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Genetic polymorphisms of glutathione *S*-transferases M1 and T1 associated with susceptibility to aflatoxin-related hepatocarcinogenesis among chronic hepatitis B carriers: a nested case–control study in Taiwan

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This study was conducted to investigate the modifying effect of glutathione *S*-transferase (GST) M1 and T1 polymorphisms on aflatoxin-induced hepatocarcinogenesis among chronic hepatitis B virus surface antigen (HBsAg) carriers. A total of 79 HBsAg-positive cases of hepatocellular carcinoma (HCC) diagnosed between 1991 and 1997 were identified and individually matched to one or two HBsAg-positive controls on age, gender, residence and date of recruitment from the same cancer screening cohort in Taiwan. Blood samples were tested for hepatitis B and C viral markers by enzyme immunoassay and for aflatoxin B_1 (AFB_1)–albumin adducts by competitive enzyme-linked immunosorbent assay. *GSTM1* and *GSTT1* genotypes were determined by PCR. There was a statistically significant relationship between detectable levels of AFB_1–albumin adducts in serum and risk of HCC among chronic HBsAg carriers, with an adjusted odds ratio (OR) of 2.0 [95% confidence interval (CI) 1.1–3.7]. In addition, the effect of aflatoxin exposure on HCC risk was more pronounced among chronic HBsAg carriers with the *GSTT1* null genotype (OR 3.7, 95% CI 1.5–9.3) than those who were non-null (OR 0.9, 95% CI 0.3–2.4). The interaction between serum AFB_1–albumin adduct level and *GSTT1* genotype was statistically significant ($P = 0.03$). For *GSTM1* the effect of aflatoxin exposure on HCC risk in those with the null genotype was also greater (adjusted OR 2.8, 95% CI 1.0–7.8) than in those with the gene present (adjusted OR 1.8, 95% CI 0.8–4.5), but the difference was not significant ($P = 0.91$). Notably, when the interaction between aflatoxin exposure and *GSTT1* genotype was considered, aflatoxin exposure by itself was not a significant determinant of HCC risk among chronic HBsAg carriers. These results demonstrate the importance of gene–environment interactions in the multifactorial development of HCC.

Abbreviations: AFB_1, aflatoxin B_1; AFP, α-fetoprotein; ALT, alanine transaminase; AST, aspartate transaminase; CI, confidence interval; GST, glutathione *S*-transferase; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; mEH, microsomal epoxide hydrolase; OR, odds ratio.

Introduction

Primary hepatocellular carcinoma (HCC) is a tumor that occurs at high frequencies in southeast Asia and tropical Africa (1). In Taiwan HCC is the leading cancer in males and the fifth in females (2). Etiologically HCC is a complex and multifactorial disease that is linked to both viral and chemical carcinogens (3–7). On the basis of epidemiological evidence, 70–90% of HCC patients in Taiwan are seropositive for hepatitis B virus surface antigen (HBsAg) or antibodies to hepatitis C virus (HCV) (5), indicating that hepatotropic viral infections are major causes of HCC in this area. Nonetheless, there are non-viral causes in 10–30% of HCC cases. It therefore seems likely that risk factors other than viral infection also contribute to the development of HCC in Taiwan.

Aflatoxin B_1 (AFB_1) is a hepatocarcinogen in several animal species (8). In humans epidemiological studies using urine and blood samples banked several years prior to diagnosis have shown that the presence of aflatoxin metabolites in urine and aflatoxin–albumin adducts was associated with a significantly elevated risk of HCC (4,9–11). In Taiwan several epidemiological studies have noted a significant aflatoxin–liver cancer link. Our previous case–control study nested in a cancer screening program with 56 cases of HCC and 220 matched controls from the same cohort of >25 000 individuals found that in hepatitis B virus (HBV)-infected males there was an adjusted odds ratio (OR) of 2.8 for detectable compared with non-detectable aflatoxin–albumin adducts and 5.5 for high compared with low levels of aflatoxin metabolites in urine (10). A nested case–control study in another cohort in Taiwan observed a dose–response relationship between urinary levels of AFM_1 (the primary oxidative metabolite of AFB_1) and HCC in chronic HBV carriers (11).

