Association between GSTM1 and GSTT1 polymorphisms and susceptibility to methamphetamine dependence

Mohammad Rashid Khalighinasab, Khyber Saify, Mostafa Saadat*

Department of Biology, College of Sciences, Shiraz University, Shiraz 71454, Iran

ABSTRACT

Glutathione S-transferases (GSTs; EC: 2.5.1.18) are ubiquitous multifunctional enzymes, which play a key role in cellular detoxification. Functional genetic polymorphisms in genes encoding GSTM1 (a member of GST class mu; OMIM: 138350), and GSTT1 (a member of GST class theta; OMIM: 600436) have been well defined. The functional null alleles of GSTM1 and GSTT1 represent deletions of GSTM1 and GSTT1 genes, respectively. The aim of the present study is to investigate the association between GSTM1 and GSTT1 polymorphisms and methamphetamine dependence. The present population-based case-control study was performed in Shiraz (southern Iran). In total, 52 methamphetamine dependence (11 females, 41 males) and 635 healthy controls (110 females, 525 males) were included in this study. The genotypes of GSTM1 and GSTT1 polymorphisms were determined by PCR. Neither GSTM1 (OR=0.92, 95% CI: 0.52-1.61, P=0.771) nor GSTT1 (OR=0.71, 95% CI: 0.33-1.54, P=0.381) null genotypes were significantly associated with risk of methamphetamine dependence. It should be noted that although there was no association between the GSTM1 null genotype and risk of methamphetamine dependence, in both genders, there was significant interaction between gender and GSTM1 polymorphism (P=0.029). The combination genotypes of the GSTM1 and GSTT1 polymorphisms revealed that the genotypes of these two polymorphisms had no additive effect in relation to the susceptibility to methamphetamine dependence. The present study revealed that genetic polymorphisms of GSTT1 and GSTM1 are not risk factors for methamphetamine dependence.

Key words: GSTM1; GSTT1; Methamphetamine dependence; Polymorphism

INTRODUCTION

Methamphetamine is now one of the major illicit drugs available worldwide [1]. A better understanding of the etiology of methamphetamine dependence is crucial for
improving the prevention and treatment of this severe type of drug dependence. Generally it has been well established that drug-dependence disorders are genetically influenced [2]. Several studies have been revealed that methamphetamine induced the oxidative stress [3-9]. Therefore, methamphetamine abuse results in numerous adverse health effects which is associated with oxidative stress, including myocardial infarction, cognitive deficits, and psychiatric disease.

Glutathione S-transferases (GSTs; EC: 2.5.1.18) are ubiquitous multifunctional enzymes, which play a key role in cellular detoxification. Human GSTs are divided into different classes; including mu and theta. Genetic polymorphisms in genes encoding GSTM1 (a member of GST class mu; OMIM: 138350), and GSTT1 (a member of GST class theta; OMIM: 600436) have been well defined [10, 11]. The functional null alleles of GSTM1 and GSTT1 represent deletions of GSTM1 and GSTT1 genes, respectively. The association studies between these functional polymorphisms and various multifactorial traits such as several types of cancers [10, 12-16], schizophrenia [17, 18], bipolar disease [19], asthma [20, 21], cataract [22, 23], and cardiovascular diseases [24] were conducted. Immunoblot analysis revealed that GSTT1 and GSTM1 were present in brain [25, 26]. There were two studies investigating the association between GSTM1 and GSTT1 polymorphisms and risk of methamphetamine dependence [27, 28], with inconsistent results. These facts sufficiently provide us with a theoretical rational to do the present study. The main aim of the present study is to investigate the association between these polymorphisms and susceptibility to methamphetamine abuse.

