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ORIGINAL ARTICLE

Association Between *GSTP1* Ile105Val Genetic Polymorphism and Dependency to Heroin and Opium

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Abstract

Relationship between glutathione S-transferase P1 (*GSTP1*, OMIM: 134660) variants and the risk of drug dependency is unknown. Chronic use of illegal drugs leads to oxidative stress, which can be alleviated by cellular detoxification mechanisms. There are several polymorphisms in the *GSTP1*, including Ile105Val (rs1695). This polymorphism leads to an Ile105Val amino acid change and may alter the GSTP1 enzyme activity. There is no study on the association between this polymorphism and risks of heroin (HD) or opium (OD) dependency. This paper consists of two case–control studies. The first study consisted of 442 HD subjects and 794 healthy controls. The second study consisted of 143 cases with OD and 565 healthy blood donors as controls. Genotyping were carried out using PCR based method. The Ile/Val (OR 0.84, 95% CI 0.65–1.07, $P=0.165$) and Val/Val (OR 0.87, 95% CI 0.56–1.36, $P=0.879$) genotypes did not show significant association with the risk of HD. Neither the Ile/Val (OR 0.72, 95% CI 0.49–1.06, $P=0.103$) nor the Val/Val (OR 0.61, 95% CI 0.29–1.30, $P=0.209$) was associated with the risk of OD. The *GSTP1* Ile105Val polymorphism was not associated with the risk of dependency to opium and heroin.

Keywords *GSTP1* · Heroin dependency · Opium dependency · Polymorphism

Introduction

Chronic use of illegal drugs leads to oxidative stress (Soykut et al. 2013; Uys et al. 2014). Oxidative stress can be alleviated by defense mechanisms including activity of superoxide dismutases, and glutathione S-transferases (GSTs; EC 2.5.1.18) (Hayes and Strange 1995). The GSTs belong to a superfamily of phase II metabolic enzymes, play important roles in the metabolism of products of oxidative stress (Hayes and Strange 1995; Schröder et al. 1995), and show significant associations

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with the risks of several multifactorial traits (Saadat 2006; Azizian-Farsani et al. 2014; Ebrahimpour and Saadat 2014; Zarei et al. 2015).

Glutathione S-transferase P1 (*GSTP1*, OMIM: 134660) is responsible for approximately 90% of the GSTs family enzyme activity and expressed in brain (Terrier et al. 1990; Moscow et al. 1989; Wahid et al. 2013). Studies revealed that GSTP knockout mice are more sensitive to neurotoxins (Castro-Caldas et al. 2012; Carvalho et al. 2013), indicating the role of GSTP1 in detoxification process.

Alteration of the mRNA levels of *GSTP1* and other members of the GSTs has been observed after exposure to methadone (Saify and Saadat 2015), morphine (Saify et al. 2016b), electromagnetic field (Mahmoudinasab et al. 2016; Mahmoudinasab and Saadat 2016), and methyl-tertiary butyl ether (Badr and Saadat 2016; Badr et al. 2016) in both in vitro and in vivo studies. The *GSTP1* mRNA levels decreased significantly in human neuroblastoma SH-SY5Y cells treated with methadone (Saify and Saadat 2015). However, the mRNA levels of *GSTP1* in morphine-treated SH-SY5Y cells showed significant increase by 24 h, decrease by 48 h, and return to normal control levels by day 18 (Saify et al. 2016b). The expression of *GSTP1* mRNA and protein levels were significantly higher in human brains obtained from the autopsy of nine heroin abusers compared to eight controls (Gutowicz et al. 2011).

Several single nucleotide genetic variations in the *GSTP1*, including Ile105Val (rs1695), have been reported. In this polymorphism, a substitution of A to G at nucleotide position 313 leads to Ile105Val change. It should be noted that this polymorphism is located near the substrate-binding site of the enzyme and alters the GSTP1 enzyme activity (Ali-Osman et al. 1997; Harries et al. 1997). Computer modeling showed deviations in the interatomic distances of amino acids of critical electrophile-binding active site as a consequence of the polymorphism (Ali-Osman et al. 1997).

To date, numerous studies have indicated that the variant allele (105Val) increased the risk of several multifactorial traits such as cancers (White et al. 2008; Xie et al. 2014; Tan and Chen 2015; Zhou et al. 2015; Song et al. 2016; Zhang et al. 2016), Alzheimer's disease (Wang et al. 2016), and type 2 diabetes mellitus (Saadat 2017). Although the association between polymorphisms of the GSTs and susceptibility to drug abuse have been reported (Koizumi et al. 2004; Hashimoto et al. 2005; Bousman et al. 2009; Nakatome et al. 2009; Khalighinasab et al. 2015a, b; Saify et al. 2016a), there is no study investigating the association between the *GSTP1* Ile105Val polymorphism and the risk of heroin (HD) and opium dependency (OD). These facts sufficiently provide us with a theoretical rationale to conduct the present study. In the current study, we evaluated the association of the rs1695 polymorphism with the risks of HD and OD.

Clinical Significance

The *GSTP1* Ile105Val polymorphism may be a potential biomarker for predicting risk of dependency to heroin and opium.

