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Plasma Carotenoids, Glutathione *S*-Transferase M1 and T1 Genetic Polymorphisms, and Risk of Hepatocellular Carcinoma: Independent and Interactive Effects

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This study was conducted to assess the role of carotenoid and glutathione *S*-transferase (GST) M1 and T1 genetic polymorphisms in the development of hepatocellular carcinoma (HCC). A total of 84 incident cases of HCC and 375 matched controls selected from a cohort of 7,342 men (4,841 chronic hepatitis B carriers and 2,501 noncarriers) who were recruited between 1988 and 1992 in Taiwan were studied. Neither GST M1/T1 polymorphisms nor plasma levels of various carotenoids were independently associated with HCC, but they modulated smoking- and/or drinking-related HCC risk. Cumulative exposure to tobacco smoke significantly increased HCC risk in a dose-dependent manner among subjects with low plasma β-carotene levels (p for trend = 0.047) but not among those with high levels. A statistically significant effect of habitual alcohol drinking on HCC risk was observed only for those with low plasma levels of β-carotene, α-carotene, or lycopene and for GST M1 null subjects. There was evidence suggesting an interaction between the GST M1/T1 genotype and certain carotenoids in HCC associated with smoking and drinking. The strongest effect of smoking and drinking was noted among GST M1 null subjects with low plasma levels of β-carotene (smoking: adjusted odds ratio (OR) = 3.54, 95% confidence interval (CI) 1.06–11.83; drinking: OR = 8.28, 95% CI 2.40–28.61). *Am J Epidemiol* 1999;149:621–9.

alcohol drinking; carcinoma, hepatocellular; carotene; glutathione transferases; smoking

Hepatocellular carcinoma (HCC) is a highly malignant neoplasm with an extremely poor prognosis. The etiologic importance of chronic infection with hepatitis B virus in HCC has been well established (1). However, only about one fifth of chronic hepatitis B virus carriers are expected to develop HCC in their lifetimes (2). Other environmental factors, including hepatitis C virus, aflatoxin exposure, and habits of alcohol drinking and cigarette smoking, have also been demonstrated as risk factors for HCC (1, 3–11).

Considerable evidence suggests that there are various biologic defense systems against carcinogenesis. Susceptibility to cancer can be due to differences in nutritional status and/or detoxification of carcinogens (4, 5, 12–22). Epidemiologic studies and laboratory investigations have demonstrated that many compounds in vegetables and fruits may have anticarcinogenic potential through a variety of biologic mechanisms (12). Although the possibility that carotenoids might modulate cancer risk has attracted the attention of many scientists, few studies examined the role of nutritional factors in the etiology of HCC (13, 14). In our previous prospective study, a strong inverse association was observed between vegetable intake and serum retinol levels and the risk of HCC (13). The relation between blood levels of various carotenoids and HCC has not yet been prospectively investigated.

The glutathione *S*-transferases (GSTs) represent a major group of detoxification enzymes catalyzing the conjugation of potential carcinogens including certain constituents of tobacco smoke with glutathione (22). GST M1 and T1 are polymorphic in humans, and deficiency in their enzyme activity is due to the inherited

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Abbreviations: CI, confidence interval; GST, glutathione *S*-transferase; HCC, hepatocellular carcinoma; OR, odds ratio; SD, standard deviation.

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homozygous deletion of the genes (22). Allelism in GST M1 has been linked with risk of various cancers, and the association between GST M1 deficiency and cancer is stronger in populations exposed to a variety of environmental carcinogens (4, 5, 15–18, 20, 21). While the relation between GST M1 and cancer has been actively studied, there is less information on the role of GST T1 in mediating cancer risk (16, 18, 19).

