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Association Analysis of a *GSTP1* Functional Polymorphism with Methamphetamine Dependence and Associated Symptoms in a Multiethnic Malaysian Population

(Analisis Perkaitan Polimorfisme Fungsian *GSTP1* dengan Pergantungan Metamfetamine dan Gejala Berkaitan dalam Populasi Berbilang Etnik di Malaysia)

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ABSTRACT

Methamphetamine (METH) is a psychostimulant that is highly addictive and has been widely linked to the adverse effect on brain. METH-induced oxidative stress can be effectively protected by glutathione S-transferases (GSTs). Genetic polymorphism of *GST* gene family may affect the susceptibility of METH users to its dependence and associated symptoms. Therefore, this study investigated the association of a functional single nucleotide polymorphism rs1695 of *GSTP1* gene with METH-induced symptoms and dependence in a Malaysian population, including Malay, Chinese, Kadazan-Dusun, and Bajau ethnic groups. Genotyping for *GSTP1* rs1695 polymorphism from 230 METH-dependent male subjects and 232 healthy male controls was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RLFP). For statistical analyses, the χ^2 test and Fisher's were performed in this research. The results showed a significant difference between *GSTP1* rs1695 polymorphism and METH dependence in the Malay and Chinese populations. Our findings suggest that the *GSTP1* rs1695 polymorphism may have possibility to methamphetamine dependence in the Malay and Chinese populations but not in other ethnicities. Furthermore, the Malay ethnic group who carried the -105G allele might have a protective role for METH-induced mania.

Keywords: GSTP1; methamphetamine; polymorphism; psychosis; stimulant

ABSTRAK

Metamfetamine (METH) ialah psikoperangsang yang sangat ketagihan dan telah dikaitkan secara meluas dengan kesan buruk kepada otak. Tekanan oksidatif aruhan METH boleh dilindungi dengan berkesan oleh glutation S-transferases (GST). Polimorfisme genetik gen famili *GST* boleh menjejaskan kerentanan pengguna METH kepada pergantungan dan gejala yang berkaitan. Oleh itu, penyelidikan ini mengkaji perkaitan polimorfisme fungsian nukleotida tunggal rs1695 gen *GSTP1* dengan gejala dan pergantungan aruhan METH dalam populasi Malaysia, termasuk kumpulan etnik Melayu, Cina, Kadazan-Dusun dan Bajau. Penjenisan gen untuk polimorfisme *GSTP1* rs1695 daripada 230 subjek lelaki pergantungan METH dan 232 lelaki sihat untuk kawalan telah dijalankan menggunakan polimorfisme panjang serpihan tindak balas rantai polimerase (PCR-RLFP). Untuk analisis statistik, ujian χ^2 dan Fisher telah dijalankan dalam penyelidikan ini. Keputusan menunjukkan perbezaan yang signifikan antara polimorfisme *GSTP1* rs1695 dan pergantungan METH dalam populasi Melayu dan Cina. Penemuan kami mencadangkan bahawa polimorfisme *GSTP1* rs1695 mungkin mempunyai kemungkinan pergantungan methamphetamine dalam populasi Melayu dan Cina tetapi tidak dalam etnik lain. Tambahan pula, kumpulan etnik Melayu yang membawa alel -105G mungkin mempunyai peranan perlindungan untuk aruhan METH mania.

Kata kunci: GSTP1; metamfetamine; perangsang; polimorfisme; psikosis

Introduction

Methamphetamine (METH) is one of the addictive drugs that is highly abused (Szumlinski et al. 2017). Therefore, METH abuse and its psychiatric consequences are uncontrollably increasing worldwide (United Nations Office on Drugs and Crime (UNODC) 2011). The Malaysian National Anti-Drug Agency reported that the cases of METH use contributed 85% of the total drug cases in the country (NADA 2021). Chronic METH use can have negative impacts on METH users, particularly exerting oxidative stress-induced neurotoxic effects on the brain (Sulaiman et al. 2014). These effects may result in METH dependence and associated symptoms as well as increased risk of depression and other cognitive decline related to METH misuse (Johnson et al. 2015; Sim et al. 2013).

