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BRIEF REPORT

No Association Between the Val66Met Polymorphism of the Brain-Derived Neurotrophic Factor Gene and Bipolar Disorder in a Japanese Population: A Multicenter Study

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Background: *Two previous studies reported a significant association between a missense polymorphism (Val66Met) in the brain-derived neurotrophic factor (BDNF) gene and bipolar disorder; however, contradictory negative results have also been reported, necessitating further investigation.*

Methods: *We organized a multicenter study of a relatively large sample of 519 patients with bipolar disorder (according to DSM-IV criteria) and 588 control subjects matched for gender, age, and ethnicity (Japanese). Genotyping was done by polymerase chain reaction–based restriction fragment length polymorphism or direct sequencing.*

Results: *The genotype distributions and allele frequencies were similar among the patients and control subjects. Even if the possible relationships of the polymorphism with several clinical variables (i.e., bipolar I or II, presence of psychotic features, family history, and age of onset) were examined, no variable was related to the polymorphism.*

Conclusions: *The Val66Met polymorphism of the BDNF gene is unrelated to the development or clinical features of bipolar disorder, at least in a Japanese population.*

Key Words: Association study, bipolar disorder, brain-derived neurotrophic factor, genetics, single nucleotide polymorphism, susceptibility

Brain-derived neurotrophic factor (BDNF) belongs to the neurotrophin family and promotes the development, regeneration, survival, and maintenance of function of neurons (Maisonpierre et al 1991). It modulates synaptic plasticity and neurotransmitter release across multiple neurotransmitter systems, as well as the intracellular signal-transduction pathway (Thoenen 1995). Growing evidence has suggested important roles of BDNF in the pathogenesis of mood disorders and in the mechanism of action of therapeutic agents, such as mood stabilizers and antidepressants (reviewed by Duman 2002). In postmortem brains of patients with bipolar disorder, BDNF protein was reduced compared with control subjects (Knable et al 2004). Chronic electroconvulsive seizure or antidepressant drug treatments increase messenger ribonucleic acid of BDNF and its receptor, tyrosine kinase receptor B (Nibuya et al 1995). Lithium might also exert its neuroprotective effect through enhancing expression of BDNF and trkB (Hashimoto et al 2002).

The BDNF gene is, therefore, an attractive candidate gene that might cause susceptibility to bipolar disorder or influence the clinical phenotype of the illness. Indeed, at least two previous studies reported a significant association between a missense polymorphism (Val66Met; National Center for Biotechnology Information Database of Single Nucleotide Polymorphisms reference number rs6265) of the BDNF gene and bipolar disorder (Neves-Pereira et al 2002; Sklar et al 2002); however, contradictory negative results have also been reported (Hong et al 2003; Nakata et al 2003). One possible reason for this inconsistency is the lack of statistical power due to small sample size. To draw any conclusion with respect to this possible association, we organized a multicenter study in which six laboratories combined their data to ensure adequate statistical power.

Methods and Materials

Subjects

Six laboratories (National Institute of Mental Health, two laboratories of the Brain Science Institute, Showa University, Tokyo Women's Medical College, and Fujita Health University) collected deoxyribonucleic acid (DNA) samples from patients with bipolar disorder and healthy control subjects. Each institute provided DNA samples of patients and control subjects matched for gender, age, and geographic area, which yielded a combined sample of 519 patients with bipolar disorder (244 male) and 588 control subjects (287 male). Mean age (± SD) for the patients was 49.3 ± 14.3 years and for the control subjects was 48.4 ± 12.7 years. All the patients and control subjects were Japanese and biologically unrelated. Consensus diagnosis of bipolar disorder was made for each patient by at least two experienced psychiatrists according to DSM-IV criteria (American Psychiatric Association 1994) on the basis of unstructured interviews and medical records. Among the patients, 347 were diagnosed as bipolar I

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Table 1. Genotype Distributions and Allele Frequencies for the Val66Met Polymorphism of the BDNF Gene Among the Patients with Bipolar Disorder and Control Subjects

	Genotype Distribution				Allele Frequency	
	n	Val/Val	Val/Met	Met/Met	*n*	Val	Met
Patients						
Total	519	188 (36.2)	239 (46.1)	92 (17.7)	1038	615 (59.2)	423 (40.8)
Bipolar I	347	123 (35.4)	166 (47.8)	58 (16.7)	694	412 (59.4)	282 (40.6)
Bipolar II	172	65 (37.8)	73 (42.4)	34 (19.8)	344	203 (59.0)	141 (41.0)
Control subjects	588	216 (36.7)	270 (45.9)	102 (17.3)	1176	702 (59.7)	474 (40.3)

Values in parentheses are percentages. Genotypewise comparisons: total patients vs. control subjects: χ^2 (2) = .0, p = .98; bipolar I vs. control subjects: χ^2 (2) = .3, p = .86; bipolar II vs. control subjects: χ^2 (2) = .8, p = .69. Allelewise comparisons: total patients vs. control subjects: χ^2 (1) = .0, p = .83; bipolar I vs. control subjects: χ^2 (1) = .0, p = .96; bipolar II vs. control subjects: χ^2 (1) = .0, p = .94.

and the remaining 172 as bipolar II. Control subjects were healthy volunteers who had no current or past contact with psychiatric services. The control subjects were recruited from the hospital staffs and their associates at each institution who showed good social functioning and reported themselves to be in good health. They were interviewed, and those individuals who had current or past contact with psychiatric services were excluded. Written informed consent for participation in the study was obtained from all subjects. The study protocol was approved by the institutional ethics committees.

Methods

Venous blood was drawn, and genomic DNA was extracted according to standard procedures. Genotyping was performed according to Neves-Pereira et al (2003). Briefly, the polymorphic site was amplified by polymerase chain reaction (PCR) and then digested with a restriction enzyme, Eco72I. The digested PCR products were visualized with gel electrophoresis and subsequent ethidium bromide staining. Genotyping for a portion of subjects was done by direct sequencing of PCR products encompassing the polymorphic site with an autosequencer (CEQ 8000; Beckman Coulter, Fullerton, California). Genotype data were read blind to the case–control status.

To examine the possible relationships of the Val66Met polymorphism with clinical variables, information on age of onset, family history, and presence of psychotic features (i.e., current or past episode with delusions or hallucinations) was obtained. We defined positive family history as having at least one first-degree relative with a history of contact with psychiatric services with a diagnosis of mood disorder or who was a suicide victim. Individuals with ambiguous clinical data were excluded from statistical analyses.

The presence of Hardy-Weinberg equilibrium for the genotype distributions in the patients and control subjects was examined with the χ^2 test for goodness of fit. The differences in the genotype and allele distributions between patients and control subjects were examined with the χ^2 test for independence. The possible relationships between the polymorphism and clinical variables were examined with the χ^2 test for independence or analysis of covariance (ANCOVA) within the patient group. All p values reported are two-tailed.

Results

Genotype and allele distributions of the Val66Met polymorphism in the patients and control subjects are shown in Table 1. The genotype distributions in the two groups were both in Hardy-Weinberg equilibrium [patients: $\chi^2(1) = 1.1$, $p = .29$; control subjects: $\chi^2(1) = 1.2$, $p = .27$]. The genotype and allele distributions for the patients were quite similar to those for the control group (see Table 1). The genotype and allele distributions of the patients with bipolar I and those with bipolar II were also similar.