Recently, we reported no association between the GST M1 genotype and cigarette smoking-related HCC risk on the basis of 30 HCC cases and 150 controls in a nested case-control study (10). Since various carotenoids rich in vegetables and fruits are antioxidants and likely to have an inhibitory effect on DNA damage caused by exposure to tobacco smoke (12), they may influence the importance of GST status in the etiology of smoking-related cancers. In this study, we have expanded our initial study to include 84 HCC cases and 375 control subjects. This study was done to examine the independent and interactive effects of various carotenoids and genotypes of GST M1 and T1 on the development of HCC.

MATERIALS AND METHODS

Study subjects

Between August 1988 and June 1992, a cohort of 4,841 male asymptomatic hepatitis B virus surface antigen carriers and 2,501 male noncarriers aged 30–65 years who were free of diagnosed HCC was recruited from the Government Employee Central Clinics and the Liver Unit of Chang-Gung Memorial Hospital in Taipei, Taiwan. The study was restricted to men because the incidence of HCC is from two to three times higher in men than in women (1). Compared with a community-based cohort study in Taiwan (13), the study subjects in this cohort study have higher educational backgrounds and a lower prevalence of cigarette smoking and alcohol drinking. At recruitment, each study participant was interviewed personally to obtain information on demographic characteristics, habits of cigarette smoking and alcohol drinking, consumption frequency of various food items, and personal and family history of major chronic diseases. Only 0.3 percent of the study subjects reported having a history of liver cirrhosis diagnosed by physicians. Because the average quantity of alcohol consumed by Chinese is not large in Taiwan, habitual alcohol drinking was defined as consuming any alcoholic beverage at least once a week for more than 1 year. Fasting blood samples were also collected from each study subject. Aliquots of plasma, serum, and buffy coat separated from blood samples were stored at −70°C until subsequent analyses. This study was approved by Executive Yuan from the Department of Health.

Follow-up of the study subjects was performed through various channels: annual α-fetoprotein measurement and ultrasonography examination, personal telephone interview, and data linkage with computer files of national cancer and death registry systems. When a case of HCC was identified, permission was sought from the hospital where the subject was diagnosed with cancer to obtain the medical charts and pathology reports. The diagnosis of HCC was based on 1) positive findings on pathologic or cytologic examinations and/or 2) an elevated α-fetoprotein level (≥400 ng/ml) combined with at least one positive image on angiography, sonography, and/or computed tomography. By December 31, 1996, we had carried out approximately 44,052 person-years of follow-up, an average of 6 years per person. There were 88 incident cases of HCC identified during the follow-up period. Four cases with no available blood samples were excluded from the analysis, which left a total of 84 HCC cases in this study. In 47.6 percent (40 of 84) of these cases, the diagnosis was based on the pathologic or cytologic examination, and in 52.4 percent, on the elevated α-fetoprotein level combined with at least one positive image on angiography, sonography, and/or computed tomography. Ninety-six percent (81 of 84) of the HCC cases studied were hepatitis B surface antigen carriers. Two hepatitis B surface antigen-negative cases were positive for antibodies against hepatitis C virus. Only one case was negative for both hepatitis B surface antigen and antibodies against hepatitis C virus.

The control subjects consisted of 375 persons (153 and 222 hepatitis B surface antigen-negative and -positive subjects, respectively). From four to six controls were selected for each HCC case from cohort members who remained unaffected throughout the follow-up. The controls were matched to the index case on age (±5 years), recruitment clinic, and date of questionnaire interview and blood collection (±3 months).

Laboratory analyses

The tests for plasma carotenoids and GST M1/T1 genotypes were performed on banked blood samples collected at recruitment of the cohort. Prediagnostic plasma levels of β-carotene, α-carotene, and lycopene were measured by high-performance liquid chromatography using a modification of the method described by Miller and Yang (23). Coefficients of variation for plasma assays were 5–6 percent for α-carotene and β-carotene and 9–10 percent for lycopene. GST M1 and T1 polymorphisms were determined in peripheral leukocyte DNA using polymerase

chain reaction-based methods described previously. Amplification of the β-globin gene was used as an internal control (4). All the tests for each case-control set were assayed on the same day. Laboratory personnel were kept blind to case-control status.

