

82233\_Auto\_Edited.docx

**Name of Journal:** *World Journal of Gastrointestinal Pathophysiology*

**Manuscript NO:** 82233

**Manuscript Type:** ORIGINAL ARTICLE

*Case Control Study*

**Polymorphism of genes encoding drug-metabolizing and inflammation-related enzymes for susceptibility to cholangiocarcinoma in Thailand**

CYP2E1, GSTT1 and risk for CCA

Gyokukou You, Lu Zeng, Hideaki Tanaka, Emi Ohta, Takahiro Fujii, Kazuhiko Ohshima, Masakazu Tanaka, Nobuyuki Hamajima, Chutiwan Viwatthanassitphong, Mantana Muangphot, Dhiraphol Chenvidhya, A Jedpiyawongse, Banchob Sripana, Masanao Miwa, Satoshi Honjo

## **Abstract**

### **BACKGROUND**

Cholangiocarcinoma (CCA) is an intractable cancer, and its incidence in northeastern Thailand is the highest worldwide. Infection with the liver fluke *Opisthorchis viverrini* (OV) has been associated with CCA risk. However, animal experiments have suggested that OV alone does not induce CCA, but its combination with a chemical carcinogen like nitrosamine can cause experimentally induced CCA in hamsters. Therefore, in humans, other environmental and genetic factors may also be involved.

### **AIM**

To examine relations between risk for CCA and genetic polymorphisms in carcinogen-metabolizing and inflammation-related genes.

### **METHODS**

This hospital-based case-control study enrolled 95 case-control pairs matched by age ( $\pm 5$  years) and sex. We examined relations between risk for CCA and genetic polymorphisms in carcinogen-metabolizing and inflammation-related genes, serum anti-OV, alcohol consumption, and smoking. Polymorphisms of CYP2E1, IL-6 (-174 and -634), IL-10 (-819), and NF- $\kappa$ B (-94) and their co-occurrence with polymorphisms in the drug-metabolizing enzyme gene GSTT1 or GSTM1 were also analyzed.

### **RESULTS**

Although CCA risk was not significantly associated with any single polymorphism, persons with the GSTT1 wild-type and CYP2E1 c1/c2+c2/c2 genotype had an increased risk (odds ratio = 3.33, 95% confidence interval: 1.23–9.00) as compared with persons having the GSTT1 wild-type and CYP2E1 c1/c1 wild genotype. The presence of anti-OV in serum was associated with a 7- to 11-fold increased risk, and smoking level was related to an OR of 1.5–1.8 in multivariable analyses adjusted for each of the seven genetic polymorphisms.

## CONCLUSION

In addition to infection with OV, gene-gene interactions may be considered as one of the risk factors for CCA development.

**Key Words:** Opisthorchis; Glutathione Transferase; Cytochrome P-450 CYP2E1; Case-Control Study

You G, Zeng L, Tanaka H, Ohta E, Fujii T, Ohshima K, Tanaka M, Hamajima N, Viwatthanassittiphong C, Muangphot M, Chenvidhya D, Jedpiyawongse A, Sripa B, Miwa M, Honjo S. Polymorphism of genes encoding drug-metabolizing and inflammation-related enzymes for susceptibility to cholangiocarcinoma in Thailand. *World J Gastrointest Pathophysiol* 2023; In press

**Core Tip:** Cholangiocarcinoma (CCA) is an intractable cancer, and its prevalence in northeastern Thailand is the highest worldwide.

An inflammatory condition produced by infection with the liver fluke *Opisthorchis viverrini* (OV) has been associated with CCA risk, but the susceptibility of individuals has not been fully examined.

Our study revealed that persons with the GSTT1 wild-type and CYP2E1 c1/c2+c2/c2 genotype had an increased risk for developing CCA (OR = 3.33 (95%CI :1.23–9.00). Therefore, both gene-gene interactions and OV infection should be considered as risk factors for cholangiocarcinogenesis.