Accumulating evidence indicates that susceptibility to cancer is mediated by genetically determined differences in the effectiveness of detoxification of potential carcinogens (12). Indeed, it has been recognized that the carcinogenic potential of AFB_1 may vary with the exposed individual's capacity to detoxify its mutagenic metabolite, aflatoxin 8,9 epoxide. Epidemiological studies have suggested that genetic polymorphisms in AFB_1 metabolizing enzymes are a factor in individual susceptibility to aflatoxin-related HCC (13,14). Members of the glutathione transferase (GST) family, such as GST-μ (*GSTM1*) and GST-θ (*GSTT1*), are important candidates for involvement in susceptibility to aflatoxin-associated liver cancer because they may regulate an individual's ability to metabolize the ultimate carcinogen of aflatoxins, the *exo*-epoxide (15). To date the human cytosolic GST superfamily contains at least 16 genes subdivided into eight distinct classes designated α, Φ, μ, π, σ, θ, ζ and ω (16). A number of recent reviews have summarized the role of GST-μ (*GSTM1*) and GST-θ (*GSTT1*) in the metabolism of and induction by numerous known or suspected carcinogenic compounds (17,18). For *GSTM1* there are four alleles *GSTM1**A, *GSTM1**B, *GSTM1**0, and *GSTM1**1χ2 (16). To date there is no evidence

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that the *GSTM1**A and *GSTM1**B alleles are functionally different from one another, while *GSTM1**0 exhibits absence of enzymatic activity. Collectively, *GSTM1* genotypes can be categorized into two classes: the homozygous deletion genotype (denoted *GSTM1* null genotype) and genotypes with one or two undeleted alleles (denoted *GSTM1* non-null genotype). Similarly, two functionally different *GSTT1* genotypes have been identified (19): the homozygous deletion genotype (denoted *GSTT1* null genotype) and genotypes with one or two undeleted alleles (denoted *GSTT1* non-null genotype). These deletion variants have been useful for molecular epidemiological studies of cancer because they divide study subjects into two well-defined susceptibility classes: those who are and those who are not able to detoxify potential carcinogens by the metabolic pathways regulated by *GSTM1* and *GSTT1*. Accordingly, a report by Chen *et al*. documented that a biological gradient between serum AFB_1–albumin adduct levels and HCC risk was observed among chronic HBsAg carriers who had null *GSTM1* and *GSTT1* genotypes but not among those who had non-null genotypes (14). In the present work we have continued to follow up the cohort, expanding the number of HCC cases analyzed for AFB_1–albumin adducts as well as determining the effects of *GSTM1* and *GSTT1* genotypes on aflatoxin-related hepatocarcinogenesis among chronic HBV carriers.

Materials and methods

Study cohort

This nested case–control study was conducted within a cancer screening cohort of individuals who were between 30 and 64 years old and lived in seven townships in Taiwan. The cohort characteristics and methods of screening and follow-up have been described previously (10,20). From July 1990 to June 1992 a community-based cancer screening project was carried out in seven townships. Makung, Huhsi and Paihsa Townships are located on the Penghu Islets, an area with the highest mortality rate of HCC in Taiwan (21). The other four townships, Sanchi, Chutung, Potzu and Kaoshu, are located on the main Taiwan Island. There were 47 079 male and 42 263 female eligible individuals who were invited by letter to participate. A total of 12 024 males and 13 594 females enrolled. All study subjects participated on a voluntary basis after giving informed consent.

Participants were personally interviewed based on a structured questionnaire at recruitment. Blood samples were collected from each study subject. Aliquots of serum, buffy coat, plasma and red blood cells were separated and stored at –70°C. Specimens were transported in dry ice to the central laboratory at the National Taiwan University and were kept in deep freezers until examination.

