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

**ESPS Manuscript NO: 9433**

**Columns: MINIREVIEW**

**Genetic and environmental determinants of risk for cholangiocarcinoma in Thailand**

Miwa M *et al.* Risk factors of cholangiocarcinoma

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**Supported by** Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology Japan

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**Received:** February11, 2014 **Revised:** May 1, 2014

**Accepted:** September 6, 2014

**Published online:**

**Abstract**

Cholangiocarcinoma (CCA) is a difficult cancer to diagnose in the early stage and to treat by curative resection. The incidence of CCA in the northeast of Thailand is the highest in the world. To make progress in detecting a high risk group, and the prevention and detection of CCA, we have been analyzing the risk factors for CCA. Although the liver fluke infection is known to be the risk factor, there are patients who are not infected with the liver fluke, and not all people infected with the liver fluke will suffer from the disease. Therefore it is of the utmost importance to analyze the risk factors and the mechanism to prevent the disease and also to detect the disease in it’s early stage to save the patients’ lives. Through collaboration among Thai and Japanese researchers, we analyzed the genetic and environmental determinants of risks for CCA. Also, we have been trying to develop the methods to detect the disease in a non-invasive way. Not repeating findings reported in various reviews on CCA, we will first discuss the environmental and genetic determinants of the risks for CCA. Second, we will discuss the properties of CCA including the etiological agents and the mechanism of cholangiocarcinogenesis, and finally we will discuss the future approaches to prevent and to cure CCA from the standpoint of evidence-based medicine. We will discuss these points by including the data from our laboratories. We would like to emphasize the importance of the genetic data, especially whole genome approaches to understand the properties of CCA, to find a high risk population for CCA, and to develop effective preventative methods to stop the carcinogenic steps toward CCA in the near future. In addition, it is of the upmost importance to develop a non-invasive, specific and sensitive method to detect CCA in it’s early stage for the application of modern medical approaches to help patients with CCA.

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**Key words:** Alcohol drinking; Cholangiocarcinoma; DNA polymorphism; Glutathione S transferase; 8-oxoguanine glycosylase 1; Liver fluke; *Opisthorchis viverrini*; Thailand

**Core tip:** Cholangiocarcinoma (CCA) is an intractable cancer due to the difficulty of diagnosis in it’s early stage. The incidence of CCA in the northeast of Thailand is the highest in the world. It is of the utmost importance to analyze the risk factors and the mechanism to prevent the disease and to also detect the disease in it’s early stage to save the patients’ lives. We analyzed the genetic and environmental determinants of risks for CCA and discussed this with the findings already published by other researchers. It is of the utmost importance to develop a non-invasive, specific and sensitive method to detect CCA.

Miwa M, Honjo S, You G, Tanaka M, Uchida K, Srivatanakul P, Khuhaprema T, Loilome W, Techasen A, Wongkham C, Limpaiboon T, Yongvanit P, Wongkham S. Genetic and environmental determinants of risk for cholangiocarcinoma in Thailand. *World J Gastrointest Pathophysiol* 2014; In press

**INTRODUCTION**

The age standardized rates (ASR World) of the incidence of liver and bile duct cancer in Thailand, between 2001 and 2003, are 38.6 and 14.6 for men and women respectively. Most remarkably, ASR World of liver and bile duct cancer in Udon Thani, Khon Kaen, Nakorn Phanom, Ubon Ratchathani, Bangkok, and Songkhla provinces for men are 115.0, 87.7, 78.4, 74.9, 21.5, and 10.9, respectively, and for women are 52.7, 36.3, 43.2, 34.7, 6.4, and 2.9, respectively. Cholangiocarcinoma (CCA) among the liver and bile duct cancer in the above provinces for men are 80.5%, 81.1%, 55.9%, 81.0%, 32.6%, and 33.3%, respectively, and for women are 86.7%, 82.3%, 60.8%, 76.6%, 56.7%, and 43.5%, respectively[1]. Thus the incidence of CCA in the northeast of Thailand is extremely high in comparison to the rest of the world.

It was previously reported that the liver fluke, *Opisthorchis viverrini* (OV) and endogenous nitrosamines are the important risk factors for CCA in Thailand[2,3]. Multiple pathways on the tumorigenic OV infection to cause CCA from Thailand are nicely summarized in the recent review[4].

