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Glutathione S-Transferase P1 Polymorphism on Exon 6 and Risk of Hepatocellular Carcinoma in Thai Male Patients

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Keywords
GSTP1 polymorphism · Thai male hepatocellular carcinoma patients · Hepatocellular carcinoma

Abstract
Introduction: Hepatocellular carcinoma (HCC) is the fifth most common malignancy and the fourth leading cause of cancer-related deaths worldwide. HCC cases are two to four times more common in males than in females, and the highest incidence is found in Asia and Sub-Saharan Africa. This gender disparity is the result of different behavioral risk factors, such as smoking and drinking alcohol. Glutathione S-Transferase P1 (GSTP1) is an enzyme that is involved in the detoxification of carcinogenic electrophiles. GSTP1 codon 105 in exon 5 and codon 114 in exon 6 polymorphisms result in decreased enzyme detoxification activity, which is the cause of many cancers. ***Objectives:*** This study aims to investigate the associations between GSTP1 polymorphism, HCC patients, and the risk factors for HCC. It is hoped that this research will provide useful knowledge on the effects of genetic GSTP1 polymorphism in Thai HCC patients. ***Methods:*** DNA from 44 Thai HCC patients and 52 healthy controls was analyzed for GSTP1 exon 5 and exon 6 polymorphisms by PCR-RFLP. The associations between GSTP1 polymorphism, the control group, and clinicopathological parameters were determined. ***Results:*** The results show that GSTP1 exon 6 polymorphism genotypes (C/T) were correlated with an increased risk of HCC susceptibility (OR = 4.40). Moreover, exon 6 polymorphism genotypes (C/T) were associated with the gender of patients ($p = 0.015$), but no relationships were found between GSTP1 exon 5 polymorphism and the clinicopathological data of patients. ***Conclusions:*** The results suggest that the GSTP1 exon 6 polymorphism genotype was associated with an increase in the risk of HCC in male patients and that it tended to be related to cancer differentiation. No association was found between GSTP1 exon 5 polymorphism and the risk of HCC.

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignancy in the world and the fourth leading cause of cancer-related deaths worldwide [1]. The risk factors for HCC include chronic hepatitis virus infections, cirrhosis, alcohol, nonalcoholic fatty liver disease,

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smoking, and consuming food contaminated with aflatoxin [2, 3]. HCC is two to four times more likely to be found in males than females, and the highest incidence is found in Asia and Sub-Saharan Africa [4, 5]. In Thailand, HCC is the leading cause of cancer in males, and the third leading cause of cancer in females [6]. This gender disparity is the result of differences in behavioral risk factors, such as smoking and drinking alcohol [7].

Glutathione S-Transferase P1 (GSTP1) is an enzyme located on chromosome 11q13 that is involved in the detoxification of carcinogenic electrophiles. Research showed that GSTP1 genetic polymorphism results in decreased enzyme detoxification activity, which is the cause of many cancers. Prior studies also found that the polymorphism at codon 105 in exon 5 results in a substitution of isoleucine for valine [8], and codon 114 in exon 6 causes the substitution of alanine for valine [9]. The GSTP1 polymorphism at codon 105 may be associated with susceptibility to breast cancer [10], and codon 114 may be associated with lung cancer [11]. In Thailand, recent research showed that GSTP1 polymorphism was related to cancer progression in breast cancer patients [12].

PCR-RFLP was used to analyze the genetic polymorphism of GSTP1 in Thai HCC patients and to investigate the association between GSTP1 polymorphism and the clinicopathological parameters of HCC patients. The risk factors were determined by applying binary logistic regression to compare the frequencies of GSTP1 polymorphism in the control group and the patients. The survival status was determined using the Kaplan-Meier survival curve. Statistical significance was set at $p \leq 0.05$. It is hoped that this research will provide useful knowledge on the effects of genetic GSTP1 polymorphism in Thai HCC patients.

Materials and Methods

Sample Collection and DNA Isolation

Forty-four HCC DNA samples were extracted from formalin-fixed, paraffin-embedded tissues obtained from the National Cancer Institute of Thailand. The DNA was collected using the High Pure PCR Template Preparation Kit (Roche Diagnostics, Germany) from the peripheral blood of 52 healthy control subjects who had no history of cancer.