Furthermore, chronic use of METH may also exhibit various symptoms, such as violent behaviour, mood disturbances, confusion, and insomnia (Rusyniak 2013). Long-term use of METH may develop psychiatric symptoms because of depletion of dopamine in the striatum. According to a cross-country study involving Thailand, Philippines, Japan, and Australia, are most common lifetime psychotic symptoms among the patients with METH-induced psychosis are auditory hallucinations, persecutory delusion thought reading and unusual beliefs (Ali et al. 2010). Studies also reported that METH-induced psychosis is common among the Japanese and Malaysian populations, and they found that the risk of patients predisposed to psychosis is increasing (Rognli & Bramness 2015; Sim et al. 2013).

While the environment around a person, along with a person's behaviour and personality, influences whether the person becomes addicted to drugs, genetics also plays an important role. Science has shown that at least half of a person's susceptibility to drug addiction can be highly associated with genetic factors. Studies reported that polymorphisms of the glutathione S-transferase (GST) gene family, such as *GSTM1, GSPT1*, and *GSTT1* have been highly associated with the risk of dependence or psychosis on METH (Nakatome et al. 2009), cocaine (Guindalini et al. 2005) heroin, and opium (Khalighinasab, Saify & Saadat 2015b). The GSTs is one of the multifunctional enzymes that catalyse the conjugation of reduced glutathione with electrophilic groups. GSTs are important detoxification enzymes in the body. They provide defense mechanisms against oxidative stress caused by chronic use of illegal drugs (Uys, Mulholland & Townsend 2014; Wahid et al. 2013). This important role in the cellular defence leads the GSTs involved with METH use and dependence (Guindalini et al. 2005) because an impaired antioxidant system can cause damage to the brain cells, which consecutively affects the response of the brain towards METH use (Board, Webb & Coggan 1989). Furthermore, a previous study showed the neuroprotective effects of glutathione dopamine-induced neurotoxicity (Nakatome et al. 2009). Thus, dopaminergic pathways in the mesocorticolimbic systems have an important role in drug reward (Guindalini et al. 2005).

The *GSPT1* gene is primarily positioned in the brain and lungs (Wahid et al. 2013). Specifically, this gene is located on chromosome 11q13 and consists of 7 exons and 6 introns (Nebert & Vasiliou 2004). It is expressed in the brain and is responsible for approximately 90% of the activity of GST enzymes (Board, Webb & Coggan 1989). One of the functional polymorphisms, *GSPT1* rs1695 is a widely studied polymorphism of *GSPT1* gene in which guanine (G) replaces adenine (A) at 313 bases of exon 5 in the coding region, leading to the substitution of valine (Val) for isoleucine (Ile) at 105 (Hayes & Strange 2000; Sim et al. 2014). The changes of catalytic activity, thermal stability, and decreased substrate specificity present in the encoded GSTP1 protein (Nakatome et al. 2009). Previous studies also have reported that *GSPT1* has functional significance on reduced GST enzyme activity (Khalighinasab, Saify & Saadat 2015a). Another study has shown individuals with *GSPT1* rs1695 polymorphism may be more susceptible to METH abuse due to a deficiency in the causative GST enzymatic activity which contributes to the development of METH dependence and related symptoms (Mandal & Mittal 2020). Therefore, this study objective is to determine whether the rs1695 genetic polymorphism in the *GSPT1* gene could lead to METH dependence and its related symptoms in a multiethnic Malaysian population.

Materials and Methods

Subject recruitment and sample collection

This case-control study was approved by the Ethics Committee of UMMC (MREC ID No.: 2017105-5641) and conducted in a drug rehabilitation Centre in Papar, Sabah, in East Malaysia, involving male METH-dependent users. The sample size was calculated using the Power for Association with Errors (PAWE) software (Biometrics, USA) (power of study = 80%) (Sheehan et al. 1998). A total of 230 cases and 232 controls consisting of Malay, Chinese, Kadazan-Dusun, and Bajau ethnic groups were recruited for this study, and their consent was obtained before the study was performed.

Patients who fulfilled the criteria listed in the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) for amphetamine and METH dependence were selected for this study (Soykut et al. 2013). Meanwhile, the experimental control group comprised healthy volunteers who did not meet the DSM-IV criteria for amphetamine and METH dependence and had no history of chronic medical and psychiatric conditions. They were recruited at the Luyang Health Clinic, Sabah and University Malaya Medical Centre (UMMC), Kuala Lumpur.