**MATERIALS AND METHODS**

**Participants:** The present study was performed in Shiraz (Fars province, southern Iran). In total, 52 methamphetamine dependence (11 females, 41 males) and 635 healthy controls (110 females, 525 males) were included in this study. The patients were in methadone maintenance for treating methamphetamine dependence and all of them reported methamphetamine as their primary drug of choice. Control individuals were blood donors, who declared that they did not suffer substance abuse. The mean age (SD) of the patients and the controls were 35.0 (8.5) and 33.6 (9.0) years, respectively. There was no statistically significant difference with regard to age (t=1.06, df=685, P=0.288) between the patients and the controls. There was no significant difference between the two study groups for their gender distribution (χ²=0.48, df=1, P=0.486). Considering the high heterogeneity of the Iranian population [29, 30], the participants were selected from Persian Muslims (Caucasians) living in Shiraz (Fars province, southern Iran). Informed consent was obtained from each subject before the study, which was approved by the institutional review board of our university.

At the time of blood donation, a brief questionnaire that ascertained age, dependency to any drug, age at first time used drug, marital status, history of cancers, cataract, and asthma, and history of drug dependency in the first degree relatives was completed. Considering that the polymorphisms of GSTT1 and GSTM1 are associated
with several types of cancers, asthma, and cataract, the subjects of the both groups had negative history of cancers, asthma, and cataract.

**Genotyping:** Peripheral blood samples were collected from the participants. Genomic DNA was isolated from EDTA treated blood samples. The PCR conditions for determining the genotypes of GSTT1 and GSTM1 polymorphisms were the same as that reported previously [14]. Successful amplification with β-globin specific primers confirmed the proper function of the PCR reaction. To test for contamination, negative controls (tubes containing the PCR mixture, without the DNA template) were incubated in every run. Any sample with ambiguous result due to low yield was retested and a random selection of 15% of all samples was repeated. No discrepancies were discovered upon replicate testing.

**Statistical analysis:** The association between the genotypes of the study polymorphisms and methamphetamine dependence risk were assessed by calculating odds ratios (ORs) and 95% confidence intervals (CIs). The reference group consisted of individuals with positive genotypes of GSTM1 and GSTT1. A probability of p<0.05 was considered as statistically significant.

Using the GPOWER (wwwpsychouni-duesseldorfde/aap/projects/gpower) software (version 3.1.3), to detect a real difference in allelic frequency with a power of 0.95, α=0.05, df=1, Lambda=13.0, and an effect size of 0.2 (small-medium effect); a minimum sample of 325 would be necessary. The present study is more than sufficiently powered with an N=687 to detect a small-medium effect in genotype frequency between the two groups.

**RESULTS AND DISCUSSION**

Table 1 shows the genotypic prevalence of the study polymorphisms between the cases and healthy controls. The prevalence of GSTM1 null genotype was 51.9 and 54.0 percent among patients and controls, respectively. Statistical analysis revealed that the null genotype of GSTM1 was not associated with the risk of methamphetamine abuse (OR=0.92, 95% CI: 0.52-1.61, P=0.771). After stratification of the participants according their genders, we observed the same finding. It should be noted that although there was no association between the GSTM1 null genotype and risk of methamphetamine dependence, in both genders, there was significant interaction between gender and GSTM1 polymorphism (P=0.029).

The frequency of null genotype of GSTT1 was 15.4 and 20.5 percent in patient and control groups, respectively. Statistical analysis revealed that polymorphism of GSTT1 was no statistically associated with susceptibility to methamphetamine dependence (OR=0.71, 95% CI: 0.33-1.54, P=0.381) (Table 1). After stratification of the participants according to their genders, the same result was observed.
Table 1: Association between genetic polymorphisms of \textit{GSTM1} and \textit{GSTT1} and risk of methamphetamine dependence