**ENVIRONMENTAL DETERMINANTS**

From the epidemiological study, it was previously known that the infection of the liver fluke, *Opisthorchis viverrini* (OV), is an important risk factor of CCA[2] (Table 1). In addition to OV infection, some of the chemical carcinogens like nitrosamine are also suggested to be factors in the risk for CCA[3]. We performed a population-based case-control study in which sex, age and place of residence were matched individually. We confirmed that the presence of the antibody against OV significantly increased the risk for CCA; odds ratio (OR) = 27.09 (95% confidence interval (CI): 6.30-116.57). The results confirmed the previously reported data by Parkin *et al*[2]. In addition we found that alcohol drinking is another risk factor for CCA. Ex-regular and regular alcohol drinkers showed OR = 6.23 (95%CI: 1.23-31.57) and OR = 4.31 (95%CI: 1.12-16.57), respectively (Table 1)[5]. We examined the possibility that alcohol consumption affects the ;risk for CCA due to OV infection, and also smoking and dietary habits during the past 10 years, and found only the risks due to smoking and eating fermented fish (*pla-ra* and/o*r pla-chao*) were altered with alcohol consumption (*P* for interaction < 0.01 and 0.07, respectively). The interactions between alcohol drinking and selected variables are shown in Table 2. The odds ratios are slightly different from those appearing in our previous paper[5] due to a typing error although the conclusion is materially the same. The increased risk for CCA due to ever-smoking was more prominent among ever-drinkers than among never-drinkers, and a similar observation was made for the risk by eating *pla-ra* and/or *pla-chao*. Conversely vitamin C was suggested to reduce the risk [3]. Recently Songserm *et al*[6] confirmed that alcohol drinking increased the risk for CCA. And they reported that the consumption of fruits and vegetables decreased the risk for CCA (Table 1). Manwong *et al*[7] also reported that family history of cancer was a significant risk factor (Table 1).

**INTERACTION BETWEEN GENETIC AND ENVIRONMENTAL DETERMINANTS**

Since not all patients with CCA are infected with OV and not all individuals infected by OV develop CCA, it is possible that some other environmental and genetic determinants are involved in the pathogenesis of CCA. We examined the genetic polymorphism on the risk for CCA. We first examined the effect of carcinogen detoxification enzyme gene polymorphisms, namely GSTM1 and GSTT1, which are well-known. DNA polymorphism of GSTM1 or GSTT1 alone was not associated with the risk of CCA. However the null genotype of GSTM1 enhanced odds ratio (OR) of the risk for CCA in anti-OV antibody positive subjects to 18.00 (95%CI: 3.33-97.40) as compared to that of GSTM1 wild in anti-OV antibody positive subjects of 10.34 (95%CI: 1.31-81.63). The null genotype of GSTT1 enhanced OR in ex-regular alcohol drinkers to OR = 27.93 (95%CI: 1.84-424.60) as compared to that of GSTT1 wild in ex-regular drinkers of OR=1.28 (95%CI: 0.12-14.08)[5].

Songserm *et al*[6] analyzed methylenetetrahydrofolate reductase gene polymorphism (*MTHFR*) at 677 and at 1298 for interaction with beef sausage consumption (Table 3). They found that *MTHFR*677 TT variants and *MTHFR*1298 CC variants showed increased risks when the individuals ate beef sausage daily. The data attained by the above researchers which showed interaction are listed in Table 3.

**EFFECTS OF GENETIC DETERMINANTS, DNA POLYMORPHISM, ON RISK FOR CCA**

There are several reports of the effects of DNA polymorphisms on the risk of CCA. Among various enzymes involved in carcinogen metabolism, CYP1A2, one of the phase I enzymes related in the activation of such carcinogen in cigarette smoke, has DNA polymorphism. *CYP1A2* polymorphism, found in the intron 1, might be involved in the risk of CCA. Prawan *et al*[8] found that *CYP1A2\*1A/\*1A* polymorphism had the protective effect on the risk of CCA in men but not in women (Table 4). Since men smoke more than women in Thailand, it is considered that in the individuals with *CYP1A2\*1A* polymorphism, the CYP1A2 enzyme might be less inducible as compared to that with *CYP1A2\*1F*, although the effect of these mutations on the induction of the enzyme is not clear.

Arylamine *N*-acetyltransferase (NAT) catalyzes *N*- and *O*-acetylatioin of various arylamines and heterocyclic amines thereby regulating the metabolic activation and detoxification of xenobiotics and carcinogen. Individuals with three *NAT2* alleles, *NAT2\*13*, *\*6B* and *\*7A*, are associated with decreased risk for CCA, while those with *NAT2\*4, \*5, \*6A* and *\*7B* were not, suggesting that the *NAT2* polymorphism may modify the risk of CCA (Table 4)[8].

Glutathione *S*-transferases (GSTs), a family of Phase II detoxifying enzymes, can conjugate reduced glutathione to various compounds. Concerning polymorphism of *GSTO1* and *GSTO2,* Marahatta *et al*[9] found that the individuals having *GSTO1\*D140* had significantly increased risk for CCA, hepatocellular carcinoma and breast cancer (Table 4). A study with a larger sample size will better clarify the function of GSTO1.