Materials and Methods

Subjects

This paper consisted of two case–control studies (Fig. 1). The first study consisted of 442 HD subjects (42 females, 400 males) and 794 healthy controls (136 females, 658 males). There was a statistical difference between the study groups for gender of participants ($P < 0.001$). The mean ± SD of age of the patients and controls were 38.2 ± 10.2 and 38.6 ± 12.7 years, respectively ($P = 0.608$). The mean of years of dependency to heroin was 16.6 ± 9.1. A comprehensive description of the participants has been reported previously (Saify et al. 2014).

The second study consisted of 143 OD patients (12 females, 131 males) and 565 (55 females, 510 males) healthy blood donors as control group. There was no statistical difference between these groups for gender of participants ($P = 0.624$). The mean ± SD of age of the patients and controls were 40.8 ± 10.7 and 39.6 ± 11.4 years, respectively ($P = 0.245$). The mean of years of dependency to opium was 14.5 ± 9.6. Detailed description of these groups was reported previously (Khalighinasab et al. 2015b).

It should be noted that the patients were assessed using the Structured Clinical Interview based on *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition criteria for drug dependency. All patients were interviewed by a senior psychiatrist. Urine drug screens were obtained. Control individuals were blood donors, who declared that they did not use any illegal drugs.

Considering that Iranian population showed high level of heterogeneity (Rafiee et al. 2010; Nasseri et al. 2015; Saadat 2015), the participants were selected from Persian (Caucasians) Muslims living in Shiraz (Fars province, Iran). Informed consent was obtained from each subject before the study. The study protocol complies with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee on Biological Research of the Shiraz University (Iran).

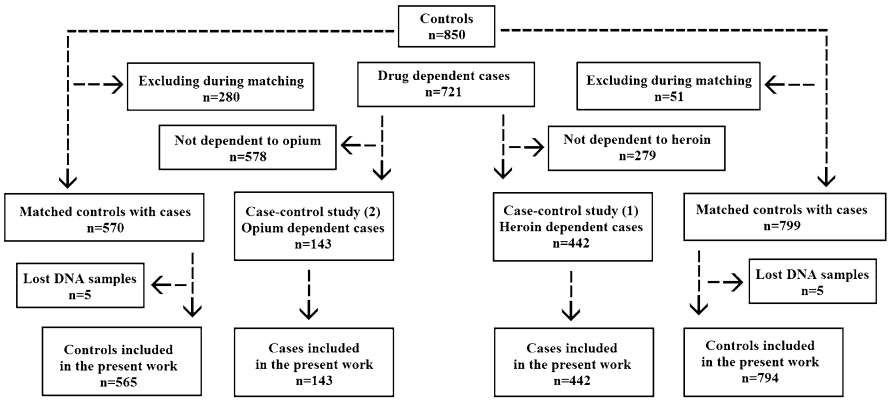

Fig. 1 STARD flow diagram demonstrating the selection cases and controls

Genotyping

Genomic DNA was extracted from whole blood samples using boiling method (Newton 1995). For genotyping of the *GSTP1* Ile105Val polymorphism, following primers were used: forward primer 5′-GTA GTT TGC CCA AGG TCA AG-3′ and reverse primer 5′-AGC CAC CTG AGG GGT AAG-3′ (Hashimoto et al. 2005). The PCR setting consisted of an initial denaturation step of 94 °C for 5 min, followed by 35 cycles of 94 °C for 60 s, of 60 °C for 30 s, and of 72 °C for 30 s, with a final extension of 72 °C for 5 min.

For quality control, tubes containing the PCR mixture without the template DNA (negative controls) were incubated in every run. Samples with uncertain result due to low yield were retested, and about 10% of all samples were evaluated twice. No discrepancy was observed upon replication.

Statistical Analysis

Chi-square test was used to investigate the similarity between observed frequencies and expected frequencies for the genotypes of *GSTP1* Ile105Val based on the Hardy–Weinberg equilibrium (HWE). The associations between the genotypes and the risks of HD and OD were expressed by odds ratios (ORs) and 95% confidence intervals (CIs). Odds ratios (and its 95% CI) adjusted for age and gender of participants were estimated using logistic regression analysis. In the analysis, the genotype of Ile/Ile was used as the reference group. Analyses were performed using the SPSS software (Chicago, IL, USA) (version 11.5). $P<0.05$ was considered statistically significant.

Results

The controls and HD patients were initially divided into two gender groups. The genotypic frequencies showed no significant differences between gender groups (control group: $\chi^2=2.17$, df = 2, $P=0.337$; HD group: $\chi^2=0.70$, df = 2, $P=0.703$); therefore, gender groups were pooled (data not shown). Table 1 shows the genotypic frequencies in HD cases and controls. The minor allele frequencies (MAFs) in the HD and control groups were 0.272 and 0.295, respectively. These frequencies are

Table 1 Association between *GSTP* Ile105Val polymorphism and risks of heroin dependency

Genotypes	Controls *N* (%)	Cases *N* (%)	OR	95% CI	*P*	OR[a]	95% CI	*P*
Ile/Ile	391 (49.2)	236 (53.4)	1.0	–	–	1.0	–	–
Ile/Val	337 (42.4)	171 (38.7)	0.84	0.65–1.07	0.165	0.83	0.65–1.06	0.152
Val/Val	66 (8.3)	35 (7.9)	0.87	0.56–1.36	0.879	0.85	0.54–1.32	0.471
Ile/Val + Val/Val	403 (50.8)	206 (46.6)	0.84	0.67–1.06	0.162	0.83	0.66–1.05	0.139

[a]Adjusted ORs for age and gender of participants

very similar with the allelic frequencies among Caucasian populations (see Tan and Chen 2015; Saadat 2017).