When relationships between genotype and clinical variables were examined, genotype and allele distributions were not different according to presence of psychotic features (frequency of the Val66 allele for psychotic patients: .567; for nonpsychotic patients: .579) or family history (positive family history: .602; negative: .603). Age of onset was also similar, irrespective of the genotype (Val/Val: 35.3 ± 13.5 years; Val/Met: 37.7 ± 14.6 years; Met/Met: 36.3 ± 14.0 years). Even when ANCOVA controlling for age and gender was performed, there was no significant difference in age of onset across the three genotypic groups [$F(2) = .99$, $p = .37$].

Discussion

We tried to replicate the studies of Sklar et al (2002) and Neves-Pereira et al (2002), who found a significant association between the Val66Met polymorphism of the BDNF gene with bipolar disorder. They reported excess transmission of the Val66 allele to the patients in their family-based association studies. Contrary to these findings, the genotype and allele frequencies among the patients and control subjects were similar in our sample, which is in turn consistent with more recent studies (Hong et al 2003; Nakata et al 2003), suggesting that the Val66Met polymorphism of the BDNF gene is unrelated to the development of bipolar disorder in our sample. Because our study had adequate statistical power (more than 90% to detect an odds ratio of 1.33 or more in allelic association; power analysis was performed according to Armitage and Berry 1994), the potential type II error due to lack of statistical power is unlikely. One possible explanation for this inconsistency might be a differential effect of the polymorphism depending on ethnicity, given that the majority of the subjects of Sklar et al (2002) and Neves-Pereira et al (2002) were Caucasian, whereas those of Hong et al (2003), Nakata et al (2003), and in our study were Asian. Alternatively, the positive results of Sklar et al (2002) and Neves-Pereira et al (2002) might have arisen by chance.

Concerning the possible effect of the polymorphism on clinical features, Rybakowski et al (2003) reported an earlier age of onset in Val/Val than Val/Met genotype (27 years vs. 38 years) among patients with bipolar disorder. They also found that the performance in all domains of the Wisconsin Card Sorting Test was significantly better for bipolar patients with Val/Val than for those with Val/Met genotype, suggesting a role of the Val66Met polymorphism in prefrontal cognitive function in bipolar disorder. This accords with the findings of Egan et al (2003), who

reported that the Met66 allele was associated with lower activity-dependent secretion of BDNF and poorer human memory and hippocampal function; however, we could not find any significant effect of the genotype on clinical variables of age of onset, subtype (bipolar I or II), psychotic features, or family history. Hong et al (2003) also failed to find significant difference in age of onset or suicidal history across genotypic groups in their Chinese subjects with bipolar disorder.

In conclusion, our results, together with previous two studies (Hong et al 2003; Nakata et al 2003), suggest that the Val66Met polymorphism of the BDNF gene is unrelated to the development or clinical features of bipolar disorder at least in Asian populations; however, the possibility remains that other variants of the BDNF gene might be associated with bipolar disorder in Asian populations, which requires further investigation.

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Analysis of Enhancer Activity of a Dinucleotide Repeat Polymorphism in the Neurotrophin-3 Gene and Its Association with Bipolar Disorder

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Key Words

Neurotrophin-3 · Dinucleotide repeat · Polymorphism · Enhancer activity · Luciferase assay · Association study · Bipolar disorder

Abstract

Growing evidence has implicated the possible involvement of neurotrophins in the pathogenesis of functional psychoses such as schizophrenia and bipolar disorder. Previous studies reported a significant association of a dinucleotide repeat polymorphism of the neurotrophin-3 (NTF3) gene with schizophrenia. The aims of the present study were to examine whether this polymorphism is associated with bipolar disorder and whether the polymorphic region has an enhancer/silencer effect on transcriptional activity in an allele-dependent manner. In an association analysis between the polymorphism and bipolar disorder in a Japanese sample of 88 patients and 98 controls matched for age, sex, and ethnicity, the distribution of alleles did not differ significantly between the two groups. pGL3-promoter luciferase reporter vectors containing the polymorphic region increased luciferase activity relative to empty pGL3-promoter vector in HeLa, IMR-32 (neuroblastoma) and Hs683 (glioma) cell lines; however, no significant difference was detected between alleles for either cell line. Our results suggest that the examined polymorphism has no major role in giving susceptibility to bipolar disorder. Although the polymorphic region may have an enhancer-like effect on transcriptional activity, we obtained no evidence for allele-dependent differential effects.

Introduction

Growing evidence has implicated the possible involvement of neurotrophins in the pathogenesis of functional psychoses such as schizophrenia [1] and bipolar disorder [2]. Neurotrophin-3 (NTF3) is a member of the neurotrophin family, which is highly expressed in the hippocampi of newborns and in immature neocortical regions of the fetus [3]. A previous study reported a significant association between schizophrenia and a dinucleotide repeat $(CA)_n$ polymorphism in the first intron of the NTF3 gene: the 'A3' allele, $(CA)_{23}$, was significantly more common in schizophrenics than in controls [4, 5]. Subsequently, sev-

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eral studies obtained evidence supporting this association [6–9], although contradictive negative results have also been reported [10–12]. Interestingly, individuals with schizophrenia who carried the A3 allele had smaller hippocampi than those who did not [13].

With respect to bipolar disorder, there is evidence for a significant association of the illness with a single nucleotide polymorphism (Val66Met) and a dinucleotide repeat polymorphism of the brain-derived neurotrophic factor (BDNF) gene [14, 15]. BDNF also belongs to the neurotrophin family and its primary structure is highly homologous to that of NTF3. Thus, NTF3 is an attractive candidate gene that may give susceptibility to bipolar disorder as well as to schizophrenia, which accords with substantial evidence that these disorders share familial risk characteristics [16].

Recently, micro- and minisatellite polymorphisms, even located in intron, have been shown to play a role in the expression of many genes [17]. In this study, we tested the hypotheses that the dinucleotide repeat polymorphism of the NTF3 gene may give susceptibility to bipolar disorder and that the polymorphism may have functional effects on transcriptional activity in an allele-dependent manner.

Materials and Methods

Subjects

To examine whether the dinucleotide repeat polymorphism of the NTF3 gene is associated with bipolar disorder, we genotyped 88 patients [38 men and 50 women; mean age 53 years, standard deviation (SD) 15] with bipolar disorder and 98 controls (44 men and 54 women; 51 years, SD 10). The patients were under treatment at Showa University Hospital, Tokyo, Japan. Consensus diagnosis was made for each patient by at least 2 psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders (ed 4, DSM-IV) criteria [18] based on unstructured interviews and all the available medical records. Forty-six patients (52%) were diagnosed as having bipolar I and the remaining 42 bipolar II disorder. The controls were healthy volunteers recruited from the hospital staff who had no current or past contact to psychiatric services for psychiatric problems. All the participants were unrelated Japanese who resided in Tokyo metropolitan area. Written informed consent was obtained from every subject after detailed description of the study. The study protocol was approved by institutional ethical committees.

Genotyping

Venous blood was drawn from the subjects and genomic DNA was extracted from whole blood according to standard procedures. The dinucleotide repeat polymorphism of the NTF3 gene was genotyped by polymerase chain reaction (PCR) amplification followed by determination of PCR fragment sizes by using an autosequencer (CEQ8000, Beckman Coulter) and a CEQ Fragment Analysis System (Beckman Coulter). Primer sequences for the PCR were 5′-(dye D4) GTTTGCGCTGGGATCTGCTCA-3′ (forward) and 5′-CCCCCA-CCCTTTCCAATCCA-3′ (reverse). Thermal cycling for the PCR was an initial denaturing stage at 95 °C for 9 min, 34 cycles of 95 °C for 45 s, 62 °C for 45 s, and 72 °C for 30 s, followed by the final extension stage at 72 °C for 10 min. Genotype data were read blind to the case-control status.