## INTRODUCTION

Cholangiocarcinoma (CCA) is a cancer of the hepatobiliary tract, and its incidence is extremely high in northeastern Thailand (1). This is related to the lifestyle of the people of this area who often consume raw fish, which carries the risk of ingesting fish-borne parasites (2). Infection with the liver fluke, *Opisthorchis viverrini* (OV), is a known CCA

risk factor for inhabitants of northeastern Thailand (3-5), where approximately one-third of the population has been infected with OV (6, 7). Although infection with OV has been listed as a carcinogen to humans by IARC (8), infection with OV alone is not sufficient for CCA development. Indeed, experiments with hamsters have suggested that parasitic infection alone is not sufficient to develop CCA in fact, co-administration of a chemical carcinogen such as N-nitrosodimethylamine is necessary to induce CCA in hamsters (9). In addition, genetic background related to the activation or detoxification of chemical carcinogens is reported to be involved in CCA risk (10). Also, elevated plasma IL-6 was associated with increased risk of CCA in patients infected with OV. Thus, maintenance of chronic infection, exposure to a chemical carcinogen(s), and genetic background may explain CCA risk in northeastern Thailand. We previously reported that infection with OV and genetic polymorphism of a drug-metabolizing enzyme gene, namely GSTM1, is related to CCA risk (5) and that the combined effect of polymorphisms of the genes 8-oxoguanine glycosylase 1 and GSTT1 is also relevant CCA risk (12) in northeastern Thailand. Here, we report our analysis of the association between polymorphisms of inflammation-related genes (IL-6, IL-10 and NF-kB), and CYP2E1, GSTT1 and GSTM1 with risk of developing CCA. We also analyzed potential interactions among genetic polymorphisms of these genes.

## **MATERIALS AND METHODS**

This work was conducted after receiving the approval from the ethics committees of the Nagahama Institute of Bio-Science and Technology, Shiga, Japan, and the National Cancer Institute, Bangkok, Thailand.

### **Study subjects**

All cases with CCA were identified between 1999 and 2005 upon a visit to the Ubon Ratchathani Cancer Centre in the northeastern province of Thailand, one of the cancer centers administered by the National Cancer Institute of Thailand. Diagnosis was based on abdominal ultrasonography by a single radiologist (C. V.) at Ubon Ratchathani Cancer Centre with serological supportive evidence including an elevated CA 19-9

Level ( $\geq 40$   $\mu\text{g}/\text{mL}$ ). Each case was matched by sex and age (within 5 years) with each control subject who lived in the same Ubon Ratchathani area and visited Ubon Ratchathani Cancer Centre for health check-up. All control subjects were without any clinical, ultrasonographical, or serological abnormalities. Finally, the 95 case-control pairs having data for an antibody against infection with OV were employed although the number of subjects for each of the analyses involving genetic polymorphisms was not equal because the amount of blood samples was limited. Still, there was a narrow range (91–95) for the number of case-control pairs for each of the genetic polymorphisms examined (Table 1).

#### OV infection

Infection with OV was determined with an enzyme-linked immunosorbent assay using an antibody (“anti-OV”) raised against an OV antigen (13).

#### DNA extraction

Blood samples were frozen and stored at  $-80^{\circ}\text{C}$ . DNA was extracted from 2 mL blood with the QIAGEN DNA Blood Midi kit (14).