In the program study subjects were first screened by serological markers, including alanine transaminase (ALT), aspartate transaminase (AST), α-fetoprotein (AFP), HBsAg and anti-HCV. Any subject who had an elevated level of ALT (⩾45 IU/ml), AST (⩾40 IU/ml) or AFP (⩾20 ng/ml), positivity for HBsAg or anti-HCV or a family history of HCC or liver cirrhosis among first degree relatives was referred for upper abdominal ultrasonography examination by board certified gastroenterologists. Subsequently, participants who were found to be affected with liver cirrhosis by ultrasonography were intensively followed up every 3 months, whereas others were examined annually. Any suspected HCC cases thus identified were referred to teaching medical centers for confirmatory diagnosis by computerized tomography, digital subtracted angiogram, aspiration cytology and pathological examination. Therefore, a final diagnosis of HCC was based on either histological/cytological findings or elevated AFP levels >400 ng/ml combined with at least one positive image on an angiogram or by ultrasonography and/or computerized tomography.

Study subjects

There were 99 confirmed cases of HCC, including 79 HBsAg-positive and 20 HBsAg-negative individuals, identified during the follow-up period between 1991 and 1997. To examine the modulatory effect of *GSTM1* and *GSTT1* genotype profiles on aflatoxin-related HCC risk among chronic HBsAg carriers we selected 79 HCC cases who were chronic HBsAg carriers as the case group for this nested case–control study. On the basis of the availability of control subjects one or two HBsAg-positive individuals who were not suspected to be affected with HCC by ultrasonography were randomly selected as controls and were matched with each case on age (±5 years), gender, residential township and date of recruitment (±3 months). There were nine case–control sets with one case matched to one control and 70 sets with one case and two controls, for a total of 79 HCC cases and 149 controls.

AFB1–albumin adducts in serum

An enzyme-linked immunosorbent assay was used to determine the level of AFB_1–albumin adducts in serum as previously described (9,10). Briefly, 50 µl of albumin extracts, equivalent to 200 µg albumin, were added to 96-well plates (Easywash, Corning, NY) previously coated with 3 ng AFB_1 epoxide-modified human serum albumin. Polyclonal antiserum 7 was used at $1{:}2\times10^5$ dilution and the secondary antiserum, goat anti-rabbit IgG–alkaline phosphatase conjugate (Boehringer Mannheim), was used at 1:750 dilution. The substrate was *p*-nitrophenyl phosphate (Sigma, St Louis, MO) and absorbance at 405 nm was read on a Dynatech MR5000 96-well plate reader (Dynatech, Chantilly, VA). This assay had 50% inhibition of antiserum binding at 10–20 fmol AFB_1 adduct/well. The limit of sensitivity (20% inhibition) when assaying the equivalent of 200 µg albumin/well was 0.01 fmol/µg. Samples were assayed by duplicate analysis in duplicate wells; samples with <20% inhibition were considered non-detectable. Two control samples were analyzed with each batch of sera, a pooled sample of plasma from non-smoking US subjects and a positive control of serum from a rat treated with 1.5 mg AFB_1.

In this study serum specimens were grouped into case–control pairs and were assayed on the same day to minimize any effects of day-to-day laboratory variation. Because specimens from cases and controls were treated similarly, differential losses should not have occurred, so case–control comparison on a relative scale should not be affected. In addition, laboratory personnel were kept blind as to case or control status.

GSTM1 and GSTT1 genotypes

GSTM1 genotyping for gene deletion was performed by PCR amplification with primers for exons 6 and 7, which produced a 210 bp band, according to the method of Bell *et al*. (22). *GSTT1* genotype was determined using the technique of Pemble *et al*. (19), with the modification that β-globin primers were added to the PCR.