Luciferase Reporter Gene Assay

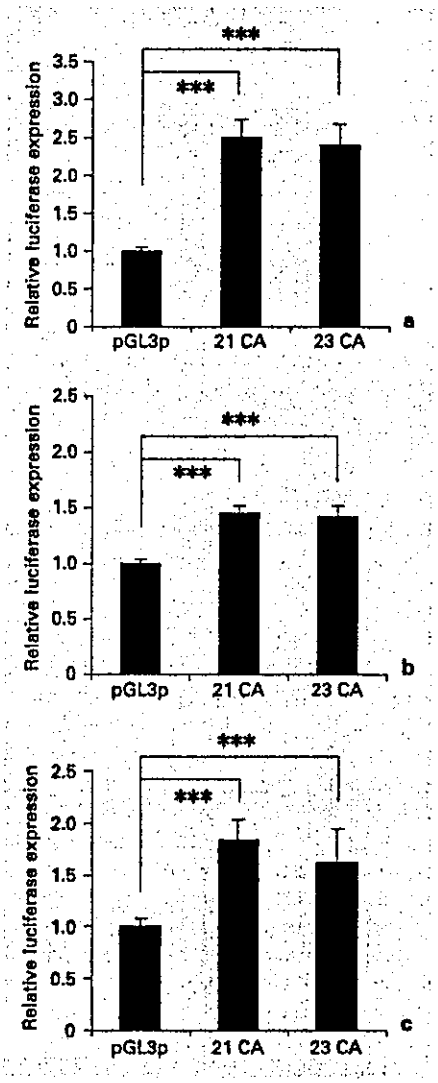
The CA repeat region in the first intron of the NT3 gene was amplified by PCR. The oligonucleotide primers were designed to incorporate Mlu I (forward) and Bgl II (reverse) restriction sites to the primers of the previous report [4], and the PCR product was inserted into the polylinker site upstream of the SV40 promoter in the pGL3-promoter vector (Promega). The primer sequences were 5′-GCACGCGTGGCTTGTGTCTTCCCCAAAGTT-3′ (forward; restriction site is underlined) and 5′-GCAGATCTAGGGGAG-GAGGTGGAGAA-3′ (reverse). According to allele frequency (see 'Results'), the major alleles were the A3 allele of $(CA)_{23}$ and A5 of $(CA)_{21}$ (153 and 149 bp, respectively), and thus these two alleles were inserted into the vector for comparison. The insert sequence was confirmed with the autosequencer (CEQ8000, Beckman Coulter) in both directions using primers supplied by the manufacturer (Promega; RV primer 3 and GL primer 2).

Human neuroblastoma IMR-32 cells and HeLa cells were cultured in MEM (Sigma) containing 10% fetal bovine serum (GIBCO). Human glioma Hs683 cells were cultured in DMEM (Sigma) containing 10% fetal bovine serum. Hs683 and IMR-32 cell lines were chosen according to previous reports on promoter assays of the NTF3 gene [19, 20]. Cells in 24-well plates were cotransfectioned in triplicate with 800 ng of pGL3-promoter firefly luciferase reporter vectors that included two major alleles of the $(CA)_n$ repeats ($n = 21$ and 23) and 25 ng of renilla luciferase expression vector (phRL-TK vector; Promega) as an internal control using Lipofectamine 2000 Regent (Invitogen). Empty pGL3-promoter vector was transfected simultaneously in the experiments. Experiments were repeated three times in all cells.

At 24 h after transfection, cells were washed with phosphate-buffered saline and then harvested with luciferase lysis buffer (Promega). Luciferase reporter assay was performed using Dual-Luciferase Reporter Assay System (Promega) and a luminometer (Lumat LB 9507, Berthold). Firefly and renilla luciferase activities were quantified sequentially as relative light units (RLU) by addition of their respective substrates according to the protocol of the supplier. The ratio of a firefly RLU to renilla RLU of each sample was automatically computed. Then the activity of each construct was expressed as the relative value compared to that of empty pGL3-promoter vector (relative luciferase expression, RLE), and these relative values were used for statistical analysis.

Statistical Analysis

For the association study, the presence of Hardy-Weinberg equilibrium was examined by using the χ^2 test for goodness of fit. Allele frequency was compared between patients and controls and the significance was assessed with a Monte Carlo approach using the CLUMP software with 10,000 simulations [21]. For luciferase reporter gene assay, analysis of variance (ANOVA) was used for comparison of RLE between alleles inserted into vectors. All p values reported are two-tailed.



Results

Association Analysis

The genotype distributions for the patients and controls did not significantly deviate from Hardy-Weinberg equilibrium (data not shown). Allele frequencies for the dinucleotide repeat polymorphism in the NTF3 gene among the patients and controls are shown in table 1. The observed allele frequencies among the two groups were quite similar. Based on the Monte Carlo approach with the CLUMP software, there was no significant difference in allele distribution between the patients and controls (T1 statistic: 0.82, p = 0.99; T2: 0.82, p = 0.99; T3: 0.50, p = 0.98; T4: 0.35, p = 0.99).

Luciferase Reporter Gene Assay

Figure 1 shows observed RLE levels for two major alleles of the dinucleotide repeat polymorphism of the NTF3 gene [the A5 allele of $(CA)_{21}$ and the A3 of $(CA)_{23}$], compared to RLE without insertion of such alleles (empty pGL3-promoter vector). For all the cell lines examined (HeLa, IMR-32, and Hs683), RLE was significantly increased ($p < 0.001$) due to insertion of the polymorphic region for both alleles [$(CA)_{21}$ and $(CA)_{23}$] compared to

Fig. 1. RLE levels are shown for the major two alleles of dinucleotide repeat polymorphism of the NTF3 gene [the A5 allele of $(CA)_{21}$ and the A3 of $(CA)_{23}$], compared to RLE level without insertion of these alleles (empty pGL3-promoter vector). Error bars represent SD. *** $p < 0.001$. **a** HeLa cells. **b** IMR-32 cells. **c** Hs683 cells.

Table 1. Allele distributions of the dinucleotide repeat polymorphism of the NTF3 gene among patients with bipolar disorder and controls

	n	A0 CA_{26}	A1 CA_{25}	A2 CA_{24}	A3 CA_{23}	A4 CA_{22}	A5 CA_{21}	A6 CA_{20}	A7 CA_{19}
Patients	176	1 0.6%	6 3.4%	5 2.8%	60 34.1%	11 6.3%	71 40.3%	14 8.0%	8 4.5%
Controls	196	1 0.5%	6 3.1%	5 2.6%	69 35.2%	16 8.2%	77 39.3%	13 6.6%	9 4.6%

the empty pGL3-promoter vector; whilst there was no significant difference in RLE between the $(CA)_{21}$ and $(CA)_{23}$ alleles.

Discussion

Against our study hypothesis, we obtained no evidence for an association between bipolar disorder and the CA repeat polymorphism of the NTF3 gene in our sample. Since the sample size (88 patients and 98 controls) was not very large, we may have obtained a false negative result due to the lack of statistical power. However, the observed allele frequencies were quite similar for the patients and controls; therefore, the possibility of type II error might be minimal. Considering the previous reports of positive association between the NTF3 gene and schizophrenia [5–9], the polymorphism might have differential effects on susceptibility to schizophrenia and bipolar disorder.

