REVIEW

# Genetic Polymorphism of Drug Metabolizing Enzymes in Association with Risk of Bile Duct Cancer

**Veerapol Kukongviriyapan**

## Abstract

**Cholangiocarcinoma (CCA) is the most common cancer in endemic areas of liver fluke infection. Although the liver fluke is recognized as a carcinogenic agent in cholangiocarcinogenesis, other factors may play important roles in bringing about the high prevalence of the cancer in populations of this region. Drug metabolizing enzymes (DME) are essential for detoxification of toxic and carcinogenic chemicals. Moreover, DME can play an alternative role by activating chemicals to more toxic metabolites. The large variation of DME activity among individuals is partly due to polymorphism of the genes encoding enzymes. Defective or variant alleles of DME genes may modify the risk of cancer in those who are exposeed to carcinogenic agents. The focus in this review is on DME genes which have been reported to be associated with CCA risk. These include *CYP1A2*, arylamine-N-acetyltransferase-1 (*NAT1*) and *NAT2*, NADPH-quinone oxidorecutase-1 (*NQO1*), glutathione-S-transferase M1 (*GSTM1*), *GSTT1*, *GSTO1* and methylenetetrahydrofolate reductase (*MTHFR*). Mutant alleles which have been reportedly associated with an increased risk include *CYP1A2*1F*, *GSTT1 null*, *GSTO1* and *MTHFR 677C>T*, whereas, slow *NAT2* and *NQO1*2* decrease risk and *NAT1* variants and *GSTM1 null* have no effect. These genes modify the risk of cancer potentially by interaction and exposure with certain environmental conditions, thereby altering the metabolism of causative agents.**

**Keywords:** Pharmacogenetics - drug metabolizing enzymes - cholangiocarcinoma - *CYP1A2* - glutathione-S-transferase

*Asian Pacific J Cancer Prev,* **13**, 7-15

## Introduction

Cancer resulting from exposure to chemicals in the environment is well established. A long list of human carcinogenic chemicals is reported by various world authorities, such as the International Agency for Research on cancer (IARC), Globally Harmonized System and the American Conference of Governmental Industrial Hygienists (ACGIH) or WHO. The discovery of chemical carcinogenesis in modern time was described by an eminent English surgeon, Sir Percivall Pott in 1775 over the occurrence of cancer of the scrotum in chimney sweeps. His report suggested that the occupational exposure to soot since their childhood period was the causative agent of cancer (Klaassen, 2007). The socio-economic conditions and child labor during the industrial revolution in England were important factors relating to incidence of the cancer, as this cancer in chimney sweeps did not happen in the European continent. Other occupational and environmental exposure to chemicals in relation to cancers have been emerging, for instance, aromatic hydrocarbons, aromatic and heterocyclic amines, and also various natural products, such as mycotoxins, aflatoxins and plant toxins such as cycasin (Loeb and Harris, 2008).

Epidemiological studies provide the first clues and evidence of carcinogenicity of chemicals which are supported by laboratory studies using various *in vitro* and *in vivo* models. It has been estimated that about 75% of carcinogenic chemicals required metabolic activation to reactive electrophilic intermediates, such as benzo[a] pyrene, β-naphthylamine or IQ to be mutagenic and carcinogenic, whereas only 25% are directly carcinogenic, such as TCDD (Nebert and Dalton, 2006). Indeed, a number of complex metabolic pathways are involved in metabolic activation and metabolic detoxification of carcinogenic chemicals. The reactions are conducted largely by a drug metabolizing enzyme (DME) system which is made up of phase I, and phase II enzymes. Phase I enzymes catalyze oxidation, reduction and hydrolysis of xenobiotics resulting in formation of a functional group (-OH, -NH2, -SH, or -COOH), whereas phase II enzymes involve conjugation reactions including glucuronidation, acetylation, sulfation and methylation of chemicals producing more water-soluble metabolites readily for renal and biliary excretion (Parkinson, 1996).

*Department of Pharmacology, Faculty of Medicine, Liver Fluke and Cholangiocarcinoma research Center, Khon Kaen University, 40002, Thailand *For correspondence: veerapol@kku.ac.th*

## Roles of Drug Metabolizing Enzymes in Cancer

DME play important roles in metabolic activation of procarcinogenic compounds including phase I enzymes, for instance some CYP isoforms, such as CYP1A1, 1A2, 2E1, 2A6 and 2A13. A number of the phase II enzymes namely, arylamine-*N*-acetyltransferases (NAT), sulfotransferases (SULT), glutathione-S-transferases (GST) and UDP-glucuronosyl-transferases (UGT) may also participate in metabolic activation of particular carcinogenic compounds. Some DME act predominately as drug detoxifying enzymes include, GSTs, NADPH-quinone oxidoreductase-1 (NQO1), UGTs, epoxide hydrolase (EHs). It has been noted that the role of DME in metabolic activation or detoxification is not absolute, but depending on type of compounds and metabolic pathways.