Statistical methods

In the present report the serum level of AFB_1–albumin adducts was analyzed as a binary rather than continuous variable. Adjusted ORs and 95% confidence intervals (CI), which were derived from conditional logistic regression models, were used to indicate the magnitude of the association between serum levels of AFB_1–albumin adducts and HCC risk. The relationship between AFB_1–albumin adduct level and HCC risk was further examined through stratified analyses for different groups categorized by *GSTM1* and *GSTT1* genotype. The statistical significance for the interaction terms between serum AFB_1–albumin adduct level and *GSTM1* and *GSTT1* genotype was also examined through logistic regression analysis. All analyses were performed with SAS software (SAS Institute, Cary, NC).

In the present study four cases and nine controls were excluded from the analysis because of inadequate blood samples for detection of AFB_1–albumin adducts in serum. Additionally, due to the failure of *GSTM1* genotyping in 10 cases and 21 controls and of *GSTT1* genotyping in 12 cases and 21 controls, these subjects were excluded from data analyses for the relevant variables. Nevertheless, in their distributions by sex, age and residential area subjects for whom specimens were available for study were similar to those for whom specimens were unavailable.

Results

This study included 79 (66 male and 13 female) cases and 149 (122 male and 27 female) controls (Table I). The mean age of both case and control subjects was 53 ± 7 years. The distribution of residential area was similar for cases and controls. In addition, cases and controls had non-significant differences in frequency distributions for cigarette smoking (53.2 and 44.3%, respectively) and alcohol consumption (21.8 and 18.1%, respectively). Anti-HCV was detected in 10.8% (8/74) of HCC cases and 7.9% (11/140) of controls.

The risk of HCC associated with serum level of AFB_1–albumin adducts and of *GSTM1* and *GSTT1* genotype is shown in Table II. HCC cases had a higher percentage of detectable AFB_1–albumin adducts than controls (62.7 versus 45.7%), resulting in a statistically significantly adjusted OR of 2.0

Table I. Baseline characteristics of 79 cases with HCC and 149 matched controls

Variable	HCC cases		Controls		*P*
	No.	Percent	No.	Percent	
Sex					
Male	66	83.5	122	81.9	0.856
Female	13	16.5	27	18.1	
Age					
30–39	5	6.3	9	6.0	0.779
40–49	14	17.7	32	21.5	
50–59	41	51.9	80	53.7	
60–64	19	24.1	28	18.8	
Residence					
Taiwan Island	24	30.4	48	32.2	0.881
Penghu Islets	55	69.6	101	67.8	
Smoking status					
Non-smokers	37	46.8	83	55.7	0.213
Smokers	42	53.2	66	44.3	
Drinking habit[a]					
Non-drinkers	61	78.2	122	81.9	0.311
Drinkers	17	21.8	27	18.1	
Anti-HCV[b] status[c]					
Negative	66	89.2	129	92.1	0.490
Positive	8	10.8	11	7.9	

[a]Data not available for one case.
[b]Anti-HCV, antibodies against hepatitis C virus.
[c]Data not available for five cases and nine controls.

Table II. Risk of HCC in relation to detectable levels of aflatoxin–albumin adducts and *GSTM1* and *8GSTT1* genotypes among chronic hepatitis B virus carriers

Variable	HCC cases		Controls		Adjusted OR[a]	95% CI
	No.	Percent	No.	Percent		
Aflatoxin–albumin adduct[b]						
Non-detectable	28	37.3	76	54.3	1.0	
Detectable	47	62.7	64	45.7	2.0	1.1–3.7
GSTM1[c]						
Non-null	43	62.3	51	39.8	1.0	
Null	26	37.7	77	60.2	0.4	0.2–0.7
GSTT1[c]						
Non-null	37	55.2	51	39.8	1.0	
Null	30	44.8	77	60.2	0.5	0.2–0.9

[a]Adjusted for sex, age and residence.
[b]Four cases and nine controls had inadequate serum samples for detection of AFB_1–albumin adducts.
[c]DNA samples were inadequate to do the *GSTM1* genotyping in 10 cases and 21 controls and the *GSTT1* genotyping in 12 cases and 21 controls.

(95% CI 1.1–3.7). In contrast to the expected distribution, the frequencies of the *GSTM1* and *GSTT1* null genotypes were significantly lower in HCC cases (37.7 and 44.8%, respectively) than in controls (60.2% for both the *GSTM1* and *GSTT1* null genotypes).

