

• BRIEF REPORTS •

Polymorphism of glutathione *S*-transferase mu 1 and theta 1 genes and hepatocellular carcinoma in southern Guangxi, China

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Supported by The Natural Scientific Foundation of China No. 39860032; by the Education Department of Guangxi Zhuang Autonomous Region No. 98-2-8

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Received: 2004-03-06 **Accepted:** 2004-04-13

Abstract

AIM: Glutathione *S*-transferase mu 1 (GSTM1) and theta 1 (GSTT1) genes are involved in the metabolism of a wide range of carcinogens, but deletions of the genes are commonly found in the population. The present study was undertaken to evaluate the association between GSTM1 and GSTT1 gene polymorphisms and hepatocellular carcinoma (HCC) risk.

METHODS: The genetic polymorphisms were studied at an aflatoxin highly contaminated region in Guangxi, China. Polymerase chain reaction (PCR) technique was used to detect the presence or absence of the GSTM1 and GSTT1 genes in blood samples. The case group was composed of 181 patients of HCC identified by the pathologists and the control group was composed of 360 adults without any tumor.

RESULTS: The frequencies of GSTM1 and GSTT1 null genotypes in the control were 47.8% and 42.7%, while those in the HCC group were 64.6% and 59.7%, respectively. The differences between HCC group and control group were very significant ($P < 0.01$). GSTM1 and GSTT1 combined null genotypes in HCC group and control group were 38.2% and 18.5% respectively, and the difference was significant ($P < 0.05$).

CONCLUSION: The GSTM1 and GSTT1 null genotypes are associated with an increased risk of HCC in a special geographic environment. Combination of the two null genotypes in an individual is substantially increased twice the risk of HCC.

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Key words: Hepatocellular carcinoma; Glutathione *S*-transferase mu 1; Glutathione *S*-transferase theta 1; Polymorphism

Deng ZL, Wei YP, Ma Y. Polymorphism of glutathione *S*-transferase mu 1 and theta 1 genes and hepatocellular carcinoma in southern Guangxi, China. *World J Gastroenterol* 2005; 11(2): 272-274

<http://www.wjgnet.com/1007-9327/11/272.asp>

INTRODUCTION

Glutathione *S*-transferases (GSTs) M1 and T1 are most important

detoxified enzymes in the body that participate in the metabolism of a wide range of chemical carcinogens that are ubiquitous in the environment^[1-3], but the enzyme deficiency is common in humans. Are there any cause and effect on the high prevalence of hepatocellular carcinoma (HCC) in a local region? Epidemiological investigations and animal experiments have identified that the major risk factors involved in the prevalence of HCC in southern Guangxi are hepatitis B virus (HBV) infection and aflatoxin B1 (AFB1) exposure^[4,5], and etiological studies have given evidence to support the existence of a synergistic relationship between HBV and AFB1^[6-8]. AFB1 is a mycotoxin that is produced by *Aspergillus flavus*. Since aflatoxin was discovered in food in early 1960s, the search for mycotoxins has led to the identification of over 100 toxicogenic fungi and more than 300 mycotoxins. While AFB1 is the most vigorous carcinogen, its mutagenic metabolites binding to DNA are capable of inducing G to T transversions and the liver is a primary target organ^[9,10]. It has been found that AFB1 could contaminate foods such as corn and peanut in the world, and it is known that the southwest of Guangxi is one of the areas with a rather high level of contamination and a high prevalence of HCC^[10]. Therefore, study need to lay stress on detoxification of AFB1 by enzymes in an individual and susceptibility to HCC. AFB1 is not harmful prior to metabolic activation via oxidase of cytochrome P450 to form a DNA damaging agent, AFB1-8, 9-epoxide^[11]. Several enzymes in the body can resist this toxin and are good for health. The present study emphasized the importance of polymorphisms of GSTM1 and GSTT1, especially their genetic deletion polymorphisms and susceptibility to HCC.