Statistical methods

The significance of the difference in the distribution of categorical variables between cases and controls was determined by the chi-square test. A *t* test was used to assess the mean difference in plasma carotenoid levels between groups. Plasma carotenoid levels were categorized into quartiles or dichotomized by their medians (depending on the available number of cases) based on values among healthy subjects in a population-based survey in Taiwan. Conditional logistic regression models were used to derive the matched odds ratios and their 95 percent confidence intervals. In the stratified analyses, the odds ratios of HCC were estimated using unconditional logistic regression models. Unadjusted and adjusted odds ratios were similar; adjusted odds ratios are shown. Tests for trend in the logit of HCC risk across levels of cumulative exposure to cigarette smoke were computed based on likelihood ratio tests, with scores of 1–3 assigned to nonsmokers and to those with ≤15 and >15 pack-years of cigarette smoking, respectively. We also used likelihood ratio tests to determine interaction among certain variables with respect to HCC risk. Statistical significance of the observed two-way interaction was determined by comparing the fit of the logistic model that included only the main effects with that of the model that included a two-factor interaction term for the variables of interest. The three-way interaction hypothesis was assessed by comparing the fit of the logistic model that included the main effects and all possible two-factor interaction terms for the variables of interest with a fully parameterized model containing a three-factor interaction term. All *p* values were calculated from two-sided statistical tests.

RESULTS

Baseline characteristics of HCC cases and matched controls are presented in table 1. HCC cases had significantly lower educational levels ($p = 0.017$) and a higher prevalence of hepatitis B surface antigen ($p = 0.000$) than did controls. The prevalence of hepatitis B surface antigen in controls was high because a large proportion of cohort members were carriers of hepatitis B surface antigen. Although none of the case-control differences in the distributions of age, ethnic groups, season of blood sample collection, and blood storage time were statistically significant, the age at recruitment was slightly younger for controls than cases.

TABLE 1. Baseline characteristics for hepatocellular carcinoma (HCC) cases and matched controls, Taipei, Taiwan, 1988–1992

Variable	Controls		HCC cases	
	No.	%	No.	%
Age (years)				
30–39	57	15.2	8	9.5
40–49	103	27.5	26	31.0
50–59	102	27.2	22	26.2
≥60	113	30.1	28	33.3
Ethnicity				
Fukien Taiwanese	189	50.4	42	50.0
Hakka Taiwanese	49	13.1	10	11.9
Mainland Chinese	137	36.5	32	38.1
Education*				
Preliminary school and below	28	7.5	13	15.5
Junior high school	28	7.5	11	13.1
Senior high school	88	23.5	20	23.8
Junior college and above	230	61.3	39	46.4
Missing	1	0.2	1	1.2
Month of study participation				
January-March	77	20.6	19	22.6
April-June	99	26.4	20	23.8
July-September	98	26.1	25	29.8
October-December	101	26.9	20	23.8
	Controls (mean years)		HCC cases (mean years)	
Time since blood collection	5.7 (1.1)†		5.8 (1.3)	

* $p = 0.017$ for the chi-square test for the difference in the distribution of education between cases and controls.
† Numbers in parentheses, standard deviation.

The mean of prediagnostic plasma levels of retinol, β-carotene, α-carotene, and lycopene in cases was 37.32 (standard deviation (SD), 16.02), 19.47 (SD, 16.33), 3.67 (SD, 3.36), and 18.65 (SD, 10.26) μg/dl, respectively. The corresponding value for controls was 50.63 (SD, 20.15), 19.52 (SD, 19.60), 3.64 (SD, 3.28), and 20.79 (SD, 14.31) μg/dl. There were no significant differences between cases and matched controls with respect to the mean values of any of the nutrients examined except for retinol, which was significantly lower in HCC cases than controls ($p = 0.0001$).

