les were overrepresented in schizophrenics [8]. In this study, we seek to confirm this association between the *GRIN2A* promoter $(GT)_n$ polymorphism and schizophrenia by analyzing extended case-control samples while excluding potential population stratification.

All subjects examined in this study were derived from a geographic area located in the mid region of Japan. In this case-control study, subjects were increased from 375 schizophrenics and 378 controls, to 672 unrelated schizophrenics (mean age 45.4 ± 10.6 years) and 686 mentally healthy control subjects (mean age 44.8 ± 11.0 years), almost doubling the size of samples. Consensual diagnosis was made by at least two experienced psychiatrists according to criteria from the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) [1]. The present study was approved by the Ethical Committee of RIKEN. Written informed consent was obtained from all participants after an explanation of the study.

DNA was extracted from whole blood according to a standard protocol. Amplification of the $(GT)_n$ repeat region was performed using the following primers: a FAM-labeled upstream primer, 5′-GAAGGAAGCATGTGGGAAATGCAG-3′ (the 3′ end is 88 bp upstream of the 5′ end of the GT repeat; see GenBank accession no. AF443855) and a non-labeled downstream primer, 5′-gtttcttGCTGGGTACAGTTATCCCCCT-3′ (the 3′ end is 19 bp downstream of the 3′ end of the GT repeat). The underlined tail sequence was added because Taq DNA polymerase catalyzes the non-templated addition of adenosine to the 3′ end of PCR products affecting the degree of product adenylation. By placing the sequence GTTTCTT at the 5′ end of reverse primers, nearly 100% adenylation of the 3′ end of the forward strand is achieved, facilitating accurate genotyping [7,8]. PCR was performed with an initial denaturation at 95 °C for 12 min, followed by 35 cycles of 94 °C for 15 s, 58 °C for 15 s and 72 °C for 30 s, and a final extension at 72 °C for 30 min, using AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA). PCR products were analyzed using an ABI 3700 sequencer equipped with GeneScan software (Applied Biosystems).

We assessed the population structure of case-control samples by genotyping 21 single nucleotide polymorphisms (SNPs). The markers were selected from a database of Japanese Single Nucleotide Polymorphisms (JSNP: http://snp.ims.u-tokyo.ac.jp/), Celera Discovery System (Celera: http://www.celeradiscoverysystem.com/) and Entrez SNP on NCBI (dbSNP: http://www.ncbi.nlm.nih.gov/SNP/) (these SNP information are available upon request). SNPs were scored using the Assays-on-Demand™ SNP genotyping system (Applied Biosystems, Foster City, CA), based on TaqMan assay methods [15]. Genotypes were determined using an ABI7900 sequence detection instrument (Applied Biosystems) and the SDS v2.0 software package (Applied Biosystems).

Fig. 1 shows the allelic distributions of the $(GT)_n$ in schizophrenia and control groups. In concordance with the previous finding, the allele distribution histogram of schizophrenic patients was shifted to the right, with longer alleles over-represented in schizophrenics. Because of the length-dependent suppressive effect of the $(GT)_n$ repeat on gene transcription [8] and the observed allele distribution patterns (Fig. 1), we divided the tandem repeat alleles into two classes according to length: short (25 or fewer repeats) and long (more than 25 repeats). The distributions of these two categories were significantly different between schizophrenia and control groups (Fisher's exact test, two-sided, $P = 0.014$, odds ratio = 1.23, 95% CI = 1.04–1.45). When we set the boundary of "short" alleles at 24 and "long" alleles at 25 repeats (cf. mean repeat sizes were 24.71 in schizophrenics and 24.66 in controls), the P value was 0.094. We assessed the overall differences of allelic distributions between the two groups using the CLUMP program (http://www.mds.qmw.ac.uk/statgen/dcurtis/software.html) [18]. We performed 10,000 Monte Carlo permutations to simulate the empirical significance levels of the statistics produced by the program, and this analysis gave significant

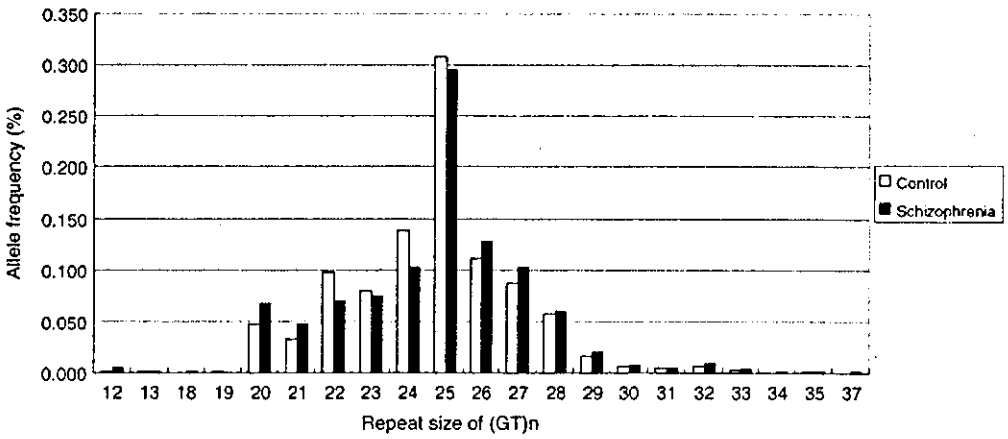

Fig. 1. Allele frequency distribution of the *GRIN2A* $(GT)_n$ repeat in schizophrenic patients and controls. Allele size is expressed as the number of GT repeats.

Table 1
Comparison of $(GT)_n$ allele distributions by the CLUMP program

	T1	T2	T3	T4
Chi-squared	34.75	28.86	8.25	19.32
d.f.	20	12	1	1
P value	0.022	0.0042	0.0041	0.000011
Empirical *P* value	0.011	0.011	0.045	0.011

P values are uncorrected for multi-allele testing. Empirical *P* values were simulated from 10,000 Monte Carlo permutations.

results in all T1–T4 statistics (empirical $P<0.05$) (Table 1). There was no deviation of genotypes from Hardy–Weinberg equilibrium, which was examined using Arlequin software (http://anthropologie.unige.ch/arlequin/methods.html) [17]. When we tested for single allelic association of common alleles [frequency > 3%: $(GT)_{20}$ to $(GT)_{28}$], Fisher's exact test gave the following *P* values: 0.029 for $(GT)_{20}$, 0.074 for $(GT)_{21}$, 0.009 for $(GT)_{22}$, 0.612 for $(GT)_{23}$, 0.004 for $(GT)_{24}$, 0.499 for $(GT)_{25}$, 0.187 for $(GT)_{26}$, 0.186 for $(GT)_{27}$ and 0.869 for $(GT)_{28}$.

We used the computer program STRUCTURE [14] to group individuals, in an attempt to identify genetically similar diploid subpopulations. Employing this Markov chain Monte Carlo method, 1,000,000 replications were used for the burn-in period of the chain and for parameter estimation. The number of populations present in the sample (K) is unknown, therefore, we ran the analysis at $K=1, 2, 3, 4$ and 5. The best estimate of K was found by calculating the posterior probabilities, $\Pr(K=1, 2, 3, 4, 5)$, as described by Pritchard et al. [14]. The Japanese case-control samples in this study showed no evidence of a cryptic population structure, as demonstrated by $\Pr(K=1)>0.99$.