To address the hypothesis that the effect of detectable levels of AFB_1–albumin adducts in serum on HCC risk might vary according to *GSTM1* and *GSTT1* genotype data were further examined within categories stratified by *GSTM1* and *GSTT1* genotype. As shown in Table III, the effect of aflatoxin exposure on HCC risk was more pronounced among chronic HBsAg carriers with a *GSTT1* null genotype (OR 3.7, 95% CI 1.5–9.3) than those who had a non-null genotype (OR 0.9, 95% CI 0.3–2.4). The interaction between serum AFB_1–albumin adduct level and *GSTT1* genotype was statistically significant ($P = 0.03$). In addition, a borderline significant effect of detectable concentrations of AFB_1–albumin adducts on HCC risk was observed for chronic HBsAg carriers with a *GSTM1* null genotype (OR 2.8, 95% CI 1.0–7.8), but not for those with a *GSTM1* non-null genotype (OR 1.8, 95% CI 0.8–4.5). There was no significant interaction between aflatoxin exposure and *GSTM1* genotype in relation to HCC risk among chronic HBsAg carriers ($P = 0.91$).

The final multiple logistic regression model is shown in Table IV. This analysis revealed that there was a significant interaction of aflatoxin exposure with *GSTT1* genotype. It can be seen from the positive coefficient that a significant aflatoxin–HCC link was observed among chronic HBsAg carriers with a *GSTT1* null genotype but not among those who had a non-null genotype. In addition, individuals with *GSTM1* and *GSTT1* null genotypes appear to have a reduced risk of developing HCC. Of note, when the interaction between aflatoxin exposure and *GSTT1* genotype was entered into the model aflatoxin exposure by itself was not statistically significant.

Discussion

AFB_1 has long been suspected to play an etiological role in human hepatocellular carcinogenesis, largely because it produces liver tumors when administered to animals (23). Human exposure to aflatoxin has been assessed in the context of epidemiological studies either by questionnaire or by laboratory measurements on biospecimens, such as urine or peripheral blood samples. It has been noted that questionnaire assessment of individual exposures to aflatoxin is imprecise. However, the development of biomarker methods provides a more accurate means of determining an individual's exposure to aflatoxin. Among the various possible biomarkers of aflatoxin exposure the measurement of urinary aflatoxin metabolites reflects short-term exposure, while serum AFB_1–albumin adducts indicate exposure over longer time periods (24). Accordingly, epidemiological studies using biomarkers of aflatoxin exposure have noted a significant aflatoxin–liver cancer link in human populations. The data from a nested case–control study conducted in Shanghai revealed a highly significant increase in the relative risk (relative risk 3.4) for HCC cases in which urinary aflatoxins were detected (4). It has been noted that peanuts and peanut products are major sources of aflatoxins in foodstuffs in Taiwan (25). A non-significantly positive association between peanut consumption derived from questionnaire data and risk of HCC, as indicated by an OR of 1.4–1.9, was observed in previous epidemiological studies conducted in Taiwan (26,27). This weak association may result from the inadequacy of the questionnaire in measuring aflatoxin exposure. Subsequently, several nested case–control studies using biomarkers of aflatoxin exposure have been undertaken in Taiwan to examine the relationship between aflatoxin exposure and incidence of HCC, and have confirmed the results of the Shanghai study (10,11). Urinary levels of AFM_1 were linked to the risk of HCC in a dose-dependent fashion (11) and high levels of total urinary aflatoxin metabolites, as compared with low levels, were associated with a 5-fold increase in HCC risk in chronic HBV carriers (10). In addition, the presence of AFB_1–albumin adducts in serum resulted in a 3- to 5-fold elevation in the risk of developing HCC (9,10). In the present study we found

Table III. OR of HCC in relation to levels of aflatoxin–albumin adducts among chronic hepatitis B virus carriers, stratified by *GSTM1* and *GSTT1* genotypes