There were no clear patterns of HCC risk associated with plasma levels of various carotenoids. Adjustment for hepatitis B surface antigen carrier status, age, plasma retinol levels, and habits of cigarette smoking and alcohol drinking made no substantial change in the crude odds ratios of HCC with various plasma carotenoids. Allelism in GST M1 and T1 was not significantly associated with HCC, even after adjustment was made for other risk factors (table 2).

TABLE 2. Distributions of plasma carotenoid levels and glutathione *S*-transferase (GST) M1 and T1 genotypes in hepatocellular carcinoma (HCC) cases and matched controls, Taipei, Taiwan, 1988–1992*

Variable	Group	HCC cases		Controls		Multivariate-adjusted OR†	95% CI†
		No.	%	No.	%		
β-Carotene	q1† (≤9.2)‡	25	29.7	91	24.2	1.0	
	q2 (≤13.0)	13	15.5	79	21.1	1.06	0.46–2.45
	q3 (≤21.3)	15	17.9	99	26.4	0.78	0.34–1.76
	q4 (>21.3)	31	36.9	106	28.3	1.42	0.66–3.05
α-Carotene	q1 (≤2.0)	26	30.9	101	26.9	1.0	
	q2 (≤2.6)	15	17.9	82	21.9	0.86	0.37–2.03
	q3 (≤4.0)	21	25.0	92	24.5	1.41	0.64–3.13
	q4 (>4.0)	22	26.2	100	26.7	1.06	0.49–2.31
Lycopene	q1 (≤12.1)	20	23.8	91	24.3	1.0	
	q2 (≤16.1)	21	25.0	86	22.9	1.65	0.72–3.79
	q3 (≤24.5)	25	29.8	106	28.3	1.61	0.72–3.63
	q4 (>24.5)	18	21.4	92	24.5	1.40	0.54–3.60
GST M1	Non-null	42	50.0	159	42.4	1.0	
	Null	42	50.0	216	57.6	0.95	0.56–1.62
GST T1§	Non-null	42	50.6	194	51.7	1.0	
	Null	41	49.4	181	48.3	0.82	0.47–1.42

* The odds ratios and 95% confidence intervals were calculated by a conditional logistic regression model. In analysis of plasma carotenoid levels and HCC, hepatitis B surface antigen (HBsAg) carrier status, age, plasma retinol levels, alcohol drinking, and cigarette smoking were also included in the model. In analysis of GST M1 and T1 genotypes and HCC, HBsAg carrier status, age, ethnicity, plasma retinol, alcohol drinking, and cigarette smoking were also included in the model.

† OR, odds ratio; CI, confidence interval; q1, quartile 1 (other quartiles treated similarly).

‡ Categorized according to the quartile distribution of plasma carotenoid levels (boundaries in μg/dl) among healthy subjects in a population-based survey in Taiwan.

§ One case had no available data on the GST T1 genotype.

Table 3 shows the odds ratios of HCC associated with pack-years of cigarette smoking stratified by plasma levels of various carotenoids and GST M1/T1 genotypes. Since only three HCC cases were hepatitis B surface antigen negative, the only way to control for the effect of hepatitis B surface antigen seropositivity in stratified analyses was to restrict the analyses to hepatitis B surface antigen carriers if the carrier status was a confounder. However, there was no association between hepatitis B surface antigen status and cigarette and alcohol use. Thus, it was reasonable to include all study subjects in the following analyses in order to increase statistical power and to improve the precision of the estimation of relative risk. Among the total group of subjects, no significant association was observed between cumulative exposure to tobacco smoke and HCC. Stratified analysis showed a statistically significant modification of the smoking effect by plasma β-carotene levels. There was a significant upward trend of HCC risk with increasing pack-years of cigarette smoking among subjects with low plasma levels of β-carotene (p for trend = 0.047), showing a multivariate-adjusted odds ratio associated with HCC of 2.41 (95 percent confidence interval (CI) 1.02–5.65) for smokers of more than 15 pack-years compared with nonsmokers. In contrast, no increase in HCC risk was observed among smokers who had high levels of β-carotene. There was no significant interaction of cigarette smoking with GST genotypes and plasma levels of α-carotene or lycopene.