In this study, we have replicated the previous association finding under more stringent conditions, by analyzing a larger panel of age-/sex-matched case-control samples and excluded the potential confounding factor of population stratification. Our recent genome-wide linkage disequilibrium scan of schizophrenia in Japanese pedigrees has revealed evidence of significant association with the marker *D16S423*, located at chromosomal region 16p13.1 and 3.8 Mb upstream from the *GRIN2A* $(GT)_n$ polymorphism [23]. Moreover, the Japanese Schizophrenia Sib-pair Linkage Group, a multisite collaborative, has identified 16p13.13 as a susceptibility region in schizophrenia (*D16S748*, located 1.9 Mb downstream of the $(GT)_n$ polymorphism) in a first-stage genome-wide scan [9]. Previously reported linkage data suggested that 16p13 might encode a susceptibility gene for bipolar disorder [4]. Edenberg et al. [5] have reported evidence of linkage to mood disorders with markers *D16S2618*, *D16S2619* and *D16S749* located at 16p12–13 in the National Institute of Mental Health (NIMH) initiative pedigrees. These markers map within 6.6 Mb upstream and 9.6 Mb downstream of the $(GT)_n$ repeat. We have recently examined the *GRIN2A* in the same NIMH panel and found evidence of a modest association [7]. Therefore, *GRIN2A* may be a potential susceptibility gene on 16p for both schizophrenia and affective disorders, consistent with the notion of a shared pathway in the predisposition to schizophrenia and bipolar disorder [3,21]. Further phenotypically defined examination of the *GRIN2A* repeat polymorphism is warranted.

Acknowledgements

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References

[1] American Psychiatric Association, Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, American Psychiatric Press, Washington, DC, 1994.
[2] O. Andersson, A. Stenqvist, A. Attersand, G. von Euler, Nucleotide sequence, genomic organization, and chromosomal localization of genes encoding the human NMDA receptor subunits NR3A and NR3B, Genomics 78 (2001) 178–184.
[3] W.H. Berrettini, Susceptibility loci for bipolar disorder: overlap with inherited vulnerability to schizophrenia, Biol. Psychiatry 47 (2000) 245–251.
[4] S.D. Detera-Waldleigh, Chromosomes 12 and 16 Workshop, Am. J. Med. Genet. 88 (1999) 255–259.
[5] H.J. Edenberg, T. Foroud, P.M. Conneally, J.J. Sorbel, K. Carr, C. Crose, C. Willig, J. Zhao, M. Miller, E. Bowman, A. Mayeda, N.L. Rau, C. Smiley, J.P. Rice, A. Goate, T. Reich, O.C. Stine, F. McMahon, J.R. DePaulo, D. Meyers, S.D. Detera-Wadleigh, L.R. Goldin, E.S. Gershon, M.C. Blehar, J.I. Nurnberger Jr., Initial genomic scan of the NIMH genetics initiative bipolar pedigrees: chromosomes 3, 5, 15, 16, 17 and 22, Am. J. Med. Genet. 74 (1997) 238–246.
[6] U. Heresco-Levy, D.C. Javitt, M. Ermilov, C. Mordel, A. Horowitz, D. Kelly, Double-blind, placebo-controlled, crossover trial of glycine adjuvant therapy for treatment-resistant schizophrenia, Br. J. Psychiatry 69 (1996) 610–617.
[7] M. Itokawa, K. Yamada, Y. Iwayama-Shigeno, Y. Ishitsuka, S. Detera-Wadleigh, T. Yoshikawa, Genetic analysis of a functional *GRIN2A* promoter $(GT)_n$ repeat in bipolar disorder pedigrees in humans, Neurosci. Lett. 345 (2003) 53–56.
[8] M. Itokawa, K. Yamada, K. Yoshitsugu, T. Toyota, T. Suga, H. Ohba, A. Watanabe, E. Hattori, H. Shimizu, T. Kumakura, M. Ebihara, J.M.A. Meerabux, M. Toru, T. Yoshikawa, A microsatellite repeat in the promoter of the *N*-methyl-D-aspartate receptor 2A subunit (*GRIN2A*) gene suppresses transcriptional activity and correlates with chronic outcome in schizophrenia, Pharmacogenetics 13 (2003) 271–278.
[9] Japanese Schizophrenia Sib-Pair Linkage Group, Initial genome-wide scan for linkage with schizophrenia in the Japanese Schizophrenia Sib-Pair Linkage Group (JSSLG) families. Am. J. Med. Genet. (Neuropsychiatr. Genet.) 120B (2003) 22–28.
[10] D.C. Javitt, S.R. Zukin, Recent advances in the phencyclidine model of schizophrenia, Am. J. Psychiatry 148 (1991) 1301–1308.
[11] D.C. Javitt, I. Zylberman, S.R. Zukin, U. Heresco-Levy, J.P. Lindenmayer, Amelioration of negative symptoms in schizophrenia by glycine, Am. J. Psychiatry 151 (1994) 1234–1236.
[12] Y. Miyamoto, K. Yamada, Y. Noda, H. Mori, M. Mishina, T. Nabeshima, Hyperfunction of dopaminergic and serotonergic neuronal systems in mice lacking the NMDA receptor epsilon 1 subunits, J. Neurosci. 21 (2001) 750–757.

[13] R. Mohrmann, H. Hatt, K. Gottmann, Developmental regulation of subunit composition of extrasynaptic NMDA receptors in neocortical neurones, Neuroreport 11 (2000) 1203–1208.
[14] J.K. Pritchard, M. Stephens, P. Donnelly, Inference of population structure using multilocus genotype data, Genetics 155 (2000) 945–959.
[15] K. Ranade, M.S. Chang, C.T. Ting, D. Pei, C.F. Hsiao, M. Olivier, R. Pesich, J. Hebert, Y.D. Chen, V.J. Dzau, D. Curb, R. Olshen, N. Risch, D.R. Cox, D. Botstein, High-throughput genotyping with single nucleotide polymorphisms, Genome Res. 11 (2001) 1262–1268.
[16] B. Scatton, The NMDA receptor complex, Fundam. Clin. Pharmacol. 7 (1993) 389–400.
[17] S. Schneider, D. Roessli, L. Excoffier, Arlequin Ver. 2.000: A Software for Population Genetics Data Analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland, 2000.
[18] P.C. Sham, D. Curtis, Monte Carlo tests for associations between disease and alleles at highly polymorphic loci, Ann. Hum. Genet. 59 (1995) 97–105.
[19] G. Tsai, P. Yang, L.C. Chung, N. Lange, J.T. Coyle, Glutamatergic neurotransmission involves structural and clinical deficits of schizophrenia, Biol. Psychiatry 44 (1998) 667–674.
[20] M. Watanabe, Y. Inoue, K. Sakimura, M. Mishina, Distinct spatiotemporal distributions of the NMDA receptor channel subunit mRNAs in the brain, Ann. N.Y. Acad. Sci. 707 (1993) 463–466.
[21] D.B. Wildenauer, S.G. Schwab, W. Maier, S.D. Detera-Wadleigh, Do schizophrenia and affective disorder share susceptibility genes? Schizophrenia Res. 39 (1999) 107–111.
[22] N.M. William, T. Bowen, G. Spurlock, N. Norton, H.J. William, B. Hoogendoorn, M.J. Owen, M.C. O'Donovan, Determination of the genomic structure and mutation screening in schizophrenic individuals for five subunits of the *N*-methyl-D-aspartate glutamate receptor, Mol. Psychiatry 7 (2002) 508–514.
[23] K. Yamada, Y. Iwayama-Shigeno, Y. Yoshida, T. Toyota, M. Itokawa, E. Hattori, H. Shimizu, T. Yoshikawa, Family-based association study of schizophrenia with 444 markers and analysis of a new susceptibility locus mapped to 11q13.3, Am. J. Med. Genet. (Neuropsychiatr. Genet.) 127 (2004) 11–19.

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Association analysis of the −308G>A promoter polymorphism of the tumor necrosis factor alpha (TNF-α) gene in Japanese patients with schizophrenia

Short Communication

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Summary. Two research groups have thus far reported a significant association between schizophrenia and a promoter polymorphism (−308G>A) of the gene encoding tumor necrosis factor alpha (TNF-α), while contradictive negative results have also been reported. We examined the possible association in a Japanese sample of 297 schizophrenia cases and 458 controls. Allele frequencies of both the patients and controls were very low (1.5% and 0.8%, respectively), and the difference was not statistically significant. We conclude that the effect of the −308G>A polymorphism on the development of schizophrenia is, if any, weak and the majority of Japanese schizophrenics are unrelated to the −308G>A polymorphism of the TNF-α gene.