DNA PREPARATION AND PCR ANALYSIS

Blood samples (± 3 mL) were collected from each participant using the standard method. Subsequently, the samples were subjected to DNA extraction using QIAamp DNA Blood Mini Kit (Qiagen, Germany) as per the manufacturer's guidelines. Alternatively, buccal swab tissues were obtained from participants unwilling to provide blood samples, followed by DNA extraction using DNA Mini Kit (Qiagen, Germany). Genotyping of *GSPT1* rs1695 genetic polymorphism was performed using polymerase chain reaction (PCR)-based methods (forward primer: 5'-GTAGTTTGCCCAAGGTCAAG-3'; reverse primer: 5'-AGCCACCTGAGGGGTAAG-3'). The PCR was performed using the PCR Master Mix (Fermentas, Canada) under the following conditions: 95 °C for 5 min, 36 denaturing cycles at 95 °C for 30 s, annealing at 65 °C for 30 s; elongation at 72 °C for 30 s, and final extension at 72°C for 5 min.

RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP)

The RFLP method was conducted with a restriction enzyme, BsmAI (NEB, USA), following PCR. First, a mixture of 40.5 µL of nucleus-free water, 1x NE Buffer, restriction enzyme (1µL), Bovine serum albumin (BSA) with 0.5 µL, and 5 µL of PCR product was prepared. The mixture was thoroughly mixed, spun down briefly, and incubated on a dry heating block (± 4 h) for enzymatic digestion of the PCR product at the targeted region to produce specific fragments. The optimum digestion temperature for BsmAI was 55 °C. After incubation, the enzymes were inactivated by incubation at 65 °C for 20 min. Finally, the RFLP product was subjected to gel electrophoresis on a 2.5% agarose gel and visualised under UV light.

The resulting bands were compared to a standard ladder (Thermo Fisher Scientific, USA), and the restriction profiles are detailed in Figure 1. The wild-type (A/A), heterozygous genotype (A/G), and mutant genotype (G/G) yielded two bands (328-bp and 105-bp), three bands (328-bp, 222-bp, 106-bp - 105-bp), and two bands (222-bp, 106-bp - 105-bp), respectively. The RFLP products were then selected randomly for DNA sequencing for validation purposes.

STATISTICAL ANALYSIS

The cases of each ethnic group were compared with ethnically matched healthy controls for the genotype frequencies of *GSPT1* rs1695 wild type (A/A), heterozygous genotype (A/G), and mutant (G/G). Statistical analyses were performed via SPSS software version 27 (IBM, USA) using the Chi-square (χ^2) test and the Fisher's exact test. The level of statistical significance was set at $p < 0.05$, and the results were considered significant when $p < 0.016$ after the Bonferroni correction for three subtests.

RESULTS

A total of 462 male subjects consisting of 230 METH-dependent and 232 healthy control subjects were recruited for this study. The demographic data is summarised in Table 1. One of the parameters investigated in the present study is the effect of *GSPT1* rs1695 single nucleotide polymorphism (SNP) on the onset age of METH dependence. The mean onset age of being METH dependent in the pooled subjects was found to be 24 ± 9 years. Interestingly, the mean onset age was younger among the Kadazan-Dusun (22 ± 7.4 years) and Bajau (22 ± 8.1 years) ethnic groups. Comparatively, Malay and Chinese ethnic groups recorded significantly older mean onset ages at 25 ± 9.1 and 30 ± 9.6 years, respectively.

Genotype and allelic frequencies of *GSPT1* rs1695 SNP in METH-dependent subjects and healthy controls are shown in Table 2. Overall, the genotype distribution for all subjects fulfilled the Hardy-Weinberg equilibrium. After stratification into the four ethnic groups, the SNP demonstrated a significant association between genotype frequency and METH dependence in the Malay ($p = 0.043$) and Chinese ($p = 0.040$) ethnic groups. In contrast, only the Malay ethnic group exhibited a significantly strong association between the SNP and METH dependence [odds ratio (OR): 1.960; $p = 0.015$] in allelic frequency. Furthermore, this study discovered that the -105G allelic frequencies were higher in METH-dependent subjects than in healthy controls. Conversely, the -105A allele demonstrated a relatively higher frequency among healthy controls than METH-dependent subjects.

