene encoding the human frizzled-3 (FZD3) protein is of interest in the study on schizophrenia susceptibility. The *FZD3* gene consists of eight exons, is approximately 70 kb long, and is located on chromosome 8p21 (OMIM*606143) [10,14]. Several linkage studies have repeatedly suggested that chromosome 8p21 is a putative locus for the development of schizophrenia [2,8,9,12], and that genes mapped to this region, if

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they have neurobiological significance, can be considered as candidates involved in the pathogenesis of schizophrenia. Therefore, we assume that the *FZD3* gene is an attractive candidate for responsibility for the susceptibility to schizophrenia. We studied the genetic association between the *FZD3* gene polymorphisms and schizophrenia in a Japanese population.

Two hundred unrelated Japanese schizophrenia patients (108 men and 92 women; mean age 45.1 ± 12.5 years) fulfilling the international statistical classification of the disease, revision 10 (ICD-10) diagnostic criteria for schizophrenia, participated in the present study. Assessment for diagnosis and subtype of schizophrenia was performed by trained psychiatrists on the basis of all available information, including hospital notes. Of the patients in the study, 91 (47 males and 44 females; mean age 44.3 ± 11.9 years) were diagnosed with paranoid type, 107 (60 males and 47 females; mean age 45.9 ± 12.9 years) with hebephrenic type, and two with catatonic type schizophrenia. Two hundred and eighteen healthy volunteers (112 men and 106 women; mean age 46.5 ± 15.7 years), mostly from the medical staff, were recruited as control subjects. Within the control group, subjects with a positive personal or familial history of major psychiatric disorders were excluded. The patients and the controls were unrelated Japanese, and were individually matched for gender, age, and geographical origin. After being provided with a complete description of the study, written informed consent to participate was obtained from all participants prior to examination. This study protocol was approved by the Ethics Committee of Okayama University Graduate School of Medicine and Dentistry.

About 40 single nucleotide polymorphisms (SNPs) are registered in the SNP database of NCBI. Among them, SNPs in the exon, in the 5′-flanking region and in the intron close to the exon–intron boundary were selected, because those may have possibilities to be functional. Three kinds of SNPs of the *FZD3* gene, −11493A > G (rs3757888), IVS3 + 258T > C (rs960914), and 435G > A (rs2241802), were selected, which are located in the 5′-flanking region, intron 3, and coding regions of exon 5, respectively. The 435G > A is a synonymous mutation that does not alter the amino acid sequence of leucine at codon 145. The −11493A > G is separated from IVS3 + 258T > C and 435G > A by about 11.7 and 34.7 kb, respectively. The adenine of the ATG-translation initiation codon is denoted nucleotide +1. The nucleotide 5′-adjacent to +1 is numbered −1. Exon SNPs are numbered according to their position in the coding sequence. For SNPs in intron X, the guanine of the donor site invariant GT is denoted IVSX + 1G.

The genomic DNA was extracted from peripheral leukocytes by standard procedures. Polymerase chain reaction (PCR) and the PCR-based restriction fragment length polymorphism assays were performed to genotype the DNA sequence variants of the *FZD3* gene. PCR was carried out in a total volume of 15 μl with 3% dimethylsulfoxide and 0.75 units of Super*Taq* DNA polymerase (Sawady Technology Co., Japan) in the reaction mixture. The primer sequences designed and used for the analysis of −11493A > G were the forward primer 5′-TGGTTTCGAACTCCTGACCACAG-3′ and the reverse primer 5′-AACCCAAGGAAAGCAACAACTTC-3′. Those of IVS3 + 258T > C were 5′-CTTAAATTTTAAACTTCTGAATGGGT-3′ and 5′-TAAAAATGTATATGTTTTGCTTCAG-3′. Those of 435G > A were 5′-GAATAAATAAGGTTGTGAGTGC-3′ and 5′-TAAGGATACCATCATGTAGCAG-3′. The amplification was initiated at 95 °C for 5 min, followed by 35 cycles consisting of denaturation at 95 °C for 30 s, annealing at the appropriate primer-pair annealing temperature for 1 min and extension at 72 °C for 30 s, with a final extension step of 10 min at 72 °C. The PCR products were digested at 37 °C with the corresponding restriction enzyme, *Dde* I (−11493A > G), *Rsa* I (IVS3 + 258T > C) and *Alu* I (435G > A), and then subsequently electrophoresed on 3.0% agarose gels and stained with ethidium bromide.