Natural killer cell receptor G2D (NKG2D) haplotypes were found to be associated with the natural cytotoxic activity of individuals. NKG2D triggers cell-mediated cytotoxicity in natural killer cells. Various NKG2D haplotype alleles showed a significant difference between cases and controls[10]. Primary sclerosing cholangitis (PSC) is an inflammatory bowel disease and is suggested to be a predisposing disease to hepatobiliary malignancy. Thirteen percent of the patients with primary sclerosing cholangitis developed CCA[11]. When NKG2D single nucleotide polymorphisms (SNPs) were compared between the PSC patients with CCA and the PSC patients without CCA in a Norweigian population, there was significantly increased allele frequencies in two SNPs, namely rs11053781 and rs11053781, both of which are non-coding. The odds ratio for G versus A in the rs11053781 was 2.08 (95%CI: 1.31-3.29) and that for A versus G in rs2617167 was 2.32 (95%CI: 1.47-3.66). When they were compared between the PSC patients with CCA and the healthy controls, there was also a significant increase of allele frequencies in the above two SNPs. The odds ratio for G versus A in the rs11053781 was 1.95 (95%CI: 1.23-3.07) and that for A versus G in rs2617167 was 2.20 (95%CI: 1.40-3.44) (Table 4)[12]. The functional role of the changes of these SNPs on the susceptibility to CCA remains to be elucidated.

Multidrug resistance-associated protein 2 (MRP2/ABCC2), one of the ATP-binding cassette transporter proteins, is suggested to be involved in the excretion of the conjugates of carcinogens into the bile, a metabolic step classified as so called “Phase III metabolism”. Thus it might play an important role in cellular defense against toxic substances. The frequency of the *c.3972C > T ABCC2* gene variant (synonymous SNP) was compared between the patients with CCA and the healthy individuals. There was a significant association between the SNP and the risk in a Caucasian population (Table 4)[13].

DNA repair mechanism is protecting DNA damage caused by various kinds of carcinogenic factors. Among them base excision repair (BER) plays an important role in the oxidative DNA damages caused by reactive oxygen species. MutY homolog, MYH, is involved in BER and functions as a DNA glycosylase, which removes adenine paired with 8-hydroxy-2’-deoxyguanine residue. Individuals with T/G genotype in MYH rs3219476 had a reduced risk (OR = 0.478, 95%CI: 0.17-0.758, P=0.006). Individuals with A/A genotype in MYHrs3219472 had an increased risk (OR = 2.816, 95%CI: 0.992-7.999, *P* = 0.047) (Table 4)[14].

Concerning other variants or mutation related to the risk for CCA, a mutation in bile salt export pump (ABCB11) was found in two children with progressive familial intrahepatic cholestasis and cholangiocarcinoma[15]. Biliary papillomatosis is considered to be a premalignant lesion with high probability to develop to CCA, although the genetic changes have not been clarified[16].

**INTERACTION AMONG GENETIC DETERMINANTS**

Susceptibility to cancer might be regulated not only by one gene or one environmental determinant. Thus interaction of genetic determinants could easily be imagined in regulating various cellular processes. However there are few reports on the interaction among genetic determinants. Ko *et al*[17] reported the interaction of polymorphisms of 5,10-methylenetetrahydrofolate reductase (MTHFR *C677T*) and thymidylate synthase enhancer region (TSER) and the risk for CCA in a South Korean population (Ko *et al*[17] 2006). MTHFR is involved in the pathway of folate metabolism and DNA methylation. Thymidylate synthase (TS) catalyzes the formation of dTMP from dUMP, an important step for production of dTTP for use in DNA synthesis. Both TS and MTHFR use the common substrate, 5,10-methylanetetrahydrofolate, and might affect DNA synthesis and DNA repair. Therefore, the interaction between *MTHFR C677T* and *TSER* polymorphisms were analyzed. Ko *et al*[17] found that the individuals with *MTHFR 677CC* with *TSER 2R(+*) genotypes (2R2R, 2R3R, 2R5R) showed an increased risk for CCA as compared to *677CC* with *TSER 2R(-*) genotypes (3R3R, 3R4R, 3R5R) (*P* = 0.0257) (Table 5)[17]. There was no association between *MTHFR C677T* polymorphism or *TSER* polymorphism alone and the risk for CCA.