Analysis of the GSTP1 Polymorphism by PCR-RFLP

Gene polymorphisms in GSTP1 exon 5 and exon 6 (rs1695:313 A > G, and rs1138272:341C > T) were analyzed using PCR-RFLP. For GSTP1 exon 5, the 206-bp amplicon fragment was amplified using forward primer (5′-GGC TCT ATG GGA AGG ACC AGC-3′) and reverse primer (5′-AAG GGG TCA GCC CAA GCC A-3′) in a final volume of 25 μL, consisting of 1.5 mm $MgCl_2$, 0.3 mm of each dNTPs, 0.2 μm of each primer, 2.5 U Taq DNA polymerase (PL1202; Vivantis Technologies Sdn. Bhd., Selangor Darul Ehsan, Malaysia) in PCR buffer containing 500 mm KCl, 100 mm Tris-HCl (pH 9.1) and 0.1% Triton™ X-100, and DNA template 100 ng. The thermal cycling conditions were initial denaturation at 94°C for 2 min, followed by 35 cycles of amplification as follows: a denaturing step at 94°C for 5 s, a primer annealing step at 60°C for 5 s, then an extension step at 72°C for 15 s, and a final extension step was done at 72°C for 5 min. For GSTP1 exon 6, the 280-bp amplicon fragment was amplified using forward primer (5′-GCA GAG GAG AAT CTG GGA CTC T-3′) and reverse primer (5′-GGC TCA CAC CTG TGT CCA TCT G-3′) in a final volume of 25 μL, consisting of 1.5 mm $MgCl_2$, 0.3 mm of each dNTPs, 0.2 μm of each primer, 2.5 U Taq DNA polymerase (PL1202; Vivantis Technologies Sdn. Bhd., Selangor Darul Ehsan, Malaysia) in PCR buffer containing 500 mm KCl, 100 mm Tris-HCl (pH 9.1) and 0.1% Triton™ X-100, and DNA template 100 ng. The thermal cycling conditions were initial denaturation at 95°C for 2 min, followed by 35 cycles of amplification as follows: a denaturing step at 95°C for 30 s, a primer annealing step at 67°C for 30 s, then an extension step at 72°C for 30 s, and a final extension step was done at 72°C for 5 min.

The amplicon polymorphism fragments of GSTP1 exon 5 and exon 6 were analyzed by digesting with *Bsm*AI (R0529; New England Biolabs, Ipswich, MA, USA) and *Aci*I (R0551; New England Biolabs, Ipswich, MA, USA), respectively. For GSTP1 exon 5 polymorphism, homozygous A/A (wild type) individuals had a single fragment length of 206 bp, heterozygous A/G individuals had three fragments of 206, 120, and 86 bp and homozygous G/G individuals had two fragments of 120 and 86 bp. For GSTP1 exon 6 polymorphism, homozygous C/C (wild type) individuals had two fragments of 170 and 110 bp, heterozygous C/T individuals had three fragments of 280, 170, and 110 bp, and homozygous T/T individuals had a single fragment of 280 bp. All the PCR-RFLP reaction products were analyzed using 2% agarose gel electrophoresis.

Statistical Analysis

The GSTP1 genotype frequencies of the control group and patients were compared using the χ^2 test. The association between the control genotype and the disease was evaluated using binary logistic regression. The relationship between the clinicopathological parameters of the patients and the GSTP1 polymorphism was determined using the χ^2 test. Survival status was determined using the Kaplan-Meier survival method and the log-rank test. A p value lower than 0.05 was considered statistically significant.

Results

The Relationship between the GSTP1 Polymorphism Frequencies of the Control Group and the Patients

The GSTP1 genetic polymorphism was analyzed using the PCR-RFLP technique. The overall mutant exon 5 (A/G) and exon 6 (C/T) frequencies of the control samples were 32.7 and 0.00%, respectively, and those of the patients were 19.4 and 77.3%, respectively. After analysis

Table 1. *GSTP1* polymorphism status in hepatocellular carcinoma (HCC) patients and the control group

GSTP1 status	Exon 5		Exon 6	
	control	HCC	control	HCC
Wild type	35 (67.3%)	29 (80.6%)	49 (100%)	10 (22.7%)
Mutant	17 (32.7%)	7 (19.4%)	0 (0.0%)	34 (77.3%)
Total	52	36	49	44
Odds ratio (95% CI)	0.50 (0.18–1.36)		**4.40** (2.55–7.59)	
p value	0.170		**0.000***	

* Significant.