The second hypothesis that we tested in the present study was that the polymorphic region of the NTF3 gene may have functional effects on transcriptional activity in an allele-dependent manner. Since the 'A3' allele was significantly increased, whilst the most frequent allele 'A5' was decreased, in schizophrenics as compared to controls [5–9], we examined these two major alleles for possible effects on the transcriptional activity by using luciferase reporter gene assay. Experiments for three cell lines yielded quite consistent results. Insertion of the polymorphic region resulted in increased RLE, compared to that without such an insertion to the pGL3-pomoter vector, suggesting that the polymorphic region may have an enhancer-like effect on the transcriptional activity. However, there was no significant difference in RLE for either cell line between the A3 and A5 alleles, which does not support our study hypothesis. Although our results cannot entirely exclude the possibility that the alleles may have differential effects on transcriptional activity in other cell lines and conditions of cell culture, our results from three cell lines suggest that there is little difference in effect on transcriptional activity between alleles. Thus the previously reported association between this polymorphism and schizophrenia may have arisen by linkage disequilibrium of the dinucleotide repeat polymorphism with other functional polymorphism.

In conclusion, our results suggest that the examined polymorphic region might have an enhancer-like effect on the transcriptional activity of the NTF-3 gene. However, we obtained no evidence for allele-dependent differential effects of the polymorphic region of the NTF-3 gene on its transcriptional activity or susceptibility to bipolar disorder.

Acknowledgments

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No Association Found between the Type 1 Sigma Receptor Gene Polymorphisms and Methamphetamine Abuse in the Japanese Population

A Collaborative Study by the Japanese Genetics Initiative for Drug Abuse

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ABSTRACT: **It has been suggested that individual genetic factors are involved in susceptibility to drug dependence and the manifestation of drug-induced psychosis. The aim of this study was to examine the relation between methamphetamine abusers/psychosis and the type 1 sigma receptor gene polymorphisms. Subjects comprised 143 MAP abusers and 181 healthy controls. Two polymor-**

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phisms in the type 1 sigma receptor gene, GC-241-240TT and A61C (Gln2Pro), were examined in the present study. No significant differences were observed in either polymorphism between healthy controls and MAP abusers/psychosis. In the subgroup analyses, the rate of CC genotype of A61C tended to be higher in MAP patients who had experienced spontaneous relapse without MAP use than in those who had not ($P = .06$, OR = 3.02 95% CI = 0.92–9.92). However, the level of this significant trend did not remain after the Bonferroni's multiple correction. This study suggests that type 1 sigma receptor gene is unlikely to play a major role in substance abuse liability and/or the development of MAP psychosis.

KEYWORDS: **type 1 sigma receptor gene; methamphetamine psychosis; drug dependence; Japanese; polymorphism**

INTRODUCTION

From the results of family, twin, and adoption studies, genetic factors have been reported to be involved in the susceptibility to drug dependence and the manifestation of drug-induced psychosis.[1–4] Of the compounds that induce drug dependence, methamphetamine (MAP) is one of the most widely used psychostimulants in Japan. MAP induces a strong psychological dependence, and its repeated consumption is known to result frequently in the development of psychiatric symptoms that resemble the paranoid type of schizophrenia.[5]

Sigma receptors are unique binding sites that interact with a variety of psychotomimetic drugs, including cocaine and amphetamine. Several subtypes are present in high densities in the limbic structures as well as in motor-related areas of the central nervous system. Although the exact physiological functions of the sigma receptors remain to be clarified, several lines of evidence suggest the possible involvement of sigma receptors in the development of psychosis.[6] The initial suggestion of this emerged from the observations that several of the earliest sigma ligands induced psychiatric symptoms such as delusions, hallucinations, and depersonalization. This link was later reinforced with the demonstration that several antipsychotic drugs such as haloperidol have a high affinity for sigma receptors. In addition, it has been reported that the sigma binding sites in the brain are significantly decreased in schizophrenic patients,[7] whose psychotic symptoms are similar to those seen in MAP psychosis. Therefore, it can be hypothesized that the sigma receptor gene is an important candidate gene for schizophrenia and psychostimulant-induced psychosis. The type 1 sigma receptor gene is located on human chromosome 9p13 and contains four exons.[8] Two polymorphisms—GC-241-240TT in the 5′ flanking region and A61C (Gln2Pro) in exon 1—have been identified in this gene.[9,10]

In this study, the relation between the type 1 sigma receptor gene polymorphisms and MAP abusers/psychosis was examined in the Japanese population to investigate a possible genetic influence of the type 1 sigma receptor gene polymorphisms on the development of MAP abusers/psychosis. This study was carried out as one of the collaborative studies by the Japanese Genetics Initiative for Drug Abuse (JGIDA) organized to facilitate the sample collection of MAP abusers/psychosis to investigate genetic factors related to the substance abuse liability and the characteristics of MAP psychosis.

SUBJECTS AND METHODS

Ethical Considerations

This study was initiated after the approval of the ethical committee in each attending institution of JGIDA. Written informed consent was obtained from all participating patients.

Subjects

The subjects consisted of 143 unrelated patients with MAP dependence and psychotic disorder (112 males and 31 females, average age 36 years, range 19–69 years) who met the ICD-10-DCR criteria (F15.2 and F15.5) and who were outpatients or inpatients of psychiatric hospitals of JGIDA; 183 age-, gender-, and geographical origin–matched unrelated healthy controls (143 males and 40 females, age 37 years, range 19–73 years) mostly consisted of medical staff who had no past history and no family history of drug dependence or psychotic disorders. All subjects were Japanese, born and living in areas restricted to Japan including northern Kyusyu, Setouchi, Toukai, and Kantou.

Clinical Characteristics

The patients with MAP psychosis were divided into two subgroups according to the following clinical characteristics: age at first MAP use, duration of MAP use until onset of psychosis, prognosis of psychosis (transient type vs. prolonged type), and presence or absence of the spontaneous relapse without MAP use. The rationales of why we focus on these clinical features were described previously.[11] In dividing the subgroups, the median values were adopted as the cutoff point.

Genomic Procedure

Genomic DNA was extracted from the leukocyte nuclei of peripheral blood. The type 1 sigma receptor gene polymorphisms examined in this study were GC-241-240TT in the 5′ flanking region and A61C (Gln2Pro) in exon 1. Genotyping was carried out according to the standard protocol, slightly modified from the published methods.[9,10] Briefly, 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

The presence of the Hardy-Weinberg equilibrium was tested using a chi-square goodness-of-fit test. Comparisons of allele and genotype frequencies were carried out using the chi-squared test for 2×2 and 2×3 contingency tables. Significance level and significant trend level were defined when the P value was less than .05 and .1, respectively.

TABLE 1. Genotype and allele frequencies of the type 1 sigma receptor gene polymorphisms in MAP abusers/psychosis and healthy controls

(a) GC-241-240TT in the 5′ flanking region

	Genotype[a]				Allele[a]		
Group	GC/GC	GC/TT	TT/TT	*P* Value[b]	GC	TT	*P* Value[b]
Controls	90	78	13		258	104	
(*n* = 181)	49.7%	43.1%	7.2%		71.3%	28.7%	
MAP abusers	70	61	12	0.92	201	85	0.78
(*n* = 143)	49.0%	42.7%	8.4%		70.3%	29.7%	
MAP psychosis	56	50	12	0.66	162	74	0.49
(*n* = 118)	47.5%	42.4%	10.2%		68.6%	31.4%	

(b) A61C (Gln2Pro) in exon 1

	Genotype[a]				Allele[a]		
Group	AA	AC	CC	*P* Value[b]	A	C	*P* Value[b]
Controls	86	83	14		255	111	
(*n* = 181)	47.0%	45.4%	7.7%		69.7%	30.3%	
MAP abusers	61	65	17	0.40	187	99	0.25
(*n* = 143)	42.7%	45.5%	11.9%		65.4%	34.6%	
MAP psychosis	49	55	14	0.39	153	83	0.77
(*n* = 118)	41.5%	46.6%	11.9%		64.8%	35.2%	

[a]*Upper row*: number of subjects; *lower row*: frequency.
[b]Compared with controls.

