CHOLANGIOCARCINOMA: EPIDEMIOLOGY AND RISK FACTORS

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List of abbreviations in order of Appearance:

CCA, cholangiocarcinoma; iCCA, intrahepatic CCA; pCCA, perihilar CCA; dCCA, distal CCA; HCC, hepatocellular carcinoma; WHO, World Health Organization; PLC, primary liver cancer; EU, European Union; UK, United Kingdom; CUP, cancer of unknown primary; APC, annual percentage change; ICD, International Classification of Disease ; ICD-O, ICD-Oncology; IARC, International Agency for Research on Cancer; SEER, Surveillance, Epidemiology, and End Results; ASIR, age-standardized incidence rates; OR, odds ratio; CI, confidence interval ; eCCA, extrahepatic CCA; PSC, primary sclerosing cholangitis; HR,

hazard ratio; IBD, inflammatory bowel disease; HBV, hepatitis B virus; HCV, hepatitis C virus; RR, relative risk; ROS, reactive oxygen species; NAFLD, nonalcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; GSTs, glutathione S-transferases; MTHFR, methylenetetrahydrofolate reductase; ABC, ATP-binding cassette; CHC, combined hepatocellular-cholangiocarcinoma

ABSTRACT

Cholangiocarcinoma (CCA) is a heterogeneous disease arising from a complex interaction between host-specific genetic background and multiple risk factors. Globally, CCA incidence rates exhibit geographical variation, with much higher incidence in parts of the Eastern world compared to the West. These differences are likely to reflect differences in geographical risk factors as well as genetic determinants. Of note, over the past few decades, the incidence rates of CCA appear to change and subtypes of CCA appear to show distinct epidemiological trends. These trends need to be interpreted with caution given the issues of diagnosis, recording and coding of sub-types of CCA. Epidemiological evidences suggest that in general population some risk factors are less frequent but associated with a higher CCA risk, while others are more common but associated with a lower risk. Moreover, while some risk factors are shared by intrahepatic and both extrahepatic forms, others seem more specific for one of the two forms. Currently some pathological conditions have been clearly associated with CCA development, and other conditions are emerging; however, while their impact in increasing CCA risk as single etiologic factors has been provided in many studies, less is known when two or more risk factors co-occur in the same patient. Moreover, despite the advancements in the knowledge of CCA aetiology, in Western countries about 50% of cases are still diagnosed without any identifiable risk factor. It is therefore conceivable that other still undefined etiologic factors are responsible for the recent increase of CCA (especially iCCA) incidence worldwide.

KEY POINTS

- Cholangiocarcinoma incidence varies globally, presumably reflecting differences in geographical risk factors as well as genetic determinants.

- Rising rates for intrahepatic CCA are widely reported but these trends are complex and need to be interpreted with caution as misclassification may be an issue.

- Several potential risk factors have increased globally over recent decades and may be contributing to rising CCA rates.

- Recognised risk factors for CCA account for approximately half of cases only.

- Further studies elucidating risk factors and the mechanisms underlying malignant change in the biliary tree are required, in addition to uniform and accurate recording of epidemiological data.

EPIDEMIOLOGY

Cholangiocarcinoma are a diverse group of malignancies arising from the biliary epithelium. In most parts of the world, particularly the Western countries, the peak age of incidence for CCA is the seventh decade and the disease affects both genders, with a slight male preponderance[1, 2]. CCA represent an estimated 3% of all gastrointestinal system malignancies and are classically sub-divided into three groups depending on the anatomical site of origin: intrahepatic CCA (iCCA), perihilar CCA (pCCA) and distal CCA (dCCA).[3, 4] iCCAs arise above the second-order bile ducts, whereas the anatomical point of distinction between perihilar cholangiocarcinomas (pCCAs) and distal cholangiocarcinomas (dCCAs) is the cystic duct.[4] pCCA account for approximately 50–60% of all CCA, dCCA 20–30%; and iCCA.[4-6] iCCA comprises approximately 10% of all primary liver cancers, making it the second most common primary hepatic malignancy after hepatocellular carcinoma (HCC).[4-6]

Globally, incidence and mortality rates of CCA show substantial geographical variation. The incidence of CCA is manifold higher in parts of the Eastern world compared to the West, with significant difference between regions of the same country too (Table 1). Presumably, these variations in incidence reflect, at least partly, differences in geographical risk factors as well as genetic determinants.[1,3,4] Of note, over the past few decades, the incidence rates of CCA appear to be changing. However, in addition to disparate risk factors, pathobiology,

clinical presentations, management and prognoses,[3] subtypes of CCA also appear to show distinct epidemiological trends.