Table 2. *GSTP1* Exon 5 polymorphism status and clinicopathological parameters of hepatocellular carcinoma patients

Parameter	*GSTP1* status		*p* value
	wild-type A/A, *n* (%)	mutant A/G, *n* (%)	
Stage			0.356
I + II	18 (78)	2 (8)	
III	2 (8)	1 (4)	
Tumor size			0.566
≤3 cm	4 (13)	0 (0)	
>3 cm	25 (69)	7 (18)	
Differentiation			0.485
WD	2 (9)	0 (0)	
MD	16 (68.5)	2 (9)	
PD	2 (9)	1 (4.5)	
Sex			1.000
Female	6 (17)	1 (2)	
Male	23 (64)	6 (17)	
Age			0.674
≤50 years	13 (36)	2 (6)	
>50 years	16 (44)	5 (14)	

WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated.

Table 3. *GSTP1* Exon 6 polymorphism status and clinicopathological parameters of hepatocellular carcinoma patients

Parameter	*GSTP1* status		*p* value*
	wild-type C/C, *n* (%)	mutant C/T, *n* (%)	
Stage			0.574
I + II	7 (25)	17 (61)	
III	2 (7)	2 (7)	
Tumor size			0.624
≤3 cm	2 (5)	5 (12)	
>3 cm	7 (16)	29 (67)	
Differentiation			**0.070**
WD	3 (11)	1 (4)	
MD	4 (16)	16 (53)	
PD	2 (8)	2 (8)	
Sex			**0.015***
Female	6 (14)	6 (14)	
Male	4 (9)	28 (63)	
Age			0.452
≤50 years	2 (5)	13 (30)	
>50 years	8 (18)	21 (47)	

WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated. * Significant.

using the χ^2 test, there were no differences between the control group and the patients in GSTP1 exon 5 ($p \geq 0.05$), but the data showed a statistical difference in GSTP1 exon 6 ($p = 0.00$).

Only GSTP1 exon 6 polymorphism (C/T) was shown to be associated with a high risk of hepatocellular carcinogenesis (OR = 4.40). While the GSTP1 exon 5 polymorphism (A/G) was not related to the risk factors for HCC (OR = 0.50), the data are summarized in Table 1.

Statistical Analysis of GSTP1 Frequencies and the Clinicopathological Parameters of the Patients

The relationship between the clinicopathological parameters of the HCC patients and GSTP1 polymorphisms was observed, as shown in Tables 2 and 3. The GSTP1 exon 5 genotype showed no significant difference in stage, size of tumor, differentiation, gender, and the patients' age at diagnosis ($p > 0.05$). Recently, the GSTP1 exon 6 genotype was found to be correlated with male patients ($p = 0.015$), and it tended to be associated with cancer differentiation ($p = 0.070$).

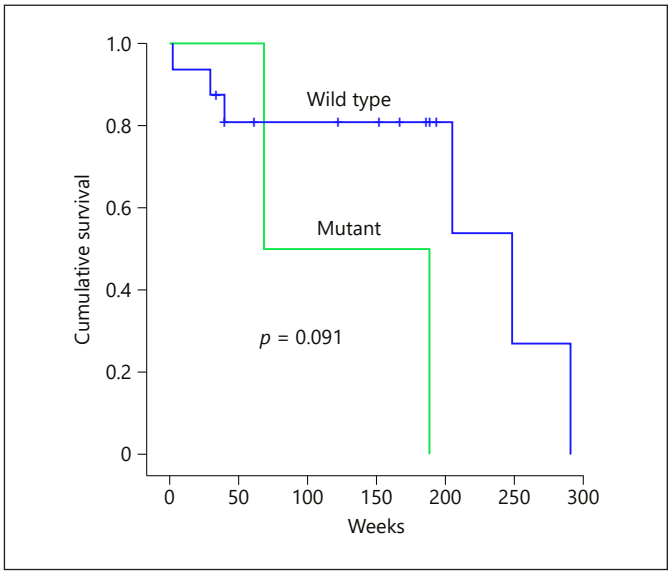

Fig. 1. Kaplan-Meier survival curve for the patients with hepatocellular carcinoma according to GSTP1 exon 5 polymorphisms.