#### Analysis of GSTM1 and GSTT1 polymorphisms

Polymorphisms in each of GSTM1 and GSTT1 were determined with PCR (5) using the following primers: GSTM1, 5'-GAA CTC CCT GAA AAG CTA AAG C-3' and 5'-GTT GGG CTC AAA TAT ACG GTG G-3'; GSTT1, 5'-TTC CTT ACT GGT CCT CAC ATC TC-3' and 5'-TCA CCG GAT CAT GGC CAG CA-3'; CYP1A1 (internal control), 5'-GAA CTG CCA CTT CAG CTG TCT-3' and 5'-CAG CTG CAT TTG GAA GTG CTC-3'. Each 20- $\mu\text{L}$  reaction contained 0.2  $\mu\text{M}$  of each primer, 200  $\mu\text{M}$  dNTPs, 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.5), 1.5 mmol/L  $\text{MgCl}_2$ , approximately 6 ng DNA, and Taq DNA polymerase (AmpliTaqGold; Cetus PerkinElmer, Norwalk, CT). The PCR cycling parameters were 3 min at  $95^{\circ}\text{C}$  (initial denaturation) followed by 45 cycles of 1 min at  $95^{\circ}\text{C}$ , 30 sec at  $59^{\circ}\text{C}$ , and 1 min at  $72^{\circ}\text{C}$ , with a final 10-min extension step. The PCR products (299 bp for GSTM1, 507 bp for GSTT1) were separated on a 2% agarose gel.

#### Analysis of CYP2E1 polymorphisms

The 5'-flanking RsaI site polymorphism of CYP2E1 was detected by PCR combined with restriction-fragment-length polymorphism analysis (PCR-RFLP). The forward and reverse primers were 5'-CCA GTC GAG TCT ACA TTG TCA-3' and 5'-TTC ATT CTG TCT TCT AAC TGG-3', respectively. Each 20- $\mu$ l reaction contained 0.2  $\mu$ M of each primer, 200  $\mu$ M dNTPs, 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.5), 1.5 mmol/L MgCl<sub>2</sub>, approximately 6 ng DNA, and Taq DNA polymerase. The PCR cycling parameters were 12 min at 95°C (initial denaturation) followed by 40 cycles of 1 min at 95°C, 30 sec at 60°C, and 1 min at 72°C, with a final 10-min extension step at 72°C. A 10- $\mu$ l aliquot of the PCR products was digested with 0.2 U of the restriction enzyme RsaI (GTaU) (TOYOBO Co., Ltd., Japan) for 16 h at 37°C. The resulting fragments were separated by 3% agarose gel electrophoresis and stained with ethidium bromide. The expected PCR product was 410 bp. Upon digestion with RsaI, the c1/c1 wild-type homozygotes had fragments of 360 and 50 bp, the c1/c2 heterozygotes had fragments of 410, 360 and 50 bp, and the c2/c2 variant homozygotes had a fragment of 410 bp (15).

#### Analysis of IL-6 (rs1800795) polymorphisms

Polymorphism -174 (G/C) (rs1800795) of IL-6 was detected by PCR-RFLP with forward primer 5'-ATG CCA AGT GCT GAG TCA CTA-3' and reverse primer 5'-TCG AGG GCA GAA TGA GCC TC-3'. Each 20- $\mu$ l reaction contained 0.2  $\mu$ M of each primer, 200  $\mu$ M dNTPs, 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.3), 1.5 mmol/L MgCl<sub>2</sub>, approximately 6 ng DNA, and Taq DNA polymerase. The PCR cycling parameters were 10 min at 95°C (initial denaturation) followed by 40 cycles of 30 sec at 95°C, 30 sec at 54°C, and 40 sec at 72°C, with a final 10-min extension step at 72°C. A 10- $\mu$ l aliquot of the PCR products was digested with 0.2 U of the restriction enzyme NlaIII (CATG↓) (New England Biolabs, Japan) at 37°C for 16 h and separated by PAGE (10% polyacrylamide gel). The expected PCR product was 308 bp. Upon digestion with NlaIII, the G/G wild-type homozygotes had fragments of 233 and 75 bp, the G/C heterozygotes had fragments of 233, 121, 112 and 75 bp, and the C/C variant homozygotes had fragments of 121, 112 and 75 bp (16).