Various combinations of genotype frequencies of different ethnic groups were also compared in this

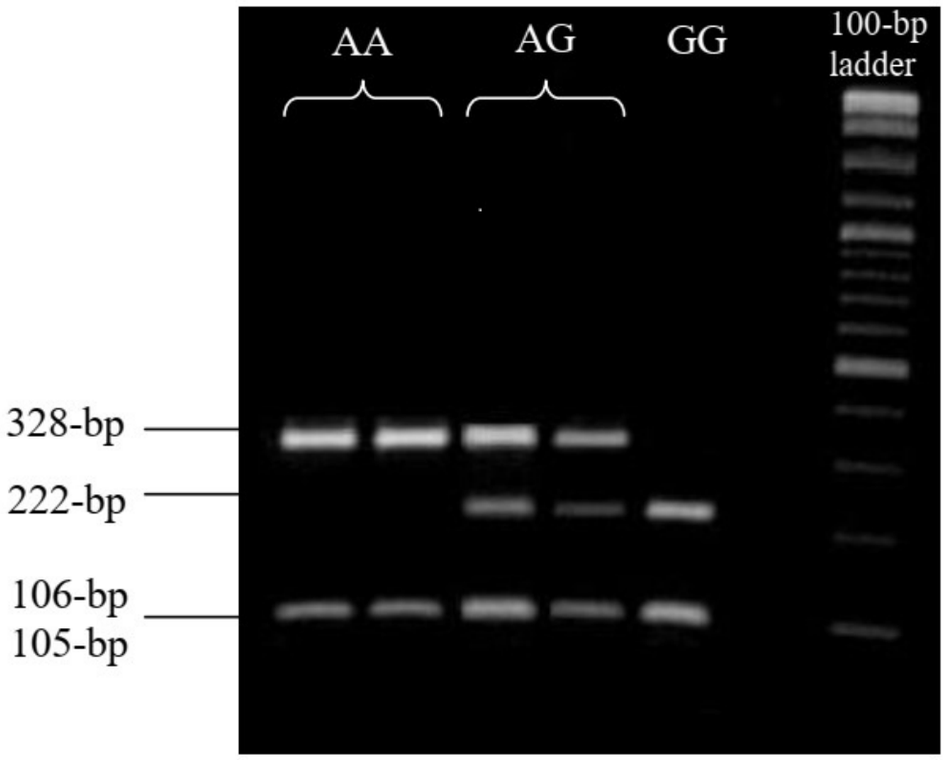

FIGURE 1. Gel photograph of a 2.5% (w/v) agarose gel following electrophoresis for *GSPT1* rs1695 polymorphism

study and presented in Table 5. In the pooled data, the probability of being METH-dependent in the homozygous -105G carriers was relatively higher (OR: 1.462) than in the homozygous -105A carriers. Meanwhile, the homozygous -105G plus heterozygous -105A/G carriers were highly prone to METH dependence (OR: 1.367) than those with homozygous -105A. Malay subjects with homozygous -105G had a higher probability (OR: 3.97) of being METH-dependent compared to their homozygous -105A counterpart. Moreover, homozygous -105G and heterozygous -105A/G carriers of Malay (OR: 2.142) and Chinese (OR: 2.968) descent were more likely to be METH-dependent compared to those with homozygous -105A. Other ethnic groups did not show significant effects between the different combinations of genotype frequencies (Table 5).

There were no significant differences in METH-induced psychosis for genotype and allelic frequencies among METH-dependent subjects, either in the pooled subjects or after stratification into four ethnic groups. Notably, no homozygous -105G genotype was observed in Chinese METH-dependent subjects with psychosis (Table 3). Similarly, there were no significant differences for different combinations of genotype frequencies for subjects with and without METH-induced psychosis (Table 5). Among the four ethnic groups, the homozygous -105G Malay carriers in the Malay were more susceptible to developing METH-induced psychosis (OR: 3.5) than their homozygous -105A counterpart. The Bajau homozygous -105G carriers have a higher risk of METH-induced psychosis (OR: 3.2) than the homozygous -105A plus heterozygous -105A/G carriers of the same ethnic group.

No significant differences were observed in METH-induced mania among METH-dependent subjects for genotype and allelic frequencies, either in the pooled subjects or after stratification into the four ethnic groups. Additionally, no homozygous -105G genotype was detected in Malay and Chinese METH-dependent subjects with manic episodes (Table 4). Lastly, no significant difference was identified among different combinations of genotype frequencies in METH-induced mania subjects and healthy controls (Table 5). Nevertheless, the Bajau homozygous -105G allele carriers were more likely to develop METH-induced mania (OR: 6 & 6.875) than the homozygous -105A and homozygous -105A plus heterozygous -105A/G carriers.