No significant association was found between alcohol drinking and HCC among the total group of subjects. However, there was evidence for an interaction of alcohol drinking and carotenoid and GST M1 genotype in HCC risk. Among subjects with low plasma levels of various carotenoids and those with the GST M1 null genotype, drinkers were at a two- to threefold increased risk of HCC relative to nondrinkers. Conversely, among subjects who had high levels of carotenoids and those with GST M1 non-null genotypes, no elevated HCC risk associated with alcohol drinking was found. There was no notable difference in the odds ratios of alcohol drinking by GST T1 genotypes (table 4).

To address the hypothesis that the modification effect of plasma carotenoid levels on the HCC risk associated with smoking and drinking might vary according to the status of GST, the effects of smoking and drinking in

TABLE 3. Odds ratios of hepatocellular carcinoma (HCC) in relation to pack-years of cigarette smoking by category of plasma carotenoid levels and glutathione *S*-transferase (GST) M1 and T1 genotypes, Taipei, Taiwan, 1988–1992*,†

Variable	Group	Nonsmokers		≤15.0 pack-years‡		>15.0 pack-years	
		Cases/controls (no.)	Odds ratio	Cases/controls (no.)	Odds ratio	Cases/controls (no.)	Odds ratio
All subjects		49/223	1.0	14/73	0.90 (0.45–1.81)§	21/78	1.26 (0.67–2.37)
β-Carotene	Low	16/92	1.0	6/37	1.31 (0.44–3.90)	16/41	2.41 (1.02–5.65)¶
	High	33/131	1.0	8/36	0.67 (0.27–1.64)	5/37	0.54 (0.19–1.58)
				Test for interaction: chi-squared = 6.68 (2 df); $p < 0.05$			
α-Carotene	Low	19/95	1.0	6/38	0.89 (0.31–2.56)	16/49	1.74 (0.78–3.91)
	High	30/128	1.0	8/35	0.82 (0.33–2.03)	5/29	0.70 (0.23–2.12)
				Test for interaction: chi-squared = 3.36 (2 df); $p > 0.05$			
Lycopene	Low	18/93	1.0	6/38	1.00 (0.35–2.85)	17/46	1.86 (0.83–4.15)
	High	31/130	1.0	8/35	0.73 (0.29–1.83)	4/32	0.54 (0.17–1.78)
				Test for interaction: chi-squared = 5.18 (2 df); $0.05 < p < 0.1$			
GST M1	Null	23/132	1.0	9/39	1.33 (0.55–3.22)	10/45	1.26 (0.53–3.02)
	Non-null	26/91	1.0	5/34	0.43 (0.14–1.29)	11/33	1.18 (0.50–2.82)
				Test for interaction: chi-squared = 3.04 (2 df); $p > 0.05$			
GST T1#	Null	24/113	1.0	7/30	0.95 (0.35–2.58)	10/37	1.26 (0.52–3.06)
	Non-null	25/110	1.0	7/43	0.71 (0.28–1.82)	10/41	0.99 (0.42–2.36)
				Test for interaction: chi-squared = 0.27 (2 df); $p > 0.05$			

* One control subject was excluded from analysis because of missing information on cigarettes smoked per day.
† Controlled for age and plasma retinol levels.
‡ Categorized on the basis of the median of smoking controls.
§ Numbers in parentheses, 95% confidence interval.
¶ p for trend = 0.047.
# One case had no available data on the GST T1 genotype.