Keywords: Schizophrenia, tumor necrosis factor α, association study, genetics, single nucleotide polymorphism (SNP), promoter.

Introduction

Schizophrenia is a complex disorder characterized by profound disturbances of perception, thought, cognition, emotion and social functioning. It affects

approximately 0.5–1% of the general population worldwide. The pathogenesis of schizophrenia is unclear; however, immune alterations in schizophrenia have been described for decades (for review; Muller et al., 2000). Tumor necrosis factor alpha (TNF-α) is one of the major factors playing a central role in immune systems. As TNF-α serum levels were significantly higher in schizophrenic patients compared with healthy controls (Naudin et al., 1997; Erbagci et al., 2001; Kowalski et al., 2001; Theodoropoulou et al., 2001), abnormal production of TNF-α could increase the risk of developing schizophrenia. The level of TNF-α production is under genetic control and determined in part by alleles of a single nucleotide polymorphism (SNP), −308G>A, in the promoter region of the TNF-α gene (Wilson et al., 1997). Furthermore, the TNF-α gene is located on chromosome 6p21.1–21.3 where linkage and association studies have suggested a locus of susceptibility to schizophrenia (Wright et al., 2001).

Recently, Boin et al. (2001) found a significant association between the −308G>A polymorphism of the TNF-α gene and schizophrenia in an Italian population. Subsequently, Meira-Lima et al. (2003) obtained evidence supporting this association in a Brazilian sample; whilst other research groups from Germany, Australia, and Korea failed to find such an association (Riedel et al., 2002; Handoko et al., 2003; Pae et al., 2003). This inconsistency requires further investigations. Here we examined the −308G>A polymorphism of the TNF-α gene for allelic association with schizophrenia in a Japanese sample.

Materials and methods

Subjects

Subjects were 297 patients (164 males, mean age: 43.7 years [SD 14.1]) with schizophrenia and 458 healthy controls (235 males, 36.4 years [12.6]). All the subjects were biologically unrelated Japanese. Consensus diagnosis was made for each patient by at least two psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria. The controls were not assessed with any structured psychiatric interviews; however, they showed good social functioning (i.e. no history of long-term absence from school or work) and reported themselves to be in good health. After description of the study, written informed consent was obtained from every subject. The study protocol was approved by institutional ethical committees (Fujita Health University School of Medicine, Showa University School of Medicine, and National Center of Neurology and Psychiatry).

Genotyping

Venous blood was drawn from the subjects and genomic DNA was extracted from whole blood according to the standard procedures. Genotypes for the −308G>A polymorphism were determined using the TaqMan 5′-exonuclease allelic discrimination assay, described previously (Hashimoto et al., 2003). Two fluorescent dyes, a reporter and a quencher, are attached to the probes used with the TaqMan PCR Reagent Kit. When both dyes are attached to the probe, reporter dye emission is quenched. During each extension cycle, Taq DNA polymerase cleaves the reporter dye from the probe via its 5′–3′ exonuclease. Once separated from the quencher, the reporter dye emits its characteristic fluorescence which can then be measured by the ABI PRISM7000 (Applied Biosystems, Japan). The amount of fluorescence measured is proportional to the amount of PCR product made.

Primers and probes for detection of the SNP are: 5′-GGACCCTGGAGGCTGAAC-3′ (forward primer), 5′-CCAAAAGAAATGGAGGCAATAGGTT-3′ (reverse primer), 5′-VIC-CCCGTCCCCATGCC-MGB-3′ (probe 1), and 5′-FAM-CCCGTCCTCATGCC-MGB-3′ (probe 2). Thermal cycling conditions for polymerase chain reaction were: at 95°C for 10 minutes, 45 cycles of 92°C for 15 seconds and 60°C for 1 minute.

Statistical analysis

The presence of Hardy-Weinberg equilibrium was examined by the χ^2 test for goodness of fit. Genotype and allele distributions between patients and controls were analyzed by the χ^2 test for independence. The critical p-value was set at 0.05 (two-tail).

Results

Genotype distributions and allele frequencies of the −308G>A polymorphism of the TNF-α gene among the patients and controls are shown in Table 1. The genotype distributions for the two groups were both in Hardy-Weinberg equilibrium (patients: $\chi^2=0.07$, $df=1$, $p=0.79$; controls: $\chi^2=0.03$, $df=1$, $p=0.87$). We found that the minor allele (−308A) was rare in our Japanese sample (0.8% in the controls), which was substantially different from that (6∼7%) reported in a Korean sample (Pae et al., 2003). There was no individual homozygous for the minor allele among the patients and controls. Although the frequency of the minor allele was a bit increased in the schizophrenics compared to the controls, the difference in genotype distribution (proportion of heterozygous individuals) or allele frequency between the two groups was not statistically significant (genotype: $\chi^2=2.0$, $df=1$, $p=0.16$; allele: $\chi^2=1.9$, $df=1$, $p=0.16$).

Discussion

We failed to find a statistically significant association between the −308G>A polymorphism of the TNF-α gene and schizophrenia in our Japanese sample, suggesting that the examined polymorphism has no major role in the pathogenesis of schizophrenia. However, a possibility remains that we may have yielded a false negative result due to an inadequate statistical power resulting from the very low frequency of the minor allele (−308A) in our Japanese

Table 1. Genotype and allele frequencies of the −308G>A polymorphism of the tumor necrosis factor alpha gene among the patients with schizophrenia and controls

	Genotype distribution				Allele frequency			Odds ratio	95% CI
	N	G/G	G/A	A/A	N	G	A		
Patients	297	288 (97.0%)	9 (3.0%)	0 (0%)	594	585 (98.5%)	9 (1.5%)	2.03	0.74–6.54
Controls	458	451 (98.5%)	7 (1.5%)	0 (0%)	916	909 (99.2%)	7 (0.8%)		

sample (approximately 1%), since the frequency of the −308A allele was somewhat increased in the schizophrenics (1.5%) than in the controls (0.8%). The obtained odds ratio (2.03) in the current study was similar to that reported by Boin et al. (2001) (2.24). When power analysis was performed, an extraordinarily large sample (approximately 1500 cases and 1500 controls) is required for Japanese to detect a significant association between the risk allele (−308A) and schizophrenia with a power of 90% when odds ratio was assumed to be 2.0 and the critical p-value was set at 0.05. Thus multi-center study or meta-analysis of multiple data would be required to draw any conclusion. However, even if such an association exists, the minor allele was very rare, and accordingly the population attributable risk for the possible association was estimated to be less than 0.01. We conclude that the effect of the −308G>A polymorphism on the development of schizophrenia is, if any, weak and the majority of Japanese schizophrenics are unrelated to the polymorphism of the TNF-α gene.