| Genotypes | Patients | Controls | OR* | 95% CI     | P    |
|-----------|---------|----------|-----|------------|------|
| **Both Genders** |         |          |     |            |      |
| Positive  | 25      | 292      | 1.0 | -          | -    |
| Null      | 27      | 343      | 0.92| 0.52-1.61  | 0.771|
| **Males** |         |          |     |            |      |
| Positive  | 24      | 246      | 1.0 | -          | -    |
| Null      | 17      | 279      | 0.62| 0.321.19   | 0.152|
| **Females** |        |          |     |            |      |
| Positive  | 1       | 46       | 1.0 | -          | -    |
| Null      | 10      | 64       | 7.18| 0.89-58.1  | 0.064|
| **Both Genders** |         |          |     |            |      |
| Positive  | 44      | 505      | 1.0 | -          | -    |
| Null      | 8       | 130      | 0.71| 0.33-1.54  | 0.381|
| **Males** |         |          |     |            |      |
| Positive  | 35      | 411      | 1.0 | -          | -    |
| Null      | 6       | 114      | 0.61| 0.25-1.50  | 0.290|
| **Females** |        |          |     |            |      |
| Positive  | 9       | 94       | 1.0 | -          | -    |
| Null      | 2       | 16       | 1.30| 0.25-6.60  | 0.747|

To investigate whether one null genotype could be compensated by an active genotype for the other isoenzymes in relation to substance abuse, we considered the association between combinations of the genotypes and risk of methamphetamine dependency. The reference group consisted of individuals with “positive genotypes of \textit{GSTM1} and \textit{GSTT1}”. In overall (and also in males), there was no significant association between combined genotypes and susceptibility to methamphetamine abuse (Table 2). There was no linear trend in risk associated with zero, one and two null genotypes ($\chi^2=0.59; P=0.441$).

In the present case-control study, we found that there was no statistically significant association between \textit{GSTM1} polymorphism and risk of methamphetamine dependence. Previously, only one study investigated the association between \textit{GSTM1} polymorphism and susceptibility to methamphetamine abuse in Japan [27]. They reported that the risk of methamphetamine dependence associated with \textit{GSTM1} null genotype was significantly higher only in females than in subjects with the \textit{GSTM1} genotype. Considering that we found that there was significant interaction between gender and \textit{GSTM1} polymorphism, our present findings were partially consistent with that report.
However, this discrepancy might be at least in part interpreted by our small sample size of female cases.

### Table 2: Associations between combination genotypes of polymorphisms of \textit{GSTM1} and \textit{GSTT1} and risk of methamphetamine dependence

| Combinations | Patients | Controls | OR* | 95% CI   | P       |
|---------------|----------|----------|-----|----------|---------|
| **Both genders** |          |          |     |          |         |
| \textit{GSTM1} | \textit{GSTT1} |          |     |          |         |
| Positive Positive | 20 | 236 | 1.0 | - | - |
| Positive Null | 5 | 56 | 1.05 | 0.38-2.93 | 0.920 |
| Null Positive | 24 | 269 | 1.05 | 0.57-1.95 | 0.871 |
| Null Null | 3 | 74 | 0.48 | 0.14-1.66 | 0.244 |
| **Males** |          |          |     |          |         |
| \textit{GSTM1} | \textit{GSTT1} |          |     |          |         |
| Positive Positive | 20 | 195 | 1.0 | - | - |
| Positive Null | 4 | 51 | 0.76 | 0.25-2.33 | 0.765 |
| Null Positive | 15 | 216 | 0.67 | 0.33-1.35 | 0.273 |
| Null Null | 2 | 63 | 0.31 | 0.07-1.36 | 0.121 |

There were two published studies investigating the association between \textit{GSTT1} polymorphism and risk of methamphetamine dependence [27, 28]. Our present finding (no significant association between null genotype of \textit{GSTT1} and susceptibility to methamphetamine dependence) was in agreement with one of them [28].

It is well established that the GSTs are involved in detoxification of a variety of compounds, some of which overlap between these enzymes and some of which are highly specific [31]. Previous studies showed that the null genotypes of \textit{GSTM1} and \textit{GSTT1} polymorphisms may have additive effect on the risk of multifactorial traits [14, 20]. However, we found that combinations of \textit{GSTT1} and \textit{GSTM1} polymorphisms are not associated with risk of methamphetamine dependence (Table 2). This finding is not consistent with one of the previous published study [27].