RESULTS

The genotype distribution was not significantly different from that expected according to the Hardy-Weinberg equilibrium in any group and in any subgroup examined in the present study.

The genotypic distribution and the allelic frequency of two polymorphisms—that is, GC-241-240TT in the 5′ flanking region and A61C (Gln2Pro) in exon 1, in the type 1 sigma receptor gene between healthy controls and the patients with MAP abusers/psychosis—are shown in TABLE 1. No significant differences were observed in either polymorphism between healthy controls and MAP abusers/psychosis.

The genotypic distribution and the allelic frequency of two polymorphisms, that is, GC-241-240TT in the 5′ flanking region and A61C (Gln2Pro) in exon 1, in the type 1 sigma receptor gene between the subgroups in patients with MAP psychosis, are shown in TABLE 2 and TABLE 3, respectively.

In the subgroup analyses, no significant differences were observed in genotype, allele, or homozygosity frequencies between any subgroup of the patients with MAP psychosis. Although the rate of CC genotype of A61C tended to be higher in MAP patients who have experienced spontaneous relapse without MAP use than in those who have not ($P = .06$, OR = 3.02 95%CI = 0.92–9.92), this significant trend level did not remain after the Bonferroni's multiple correction.

TABLE 2. Genotype and allele frequencies of the GC-241-240TT polymorphism in the type 1 sigma receptor gene between subgroups in MAP psychosis

Subgroups in MAP psychosis	Genotype[a]			*P* Value[b]	Allele[a]		*P* Value
	GC/GC	GC/TT	TT/TT		GC	TT	
(a) Age at first MAP use (in years)							
≧ 20	27	25	4		79	33	
(*n* = 56)	48.2%	44.6%	7.1%	0.64	70.5%	29.5%	0.63
< 20	27	23	7		77	37	
(*n* = 57)	47.4%	40.4%	12.3%		67.5%	32.5%	
(b) Duration of MAP use until onset of psychosis							
≧ 3 years	29	22	4		80	30	
(*n* = 55)	52.7%	40.0%	7.3%	0.30	72.7%	27.3%	0.13
< 3 years	18	23	6		59	35	
(*n* = 47)	38.3%	48.9%	12.8%		62.8%	37.2%	
(c) Disappearance type psychosis[b]							
Transient type	31	28	6		90	40	
(*n* = 65)	47.7%	43.1%	9.2%	0.94	69.2%	30.8%	0.73
Prolonged type	21	21	5		63	31	
(*n* = 47)	44.7%	44.7%	10.6%		67.0%	33.0%	
(d) Spontaneous relapse without MAP use							
Present	21	16	6		58	28	
(*n* = 43)	48.8%	37.2%	14.0%	0.39	67.4%	32.6%	0.72
Absent	33	33	5		99	43	
(*n* = 71)	46.5%	46.5%	7.0%		69.7%	30.3%	

[a]*Upper row*: number of subjects; *lower row*: frequency.
[b]*Transient type*: psychotic symptoms improved within one month after discontinuation of METH, along with initiation of treatment with neuroleptics; *prolonged type*: psychotic symptoms continued for more than one month even after discontinuation of METH, along with initiation of treatment with neuroleptics.

DISCUSSION

The present results did not show any allelic association between the type 1 sigma receptor gene polymorphisms and the patients with MAP abusers/psychosis. Moreover, no allelic association with any of the subgroup analyses on the clinical characteristics examined in the present study was detected, indicating that the type 1 sigma receptor gene is unlikely to play a major role in substance abuse liability and/or development of MAP psychosis.

As far as we know, this is the first report on the association study between the type 1 sigma receptor gene polymorphism and the patients with MAP psychosis, whose psychiatric symptoms are similar to those observed in patients with schizophrenia. There are two studies examining the association between the type 1 sigma receptor

TABLE 3. Genotype and allele frequencies of the A61C (Gln2Pro) polymorphism in the type 1 sigma receptor gene between subgroups in MAP psychosis

Subgroups in MAP psychosis	Genotype[a] AA	AC	CC	*P* Value	Allele[a] A	C	*P* Value
(a) Age at first MAP use (in years)							
≧ 20	24	27	5		75	37	
(*n* = 56)	42.9%	48.2%	8.9%	0.68	67.0%	33.0%	0.64
< 20	24	25	8		73	41	
(*n* = 57)	42.1%	43.9%	14.0%		64.0%	36.0%	
(b) Duration of MAP use until onset of psychosis							
≧ 3 years	26	24	5		76	34	
(*n* = 55)	47.3%	43.6%	9.1%		69.1%	30.9%	
< 3 years	16	24	7	0.35	56	38	0.16
(*n* = 47)	34.0%	51.1%	14.9%		59.6%	40.4%	
(c) Disappearance type psychosis[b]							
Transient type	27	30	8		84	46	
(*n* = 65)	41.5%	46.2%	12.3%		64.6%	35.4%	
Prolonged type	19	23	5	0.94	61	33	0.97
(*n* = 47)	40.4%	48.9%	10.6%		64.9%	35.1%	
(d) Presence or absence of the spontaneous relapse without MAP use							
Yes	19	16	8		54	32	
(*n* = 43)	44.2%	37.2%	18.6%		62.8%	37.2%	
No	30	36	5	0.12	96	46	0.46
(*n* = 71)	42.3%	50.7%	7.0%		67.6%	32.4%	

[a] *Upper row*, number of subjects; *lower row*, frequency.
[b] *Transient type*: psychotic symptoms improved within one month after discontinuation of METH along with initiation of treatment with neuroleptics; *prolonged type*: psychotic symptoms continued for more than one month even after discontinuation of METH, along with initiation of treatment with neuroleptics.

gene polymorphisms and schizophrenia in the Japanese population. Ishiguro *et al.*[9] first observed a significant association between the presence of the TT/Pro2 haplotype and schizophrenia (odds ratio = 1.27, $P = .04$), and they suggested a possible role in the pathogenesis of schizophrenia. Subsequently, Ohmori *et al.*[10] also observed a weak association between homozygosity for TT/Pro2 and schizophrenia ($P = .045$). However, they reported that this significance did not remain when a Bonferroni's correction was made ($P = .135$) and concluded that the type 1 sigma receptor gene is unlikely to play a major role in the pathogenesis of schizophrenia.

However, considering the previous reports on the close relationship between sigma receptor and psychiatric symptoms, the possibility of some involvement in the sigma receptor gene polymorphism for the development of psychiatric conditions such as MAP psychosis and schizophrenia cannot be completely ruled out. Moreover, because the sample size for the present study is fairly small, there is the possi-

bility of a type II error to detect the significant difference. Therefore, further research is needed to clarify the exact role of these polymorphic sites in determining certain phenotypes, using a larger number of samples, that can be related to some forms of psychosis.