Evolving epidemiology of Cholangiocarcinoma

Multiple studies have shown rising rates of iCCA. This was first reported in the United Kingdom, where a 15-fold increase in age specific mortality rates for iCCA in ages 45 and above was found between 1968 and 1996.[7] There was a steady decrease in extrahepatic CCA over the same period.[7] Further studies published in the early 2000's showed similar findings, i.e. rising iCCA and falling extrahepatic CCA in both genders across many European countries, the US, Australia and Japan.[2,8] More recent studies report these patterns are continuing. Bertuccio et al. used data from the World Health Organization (WHO) to compute age-standardized (world population) mortality rates in primary liver cancer (PLC) and employed join-point analysis to identify substantial changes.[9] Between 2002 and 2007, PLC rates across 12 selected European Union (EU) countries overall declined from 3.9 to 3.6/100,000 in men. In women, mortality was lower (0.8/100,000 in 2007 in the EU), and showed more favourable trends, with a decline of over 2% per year over the last two decades as compared with 0.4% in men. In contrast, mortality from iCCA increased by around 9% in both sexes from 1990 to 2008, reaching rates of 1.1/100,000 men and 0.75/100,000 women, the highest rates occurring in the United Kingdom (UK), Germany, and France.[9] Data for the United States, Japan, and Australia was also analysed for comparison, and similar trends were found.[9]

A recent US study analysed Surveillance, Epidemiology, and End Results data to assess 40-year trends in the age-standardized incidence of intrahepatic and extrahepatic CCA between 1973 and 2012.[10] As iCCA may potentially be misdiagnosed as cancer of unknown primary (CUP), trends in the incidence of CUP were also analysed. Between 1973 and 2012, the reported U.S. incidence of iCCA increased from 0.44/100,000 to 1.18/100,000, representing an annual percentage change (APC) of 2.30%. This trend accelerated during the last decade to an APC of 4.36%; whereas the incidence of extrahepatic CCA increased modestly from 0.95/100,00 to 1.02/100,000 during the 40-year period (APC, 0.14%). The incidence of CUP with histologic features potentially consistent with cholangiocarcinoma decreased by 51% between 1973 and 2012 (APC, -1.87%). Thus, although the incidence of iCCA in the U.S. rose, the incidence of CUP fell during the same period.[10] In a phase II trial of patients with previously untreated CUP, molecular tumour profiling enabled determination of the tissue of

origin in 98% of cases. Of 289 patients, 18% were found to have biliary tract cancer.[11] Hence, the improved clinical distinction between CUP and iCCA might be another factor contributing to the apparent increase in iCCA incidence.[10]

However, other single nation studies reported different CCA incidence trends. The incidence of both intra- and extrahepatic CCA remained stable in Burgundy, France,[12] and iCCA incidence reportedly actually decreased in Denmark over recent decades.[13] Furthermore, data from the North American Association of Central Cancer Registries indicate that the incidence of iCCA fell between 1998 and 2003 ([APC -8% per year), then rose between 2003 and 2009 (APC 6% per year); the incidence of extrahepatic CCA increased between 1998 and 2003 (APC 9% per year), before plateauing from 2003 to 2009.[14]

CCA Coding and Misclassification

The reasons for these changes in trends in CCA are unclear. iCCA is a primary liver cancer and shares several similar underlying risk factors with HCC.[4] Several of these risk factors are also known to be increasing globally and are discussed in detail below. Improvements in the accuracy and availability of diagnostic tools over the past few decades may also have contributed to diverging incidence rates of various hepatobiliary malignancies, but it is exceptionally difficult to measure this effect.[15]

Another important issue that requires consideration when interpreting reported epidemiological trends in CCA is the ever evolving WHO International Classification of Disease (ICD) coding system, which is used by cancer registries internationally to record different cancers and thus feeds into national datasets which are analysed in published studies. Multi-disciplinary specialists involved in CCA clinical care and research generally agree that CCA should be divided into three distinct sub-types: iCCA, pCCA, and distal/extrahepatic (dCCA), as these three sub-types have distinct epidemiology, biology, prognosis and clinical management approaches.[1,3,16] Although pCCA (historically often referred to as "Klatskin" tumours) make up the bulk of CCA, unfortunately, to date no version of the ICD coding system distinguishes between pCCA and dCCA. The main form of ICD has codes for all known diagnoses, cancer and non-cancer, and the current version in use at the time of writing is ICD-10. ICD-10 lists topography codes, which describe the anatomical site of origin, or organ, of a tumour. A separate ICD exists for cancers only, ICD-

Oncology (ICD-O), overseen by the International Agency for Research on Cancer (IARC), the specialized cancer agency of the WHO. ICD-O-3, the third iteration of ICD-O, is currently in use and consists of two coding systems, which together describe the tumour: 1) the topographical code, which describes the anatomical site of origin (or organ system) of the tumour, and 2) the morphological code, which describes the cell type (or histology) of the tumour, together with the behaviour (malignant or benign). ICD-10 (and previous versions of ICD) have separate topography codes for iCCA (C22.1) and dCCA (C24.0), but none for pCCA. ICD-O also has no topographical code for pCCA. However, ICD-O has a morphological code for pCCA, but does not have specific morphological codes for iCCA or dCCA. Thus, although multiple studies report rising incidence rates of iCCA and falling rates of extrahepatic CCA, we do not know what is happening with incidence/mortality rates of pCCA, the commonest form of CCA, which could have been incorrectly coded as either iCCA or dCCA in current and previous versions of ICD coding.[17]