We stratified our participants by gender, which reduced sample sizes especially for females; therefore the present analyses on females may have been statistically underpowered. Considering the fact that ethnicity may influence the observed associations in multifactorial diseases, differences between our ethnicity and Japanese ethnicity might be involved. In order to address the involvement of the polymorphisms of \textit{GSTT1} and \textit{GSTM1} on susceptibility to methamphetamine abuse replication of this study in other countries is recommended.

**Acknowledgements:** The authors are indebted to the participants for their close cooperation. This study was supported by Shiraz University.

**Conflicts of Interest:** No competing interests are declared by any of the authors.

http://mbrc.shirazu.ac.ir
REFERENCES

1. Singleton J, Degenhardt L, Hall W, Zabransky T. Mortality among amphetamine users: A systematic review of cohort studies. Drug Alcohol Depend 2009;105:1-8.
2. Gelernter J, Kranzler HR. Genetics of drug dependence. Dialogues Clin Neurosci 2010;12:77-84.
3. Solhi H, Malekird A, Kazemifar AM, Sharifi F. Oxidative stress and lipid peroxidation in prolonged users of methamphetamine. Drug Metab Lett 2014;7:79-82.
4. Ghazavi A, Mosayebi G, Solhi H, Rafiei M, Moazzeni SM. Serum markers of inflammation and oxidative stress in chronic opium (Taryak) smokers. Immunol Lett 2013;153:22-26.
5. Tata DA, Yamamoto BK. Interactions between methamphetamine and environmental stress: role of oxidative stress, glutamate and mitochondrial dysfunction. Addiction 2007;102 Suppl 1:49-60.
6. Brown JM, Yamamoto BK. Effects of amphetamines on mitochondrial function: role of free radicals and oxidative stress. Pharmacol Therapeut 2003;99:45-53.
7. Castro AA, Moretti M, Casagrande TS, Martinello C, Petronilho F, Steckert AV, Guerrini R, Calo G, Dal Pizzol F, Quevedo J, Gavioli EC. Neuropeptide S produces hyperlocomotion and prevents oxidative stress damage in the mouse brain: A comparative study with amphetamine and diazepam. Pharmacol Biochem Behav 2009;91:636-642.
8. Shin EJ, Duong CX, Nguyen XKT, Li Z, Bing G, Bach JH, Park DH, Nakayama K, Ali SF, Kanthasamy AG, Cadet JL, Nabeshima T, Kim HC. Role of oxidative stress in methamphetamine-induced dopaminergic toxicity mediated by protein kinase. Behav Brain Res 2012;232:98-113.
9. Perfeito R, Olivei TC, Rego AC. Revisiting oxidative stress and mitochondrial dysfunction in the pathogenesis of Parkinson disease, resemblance to the effect of amphetamine drugs of abuse. Free Radic Biol Med 2012;53:1791-1806.
10. Harada S, Misawa S, Nakamura T, Tanaka N, Ueno E, Nozoe M. Detection of GST1 gene deletion by the polymerase chain reaction and its possible correlation with stomach cancer in Japanese. Hum Genet 1992;90:62-64.
11. Pemble S, Schroeder KR, Spencer SR, Meyer DJ, Hallier E, Bolt HM, Ketterer B, Taylor JB. Human glutathione S-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. Biochem J 1994;300:271-276.
12. Kelada SN, Kardia SL, Walker AH, Wein AJ, Malkowicz SB, Rebbeck TR. The glutathione S-transferase-mu and -theta genotypes in the etiology of prostate cancer: genotype-environmental interaction with smoking. Cancer Epidemiol Biomarkers Prev 2000;9:1329-1334.
13. Saadat I, Saadat M. The glutathione S-transferase mu polymorphism and susceptibility to acute lymphocytic leukemia. Cancer Lett 2000;158:43-45.
14. Saadat I, Saadat M. Glutathione S-transferase M1 and T1 null genotypes and the risk of gastric and colorectal cancers. Cancer Lett 2001;169:21-26.
15. Hashibe M, Brennan P, Strange RC, Bhisey R, Cascorbi I, Lazarus P, Oude Ophuis MB, Benhamou S, Foulkes WD, Katoh T, Coutelle C, Romkes M, Gaspari L, Taioli E, Boffetta P. Meta- and pooled analyses of GSTM1, GSTT1, GSTP1 and CYP1A1 genotypes and risk of head and neck cancer. Cancer Epidemiol Biomarkers Prev 2003;12:1509-1517.