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References

Boin F, Zanardini R, Pioli R, Altamura CA, Maes M, Gennarelli M (2001) Association between −G308A tumor necrosis factor alpha gene polymorphism and schizophrenia. Mol Psychiatry 6: 79–82

Erbagci AB, Herken H, Koyluoglu O, Yilmaz N, Tarakcioglu M (2001) Serum IL-1beta, sIL-2R, IL-6, IL-8 and TNF-alpha in schizophrenic patients, relation with symptomatology and responsiveness to risperidone treatment. Mediators Inflamm 10: 109–115

Handoko HY, Nancarrow DJ, Hayward NK, Ohaeri JU, Aghanwa H, McGrath JJ, Levinson DF, Johns C, Walters MK, Nertney DA, Srinivasan TN, Thara R, Mowry BJ (2003) Tumor necrosis factor haplotype analysis amongst schizophrenia probands from four distinct populations in the Asia-Pacific region. Am J Med Genet 121B: 1–6

Hashimoto R, Straub RE, Weickert CS, Hyde TM, Kleinman JE, Weinberger DR (2003) Expression analysis of neuregulin-1 in the dorsolateral prefrontal cortex in schizophrenia. Mol Psychiatry (in press)

Kowalski J, Blada P, Kucia K, Madej A, Herman ZS (2001) Neuroleptics normalize increased release of interleukin-1 beta and tumor necrosis factor-alpha from monocytes in schizophrenia. Schizophr Res 50: 169–175

Meira-Lima IV, Pereira AC, Mota GF, Floriano M, Araujo F, Mansur AJ, Krieger JE, Vallada H (2003) Analysis of a polymorphism in the promoter region of the tumor necrosis factor alpha gene in schizophrenia and bipolar disorder: further support for an association with schizophrenia. Mol Psychiatry 8: 718–720

Muller N, Riedel M, Gruber R, Ackenheil M, Schwarz MJ (2000) The immune system and schizophrenia. An integrative view. Ann NY Acad Sci 917: 456–467

Naudin J, Capo C, Giusano B, Mege JL, Azorin JM (1997) A differential role for interleukin-6 and tumor necrosis factor-alpha in schizophrenia? Schizophr Res 26: 227–233

Pae CU, Chae JH, Bahk WM, Han H, Jun TY, Kim KS, Kwon YS, Serretti A (2003) Tumor necrosis factor-alpha gene polymorphism at position −308 and schizophrenia in the Korean population. Psychiatry Clin Neurosci 57: 399–403

Riedel M, Kronig H, Schwarz MJ, Engel RR, Kuhn KU, Sikorski C, Sokullu S, Ackenheil M, Moller HJ, Muller N (2002) No association between the G308A polymorphism of the tumor necrosis factor-alpha gene and schizophrenia. Eur Arch Psychiatry Clin Neurosci 252: 232–234

Theodoropoulou S, Spanakos G, Baxevanis CN, Economou M, Gritzapis AD, Papamichail MP, Stefanis CN (2001) Cytokine serum levels, autologous mixed lymphocyte reaction and surface marker analysis in never medicated and chronically medicated schizophrenic patients. Schizophr Res 47: 13–25

Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW (1997) Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc Natl Acad Sci USA 94: 3195–3199

Wright P, Nimgaonkar VL, Donaldson PT, Murray RM (2001) Schizophrenia and HLA: a review. Schizophr Res 47: 1–12

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Association Between the Glutathione S-Transferase M1 Gene Deletion and Female Methamphetamine Abusers

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Several lines of evidence suggest that increased generation of auto-oxidized dopamine (DA) *o*-quinone is associated with the neurotoxicity of methamphetamine (MAP) in the brain, and that, as a cellular defenses against DA-derived quinines, glutathione S-transferase (GST) detoxifies auto-oxidized DA *o*-quinone in the brain. Glutathione S-transferase M1 (GSTM1) of the mu-class of GSTs catalyzes reaction between glutathione and catecholamine *o*-quinones under physiological conditions. This study was undertaken to investigate the role of the *GSTM1* gene deletion polymorphism in the neuropathology of MAP abuse. One hundred fifty-seven MAP abusers and 200 healthy comparison subjects were tested for a genetic polymorphism of GSTM1. The difference in the frequency of deletion (D)/non-deletion (N) alleles between the female abusers and female controls was close to statistical significance ($P = 0.071$), although there was no statistical difference ($P = 0.651$) between male abusers and male controls. Furthermore, the number of female abusers with deletion alleles was significantly ($P = 0.007$, odds ratio: 2.77, 95% CI 1.30–5.89) higher than that of male abusers with deletion alleles. These findings suggest that *GSTM1* gene deletion may contribute to a vulnerability to MAP abuse in female subjects, but not in male subjects.

KEY WORDS: methamphetamine; drug abuse; glutathione S-transferase; gender difference

INTRODUCTION

Abuse of methamphetamine (MAP) is a growing problem worldwide. Some lines of evidence have suggested strong genetic contributions to drug abuse vulnerability [Uhl et al., 2002]. The application of brain imaging techniques to the study of drug abuse have demonstrated that the density of dopamine (DA) transporters is significantly reduced in the caudate/putamen of MAP abusers [Sekine et al., 2001; Volkow et al., 2001], suggesting that long-term use of MAP causes damage to dopaminergic neurons in the human brain. Furthermore, it has been shown that MAP-induced neurotoxicity in the brain has been shown to require striatum DA and to involve mechanisms associated with oxidative stress [Cadet and Brannock, 1998]. It is also known that DA is auto-oxidized and the corresponding DA *o*-quinone (aminochrome) is subse-

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quently generated; moreover, aminochrome and its subsequent product, DA *o*-semiquinone, elicit redox cycling which leads to the generation of reactive oxygen species, which in turn degenerate dopaminergic neurons [Graham et al., 1978; Smythies and Galzigna, 1998]. DA oxidation also results in the formation of DA *o*-quinone, which readily participates in nucleophilic addition reactions with sufhydryl groups on free cysteine, glutathione, or cysteine found in protein including DA transporter [Graham et al., 1978; Hastings and Zigmond, 1994; Smythies and Galzigna, 1998; Whitehead et al., 2001]. In addition, it has been reported recently that DA auto-oxidation contributes to MAP-induced neurotoxicity to DA terminals, adding support to the role of DA and oxidative stress in this model [LaVoie and Hastings, 1999]. Taken together, it is likely that increased generation of DA *o*-quinone by DA auto-oxidation is associated with the neurotoxicity of MAP in the brain.

Glutathione S-transferase M1 (GSTM1) is a subtype of GSTs that detoxify xenobiotics by conjugating glutathione. It has been reported that GSTM1 catalyzes a glutathione conjugate of catecholamine *o*-quinones such as aminochrome [Smythies and Galzigna, 1998]. GSTM1 has an entire gene deletion polymorphism and its enzymatic activity is classified into three grades, i.e., a highly active genotype (homozygous non-deletion alleles; NN), a moderately active genotype (heterozygous non-deletion alleles; DN), and a null genotype (homozygous deletion alleles; DD) [McLellan et al., 1997]. Recently, it has been reported that the frequency of D allele of *GSTM1* gene in the patients with schizophrenia was significantly ($P = 0.0075$) higher than that of normal controls, suggesting that *GSTM1* gene may be associated with an increased susceptibility to schizophrenia [Harada et al., 2001a]. Thus, it seems that differences in the GSTM1 genotype may contribute to the development of MAP abuse. In order to verify a potential role of the *GSTM1* gene in the neuropathology by MAP abuse, we analyzed a polymorphism of the *GSTM1* gene in subjects with diagnosed MAP-related disorders and in control groups.

MATERIALS AND METHODS

The research was performed after obtaining approval from the ethics committees of each institute of Japanese Genetics Initiative for Drug Abuse (JGIDA), and all subjects provided written informed consent for the use of their DNA samples for this research. The subjects were 157 patients (125 males, age: 37 ± 11 years (mean ± SD), age range: 19–69 years; and 32 females, age: 28 ± 5 years (mean ± SD), age range: 21–47 years) with MAP dependence and psychotic disorder meeting ICD-10-DCR criteria (F15.2 and F15.5) who were outpatients or inpatients of psychiatric hospitals of JGIDA, and 200 age-, gender- and geographical origin-matched normal controls (157 males, age: 37 ± 11 years (mean ± SD), age range: 19–69 years; and 43 females, age: 36 ± 10 years (mean ± SD), age range: 21–58 years) mostly consisted of medical staffs who had no past history and no family history of drug dependence or psychotic disorders. All subjects were Japanese, born and living in restricted arrears of Japan including northern Kyusyu, Setouchi, Tyukyou, Toukai, and Kantou.