Human 8-oxoguanine glycosylase 1 (hOGG1) is involved in the repair of 8-hydroxy-2’deoxyguanine residue in oxidatively damaged DNA, one of the most mutagenic lesions among base modification, produced by reactive oxygen species. While polymorphisms of DNA repair enzymes including hOGG1 (codon 326), XRCC1 (codon 194, 280 and 399) and PARP1 (codon 762) alone had no association with the risk for CCA[18], there is a significant interaction between hOGG1 and GSTM1 polymorphisms for the risk for CCA. When GSTM1 polymorphism was considered, the hOGG1 codon 326 polymorphism was related to the decreased risk: OR = 1.00 (reference), OR = 0.06 (95%CI: 0.01-0.53), OR = 0.06 (95%CI: 0.01-0.54), and OR = 0.14 (95%CI: 0.02-1.08) for subjects with hOGG1 Ser/Ser and GSTM1 wild, ones with Ser/Ser and GSTM1 null, ones with Ser/Cys or Cys/Cys and GSTM1 wild, and ones with Ser/Cys or Cys/Cys and GSTM1 null, respectively (*P* for interaction < 0.01) (Table 5). Although the effect of hOGG1 polymorphism is not clear when amino acid Ser 326 is changed to Cys, the DNA repair capacity might decrease. However the above data showed the decreased risk of CCA. It could be considered that if DNA repair capacity is inhibited when relatively abundant DNA damages are present in the presence or absence of GSTM1 enzyme, the cells would die before malignant transformation[18]. Kim *et al*[19] reported that hOGG1 326 Cys/Cys genotypes were associated with lowered risk of bladder cancer occurrence and recurrence in South Korean subjects, while hOGG1 326 Ser/Cys genotype was a risk factor. The protective effect of GSTM1 null variant could be due to the slow metabolism, caused by GSTM1 deficiency, of some dietary materials, such as isothiocyanates contained in cruciferous vegetables, which is known to be a chemopreventive compound. The protective effects of GSTM1 null variant were reported in breast carcinoma[20] and hepatocellular carcinoma[21]. The concerted action of a DNA-repair enzyme and GSTM1 on the risk for CCA should give a new insight in understanding the mechanism of the carcinogenesis of CCA.

**ETIOLOGICAL AND ENHANCING AGENTS FOR CHOLANGIOCARCINOGENESIS AND THEIR PATHOGENICITY**

Concerning the etiological agents for CCA, epidemiological studies implicated various chemicals and occupational risks. One of the examples is thorium dioxide (thorotrast) used for radiological examination[22]. Animal experiments showed that *N*-nitrosodimethylamine could induce CCA in the Syrian Golden hamster[23]. Although OV infection alone did not induce CCA, the OV infection enhanced CCA production by *N-*nitrosodimethylamine in the hamsters[24,25]. Actually a small amount of nitrosamine was detected in the food[4]. Quite recently 1, 2-dichloropropane and/or dichloromethane used in the color proof-printing factory were considered to be the etiological agents from precise epidemiological study in Japan[26]. Other than the liver fluke, viral infections like hepatitis B and C virus infections, are also related to the increased risk for CCA[27].

There have been many findings on the abnormalities of gene expression caused by the reorganization of the genome through endogenous and environmental factors in many types of cancers[28]. It is also true for CCA that many genetic changes are found in CCA. One of the examples from our laboratory is the mutation of the tumor suppressor protein genes, *p16Ink4/CDKN2* and *p15Ink4B/MTS2*[29]. However the precise mechanisms of cholangiocarcinogensis are not well clarified. We have been using a hamster model of cholangiocarcinoma, and found that a molecule, protein kinase A regulatory subunit 1 alpha (Prkar1a), is overexpressed in the cholangiocarcinoma tissues[30,31].　PRKAR1A gene overexpression is also found in humans and this is associated with production of extracellular protein kinase A (ECPKA) especially its catalytic subunit (PRKACA)[31] as found in prostate cancer. Although the function of the extracellular protein kinase A is not clear it might contribute for the development of cancer cells[32].

The precise mechanism of the liver fluke infection to cause CCA (cholangiocarcinogenesis) is also not known. OV produces mechanical injury to the biliary epithelia by attachment with suckers, inflammation caused by OV and mitogenic factors secreted by OV to help the biliary epithelial cells transform to CCA[33]. In particular, TGF-beta and EGF signal transduction pathways are indicated as the possible pathways of OV-induced cell proliferation of fibroblasts[34]. It could be speculated that CCA-associated fibroblasts induce tumor progression of the initiated epithelium as found in human prostate epithelium[35]. And this would be a novel target for chemoprevention and treatment of fibrosis in CCA which might delay the formation of CCA. Gene expression profile of OV infection-related CCA and non-OV associated CCA was reported by Jinawath *et al*[36]. Also enhanced expression of RAD51 associating protein-1 was involved in the growth of CCA cells[37]. These genes upregulated in CCA would be expected to serve as diagnostic and therapeutic targets for CCA. The up-to-date findings of the mechanism of tumorigenesis by OV infection and the prevention of OV infection, including the education and trial for vaccine development against OV, is reviewed by Sripa *et al*[4].