Examples of carcinogenic chemicals and roles of DME in activation are shown in Table 1. The activation of reactive metabolites usually involves the formation of unstable electrophilic groups by oxidation or reduction by CYPs. CYP1A1, and 1B1 which participate in metabolic activation of polycyclic aromatic hydrocarbons, such as benzo[a]pyrene yielding epoxide intermediates and those epoxides are further converted to dihydrodiol and then to highly reactive epoxide derivatives by epoxide hydrolase and CYP2B1, respectively (Parkinson, 1996) (Table 1). Alternatively, CYP1A2 carries out the *N*-hydroxylation of arylamines and heterocyclic amines food mutagens; MeIQ or PhIP, and reactions follow with *O*-esterification by acetate or sulfate via transferase enzymes, i.e. NAT and SULT, resulting in unstable electrophilic intermediates (Kim and Guengerich, 2005). Moreover, CYP1A2 also metabolizes aflatoxin B1 to the reactive epoxides. CYP2E1 mainly metabolizes small aliphatic and aromatic molecules such as ethanol, vinyl halides, aniline, *N*-nitrosomethylamine and yields strong electrophilic compounds. The tobacco-specific nitrosamines, i.e. 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and *N*''-nitrosonornicotine (NNN) are activated to their carcinogenic metabolites by hepatic CYP2E1, 2A6 and lung CYP2A13. CYP2A13 is not expressed in the liver but expressed in the lung and is highly efficient in metabolizing NNN, NNK and aflatoxin B1. The reactive electrophiles react rapidly with nucleophilies in the vicinity; e.g. nucleic acids, proteins and lipids, forming adducts, causing the lost of functional molecules and leading to cytotoxicity, cell death or cell transformation. A schematic of possible events in metabolic activation and detoxification of reactive metabolites, which lead to cell injury, cell death, transformation and carcinogenesis, is shown in Figure1.

## Drug Metabolizing Enzymes and Cholangiocarcinogenesis

Cancers of the bile duct in populations of northeast Thailand, and neighboring countries are associated with liver fluke infection (Kurathong et al., 1985; IARC, 1994). An animal model of opischorchiasis-induced cholangiocarcinoma suggests that the concurrent exposure to carcinogens with liver fluke infection may be necessary to enhance the carcinogenesis process of cholangiocarcinoma (Thamavit et al., 1978). Carcinogenic agents identified in relation to cancer in this region so far include nitroso compounds, tobacco, mycotoxins, heterocyclic amines from food mutagens, ethanol and hepatitis viruses (Srivatanakul et al., 1991b; Mitacek et al., 2008; Shin et al., 2010). *N*-nitrosodimethylamine (NDMA) induces liver damage and liver cancer, however, when administered in liver fluke-infected hamster, NDMA

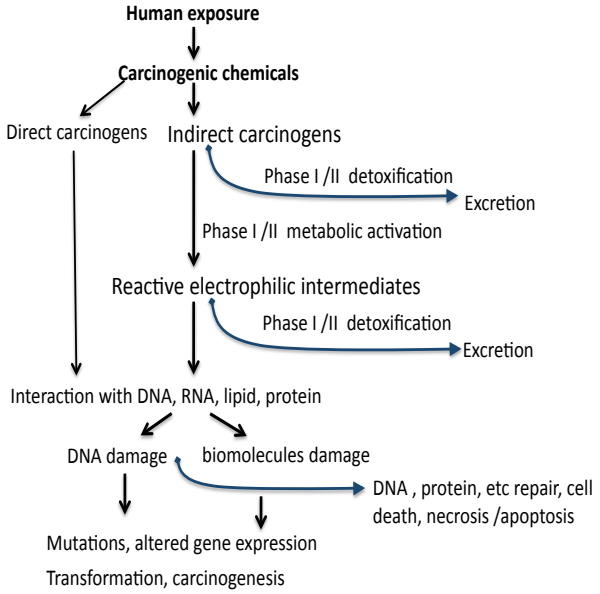


**Figure 1. Sequence of Events in Metabolic Transformation of Chemicals Leading to Cell Death, Transformation and Carcinogenesis**