TABLE 1. Demographic data for METH-dependent and control subjects

Characteristics	Malay (n = 138)		Chinese (n = 93)		Kadazan-Dusun (n = 124)		Bajau (n = 107)		Total (n = 462)	
	Case (n = 70)	Control (n = 68)	Case (n = 35)	Control (n = 58)	Case (n = 58)	Control (n = 66)	Case (n = 67)	Control (n = 40)	Case (n = 230)	Control (n = 232)
Age (years), mean (SD)	32 (8.1)	34 (10.1)	39 (9.3)	32 (9.4)	28 (6.4)	33 (10.6)	28 (6.8)	33 (11.8)	31 (8.3)	33 (10.4)
Onset age of METH dependence (years), mean (SD)	25 (9.1)		30 (9.6)		22 (7.4)		22 (8.1)		24 (9.0)	
METH-dependence										
With psychosis, n (%)	33 (47.1)		16 (45.7)		18 (31.0)		17 (25.4)		84 (36.5)	
Without psychosis, n (%)	37 (52.9)		19 (54.3)		40 (69.0)		50 (74.6)		146 (63.4)	
With mania, n (%)	12 (17.1))		6 (17.1)		12 (20.7)		10 (14.9)		40 (17.4)	
Without mania, n (%)	58 (82.9)		29 (82.9)		46 (79.3)		57 (85.1)		190 (82.6)	

SD: Standard deviation

TABLE 2. Genotype and allelic frequencies of the *GSTP1* rs1695 polymorphism in male controls and male METH dependent subjects

Ethnicity	Participant	Genotype, n (frequency)			*p*-value	Allele, n (frequency)		*p*-value	OR (95% CI)
		A/A	A/G	G/G		A	G		
Malay	Case	27 (0.386)	32 (0.457)	11 (0.157)	**0.043**	86 (0.614)	54 (0.386)	**0.015**	1.960 (1.166-3.294)
	Control	39 (0.574)	25 (0.368)	4 (0.059)		103 (0.758)	33 (0.243)		
Chinese	Case	18 (0.514)	15 (0.429)	2 (0.057)	**0.040**	51 (0.729)	19 (0.272)	0.058	2.170 (1.039-4.531)
	Control	44 (0.759)	11 (0.190)	3 (0.052)		99 (0.853)	17 (0.147)		
Kadazan-Dusun	Case	32 (0.552)	18 (0.310)	8 (0.138)	0.205	82 (0.707)	34 (0.293)	0.158	0.659 (0.387-1.121)
	Control	26 (0.394)	29 (0.439)	11 (0.167)		81 (0.613)	51 (0.386)		
Bajau	Case	35 (0.522)	28 (0.418)	4 (0.060)	0.952	98 (0.731)	36 (0.269)	0.888	1.102 (0.585-2.078)
	Control	22 (0.550)	16 (0.400)	2 (0.050)		60 (0.750)	20 (0.250)		
Total	Case	112 (0.487)	93 (0.404)	25 (0.109)	0.239	317 (0.690)	143 (0.311)	0.107	1.279 (0.960-1.703)
	Control	131 (0.565)	81 (0.350)	20 (0.086)		343 (0.739)	121 (0.261)		

Boldface show a significant difference after being adjusted by the Bonferroni correction test
OR: Odds ratio; CI: Confidence Interval

TABLE 3. Genotype and allelic frequencies of the *GSTP1* rs1695 polymorphism in METH dependent subjects with and without psychosis episodes

Ethnicity	Participant	Genotype, n (frequency)			*p*-value	Allele, n (frequency)		*p*-value	OR (95% CI)
		A/A	A/G	G/G		A	G		
Malay	Psychosis	9 (0.273)	17 (0.515)	7 (0.212)	0.155	35 (0.530)	31 (0.470)	0.079	1.964 (0.985-3.915)
	No psychosis	18 (0.486	15 (0.405)	4 (0.108)		51 (0.689)	23 (0.311)		
Chinese	Psychosis	6 (0.375)	10 (0.625)	0 (0.000)	0.066	22 (0.688)	10 (0.313)	0.941	1.098 (0.402-3.003)
	No psychosis	12 (0.632)	5 (0.263)	2 (0.105)		29 (0.707)	12 (0.293)		
Kadazan-Dusun	Psychosis	13 (0.722)	3 (0.167)	2 (0.111)	0.197	19 (0.731)	7 (0.269)	0.237	0.501 (0.191-1.320)
	No psychosis	19 (0.475)	15 (0.375)	6 (0.150)		49 (0.576)	36 (0.424)		
Bajau	Psychosis	9 (0.529)	6 (0.353)	2 (0.118)	0.469	24 (0.706)	10 (0.294)	0.970	0.891 (0.376-2.115)
	No psychosis	26 (0.520)	22 (0.440)	2 (0.040)		70 (0.729)	26 (0.271)		
Total	Psychosis	37 (0.440)	36 (0.429)	11 (0.131)	0.501	110 (0.655)	58 (0.345)	0.270	1.284 (0.856-1.927)
	No psychosis	75 (0.514)	57 (0.390)	14 (0.096)		207 (0.709)	85 (0.291)		