**FUTURE PROSPECTS FOR PREVENTION AND EARLY DETECTION OF CCA**

The present work is intended to analyze the effects of environmental and genetic determinants on the risk of CCA, and to know the mechanisms of CCA to prevent the disease. At the same time, it is also important to detect the disease during it’s early phase so that medical intervention could possibly prevent the death of the patients with cholangiocarcinoma. Therefore, the method to detect the high risk population and the patients with cholangiocarcinoma using non-invasive procedure is quite important. To find out the possible tumor marker of CCA, we are using the sera and labeling the compounds with fluorescent chemicals to try and find a certain compound that is found in the serum of the patients with cholangiocarcinoma. One of our preliminary results showed that a new peak (named peak B) was found in 50% of CCA patients but 6.3% in the normal individuals[38]. In addition, Loilome *et al*[31] found that in liver fluke-associated CCA, PRKR1A overexpression is associated with increased extracellular PKA autoantibody. The antibody titers in the sera from patients with CCA (0.154 ± 0.077), adenocarcinoma (0.150 ± 0.061) and OV infected individuals with fibrosis (0.157 ± 0.045) were significantly higher than that in healthy control subjects (0.129 ± 0.028), while there was no significant difference between the sera from OV infected individuals without fibrosis (0.139 ± 0.053) and that of the healthy control subjects[31]. Recently, Matsuda *et al*[39] found the *Wisteria floribunda* agglutinin-positive mucin 1 and the L1 cell adhesion molecule[40] were sensitive biliary biomarkers for CCA. Silsirivanit A *et al*[41] reported a novel Lewis a associated carbohydrate epitope, CA-S27, as a diagnostic and prognostic biomarker for CCA.

Although the prognosis of CCA is not good, there are several reports on the relationship of genetic changes and the prognosis of the patients with CCA. One example would be with the classical comparative genomic hybridization studies. It was suggested that amplification of the D22S283 region of the chromosome was a favorable prognostic marker[42].

With recent rapid advancement of DNA sequencing technology, it becomes possible to analyze the whole genome sequence relatively less expensively. Therefore it should be possible to search the responsible chromosomal region involved in the genetic determinants of the risk for CCA, and the progression or inhibition of the growth of CCA in more detail. With the technology of genomics, proteomics and glycobiology, one can expect to find the high risk population for CCA more easily, to help the population better adjust their life styles for prevention of CCA and also to detect the patients with CCA in it’s early phase.

**ACKNOWLEDGMENTS**

We thank the cases and controls for their participation in our study. We are grateful to Dr. Takeshi Todoroki of University of Tsukuba, Japan, Dr. Kiti Chindavijak, Dr. Somyos Deerasamee, Dr. Anant Karalak, Dr. Suleeporn Sangrajrang, Ms. Adisorn Jedpiyawongse, Ms. Nuntana Meesiripan of National Cancer Institute of Thailand, Dr. Patcharin Kittiwatanachot of Nakhon Phanom Hospital of Thailand, Dr. Hutcha Sriplung of Prince of Songkla University of Thailand, Dr. Dhiraphol Chenvidhya, Dr. Chutiwan Viwatthanasittiphong, Ms. Mantana Matharit of Ubon Cancer Center of Thailand, Dr. Upama Liengswangwong of Chulalongkorn University of Thailand, Dr. Thong-Ueb Uttaravichien, Dr. Banchob Sripa, Dr. Vatcharabhongsa Bhudhisawasdi, Dr. Chawalit Pai-Rojkul, Dr. Paiboon Sithithaworn, Dr. Wanchai Maleewong, Dr. Nisana (Tepsiri) Namwat, Dr. Chanitra Thuwajit, Dr. Peti Thuwajit, and Dr. Jongkonee Thanasai of Khon Kaen University for generous support of our work.

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**P-Reviewer:** Lau WY, Plentz RR, Petmitr S, Wang DS, Xu R