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Association Between Chromogranin B Gene Polymorphisms and Schizophrenia in the Japanese Population

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Background: *We found in previous work a significant association between schizophrenia and D20S95 on chromosome 20p12.3. In this study, we analyzed 10 microsatellite markers and found an association of schizophrenia with D20S882 and D20S905 that flank D20S95. The chromogranin B gene (*CHGB*) is 30 kb from D20S905. The chromogranin B (secretogranin I) belongs to a series of acidic secretory proteins that are widely expressed in endocrine and neuronal cells, and its cerebrospinal fluid levels have been reported to decrease in patients with chronic schizophrenia.*

Methods: *We screened for polymorphisms in* CHGB *with polymerase chain reaction direct sequencing methods in 24 Japanese schizophrenic patients and identified a total of 22 polymorphisms. Allelic and genotypic distributions of detected polymorphisms were compared between unrelated Japanese schizophrenic patients (*n = *192) and healthy control subjects (*n = *192).*

Results: *Statistically significant differences in the allelic distributions were found between schizophrenic patients and control subjects for 1058C/G (A353G) (corrected* p = 7.7×10^{-5}*) and 1104A/G (E368E) (corrected* p = 8.1×10^{-6}*). The 1058C/G and 1104A/G alleles were in almost complete linkage disequilibrium and were in linkage disequilibrium with D20S95.*

Conclusions: *Results suggest that the* CHGB *variations are involved in the susceptibility to schizophrenia in our study population.*

Key Words: Schizophrenia, chromogranin B, association study, neuropeptide

The region of 20p12.3-p11 may contain a locus of predisposition to schizophrenia (Lewis et al 2003). In our previous screening association study, we observed a significant association with schizophrenia at the locus of D20S95 ($p = 5 \times 10^{-6}$, corrected p value after Bonferroni correction, .00035) on 20p12.3 (Kitao et al 2000). The marker D20S95 is approximately 2 megabases (Mb) outside the 21.2 to 47.5 cM region of chromosome 20 highlighted in the meta-analysis of Lewis et al (2003), though quite possibly within its confidence bounds. In the individual genome scans, Moises et al (1995) reported a p-value of .009 with marker D20S40 and Hovatta et al (1998) found a maximum lod score of 1.22 with marker D20S172. These markers are located approximately 7 Mb and 12 Mb centromeric to D20S95.

The only known gene within 180 kb from D20S95 is the gene encoding chromogranin B, a tyrosine-sulfated secretory protein found in a wide variety of peptidergic endocrine cells. The granins (secretogranins/chromogranins) belong to a family of soluble proteins stored and released from the large dense-core secretory vesicles of the synapse (Benedum et al 1987; Winkler and Fischer-Colbrie 1992). A number of studies have compared the chromogranin levels in cerebrospinal fluid (CSF) between healthy control subjects and schizophrenic patients at various stages of this disease (Landen et al 1999; Miller et al 1996; van Kammen et al 1991, 1992, 1994). Landen et al (1999) reported that levels of chromogranin A and chromogranin B were lower in chronic schizophrenic patients and pointed out the possibility that an acute increase of chromogranin levels reflects an active disease process and a chronic decline indicates an advanced neurodegenerative process. Reduction of chromogranin B-like immunoreactivity in distinct subregions of the hippocampus from individuals with schizophrenia was reported (Nowakowski et al 2002). An association between some *CHGB* polymorphisms and schizophrenia was reported in the Chinese Han population (Zhang et al 2002).

In the present study, we performed additional dense-mapping analyses using 10 microsatellite markers close to D20S95 and the mutation search of the *CHGB*, followed by the case-control association studies on its detected polymorphisms in schizophrenic patients, using the same sample set as we previously used in the screening study in which a significant association with schizophrenia was detected at D20S95.

Methods and Materials

Ethical Considerations

The present study was approved by the Ethical Committee of the Kohnodai area, National Center of Neurology and Psychiatry, University of Tsukuba, and Nagoya University. Written informed consent was obtained from all subjects.

Subjects

The DNA samples were all selected from those of the subjects enrolled in our previous screening study (Kitao et al 2000). The subjects consisted of 192 schizophrenic patients and 192 healthy control subjects. The schizophrenic patients (91 men, 101 women; aged 19–90 years [mean 57.2 years]) were all inpatients recruited from several psychiatric facilities located around the Tokyo area. They were interviewed several times during a hospitalization period of 6 months or more by experienced psychiatrists who were familiar with the structured clinical interview for DSM-III-R (SCID) rating system (Spitzer et al 1992). The diagnosis of schizophrenia was assigned on the basis of clinical interviews and chart review of medical records, accord-

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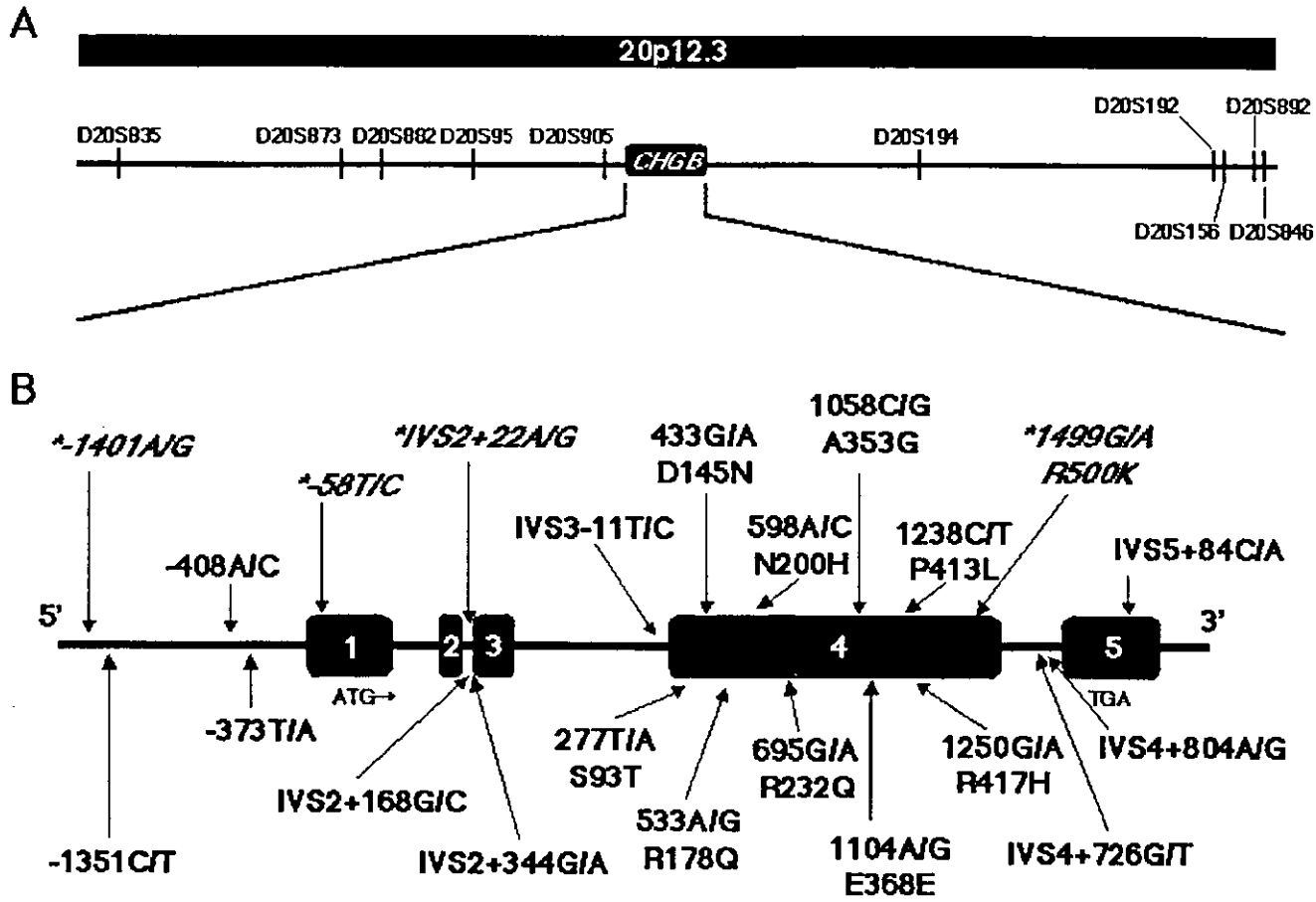

Figure 1. (A) The positions of the microsatellite markers in relation to chromogranin B gene (*CHGB*). **(B)** Genomic organization and positions of the SNPs in *CHGB*. *Italics are newly identified SNPs. SNP, single nucleotide polymorphism.