OR: Odds ratio; CI: Confidence Interval

TABLE 4. Genotype and allelic frequencies of the *GSTP1* rs1695 polymorphism in METH-dependent subjects with and without manic episodes

Ethnicity	Participant	Genotype, n (frequency)			*p*-value	Allele, n (frequency)		*p*-value	OR (95% CI)
		A/A	A/G	G/G		A	G		
Malay	Mania	6 (0.500)	6 (0.500)	0 (0.000)	0.244	18 (0.750)	6 (0.250)	0.204	0.472 (0.175-1.277)
	No mania	21 (0.362)	26 (0.448)	11 (0.190)		68 (0.586)	48 (0.414)		
Chinese	Mania	3 (0.500)	3 (0.500)	0 (0.000)	0.778	9 (0.750)	3 (0.250)	0.945	0.812 (0.194-3.396)
	No mania	15 (0.517)	12 (0.414)	2 (0.069)		39 (0.709)	16 (0.291)		
Kadazan-Dusun	Mania	8 (0.667)	2 (0.167)	2 (0.167)	0.482	18 (0.750)	6 (0.250)	0.788	0.762 (0.273-2.124)
	No mania	24 (0.522)	16 (0.348)	6 (0.130)		64 (0.696)	28 (0.304)		
Bajau	Mania	5 (0.500)	3 (0.300)	2 (0.200)	0.118	13 (0.650)	7 (0.350)	0.538	1.578 (0.574-4.337)
	No mania	30 (0.526)	25 (0.439)	2 (0.035)		85 (0.746)	29 (0.254)		
Total	Mania	22 (0.550)	14 (0.350)	4 (0.100)	0.676	58 (0.725)	22 (0.275)	0.529	0.812 (0.475-1.388)
	No mania	90 (0.474)	79 (0.416)	21 (0.111)		259 (0.682)	121 (0.318)		

OR: Odds ratio; CI: Confidence Interval

TABLE 5. Odds ratio in various genetic models for the different ethnic groups in METH dependence, methamphetamine-induced psychosis and METH-induced manic episodes

Genotype	Malay		Chinese		Kadazan-Dusun		Bajau		Total	
	Odds ratio (95% CI)	*p*-value	Odds ratio (95% CI)	*p*-value	Odds ratio (95% CI)	*p*-value	Odds ratio (95% CI)	*p*-value	Odds ratio (95% CI)	*p*-value
METH dependence										
AA vs GG	3.972 (1.144-13.797)	**0.047**	1.630 (0.251-10.588)	0.994	0.591 (0.207-1.684)	0.467	1.257 (0.212-7.449)	0.585	1.462 (0.771-2.772)	0.315
AA vs (AG + GG)	2.142 (1.085-4.228)	**0.042**	2.968 (1.213-7.265)	**0.028**	0.528 (0.258-1.080)	0.115	1.118 (0.509-2.453)	0.920	1.367 (0.947-1.971)	0.114
(AA + AG) vs GG	2.983 (0.900-9.883)	0.114	1.111 (0.176-7.000)	0.717	0.96 (0.360-2.563)	0.863	1.206 (0.211-6.904)	0.600	1.402 (0.756-2.600)	0.357
METH-induced psychosis										
AA vs GG	3.500 (0.808-15.163)	0.176	NA	0.479	0.4872 (0.085-2.801)	0.350	2.889 (0.353-23.626)	0.313	1.593 (0.659-3.849)	0.420
AA vs (AG + GG)	2.526 (0.928-6.876)	0.112	2.8571 (0.722-11.312)	0.240	0.348 (0.104-1.160)	0.143	0.963 (0.320-2.900)	0.823	1.342 (0.782-2.301)	0.351
(AA + AG) vs GG	2.221 (0.586-8.412)	0.387	NA	0.287	0.708 (0.129-3.905)	0.522	3.200 (0.415-24.701)	0.265	1.421 (0.613-3.291)	0.547
METH-induced manic episodes										
AA vs GG	NA	0.225	NA	0.716	1.000 (0.167-5.984)	0.688	6.000 (0.681-52.902)	0.141	0.779 (0.243-2.502)	0.890
AA vs (AG + GG)	0.568 (0.162-1.985)	0.570	1.071 (0.185-6.217)	0.642	0.546 (0.144-2.067)	0.286	1.111 (0.290-4.261)	0.573	0.736 (0.371-1.461)	0.482
(AA + AG) vs GG	NA	0.227	NA	0.682	1.333 (0.233-7.627)	0.529	6.875 (0.846-55.897)	0.103	0.894 (0.289-2.763)	0.932

