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

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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 ($P_{\text{trend}} = .01$). 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 between XRCC1 with HCC ($P_{\text{interaction}} = .005$); 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.

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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...
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 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′-CAACACCCCCAAGT ACAGCC-3′) and 28265R (5′-GGCTG GGACCACCTGTGTT-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, \( P = .947 \)). 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) (\( P_{\text{trend}} = .01 \)) 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 (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 (\( P_{\text{interaction}} = .005 \)). 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 pancreaticitis 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...
Late-onset case patients versus control subjects born in or before 1948

Early-onset case patients versus control subjects born after 1948

All case patients versus all control subjects

GSTM1/GSTT1

Test for interaction:

GSTT1

Test for interaction:

GSTM1

Dichotomization (born in or before 1948 or later) according to their median year of birth.

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 was 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.

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)–positive case patients with hepatocellular carcinoma (HCC) and HBsAg–positive control subjects

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Arg/Arg</th>
<th>Arg/Gln</th>
<th>Gln/Gln</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n*</td>
<td>OR (95% CI)†</td>
<td>n</td>
</tr>
<tr>
<td>GSTM1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present‡</td>
<td>123/90</td>
<td>1.00 (referent)</td>
<td>104/56</td>
</tr>
<tr>
<td>Null</td>
<td>178/128</td>
<td>1.00 (referent)</td>
<td>119/87</td>
</tr>
<tr>
<td>Test for interaction: P = .378§</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSTT1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>146/112</td>
<td>1.00 (referent)</td>
<td>114/58</td>
</tr>
<tr>
<td>Null‡</td>
<td>155/106</td>
<td>1.00 (referent)</td>
<td>109/85</td>
</tr>
<tr>
<td>Test for interaction: P&lt;.001§</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSTM1/GSTT1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present/ Present</td>
<td>63/46</td>
<td>1.00 (referent)</td>
<td>54/19</td>
</tr>
<tr>
<td>Present/ Null‡</td>
<td>60/44</td>
<td>1.00 (referent)</td>
<td>50/37</td>
</tr>
<tr>
<td>Null/ Present</td>
<td>83/86</td>
<td>1.00 (referent)</td>
<td>60/39</td>
</tr>
<tr>
<td>Null/ Null</td>
<td>95/62</td>
<td>1.00 (referent)</td>
<td>59/48</td>
</tr>
<tr>
<td>Test for interaction: P = .005]</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*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.
¶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 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.
||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.

B1-DNA adduct levels (1/1), and null genotypes for GSTM1 and GSTT1 may increase the risk for aflatoxin B1-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.

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NOTES

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