**Table 1. Carcinogenic Agents, Cancer Sites and Enzymes in Metabolic Pathways**

| Chemical | Cancer sites | Activating enzymes | Reference |
|---|---|---|---|
| 1. Polycyclic aromatic hydrocarbons (PAH); benzo[a]pyrene, dibenz[a,h]anthracene, chrysene, 3-methylcholanthrene | Lung, skin, bladder | CYP1A1, 1B1, EHs | Shimada,2006; coal tar,tobacco; Irigaray and Belpomme, 2010 |
| 2. Polyhalogenated aromatic hydrocarbon; dioxins, polychlorinated biphenyls (PCBs) | Liver, biliary tract | CYP1A1, 1B1 | Shimada, 2006; Irigaray and Belpomme, 2010 |
| 3. Heterocyclic amines: food mutagens; MeIQ, PhIP, arylamnes: benzidine, 2-naphthylamine, 2-aminofluorene | Colon, bladder | CYP1A2, 1A1, 1B1, NQO1, NAT1, NAT2, SULTs | Williams et al., 2001; Suzuki et al., 2008 |
| 4. Nitroso compounds: N-nitrosodimethylamine (NDMA) | Liver | CYP2E1, CYP2A6, | Kushida et al., 2000 |
| 5. Tobacco-specific: NNN, NNK | Lung | CYP2A13, NQO1, EHs | Wang et al., 2003; Zhu et al., 2006 |
| 6. Mycotoxins; aflatoxins, | Liver, lung | CYP1A2, CYP3A4, | Guengerich et al., 1998; Chen et al., 2006 |

specifically induces cholangiocarcinoma (Thamavit et al., 1978). NDMA was found to be a food contaminant in region of the high prevalence of liver fluke infection. Moreover, subjects with liver fluke infection showed much higher endogenous nitrosation rate than subjects without liver fluke infection, as assessed by ingestion of proline and measurement of nitrosation product of N-nitrosothioproline (Srivatanakul et al., 1991a). It is possible that endogenous nitrosation may be another source of formation of mutagenic nitroso-compounds. NDMA and other nitro-compounds, aflatoxins, and many other agents are required for metabolic activation to yield ultimate reactive carcinogenic compounds. It has been suggested that metabolic capacity of DME in individuals may contribute to susceptibility to toxic responses of carcinogenic agents.

## Genetic polymorphism of drug metabolizing enzymes

It is well-known that there is considerable individual variation in activity of DME and the variation may be accountable for individual susceptibility to toxic chemicals. It is apparent that variability in gene encoding DME often affect outcome to a large extent in drug efficacy and toxicity (Eichelbaum et al., 2006). Most genes exist in many physical forms or alleles, and those alleles differ in certain nucleotides positions (or single nucleotide polymorphism: SNP) on coding and non-coding regions of the genes. The presence of several forms or polymorphism of DME genes result in a variation of DME activity or phenotype polymorphism. Phenotypes of DME in individuals may be classified as: 1. absent or very low enzymatic activity, which are designated as Slow metabolizers, resulting from the presence of the severe defective alleles including gene deletion, nonsense, frame shift or missense mutations in both alleles, 2. Intermediate metabolizers, the presence of only one active allele, 3. Rapid metabolizers, the presence of two active alleles, and 4. Ultra-rapid metabolizers, the presence of multiple copy of active alleles. Frequency of allelic variants of given genes could vary widely resulting in inter-individual variation in a population and variation among populations in susceptibility to toxic chemicals and carcinogenesis. Allele frequency of important alleles of DME among populations is shown in Table 2.

## Polymorphism of DME and Cholangiocarcinoma

Epidemiological studies have shown that liver fluke infection is a major risk factor in cholangiocarcinoma. However, as not all subjects with liver fluke infection develop cancer, some individuals may be more susceptible to develop this disease. The individual susceptibility has been related to polymorphism in DME genes. As exposure to carcinogenic compounds is probably related to cancer risk, genetic polymorphism of DME genes may contribute to individual susceptibility. Candidate DME genes have been identified based on the discovery of tumor-specific mutations through genotyping of CCA patients. Epidemiological studies and meta-analyses have revealed several tumor susceptible genes and identified associated risk factors of tumor development (Prawan et al., 2005; Ko et al., 2006; Marahatta et al., 2006; Songserm et al., 2012). The susceptibility genes are usually weakly associated with cancer or have low penetrance in causing cancer.

Environmental factors may also play crucial part in carcinogenesis. Non-genetic factors associated with increased cancer risk include consumption of food contaminated with liver fluke (*Opisthorchis viverrini*), HBV or HCV infection, alcoholic drinking, processed meat, whereas consumption of vegetables and fruits decreased the risk. Following is a discussion of polymorphism of DME genes in association with the risk of CCA.

