Polymorphisms in XRCC1 and Glutathione *S*-Transferase Genes and Hepatitis B–Related Hepatocellular Carcinoma

Ming-Whei Yu, Shi-Yi Yang, I-Jen Pan, Chih-Lin Lin, Chun-Jen Liu, Yun-Fan Liaw, Shi-Ming Lin, Pei-Jer Chen, Shou-Dong Lee, Chien-Jen Chen

Chronic infection with hepatitis B virus (HBV) causes DNA damage. An arginine (Arg)-to-glutamine (Gln) polymorphism at codon 399 in the XRCC1 gene is putatively associated with DNA damage. In a case–control study of 577 HBV surface antigen carriers with hepatocellular carcinoma (HCC) and 389 HBV carrier control subjects, we investigated the association between this polymorphism and the risk of HCC and assessed whether this association varied with glutathione *S*-transferase (GST) status; GSTs are involved in carcinogen metabolism. All statistical tests were two-sided. The XRCC1 Gln allele was associated with a dose-dependent increased risk of early-onset HCC (<50 years) but not with the risk of late-onset HCC (P_{trend} = .01). The GSTT1-null genotype alone did not affect risk, but the GSTM1-null genotype was associated with a decreased risk for early-onset HCC. Various combinations of GSTM1 and GSTT1 genotypes differentially modified the association of XRCC1 with HCC ($P_{interaction}$ = .005); e.g., for individuals with the GSTT1-null/GSTM1-present genotype, the risk of HCC was greater for those with the Gln/Gln genotype (odds ratio = 8.07, 95% confidence interval = 1.67 to 38.93) than for those with the Arg/Arg genotype. Thus, GST status appears to affect the risk of HCC associated with this XRCC1 polymorphism. [J Natl Cancer Inst 2003;95:1485–8]

Chronic infection with hepatitis B virus (HBV) is the most important cause of hepatocellular carcinoma (HCC) in Taiwan, where the predominant mode of HBV transmission is perinatal *(1,2).* Although the relative risk is about 20 for HBV surface antigen (HBsAg) carriers compared with noncarriers, only a fraction of HBsAg carriers eventually develop HCC. The age at onset of HCC also varies over a wide range among HBsAg carriers *(1,3).*

Persistent HBV infection can cause genomic damage directly, through a mechanism of chromosomal integration, or indirectly, through increased oxidative stress and free-radical generation during the course of chronic hepatitis *(1,4–7).* The XRCC1 protein is required for genomic stability and participates in the repair of endogenous oxidative DNA damage and DNA single-strand breaks *(8–10).* The XRCC1 gene has an arginine (Arg)-to-glutamine (Gln) polymorphism at codon 399 (XRCC1-Arg399Gln polymorphism) that has been associated with DNA damage phenotypes and cancer risk *(11–15).* We conducted a multicenter case–control study to examine whether this polymorphism influences risk for HCC among HBsAg carriers. We specifically investigated whether this association varied by age at onset of HCC and the status of glutathione *S*-transferase genes GSTM1 and GSTT1, which are involved in the metabolism of potential carcinogens, including endogenously generated cancer-causing reactive oxygen species continuously produced through HBV-induced inflammatory disease *(16,17).*

The study included 577 HBsAg carriers with incident HCC selected among 1572 patients who participated in our ongoing genetic epidemiology study of

Affiliations of authors: M.-W. Yu, S.-Y. Yang, I-J. Pan, C.-J. Chen, Graduate Institute of Epidemiology, College of Public Health, National Taiwan University, Taipei, Taiwan; C.-L. Lin, Division of Gastroenterology, Department of Internal Medicine, Taipei Municipal Jen-Ai Hospital, Taipei; C.-J. Liu, P.-J. Chen, Hepatitis Research Center, National Taiwan University Hospital, Taipei; S.-D. Lee, Department of Medicine, Veterans General Hospital and School of Medicine, National Yang-Ming University, Taipei; Y.-F. Liaw, S.-M. Lin, Liver Research Unit, Chang-Gung Memorial Hospital, Chang-Gung University, Taoyuan, Taiwan.