Furthermore, ICD and ICD-O editions change every few years, but are adopted by different countries at different times, which again could potentially contribute to differences between countries' reported rates. The second edition of the ICD-O (ICD-O-2) assigned 'Klatskin' tumours (pCCA) a unique histology code, but this was cross-referenced to the topography code for intrahepatic rather than extrahepatic CCA.[17] With the advent of ICD-O-3, however, 'Klatskin' tumours can be cross-referenced to either intrahepatic or extrahepatic cholangiocarcinoma. A study of UK data and US Surveillance, Epidemiology, and End Results (SEER) data examined whether a change in ICD-O coincided with changes in recorded rates of different types of CCA.[17]. In the USA, the switch from ICD-O-2 to ICD-O-3 occurred in 2001, whereas in the UK, this switch did not occur until 2008.[17] Age-standardised incidence rates (ASIR) in England and Wales between 1990 and 2008 markedly increased for iCCA and decreased for pCCA/dCCA. This trend was still evident after transferring all CCA recorded as "Klatskin" from intrahepatic to extrahepatic codes.[17] Remarkable however, only 1% of all CCA were reportedly Klatskin, which cannot be a true reflection of all pCCA cases. Of note, on direct questioning, most UK cancer registries reported that if a tumour site is unspecified, most would classify CCA as intrahepatic.[17] The analysis of US SEER data found that ASIR of iCCA rose from 0.6 per 100,000 individuals in 1990 to 0.9 per 100,000 individuals in 2001. But from 2001, when ICD-O-3 was adopted in the US, the ASIRs for iCCA began to decrease, before plateauing at 0.6 per 100,000 individuals by 2007. Conversely, ASIRs for pCCA/dCCA remained stable at around 0.8 per

100,000 individuals until 2001, and then began increasing, reaching 1.0 per 100,000 individuals by 2007.[17] Other studies have highlighted the potential for misclassification of CCA.[18,19] Systematic under-reporting of the incidence of CCA may be another confounding issue. This was noted in a study of the concordance between Swedish cancer registries and patient registries, which found that between 1990 and 2009, 44% of CCA were reported only in the patient registries.[20]

In conclusion, potential explanations behind the trends in CCA incidence are complex and reported changes in incidence rates need to be interpreted with caution. For example, it is quite possible that pCCA, the most-common subtype of CCA, is regularly being misclassified as iCCA, the least common subtype, thereby falsely skewing the reported rates of iCCA. Going forward, diagnoses and epidemiological data need to be recorded uniformly and accurately. This responsibility resides with both clinicians and cancer registries, as well as with ICD coding system, which needs to more accurately reflect the different types of CCA. There is a need for both ICD-11 and subsequent iterations of ICD-O to have separate topography and morphology codes for iCCA, pCCA and dCCA.

RISK FACTORS

Cholangiocarcinoma: a heterogeneous disease arising from multiple risk factors

CCA encompasses an assorted group of malignancies lacking a stereotyped phenotype and molecular signature.[1,3,4] Compelling evidence supports the notion that CCA heterogeneity is the result of a complex interaction between the host-specific genetic background and a different geographical distribution of the risk factors (Table 2) associated with this disease. Epidemiological studies suggest that multiple risk factors are involved in cholangiocarcinogenesis, and that some of them are less frequent but associated with a higher risk of CCA, whereas others are more common but associated with a lower risk. Moreover, while some risk factors seem to be shared by iCCA and the two extrahepatic forms (hereafter referred to eCCA), others seem more specific for iCCA or eCCAs.[21] This last observation is also reinforced by the broad geographic variations in iCCA and eCCA incidence, a phenomenon that suggests a spatial-temporal segregation of the underlying etiologic factors. The existence along the biliary tree of two distinct stem cell niches (the canals of Hering and the peribiliary glands) susceptible to different injuries may add a further level of complexity in the identification of the risk factors linked to CCA.[22]

Currently, some pathological conditions have been clearly linked to CCA development, and other conditions are emerging from recent studies. However, while their impact as single agents in increase CCA risk has been established, less clear is when two or more risk factors co-occur in the same patient. Moreover, despite the advancements in the knowledge of CCA aetiology, in Western countries about 50% of cases are still diagnosed without any identifiable risk factor. It is therefore conceivable that other still undefined factors are responsible for the recent reported increases in CCA (especially iCCA) incidence worldwide, a phenomenon that justifies the increasing scientific attention towards this disease.

Cholangiocarcinoma misclassification and possible biases on risk factor aetiology

CCA incidence shows wide geographic differences worldwide.[1] However, while these differences are expected among populations exposed to different risk factors, epidemiological discrepancies observed among populations exposed to similar risk factors are less expected. Likely, such discrepancies rely not only on possible errors in cancer registers, but also on misclassification of some CCA forms. Indeed, some iCCAs may be misdiagnosed as CUP, HCC or mixed HCC-iCCA, whereas some Klatskin tumors can be topographically ascribable to iCCA or eCCA;[18] moreover, the diagnosis of CCA at an advanced stage makes sometimes difficult to identify its anatomical origin.

In this scenario, as CCA still remains a relatively rare cancer, misclassification can introduce significant biases in the identification of the risk factors associated with this disease. A more refined CCA classification, along with an accurate diagnosis and patient anamnesis, is therefore required to better clarify the underlying aetiology of this disease.

