Combined Effects of Polymorphisms of DNA-repair Protein Genes and Metabolic Enzyme Genes on the Risk of Cholangiocarcinoma

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Objective: Although *Opisthorchis viverrini* is a risk factor for cholangiocarcinoma, not all the infected individuals develop cholangiocarcinoma. We investigated whether the base excision repair enzyme gene polymorphisms with differentiated repair capacities of inflammation-related deoxyribonucleic acid damage may play a key role and such possible effects from those genes may be increased or diminished in co-existence of polymorphisms of metabolic enzymes, including glutathione-S-transferases mu 1 and glutathione-S-transferases u 1.

Methods: We genotyped five non-synonymous single-nucleotide polymorphisms of three genes, including the human homolog of the 8-oxoguanine glycosylase 1 Ser326Cys, X-ray repair cross-complementing protein 1 Arg194Trp, Arg280His and Arg399Gln and poly (adenosine diphosphate ribose) polymerase 1 Val762Ala in 87–94 matched case–control pairs, and examined relations between those polymorphisms and the risk of cholangiocarcinoma.

Results: Any single polymorphism did not have a measurable association with the risk of cholangiocarcinoma. However, when considering glutathione-S-transferases mu 1 polymorphism together, the human homolog of the 8-oxoguanine glycosylase 1 codon 326 polymorphism was related to the decreased risk; odds ratios were 1.00 (reference), 0.06 (95% confidence interval 0.01–0.53), 0.06 (0.01–0.54) and 0.14 (0.02–1.08) for persons with human homolog of the 8-oxoguanine glycosylase 1 Ser/Ser and glutathione-S-transferases mu 1 wild, ones with Ser/Ser and glutathione-S-transferases mu 1 null, ones with Ser/Cys or Cys/Cys and glutathione-S-transferases mu 1 wild and ones with Ser/Cys or Cys/Cys and glutathione-S-transferases mu 1 null, respectively (P for interaction < 0.01). Further adjustment for the presence of anti-*Opisthorchis viverrini* antibody, smoking and alcohol drinking did not change the decreased risk. Other combinations of deoxyribonucleic acid-repair gene polymorphism and glutathione-S-transferases were not associated with the risk of cholangiocarcinoma.

Conclusions: The present findings suggested that decreased capacity of deoxyribonucleic acid-repair gene, human homolog of the 8-oxoguanine glycosylase 1, may be related to decreased risk if much damaged cells die before malignant transformation.

Key words: epidemiology-prevention – carcinogenesis – genetics-carcinogenesis – GI-hepatobiliary-basic

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INTRODUCTION

The incidence of cholangiocarcinoma (CCA) in northeast Thailand is higher than that in other countries in Asia (1). Infection with the liver fluke, *Opisthorchis viverrini* (OV), has been known as an important risk factor in northeast Thailand, where approximately one-third of the population is infected with OV (1). But not all people infected with liver fluke develop CCA. Animal experiments suggest that the liver fluke infection alone does not cause CCA, but combined application of nitrosamines does cause the disease, similar to human pathology, in hamster (2). Involvement of endogenous carcinogens, especially N-nitrosamines, was also suggested in a human study using participants infected by OV (3). Thus, other environmental carcinogens and their metabolizing enzymes may also play a role in causing CCA. The genetic susceptibility to cancer may also result from the capacity to repair DNA damages caused by endogenous and exogenous mutagens and carcinogens. Therefore, it is hypothesized that some inherited polymorphisms in the genes involved in DNA damage repair are important as a risk factor for CCA (4). Among several DNA-repair mechanisms, base excision repair (BER) is an important one that repairs DNA damage resulting from chemical alterations in a single base caused by nitrosamines, and rectifies single-strand interruptions in DNA (5,6). It is well known that a number of proteins are involved in these steps, including human homolog of the 8-oxoguanine glycosylase 1 (*hOGG1*), X-ray repair cross-complementing protein 1 (*XRCC1*) (7) and poly (ADP-ribose) polymerase 1 (*PARP-1*) (8). *hOGG1* gene encodes a DNA glycosylase that repairs one of the most mutagenic lesions among base modification, 8-hydroxy-2′-deoxyguanine (8-OH-dG), produced in oxidatively damaged DNA by reactive oxygen species (9). *XRCC1* acts as a scaffolding intermediate facilitating or coordinating BER, through its interaction with *PARP-1*, DNA polymerase β and DNA ligase III (8,10–12).