Table 1. Results of the Additional Dense-Mapping Analyses Using 10 Microsatellite Markers Close to D20S95

Microsatellite Markers[a]	Distance from *CHGB* (kb)	Number of Subjects		Statistics[b]	
		Schizophrenia	Control	H-W[c]	S vs. C[d]
D20S835	−577	172	192	ns	ns
D20S873	−295	175	191	ns	ns
D20S882	−257	203	196	.02	.02
D20S95	−176	230	217	ns	.002
D20S905	−29	184	190	ns	.04
CHGB	0				
D20S194	251	164	135	ns	ns
D20S192	805	171	192	ns	ns
D20S156	809	171	192	ns	ns
D20S892	858	174	192	ns	ns
D20S846	873	176	192	ns	ns

Details are shown in Appendix 1.

CHGB, chromogranin B gene; kb, kilobase; H-W, Hardy-Weinberg equilibrium; S, schizophrenia; C, control.

[a]These markers are listed in the order from telomeric (D20S835) to centromeric (D20S846) site. The average distance between the markers is about 120 kb.

[b]*p* value is denoted when $p < .05$.

[c]Deviations from the Hardy-Weinberg equilibrium.

[d]Comparison between schizophrenia vs. control (empirical *p*).

ing to the DSM-III-R criteria (American Psychiatric Association 1987). We actually did not use SCID itself at the clinical interview because: (1) a validated Japanese translation version of SCID was not available at the time of clinical evaluation in this study, and (2) we considered that diagnosis by repeated interviews by trained attendant physicians seems to be more reliable than SCID performed by a nonattendant physician only at once. Control subjects (96 men, 96 women; aged 24–87 years [mean 49.8 years]) were recruited mostly from the medical staff working in the psychiatric facilities and had no history of psychoses. All subjects were of Japanese descent, born to Japanese parents. The sampling methods of the subjects were satisfied with the criteria of the proposed checklists for gene-disease association study (Colhoun et al 2003; Little et al 2002).

Genomic Procedure

Genomic DNA was extracted from leukocytes in venous blood samples. Ten microsatellite markers on chromosome 20p12.3, D20S835 (Genome Database [GDB]:603368), D20S873 (GDB:609813), D20S882 (GDB:610170), D20S905 (GDB:612744), D20S916 (GDB:614700), D20S194 (GDB:200327), D20S192 (GDB:200199), D20S156 (GDB:198517), D20S892 (GDB:611556),

Table 2. Pair-Wise Linkage Disequilibrium in the *CHGB*

		4 −1401A/G	5 −1351C/T	6 −408A/C	7 −373T/A	8 −58T/C	9 IVS3-11T/C	10 277T/A	11 433G/A	12 533A/G	13 598A/C	14 695G/A	15 1058C/G	16 1104A/G	17 1238C/T	18 1250G/A	19 1499G/A
4	−1401A/G		1.00	1.00	.73	.51	.60	1.00	1.00	1.00	1.00	.50	.48	.37	.69	.84	.60
5	−1351C/T	.10		.90	.72	.43	1.00	.89	1.00	.98	.85	.92	.64	.64	.57	.58	.93
6	−408A/C	.01	.35		1.00	.96	.77	.86	.45	.88	1.00	.67	.84	.84	.77	.83	.90
7	−373T/A	.00	.09	.27		.58	1.00	.72	.85	.76	.53	.51	.68	.70	.55	1.00	1.00
8	−58T/C	.00	.02	.45	.01		.50	.36	.44	.42	.74	.43	.64	.63	1.00	.66	1.00
9	IVS3-11T/C	.00	.07	.07	.02	.00		1.00	1.00	1.00	1.00	.98	.61	.62	.83	.74	.51
10	277T/A	.01	.70	.23	.07	.01	.06		.99	.99	1.00	.90	.58	.57	.56	.51	.92
11	433G/A	.01	.38	.20	.08	.08	.17	.37		1.00	1.00	.92	.47	.46	.23	.43	.67
12	533A/G	.02	.58	.49	.14	.04	.11	.60	.60		1.00	.94	.63	.62	.32	.42	.83
13	598A/C	.13	.06	.10	.02	.04	.01	.08	.07	.12		1.00	.79	.79	.54	.43	.90
14	695G/A	.00	.06	.06	.01	.02	.87	.06	.16	.09	.01		.58	.59	.74	.69	.48
15	1058C/G	.00	.13	.57	.16	.29	.05	.08	.15	.21	.04	.02		1.00	1.00	.98	.77
16	1104A/G	.00	.13	.57	.16	.29	.05	.08	.15	.21	.04	.04	1.00		1.00	.98	.77
17	1238C/T	.02	.04	.08	.02	.04	.00	.04	.00	.01	.29	.00	.10	.10		.75	.81
18	1250G/A	.01	.25	.34	.11	.19	.04	.16	.12	.17	.02	.03	.66	.66	.03		.82
19	1499G/A	.01	.05	.05	.02	.03	.31	.04	.09	.07	.01	.24	.10	.10	.01	.04	

Upper diagonal figures are *D'* and lower diagonal figures are r^2.
CHGB, chromogranin B gene.

and D20S846 (GDB:606163), were amplified by polymerase chain reaction (PCR). The positions of these markers in relation to *CHGB* is shown in Figure 1A. The PCR primers are available on the GDB (http://gdbwww.gdb.org). Genotyping to determine the lengths of the microsatellite alleles was done with an ABI PRISM 3100 DNA sequencer (Applied Biosystems, Foster City, California). The sequence and genomic structure of *CHGB* were obtained from the high throughput genome sequences (HTGs) database of the Blast server at the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/BLAST/). All exons containing exon-intron junctions and a 5′-side regulatory region of *CHGB* were amplified by the PCR method. The primers are available on request. Polymorphisms were screened in 24 schizophrenic patients by direct sequencing of PCR products with the Big Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, California) and on the ABI PRISM 3100 DNA sequencer. All nucleotide variants detected in this study were genotyped by direct sequencing after PCR amplification. Trace data were aligned by Sequencher software (Gene Codes Corp, Ann Arbor, Michigan) and scanned for polymorphisms.

Statistical Procedures

Microsatellite Marker Analyses. Assessing the deviations from Hardy-Weinberg equilibrium and the case-control comparison of the distribution of the microsatellite allele frequencies between control subjects and schizophrenic patients were performed by the program implemented in MEGA2 (Guo and Thompson 1992).