MATERIALS AND METHODS

Patients and controls

One hundred and eighty-one HCC patients and 360 controls participated in the present investigation. The HCC cases were recruited from the Affiliated Hospital of Guangxi Medical University from January 1998 to December 2002. All HCCs were confirmed by pathologic diagnosis. There were 145 male and 36 female patients aged from 28 to 70 years with an average age of 49 years. The control group came from the same hospital with their age and sex matched for the case group.

Blood samples

Three mL of blood was taken by venous puncture. The blood was used for lymphocyte isolation using buffy coat extraction kit. Genomic DNA was prepared by standard phenol-chloroform extraction.

Genotypes for the GSTM1 and GSTT1 deletions were determined by polymerase chain reaction (PCR) on the genomic DNA. Primers binding to the 5' region of exon 4 (5'-CTGCCCTA CTTGATTGATGGG-3') and the 3' region of exon 5 (5'-CTGGAT TGTAGCAGATCATGC-3') of GSTM1 were used to amplify a 273 base pair (bp) fragment. Primers for the 5' region of GSTT1 (5'-TTCCTTACTGGTCCTCACATCTC-3') and the 3' region (5'-TCACCGGATGGCCAGCA-3') were used to amplify a 480 bp fragment. In both assays, the absence of PCR products was

indicative of the null genotypes^[12,13]. In the cases of GSTM1 or GSTT1 null genotypes, the samples must have internal controls by a pair of β -globin or p53 gene primer co-amplification repeat analysis to monitor the quality control to exclude possible pseudo-negative reactions^[13].

PCR reaction was carried out in a "BIO-RAD" amplified instrument. A commercial PCR kit was used with 5 μ g of DNA, 2.5 mmol/L of dNTP, 5 μ mol/L of each primer, 25 mmol/L of $MgCl_2$ and 0.5 units of Taq polymerase in a total volume of 25 μ L, then overlaid with a drop of mineral oil and proceeded to amplification.

PCR amplified conditions

The samples were denatured at 94 °C for 5 min, then treated in a different way for GSTM1 and GSTT1 gene amplification. As for the amplification of GSTM1 gene, the best condition was for 30 s at 93 °C and for 45 s at 50 °C and at 72 °C for 45 s, while for the GSTT1 gene, the best condition was for 45 s at 94 °C and 50 s at 61 °C and at 72 °C for 60 s, respectively. After 35 cycles, a final step of extension at 72 °C for 10 min was followed. The amplified products were subjected to electrophoresis on a 2% agarose gel and stained with ethidium bromide, observed under violet light.

Statistical analysis

The experimental results were analyzed by χ^2 -test.

RESULTS

PCR products from amplification of GSTT1 (480 bp) and GSTM1 (273 bp) on agarose gels are shown in Figure 1.

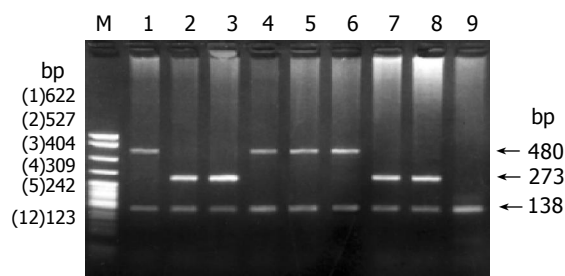


Figure 1 Amplification of GSTT1 (480 bp), GSTM1 (273 bp), and the control p53 (138 bp) gene products illustrated in agarose gel electrophoresis. M: pBR322 DNA/MspI. Lane 1: positive reaction of GSTT1 in a control case; Lane 2-3: positive reaction of GSTM1 in a control case; Lane 4-6: positive reaction of GSTT1 in HCC cases; Lane 7-8: positive reaction of GSTM1 in HCC cases; Lane 9: negative reaction of GSTM1 in a HCC case, p53 positive reaction in each case from lanes 1 to 9.