Here, we have analyzed the association between the polymorphisms in DNA-repair-related genes, including *hOGG1* Ser326Cys, *XRCC1* Arg194Trp, *XRCC1* Arg280His, *XRCC1* Arg399Gln and *PARP1* Val762Ala, and the risk of CCA. We have also examined whether effects of those repair genes may be modified in relation to genetic polymorphisms of glutathione-S-transferase mu1 (*GSTM1*) and glutathione-S-transferase θ 1 (*GSTT1*) that can detoxify mutagens and carcinogens (13). Our hypothesis for the present examination was that null variants of GSTM1 and GSTT1 genes may be associated with the increased risk of CCA, especially when the DNA-repair proteins do not work well.

STUDY SUBJECTS

All cases with CCA were identified between 1999 and 2005 at Ubon Ratchathani Cancer Centre Hospital, Ubon Ratchathani Province of northeast Thailand. Diagnosis was based on abdominal ultrasonography by a single radiologist (C.V.) at the cancer center with serological supportive evidence including a raised CA 19–9 (>40 µg/ml) and a normal level of alpha fetoprotein (AFP; <20 ng/ml), although the latter was not obligatory. For each patient, each control subject was selected among visitors to the cancer center for general health check-up being matched by sex and age (within 5 years). Each control candidate underwent an abdominal ultrasonographic examination by the same radiologist examining the cases. Control candidates who had no ultrasonographic findings suggestive of CCA or hepatocellular carcinoma were verified as controls. Thus, 29 female and 76 male patient–control pairs were recruited, although the number of subjects for which genetic polymorphisms analyzed was not equal between the genes because the amount of blood sample was limited.

INFECTION BY THE OV

OV-specific IgG antibody titer was determined by an enzyme-linked immunosorbent assay (14). Although the raised OV antibody has been considered to continue over years, the length of the time period has not been precisely determined.

GENETIC POLYMORPHISM ANALYSIS

The hOGG1 Ser326Cys polymorphism was detected by a polymerase chain reaction (PCR) with the forward primer 5′-TGAAATTCGGAAGGTGCTTGGGGAAT-3′ and the reverse primer 5′-ACTGTCACTAGTCTCACCAG-3′. The PCR amplification parameters were a 12 min initial denaturation at 95°C, and 40 cycles each of 30 s at 95°C, 8°C and 1 min at 95°C, 30 s at 62°C and 1 min at 72°C, followed by a 10 min final elongation at 72°C. The 200 bp PCR product was digested with *Fnu4HI* (GC↓NGC) at 37°C. The Cys/Cys homozygotes gave a fragment length of 100 bp and the heterozygotes gave 200 and 100 bp, whereas the Ser/Ser homozygotes gave 200 bp. Restriction fragments were analyzed on 5% polyacrylamide gel.

The XRCC1 Arg194Trp polymorphism was detected by PCR with the forward primer 5′-GCCCCGTCCCAGGTA-3′ and the reverse primer 5′-AGCCCCAAAGACCCTTTCACT-3′. The PCR amplification parameters were a 10 min initial denaturation at 95°C, and 40 cycles each of 30 s at 95°C, 30 s at 55°C and 45 s at 72°C, followed by a 10 min final elongation at 72°C. The 491 bp PCR product was digested with *PvuII* (CAG↓CTG) at 37°C for 16 h. The Arg/Arg homozygotes gave a fragment length of 491 bp, and the heterozygotes gave 491, 294 and 197 bp, whereas the Trp/Trp homozygotes gave 294 and 240 bp. Restriction fragments were analyzed on 2% agarose gel.