The polymorphism studied in this project was the deletion of the entire *GSTM1* gene. Genotyping for this gene was performed by a combination of two types of polymerase chain reaction (PCR) amplification as reported previously [Harada et al., 2001a,b]. The first type of PCR was used for the detection of a non-deletion allele with the appropriate primers (forward: 5′-CTTCACGTGTTATGGAG GTTC-3′, reverse: 5′-GCGAGTTATTCTGTGTGTAGC-3′). The other type of PCR was used for the detection of a deletion allele with suitable primers (forward: 5′-ACAGAGGAAGGGTG-CATTTGATA-3′, reverse: 5′-GACATTCATTCCCAAA-GCGACCA-3′); both types of PCR were followed by agarose gel electrophoresis with ethidium bromide staining. Allele frequencies were calculated by gene counting and the differences between groups were evaluated by Fisher's exact test. The odds ratio (OD) and 95% confidence intervals were calculated to evaluate the effects of the different genotypes.

RESULTS

The GSTM1 genotypes and the allele frequencies in MAP abusers and controls are shown in Table I. The genotype distribution in both abusers and controls was within the Hardy–Weinberg equilibrium. We found that a difference in the frequency of deletion (D)/non-deletion (N) alleles between the female abusers and female controls was a trend toward a statistical significance ($P = 0.071$). In contrast, there was no significant difference between male abusers and male controls ($P = 0.651$). The frequency of carrying the D allele among female abusers was significantly higher than that in male abusers ($P = 0.007$, odds ratio: 2.77, 95% CI 1.30–5.89), whereas no gender difference was shown among control subjects ($P = 0.297$, odds ratio: 1.36, 95% CI 0.80–2.31). The genotype distribution difference between female abusers and female controls was significant ($P = 0.032$), whereas no significant difference between male abusers and male controls was shown ($P = 0.819$).

DISCUSSION

Our findings suggest that a deletion of the *GSTM1* gene may contribute to MAP abuse vulnerability in female, but not in male, subjects. Based on the role played by GSTM1 in the antioxidant system preventing neurotoxicity, *GSTM1* gene deletion might lead to an excess of catecholamine *o*-quinones (e.g., aminochrome) that are neurotoxic in the brain, including DA neurons. The reason underlying this gender difference is currently unclear. However, recent evidence has been suggestive of gender differences in course of drug dependence and drug abuse [Lynch et al., 2002]. It has been reported that females enter treatment programs after fewer years of amphetamine use, and that females also take less time to become addicted after initial use than do males [Westermeyer and Boedicker,

TABLE I. Allele and Genotype Frequencies of the *GSTM1* Gene Deletion Polymorphism in MAP Abusers and Controls

	Male		Female	
	Abusers (n = 125)	Controls (n = 157)	Abusers (n = 32)	Controls (n = 43)
GSTM1 allele frequency				
D	172 (68.8%)	210 (66.9%)	55 (85.9%)	63 (73.3%)
N	78 (31.2%)	104 (33.1%)	9 (14.1%)	23 (26.7%)
	$P = 0.651$		$P = 0.071$	
GSTM1 genotype frequency				
DD	58 (46.4%)	67 (42.7%)	24 (75.0%)	21 (48.8%)
DN	56 (44.8%)	76 (48.4%)	7 (21.9%)	21 (48.8%)
NN	11 (8.8%)	14 (8.9%)	1 (3.1%)	1 (2.3%)
	$P = 0.819$		$P = 0.032$*	

GSTM1, glutathione S-transferase M1; MAP, methamphetamine; D, deletion allele; N, non-deletion allele.
*$P < 0.05$.

2000]. In addition, positive subjective effects of D-amphetamine are enhanced during the follicular phase, which correlates with changes in estrogen levels [Lynch et al., 2002].

It has been suggested that gonadal hormones such as estrogen play a role in the differences between males and females regarding responses to drugs of abuse [Lynch et al., 2002]. In females, there is an accelerated transition from controlled to uncontrolled use, namely, dependence, and that gonadal hormones, particularly estrogen, may play a role in these processes [Justice and De Wit, 2000]. In studies using rats, estrogen has been revealed to enhance the behavioral and neurochemical responses to MAP by increasing stimulated DA release [Becker, 1999]. Furthermore, recent studies using brain imaging technique revealed that women have higher levels of DA transporters [Mozley et al., 2001] and lower DA D_2 receptor affinity in the striatum than men [Pohjalainen et al., 1998], suggesting a lower baseline of dopaminergic tone and elevated levels of DA released by MAP in females. Therefore, it is likely that gonadal hormones and gender differences in dopaminergic systems may be implicated in gender differences related to susceptibility to addiction to psychomotor stimulants. Thus, it appears that excess DA released by MAP might generate an excess of DA *o*-quinone, rendering it especially difficult for persons with low-activity GST to detoxify a sufficient amount of DA *o*-quinone. Furthermore, the GSTM1 deletion would influence the susceptibility of females to MAP abuse.

In conclusion, our findings suggest that *GSTM1* gene deletion may contribute to a vulnerability to MAP abuse in female subjects, but not in male subjects.

REFERENCES

Becker JB. 1999. Gender differences in dopaminergic function in striatum and nucleus accumbens. Pharmacol Biochem Behav 64:803–812.

Cadet JL, Brannock C. 1998. Free radicals and the pathobiology of brain dopamine systems. Neurochem Int 32:117–131.

Graham DG, Tiffany SM, Bell WR Jr, Gutknecht WF. 1978. Auto-oxidation versus covalent binding of quinones as the mechanism of toxicity of dopamine, 6-hydroxydopamine, and related compounds toward C1300 neuroblastoma cells in vitro. Mol Pharmacol 14:644–653.

Harada S, Fujii C, Hayashi A, Ohkoshi N. 2001a. An association between idiopathic Parkinson's disease and polymorphisms of phase II detoxification enzymes: Glutathione S-transferase M1 and quinone oxidoreductase 1 and 2. Biochem Biophys Res Commun 288:887–892.

Harada S, Tachikawa H, Kawanishi Y. 2001b. Glutathione S-transferase M1 gene deletion may be associated with susceptibility to certain forms of schizophrenia. Biochem Biophys Res Commun 281:267–271.

Hastings TG, Zigmond MJ. 1994. Identification of catechol–protein conjugates in neostriatal slices incubated with [^{3}H]dopamine: Impact of ascorbic acid and glutathione. J Neurochem 63:1126–1132.

Justice AJ, De Wit H. 2000. Acute effects of D-amphetamine during the early and late follicular phases of the menstrual cycle in women. Pharmacol Biochem Behav 66:509–515.

LaVoie MJ, Hastings TG. 1999. Dopamine quinone formation and protein modification associated with the strial neurotoxicity of methamphetamine: Evidence against a role for extracellular dopamine. J Neurosci 19:1484–1491.

Lynch WJ, Roth ME, Carroll ME. 2002. Biological basis of sex differences in drug abuse: Preclinical and clinical studies. Psychopharmacol 164:121–137.

McLellan RA, Oscarson M, Alexandrie AK, Seidegård J, Evans DAP, Rannug A, Ingelman-Sundberg M. 1997. Characterization of a human glutathione S-transferase μ cluster containing a duplicated *GSTM1* gene that causes ultrarapid enzyme activity. Mol Pharmacol 52:958–965.

Mozley LH, Gur RC, Mozley PD, Gur RE. 2001. Striatal dopamine transporters and cognitive functioning in healthy men and women. Am J Psychiatry 158:1492–1499.

Pohjalainen T, Rinne JO, Nagren K, Lehikoinen P, Anttila K, Syvalahti EK, Hietala J. 1998. The A1 allele of the human D2 dopamine receptor gene predicts low D2 receptor availability in healthy volunteers. Mol Psychiatry 3:256–260.

Sekine Y, Iyo M, Ouchi Y, Matunaga T, Tsukada H, Okada H, Yoshikawa E, Futatsubashi M, Takei N, Mori N. 2001. Methamphetamine-related psychiatric symptoms and reduced brain dopamine transporters studied with PET. Am J Psychiatry 158:1206–1214.