Bile duct disorders

• *Bile duct cysts*

Bile duct cysts are a rare congenital disorder characterized by cystic dilatation of the intrahepatic and/or extrahepatic biliary tree; according to the classification, they can be divided into type I, type II, type III, type IV and type V (Table 3).[23] The frequency of bile duct cysts is high in females of Asian countries, especially China and Japan, while is relatively low in Western populations.[24] The association between bile duct cysts and CCA is well established and, when they are undetected or treated inappropriately, tumour can arise from both cysts and undilated parts of the biliary tree.[25] A recent analysis based on the US SEER registry reported an odds ratio (OR)=15.66 (95% confidence interval [CI]11.58–

21.18) for iCCA and an OR=27.12 (95% CI 22.06–33.34) for extrahepatic CCA (eCCA).[26] Typically, these patients develop CCA at a mean age of 32 years (much lower than in the general population), and the higher incidence has been documented among subjects with type I and IV bile duct cysts.[27] Surgical treatment usually decreases the risk of CCA in these patients; however, also after surgery such risk remains higher than the general population.[28] Reflux of pancreatic enzymes, bile stasis and increased intraductal concentration of bile acids may contribute to malignant transformation of the epithelium lining the cystic bile duct wall.[27] According to preliminary findings, bacterial infection could also play a role in CCA development in patients with bile duct cysts.[29]

Caroli's disease is a rare autosomal recessive disorder characterized by non-obstructive gross dilatation of the segmental intrahepatic bile ducts and has been included in the classification of type V choledochal cysts. The associated bile stasis, chronic inflammation and cholangitis have been suggested as conditions linked to the increased cancer risk in these patients.[30] Caroli's diseases has been reported as one of the strongest risk factors for both iCCA and eCCA, conferring a 38-fold higher risk for iCCA (OR=38.13, 95% CI 14.20–102.38) and a 97-fold higher risk for eCCA (OR=96.81, 95% CI 51.02–183,68).[26] The risk of malignant transformation associated with Caroli's diseases mostly occurs after the second decade of life, although some cases have been reported among teenagers.[31]

• *Primary sclerosing cholangitis/cholangitis*
Primary sclerosing cholangitis (PSC) is an autoimmune disease affecting bile ducts, leading to inflammation and subsequent obstruction of both intra- and extrahepatic bile ducts. Patients with PSC carry a 400-fold higher risk for CCA than the general population (standardized incidence rate, 398, 95% CI 246-608), with a reported overall incidence of about 7%.[32] In these patients, CCA is usually diagnosed in the fourth decade of life compared to the seventh decade in general population, and longitudinal studies have shown that up to 50% of CCAs are detected within the first year of PSC diagnosis.[21] Results from the US SEER registry also reported a strong association between cholangitis and CCA development, with an OR=21.52 (95% CI 7.21–26.90) for iCCA and an OR=40.80 (95% CI 34.96–47.60) for eCCA.[26] However, this analysis did not distinguish the impact of the autoimmune forms from that arising from the others forms of cholangitis.

The causal link between PSC/cholangitis and CCA development likely includes chronic inflammation, proliferation of biliary epithelium, production of endogenous bile mutagens and bile stasis.[21,33] The presence of some inflammatory conditions, such as inflammatory bowel disease (IBD), have been reported in some studies to significantly increase the risk of CCA in PSC, compared to non-PSC-IBD subjects (Hazard ratio, HR= 190, 95% CI 54.8-660), with the highest incidence of CCA occurring within the first year after diagnosis of IBD.[34,35] However, no additional risk of CCA in PSC patients was reported in a US study.[36] Therefore, the impact of IBD in increasing the risk of CCA in PSC patients remains to be fully clarified.

● *Hepatolithiasis, cholelithiasis and choledocholithiasis*

Hepatolithiasis refers to the presence of calculi in the intrahepatic biliary tree. This condition is rare in Western Countries (0.6-1.3%), while fairly common in the East Asia (up to 25%).[37] In patients with hepatolithiasis, the association with iCCA has been well documented, with an overall incidence of 5-13%.[37,38] Hepatolithiasis has been found to represent a strong risk factor for iCCA in a Korean case-control study (OR = 50.0, 95% CI 21.2–117.3).[39] The role of hepatolithiasis in the genesis of iCCA has been also confirmed outside Asia; an OR=6.7 (95% CI 1.3–33.4) was indeed observed in an Italian case-control study.[40] The association between hepatolithiasis and iCCA is likely linked to chronic inflammation, bile stasis and bacterial infections.[37] Concurrence of hepatolithiasis and parasitic infestations has been documented in Asia;[41] in addition, smoking, family history of cancer and duration of symptoms longer than 10 years have been suggested as risk factors for iCCA in patients with hepatolithiasis.[42]

Cholelithiasis and choledocholithiasis are both conditions that have been linked to increased risk for eCCA and the risk seems to increase with gallstones size, epithelium calcification and disease duration.[43] Conversely, their role in iCCA pathogenesis is less clear. A recent analysis based on the US SEER registry reported a significant association between CCA development and cholelithiasis/choledocholithiasis; this association was stronger for eCCA (cholelithiasis: OR=5.29, 95% CI 4.83-5.80; choledocholithiasis: OR=14.22, 95% CI 12.48-16.20) than for iCCA (cholelithiasis: OR=3.93, 95% CI 3.49-4.43; choledocholithiasis: OR=6.94, 95%CI 5.64-8.54).[26] In addition, a meta-analysis of seven case-control studies suggested that choledocholithiasis without hepatolithiasis associates with a high risk of iCCA, whereas the evidence for cholelithiasis seems less clear.[44]