Boldface show a significant difference, $p < 0.05$.
OR: Odds ratio; CI: Confidence Interval

DISCUSSION

Earlier studies have proven that METH-dependent individuals are susceptible to cellular oxidative stress, resulting from the imbalance between the activity of antioxidant enzymes and the generation of free radicals (Hayes & Strange 2000). Theoretically, individuals carrying the -105G allele possess lower functional *GSTP1* enzyme (Rezaei & Saadat 2019); thus, *GSTP1* genetic polymorphism possibly results in excessive metabolic by-products in the METH-induce oxidative process (Sim et al. 2013).

In this study, the potential link between *GSTP1* rs1695 SNP and METH dependence and associated symptoms in four Malaysian ethnic groups (Malay, Chinese, Kadazan-Dusun, and Bajau) were investigated. The Kadazan-Dusun and Bajau METH-dependent subjects had significantly lower mean onset age than Malay and Chinese ethnic groups, suggesting early METH exposure at a younger age in these populations (Table 1). This finding is consistent with Sim *et al.* (2013), who reported that Kadazan-Dusun and Bajau have younger mean age and the onset age of METH dependence associated with functional FAAH polymorphism. Besides, data from NADA state that number of abusers and drug and substances addicts for 2019 among youths aged 19 to 39 years old was alarming, with the highest percentage of 68.1 percent in adult and adolescents at 29.5 percent and 2.3 percent, respectively.

This study outcome also indicated that rs1695 SNP of the *GSTP1* gene had no significant association with METH dependence in the pooled Malaysian population (Table 2). This finding aligned with a study conducted in the Iranian population, where the Ile105Val polymorphism of the *GSTP1* gene was not associated with the risk of heroin and opium dependency (Mcketin et al. 2006). On the contrary, a Brazilian study found a significant association between this genetic polymorphism and cocaine dependence (Sim et al. 2014).

After the ethnic stratification, this study showed that the *GSTP1* rs1695 SNP significantly differed in genotype frequency among Malay and Chinese METH-dependent subjects. Conversely, no significant difference was found in the Kadazan-Dusun and Bajau ethnic groups (Table 2). This finding indicated that the Kadazan-Dusun and Bajau ethnic groups have different population-attributable risks compared to Chinese and Malay, such as different lifestyles, cultures, geography, and dietary habits attributed to their diverse gene pool of origin. In addition, there was a significant difference in *GSTP1* rs1695 SNP allelic frequency for the Malay ethnic group (OR: 1.960), proposing that this SNP may be associated with METH-dependence in Malay males. Despite the lack of significant difference in the SNP allelic frequency with METH-dependence in other ethnic groups, the Chinese -105G allele carrier showed a higher METH dependence (OR: 2.2) than their -105A allele counterpart. This finding suggests that the -105G allele is the risk factor for METH dependence in Chinese males.

The -105A allele was more frequently observed in healthy male controls than METH-dependent subjects in this study (Table 2). In contrast, the -105A allele was more common in male cocaine-dependent subjects than healthy controls in the Brazilian population (Guindalini et al. 2005). These discrepancies are potentially attributed to the difference in population and drug dependence trends. Additionally, the risk for METH dependence in the Malay ethnic group possessing the homozygous -105G was significantly higher (OR: 3.97) than the homozygous -105A carriers. Similarly, the homozygous -105G plus heterozygous -105A/G Malay carriers had a significantly higher risk of METH dependence (OR: 2.142), suggesting the association between the -105G allele of rs1695 polymorphism and METH dependence in the Malay ethnic group (Table 5).