Correspondence to: Ming-Whei Yu, PhD, Graduate Institute of Epidemiology, College of Public Health, National Taiwan University, No. 1 Jen-Ai Rd., Sec. 1, Rm. 1550, Taipei 100, Taiwan (e-mail: mingwhei@ha.mc.ntu.edu.tw).

See "Notes" following "References."

DOI: 10.1093/jnci/djg051

Journal of the National Cancer Institute, Vol. 95, No. 19,

HCC between September 1, 1997, and December 31, 2001 *(3)*. The 1572 patients were recruited from three major hospitals (Chang-Gung Memorial Hospital, Taipei Veterans General Hospital, and National Taiwan University Hospital) in northern Taiwan, representing 90% of the patients originally contacted. The diagnosis of HCC was confirmed by liver biopsy or the combination of increased α-fetoprotein (≥400 ng/mL) plus typical features on angiography, sonography, or computed tomography. To include sufficient numbers of early- and late-onset patients, we selected approximately equal numbers of case patients in the following five age groups: younger than 30, 30–39, 40–49, 50–59, and 60–69 years old. Because early-onset HCC patients (<30 years old at diagnosis) are rare, only 43 patients younger than 30 years were included. Finally, the proportions of case patients in the five age groups were 7.5%, 21.0%, 21.3%, 28.2%, and 22.0%, respectively.

Control subjects selected from a pool of HBsAg carriers were frequency-matched to the case patients by sex and year of birth (±10 years). This HBsAg carrier pool consisted of the following two groups: 1) 5508 individuals born in or before 1958 who were enrolled during regular physical examinations at the Chang-Gung Memorial Hospital or the Government Employee Central Clinics (Taipei, Taiwan) between August 1, 1988, and June 30, 1992 *(3,18)* and 2) 418 individuals born between January 1, 1928, and December 31, 1987, who were biological or non-biological relatives selected from different families of patients with HCC, resided in northern Taiwan, and participated in our ongoing genetic epidemiology study for clinical evaluation. All the HBsAg carriers are being followed up through various channels, including a clinical evaluation every 6–12 months and a data linkage with Taiwan's national cancer registry and death certification systems. More case patients (143 males and 19 females) than cancer-free HBV carriers (68 males and 59 females) in the pool were born after 1958. Consequently, to achieve frequency matching, we calculated the expected number of control subjects needed in each sex-year of birth stratum. There were 389 control subjects selected by stratified random sampling. The study was approved by the research ethics committee at the College of Public Health, National Taiwan University. All study participants provided written informed consent.

GSTM1 and GSTT1 genotypes were determined by a multiplex polymerase chain reaction that used primers as described previously *(19,20)*. The XRCC1-Arg399Gln polymorphism was determined by a polymerase chain reaction–restriction fragment length polymorphism method that used primers X1–399NF (5′-CAACACCCCCAAGTACAGCC-3′) and 28265R (5′-GGCTGGGACCACCTGTGTT-3′). Odds ratios and 95% confidence intervals from unconditional logistic regression models were used to evaluate relative risks. All statistical tests were two-sided.

The mean age at enrollment in this case–control study (i.e., in 2002) was 52.3 ± 12.7 years (± standard deviation) for case patients and 53.0 ± 12.5 years for control subjects. Case patients and control subjects had identical sex distributions (14% were women). Cigarette smoking and alcohol consumption were more frequent among case patients (57.4% and 35.9%, respectively) than among control subjects (34.2% and 17.0%, respectively). Twenty-eight percent of case patients and 19.3% of control subjects had a first-degree relative with a history of HCC.

Among control subjects, the frequency of the XRCC1 genotype was consistent with the Hardy–Weinberg equilibrium (chi-square goodness of fit, $P = .497$). The risk of HCC was statistically nonsignificantly greater for the Gln/Gln XRCC1 genotype than for the Arg/Arg genotype (Table 1). The mean age at diagnosis of HCC was lower for case patients with the Gln/Gln XRCC1 genotype than for case patients with the Gln/Arg or Arg/Arg XRCC1 genotype (43.8 years versus 48.9 years; $P = .004$ by Student's t test). After stratification by age at diagnosis of HCC, the Gln XRCC1 allele was associated with a dose-dependent increased risk of early-onset HCC (<50 years) ($P_{trend} = .01$) but not with the risk of late-onset HCC, although this difference was not statistically significant ($P = .098$). No evidence indicates that this association varies by family history of HCC or by the status of cigarette smoking and alcohol consumption (data not shown).