Liver diseases

• *Cirrhosis*

Liver cirrhosis is a manifestation of advanced liver disease. In cirrhotic livers, the architecture of hepatic parenchyma is subverted by fibrosis and regenerative nodules that determine progressive loss of liver function. Cirrhosis is a well-established risk factor for HCC, with > 90% of HCCs developing in cirrhotic patients.[45] In a meta-analysis from seven case-control studies, liver cirrhosis was also identified as a strong risk factor for iCCA (OR=22.92, 95% CI 18.24–28.79).[46] Cirrhosis might also represent a risk factor for eCCA; an OR=5.4 (95% CI 2.9–10.2) was indeed estimated in a large case-control study conducted in the US population.[47] A recently population-based case-control study in Asian patients also reported an increased risk for iCCA (OR=8.0, 95% CI 6.6–9.8) and eCCA (OR= 3.9, 95% CI: 3.0–5.1) in cirrhotic patients.[48] The raised risk of iCCA and, possibly, eCCA, in cirrhotic patients could be explained by the increased cellular proliferation, release of inflammatory cytokines and occurrence of fibrosis in the liver.[49]

• *Viral Hepatitis*

Hepatitis B (HBV) and C (HCV) virus chronic infection is a strong risk factor for HCC. Findings from different epidemiological studies suggest that these infections may also represent a risk factor for CCA development, with a stronger association for iCCA.[50] The association between hepatitis viruses and iCCA incidence was found to vary between Western and Asian countries. Indeed, while in Western populations iCCA was stronger associated with HCV, in Asian populations this malignancy was stronger associated with HBV, where this infection is endemic.[46,51,52] According to a meta-analysis including 16 case-control and 2 cohort studies, the risk of iCCA in patients with HBV infection was more than three times higher than in patients without HBV infection (relative risk, RR=3.42, 95% CI 2.46–4,74).[53] The meta-analysis also identified signs of a small increase in eCCA risk (RR=1.46, 95% CI 0.98–2.17).[53] The association between HBV and iCCA has been also confirmed in another study, where an OR=5.10 (95% CI 2.91–8.95) was reported.[52] More recently, a meta-analysis including thirty-nine studies reported an OR=2.72 (95% CI 1.90–3.88) for the risk of CCA in HBV positive patients; in particular, an OR=3.184 (95% CI 2.356–4.302) was found for iCCA, whereas a weak association was found for eCCA (OR= 1.407, 95% CI 0.925–2.141).[54]

An OR=4.84 (95% CI 2.41–9.71) was estimated in a meta-analysis of eight case-control studies evaluating the association between HCV and iCCA.[46] In another meta-analysis of sixteen case-control studies, pooled risk estimates showed a significant increased risk for CCA in HCV positive patients (OR= 5.44, 95% CI, 2.72-10.89); notably, the pooled risk estimate of iCCA was higher than eCCA (OR=3.38, 95% CI, 2.72-4.21 *vs* OR=1.75, 95% CI, 1.00-3.05).[55] The presence of liver cirrhosis in HBV or HCV patients was shown to increase the risk of CCA; in particular, the risk of iCCA was found to increase 2.5-fold (95% CI 1.2-5.1) in HBV positive patients and 3.2-fold (95% CI 1.2-8.1) in HCV positive patients.[56] The increased risk of CCA among HBV and HCV patients likely relies not only on the presence of liver cirrhosis, but also on a direct carcinogenic effect by these viruses on target cells;[57] moreover, chronic liver inflammation resulting from virus infection triggers cellular proliferation, thus increasing the risk of malignant transformation.[57]

● *Hemochromatosis*

Hemochromatosis type 1 is a genetic disorder most commonly linked to the HFE1 mutation (C282Y) and is characterized by pathological iron accumulation in the body, particularly in the liver. Clinical manifestations include cirrhosis, polyarthropathy, adrenal insufficiency, heart failure or diabetes.[58] While hemochromatosis has been clearly reported to increase the HCC risk,[59] a definitive conclusion about its role in CCA cannot be yet provided. Some case reports and case series suggest an association between hemochromatosis and iCCA development.[60-63] Results from the US SEER registry reported an OR=2.07 (95% CI 1.33-3.22) for iCCA, whereas no increased risk was found for eCCA.[26] This last finding is not totally surprising, as iron deposition preferentially occurs in the liver. Liver cirrhosis, a common clinical manifestation of hemochromatosis, could explain the increased iCCA risk. However, some iCCA cases have been also observed in hemochromatosis patients without cirrhosis, suggesting that hemochromatosis could increase the iCCA risk independently from this disease.[64,65] The hypothesized molecular mechanisms linking hemochromatosis to iCCA are similar to those observed for HCC and include formation of reactive oxygen species (ROS) within the liver, DNA damage, lipid peroxidation and acceleration of fibrogenesis.[66] Indeed both HCC and iCCA arise from the differentiation of common hepatic progenitor cells localized in the canals of Hering, and activation of this stem cell compartment typically occurs in chronic liver diseases.[67]