Smythies J, Galzigna L. 1998. The oxidative metabolism of catecholamines in the brain: A review. Biochim Biophys Acta 1380:159–162.

Uhl GR, Liu QR, Naiman D. 2002. Substance abuse vulnerability loci: Converging genome scanning data. Trends Genet 18:420–425.

Volkow ND, Chang L, Wang GJ, Fowler JS, Leonido-Yee M, Franceschi D, Sedler MJ, Gateley SJ, Hitzemann R, Ding YS. 2001. Association of dopamine transporter reduction with psychomotor impairment in methamphetamine abusers. Am J Psychiatry 158:377–382.

Westermeyer J, Boedicker AE. 2000. Course, severity, and treatment of substance abuse among women versus men. Am J Drug Alcohol Abuse 26:523–535.

Whitehead RE, Ferrer JV, Javitch JA, Justice JB. 2001. Reaction of oxidized dopamine with endogenous cysteine residues in the human dopamine transporter. J Neurochem 76:1242–1251.

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Association Study Between Brain-Derived Neurotrophic Factor Gene Polymorphisms and Methamphetamine Abusers in Japan

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Several lines of evidence suggest that genetic factors might contribute to drug abuse vulnerability. Recent genomic scans for association demonstrated that the brain-derived neurotrophic factor (*BDNF*) gene was associated with drug abuse vulnerability. In this study, we analyzed association of two *BDNF* gene single nucleotide polymorphisms (SNPs), 132C > T (C270T named formerly) in the noncoding region of exon V and 196G > A (val66met) in the coding region of exon XIIIA, with methamphetamine (MAP) abuse in Japan. No significant differences were found in the frequency of the genotype or allele in these two SNPs between MAP abusers and controls (132C > T in exon V: genotype, $P = 0.586$, allele, $P = 0.594$; 196G > A (val66met) in exon XIIIA: genotype, $P = 0.889$, allele, $P = 0.713$). Furthermore, there was no difference between clinical parameters (e.g., prognosis psychosis, spontaneous relapse, or poly-substance abuse) and the two SNPs of *BDNF* gene. These results suggest that the two SNPs (132C > T in exon V and 196G>A (val66met) in exon XIIIA) of the *BDNF* gene may not be associated with Japanese MAP abusers. This article contains supplementary material, which may be viewed at the American Journal of Medical Genetics website at http://www.interscience.wiley.com/jpages/0148-7299:1/suppmat/index.html.

KEY WORDS: brain-derived neurotrophic factor; polymorphism; drug abuse; methamphetamine

INTRODUCTION

Family, twin, and adoption studies suggest that genetic factors are implicated in vulnerability of substance abuse [Merikangas et al., 1998; Kendler, 2001; Tsuang et al., 2001]. The genome-scanning study of poly-substance abuse vulnerability demonstrated that the brain-derived neurotrophic factor (*BDNF*) gene might be one of the strong candidate genes to drug abuse [Uhl et al., 2001]. BDNF is a member of a neurotrophin superfamily mainly expressed within the brain. BDNF interacts with TrkB receptor tyrosine kinase, playing several important roles such as promotion of survival, differentiation, and maintenance of neurons in peripheral nervous system and central nervous system; influences to axonal growth and connectivity; participation in the local responses to various types of neuronal stress or insults [Manji et al., 2003; Mattson et al., 2003]. Furthermore, it also has been reported that the gene encoding BDNF might be an important candidate for susceptibility of neuropsychiatric disorders including bipolar disorder [Neves-Pereira et al., 2002; Sklar et al., 2002; Hashimoto et al., 2004] and schizophrenia [Krebs et al., 2000]. In the studies reporting possible association of BDNF and these disorders, two single nucleotide polymorphisms (SNPs) of *BDNF* gene has been reported. One is 196G > A (val66met) SNP in exon XIIIA (GENBANK: AF411339; at position 95422) located within the propeptide region of BDNF. The A of the ATG-translation initiation codon is denoted nucleotide +1 in exon XIIIA (GENBANK: AF411339; at position 95227). Sklar et al. [2002] reported that BDNF 196G > A (val66met) is significantly associated with bipolar disorder. Interestingly, it has been demonstrated that this SNP (val66met) is strongly suspected to influence human memory and hippocampal function [Egan et al., 2003]. Several lines of evidence demonstrated that methamphetamine (MAP) dependence may cause long-term neural damage in humans, with concomitant deleterious effects on cognitive processes such as memory and attention [Nordahl et al., 2003], suggesting the possible role of BDNF secretion in the memory deficits of MAP abusers. The other SNP frequently analyzed is 132C > T in the noncoding region of exon V (GENBANK: AF411339; at position 53620). This SNP at position 132 of exon V is numbered from the start of exon V (GENBANK: AF411339; at position 53488). It was detected and named C270T by Kunugi et al. [2001] after their searching for a novel nucleotide substitution in the

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noncoding region of the *BDNF* gene reported by Shintani et al. [1992]. It has been reported that the 132C > T in exon V of the *BDNF* gene was significantly associated with late-onset Alzheimer's disease [Kunugi et al., 2001], or schizophrenia [Szekeres et al., 2003]. In addition, it has been reported that the BDNF 196G > A (val66met) is associated with personality traits in healthy subjects [Sen et al., 2003; Itoh et al., 2004], suggesting the role of *BDNF* gene in personality traits and temperament. Considering the role of personality traits in substance use disorders [Howard et al., 1997], it is likely that the *BDNF* gene may be implicated in the vulnerability of drug abuse.

MAP is the most popular abused drug in Japan. Use of MAP induces a strong psychological dependence, and repeated usage frequently results in psychotic states, which symptoms are similar to those of paranoid-type schizophrenia [Sato et al., 1992; Ujike, 2002]. It has been demonstrated that BDNF plays a role in the survival and differentiation of midbrain dopaminergic neurons in vivo [Hyman et al., 1991] and in vitro [Spina et al., 1992], and that chronic BDNF treatment enhances locomotor activity and conditioned reward to cocaine [Hoger et al., 1999]. In addition, it is likely that BDNF could modulate the release of dopamine through the activation of TrkB receptors [Blochl and Sirrenberg, 1996]. Furthermore, it has been reported that locomotor behaviors by amphetamine was increased to a greater degree in the BDNF heterozygous (+/−) knock-out mice, and that striatal dopamine concentrations were significantly higher in the BDNF heterozygous (+/−) knock-out mice [Dluzen et al., 2001]. Moreover, it has been reported recently that pretreatment with intra-nucleus accumbens injection of BDNF antibody or TrkB antibody suppressed significantly the release of dopamine and dopamine-related behaviors induced by administration of MAP, suggesting the implication of BDNF in MAP-induced dopamine release and MAP-induced abnormal behaviors [Narita et al., 2003]. Taken together, it is of interest to study the influences of the *BDNF* gene SNPs in MAP abuse vulnerability. In this study, we analyzed the frequency of two known SNPs (196G > A (val66met) in exon XIIIA and 132C > T in exon V) of *BDNF* gene between MAP abusers and healthy subjects in Japan.

MATERIALS AND METHODS

Subjects and Samples

This study was performed after obtaining the approval of the ethics committees of each affiliated institute of the Japanese Genetics Initiative for Drug Abuse (JGIDA). All subjects provided written informed consent for the use of their DNA samples for this study. The subjects were 189 patients (150 males and 39 female; age, 36.6 ± 11.9 (mean ± SD)) with MAP dependence and a psychotic disorder meeting the ICD-10-DCR criteria (F15.2 and F15.5) who were outpatients or inpatients of psychiatric hospitals of the JGIDA. Two hundred and two volunteers were recruited as healthy controls. All controls have no significant lifetime history of use of any addictive substance (158 males and 44 females; 37.2 ± 10.6 (mean ± SD)), the majority of whom were medical staff with no past history and no family history of drug dependence or psychotic disorders. Diagnoses were made by two trained psychiatrists by interview and available information including hospital records. Detailed characteristics of patients were shown in the Supplement 1 (see the online Supplement 1 at http://www.interscience.wiley.com/jpages/0148-7299:1/suppmat/index.html).