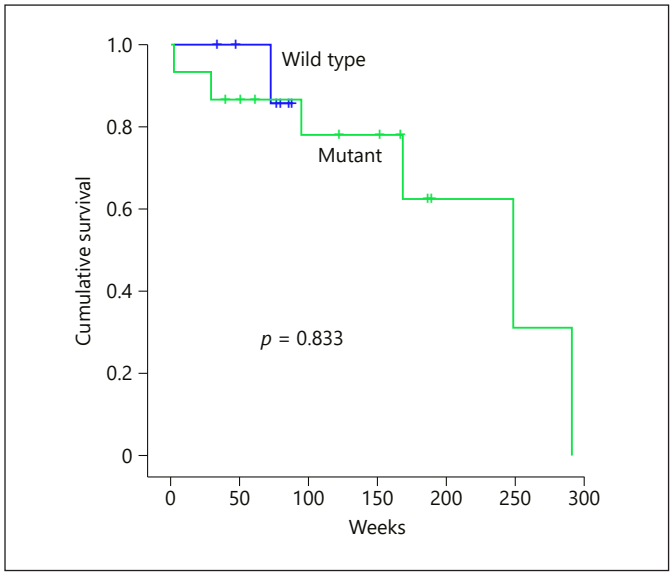

Fig. 2. Kaplan-Meier survival curve for the patients with hepatocellular carcinoma according to GSTP1 exon 6 polymorphisms.

Survival of Patients with GSTP1 Polymorphisms

The survival analysis showed that there was no association between GSTP1 polymorphism and patient survival (Fig. 1 and 2, $p = 0.091$ and 0.833, respectively).

Discussion/Conclusion

The GSTP1 gene was involved in the detoxification of carcinogenic electrophiles and genetic polymorphism led to a loss of enzyme catalytic activity, resulting in an increase in harm to the cell or DNA from various toxic agents [13]. Previous studies have indicated that GSTP1 exon 5 polymorphism increased the risk of hepatocellular carcinogenesis [14, 15]. Conversely, the results of our study found no significant difference between the control group and Thai HCC patients with wild-type Ile105/Ile105 and heterozygous genotype Ile105/Val105 ($p = 0.170$) and the risk of HCC (OR = 0.50). However, we found a relationship between the wild-type Ala114/Ala114 and heterozygous genotype Ala114/Val114 ($p = 0.00$) that increased the risk for HCC (OR = 4.40). We analyzed the relationship between GSTP1 polymorphism and the clinicopathological parameters; the results showed that the GSTP1 exon 6 mutant genotype was associated with the gender of patients ($p = 0.015$). The frequency of mutant genotype in male patients was higher than in female patients (male 63%, female 14%).

Males are more likely to develop chronic hepatitis, cirrhosis, and HCC from the hepatitis virus than females because the effect of the estrogen hormone decreases hepatic inflammation and viral production [16, 17]. However, HCC was found in males two to four times more often than in females due to behavioral risk factors such as smoking and alcohol consumption. Smoking and alcohol use are more common in males than in females [7, 18] damaging the liver tissue and DNA structure by oxidative stress and inflammation. It is already known that oxidative stress is correlated with the pathogenesis of HCC. Oxidative stress is an oxidation process that occurs after the body has been exposed to harmful factors, which produces excess reactive oxygen species. Glutathione S-transferases, especially in GSTP1, are involved in the reactive oxygen species detoxification pathway [19]. Previous research noted the correlation between GSTP1 and oxidative stress in HCC. The results showed that a decrease in the GSTP1 expression might elevate oxidative stress and promote hepatocellular carcinogenesis [20].

Research into the relationship between GSTP1 exon 6 polymorphism and overall cancer risk has been limited. Previous studies only identified an association with lung cancer susceptibility [21]. This research is the first to provide data on the effects of GSTP1 polymorphism risks for HCC in Thailand. We found that GSTP1 exon 6 polymorphism was related to an increased risk of hepatocellular carcinogenesis, and it tended to be related to cancer differentiation.

In conclusion, we found that GSTP1 exon 6 polymorphism was associated with an increased risk for HCC in male patients, and it tended to be related to cancer differentiation. However, no association was found between GSTP1 exon 5 polymorphism and the risk for HCC.

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Statement of Ethics

The study was approved by the Rangsit University Ethics Committee (No. RSUERB2019-050) and the Ethics Committee of the National Cancer Institute, Thailand (No. EC COA 017/2019). As this was a retrospective study, informed consent was not required.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

T.S. carried out the molecular studies and participated in the draft of the manuscript. P.S. performed the sample preparation and statistical analysis. T.P. conceived the project, analyzed the field data and wrote on the draft of the manuscript. All the authors contributed to the manuscript conceptualization and editing and approved the final manuscript.

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