The GSTT1 genotype was not associated with the risk of HCC, overall or stratified by age at diagnosis. After adjusting for matching factors (sex and year of birth), a family history of HCC, tobacco and alcohol use, and other polymorphisms, the GSTM1-null genotype was associated with a decreased risk for early-onset HCC (OR = 0.64, 95% CI = 0.43 to 0.97) but not for late-onset HCC (data not shown). Table 2 shows that the association between XRCC1 and HCC is stronger for individuals with the GSTT1-null genotype than for those with the GSTT1-present genotype ($P_{interaction}$<.001). Conversely, this association appeared to be stronger for individuals with the GSTM1-present genotype than for those with the GSTM1-null genotype, although the difference between the two groups was not statistically significant. We also observed that the association of XRCC1 with HCC was modified by GSTT1 and GSTM1 status ($P_{interaction} = .005$). For those with the GSTT1-null/GSTM1-present genotype, a greater risk of HCC was associated with the Gln/Gln XRCC1 genotype than with the Arg/Arg genotype (adjusted OR = 8.07, 95% CI = 1.67 to 38.93); the 95% confidence interval is wide because there were only two control subjects with the Gln/Gln genotype in this stratum.

Extensive oxidative DNA damage has been detected in hepatocytes of HBV transgenic mice with chronic active hepatitis *(5)*. The XRCC1–399Gln allele is associated with lower efficiency of DNA repair *(11)*. GSTT1 protects cells from the natural by-products of lipid peroxidation and oxidative stress, and deletion of the GSTT1 gene is associated with enhanced endogenous mutagenic processes and is implicated in susceptibility to other inflammation-related cancers, such as pancreatic cancer, for which pancreatitis is a risk factor *(17,21,22)*. We found that the XRCC1-399Gln allele appeared to modify the age-associated risk of HCC among HBsAg carriers. Moreover, a greater-than-additive risk of HCC was obtained when the combination of the Gln/Gln XRCC1 genotype and GSTT1-null genotype was analyzed. These observations suggest that the lower DNA repair capacity and the increased level of reactive oxygen species resulting from the decreased activity of detoxification enzyme may increase the risk of HBV-related HCC.

The XRCC1–399Gln allele has also been associated with elevated aflatoxin

Table 1. Frequency distribution of the XRCC1-Arg399Gln polymorphism among hepatitis B surface antigen (HBsAg)–positive case patients with hepatocellular carcinoma (HCC) and HBsAg-positive control subjects

Characteristic	XRCC1-Arg399Gln polymorphism			P_{trend}*
	Arg/Arg	Arg/Gln	Gln/Gln	
All case patients versus all control subjects				
Case patients, No. (%)	301 (52.2)	223 (38.6)	53 (9.2)	
Control subjects, No. (%)	218 (56.0)	143 (36.8)	28 (7.2)	
Univariate OR (95% CI)†	1.00 (referent)	1.13 (0.86 to 1.48)	1.37 (0.84 to 2.24)	.166
Multivariable OR (95% CI)‡§	1.00 (referent)	1.10 (0.82 to 1.46)	1.54 (0.92 to 2.58)	.129
Early-onset case patients versus control subjects born after 1948‖				
Case patients, No. (%)	142 (49.5)	112 (39.0)	33 (11.5)	
Control subjects, No. (%)	110 (59.8)	60 (32.6)	14 (7.6)	
Univariate OR (95% CI)†	1.00 (referent)	1.45 (0.97 to 2.16)	1.83 (0.93 to 3.58)	.025
Multivariable OR (95% CI)‡¶	1.00 (referent)	1.58 (1.03 to 2.42)	2.12 (1.03 to 4.35)	.01
Late-onset case patients versus control subjects born in or before 1948				
Case patients, No. (%)	159 (54.8)	111 (38.3)	20 (6.9)	
Control subjects, No. (%)	108 (52.7)	83 (40.5)	14 (6.8)	
Univariate OR (95% CI)†	1.00 (referent)	0.91 (0.62 to 1.32)	0.97 (0.47 to 2.00)	.714
Multivariable OR (95% CI)‡§	1.00 (referent)	0.66 (0.42 to 1.03)	1.07 (0.46 to 2.50)	.294

