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**Association between the Glutathione S-transferase M1 gene deletion
and female methamphetamine abusers**

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Abstract

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. 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 and 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 (Volkow et al., 2001; Sekine 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 subsequently 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 et al., 1998). DA oxidation also results in the formation of DA *o*-quinone, which readily participates in nucleophilic addition reactions with sulfhydryl groups on free cysteine, glutathione, or cysteine found in protein including DA transporter (Graham et al., 1978; Hastings and Zigmond, 1994; Smythies et al., 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 et al., 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.

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 \pm SD), age range: 19-69 years; and 32 females, age: 28 ± 5 years (mean \pm 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 \pm SD), age range: 19-69 years; and 43 females, age: 36 ± 10 years (mean \pm 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, Tyukyuu, 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; 2001b). 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'-ACAGAGGAAGGGTGCATTTGATA-3', reverse: 5'-GACATTCATTCCCAAAGCGACCA-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 1. 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 dopamine 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, 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, 1999). In studies using rats, estrogen has been revealed to enhance the behavioral and neurochemical responses to MAP by increasing stimulated DA release (Becker et al., 1999). Furthermore, recent studies using brain imaging technique revealed that women have higher levels of DA transporters (Mozley et al., 2001) and lower DA D₂ 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.

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Table 1 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*	

Note. D, deletion allele ; N, non-deletion allele. *P<0.05