**S-Editor:** Ji FF **L-Editor: E-Editor:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Table1 Effects of environmental determinants on risks for cholangiocarcinoma** |  |  | 　 |
| Environmental determinants | 　 | Cases | Controls | OR | 95%CI  LL UL | *P* value | Ref. | Ethnic group |
| Anti-OV Ab | ref: < 1/40 | 101 matched case-control pairs | 5.0 | 2.3 | 11 | <0.001 | Parkin *et al*[2] 1991 | Thai |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | AdjustedOR |  |  |  |  |  |
| Anti-OV Ab (ELISA) | < 0.200 | 61 | 119 | 1.00 | Reference |  | Honjo *et al*[5] 2005 | Thai |
|  | ≥ 0.200 | 65 | 8 | 27.09 | 6.30 | 116.57 | < 0.01 |  |  |
| Alcohol drinking | Never | 30 | 46 | 1.00 | Reference | - |  |  |
|  | Occasional | 41 | 54 | 2.20 | 0.65 | 7.45 | 0.21 |  |  |
|  | Ex-regular | 15 | 7 | 6.23 | 1.23 | 31.57 | 0.03 |  |  |
|  | Regular | 41 | 21 | 4.31 | 1.12 | 16.57 | 0.03 |  |  |
|  | Missing | 2 | - | - | - | - | - |  |  |
| Raw fish | 0 | 30 | 57 | 1.00 | Reference |  |  |  |
|  | < 2/mo | 54 | 41 | 2.70 | 1.28 | 5.68 | < 0.01 |  |  |
|  | ≥ 2/mo | 45 | 31 | 2.94 | 1.24 | 6.96 | 0.01 |  |  |
| Fermented fish or pork | 0 | 28 | 41 | 1.00 | Reference |  |  |  |
|  | < 2/mo | 58 | 63 | 2.95 | 0.98 | 8.90 | 0.06 |  |  |
|  | ≥ 2/mo | 43 | 25 | 4.50 | 1.30 | 15.54 | 0.02 |  |  |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | AdjustedOR |  |  |  |  |  |
| Alcohol drinking | Non-drinker | 57 | 254 | 1.00 | Reference |  | Songserm *et al*[6] 2012 | Thai |
| (Units of alcohol per month) | < 14 | 79 | 92 | 5.6 | 2.85 | 10.95 | < 0.001 |  |  |
|  | ≥ 14 | 83 | 92 | 9.5 | 4.55 | 19.79 | < 0.001 |  |  |
| Total vegetables | < 52 | 136 | 214 | 1.0 | Reference |  |  |  |
|  (average times/month) | ≥ 52 | 83 | 224 | 0.4 | 0.23 | 0.76 | 0.004 |  |  |
| Total fruits | < 35 | 131 | 217 | 1.0 | Reference |  |  |  |
| (average times/month) | ≥ 35 | 88 | 221 | 0.6 | 0.33 | 0.98 | 0.04 |  |  |
|  |  |  |  |  |  |  |  |  |  |
| Family history of cancer | No | 85 | 107 | 1.00 | Reference |  | Manwong *et al*[7] 2013 | Thai |
|  | Yes | 38 | 16 | 4.34 | 1.80 | 10.43 | 0.001 |  |  |
| 　 | 　 | 　 | 　 | 　 | 　 | 　 | 　 | 　 | 　 |
| OR: Odds ratio; CI: Confidence interval; LL: Lower limit; UL: Upper limit. |  |  |  |  |  |  |  |  |  |