Aflatoxin–albumin adduct[a]	Null			Non-null		
	HCC cases (*n*)	Controls (*n*)	Adjusted OR[b] (95% CI)	HCC cases (*n*)	Controls (*n*)	Adjusted OR[b] (95% CI)
GSTM1[c]						
Non-detectable	8	38	1.0	16	27	1.0
Detectable	16	34	2.8 (1.0–7.8)	25	20	1.8 (0.8–4.5)
Test for interaction: $\chi^2 = 0.01$(1 df); $P = 0.91$						
GSTT1[c]						
Non-detectable	10	48	1.0	14	18	1.0
Detectable	19	25	3.7 (1.5–9.3)	20	28	0.9 (0.3–2.4)
Test for interaction: $\chi^2 = 4.48$(1 df); $P = 0.03$						

[a]Four cases and nine controls had inadequate blood samples for detection of aflatoxin–albumin adducts in serum.
[b]Adjusted for age, sex and residence.
[c]DNA samples were inadequate to do the *GSTM1* genotyping in 10 cases and 21 controls and the *GSTT1* genotyping in 12 cases and 21 controls.

Table IV. Multivariate analysis of risk determinants for HCC in chronic hepatitis B virus carriers

Variable	Coefficient	95% CI for coefficient	*P*
Aflatoxin–albumin adduct[a]	–0.11	–1.21, 0.99	0.84
GSTM1[b]	–1.21	–1.95, –0.46	0.02
GSTT1[c]	–1.69	–2.92, –0.45	0.01
Aflatoxin-albumin adduct × *GSTT1*	1.75	0.14, 3.36	0.03

[a]Non-detectable = 0, detectable = 1.
[b]Non-null = 0, null = 1.
[c]Non-null = 0, null = 1.

a statistically significantly adjusted OR of developing HCC for detectable AFB1–albumin adducts (adjusted OR 2.0, 95% CI 1.1–3.7), which is comparable with previous studies using the same biomarker of exposure to aflatoxins (9,10,14). Taken together, this and previous nested case–control studies (9–11) using specimens banked before disease onset have provided the most reliable data on the role of AFB_1 in hepatocarcinogenesis in Taiwan.

While the present study and previous reports (4,9–11,14) have demonstrated that aflatoxin exposure is an important risk factor for HCC, several epidemiological observations have implied that the carcinogenic potential of AFB_1 varies with the exposed individual's capacity to detoxify its mutagenic metabolite, aflatoxin 8,9 epoxide (13,14). In 1995 McGlynn *et al*. (13) reported on a cross-sectional study and a case–control comparison in Ghana and China to assess serum levels of AFB_1–albumin adducts and the development of HCC in relation to genetic polymorphisms of detoxifying enzymes involved in biotransformation of aflatoxins. The results support the existence of genetic susceptibility to the carcinogenic effect of AFB_1. A subsequent study in Taiwan gave results consistent with the prior results from the HCC endemic areas of Ghana and China; a biological gradient between serum AFB_1–albumin adduct levels and HCC risk was observed among HBsAg chronic carriers who had *GSTM1* and/or *GSTT1* null genotypes but not among those who had non-null genotypes (14). In this report we observed a significantly increased risk for aflatoxin-related HCC among chronic HBsAg carriers with the *GSTT1* null genotype but not among those who had a non-null genotype. However, while a similar trend was observed for *GSTM1*, the results were not statistically significant.

Rather unexpectedly, a significant inverse relationship between both *GSTM1* and *GSTT1* null genotypes and risk of HCC was observed in the current study. This finding is in conflict with the knowledge that individuals who are deletion homozygotes exhibit absence of enzymatic activity and are hypothesized to be at increased risk for the carcinogenic effects of a wide range of environmental exposures. The mechanisms responsible for this inverse association are currently unknown.

