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No association between *GSTM1* and *GSTT1* genetic polymorphisms and susceptibility to opium sap dependence

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ABSTRACT

Glutathione S-transferases (GSTs; EC: 2.5.1.18) are a ubiquitous family of eukaryotic and prokaryotic phase II metabolic isozymes. Genes encoding *GSTM1* (OMIM: 138350), and *GSTT1* (OMIM: 600436) are members of class mu and theta, respectively. The most common polymorphism in the *GSTM1* is a deletion of the whole *GSTM1* gene with a lack of enzyme activity. A homozygous deletion in the *GSTT1* has also been reported (null genotypes of GSTT1). The aim of the present study was to investigate the association between *GSTM1* and *GSTT1* polymorphisms and risk of dependency to opium sap. The present study was performed in Shiraz (southern Iran). In total, 71 males dependent to opium sap and 590 healthy males (as a control group) were included in this study. The genotypes of *GSTM1* and *GSTT1* polymorphisms were determined by PCR. Our data indicate that neither *GSTM1* (OR=0.78, 95% CI: 0.47-1.27, P=0.325) nor *GSTT1* (OR=1.25, 95% CI: 0.70-2.21, P=0.442) null genotypes significantly associated with the risk of opium sap dependence. There is no additive effect of the null genotypes of *GSTT1* and *GSTM1* in relation to the risk of dependency to opium sap. The present study indicated that the null genotypes of *GSTT1* and *GSTM1* are not risk factor for opium sap dependence.

Keywords: *GSTM1*; *GSTT1*; Opium sap dependence; Polymorphism

INTRODUCTION

Family, adoption and twin studies have been clearly demonstrated that genetic factors are important in moderating vulnerability to drug-dependent disorders [1-4].

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Genetic influences account for 30 to 70% of addiction vulnerability [2]. Opium sap is now one of the illicit drugs used in Iran. A better understanding of the etiology of opium sap dependence is crucial for improving the prevention and treatment of this type of drug dependence. Glutathione S-transferases (GSTs; EC: 2.5.1.18) are a ubiquitous family of eukaryotic and prokaryotic phase II metabolic isozymes. The GSTs are one of the key enzymes involved in cellular detoxification. Furthermore, recent evidence has been shown that the GSTs modulate the signaling pathways of cell proliferation, cell differentiation, and apoptosis [5]. Human GSTs divided into different classes and consisting 16 genes encoding cytosolic proteins and at least 6 genes expressing membrane-associated proteins. Genes encoding *GSTM1* (OMIM: 138350), and *GSTT1* (OMIM: 600436) are members of class mu and theta, respectively. The most common polymorphism in the *GSTM1* is a deletion of the whole *GSTM1* gene with a lack of enzyme activity [6]. A homozygous deletion in the *GSTT1* has also been reported (null genotypes of GSTT1) [7]. The association studies between these genetic polymorphisms and various multifactorial diseases were conducted [8-18]. Studies indicated that the *GSTT1* and *GSTM1* were expressed in brain [19, 20]. Very recently it has been reported that the mRNA levels of several antioxidant genes (including some of the GSTs family) were significantly down-regulated in human SH-SY5Y cells exposed to morphine and/or methadone [21, 22]. The association studies between polymorphisms of *GSTM1* and *GSTT1* and risk of dependent to methamphetamine [23-25], heroin, and opium [26] have been reported, with inconsistent results. It has been reported that opium induced the oxidative stress [27, 28]. Taken together, it is hypothesized that the null genotypes of *GSTT1* and *GSTM1* might be associated with risk of dependency to opium sap. Considering that there is no any data on the association between the polymorphisms of GSTs family (including *GSTT1* and *GSTM1* polymorphisms) and risk of dependency to opium sap, the present study was carried out.

MATERIALS AND METHODS

Participants: The present case-control study was performed in Shiraz (Fars province, southern Iran). In total, 71 males dependent to opium sap and 590 healthy male controls were included in this study. The patients were in methadone maintenance for treating their dependency and all of them reported opium sap as their primary drug of choice. All patients were assessed using the Structured Clinical Interview based on *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition (DSM-IV) criteria for heroin and opium dependence. Moreover, urine drug screens were obtained. All patients were interviewed by a senior psychiatrist. 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 37.3 (10.2) and 36.1 (11.2) years, respectively. There was no statistically significant difference with regard to age (t=0.90, df=659, P=0.364) between the patients and the controls. As mentioned previously, Iranian gene pool is very heterogeneous [29, 30]. Therefore, we select the