The METH-dependent subjects with psychosis in this study also possess a lower -105G allele frequency than those without psychosis (Table 3). This result contradicted a previous study where a significantly higher frequency of -105G allele was detected in METH subjects with psychosis compared to controls (Gordon et al. 2003). Moreover, genotype and allelic frequencies were not significantly different when comparing METH-dependent subjects with and without psychosis. This outcome suggested that *GSTP1* rs1695 SNP may not be associated with METH-induced psychosis in the Malaysian population, particularly in the four studied ethnic groups. Likewise, the studies conducted in the Korean and Chinese Han populations also reported similar findings in a study on the association of the same genetic polymorphism with the risk of psychiatric disorders, such as psychotic symptoms in schizophrenia (Rognli & Bramness 2015). Nonetheless, this SNP was strongly associated with a higher risk of METH-induced psychosis in the Japanese population (Gordon et al. 2003).

A study by Kim et al. (2015) reported that METH use is highly associated with panic episodes and manic disorder in the Malaysian population, but no significant difference was detected in this study for both genotype and allelic frequencies of this SNP when comparing METH-dependent subjects with and without METH-induced mania. Furthermore, this study findings demonstrated that the -105G allele might exert a strong protective role against METH-induced manic episodes (OR: 0.472) compared to the -105A allele carriers in the Malay ethnic group, but no statistically significant difference was observed for this condition (Table 4).

The Malay METH-dependent subjects who are homozygous -105G allele carriers were also more susceptible to developing METH-induced psychosis (OR: 3.5) than the homozygous -105A carriers. Similarly, the Bajau homozygous -105G allele carriers were more vulnerable to METH-induced psychosis (OR:3.2) than the homozygous -105A plus heterozygous -105A/G carriers. These findings indicated that Malay and Bajau homozygous -105G carriers may be more vulnerable to METH-induced psychosis than other ethnic groups in Malaysia.

Apart from that, the homozygous -105G carriers in the Bajau ethnic group were more likely to develop METH-induced (OR: 6 & 6.9) than the homozygous -105A and homozygous -105A plus heterozygous -105A/G carriers. These findings suggest that the homozygous -105G carriers in the Bajau ethnic group may be more susceptible to METH-induced mania (Table 5). The literature has highlighted that the Asian populations are inherited with higher homozygous -105A genotype and less heterozygous -105A/G and homozygous -105G genotypes compared to the Caucasians (Peng et al. 2021). Besides, the -105G allele frequency of *GSTP1* rs1695 SNP in Malaysian METH-dependent subjects was only 3.1% (Table 2), which is lower than the Chinese Han (Gordon et al. 2003) and Korean (Rognli & Bramness 2015) populations.

There are several limitations in this study. First, the sample size was limited to the male subjects for all ethnic groups. Most cases of METH abuse occur among males, thus, providing greater homogeneity of the study population. Apart from that, this study only investigated a single SNP of the *GSTP1* gene. To date, this SNP is the only functional polymorphism found in the *GSTP1* gene proven to significantly alter the *GSTP1* encoded GST enzyme's functional activity. Nonetheless, other *GSTP1* gene variants may also contribute to the METH-dependence susceptibility and the associated symptoms. Finally, the sample size in this study is relatively small. Future studies should consider investigating a series of tagging SNPs with a larger sample size to confirm the genetic impact of the *GSTP1* gene on METH dependence and the associated symptoms.

Conclusion

This study findings indicated the rs1695 polymorphism in the *GSTP1* gene might not be associated with METH dependence in the pooled Malaysian population. However, the stratification into four ethnic groups demonstrated that this polymorphism may be significantly associated with METH dependence in the Malay and Chinese ethnic groups. Interestingly, the results also suggested that the male Malay and Chinese -105G allele carriers might be more vulnerable to METH dependence than other populations. Meanwhile, the study also demonstrated that this polymorphism might not be associated with METH-induced psychosis and mania. Nevertheless, the Malay -105G allele carriers might have a protective advantage against METH-induced manic episodes. Further study with a larger sample size could provide more evidence to confirm the genetic influence of the *GSTP1* rs1695 polymorphism on METH dependence.

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