16. Abbas A, Delvinquiere K, Lechevrel M, Lebailly P, Gauduchon P, Launoy G, Sichel F. GSTM1, GSTT1, GSTP1 and CYP1A1 genetic polymorphisms and susceptibility to esophageal cancer in a French population: Different pattern of squamous cell carcinoma and adenocarcinoma. World J Gastroenterol 2004;10: 3389-3393.

17. Saadat M, Mobayen F, Farrashbandi H. Genetic polymorphism of glutathione S-transferase T1: A candidate genetic modifier of individual susceptibility to schizophrenia. Psychiatry Res 2007;153:87-91.

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

19. Mohammadnejad P, Saadat I, Ghanizadeh A, Saadat M. Bipolar disorder and polymorphisms of glutathione S-transferases M1 (GSTM1) and T1 (GSTT1). Psychiatry Res 2011;186:144-146.

20. Saadat M, Saadat I, Saboori Z, Emad A. Combination of CC16, GSTM1 and GSTT1 polymorphisms is associated with asthma. J Allergy Clin Immunol 2004;113:996-998.

21. Liang S, Wei X, Gong C, Wei J, Chen Z, Chen X, Wang Z, Deng J. Significant association between asthma risk and the GSTM1 and GSTT1 deletion polymorphisms: an updated meta-analysis of case-control studies. Respirology 2013;18:774-783.

22. Saadat M, Farvardin-Jahromi M, Saadat H. Null genotype of glutathione S-transferase M1 is associated with senile cataract susceptibility in non-smoker females. Biochem Biophys Res Commun 2004;319:1287-1291.

23. Saadat M, Farvardine-Jahromi M. Polymorphism of glutathione S-transferase M1, occupational exposure to sunlight, and senile cataract risk. Occup Environ Med 2006;63:503-504.

24. Wilson MH, Grant PJ, Hardie LJ, Wild CP. Glutathione S-transferase M1 null genotype is associated with a decreased risk of myocardial infarction. FASEB J 2000;14:791-796.

25. Juronen E, Tasa G, Uuskula M, Pooga M, Mikelsaar AV. Purification, characterization and tissue distribution of human class theta glutathione S-transferase T1-1. Biochem Mol Biol Int 1996;39:21-29.

26. Listowsky I, Rowe JD, Patkovsky YV, Tchaikovskaya T, Shintani N, Novikova E, Nieves E. Human testicular glutathione S-transferases: insights into tissue-specific expression of the diverse subunit classes. Chemico-Biol Interact 1998;111-112:103-112.

http://mbrc.shirazu.ac.ir
27. Nakatome M, Miyaji A, Mochizuki K, Kishi Y, Isobe I, Matoba R. Association between the GST genetic polymorphisms and methamphetamine abusers in the Japanese population. Leg Med (Tokyo) 2009;11 Suppl 1:S468-470.

28. Koizumi H, Hashimoto K, Kumakiri C, Shimizu E, Sekine Y, Ozaki N, Inada T, Harano M, Komiyama T, Yamada M, Sora I, Ujike H, Takei N, Iyo M. Association between the glutathione S-transferase M1 gene deletion and female methamphetamine abusers. Am J Med Genet B Neuropsychiatr Genet 2004;126B: 43-45.

29. Rafiee L, Saadat I, Saadat M. Glutathione S-transferase genetic polymorphisms (GSTM1, GSTT1 and GSTO2) in three Iranian populations. Mole Biol Rep 2010;37: 155-158.

30. Saadat M, Saadat I. Prevalence of G6721T polymorphism of XRCC7 in an Iranian population. EXCLI Journal 2012;11:93-97.

31. Hayes JD, Strange RC. Glutathione S-transferase polymorphisms and their biological consequences. Pharmacology 2002;61:154-166.