participants from Persians (Caucasians/Muslims) living in Fars province. Data on ethnicity were collected using simple questions like the parental and grandparental ethnicity (and also the religion) of each participant. Participants that their mothers and fathers (and also their grandparents) did not belong to Persians or they belong to non-Muslims religious communities were excluded. At the time of blood donation, a brief questionnaire that ascertained age, dependency to any drug, history of diagnosed cancers, cataract, schizophrenia, bipolar disorder, 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 multifactorial traits [8-18], the subjects of the both groups had negative history of cancers, cataract, schizophrenia, bipolar disorder, and asthma. This study was approved by the Shiraz University ethics committee and informed consent was obtained from each subject before the study. The work has been carried out in accordance with The Code of Ethics of the World medical association (Declaration of Helsinki) for experiments in humans.

Using the QUANTO (http://biostats.usc.edu/software) software, to detect a real difference in genotypic frequency with a power of 0.80, α=0.05, OR=1.50, 45% frequency of the minor allele (null allele of the *GSTT1*), and if the ratio of case/control be equal to 1/8; a minimum sample of 286 would be necessary.

Genotyping: Genomic DNA for PCR was isolated from whole blood using the thawed blood samples [31]. The PCR conditions and quality control for determining the genotypes of *GSTT1* and *GSTM1* polymorphisms were the same as that reported previously [10].

Statistical analysis: The association between the study polymorphisms and the risk of dependency to opium sap was assessed by calculating odds ratios (ORs) and 95% confidence intervals (CIs). The reference group consisted of individuals with the positive genotypes. A probability of $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Table 1 shows the genotypic frequency of the study polymorphisms between the patients and healthy controls. Statistical analysis revealed that the *GSTM1*-null genotype was not associated with the risk of dependency to opium sap (OR=0.78, 95% CI: 0.47-1.27, P=0.325). Also, there was no significant association between the null-genotypes of *GSTT1* and susceptibility to opium sap dependency (OR=1.25, 95% CI: 0.70-2.21, P=0.442) (Table 1). Previously, a significant association between *GSTT1* polymorphism and susceptibility to opium abuse has been reported, which is not confirming by the present data [26].

It has been showed that the null genotypes of *GSTM1* and *GSTT1* polymorphisms may have additive effect on the risk of multifactorial traits [10, 15, 17]. To investigate whether the null genotypes of *GSTM1* and *GSTT1* had additive effect on the risk of

dependency to opium sap, we considered the association between combinations of the genotypes and susceptibility to opium sap dependency. The reference group consisted of individuals with the "double positive genotypes of the *GSTM1* and *GSTT1*". Statistical analysis revealed that there was no significant association between combined genotypes and the risk of dependency to opium sap (Table 1). There was no linear trend in risk associated with zero, one and two null genotypes (χ^2=0.07; P=0.788). Previously, significant associations between combined genotypes of "positive genotypes of the *GSTM1* and null genotype of the *GSTT1"* and susceptibility to heroin and opium were reported [26]. These findings indicating that dependency to opium, heroin and opium sap are not quite same traits.

Table1: Association between polymorphisms of *GSTM1* and *GSTT1* and risk of dependent to opium sap

Genotypes	Patients	Controls	OR	95% CI	P
***GSTM1* polymorphism**					
Positive	37	271	1.0	-	-
Null	34	319	0.78	0.47-1.27	0.325
***GSTT1* polymorphism**					
Positive	53	464	1.0	-	-
Null	18	126	1.25	0.70-2.21	0.442
Combination genotypes *GSTM1/GSTT1*					
Positive/Positive	26	215	1.0	-	-
Positive/Null	11	56	1.62	0.75-3.48	0.213
Null/Positive	27	249	0.89	0.50-1.58	0.707
Null/Null	7	70	0.82	0.34-1.98	0.671

Very recently it has been reported that antioxidant genes (including several GSTs) significantly were down-regulated in SH-SY5Y cells exposed to morphine and/or methadone [21, 22]. Considering that the null genotypes of *GSTT1* and *GSTM1* have no enzyme activity [6, 7], we hypothesized that these polymorphisms were associated with the risk of dependency to opium sap. However, this study did not support our hypothesis. In order to address the involvement of the polymorphisms of *GSTT1* and *GSTM1* on susceptibility to opium sap dependency replication of this study in other populations is recommended.

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