#### Analysis of IL-6 (rs1800796) polymorphisms

Polymorphism -634 G/C (rs1800796) of IL-6 was analyzed by PCR with confronting two-pair primers (PCR-CTPP) (17). The four primers used were IL-6-634 F1 5'-CCT CTA AGT TGG GCT GAA GCA GG-3' and IL-6-634 R1 5'-GTT CTG GCT CTC CCT GTG AGG-3' for amplifying the variant type, and IL-6-634 F2 5'-CCA GGC AGT TCT ACA ACA GCC G-3' and IL-6-634 R2 5'-TGA GTT TCC TCT GAC TCC ATC GC-3' for amplifying wild type. Each 25- $\mu$ l reaction contained 0.2  $\mu$ M of each primer, 200  $\mu$ M dNTPs, 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.3), 2 mmol/L MgCl<sub>2</sub>, approximately 6 ng DNA, and Taq DNA polymerase. The PCR cycling parameters were 10 min at 95°C (initial denaturation) followed by 36 cycles of 1 min at 95°C, 1 min at 65°C, and 1 min at 72°C, with a final 5-min extension step at 72°C. The PCR products were separated by PAGE (10% polyacrylamide gel). The G/G wild-type homozygotes had fragments of 157 and 240 bp, G/C heterozygotes had fragments of 125, 157, 240 and 75 bp, and the C/C variant homozygotes had fragments of 125 and 240 bp.

#### Analysis of IL-10 (rs1800871) polymorphisms

Polymorphism -819 T/C (rs1800871) of IL-10 was analyzed by PCR-RFLP. The forward and reverse primers were IL-10-819 F 5'-TCA TTC TAT GTG CTG GAG ATG G-3' and IL-10-819 R 5'-TGG GGG AAG TGG GTA AGA GT-3', respectively. Each 20- $\mu$ l reaction contained 0.2  $\mu$ M of each primer, 200  $\mu$ M dNTPs, 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.3), 2 mmol/L MgCl<sub>2</sub>, approximately 6 ng DNA, and Taq DNA polymerase. The PCR cycling parameters were 5 min at 95°C (initial denaturation) followed by 40 cycles of 30 sec at 95°C, 45 sec at 59°C, and 1 min at 72°C, with a final 10-min extension step at 72°C. A 10- $\mu$ l aliquot of the PCR products was digested with 0.03 U of the restriction enzyme MaeIII ( $\downarrow$ GTNAC) (Roche Diagnostics GmbH Germany) for 16 h at 55°C and separated by PAGE (10% polyacrylamide gel). The expected PCR product was 209 bp. Following digestion with MaeIII, the T/T wild-type homozygotes had a fragment of 209 bp, the T/C heterozygotes had fragments of 209, 125 and 84 bp, and the C/C variant homozygotes had fragments of 209 and 84 bp (18).

#### Analysis of NF- $\kappa$ B polymorphisms



Polymorphism -94 ins/del ATTG of the NF- $\kappa$ B promoter was determined by PCR-RFLP. The forward and reverse primers were 5'-TTT AAT CTG TGA AGA GAT GTG AAT-3' and 5'-CTA GCA GGG CGC TCC CGA AT-3', respectively. Each 20- $\mu$ l reaction contained 0.2  $\mu$ M of each primer, 200  $\mu$ M dNTPs, 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.3), 1.5 mmol/L MgCl<sub>2</sub>, approximately 6 ng DNA, and Taq DNA polymerase. The PCR cycling parameters were 10 min at 95°C (initial denaturation) followed by 40 cycles of 30 sec at 95°C, 1 min at 56°C, and 1 min at 72°C, with a final 10-min extension at 72°C. A 10- $\mu$ l aliquot of the PCR products was digested with 2 U of the restriction enzyme PflMI (New England Biolabs Inc., Japan) at 37°C for 16 h and separated by PAGE (10% polyacrylamide gel). The expected PCR product was 254 bp. Upon digestion with PflMI, the del/del ATTG wild-type homozygotes had a fragment of 254 bp, the ins/del heterozygotes had fragments of 254 and 206 bp, and the ins/ins variant homozygotes had a fragment of 206 bp (19).