MATERIALS AND METHODS

This study was performed after receiving approval from the ethics committees of the Nagahama Institute of Bio-Science and Technology, Nagahama, Japan and the National Cancer Institute, Bangkok, Thailand.
The XRCC1 Arg280His polymorphism was detected by PCR with the forward primer 5′-CCAGTGGTACTAACCTAATC-3′ and the reverse primer 5′-AGGCCCAAACCTTTCCTACT-3′. The PCR amplification parameters were a 5 min initial denaturation at 95°C, and 40 cycles each of 40 s at 95°C, 40 s at 55°C and 60 s at 72°C, followed by a 10 min final elongation at 72°C. The 201 bp PCR product was digested with Rsal (GT ↓ AC) at 37°C for 16 h. The Arg/Arg homozygotes gave a fragment length of 201 bp, and the heterozygotes gave 201, 145 and 56 bp, whereas the Trp/Trp homozygotes gave 145 and 56 bp. Restriction fragments were analyzed on 3% agarose gel.

The XRCC1 Arg399Gln polymorphism was detected by PCR with the forward primer 5′-TTGTGCTTTCTCTGTGTCCA-3′ and the reverse primer 5′-TTCGCACGCTTTCTGA-3′. The PCR amplification parameters were a 12 min initial denaturation at 95°C, and 40 cycles each of 30 s at 95°C, 45 s at 57°C and 45 s at 72°C, followed by a 10 min final elongation at 72°C. The 615 bp PCR product was digested with MspI (C ↓ CGG) at 37°C for 16 h. The Arg/Arg homozygotes gave a fragment length of 615 bp, and the heterozygotes gave 615, 375 and 240 bp, whereas the Gln/Gln homozygotes gave 375 and 240 bp. Restriction fragments were analyzed on 2% agarose gel.

The PI3K-AKT-1 Val762Ala polymorphism was detected by PCR with the forward primer 5′-TTCCTCCAGGCCTTGATA-3′ and the reverse primer 5′-TGCTCCAGCCTTTCTGATA-3′. The PCR amplification parameters were a 12 min initial denaturation at 95°C, and 40 cycles each of 30 s at 95°C, 45 s at 57°C and 45 s at 72°C, followed by a 10 min final elongation at 72°C. The 110 bp PCR product was digested with AccI (CG ↓ CG) at 37°C for 16 h. The Val/Val homozygotes gave a fragment length of 110 bp, and the heterozygotes gave 120, 90 and 20 bp, whereas the Ala/Ala homozygotes gave 90 and 20. Restriction fragments were analyzed on 10% polyacrylamide gel.

**RESULTS**

Any single polymorphism of the three DNA-repair-related genes was not associated with the risk of CCA (Table 1). Neither of genetic polymorphisms of the GSTM1 and the GSTTI gene alone was associated with the risk (data not shown).

Subsequently, we examined 10 combined effects consisting of each of five DNA-repair-related gene polymorphisms and each of two metabolic enzyme polymorphisms. We observed that the effect from hOGG1 genetic polymorphism on the risk of CCA was modified by polymorphism of XRCC1 (Table 2). After further adjustments for the presence of anti-OV antibody, smoking and alcohol drinking, those decreased risks were essentially unchanged; the corresponding ORs were 1.00 (reference), 0.02 (95% confidence interval: 0.001–0.39), 0.03 (0.001–0.48) and 0.06 (0.004–0.89), respectively. Because in this multivariate analysis, the OR of OV infection was 45.57 (2.63–787.33), interactions between GSTM1 polymorphism and OV infection and that between hOGG1 polymorphism and OV infection were examined and the former was found to be statistically significant (P for interaction = 0.01). However, the risk due to combination of GSTM1 polymorphism and OV infection was not explicitly determined largely because of the small sample size; ORs were 1.00 (reference), 1.36 (0.18–10.12), 2.33 × 10−8 (not determined) and 1.46 (0.25–8.35) for those with GSTM1 wild but without the raised anti-OV antibody, those with GSTM1 wild and the raised antibody, those with GSTM1 null variant but without the raised antibody and those with GSTM1 null and the raised antibody, respectively.