Genotyping

The genomic DNA was extracted from peripheral leukocytes by standard procedures. Polymerase chain reaction (PCR) and the PCR-based restriction fragment length polymorphism (RFLP) assay were performed to genotype the DNA sequence variants of the *BDNF* gene. Detailed methods for genotyping were shown in the Supplement 2 (see the online Supplement 2 at http://www.interscience.wiley.com/jpages/0148-7299:1/suppmat/index.html).

Statistical Analysis

Fisher's exact test was used for categorical comparisons, and Student's *t*-test was employed for age difference. Significance for the results was set at $P < 0.05$.

RESULTS

Both the genotype and allele frequencies for the patients and controls are shown in Tables I and II. The genotype distribution for patients groups and control groups did not deviate significantly from the Hardy–Weinberg equilibrium. No significant differences were found in the frequency of the genotype or allele in these two SNPs between patients and controls (132C > T in exon V: genotype, $P = 0.586$, allele, $P = 0.594$; 196G > A (val66met) in exon XIIIA: genotype, $P = 0.889$, allele, $P = 0.713$). As for the 132C > T substitution, there was no individual who was homozygous for the 132T allele in exon V. Within patients, we analyzed the effects of prognosis psychosis (transient or prolonged), spontaneous relapse (positive or negative), and poly-substance abuse (yes or no) on the *BDNF* gene SNPs (132C > T in exon V and 196G > A in exon XIIIA). The genotypic and allelic distribution of two SNPs was not

TABLE I. Genotype and Allele Frequencies of the Brain-Derived Neurotrophic Factor (BDNF) 132C > T (in Exon V) Gene Polymorphism of in Controls and Methamphetamine (MAP) Abusers

		Genotype				Allele		
132C > T	n	CC	CT	TT	*P*	C	T	*P*
Control	202	183 (90.6%)	19 (9.4%)	0 (0%)		385 (95.3%)	19 (4.7%)	
Abuser	189	175 (92.6%)	14 (7.4%)	0 (0%)	0.586	364 (96.3%)	14 (3.7%)	0.594
Prognosis of psychosis								
Transient	94	87 (92.6%)	7 (7.4%)	0 (0%)	0.664	181 (96.3%)	7 (3.7%)	0.671
Prolonged	66	62 (93.9%)	4 (6.1%)	0 (0%)	0.612	128 (97.0%)	4 (3.0%)	0.620
Spontaneous relapse								
Positive	64	60 (93.8%)	4 (6.3%)	0 (0%)	0.611	124 (96.9%)	4 (3.1%)	0.619
Negative	116	107 (92.2%)	9 (7.8%)	0 (0%)	0.685	223 (96.1%)	9 (3.9%)	0.692
Poly-substance abuse								
No	56	51 (91.1%)	5 (8.9%)	0 (0%)	1	107 (95.5%)	5 (4.5%)	1
Yes	122	114 (93.4%)	8 (6.6%)	0 (0%)	0.414	236 (96.7%)	8 (3.3%)	0.424

Statistical analysis was performed by a Fisher's exact test (vs. control).

TABLE II. Genotype and Allele Frequencies of the BDNF 196G > A (val66met) (in Exon XIIIA) Gene Polymorphism in Controls and MAP Abusers

		Genotype				Allele		
196G > A (val66met)	n	GG	GA	AA	*P*	G	A	*P*
Control	202	70 (34.7%)	107 (53.0%)	25 (12.4%)		247 (61.1%)	157 (38.9%)	
Abuser	189	70 (37.0%)	96 (50.8%)	23 (12.2%)	0.889	236 (62.4%)	142 (37.6%)	0.713
Prognosis of psychosis								
Transient	94	32 (34.0%)	53 (56.4%)	9 (9.6%)	0.778	117 (62.2%)	71 (37.8%)	0.856
Prolonged	66	25 (37.9%)	30 (45.5%)	11 (16.7%)	0.472	80 (60.6%)	52 (39.4%)	0.918
Spontaneous relapse								
Positive	64	27 (42.2%)	30 (46.9%)	7 (10.9%)	0.571	84 (65.6%)	44 (34.4%)	0.403
Negative	116	39 (33.6%)	62 (53.4%)	15 (12.9%)	0.972	140 (60.3%)	92 (39.7%)	0.866
Poly-substance abuse								
No	56	21 (37.5%)	27 (48.2%)	8 (14.3%)	0.791	69 (61.6%)	43 (38.4%)	1
Yes	122	47 (38.5%)	60 (49.2%)	15 (12.3%)	0.762	154 (63.1%)	90 (36.9%)	0.676

Statistical analysis was performed by a Fisher's exact test (vs. control).

significantly different between transient type of psychosis and prolonged type of psychosis (Tables I and II). Furthermore, the genotypic and allelic distribution of two SNPs was not significantly different between positive spontaneous relapse and negative spontaneous relapse (Tables I and II). Moreover, the genotypic and allelic distribution of two SNPs was not significantly different between poly-substance abuse and non-poly-substance abuse (Tables I and II). In addition, we found that two SNPs were not in linkage disequilibrium with each other.

DISCUSSION

The present study suggests that two SNPs (132C > T in exon V and 196G > A (val66met) in exon XIIIA) of the *BDNF* gene may not be susceptible to MAP abuse in Japanese samples. Using a European American sample and an African American sample, it has been reported that the *BDNF* gene could contribute to vulnerabilities to poly-substance abuse [Uhl et al., 2001]. It is possible that difference in ethnicity might contribute to discrepancy between our study and other study. Frequency of A allele of 196G > A (val66met) in Japanese population [Momose et al., 2002; Nakata et al., 2003; Itoh et al., 2004; this study] is higher than that of Caucasian population [Egan et al., 2003; Hakansson et al., 2003; Sen et al., 2003], suggesting the ethnic difference in this SNP (val66met) [Shimizu et al., 2004]. First, it has been reported that the 196G > A (val66met) of the *BDNF* gene is associated with Parkinson's disease in Japanese subjects [Momose et al., 2002]. However, lack of association between the BDNF 196G > A (val66met) and Parkinson's disease in a Swedish population was reported [Hakansson et al., 2003]. Second, it has been reported that the 196G > A (val66met) of the *BDNF* gene is associated with bipolar disorder in Caucasian [Neves-Pereira et al., 2002; Sklar et al., 2002]. However, no association between 196G > A (val66met) of the *BDNF* gene and bipolar disorder in Japanese population was detected [Nakata et al., 2003], suggesting that the *BDNF* gene may confer a susceptibility to bipolar disorder in Caucasian, but not in Japanese population. Thus, it is likely that ethnic differences may contribute to inconsistent findings between Caucasian sample and Japanese sample.

In this study, we investigated two SNPs; one (132C > T in exon V) in the noncoding region and the other (196G > A (val66met) in exon XIIIA) in the coding region. Whereas BDNF 196G > A (val66met) SNP does not affect the function of a mature BDNF protein, it has been shown to dramatically alter the intracellular trafficking and packaging of pro-BDNF, and, thus, the regulated secretion of the mature BDNF protein [Egan et al., 2003]. At cellular levels, marked deficits were observed in the intracellular distribution, processing, and secretion of met-BDNF, suggesting that pro-BDNF may play a critical role in synaptic targeting and activity-dependent secretion at synapses [Egan et al., 2003]. Remarkably, healthy human subjects with the met allele exhibit impaired hippocampal activity and memory function [Egan et al., 2003]. However, it is currently unknown whether the BDNF 132C > T SNP could affect on the function, synthesis, or secretion of BDNF. There are still other known SNPs in the *BDNF* gene sequences, and it is possible that there are more unknown SNPs. Further studies of other SNPs and unknown SNPs should be done to clarify the involvement of the *BDNF* gene in substance abuse vulnerability.