The genotypic frequencies of the *GSTP1* Ile105Val in controls of HD were consistent with the HWE ($\chi^2=0.30$, df = 1, $P=0.578$). The Ile/Val (OR 0.84, 95% CI 0.65–1.07, $P=0.165$), Val/Val (OR 0.87, 95% CI 0.56–1.36, $P=0.879$), and "Ile/Val + Val/Val" genotypes (OR 0.84, 95% CI 0.67–1.06, $P=0.162$) did not show significant association with the risk of HD. Considering that there was significant difference between cases and controls for age of participants, the ORs were reestimated after adjustment for age and gender. Adjusted ORs also indicated no relationship between the genotypes and the risk of HD (Table 1).

No statistical difference for the genotypic frequencies was observed between gender groups among controls ($\chi^2=0.16$, df = 2, $P=0.920$) and OD cases ($\chi^2=2.40$, df = 2, $P=0.300$); therefore, the gender groups were pooled (data not shown). Table 2 summarizes the genotypic distribution in OD patients and controls. The MAFs in the controls and OD patients were 0.296 and 0.241, respectively. The observed genotypic frequencies in controls did not show significant deviation from the expected values based on the HWE ($\chi^2=0.02$, df = 1, $P=0.894$). Present data indicated that the Ile/Val (OR 0.72, 95% CI 0.49–1.06, $P=0.103$), Val/Val (OR 0.61, 95% CI 0.29–1.30, $P=0.209$), and "Ile/Val + Val/Val" genotypes (OR 0.70, 95% CI 0.48–1.02, $P=0.065$) showed no significant association with the risk of OD, in comparison with the Ile/Ile genotype. Similar results were observed after adjustment of ORs for age and gender (Table 2).

Discussion

Studies have indicated that heroin- and opium-dependent persons experience oxidative stress condition, which is a reflection of unbalance between generation of free radicals and activity of antioxidant enzymes. On the other hand, oxidative stress alters the function of the Ca^{2+} channels (Hool 2008, 2015), and therefore might be involved in drugs dependency. The 105Val allele is assumed as an allele with lower activity (Ali-Osman et al. 1997; Harries et al. 1997) and showed significant associations with various multifactorial traits. In the present study, we found that the 105Val variant allele was not associated with the risk of heroin and/or opium dependency. It should be noted that a negative significant relationship between the 105Val carriers

Table 2 Association between *GSTP* Ile105Val polymorphism and risks of opium dependency

Genotypes	Controls *N* (%)	Cases *N* (%)	OR	95% CI	*P*	OR[a]	95% CI	*P*
Ile/Ile	279 (49.4)	83 (58.0)	1.0	–	–	1.0	–	–
Ile/Val	237 (41.9)	51 (35.7)	0.72	0.49–1.06	0.103	0.73	0.49–1.08	0.118
Val/Val	49 (8.7)	9 (6.3)	0.61	0.29–1.30	0.209	0.62	0.29–1.32	0.217
Ile/Val + Val/Val	286 (50.6)	60 (42.0)	0.70	0.48–1.02	0.065	0.71	0.49–1.03	0.075

[a]Adjusted ORs for age and gender of participants

and the risk of cocaine dependency has been reported (Guindalini et al. 2005). Hashimoto et al. (2005) have reported a positive association between the 105Val carriers and risk of dependency to methamphetamine in Japanese population.

We have to admit there are major limitations in the present study. As we mentioned in "Introduction" section, while there are several other genetic polymorphisms in the *GSTP1*, here we studied only one of those polymorphisms. Our study indicated that *GSTP1* Ile105Val polymorphism is not associated with susceptibility to heroin and opium dependency. Significant differences were observed between ethnicity and susceptibility to several multifactorial traits in relation to some genetic variations (Saadat 2006, 2012; Saadat and Ansari-Lari 2009) or the study polymorphism (Tan and Chen 2015; Tang et al. 2015; Song et al. 2016). It means that a specific polymorphism may be associated with the risk of a trait among Caucasians but not in Asians, or vise versa. Further large-scale studies from different ethnicities should be conducted to provide more rigorous data to validate our finding.

Conclusion

We found that the *GSTP1* Ile105Val polymorphism is not a potential genetic marker for dependency to heroin and opium in Iranians. Extrapolation of our findings to other populations would be supported by future studies utilizing large sample size, investigating the relationship between the *GSTP1* Ile105Val polymorphism and dependency to heroin and opium in various parts of the world.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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