**DISCUSSION**

We did not find significant associations of any single genetic polymorphism alone of the DNA-repair genes with the risk of
CCA. As shown in our previous case-control study to see the involvement of genetic polymorphism of drug-metabolizing enzyme genes of GSTT1 and GSTM1 (15), the polymorphisms of those genes alone were not associated with the risk of CCA, either, in the present study (data not shown). However, we found a decreased risk in persons with hOGG1 326 Ser/Cys and Cys/Cys combined genotypes in relation to the GSTM1 polymorphism. Contrary to our expectation, the risk of CCA was not increased when the GSTM1 was null and the hOGG1 326 was not Ser/Ser. Although the functional effect of hOGG1 polymorphisms is unknown in detail at present when amino acid Ser 326 is changed to Cys, the DNA-repair capacity should be assumed to decrease. However, hOGG1 326 Cys/Cys genotype was reported to be associated with lowered

### Table 1. Relations of polymorphism of selected genes to the risk of CCA, Ubon Ratchathani, Thailand: based on the conditional logistic regression model

<table>
<thead>
<tr>
<th>Gene</th>
<th>Category</th>
<th>Casesa</th>
<th>Controlsa</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P value</th>
<th>Adjusteda</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LL</td>
<td>UL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hOGG1 (codon 326 genotype)</td>
<td>Ser/Ser</td>
<td>25</td>
<td>24</td>
<td>1.00</td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ser/Cys</td>
<td>44</td>
<td>43</td>
<td>0.98</td>
<td>0.44</td>
<td>2.16</td>
<td>0.96</td>
<td>0.85</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>Cys/Cys</td>
<td>18</td>
<td>20</td>
<td>0.87</td>
<td>0.37</td>
<td>2.04</td>
<td>0.75</td>
<td>0.79</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>Ser/cys + Cys/Cys</td>
<td>62</td>
<td>63</td>
<td>0.93</td>
<td>0.45</td>
<td>1.93</td>
<td>0.85</td>
<td>0.82</td>
<td>0.32</td>
</tr>
<tr>
<td>XRCC1 (codon 194 genotype)</td>
<td>Arg/Arg</td>
<td>49</td>
<td>52</td>
<td>1.00</td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arg/Trp</td>
<td>41</td>
<td>34</td>
<td>0.82</td>
<td>0.46</td>
<td>1.46</td>
<td>0.50</td>
<td>0.89</td>
<td>0.42</td>
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<tr>
<td></td>
<td>Trp/Trp</td>
<td>3</td>
<td>7</td>
<td>2.08</td>
<td>0.52</td>
<td>8.38</td>
<td>0.30</td>
<td>2.24</td>
<td>0.44</td>
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<tr>
<td></td>
<td>Arg/Trp + Trp/Trp</td>
<td>44</td>
<td>41</td>
<td>0.88</td>
<td>0.50</td>
<td>1.55</td>
<td>0.67</td>
<td>0.99</td>
<td>0.47</td>
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<tr>
<td>XRCC1 (codon 280 genotype)</td>
<td>Arg/Arg</td>
<td>72</td>
<td>74</td>
<td>1.00</td>
<td>Reference</td>
<td></td>
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<tr>
<td></td>
<td>Arg/His</td>
<td>19</td>
<td>17</td>
<td>0.87</td>
<td>0.41</td>
<td>1.82</td>
<td>0.71</td>
<td>0.49</td>
<td>0.16</td>
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<tr>
<td>XRCC1 (codon 399 genotype)</td>
<td>Arg/Arg</td>
<td>46</td>
<td>54</td>
<td>1.00</td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arg/Gln</td>
<td>46</td>
<td>35</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Gln/Gln</td>
<td>1</td>
<td>4</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arg/Gln + Gln/Gln</td>
<td>47</td>
<td>39</td>
<td>0.70</td>
<td>0.39</td>
<td>1.27</td>
<td>0.24</td>
<td>0.63</td>
<td>0.29</td>
</tr>
<tr>
<td>PARP1 (codon 762 genotype)</td>
<td>Val/Val</td>
<td>40</td>
<td>35</td>
<td>1.00</td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Val/Ala</td>
<td>11</td>
<td>11</td>
<td>1.15</td>
<td>0.43</td>
<td>3.10</td>
<td>0.78</td>
<td>0.90</td>
<td>0.24</td>
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<tr>
<td></td>
<td>Ala/Ala</td>
<td>43</td>
<td>48</td>
<td>1.29</td>
<td>0.69</td>
<td>2.42</td>
<td>0.43</td>
<td>1.26</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>Val/Ala + Ala/Ala</td>
<td>54</td>
<td>59</td>
<td>1.26</td>
<td>0.69</td>
<td>2.31</td>
<td>0.45</td>
<td>1.18</td>
<td>0.54</td>
</tr>
</tbody>
</table>