*From Wald test for trend performed in the logistic regression model assigning scores of 1, 2, and 3, respectively, to the Arg/Arg, Arg/Gln, and Gln/Gln genotypes.

†OR = odds ratio; CI = confidence interval.

‡Adjusted for year of birth (continuous variable), sex, cigarette smoking (yes or no), alcohol consumption (yes or no), first-degree family member with history of HCC, and GSTM1 and GSTT1 genotypes.

§One case patient was excluded from analysis because of missing data on alcohol consumption.

‖The cut point for age at diagnosis between early- (<50 years) and late-onset HCC (≥50 years), 50 years old, was as defined in our previous study of familial aggregation *(3).* Because control subjects were frequency-matched to the case patients by year of birth and the earliest birth year for the early-onset case patients in this study is 1945, approaching the median year of birth in the control group, to compare separately with the early- and late-onset case patients, control subjects were dichotomized (born in or before 1948 or later) according to their median year of birth.

¶Likelihood ratio test for interaction of the stratified variable (onset age for case patients and year of birth for control subjects) and XRCC1 genotype was calculated as a test for the homogeneity of ORs across strata ($P_{interaction}$ = .098).

Table 2. Odds ratios (ORs) and 95% confidence intervals (CIs) for the association between the Arg399Gln polymorphism in the XRCC1 gene and hepatocellular carcinoma (HCC) among hepatitis B surface antigen (HBsAg) carriers, stratified by GSTM1 and GSTT1 genotypes

Genotype	XRCC1-Arg399Gln polymorphism					
	Arg/Arg		Arg/Gln		Gln/Gln	
	n*	OR (95% CI)†	n	OR (95% CI)†	n	OR (95% CI)†
GSTM1						
Present‡	123/90	1.00 (referent)	104/56	1.30 (0.82 to 2.06)	28/12	2.07 (0.95 to 4.52)
Null	178/128	1.00 (referent)	119/87	0.96 (0.66 to 1.40)	25/16	1.20 (0.60 to 2.42)
Test for interaction: P = .378§						
GSTT1						
Present	146/112	1.00 (referent)	114/58	1.49 (0.97 to 2.27)	19/20	0.75 (0.37 to 1.53)
Null‡	155/106	1.00 (referent)	109/85	0.85 (0.57 to 1.27)	34/8	3.53 (1.52 to 8.18)
Test for interaction: P<.001§						
GSTM1/GSTT1						
Present/Present	63/46	1.00 (referent)	54/19	1.91 (0.95 to 3.84)	11/10	0.92 (0.33 to 2.57)
Present/Null‡	60/44	1.00 (referent)	50/37	0.96 (0.51 to 1.81)	17/2	8.07 (1.67 to 38.93)
Null/Present	83/66	1.00 (referent)	60/39	1.20 (0.69 to 2.08)	8/10	0.54 (0.19 to 1.53)
Null/Null	95/62	1.00 (referent)	59/48	0.79 (0.47 to 1.33)	17/6	2.24 (0.81 to 6.21)
Test for interaction: P = .005‖						

*n = No. of case patients/No. of control subjects.

†Adjusted for year of birth (continuous variable), sex, cigarette smoking (yes or no), alcohol consumption (yes or no), first-degree family history of HCC, and the GSTM1 or GSTT1 genotype (when appropriate).

‡One case patient was excluded from analysis because of missing data on alcohol consumption.

§From the likelihood ratio test comparing the fit of the logistic model that included the main effects of various polymorphisms and all potential confounders (year of birth, sex, cigarette smoking, alcohol consumption, and first-degree family history of HCC) with a fully parameterized model containing all possible interaction terms of XRCC1 and GSTM1 (or GSTT1) genotypes.