*Polymorphism of CYP1A1/2 and CYP1B1*

CYP1A1 and CYP1B1 participated in reactions of substrate molecules of planar shape, such as PAH found in tobacco smoking, pollutants from combustion of fossil fuel. The heterocyclic amines, food mutagens formed during the pyrolysis of creatine, amino acids and proteins such as AIAs, IQ, MeIQ, MeIQx and PhIP, and aromatic amines, such as naphthylamine and benzidine are metabolized by CYP1A2. All have been shown to be carcinogenic in animals. *CYP1A2* is expressed in the liver, while *CYP1A1* and *CYP1B1* are expressed at

**Table 2. Polymorphism of DME Genes in Populations. Allelic Variants of Some DME Genes and their Association with Phenotypic Characteristics, and Allele Frequency in Various Populations**

| Gene | Variant alleles | Phenotype activity | Variant allele frequency (%) | | | |
|---|---|---|---|---|---|---|
| | | | Thai | Chinese | Caucasian | African |
| *CYP1A2* | *CYP1A2**1F | Higher inducibility | 72.3 | 67 | 72.3 | 61 |
| *CYP2A6* | *CYP2A6*4,*7, *9,*10* | Absence or reduced | 41 | 37.3 | 15.5 | 25.6 |
| *NAT2* | *NAT2*5, *6, *7* | Slow acetylation | 56.7 | 49 | 75 | 63 |
| *NAT1* | *NAT1**3,*10,*11,*14 | Unchanged | 49.6 | 50 | 21 | 51.5 |
| *NQO1* | *NQO1*2* | Reduced activity | 58.7 | 41.4 | 18 | 16 |
| *GSTM1* | *GSTM1 null* | Absence | 67.2 | 44 | 53.5 | 26.1 |
| *GSTT1* | *GSTT1 null* | Absence | 39.2 | 51 | 17.5 | 22 |
| *GSTO1* | *GSTO1**A140D | Unchanged | 13 | 17 | 33.4 | 8.1 |
| *MTHFR* | *MTHFR* C677T | Reduced activity | 31.1 | 32.8 | 42 | 13 |

*Data from; Kukongviriyapan et al., 2003a; Kukongviriyapan et al., 2003b; Prawan et al., 2005; Marahatta et al., 2006; Peamkrasatam et al., 2006; Ginsberg et al., 2009; Chutinet et al., 2012; Songserm et al., 2012

extra-hepatic tissues (Landi et al., 1999). All these CYPs are highly inducible by PAHs, some drugs and many phytochemicals via Ah receptor (Landi et al., 1999). To date, at least 11 allelic variants and several subvariants have been described in *CYP1A1* gene (www.cypalleles.ki.se/, access date Aug 16, 2012).

There are at least 133 SNPs described for *CYP1A1* and 23 are nonsynonymous mutations, however, for most their effect is not known. Some of SNPs in *CYP1A1* i.e 2455 A>G, *CYP1A1*2B*, and *2C, have been suggested to influence the inducibility. About 10% of the Caucasian population has a highly inducible form of the *CYP1A1*, that might present risk to individuals with high inducibility for tobacco smoke-induced lung cancer (Nakachi et al., 1993). In smokers with 2455A>G SNP, more PAH-DNA adducts were observed in white blood cells compared with smokers without SNP. Apart from lung cancer, *CYP1A1*1* is associated with breast cancer risk in individuals who are exposed to polychlorinated biphenyls, known as potent *CYP1A1* inducers, by increased metabolism forming reactive hydroxylated metabolites (Moysich et al., 1999; Modugno et al., 2003). However, there is still no reports concerning *CYP1A1* polymorphism and cholangiocarcinoma.