|  |
| --- |
| **Table 2 Effect modification of alcohol drinking on relations between smoking, eating fermented fish and risk for cholangiocarcinoma** |
| Variable | Category | 　 | Alcohol drinking | 　 |
|  | Never drinkers |  | Ever1 drinkers |
|  | Adjusted2 OR | 95%CI | *P* value |  | Adjusted2 OR | 95% CI | *P* value |
| 　 | LL | UL | 　 | LL | UL |
| Smoking | Never |  | 1.00  | Reference |  |  | 4.25  | 1.02  | 17.63  | 0.05  |
|  | Occasional |  | 4.36  | 0.40  | 47.49  | 0.23  |  | 1.07  | 0.06  | 20.66  | 0.96  |
|  | Ex-regular |  |  | 9.09  | 1.27  | 65.18  | 0.03  |
|  | Regular |  | 3.64  | 0.19  | 71.41  | 0.39  |  | 7.99  | 1.56  | 40.94  | 0.01  |
|  |  |  |  |  |  |  |  |  |  |  |  |
| *Pla-ra, Pla-chao* | < 3/d |  | 1.00  | Reference |  |  | 14.07  | 1.46  | 135.36  | 0.02  |
|  | ≥ 3/d |  | 12.34  | 1.22  | 124.75  | 0.03  |  | 20.88  | 2.27  | 192.06  | < 0.01 |
| 1Including occasional, ex- and currently regular drinkers; 2Adjusted for anti-OV Ab when calculating the OR of smoking, and adjusted for anti-OV Ab and smoking when calculating the OR of eating of fermented fish (*pla-ra* and/or *pla-chao)*. Nakorn Phanom (Thailand): based on the conditional logistic regression model. CI: Confidence interval; LL: Lower limit; UL: Upper limit. Adapted from Honjo *et al*[5] 2005. Allowing for absence of control subject in the category for occasional smoking and absence of case subject in the category for ex-regular smoking among never drinkers, we combined these two categories and confirmed the conclusion in the table is the materially unchanged from that in the table in our previous paper (Honjo *et al* [5] 2005). |
| **Table 3 Interaction between genetic and environmental determinants on risks for cholangiocarcinoma** | 　 |  |
| Genetic determinants | Environmental determinants |  OR | 95%CI  LL UL | *P* value | Ref. | Ethnic group |
|  |  |  |  | Adjusted OR |  |  |  |  |  |
| *GSTMI* | Wild | Anti-OV antibody | Negative | 1.00 | Reference |  | Honjo *et al*[5] 2005 | Thai |
|  | Wild |  | Positive | 10.34 | 1.31 | 81.63 | 0.03 |  |  |
|  | Null |  | Negative | 0.48 | 0.21 | 1.11 | 0.09 |  |  |
|  | Null |  | Positive | 18.00 | 3.33 | 97.40 | < 0.01 |  |  |
| *GSTMI* | Wild | Toilet  | Inside the house | 1.00 | Reference |  |  |  |
|  | Wild |  | Outside or none | 0.20 | 0.04 | 1.02 | 0.05 |  |  |
|  | Null |  | Inside the house | 0.22 | 0.06 | 0.88 | 0.03 |  |  |
|  | Null |  | Outside or none | 0.25 | 0.07 | 0.91 | 0.04 |  |  |
| *GSTTI* | Wild | Alcohol drinking | Never | 1.00 | Reference |  |  |  |
|  | Wild |  | Occasional | 3.58 | 0.71 | 17.95 | 0.12 |  |  |
|  | Wild |  | Ex-regular | 1.28 | 0.12 | 14.08 | 0.84 |  |  |
|  | Wild |  | Regular | 4.69 | 0.93 | 23.51 | 0.06 |  |  |
|  | Null |  | Never | 0.75 | 0.23 | 2.43 | 0.63 |  |  |
|  | Null |  | Occasional | 1.12 | 0.22 | 5.80 | 0.89 |  |  |
|  | Null |  | Ex-regular | 27.93 | 1.84 | 424.60 | 0.02 |  |  |
|  | Null |  | Regular | 3.28 | 0.35 | 30.91 | 0.30 |  |  |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | Crude OR |  |  |  |  |  |
| *MTHFR* 677 |  CC  | Beef sausage | < 1/mo | 1.0 | Reference |  | Songserm *et al*[6] 2012　 | Thai |
|  |  CT |  | < 1/mo | 1.1 | 0.51 | 2.37 | 0.82 |  |  |
|  |  TT |  | < 1/mo | 0.6 | 0.25 | 1.53 | 0.32 |  |  |
|  |  CC  |  | Weekly | 0.9 | 0.45 | 1.83 | 0.80 |  |  |
|  |  CT |  | Weekly | 1.2 | 0.57 | 2.43 | 0.65 |  |  |
|  |  TT |  | Weekly | 1.6 | 0.80 | 3.31 | 0.18 |  |  |
|  |  CC  |  | Daily | 3.3 | 1.51 | 7.07 | 0.003 |  |  |
|  |  CT |  | Daily | 3.2 | 1.33 | 7.62 | 0.01 |  |  |
|  |  TT |  | Daily | 8.3 | 2.23 | 30.82 | 0.002 |  |  |
| *MTHFR* 1298 |  AA | Beef sausage | < 1/mo | 1.0 | Reference |  |  |  |
|  |  AC |  | < 1/mo | 1.3 | 0.63 | 2.55 | 0.51 |  |  |
|  |  CC |  | < 1/mo | 0.8 | 0.28 | 2.15 | 0.63 |  |  |
|  |  AA |  | Weekly | 1.3 | 0.71 | 2.45 | 0.39 |  |  |
|  |  AC |  | Weekly | 1.0 | 0.49 | 1.79 | 0.84 |  |  |
|  |  CC |  | Weekly | 3.8 | 1.48 | 9.89 | 0.01 |  |  |
|  |  AA |  | Daily | 3.8 | 1.71 | 8.62 | 0.001 |  |  |
|  |  AC |  | Daily | 3.5 | 1.56 | 7.85 | 0.002 |  |  |