#### Data collection for lifestyle-related factors

Smoking and alcohol consumption status were ascertained alongside dietary habits at the hospital by local health personnel using a structured questionnaire used in previous studies (5, 12). Smoking status was classified into four categories: never, occasional, former, and current. A current smoker was defined as smoking at least 1 cigarette per day, whereas the former smoker was defined as having stopped smoking regularly at least 1 year prior to our study. Alcohol consumption status was classified the same as for smoking status into four categories: never, occasional, former, and current. "Current" drinker was defined as drinking more than once a week, whereas "former" drinker was defined as having stopped regular drinking at least 1 year prior to the study.

#### Statistical analysis

CCA risk attributable to infection with OV was defined as positivity for anti-OV, and the potential contributions of genetic polymorphisms of genes encoding inflammation-related enzymes were examined by calculating an odds ratio (OR). Each OR was calculated using a conditional logistic regression model, keeping matched case-control

pairs. Interactions between each of the genetic polymorphisms of the inflammation-related genes and each of the metabolic enzyme genes were first examined using a log-likelihood ratio test. The tests compared the main effect with no interaction terms with a full model that included the interaction term for the variables concerned. When the p value for the log-likelihood ratio statistic was  $<0.1$ , we calculated the OR owing to the co-occurrence of two genetic polymorphisms. Three subjects lacked data for alcohol consumption, and two lacked data for smoking status (Table 1). Most of the multivariable statistical analyses were performed with imputation for the missing data for these five subjects and produced comparable results (Tables 2–4). All statistical analyses were performed with the statistical package STATA 16.1 (College Station, TX, USA), and statistical significance was defined as  $p < 0.05$  unless indicated otherwise. Although the CCA-related risk of infection with OV among the current study subjects was reported in the multivariable analyses that included the interaction terms between the GSTT1 and hOGG1 polymorphisms in our previous study (12), a crude or adjusted OR for each polymorphism has not been reported. Data for CCA risk owing to polymorphism of GSTM1 or GSTT1 alone also has not been reported (12). Findings for those factors are, thus, reported in the present study.

## **RESULTS**

Concerning environmental factors, we confirmed the strong association between anti-OV-positive individuals and CCA risk adjusted for smoking status and alcohol consumption (Table 2). The OR values (95% confidence interval) for a one-category change in smoking category (never, occasional, former, current) were approximately 1.5 regardless of the particular genetic polymorphism(s) examined or whether imputed data were included (Table 3). Alcohol consumption was not materially related to CCA risk (Table 3).

There was no significant association between polymorphisms for any of the inflammation-related genes or drug-metabolizing genes and CCA risk. When an inflammation-related gene (IL-6, IL-10, NF- $\kappa$ B) wild-type genotype was used as the

reference, CCA risk was not significantly associated with any homozygous or heterozygous genotype (Table 4). Likewise, when the GSTT1 wild-type genotype was used as the reference, CCA risk was not significantly associated with the null genotype. Similarly, when the GSTM1 wild-type genotype was used as the reference, CCA risk was not significantly associated with the null genotype. When the CYP2E1 c1/c1 wild-type genotype was used as the reference, CCA risk was not significantly associated with c1/c2, c2/c2, or the combination of the c1/c2+c2/c2 genotypes (Table 4).

#### Gene-gene interactions between GSTT1 and CYP2E1

Interaction of the CYP2E1 polymorphism and the GSTT1 polymorphisms was suggested by a p value of 0.003, whereas other interactions between drug-metabolizing enzyme genes (GSTM1 or GSTT1) and other genetic polymorphisms were not substantiated ( $p > 0.10$ ). Persons with the GSTT1 wild-type plus CYP2E1 c1/c2+c2/c2 genotype had an increased risk for CCA (OR = 3.33, 95% confidence interval: 1.23–9.00) as compared with persons having the GSTT1 wild-type plus CYP2E1 c1/c1 wild-type genotype that was used as the reference (Table 5).