- *Wilson's disease*

Wilson's disease is an autosomal recessive hereditary disorder due to mutations in the Wilson disease (ATP7B) gene and is characterized by copper accumulation in several tissues, primarily liver, brain and other vital organs.[68] A recent cohort study on 1186 patients showed that sporadic cases of iCCA (0.5%) occurred in patients with Wilson's disease.[69] The reason for these low incidences is still debated. Indeed, while an excess of copper is known to induce DNA damage via ROS generation,[70] a protective role of this metal against malignancies has been also reported in some studies.[71-73]

Digestive diseases

• *Inflammatory bowel disease*

Inflammatory bowel disease is a known risk factor for colorectal cancer.[74,75] According to a recent meta-analysis, an increased CCA risk was reported in IBD patients (RR=2.63, 95% CI = 1.47-4.72).[36] Site-specific analyses revealed a RR= 2.61 (95% CI 1.72-3.95) for iCCA, whereas a RR= 1.47 (95% CI 1.10-1.97) for eCCA.[36] Both ulcerative colitis and Crohn's disease were found to be associated with increased CCA risk, although a stronger association was found for ulcerative colitis (RR= 3.40, 95% CI 2.50-4.62 *vs* RR=2.69, 95%CI 1.59-4.55, respectively).[36] A recent analysis based on the US SEER registry also confirmed a stronger association with iCCA for ulcerative colitis (OR=2.18, 95% CI 1.61-2.95) compared to Crohn's disease (OR=1.77, 95% CI 1.13-2.75), whereas a similar increased risk was found for eCCA (OR=1.75, 95% CI 1.32-2.33 *vs* OR=1.71, 95% CI 1.17-2.51).[26] Both pathological conditions may be related to CCA development by induction of chronic inflammation and/or microbiome dysbiosis.[76] IBD may also have extra-intestinal manifestations, including PSC, a well-known risk factor for CCA.[77] In a retrospective cohort study based on Danish national registries, the co-existence of IBD was reported to significantly increase CCA risk in PSC patients (HR=190, 95% CI 54.8-660), compared to subjects with no PSC and IBD;[35] conversely, in a US study neither IBD nor its duration conferred additional CCA risk in PSC patients.[36] Therefore, the impact of IBD in increasing the CCA risk in PSC patients remains undefined.

• *Chronic pancreatitis and duodenal/gastric ulcer*

A positive association between chronic pancreatitis and CCA has been reported, with a stronger association for eCCA (OR:=6.61, 95% CI 5.21-8.40) than iCCA (OR=2.66, 95% CI 1.72-4.10).[26] About 3–23% of patients with chronic pancreatitis develop biliary stricture, which in turn may lead to cholangitis and cholelithiasis, both representing well known risk factors for CCA.[78]

A modest association between duodenal/gastric ulcer with *Helicobacter Pylori* (*H. Pylori*) infection and CCA has been reported, either for iCCA (OR=1.42, 95% CI 1.21-1.66) or eCCA (OR=1.46, 95%CI 1.29-1.66).[26] It has been hypothesized that *H. pylori* may play a role in cholangiocarcinogenesis by increasing the cell kinetics of the biliary epithelium and inducing the formation of stones.[79] A meta-analysis of ten case-control studies suggests that other *Helicobacter* species may be also involved in CCA development (cumulative OR=8.88, 95%CI 3.67-21.49);[80] however, since CCA patients (especially those with eCCA) often undergo endoscopy, these findings should be interpreted with caution.

Parasitic infections

O. viverrini and *C. sinensis* liver flukes have been identified as strong risk factors for CCA, and in endemic areas of Eastern Asia the vast majority of CCAs are linked to these parasitic infestations.[81] It has been estimated that up to 10% of people chronically infected with these liver flukes will develop CCA, especially iCCA.[82] A meta-analysis of case-control studies reported a strong association between *O. viverrini* and *C. sinensis* infections and CCA (OR=4.8, 95% CI 2.8-8.4).[83] *O. viverrini* and *C. sinensis* are flat worms that colonize the bile ducts and infestation in humans typically occurs via the ingestion of raw, pickled, or undercooked fish. *O. viverrini* has been classified as ''carcinogenic to humans'' by IARC more than twenty years ago due to its role in the development of CCA.[84] More recently, the same definition was extended also to *C. sinensis*.[85] Infection with these parasites may lead to CCA by inducing chronic inflammation, cholangitis and fibrosis of the periportal system over the course of decades.[86] Despite anti-helminthic treatment, multiple reinfections are common and tend to be chronic, a phenomenon that may contribute to cholangiocarcinogenesis particularly when exposure to other genetic, environmental and infective factors coexists.[87,88]

Metabolic and endocrine disorders

• *Type II diabetes*

In the last years, epidemiological studies have provided evidences that some metabolic disorders may predispose to primary liver cancers.[89] A meta-analysis of ten case-control studies and five cohort studies found a positive association between type II diabetes and iCCA (RR 1.97, 95% CI 1.57–2.46), as well as eCCA (RR 1.6, 95% CI 1.29–2.05).[90] A positive association between type II diabetes and both CCA cancer types, especially iCCA, has been also reported in a more recent population-based study, where an OR=1.54 (95% CI 1.41-1.68) was observed for iCCA and an OR=1.45 (95% CI 1.34-1.56) for eCCA.[26] Diabetic patients who received metformin had a lower risk to develop iCCA (OR=0.4, 95% CI 0.2–0.9), compared to diabetic patients not treated with metformin, thus reinforcing the potential link between diabetes and iCCA risk.[91] Whether the potential association between diabetes and CCA may be direct or mediated by other intermediate risk factors, such as obesity or non-alcoholic fatty liver disease (NAFLD), remains unclear. Type II diabetes is characterized by compensatory hyperinsulinemia, and insulin has been shown to stimulate cancer cell growth by binding to insulin receptors. Furthermore, diabetes may increase the risk of biliary stones, an independent risk factor for eCCA.[90]