For *CYP1A2*, its expression is distinct from *CYP1A1*, and the enzymes have considerable overlapping substrate specificity. A number of SNPs are observed on the 5' flanking region in introns and exons, however, no common alleles have been described that cause any important change in gene expression and enzymatic activity. *CYP1A2*1C*, **1F* and **1K* are alleles that have received much interest, in that *CYP1A2*1F* is suggested to be highly inducible and *CYP1A2*1C*, **1K* to be reduced in activity (Nakajima et al., 1999). However, there are still many conflicting reports over the inducibility phenotype of *CYP1A2*1F*. This may be partly because that only SNP -163C>A in *CYP1A2*1F* is also present in haplotypes with SNPs -739C>T and 729C>T in *CYP1A2*1K*, where this allele is suggested to be a reduced activity allele (www.cypalleles.ki.se/, access date Aug 16, 2012). Allele frequency of *CYP1A2*1F* is relatively high in most populations, i.e. about 60-70%. In Thai populations, the frequency has been reported to be 72% (Prawan et al., 2005). Furthermore, our study reported that the male population with wild type *CYP1A2*1A* genotype had lower risk of developing CCA when compared with males who carried the *CYP1A2*1F/*1F* genotype with odds ratio of 0.28: 95% CI: 0.08-0.94. Individuals with wild type genotype also have lower risk of breast cancer when compared with -163A/A, which is consistent with the observation of lower plasma estrogen levels in premenopausal women with -163C/C genotype (Le Marchand et al., 2005a). A significant effects of *CYP1A2*1F* or combination of *CYP1A2*1F* and *NAT1*10* or **11* on the increased risk for pancreatic cancer was observed among non-smokers (Li et al., 2006). Similarly, light smokers with *CYP1A2**1 and *NAT2* slow acerylator genotypes were at the highest risk of lung cancer (Osawa et al., 2007). The high inducibility of *CYP1A2* genotype may confer cancer risk in individuals who are exposed to carcinogenic chemicals.

*Polymorphism of arylamine-N-acetyltransferases*

Arylamine-*N*-acetyltransferases (NATs) are phase II enzyme genes that catalyze the transfer of acetyl group to various substrates including endogenous compounds, drugs and food mutagens. There are 2 isoforms of enzymes encoded from 2 different but related genes, i.e. *NAT1* and *NAT2* (Sim et al., 2008). Individuals who carry defective *NAT2* genotypes or slow acetylators are likely to develop peripheral neuropathy when use of isoniazid for the treatment of tuberculosis. Other adverse drug reactions with use of sulfasalazine and hydrazine were observed. *NAT1* is composed of at least 20 alleles with various subvariants (http://louisville.edu/medschool/pharmacology/consensus-human-arylamine-n-acetyltransferase-gene-nomenclature/). *NAT1*3, *10, *11* common genotypes do not cause any consistent changes in enzymatic activity when compared with the

**Table 3. Polymorphism of DME and Association with Risk of CCA and Other Cancers**

| Gene | Variant allele | CCA risk | Other cancer risk | References |
|---|---|---|---|---|
| *CYP1A2* | *CYP1A2*1F* (-163C>A) | ↑(male)* | ↑ panceas (smokers) ↑lung, breast (non-smokers) | Prawan et al., 2005; Li et al., 2006; Osawa et al., 2007; |
| *NAT2* | Slow acetylators | ↓ | ↑bladder (smokers), ↓colon, ↑HCC (non viral hepatitis) | Garcia-Closas et al., 2005; Agundez, 2008; Walker et al., 2009 |
| *NAT1* | Variant alleles | ↔ | ↑ panceas (smokers), ↑bladder (slow *NAT2*+smokers), ↔colon, ↔HCC | Prawan et al., 2005; Li et al., 2006; Agundez, 2008; |
| *NQO1* | *NQO1*2* (609C>T) | ↓ | ↑breast, ↑colon (Caucasian), ↓bladder, ↓lung | Menzel et al., 2004; Zeekpudsa et al., 2009; Yang et al., 2012; Yu et al., 2012 |
| *GSTM1* | *GSTM1 null* | ↔ ↑ (Ov+ve) | ↑HCC, ↑colon, ↑lung, ↑bladder (combination with *GSTT1 null*), ↑prostate, ↔breast | Egan et al., 2004; Honjo et al., 2005; White et al., 2008; Mo et al., 2009; Economopoulos and Sergentanis, 2010; Safarinejad et al., 2011; Li et al., 2012 |
| *GSTT1* | *GSTT1 null* | ↑↔ | ↑HCC, ↑ panceas, ↔colon (Caucasian), ↔prostate, ↔breast | |
| *GSTO1* | *GSTO1**A140D | ↑ | ↑ breast (advanced), ↑bladder (high As exposure), ↑ALL, ↑HCC | Marahatta et al., 2006; Pongstaporn et al., 2009; Escobar-Garcia et al., 2012; |
| *MTHFR* | *MTHFR 677C>T* | ↑ (raw fish intake) | ↓Colon, ↑ panceas (Caucasian), | Le Marchand et al., 2005b; Ko et al., 2006; Mazaki et al., 2011; Songserm et al., 2012 |

wild type *NAT1*4* (Sim et al., 2008). On the other hand, there are at least 18 alleles of *NAT2* and 27 alleles of *NAT1* reported to date. The defective *NAT2* alleles consist of several SNPs forming SNP haplotypes designated as *NAT2*5, *6* and **7*. These are common defective alleles in most populations. Asian populations, including Thais, have lower frequencies of slow acetylator genotype and phenotype when compared with Caucasian and African populations, i.e. about 20-35% vs 60% and 40%, respectively (Kukongviriyapan et al., 2003a). The major defective alleles in Asian populations are *NAT2*6* and **7*, while those found in Caucasians and Africans are *NAT2*5* and **6*. The important *NAT1* allele, *NAT1**10, has been reported to have prevalence of 43.8% in Asian populations including Thais at about 40-50%, whereas that in white Caucasians is about 20% (Kukongviriyapan et al., 2003b) (Table 2).