‖From the likelihood ratio test comparing the fit of the logistic model that included the main effects of the XRCC1 genotype, the combinations of GSTM1 and GSTT1 genotypes, and all other factors listed above with a fully parameterized model containing all possible interaction terms of XRCC1 genotype and the combinations of GSTM1 and GSTT1 genotypes.

B_1-DNA adduct levels *(11),* and null genotypes for GSTM1 and GSTT1 may increase the risk for aflatoxin B_1-related HCC *(23,24).* However, the highest risk of HCC associated with the Gln/Gln XRCC1 genotype for HBsAg carriers was found in the GSTT1-null/GSTM1-present subgroup. Moreover, the GSTM1-null genotype was associated with

decreased risk for early-onset HCC. Although the mechanisms underlying the decreased risk associated with the GSTM1-null genotype are currently unknown, another Taiwanese study *(24)* of HCC has identified a statistically significantly inverse association with the GSTM1-null genotype despite the probable synergistic interaction between aflatoxin exposure and the null type. Conjugation with glutathione catalyzed by GSTM1 promotes elimination not only of carcinogens but also of anticarcinogenic compounds, notably isothiocyanates that occur naturally in cruciferous vegetables *(16,17,25)*. In fact, many studies *(22,26)* have evaluated the relationships between GSTM1 and GSTT1 gene deletions and other cancers, but the results are equivocal.

This is, to our knowledge, the first report that the combination of a DNA repair gene polymorphism and GST genotype is associated with the development of HCC. Given the small number of participants in certain comparisons, our findings require confirmation in larger studies. However, in light of our hypothesis, we believe that future studies on other genotypic variants involved in oxidative stress response and DNA repair are warranted.

REFERENCES

(1) Yu MW, Chen CJ. Hepatitis B and C viruses in the development of hepatocellular carcinoma. Crit Rev Oncol Hematol 1994;17: 71–91.

(2) Stevens CE, Beasley RP, Tsui J, Lee WC. Vertical transmission of hepatitis B antigen in Taiwan. N Engl J Med 1975;292:771–4.

(3) Yu MW, Chang HC, Liaw YF, Lin SM, Lee SD, Liu CJ, et al. Familial risk of hepatocellular carcinoma among chronic hepatitis B carriers and their relatives. J Natl Cancer Inst 2000;92:1159–64.

(4) Chen PJ, Chen DS. Hepatitis B virus infection and hepatocellular carcinoma: molecular genetics and clinical perspectives. Semin Liver Dis 1999;19:253–62.

(5) Hagen TM, Huang S, Curnutte J, Fowler P, Martinez V, Wehr CM, et al. Extensive oxidative DNA damage in hepatocytes of transgenic mice with chronic active hepatitis destined to develop hepatocellular carcinoma. Proc Natl Acad Sci U S A 1994;91: 12808–12.

(6) Takada S, Gotoh Y, Hayashi S, Yoshida M, Koike K. Structural rearrangement of integrated hepatitis B virus DNA as well as cellular flanking DNA is present in chronically infected hepatic tissues. J Virol 1990;64: 822–8.

(7) Yaginuma K, Kobayashi M, Yoshida E, Koike K. Hepatitis B virus integration in hepatocellular carcinoma DNA: duplication of cellular flanking sequences at the integration site. Proc Natl Acad Sci U S A 1985;82: 4458–62.

(8) Whitehouse CJ, Taylor RM, Thistlewaite A, Zhang H, Karimi-Busheri F, Lasko DD, et al. XRCC1 stimulates human polynucleotide kinase activity at damaged DNA termini and accelerates DNA single-strand break repair. Cell 2001;104:107–17.

(9) Vidal AE, Boiteux S, Hickson LD, Radicella JP. XRCC1 coordinates the initial and late stages of DNA abasic site repair through protein-protein interactions. EMBO J 2001;20: 6530–9.

(10) Lindahl T, Wood RD. Quality control by DNA repair. Science 1999;286:1897–1905.