Obesity

The role of obesity in CCA development is still controversial and current evidence is too limited to make any solid conclusions.[92] However, a meta-analysis of three case-control studies showed a pooled OR=1.56 (95% CI 1.26–1.94) for iCCA.[46] A positive association between obesity and CCA has been also reported in another meta-analysis including five cohort and five case-control studies where, compared to normal weight subjects, a pooled OR=1.52 (95% CI 1.13-1.89) was found in obese subjects. However, the analysis was not stratified according to tumor location.[93] These findings are consistent with current knowledge supporting an increased risk for many cancers with obesity. Obesity could increase the risk of cancer, including CCA, by affecting the levels of leptin, adiponectin and proinflammatory cytokines.[92]

NAFLD/NASH

Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of liver diseases ranging from fatty liver to non-alcoholic steatohepatitis (NASH) and cirrhosis. NAFLD/NASH has been identified as a risk factor for different cancer types, especially HCC;[94,95] however, few studies have investigated the possible involvement on CCA pathogenesis. A population-based study reported that NAFLD was associated with an increased risk of iCCA (OR= 3.52, 95% CI 2.87-4.32) and eCCA (OR= 2.93, 95% CI 2.42-3.55).[26] A positive association between NAFLD and CCA has been also suggested from a recent meta-analysis of seven case-control studies (pooled OR=1.95, 95%CI 1.36–2.79).[96] When classified according to CCA subtypes, NAFLD was stronger associated with iCCA (OR= 2.22, 95% CI 1.52–3.24) than eCCA (OR=1.55, 95% CI 1.03–2.33), suggesting that iCCA and HCC may share a common patho-genetic mechanism.[96] It is biologically conceivable that NAFLD may promote CCA development directly by induction of hepatic inflammation or, indirectly, *via* cirrhosis. A cohort study reported that NASH affected up to 20% of patients with iCCA. Notably, these patients were more likely obese (median body mass index 30.0 *vs* 26.0 kg/m^2) and had higher rates of diabetes mellitus (38.7 *vs* 22.0 %), compared to those ones without NASH.[97] More recently, Kinoshita et al. showed that NASH is an independent risk factor for iCCA (OR=3.36, 95% Cl 1.15-10.2).[98] Overall these findings suggest that NAFLD/NASH may represent a risk factor for iCCA. Nonetheless, further studies are warranted to better elucidate the strength of the association and the mechanisms underlying this relationship. Moreover, while a role for NASH, obesity and type II diabetes in increasing CCA risk as single etiologic factors has been provided in some studies, the relative impact of these overlapping diseases in increasing CCA risk when they co-occur in the same patient still remains an open question due to the lack of data.

Life style

• *Alcohol consumption*

Alcohol consumption has been clearly established as a risk factor for HCC;[99] conversely, its association with iCCA has been less investigated. A meta-analysis including eleven case-control studies reported that heavy alcohol consumption (about six drinks/day) associates with increased risk of iCCA (OR=2.81, 95% CI 1.52-5.21).[46]

Similarly, results from the Liver Cancer Pooling Project (including 14 US-based prospective cohort studies) showed that, compared to non-drinkers, heavy alcohol consumption (≥5

drinks/day) was associated to a 68% increased risk of iCCA (HR= 1.68, 95% CI 0.99–2.86).[100] Another prospective cohort study in Japan reported a HR=1.96 (95% CI 0.99–3.91) for iCCA in regular drinkers consuming ≥300 g/day of ethanol, compared to non-drinkers; however, these results did not reach statistical significance (p-trend = 0.065), probably because of the small number of iCCA cases included.[101] Whether the association between alcohol consumption and iCCA is related to liver disease (i.e. alcoholic liver disease and cirrhosis), or to other underlying carcinogenic mechanisms is unclear. Indeed, alcohol may contribute to carcinogenesis by induction of CYP2E1, which metabolises ethanol to acetaldehyde, increasing reactive oxygen-species production, lipid peroxidation and DNA damage. In addition, ethanol may induce enzymes that metabolize pro-carcinogens to carcinogens.[102]

As to eCCA, a meta-analysis including eleven case-control studies and one cohort study reported a similar risk between regular drinkers and non-drinkers (summary RR=1.09, 95% CI 0.87-1.37).[103] The lack of association between alcohol consumption and eCCA could rely on the protective effects of alcohol against gallstone formation (a well-known risk factor for eCCA) by inhibition of cholesterol metabolism.[104]