Polymorphism of *NAT2* was reported to be associated with risk of CCA, in which some slow acetylator genotypes, i.e. *NAT2*6* and **7* are at lower risk (OR: 0.26, 95%CI: 0.15-0.44). However, *NAT1*10* is not associated with cancer risk (Kukongviriyapan et al., 2003b). Further epidemiological studies in larger populations and environmental exposure to identify putative carcinogenic chemicals in relation to *NAT2* metabolism may be beneficial to reduce the risk of CCA in populations in this region.

Moreover, slow acetylators of *NAT2* have been shown to be associated with increased risk of bladder cancer among smokers, but not of those with *NAT1* (Garcia-Closas et al., 2005). On the other hand, rapid acetylator genotypes are at increased risk of colon cancer among smokers and individuals who consumed red meat (Walker et al., 2009). A study in patients with hepatocellular carcinoma who were not related to viral hepatitis showed a minor, but relevant association with slow *NAT2* status (Agundez, 2008). It is noted that an association of risk of various cancers with *NAT2* is strongly modified by environmental exposure, particularly, heterocyclic amines and aromatic amines from food and occupational chemicals.

*Polymorphism of NADPH-quinone oxidoreductase-1*

NADPH-quinone oxidoreductase-1 (NQO1) is the cytosolic enzyme that catalyzes the two-electron reduction of nitroaromatic, quinones and quinine-imines, to hydroquinones, thereby promoting detoxification and preventing the formation of reactive oxygen species. Moreover, NQO1 also plays a role in regenerating antioxidant forms of ubiquinone and tocopherol (vitamin E) after free radical attack (Dinkova-Kostova and Talalay, 2010). NQO1, a highly inducible enzyme, is regulated by the Nrf2-ARE signaling pathway suggesting important functions as an antioxidant and cytoprotection in the defense of oxidative stress and prevention of cancer. This is consistent with recent studies showing that defective in *NQO1* gene is associated with increased risk of cancers (Yang et al., 2012). Using immunohistochemisty, NQO1 staining is found in vascular endothelium in most tissues, i.e. respiratory, colon, and breast epithelium, thyroid follicle, and weakly expressed in the liver, bile duct and myocardium (Siegel and Ross, 2000). Interestingly, NQO1 is found highly expressed in various solid tumors, i.e. thyroid, breast, colon and lung suggesting its role in carcinogenesis, probably by conferring cytoprotection for tumors (Siegel and Ross, 2000). CCA tissues also showed high activity of NQO1, when compared with normal liver tissues (Buranrat et al., 2012). It is reasonable to suggest that inhibition of NQO1 is a novel approach to enhance anticancer agent sensitivity in cancers (Buranrat et al., 2010).

Polymorphism of human *NQO1* gene is constituted from more than 300 SNPs (http://www.genecards.org/cgi-bin/carddisp.pl?gene=*NQO1*). The most common allele is *NQO1*2*, a nonsynonymous mutation at 609C>T (P187S) which causes reduced activity due to a rapid degradation of the mutant protein (Siegel et al., 1999). Allele frequency of *NQO1* among Asian populations is relatively high i.e. 50%, and low in Caucasian and African populations, i.e. 18% and 16%, respectively (Table 2). Carriers of *NQO1*2* allele are at greater risk of cancers of the gastrointestinal tract as compared to ones who carry *NQO1*1*, the wild type allele (Yang et al., 2012) which is similar to breast cancer (Menzel et al., 2004). On the other hand, our preliminary study in patients with cancers of the bile duct suggested that *NQO1 609T* allele was associated with lower risk of CCA (Zeekpudsa et al., 2009), in the other words, 609C wild type genotype presented a risk of CCA (adjusted odds ratio: 2.57 (95% CI: 1.39-4.74) and the risk was even higher in patients who also carried null alleles of *GSTT1* genotype. The increased risk of cancers in wild type *NQO1* genotype might suggest a role in metabolic activation of carcinogenic agents in exposed populations.