Deviation of the genotype counts from the Hardy–Weinberg equilibrium was tested using a chi-square goodness-of-fit test. The statistical significance of differences in the genotype distribution and allele frequency between patients and controls was assessed by a chi-square test or Fisher's exact test at a significance level of 0.05 (two-tailed). Bonferroni correction for multiple testing was carried out to exclude type I errors. Haplotype frequencies and the level of linkage disequilibrium were estimated using the Arlequin program based on the expectation-maximization (EM) algorithm. To evaluate the statistical significance of differences between patients and control subjects in haplotype frequencies, we used a Monte Carlo method as implemented in CLUMP [16].

Both the genotype distribution and allele frequencies for the patients and control subjects are shown in Table 1. The genotype distribution of the patients and control subjects did not deviate significantly from the Hardy–Weinberg equilibrium at these polymorphic loci. As for the −11493A > G polymorphism, no significant differences were found in the frequency of the genotype or allele between patients and controls (genotype, $\chi^2 = 1.90$, d.f. = 2, $P = 0.32$; allele, $\chi^2 = 1.71$, d.f. = 1, $P = 0.21$). With regard to the subcategories of schizophrenia, no significant differences were found in the frequency of the genotype or allele between patients with hebephrenic type schizophrenia and controls (genotype, $\chi^2 = 0.30$, d.f. = 2, $P = 0.93$; allele, $\chi^2 = 0.003$, d.f. = 1, $P = 1.00$), and in the frequency of the allele between patients with paranoid type schizophrenia and controls ($\chi^2 = 3.90$, d.f. = 1, $P = 0.055$). Although there were significant differences in the frequency of the genotype between patients with paranoid type schizophrenia and controls ($\chi^2 = 5.70$, d.f. = 2, $P = 0.037$), these differences were not significant after the Bonferroni correction for multiple testing. As for the IVS3 + 258T > C polymorph-

Table 1
Genotype and allele frequencies of the polymorphisms of the FZD3 gene

	n	Genotype			P	Allele		P
− 11493A > G		AA	AG	GG		A	G	
Control	218	195 (89.4)	22 (10.1)	1 (0.5)		412 (94.5)	24 (5.5)	
Schizophrenia	200	170 (85.0)	29 (14.5)	1 (0.5)	0.32	369 (92.2)	31 (7.8)	0.21
Paranoid type	91	73 (80.2)	18 (19.8)	0 (0.0)	0.037	164 (90.1)	18 (9.9)	0.055
Hebephrenic type	107.	96 (89.7)	10 (9.4)	1 (0.9)	0.93	202 (94.4)	12 (5.6)	1.00
Catatonic type	2	1 (50.0)	1 (50.0)	0 (0.0)		3 (75.0)	1 (25.0)	
IVS3 + 258T > C		TT	TC	CC		T	C	
Control	209	52 (24.9)	111 (53.1)	46 (22.0)		215 (51.4)	203 (48.6)	
Schizophrenia	200	72 (36.0)	104 (52.0)	24 (12.0)	0.0061	248 (62.0)	152 (38.0)	0.0024
Paranoid type	91	31 (34.1)	49 (53.8)	11 (12.1)	0.071	111 (61.0)	71 (39.0)	0.033
Hebephrenic type	107	39 (36.4)	55 (51.4)	13 (12.2)	0.031	133 (62.1)	81 (37.9)	0.011
Catatonic type	2	2 (100.0)	0 (0.0)	0 (0.0)		4 (100.0)	0 (0.0)	
435G > A		GG	GA	AA		G	A	
Control	215	60 (27.9)	109 (50.7)	46 (21.4)		229 (53.3)	201 (46.7)	
Schizophrenia	197	62 (31.5)	105 (53.3)	30 (15.2)	0.27	229 (58.1)	165 (41.9)	0.16
Paranoid type	88	26 (29.5)	46 (52.3)	16 (18.2)	0.83	98 (55.7)	78 (44.3)	0.59
Hebephrenic type	107	35 (32.7)	58 (54.2)	14 (13.1)	0.18	128 (59.8)	86 (40.2)	0.13
Catatonic type	2	1 (50.0)	1 (50.0)	0 (0.0)		3 (75.0)	1 (25.0)	