TABLE 4. Odds ratios of hepatocellular carcinoma (HCC) in relation to alcohol drinking by category of plasma carotenoid levels and glutathione *S*-transferase (GST) M1 and T1 genotypes, Taipei, Taiwan, 1988–1992*

Variable	Group	Nondrinkers		Drinkers	
		Cases/controls (no.)	*Odds ratio*	Cases/controls (no.)	*Odds ratio*
All subjects		64/299	1.0	20/76	1.82 (0.97–3.42)†
β-Carotene	Low	22/131	1.0	16/39	3.48 (1.49–8.13)
	High	42/168	1.0	4/37	0.49 (0.16–1.48)
		Test for interaction: chi-squared = 9.08 (1 df); $p < 0.01$			
α-Carotene	Low	25/138	1.0	16/45	2.56 (1.16–5.66)
	High	39/161	1.0	4/31	0.62 (0.20–1.93)
		Test for interaction: chi-squared = 5.08 (1 df); $p < 0.05$			
Lycopene	Low	27/139	1.0	14/38	2.30 (1.02–5.15)
	High	37/160	1.0	6/38	0.84 (0.31–2.25)
		Test for interaction: chi-squared = 3.56 (1 df); $0.05 < p < 0.1$			
GST M1	Null	29/176	1.0	13/40	2.61 (1.18–5.78)
	Non-null	35/123	1.0	7/36	0.73 (0.28–1.90)
		Test for interaction: chi-squared = 3.67 (1 df); $0.05 < p < 0.1$			
GST T1‡	Null	30/142	1.0	11/39	1.62 (0.70–3.74)
	Non-null	33/157	1.0	9/37	1.43 (0.60–3.43)
		Test for interaction: chi-squared = 0.05 (1 df); $p > 0.05$			

* Controlled for age and plasma retinol levels.
† Numbers in parentheses, 95% confidence interval.
‡ One case had no available data on the GST T1 genotype.

relation to HCC risk were further examined within categories simultaneously stratified by plasma carotenoid levels and genotypes of GST. There was evidence suggesting that GST genotype, carotenoid, and smoking and/or drinking interacted with HCC risk, although tests for the three-way interaction were not statistically significant. In analysis of the associations of HCC with smoking within strata categorized by both GST M1 genotype and plasma carotenoid levels, the highest risk of smoking was noted among GST M1 null subjects with low plasma β-carotene levels (adjusted odds ratio (OR) = 3.54, 95 percent CI 1.06–11.83). A statistically significant effect of drinking on HCC risk was observed only for GST M1 null subjects who also had low plasma levels of various carotenoids. The strongest effect of drinking was noted in GST M1 null subjects with low plasma β-carotene levels (adjusted OR = 8.28, 95 percent CI 2.40–28.61) (table 5).

In analysis of the associations of HCC with smoking within strata categorized by both the GST T1 genotype and plasma carotenoid levels, a statistically significant odds ratio was found only among GST T1 null subjects who had low plasma levels of β-carotene (OR = 3.06, 95 percent CI 1.03–9.06). There was little evidence that the modification effect of carotenoids on drinking-related HCC risk varied with the GST T1 genotype (table 6).

DISCUSSION

Results on the association between cigarette and alcohol use and the development of HCC have been inconsistent: some studies reported smoking and drinking to be independent risk factors for HCC (7–9); others observed the significant associations of HCC with smoking and/or drinking only in specific groups (10, 11); and a few have failed to detect a significant association for either exposure (6, 14). No studies have considered both genetic and nutritional factors in susceptibility to smoking- or drinking-related HCC.

Our previous cohort study carried out in six communities of Taiwan observed that the effect of low vegetable intake on HCC risk was more striking among cigarette smokers than nonsmokers, implicating possible interactions between dietary carotenoids and cigarette smoking in relation to hepatocarcinogenesis (13). The interaction between alcohol drinking and vegetable

TABLE 5. Odds ratios of hepatocellular carcinoma (HCC) associated with cigarette smoking and alcohol drinking stratified by plasma levels of various carotenoids among subjects with different genotypes of glutathione *S*-transferase (GST) M1, Taipei, Taiwan, 1988–1992†

Variable	β-Carotene		α-Carotene		Lycopene	
	Low	High	Low	High	Low	High
			Among GST M1 null subjects			
Cigarette smoking	3.54**	0.65	1.60	1.17	3.13*	0.73
Alcohol drinking	8.28***	0.60	6.51***	1.14	4.90***	1.85
			Among GST M1 non-null subjects			
Cigarette smoking	1.34	0.48	1.40	0.44	1.10	0.51
Alcohol drinking	1.37	0.27	0.93	0.24	1.11	0.19

* $0.05 < p < 0.1$; ** $0.01 < p < 0.05$; *** $p < 0.01$.
† Controlled for age and plasma retinol levels.