## **DISCUSSION**

+ADw-html+AD4APA-p+AD4- Early diagnosis of CCA is difficult, and most patients die within a year after diagnosis (20). Identification of risk factors and means of preventing cholangiocarcinogenesis is thus highly desirable. We confirmed that positivity for anti-OV constitutes a significant risk factor for CCA, as reported previously (3, 5, 21). Smoking was not a significant risk factor for CCA, whereas alcohol consumption was in fact related to increased risk as we reported previously for subjects (and matched controls) recruited from another part of northern Thailand (5). In the present study, control subjects were persons seeking a health check-up and thus possibly may have led a relatively healthier lifestyle compared with our experimental subjects. However, the discordance between the findings on smoking and alcohol drinking of the two studies are not yet explained. Although we did not find any significant association between CCA risk and polymorphisms in inflammation-related

genes (IL-6, IL-10, NF- $\kappa$ B) or in drug metabolism-related genes (CYP2E1, GSTT1 and GSTM1), persons with the GSTT1 wild-type plus CYP2E1 c1/c2+--c2/c2 genotype had a 3-fold greater risk than persons having the GSTT1 wild-type plus CYP2E1 c1/c1 wild-type genotype that was used as the reference. Hayashi <sup>1</sup> +ADw-i+AD4-et al+ADw-/i+AD4- reported that the c2/c2 homozygous sequence placed upstream of the SV40 promoter and chloramphenicol acetyltransferase gene enhanced the expression of that gene, and the enhancement of expression by the c2/c2 sequence was about 10-fold that by the c1/c1 sequence (22). Thus, it is possible that CYP2E1 is expressed at a higher level in the presence of the c2/c2 sequence than in the presence of the c1/c1 sequence, which is consistent with the known function of CYP2E1 as a carcinogen-activating enzyme (23, 24). Because the numbers of cases and controls were not large, the conclusions from this work should be confirmed in a future study with more cases and controls. In addition, genes encoding other drug-metabolizing enzymes should also be tested with respect to gene-gene interactions.

## **CONCLUSION**

The presence of anti-OV in serum was associated with a 7- to 11-fold increased risk for independently of the genetic polymorphisms of carcinogen-metabolizing and inflammation-related genes. While any single polymorphism was not significantly associated with CCA risk, persons with the GSTT1 wild-type and CYP2E1 c1/c2+c2/c2 genotype had a 3-fold increased risk as compared with persons having the GSTT1 wild-type and CYP2E1 c1/c1 wild genotype. In addition to infection with OV, gene-gene interactions may be considered as one of the risk factors for CCA development.

## **ARTICLE HIGHLIGHTS**

### ***Research background***

Cholangiocarcinoma (CCA) is a cancer of the hepatobiliary tract, and its incidence is extremely high in northeastern Thailand. This is related to the lifestyle of the

inhabitants of this area consuming often raw fish, which carries the risk of ingesting fish-borne parasites, *Opisthorchis viverrini* (OV), a known CCA risk factor. While infection with OV has been listed as a carcinogen to humans by IARC, the parasitic infection alone is not sufficient to develop CCA; in fact, co-administration of a chemical carcinogen such as N-nitrosodimethylamine is necessary to induce CCA in animal model. In addition, genetic background related to the activation or detoxification of chemical carcinogens is reported to be involved in CCA risk. Also, elevated plasma IL-6 was associated with increased risk of CCA in patients infected with OV.

### ***Research motivation***

We already reported that infection with OV and genetic polymorphism of a drug-metabolizing enzyme gene, namely GSTM1, is related to CCA risk and that the combined effect of polymorphisms of the genes 8-oxoguanine glycosylase 1 and GSTT1 is also relevant CCA risk in northeastern Thailand. In the present study, we further investigated possible associations of maintenance of chronic infection, exposure to a chemical carcinogen(s) and genetic background with CCA risk in northeastern Thailand.