The frequencies of GSTT1 null genotype in HCC group and control group were 59.7% (108/181) and 42.7% (154/360) respectively. The frequencies of GSTM1 null genotype in HCC group and control group were 64.6% (117/181) and 47.8% (172/360) respectively. The differences were very significant ($P < 0.01$, Table 1). In some of the cases, both GSTM1 and GSTT1 null genotypes that occurred in HCC group and control group were 38.2% and 18.5% respectively, and the difference was significant ($P < 0.05$). GSTM1-positive but GSTT1-negative cases accounted for 21.8% and 20.7%, GSTM1-negative but GSTT1-positive cases were 25.5% and 28.9% respectively, and the two groups had no significant difference compared with the controls.

Table 1 Polymorphisms of GSTM1 and GSTT1 genes in HCC patients and controls

Group	n	GSTM1			GSTT1		
		Null	Present	Deletion rate (%)	Null	Present	Deletion rate (%)
HCC	181	117	64	64.6	108	73	59.7
Control	360	172	188	47.8 ¹	154	206	42.7 ²

HCC group compared with control ¹ $\chi^2 = 13.7643$, $P < 0.01$
² $\chi^2 = 13.7585$, $P < 0.01$.

DISCUSSION

GSTs are a super family, in which seven classes have been found, but only class μ (GSTM1) and class θ (GSTT1) have gene deficiency (null genotype)^[14] and completely lack of respective enzyme activity. Carcinogen AFB1-8, 9-epoxide is a substrate of both GSTM1 and GSTT1^[13,14]. GSTs are dimeric proteins that could catalyze conjugation reaction between glutathione and epoxides facilitate excretion and detoxification^[17]. As a result, GSTs may play an important role in anti-carcinogenesis. The absence of these enzymes might susceptible to several cancers^[18]. A previously report failed to detect a statistically significant relationship between GSTM1 genotype and HCC case/control status might be due to an inadequate sample size^[11]. We investigated the GSTs genotypes in the area of high prevalence of HCC with enough cases and control participants^[16]. In our previous study^[10] we found that HCC patients were significantly associated with tumor suppressor gene p53 mutation at codon 249 G to T transversion which may be involved in environmental mutagen AFB1^[19]. Here, we are interested in the GSTM1 and GSTT1 genotypes associated with susceptibility to HCC in the natives of Guangxi and whether HCC patients possess more GSTM1 or GSTT1 null genotypes than other people.

Cheng^[20] reported that smokers of deficiency in GSTM1 and GSTT1 genes were predisposed to head and neck squamous cell carcinoma. Dialyna^[21] found that GSTM1 and GSTT1 null genotypes were correlated with lung cancer in heavy smokers. The present results suggest that natives in southwest Guangxi had a rather higher level of GSTM1 and GSTT1 null genotypes, their GSTM1 null genotype accounted for 47.8%, a high level in the world. While GSTT1 null genotype accounted for 42.7%, much higher than the average level reported^[22-24]. It is known that people with high GSTs null genotypes who have never contacted with relevant chemical toxins such as AFB1 will never increase the risk of HCC. However, the people who live in an AFB1 contaminated area for a long time would fully reveal genetic defects and susceptibility to HCC. The present study showed that GSTM1 or GSTT1 null genotypes were higher in the HCC group than in control group, and the differences were very significant ($P < 0.01$). There was not any relationship between their age and sex status ($P > 0.05$). The frequency of combined GSTM1 and GSTT1 deletions in HCC group versus control group was 38.2% and 18.5%, respectively ($P < 0.05$). Actually, there was twice more the risk of HCC in the GSTM1 and GSTT1 combined null genotypes in patients than in control.

In conclusion, the risk of HCC is associated with GSTM1 and GSTT1 null genotypes, especially in people contacted with AFB1. The natives in Guangxi have a high level of GSTM1 or/and GSTT1 null genotypes. AFB1 undergoes metabolism by GSTM1 and GSTT1 enzymes, an individual lacking of these enzymes should predispose to HCC. Genetic susceptibility due to GSTM1 and GSTT1 null genotypes in humans occurs in conjunction with exposure to environmental carcinogens such as AFB1 involved in the pathogenesis of HCC, especially in an area with hepatitis B prevalence.

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Edited by Wang XL and Zhang JZ