*Polymorphism of glutathione-S-transefrases*

Human glutathione-S-transferase (GST) enzymes have been discovered as over 16 cytosolic isoforms. They are divided into 7 main classes, α (alpha, GSTA), ϰ (kappa GSTK), μ (mu, GSTM), ω (omega, GSTO), π (Pi, GSTP), θ (theta, GSTT), and ζ (zeta, GSTZ). Moreover, there are also mitochobndrial and microsomal forms (now designated as MAPEG). Cytosolic and mitochondrial GST share some similarity in their structure, but not with MAPEG. Cytosolic GSTs represent the largest family and are of interest to biomedical research, because they are relevant to several drug targets including anticancer agents, and environmental toxicants, carcinogens and by-products of oxidative stress (Hayes et al., 2005).

The GSTs play central role in xenobiotic detoxification, resulting more water-soluble conjugates facilitating excretion via the MRP efflux pumps or undergo further metabolism to mercapturic acid derivatives (Hayes et al., 2005). Metabolism by GSTs, thereby, confers cellular protection from environmental and oxidative stress, yet is also implicated in resistance to anticancer agents. Drug resistant tumor cell lines have been shown to have over expression of GST genes, which leads to an increase of drug-glutathione conjugation with subsequent excretion (Lo and Ali-Osman, 2007). Apart from catalyzing GSH conjugation reactions for chemicals, GSTs also function as ligand-binding proteins with cellular protein, such as JNK, ASK, PKC in cell signaling, apoptosis and drug

sensitivity (Lo and Ali-Osman, 2007). The polymorphism of GST isoforms that has been reported in association with cholangiocarcinoma included *GSTM*, *GSTO* and *GSTT*.

*GSTM* subfamily consists of 5 isoforms (M1- M5).The *GSTM1* is the most studied isoform, where loss of function is ascribed to a homozygous deletion of this gene resulting in the *GSTM1 null* or *GSTM1*0* allele. The *GSTM null* has been reported to be associated with various cancer risk (Hayes et al., 2005). The allele frequency of *GSTM1*0* in Thais is about 67% (Table 2) whereas in other populations it is lower. Additionally, *GSTT* consists of two subfamilies: *GSTT1* and *GSTT2*. Polymorphisms exist within both genes particularly, the gene deletion type, *GSTT1**0 or *GSTT1 null*. A number of reports implicated *GSTT1 null* and GSTM null in association with risk of various cancers. In a meta-analysis of hepatocellular carcinoma, a study suggested that a small excessive risk of liver cancer in individuals with *GSTM1 null* and possibly with *GSTT1 null* genotypes as well (White et al., 2008). In a study of lung cancer, *GSTM1* genotype was associated with cancer risk and smoking may enhance a greater risk of cancer (Li et al., 2012). In an analysis of 42 studies in colorectal cancer, *GSTM1 null* allele exhibited a small excessive risk of colorectal cancer (pooled odds ratio 1.15, 95%CI: 1.06-1.25) in Caucasian but not Chinese populations a similar result to *GSTT1 null* that exhibited increased cancer risk in Caucasian population with pooled odds ratio 1.31, 95%CI: 1.12-1.54 (Economopoulos and Sergentanis, 2010). In contrast, a large population study previously showed that *GSTM1 null* and *GSTT1 null* or the combination did not present risk of breast cancer (Egan et al., 2004). In a case-control study in an endemic area of liver fluke infection in northeast Thailand, genetic polymorphism of *GSTM1* and *GSTT1* alone did not present a risk for CCA. However, elevated serum anti-*Opischorchis viverrini* (liver fluke) indicating previous liver fluke infection was correlated with an increase in CCA risk (adjusted odds ratio 10.3, 95% CI: 1.31-81.63) and the risk was even amplified in subjects with *GSTM1 null* genotype with adjusted odds ratio 23.5, 95%CI:3.33-97.40 (Honjo et al., 2005).

The omega class of GST contains 2 members, *GSTO1* and *GTSO2*. Substrates of GSTO do not usually overlap with other classes of GSTs. The enzymes act as small stress response proteins involved in redox regulation and possess dehydroascorbate reductase activity (Schmuck et al., 2005). No synonymous mutations on *GSTO1* causes amino acid change of A140D and E208K, but these polymorphisms do not alter specific activity of the recombinant enzyme. *GSTO1-1* may play important roles in arsenic metabolism and non-enzymatic effects, in that polymorphism of *GSTO1-A140D* and *E208K* are associated with higher expression of IL-1, IL-8, TGF-β and Apaf-1 which, thereby, could increase risk of inflammatory diseases (Escobar-Garcia et al., 2012). *GSTO1* polymorphism has been suggested to be associated with increased risk of chronic obstructive pulmonary diseases, acute lymphoblastic leukemia (Pongstaporn et al., 2009), hepatocellular carcinoma and breast cancer (Marahatta et al., 2006). *GSTO1 A140D* polymorphism has been suggested to a risk factor for cholangiocarcinoma in endemic areas of liver fluke infection (Marahatta et al., 2006).