### ***Research objectives***

To examine relations between risk for CCA and genetic polymorphisms in carcinogen-metabolizing (CYP2E1, GSTT1 and GSTM1) and inflammation-related genes (IL-6, IL-10 and NF- $\kappa$ B), and potential interactions among genetic polymorphisms of these genes on the CCA risk.

### ***Research methods***

All cases with CCA were identified between 1999 and 2005 upon a visit to the Ubon Ratchathani Cancer Centre in the northeastern province of Thailand. This hospital-based case-control study enrolled 95 case-control pairs matched by age ( $\pm 5$  years) and sex. We examined relations between risk for CCA and genetic polymorphisms in

carcinogen-metabolizing and inflammation-related genes, serum anti-OV, alcohol consumption, and smoking. Smoking and alcohol consumption status were ascertained using a structured questionnaire used in previous studies. Conditional logistic regression was employed to estimate CCA risk as odds ratio (OR) due to each of genetic polymorphisms and possible interactions of those polymorphisms.

### ***Research results***

Although any single polymorphism was not significantly associated with CCA risk, persons with the GSTT1 wild-type and CYP2E1 c1/c2+c2/c2 genotype had a 3-fold increased risk as compared with persons having the GSTT1 wild-type and CYP2E1 c1/c1 wild genotype. The presence of anti-OV in serum was associated with a 7- to 11-fold increased risk for CCA independently of the genetic polymorphisms of carcinogen-metabolizing and inflammation-related genes.

### ***Research conclusions***

An inflammatory condition produced by infection with OV indicated as raised anti-OV in serum has been associated with CCA risk. Our study added the finding that persons with the GSTT1 wild-type and CYP2E1 c1/c2+c2/c2 genotype had a 3-fold increased risk for developing CCA. Therefore, both gene-gene interactions and OV infection should be considered as risk factors for cholangiocarcinogenesis in northeastern Thailand.

### ***Research perspectives***

CCA is still an intractable cancer. While our study revealed the interaction of polymorphisms of GSTT1 and CYP2E1 possibly contributes to development of CCA, the numbers of cases and controls were not large. The conclusions from this work should be confirmed in a future study with more cases and controls. In addition, genes encoding other drug-metabolizing enzymes should also be tested with respect to gene-gene interactions.

## **ACKNOWLEDGEMENTS**

We thank both the case and control subjects for their participation in our study <sup>2</sup> and all the community hospitals for their generous assistance in interviewing and collecting the specimens from all the subjects.

The late Dr. Srivatanakul had started the collaborative study with Professor Miwa in 1998 concerning an etiological investigation on CCA in Thailand and continued to be the Thai side's active organizer based on the National Cancer Institute, Bangkok, until her death in 2020. As such, she actively participated in the planning, epidemiological investigation (including patient and control subject recruitment) and giving helpful comments on data analyses.

## ORIGINALITY REPORT

1 %

SIMILARITY INDEX

## PRIMARY SOURCES

- 1

Dmitry Korolev, Konstantin V. Balakin, Yuri Nikolsky, Eugene Kirillov et al. "Modeling of Human Cytochrome P450-Mediated Drug Metabolism Using Unsupervised Machine Learning Approach", Journal of Medicinal Chemistry, 2003

Crossref

20 words — < 1 %
- 2

Satoshi Honjo, Petcharin Srivatanakul, Hutchia Sriplung, Hiroko Kikukawa et al. "Genetic and environmental determinants of risk for cholangiocarcinoma via *Opisthorchis viverrini* in a densely infested area in Nakhon Phanom, northeast Thailand", International Journal of Cancer, 2005

Crossref

19 words — < 1 %
- 3

[gut.bmj.com](http://gut.bmj.com)

Internet

12 words — < 1 %
- 4

[www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)

Internet

12 words — < 1 %

EXCLUDE QUOTES ON

EXCLUDE BIBLIOGRAPHY ON

EXCLUDE SOURCES

EXCLUDE MATCHES

< 12 WORDS

< 12 WORDS