In conclusion, we failed to detect evidence for a role of two SNPs (196G > A (val66met) in exon XIIIA and 132C > T in exon V) of the *BDNF* gene in the pathogenesis of MAP abusers in our Japanese sample. Therefore, it is unlikely that the two SNPs (196G > A (val66met) in exon XIIIA and 132C > T in exon V) of *BDNF* gene are associated with Japanese MAP abusers.

REFERENCES

Blochl A, Sirrenberg C. 1996. Neurotrophins stimulate the release of dopamine from rat mesencephalic neurons via Trk and $p75^{Lntr}$ receptors. J Biol Chem 271:21100–21107.

Dluzen DE, Gao X, Story GM, Anderson LI, Kucera J, Walro JM. 2001. Evaluation of nigrostriatal dopaminergic function in adult +/+ and +/− BDNF mutant mice. Exp Neurol 170:121–128.

Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR. 2003. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112:257–269.

Hakansson A, Melke J, Westberg L, Shahabi HN, Buervenich S, Carmine A, Klingborg K, Grundell MB, Schulhof B, Holmberg B, Ahlberg J, Eriksson E, Sydow O, Olson L, Johnels B, Nissbrandt H. 2003. Lack of association between the BDNF Val66Met polymorphism and Parkinson's disease in a Swedish population. Ann Neurol 53:823.

Hashimoto K, Shimizu E, Iyo M. 2004. Critical role of brain-derived neurotrophic factor in mood disorders. Brain Res Rev 45:104–114.

Hoger BA, Iyasere CA, Berhow MT, Messer CJ, Nestler EJ, Taylor JR. 1999. Enhancement of locomotor activity and conditioned reward to cocaine by brain-derived neurotrophic factor. J Neurosci 19:4110–4122.

Howard MO, Kivlahan D, Walker RD. 1997. Cloninger's tridimensional theory of personality and psychopathology: Applications to substance use disorders. J Stud Alcohol 58:48–66.

Hyman C, Hofer M, Barde YA, Jahasz M, Yancopoulos GD, Squinto SP, Lindsay RM. 1991. BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. Nature 350:230–232.

Itoh K, Hashimoto K, Kumakiri C, Shimizu E, Iyo M. 2004. Association between brain-derived neurotrophic factor 196 G/A polymorphism and

personality traits in healthy subjects. Am J Med Genet (Neuropsychiatr Genet) 124B:61–63.

Kendler KS. 2001. Twin studies of psychiatric illness: An update. Arch Gen Psychiatry 58:1005–1014.

Krebs MO, Guillin O, Bourdell MC, Schwartz JC, Olie JP, Poirier MF, Sokoloff P. 2000. Brain derived neurotrophic factor (*BDNF*) gene variants association with age at onset and therapeutic response in schizophrenia. Mol Psychiatry 5:558–562.

Kunugi H, Ueki A, Otsuka M, Isse K, Hirasawa H, Kato N, Nabika T, Kobayashi S, Nanko S. 2001. A novel polymorphism of the brain-derived neurotrophic factor (*BDNF*) gene associated with late-onset Alzheimer's disease. Mol Psychiatry 6:83–86.

Manji HK, Quiroz JA, Sporn J, Payne JL, Denicoff KA, Gray N, Zarate CA Jr, Charney DS. 2003. Enhancing neuronal plasticity and cellular resilience to develop novel, improved therapeutics for difficult-to-treat depression. Biol Psychiatry 53:707–842.

Mattson MP, Duan W, Guo Z. 2003. Meal size and frequency affect neuronal plasticity and vulnerability to disease: Cellular and molecular mechanisms. J Neurochem 84:417–431.

Merikangas KR, Stolar M, Stevens DE, Goulet J, Preisig MA, Fenton B, Zhang H, O'Malley SS, Rounsaville BJ. 1998. Familial transmission of substance use disorders. Arch Gen Psychiatry 55:973–979.

Momose Y, Murata M, Kobayashi K, Tachikawa M, Nakabayashi Y, Kanazawa I, Toda T. 2002. Association studies of multiple candidate genes for Parkinson's disease using single nucleotide polymorphisms. Ann Neurol 51:133–136.

Nakata K, Ujike H, Sakai A, Uchida N, Nomura A, Imamura T, Katsu T, Tanaka Y, Hamamura T, Kuroda S. 2003. Association study of the brain-derived neurotrophic factor (*BDNF*) gene with bipolar disorder. Neurosci Lett 337:17–20.

Narita M, Aoki K, Takagi M, Yajima Y, Suzuki T. 2003. Implication of brain-derived neurotrophic factor in the release of dopamine and dopamine-related behaviors induced by methamphetamine. Neuroscience 119:767–775.

Neves-Pereira M, Mundo E, Muglia P, King N, Macciardi F, Kennedy JL. 2002. The brain-derived neurotrophic factor gene confers susceptibility to bipolar disorder: Evidence from a family-based association study. Am J Hum Genet 71:651–655.

Nordahl TE, Salo R, Leamon M. 2003. Neuropsychological effects of chronic methamphetamine use on neurotransmitters and cognition: A review. J Neuropsychiatry Clin Neurosci 15:317–325.

Sato M, Numachi Y, Hamamura T. 1992. Relapse of paranoid psychotic state in methamphetamine model of schizophrenia. Schizophr Bull 18:115–122.

Sen S, Nesse RM, Stoltenberg SF, Li S, Gleiberman L, Chakravarti A, Weder AB, Burmeister M. 2003. A BDNF coding variant is associated with the NEO personality inventory domain neuroticism, a risk factor for depression. Neuropsychopharmacology 28:397–401.

Shimizu E, Hashimoto K, Iyo M. 2004. Ethnic difference of the BDNF 196G/A (val66met) polymorphism frequencies: The possibility to explain ethnic mental traits. Am J Med Genet (Neuropsychiatr Genet) 126B: 122–123.

Shintani A, Ono Y, Kaisho Y, Igarashi K. 1992. Characterization of the 5'-flanking region of the human brain-derived neurotrophic factor gene. Biochem Biophys Res Commun 182:325–332.

Sklar P, Gabriel SB, McInnis MG, Bennett P, Lim YM, Tsan G, Schaffner S, Kirov G, Jones I, Owen M, Craddock N, DePaulo JR, Lander ES. 2002. Family-based association study of 76 candidate genes in bipolar disorder: BDNF is a potential risk locus. Brain-derived neutrophic factor. Mol Psychiatry 7:579–593.

Spina MB, Squinto SP, Miller J, Lindsay RM, Hyman C. 1992. Brain-derived neurotrophic factor protects dopamine neurons against 6-hydroxydopamine and *N*-methyl-4-phenyl-pyridinium ion toxicity: Involvement of the glutathione system. J Neurochem 59:99–106.

Szekeres G, Juhasz A, Rimanoczy A, Keri S, Janka Z. 2003. The C270T polymorphism of the brain-derived neurotrophic factor gene is associated with schizophrenia. Schizophr Res 65:15–18.

Tsuang MT, Bar JL, Harley RM, Lyons MJ. 2001. The Harvard twin study of substance abuse: What we have learned. Harv Rev Psychiatry 9:267–279.

Uhl GR, Liu QR, Walther D, Hess J, Naiman D. 2001. Polysubstance abuse-vulnerability genes: Genome scans for association, using 1,004 subjects and 1,494 single-nucleotide polymorphisms. Am J Hum Genet 69:1290–1300.

Ujike H. 2002. Stimulant-induced psychosis and schizophrenia: The role of sensitization. Curr Psychiatry Rep 4:177–184.

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]				Allele[a]		
	GC/GC	GC/TT	TT/TT	*P* Value[b]	GC	TT	*P* Value
(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