Associations between *GSTM1* and *GSTT1* null genotypes and cancer of the lung, bladder and colon have been reported, but the results are inconsistent across studies (12,18). In contrast, Rebbeck *et al*. (18) recently reported case–control study results that indicate that men with *GSTT1* present are at increased prostate cancer risk. Similarly, the current study observed that individuals who have presumably expressed and functional GST-θ protein are at increased risk of HCC. These findings are consistent with the knowledge that some metabolic intermediates in the GST-θ pathway of glutathione conjugation, i.e. dichloromethane, are mutagenic (28) and the production of mutagenic intermediates in the target site may be a mechanism promoting tumorigenesis. Another potential explanation derives from a follow-up study by Kelada *et al*. (29). This study looked at the interaction between smoking and *GSTM1* and *GSTT1* genotype and risk of prostate cancer. While the presence of a functional *GSTT1* genotype was confirmed as a risk factor for prostate cancer, no main effect of smoking was observed; a significant increased risk was observed in men who were both smokers and had a non-deleted *GSTT1* genotype. The authors suggest that in addition to the production of carcinogenic metabolic intermediates, response to chronic inflammation associated with smoking may be modulated by the *GSTT1* genotype. A similar inflammatory mechanism may take place in hepatocarcinogenesis. It has been documented that chronic HBV infection induces recurrent liver injury and a resultant inflammatory reaction (30). It is known that the GSTs are, in general, mediators of the inflammatory response (31). Thus it is possible that upon exposure to aflatoxins of chronic HBsAg carriers a more vigorous or more chronic inflammatory response is mounted among individuals with non-deleted *GSTM1* and *GSTT1* genotypes, which paves the way for carcinogenesis (32). Finally, it has been noted that GST levels are regulated by a number of factors, including diet, in addition to inherited variants in genes coding for the GST enzymes (33,34). In recent literature efforts appear to concentrate on the interplay between dietary inducers of GST and *GST* genotypes in cancer risk. For example, in a prospective

study of lung cancer risk investigators found that individuals with *GSTM1* null and *GSTT1* null polymorphisms appeared not to be at increased risk of lung cancer when they had detectable isothiocyanates in the urine (35). Thus confounding dietary factors, such as isothiocyanates in broccoli conjugated to GSTs, have recently been suggested to contribute to the inconsistencies seen in studies on the role of these enzymes in lung cancer (36) and colorectal adenoma (37). If this is the case, a potential weakness of our study could be that dietary factors possibly related to GSTs could not be assessed. Although the aforementioned explanations are largely hypothesis generating, these hypotheses merit further investigation. In addition, our finding of an inverse relationship of the *GSTM1* and *GSTT1* null genotypes with risk of HCC in a Chinese population needs to be replicated in other ethnic populations.

There are a number of limitations of this study. Subjects were recruited from individuals who participated in a liver cancer screening program on a voluntary basis and there may exist a self-selection bias. However, comparability is more important than representativeness in studies designed to examine the association between risk factor exposure and health outcome. Because both cases and controls were recruited from the same screening program they were considered quite comparable in physical and psychological characteristics in relation to their decision to participate. In addition, chemically induced cancers involve successive host responses to carcinogens, in which phase I and phase II enzymes are coordinated in their metabolism of xenobiotics. It will likely be important to determine all the relevant genotypes for a given exposure to obtain a reliable picture of the potential role of metabolic polymorphisms in individual susceptibility to environmental toxicants. Such studies will require large numbers of subjects. It has been demonstrated that microsomal epoxide hydrolase (mEH) is involved in AFB_1 detoxification in hepatocytes (38). Genetic variation in mEH was identified as a susceptibility factor for HCC (13). However, data on mutant alleles of mEH and their effects on HCC risk have not been analyzed in the present study.

Despite these limitations, our study indicates that aflatoxin-related HCC risk may be modified by the *GSTM1* and *GSTT1* genotypes. More interestingly, when the effect of genetic polymorphisms in carcinogen metabolizing genes was considered, aflatoxin exposure by itself was not a significant determinant of HCC risk. These results demonstrate the importance of gene–environment interactions in the multifactorial development of HCC.

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