Numbers in parentheses indicate percentages. Statistical analysis was performed by a chi-square test or Fisher's exact test.

ism, there were significant differences in the frequency of the genotype and allele between patients and controls (genotype, $\chi^2 = 10.2$, d.f. = 2, $P = 0.0061$; allele, $\chi^2 = 9.29$, d.f. = 1, $P = 0.0024$). With regard to the subcategories of schizophrenia, significant differences were also found in the frequency of the genotype or allele between patients with hebephrenic type schizophrenia and controls (genotype, $\chi^2 = 7.01$, d.f. = 2, $P = 0.031$; allele, $\chi^2 = 6.57$, d.f. = 1, $P = 0.011$), and in the frequency of the allele between patients with paranoid type schizophrenia and controls ($\chi^2 = 4.66$, d.f. = 1, $P = 0.033$), whereas no significant differences were found in the frequency of the genotype between patients with paranoid type schizophrenia and controls ($\chi^2 = 5.22$, d.f. = 2, $P = 0.071$). Even after the Bonferroni correction, differences in the frequency of the allele between patients and controls remained significant ($P < 0.05$). As for the 435G > A polymorphism, no significant differences were found in the frequency of the genotype or allele between patients and controls (genotype, $\chi^2 = 2.69$, d.f. = 2, $P = 0.27$; allele, $\chi^2 = 1.97$, d.f. = 1, $P = 0.16$). With regard to the subcategories of schizophrenia, no association was observed between patients with paranoid type schizophrenia and controls (genotype, $\chi^2 = 0.40$, d.f. = 2, $P = 0.83$; allele, $\chi^2 = 0.30$, d.f. = 1, $P = 0.59$) and between patients with hebephrenic type schizophrenia and controls (genotype, $\chi^2 = 3.38$, d.f. = 2, $P = 0.18$; allele, $\chi^2 = 2.49$, d.f. = 1, $P = 0.13$).

We estimated pairwise linkage disequilibrium and performed haplotype analysis to calculate haplotype frequencies between patients and control subjects. We detected that IVS3 + 258T > C and 435G > A were in a high linkage disequilibrium (in either control subjects or patients with schizophrenia: $P < 0.00001$). Estimated haplotype frequencies for these two polymorphisms are listed in Table 2. The frequency of the haplotype IVS3 + 258T − 435G was significantly higher in patients than in controls ($P = 0.022$).

This study examined the possible association between the *FZD3* gene polymorphisms and schizophrenia. Although two of the three SNPs investigated (− 11493A > G and 435G > A) showed no significant differences after the Bonferroni correction, one SNP, the IVS3 + 258T > C in intron 3, showed significant differences in the frequency of the allele between patients and controls. The odds ratio of the TT genotype in intron 3 for schizophrenia is 1.70 (95% CI = 1.11–2.60) and those of the T allele for schizophrenia and hebephrenic type schizophrenia are 1.54 (95% CI = 1.17–2.04) and 1.55 (95% CI = 1.11–2.17), respectively. In addition, the haplotype including the IVS3 + 258T allele (IVS3 + 258T − 435G) was significantly higher in patients than controls. We could not draw conclusions with regard to catatonic type schizophrenia because we examined only two subjects in the study. The IVS3 + 258T > C polymorphism

Table 2
Estimated haplotype frequencies for control and schizophrenia subjects

Haplotype		Estimated frequency	
IVS3 + 258T > C	435G > A	Control	Schizophrenia
T	G	0.458915	0.539359[a]
T	A	0.060502	0.079930
C	G	0.072638	0.041859
C	A	0.407944	0.338852

[a] $P = 0.022$ for difference in haplotype frequency between control and schizophrenia.