TABLE 6. Odds ratios of hepatocellular carcinoma (HCC) associated with cigarette smoking and alcohol drinking stratified by plasma levels of various carotenoids among subjects with different genotypes of glutathione *S*-transferase (GST) T1, Taipei, Taiwan, 1988–1992†,‡

Variable	β-Carotene		α-Carotene		Lycopene	
	Low	High	Low	High	Low	High
			Among GST T1 null subjects			
Cigarette smoking	3.06**	0.37	1.41	0.88	2.08	0.62
Alcohol drinking	2.91*	0.58	2.74*	0.68	2.36	1.13
			Among GST T1 non-null subjects			
Cigarette smoking	1.24	0.69	1.17	0.67	0.95	0.64
Alcohol drinking	4.22**	0.44	2.92*	0.57	2.91*	0.54

* $0.05 < p < 0.1$; ** $0.01 < p < 0.05$.
† Controlled for age and plasma retinol levels.
‡ One case had no available data on the GST T1 genotype.

consumption was not examined in that cohort study. In the present study, neither smoking nor drinking was independently associated with HCC risk. However, smoking and drinking increased HCC risk among subjects who had low plasma levels of certain carotenoids and GST M1 and/or T1 null genotypes. The heterogeneity in HCC risk in relation to exposure to exogenous agents observed in this study implies that there is a complex gene-environment interaction in cancer development and explains previous inconsistent findings for drinking and smoking as risk factors for HCC.

Carotenoids are among the most potent dietary antioxidants. α- and β-carotene are carotenoids with provitamin A activity. Lycopene, which is often present in human blood at higher concentrations than β-carotene, is a non-provitamin A carotenoid (12). Exposure to α- or β-carotene suppressed spontaneous hepatocarcinogenesis and chemically induced lung and skin cancer in mice (24). In vitro studies demonstrated that various carotenoids inhibited chemically induced malignant transformation and enhanced cell-to-cell communication (12, 25, 26). Both case-control and cohort studies have shown that vegetable intake and/or dietary carotenoids are associated with a reduced risk of epithelial cancers (27–31). There have been some reports that the negative association between vegetable consumption and lung cancer was more pronounced among male heavy smokers (28). Prospective studies have consistently found a lower risk of lung cancer associated with elevated blood levels of β-carotene (32). However, recent intervention trials produced conflicting results concerning the utility of β-carotene as a chemopreventive agent for lung cancer in smokers and men exposed to asbestos (33–35). The lack of an effect of β-carotene supplementation on lung cancer risk was reported from one large trial (33), while an increase in lung cancer risk by β-carotene supplementation was observed in two large trials (34, 35).

This study suggests that low plasma levels of various carotenoids are not independent risk factors for HCC but may increase the HCC risk among smokers and drinkers. Furthermore, low β-carotene status appeared to be more predictive of elevated HCC risk associated with smoking and drinking than other types of

carotenoids examined. Discrepancies in the capacity of diverse carotenoids to suppress carcinogenesis have been shown in both experimental and epidemiologic studies (24–26, 29). There are differences in carotenoid levels in different types of tissues; β-carotene is the major carotenoid in liver (12). However, β-carotene was found to enhance the formation of aflatoxin B_1-DNA adducts in cultured woodchuck hepatocytes (36). In a recent cross-sectional study, we also found that plasma levels of α- and β-carotene were positively associated with urinary aflatoxin B_1-DNA adducts, a surrogate for estimating the hepatic aflatoxin B_1-DNA adduct levels, but an inverse relation was seen with lycopene (37). Our knowledge is still very limited on the metabolism and biologic functions of various carotenoids. Whether certain carotenoids may have inhibitory effects specific to smoking- and drinking-related hepatocarcinogenesis merits further study.