Single Nucleotide Polymorphism Analyses. Deviations of the genotype distributions from the Hardy-Weinberg equilibrium were assessed with the 2×3 χ^2 test. Case-control comparisons of genotype and allele frequencies of polymorphisms were done with the Armitage Trend Test and 2×2 χ^2 tests. We examined associations by permutation procedure in COCAPHASE ver 2.4 to determine the empirical significance of our findings. We did not use -EM and -missing options. The χ^2 value, the p-value, and the odds ratio (OR) were calculated with the SPSS computer package for Windows Release 11.0J (SPSS Japan Inc., Tokyo, Japan). Linkage disequilibrium analyses were conducted with the Cocaphase program: unbiased application of the transmission/disequilibrium test to multilocus haplotypes (Dudbridge et al 2000).

Results

Results of the additional dense mapping association analyses using 10 microsatellite markers close to D20S95 on 20p12.3 with schizophrenia are shown in Table 1 and supplemental data table 1. The genotype frequencies of these microsatellite markers did not significantly deviate from Hardy-Weinberg equilibrium except for D20S882 ($P = 0.02$). As shown in Table 1, possible association with schizophrenia was detected in the loci of D20S882 and D20S905, in addition to D20S95. The 109-bp allele of D20S95 was more frequent in the patient group (.10) than in the control group (.03, uncorrected $p = 7.2 \times 10^{-5}$, empirical $p = .002$). The 80-bp allele of D20S882 was less frequent in the patient group (.14) than in the control group (.22, uncorrected $p = .0009$, empirical $p = .02$). The 90-bp allele of D20S905 was less frequent in the patient group (.01) than in the control group (.05, uncorrected $p = .002$, empirical $p = .004$). Although there was a global significance for the haplotype comprising the three microsatellites ($p = 1 \times 10^{-9}$), no specific individual haplotype of these microsatellite markers was significantly associated with schizophrenia after correction for the number of haplotypes.

Table 3. Genotype and Allelic Distribution of the *CHGB* SNPs in Japanese Control Subjects and Patients with Schizophrenia

		Genotype					Allele			
					p Value[a]					
Polymorphisms Group	*N*	Genotype Count			H-W[c]	S vs. C[d]	Count (frequency)		Odds Ratio (95% CI)	*p* Value[b] S vs. C[d]
−1401A/G		AA	AG	GG			A	G		
Control	192	186	6	0	1.00	.79	378 (.98)	6 (.02)	1.22 (.40–3.65)	.73
Schizophrenia	185	178	7	0	1.00		363 (.98)	7 (.02)		
−1351C/T		CC	CT	TT			C	T		
Control	192	78	77	37	.30	.87	233 (.61)	151 (.39)	.92 (.69–1.23)	.58
Schizophrenia	186	72	89	25	.98		233 (.63)	139 (.37)		
−408A/C		AA	AC	CC			A	C		
Control	96	14	45	37	1.00	.47	73 (.38)	119 (.62)	.79 (.53–1.19)	.26
Schizophrenia	95	15	53	27	.67		83 (.44)	107 (.56)		
−373T/A		TT	TA	AA			T	A		
Control	94	71	21	2	.99	.43	163 (.87)	25 (.13)	.99 (.55–1.79)	.97
Schizophrenia	95	70	25	0	.33		165 (.87)	25 (.13)		
−58T/C		TT	TC	CC			T	C		
Control	185	106	67	12	.96	.24	279 (.75)	91 (.25)	1.22 (.88–1.69)	.24
Schizophrenia	181	94	71	16	.95		259 (.72)	103 (.28)		
IVS3-11T/C		TT	TC	CC			T	C		
Control	187	154	32	1	.84	.32	340 (.91)	34 (.09)	.66 (.38–1.14)	.14
Schizophrenia	186	164	21	1	.99		349 (.94)	23 (.06)		
277T/A (S93T)		TT	TA	AA			T	A		
Control	189	73	86	30	.91	.82	232 (.61)	146 (.39)	.94 (.70–1.26)	.68
Schizophrenia	187	73	89	25	.98		235 (.63)	139 (.37)		
433G/A (D145N)		GG	GA	AA			G	A		
Control	187	73	90	24	.96	.78	236 (.63)	138 (.37)	.99 (.73–1.33)	.94
Schizophrenia	187	77	83	27	.91		237 (.63)	137 (.37)		
533A/G (R178Q)		AA	AG	GG			A	G		
Control	188	51	90	47	.92	.35	192 (.51)	184 (.49)	.87 (.65–1.16)	.34
Schizophrenia	187	42	94	51	1.00		178 (.48)	196 (.52)		
598A/C (N200H)		AA	AC	CC			A	C		
Control	189	149	39	1	.82	.59	337 (.89)	41 (.11)	1.12 (.72–1.76)	.61
Schizophrenia	187	143	43	1	.57		329 (.88)	45 (.12)		
695G/A (R232Q)		GG	GA	AA			G	A		
Control	186	157	28	1	1.00	.45	342 (.92)	30 (.08)	.87 (.50–1.50)	.61
Schizophrenia	184	161	20	3	.50		342 (.93)	26 (.07)		
1058C/G (A353G)		CC	CG	GG			C	G		
Control	192	49	89	54	.80	1.3×10^{-5}	187 (.49)	197 (.51)	1.96 (1.46–2.63)	5.9×10^{-6}
Schizophrenia	190	21	82	87	.97		124 (.33)	256 (.67)		
1104A/G (E368E)		AA	AG	GG			A	G		
Control	192	49	89	54	.80	2.0×10^{-6}	187 (.49)	197 (.51)	2.11 (1.57–2.84)	6.2×10^{-7}
Schizophrenia	187	20	76	91	.89		116 (.31)	258 (.69)		
1238C/T (P413L)		CC	CT	TT			C	T		
Control	192	155	36	1	.82	.54	346 (.90)	38 (.10)	1.15 (.72–1.83)	.55
Schizophrenia	187	147	38	2	1.00		332 (.89)	42 (.11)		
1250G/A (R417H)		GG	GA	AA			G	A		
Control	192	71	84	37	.67	.006	226 (.59)	158 (.41)	1.51 (1.13–2.01)	.005
Schizophrenia	187	42	98	47	.90		182 (.49)	192 (.51)		
1499G/A (R500K)		GG	GA	AA			G	A		
Control	191	160	29	2	.84	.270	349 (.91)	33 (.09)	.67 (.38–1.16)	.15
Schizophrenia	186	166	18	2	.75		350 (.94)	22 (.06)		

CHGB, chromogranin B gene; H-W, Hardy-Weinberg equilibrium; S, schizophrenia; C, control; CI, confidence interval.
[a]Armitage's trend test.
[b]Chi-squared test.
[c]H-W: Observed genotype vs. expected genotype according to Hardy-Weinberg equilibrium.
[d]S vs. C: Genotype comparison between schizophrenia vs. control.

All exons containing exon-intron junctions and a 5′-side regulatory region of *CHGB* were screened for polymorphisms in 24 schizophrenic patients. A total of 22 single nucleotide polymorphisms (SNPs) were identified (Figure 1B). Eighteen SNPs, −1351C/T (rs236139), −408A/C (rs236140), −373T/A (rs236141), IVS2+168G/C (rs236145), IVS2+344G/A (rs236146), IVS3−11T/C (rs6139872), 277T/A(S93T) (rs6085324), 433G/A(D145N) (rs6133278), 533G/A(R178Q) (rs910122), 598A/C(N200H) (rs881118), 695G/A(R232Q) (rs6139873), 1058G/C(A353G) (rs236152), 1104G/A(E368E) (rs236153), 1238C/