(11) Lunn RM, Langlois RG, Hsieh LL, Thompson CL, Bell DA. XRCC1 polymorphisms: effects on aflatoxin B1-DNA adducts and glycophorin A variant frequency. Cancer Res 1999;59:2557–61.

(12) Kim SU, Park SK, Yoo KY, Yoon KS, Choi JY, Seo JS, et al. XRCC1 genetic polymorphism and breast cancer risk. Pharmacogenetics 2002;12:335–8.

(13) Park JY, Lee SY, Jeon HS, Bae NC, Chae SC, Joo S, et al. Polymorphism of the DNA repair gene XRCC1 and risk of primary lung cancer. Cancer Epidemiol Biomarkers Prev 2002;11:23–7.

(14) Shen H, Xu Y, Qian Y, Yu R, Qin Y, Zhou L, et al. Polymorphisms of the DNA repair gene XRCC1 and risk of gastric cancer in a Chinese population. Int J Cancer 2000;88: 601–6.

(15) Nelson HH, Kelsey KT, Mott LA, Karagas MR. The XRCC1 Arg399Gln polymorphism, sunburn, and non-melanoma skin cancer: evidence of gene-environment interaction. Cancer Res 2002;62:152–5.

(16) Hayes JD, Pulford DJ. The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit Rev Biochem Mol Biol 1995;30: 445–600.

(17) Ketterer B. Glutathione S-transferases and prevention of cellular free radical damage. Free Radic Res 1998;28:647–58.

(18) Yu MW, Chang HC, Chen PJ, Liu CJ, Liaw YF, Lin SM, et al. Increased risk for hepatitis B-related liver cirrhosis in relatives of patients with hepatocellular carcinoma in northern Taiwan. Int J Epidemiol 2002;31: 1008–15.

(19) Pemble S, Schroeder KR, Spencer SR, Meyer DJ, Hallier E, Bolt HM, et al. Human glutathione S-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. Biochem J 1994;300: 271–6.

(20) Bell DA, Taylor JA, Paulson DF, Robertson CN, Mohler JL, Lucier GW. Genetic risk and carcinogen exposure: a common inherited defect of the carcinogen-metabolism gene glutathione S-transferase M1 (GSTM1) that increases susceptibility to bladder cancer. J Natl Cancer Inst 1993;85:1159–64.

(21) Wiencke JK, Pemble S, Ketterer B, Kelsey KT. Gene deletion of glutathione S-transferase theta: correlation with induced genetic damage and potential role in endogenous mutagenesis. Cancer Epidemiol Biomarkers Prev 1995;4:253–9.

(22) Duell EJ, Holly EA, Bracci PM, Liu M, Wiencke JK, Kelsey KT. A population-based, case-control study of polymorphisms in carcinogen-metabolizing genes, smoking, and pancreatic adenocarcinoma risk. J Natl Cancer Inst 2002;94:297–306.

(23) Chen CJ, Yu MW, Liaw YF, Wang LW, Chiamprasert S, Matin F, et al. Chronic hepatitis B carriers with null genotypes of glutathione S-transferase M1 and T1 polymorphisms who are exposed to aflatoxin are at increased risk of hepatocellular carcinoma. Am J Hum Genet 1996;59:128–34.

(24) Sun CA, Wang LY, Chen CJ, Lu SN, You SL, Wang LW, et al. 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. Carcinogenesis 2001;22: 1289–94.

(25) Kolm RH, Danielson UH, Zhang Y, Talalay P, Mannervik B. Isothiocyanates as substrates for human glutathione transferases: structure-activity studies. Biochem J 1995; 311:453–9.

(26) Rebbeck TR. Molecular epidemiology of the human glutathione S-transferase genotypes GSTM1 and GSTT1 in cancer susceptibility. Cancer Epidemiol Biomarkers Prev 1997;6: 733–43.

NOTES

Supported by grants NSC 90-2314-B-002-100 (to M.-W. Yu) from the National Science Council and DOH91-TD-1003 (National Research Program for Genomic Medicine) (to M.-W. Yu) from the Department of Health, Executive Yuan, Taiwan.

Manuscript received February 21, 2003; revised July 8, 2003; accepted July 15, 2003.