|  |  CC |  | Daily | 18.3 | 3.68 | 90.8 | <0.001 |  |  |
|  | 　 | 　 | 　 | 　 | 　 | 　 | 　 | 　 | 　 |
| **Table 4 Effects of genetic determinants on risks for cholangiocarcinoma** |  |  |  |  |  |
| Genotype | No. CCA (%) | No. control (%) | OR | 95%CI  LL UL | *P* value | Ref. | Ethnic group |
|  |  |  |  | Adjusted OR |  |  |  |  |  |
| *CYP1A2*, Male |  *\*1F/\*1F* | 85 (57.4) | 88 (51.2) | 1.00 | Reference |  | Prawan *et al*[8] 2005 | Thai |
|  |  *\*1A/\*1F* | 59 (39.9) | 69 (40.1) | 0.90 | 0.55 | 1.47 | 0.677 |  |  |
|  |  *\*1A/\*1A* | 4 (2.7) | 15 (8.7) | 0.28 | 0.08 | 0.94 | 0.039 |  |  |
| *NAT2* | *All,* except *\*6B, \*7A* and *\*13* | 193 (89.4) | 162 (69.5) | 1.00 | Reference |  |  |  |
|  | One or two *alleles (All,* except *\*6B, \*7A* and *\*13)* | 23 (10.6) | 71 (30.5) | 0.26 | 0.15 | 0.44 | <0.001 |  |  |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | Crude OR |  |  |  |  |  |
| *GST01* | A140/A140 | 13 (43.33) | 26 (86.67) | 1.00 | Reference |  | Marahatta *et al*[9] 2006　 | Thai |
|  | A140/D140 + D140/D140 | 17 (56.67) | 4 (13.33) | 0.86 | 2.07 | 37.85 |   |  |  |
|  |  |  |  |  |  |  |  |  |  |
|  |  | Minor allele frequency |  |  |  |  |  |  |
|  | Alleles | PSC2 with CCA (*n* = 49) | PSC without CCA (*n* = 316) | OR |  |  | Corrected *P* |  |  |
|  *NKG2D*1 | rs11053781 (Intron 5) G versus A | 0.66 | 0.49 | 2.08 | 1.31 | 3.29 | 0.011 | Melum *et al*[12] 2008 | Norwegian |
|  | rs2617167 (Intron 1) A versus G  | 0.39 | 0.22 | 2.32 | 1.47 | 3.66 | 0.002 |  |  |
|  |  | PSC with CCA (*n* = 49) | Healthy controls (n=368) |  |  |  |  |  |  |
|  | rs11053781 (Intron 5) G versus A | 0.66 | 0.5 | 1.95 | 1.23 | 3.07 | 0.021 |  |  |
|  | rs2617167 (Intron 1) A versus G  | 0.39 | 0.23 | 2.20 | 1.40 | 3.44 | 0.003 |  |  |
|  |  |  |  |  |  |  |  |  |  |
|  |  | Counts (frequencies) of alleles/genotypes |  |  |  |  |  |
|  |  | 2*n* = 120 | 2*n* = 146 | Crude OR |  |  |  | Hoeblinger *et al*[13] 2009 | Caucasian  |
|  *MRP2/ABCC2*3 | *ABCC2* c.3972 C (exon 28, synonymous SNP) | 73 (0.61) | 108 (0.74) |  |  |  |  |  |  |
|  | *ABCC2* c.3972 T | 47 (0.39) |  38 (0.26) | 1.830 | 1.087 | 3.080 | 0.022 |  |  |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | OR |  |  |  |  |  |
| *MYH* rs3219476 | T/T | 25 (42.4) | 26 (26.0) | 1.00 | Reference |  | You *et al*[14] 2013　 | Han Chinese |
|  | T/G | 20 (33.9) | 58 (58.0) | 0.359 | 0.17 | 0.758 | 0.006 |  |  |
|  | G/G | 14 (23.7) | 16 (16.0) | 0.91 | 0.369 | 2.246 | 0.838 |  |  |
|  | T/G + G/G | 34 (57.6) | 74 (74.0) | 0.478 | 0.241 | 0.946 | 0.033 |  |  |
| *MYH* rs3219472 | G/G | 28 (47.5) | 46 (46.0) | 1.00 | Reference |  |  |  |
|  | G/A | 19 (32.2) | 47 (47.0) | 0.664 | 0.326 | 1.351 | 0.258 |  |  |
|  | A/A | 12 (20.3) | 7 (7.0) | 2.816 | 0.992 | 7.999 | 0.047 |  |  |
| 　 | G/A + A/A | 31 (52.5) | 54 (54.0) | 0.943 | 0.495 | 1.797 | 0.859 | 　 | 　 |
| 1Natural killer cell receptor G2D; 2Primary sclerosing cholangitis; 3Multidrug resistance-associated protein 2 gene. |
| **Table 5 Interaction among genetic determinants on risks for cholangiocarcinoma** |
| Genetic determinant | Genetic determinant | OR | 95%CI  LL UL | *P* value | Ref. | Ethnic group |
| *MTHFR C677T*1 | CC | *TSER*2 | *2R* (-) | 1.00 |  Reference |  | Ko *et al*[17] 2006 | South Korean  |
|  | CC |  | *2R* (+)3 | 5.38 | 1.23 | 23.56 | 0.0257 |  |  |
|  | CT |  | *2R* (-) | 1.08 | 0.68 | 1.07 |  |  |  |
|  | CT |  | *2R* (+)3 | 1.19 | 0.71 | 2.01 |  |  |  |
|  | TT |  | *2R* (-) | 1.02 | 0.7 | 1.5 |  |  |  |
|  | TT |  | *2R* (+)3 | 1.24 | 0.9 | 1.71 |  |  |  |
|  |  |  |  |  |  |  |  |  |  |
| *hOGG1(Codon326)* | Ser/Ser | *GSTM1* | wild | 1.00 |  Reference |  | Zeng *et al*[18] 2013 | Thai |
|  | Ser/Ser + Cys/Cys |  | wild | 0.06 | 0.01 | 0.54 | 0.01 |  |  |
|  | Ser/Ser |  | null | 0.06 | 0.01 | 0.53 | 0.01 |  |  |
|  | Ser/Ser + Cys/Cys |  | null | 0.14 | 0.02 | 1.08 | 0.06 |  |  |
| 　 | 　 | 　 | 　 | 　 | 　 | 　 | 　 | 　 | 　 |
| 15,10-Methylenetetrahydrofolate reductase;2Thymidylate synthase enhancer region; 3Including 2R2R and 2R3R.  |