*Polymorphism of Methylenetetrahydrofolate reductase*

The enzyme methylenetetrahydrofolate reductase (MTHFR) plays a central role in folate metabolism, by regulation of the synthesis of thymidylate and purines, and supply of methyl groups for the synthesis of methionine and DNA methylation (Powers, 2005). Polymorphism of *MTHFR* gene at *677C>T* generates a change of amino acid from alanine to valine resulting in a thermolabile enzyme with decreased activity. The defective allele frequency in Thais is comparable with other Asian and European populations (Table 2) (Chutinet et al., 2012; Songserm et al., 2012). The second common mutation in coding region of *MTHFR* is *1298A>C*, which causes a glutamic acid to alanine substitution with decreased enzymatic activity. The prevalence of this allele in Thai populations is 28.1% (Songserm et al., 2012). The polymorphism of *MTHFR* in coding region causing decreased *MTHFR* activity has been suggested to be associated with increased homocysteine levels and abnormal DNA methylation, an important epigenic feature of DNA, which is associated with a number of diseases, including, hypertension, neuro-behavioral abnormalities and cancers (Powers, 2005; Cheng et al., 2010; Kim et al., 2012). It should be noted that the levels of folate and homocysteine are strong modifying factors of the diseases, as low folate and hyperhomocysteinaemia are known to be causally related to cancers and cardiovascular diseases (Powers, 2005; Kim et al., 2012).

The common *MTHFR 677C>T* allele was investigated in a large case-control study in association with colorectal cancer. The TT genotype was associated with decreased risk with adjusted odds ratio 0.77, 95%CI: 0.58-1.03, especially at high levels of folate and low levels of ethanol intake (Le Marchand et al., 2005b; Powers, 2005). In contrast, *MTHFR 677C>T* or T genotype had a tendency to have a higher risk to develop pancreatic cancer, especially in the condition of depletion of folate, vitamin B6, B12 (Mazaki et al., 2011). An analysis for CCA risk in Thai popluations, showed that *MTHFR 677TT* and *1298TT* genotypes alone did not present significant risk, however, the risk was significantly elevated in individuals with high intake of raw fresh water fish, which are at risk of liver fluke infection, and other meat products (Songserm et al., 2012). The study suggested that the gene-environment interaction plays crucial part in cancer risk. In a study in Korean populations, which is not an endemic area of liver fluke infection, *MTHFR* and thymidylate synthase enhancer region (TSER) polymorphisms were analyzed. A combination of *MTHFR 677CC* with *TSER 2R(+)* genotype had a higher risk for developing CCA compared to *MTHFR 677CC* with *TSER3R* genotype (odds ratio 5.4, 95%CI: 1.2-23.5) (Ko et al., 2006). The studies suggest that *MTHFR* may be a risk modifier of environmental exposure to toxic agents.

## Conclusion

Cumulating evidence illustrates an association of genetic polymorphism of drug metabolizing genes with

risk of developing cancer of the bile duct. The frequency of important allelic variants of various DME genes in high risk populations of the northeast Thailand is similar to most Asian populations. The mutant alleles may encode defective enzymes, which alter the metabolism of carcinogenic chemicals probably due to environmental exposure with subsequent modifations of the risk of CCA. Polymorphism of DME is similar to other cancer susceptible genes in that these genes generally present only a small excessive risk, however, the risk is modified by environmental exposure. The well recognized and important environmental factor is liver fluke infection, which causes chronic inflammation of the biliary tract. However, liver fluke alone may not trigger cancer. Other causative agents, which may be affected by various DME, are yet to be identified. Alcohol, tobacco, nitroso compounds, raw fresh water fish, fermented meat and mycotoxins are among chemicals/substances possibly related to tumor development. Comprehensive studies of the complex interactions of genes and the environment are required to better understand the nature of chemical exposure and impact of genetic polymorphism in individual settings.

## Acknowledgement

Research works were supported by grant-in-aid from Khon Kaen University and Thailand Research Fund. We wish to acknowledge the support of the Khon Kaen University Publication Clinic, Research and Technology Transfer Affairs, Khon Kaen University, for their assistance.

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