CCA, cholangiocarcinoma; OR, odds ratio; CI, confidence interval; LL, lower limit; UL, upper limit.
aAnalysis was done keeping matched pairs. Number of pairs used in each of the five polymorphisms was varied because of the limited volume of the samples used for genetic analysis.
bAdjusted for serum anti-OV antibody, alcohol drinking and smoking.

### Table 2. Risk of CCA due to combination of polymorphisms of GSTM1 and hOGG1 at codon 326: matched case-control study, Ubon Ratchathani, Thailand

<table>
<thead>
<tr>
<th>GSTM1</th>
<th>Ser/Ser</th>
<th>Ser/Cys + Cys/Cys</th>
<th>GSTM1</th>
<th>Ser/Ser</th>
<th>Ser/Cys + Cys/Cys</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Number</td>
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<td>Number</td>
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<td>Case</td>
<td>Control</td>
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<td>Control</td>
</tr>
<tr>
<td>Wild</td>
<td>11</td>
<td>3</td>
<td>Wild</td>
<td>17</td>
<td>24</td>
</tr>
<tr>
<td>Null</td>
<td>16</td>
<td>22</td>
<td>Null</td>
<td>59</td>
<td>42</td>
</tr>
</tbody>
</table>

GSTM1, glutathione-S-transferases mu 1; hOGG1, human homolog of the 8-oxoguanine glycosylase 1.
aBased on the conditional logistic regression model.
risk of bladder cancer occurrence and of its recurrence in Korean subjects, although hOGG1 326 Ser/Cys genotype was reported to be a risk factor (18). It may be proposed that the impaired detoxification due to GSTM1 null variant may behave independently from and/or jointly with ample carcinogens beyond the capacity of DNA-repair proteins, and that cells with too much damaged DNA may die before malignant transformation although no direct evidence has been available for irreparable DNA lesions (19). In such a circumstance, the risk of CCA can be decreased regardless of the hOGG1 326 polymorphism. When GSTM1 genotype is wild and hOGG1 326 has Cys allele, the risk of CCA can also be decreased if the DNA capacity is too small to repair DNA lesions potentially leading to cell death.

The protective effect of GSTM1 null variant could be due to the slow metabolism of some dietary materials, such as isothiocyanates derived from cruciferous vegetables, which is known to be a chemopreventive compound, by the deficient GSTM1, and its accumulation (20) although we could not examine the association between dietary isothiocyanates and the risk of CCA in the present study. The protective effects of GSTM1 null variant was also reported in hepatocellular carcinoma (21) and breast carcinoma (22).

Findings of the concerted action of DNA-repair protein gene and GSTM1 on the risk of CCA in the present study should give a novel insight into understanding the mechanism of carcinogenesis of CCA.

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**Conflict of interest statement**

None declared.

**References**