GST M1 gene deletion is one of several common genetic polymorphisms that confer an excess risk of cancer associated with exposure to environmental carcinogens (4, 5, 15–18, 20, 21). It has been associated with other smoking-related cancers showing an odds ratio in the range of 1.4–2.0 (15–18, 20). The modest increased genetic risk for developing cancer may be masked because of small sample size and/or unidentified confounders, such as other polymorphic loci encoding enzymes involved in the metabolism of carcinogens and/or dietary factors that may have critical effects on DNA damage induced by tobacco smoke. The lack of an independent effect of GST M1 deficiency on the smoking-related HCC risk observed in this study is in agreement with our earlier report involving only 30 HCC cases and 150 controls (10). However, in this study there was evidence suggesting that the GST M1 genotype modified the association between cigarette and alcohol use and HCC among subjects with low levels of certain carotenoids. The effect of smoking and drinking was strongest among GST M1 null subjects with low plasma β-carotene. In contrast, neither smoking nor drinking significantly increased HCC risk among GST M1 non-null subjects with high or low levels of various carotenoids.

To our knowledge, no study has been carried out to examine the interaction between the GST M1/T1 genotype and alcohol drinking in cancer risk; recent reviews do not mention it (17, 18). This study for the first time revealed that the GST M1 null genotype influenced the role of alcohol drinking in hepatocarcinogenesis. Alcohol per se is unlikely to be carcinogenic. However, habitual alcohol drinking has long been postulated as a risk factor for HCC because of its relation to cirrhosis. The hepatic injury and nonspecific regenerative proliferation caused by habitual alcohol drinking may allow an accumulation of multiple mutational events and provide a growth stimulation to a rare cell that already has a genetic change due to random mutation or the integration of the hepatitis B virus genome. However, the average quantity of alcohol consumed by habitual alcohol drinkers in this study was only 241.3 g/week. This amount may be insufficient to induce liver cirrhosis. No overall association between alcohol drinking and HCC was found. Habitual alcohol drinking may also increase cancer risk through induction of microsomal enzymes that activate procarcinogens (38). Cigarette smoke contains a number of procarcinogens (39). There was a synergistic interaction between alcohol drinking and cigarette smoking in HCC (8). Because of the limited sample size, it was not possible to assess the complex interactions among GST genotypes, drinking, and smoking or other potential hepatocarcinogens in this study.

Certain alkyl halides have been recognized as substrates for GST T1 (22). While fewer studies of GST T1 and cancer have been undertaken than for GST M1 (16, 18, 19), a significant effect of the GST T1 null genotype on colorectal cancer, astrocytoma, meningioma, and myelodysplasia has been reported (18, 19). No effect was found for lung, oral, and gastric cancer (19). A recent case-control study reported that the GST T1 non-null genotype was a risk factor for bladder cancer in nonsmokers (16). Although this study suggests a GST T1-mediated HCC risk in relation to smoking among subjects with a low dietary intake of β-carotene, xenobiotics in tobacco smoke metabolized by this enzyme that may contribute to hepatocarcinogenic action remain to be identified.

Hepatitis B surface antigen carrier status is the most important determinant of HCC in Taiwan (1–3, 8). A strong synergistic interaction of chronic hepatitis B virus infection with smoking and alcohol drinking in HCC risk has been reported in this high-risk area. The risk of HCC was rather low in the absence of chronic hepatitis B virus infection (8). Since a large proportion of HCC cases were chronic hepatitis B virus carriers in this study, whether carotenoids and the GST genotype are significant susceptibility factors for HCC in hepatitis B virus noncarriers requires further studies.

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