• *Cigarette smoking*

Cigarette smoking has been investigated as a risk factor for CCA. A meta-analysis of case-control studies conducted in 2012 showed marginal evidence of association between smoking and iCCA (OR 1.31, 95% CI 0.95–1.82). However, there was high heterogeneity among the studies included.[46] More recently, two different studies reported a positive association between smoking and iCCA (HR=1.47, 95% CI 1.07–2.02 and OR=1.46, 95% CI 1.28-1.66, respectively).[26,100] A meta-analysis of eleven case-control studies reported an increased risk also for eCCA in smokers, compared to non-smokers (summary RR =1.23; 95% C 1.01-1.50).[103] Similarly, an analysis based on the US SEER registry reported a 77% increased risk of eCCA in smokers (OR 1.77, 95% CI 1.59-1.96) compared to non-smokers.[26] Early studies suggest that tobacco may exert carcinogenic effects on biliary epithelial cells since carcinogenic compounds (e.g. benzopyrene, formaldehyde, benzene and chromium) are metabolized by hepatic microsomes and excreted to bile.[105,106] However, the causal role of smoking in determining the risk of CCA still remains unclear and further studies are warranted.

Environmental exposure

Epidemiological studies suggest a positive association between CCA and exposure to some environmental carcinogens, with varying strength of evidence. A three-hundred-fold increase in CCA risk has been reported in subjects exposed to the radiographic contrast agent Thorotrast, due to the emission of alpha-radiations;[107,108] however, as this compound has been banned since 1969, the number of CCAs currently linked to exposure to thorotrast is negligible.

Chronic exposure to 1,2-dichloropropane, an organic solvent used in printing, has been also implicated as a causative factor for CCA in a recent study (adjusted RR=14.9, 95%CI 4.1-54.3 for middle exposure category and adjusted RR=17.1, 95% CI 3.8-76.2 for high exposure category).[109]

Several cohort studies also suggested an increased risk of liver cancer in subjects exposed to asbestos.[110,111] However, most of these studies reported estimates for the broad category of liver cancers, without reporting specific data on CCA. There are several reasons behind the lack of specific data on CCA. First, iCCA comprises approximately 10-20% of all primary liver cancers[4,6] therefore, estimated relative risks are driven by the vast majority of HCCs, with iCCAs playing a minor role. Secondly, only very large cohorts (e.g., those based on nationwide registers) have enough statistical power to study iCCA as a specific disease. Recently, a link between asbestos exposure and CCA has been provided in two different case-control studies. In the first study, an OR=4.81 (95% CI 1.73–13.33) for iCCA risk was reported among subjects occupationally exposed to asbestos for over 30 years; a limited evidence was instead reported for eCCA (OR=2.09, 95% CI 0.83-5.27).[112] These findings have been confirmed in a case-control population-based study on the Nordic Occupational Cancer cohort, where an increased risk of iCCA, but not of eCCA, was observed by cumulative exposure to asbestos: 0.1–4.9 f/ml x years, OR=1.1 (95% CI 0.9–1.3); 5.0–9.9 f/ml x years, OR=1.3 (95% CI 0.9–2.1); 10.0–14.9 f/ml x years, OR=1.6 (95% CI 1.0–2.5); ≥15.0 f/ml x years, OR=1.7 (95% CI 1.1–2.6).[113] Overall these findings provide evidence that asbestos may represent a risk factor for iCCA. Although how asbestos fibers may reach the biliary tract remains an open question, these fibers have been detected in this body region.[114] It can be hypothesized that, after crossing the alveolar barrier after inhalation or penetrating the gastrointestinal mucosa after ingestion, they may reach the interstitial environment and circulatory system through lymphatic vessels, and finally be delivered to all body districts.[115]

In the biliary tract, they could remain trapped in the smaller bile ducts, thus explaining why asbestos exposure seems to be mainly involved in iCCA, and not eCCA, pathogenesis. Taking into account the number of subjects occupationally or environmentally exposed to asbestos, this risk factor is likely one of the most responsible for iCCA increasing incidence worldwide. In our case series (G.B) of about 600 CCAs, about 40% of cases were related to asbestos exposure (unpublished data).

Genetic polymorphisms

Host genetic polymorphisms have been shown to modulate CCA risk. Preliminary evidences support an association between CCA and polymorphisms in genes codifying for glutathione S-transferases (GSTs). The GSTO1*D140 polymorphism was reported to increase CCA risk (OR=8.5, CI 95%: 2.07-37.85).[116] Similarly, polymorphisms in the carcinogen detoxification enzymes GSTM1 and GSTT1 have been linked to CCA development. Indeed, ex-regular alcohol drinkers harbouring the GSTT1$^{-/-}$ genotype were found to associate with a higher CCA risk compared to those ones harbouring the GSTT1$^{+/+}$ genotype (OR=27.93, 95% CI 1.84-424.60 *vs* OR=1.28, 95% CI 0.12, respectively).[117] In addition, in anti-*O.Viverrini* positive subjects, the GSTM1$^{-/-}$ genotype was found to increase the risk for CCA compared to GSTM1$^{+/+}$ genotype (OR=18.00, 95% CI 3.33-97.40 *vs* OR=10.34, 95% CI 1.31-81.63).[117]

Another study provided evidence that 1298CC homozygous variants in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene (that codifies a pivotal enzyme involved in folate metabolism and DNA methylation) may increase the risk of CCA in subjects positive for *O.Viverrini* infection, when compared to wild-type subjects (OR=2.0, 95% CI 1.14-3.48).[118] Polymorphisms in MTHFR gene have been also reported to increase the risk of CCA when combined with polymorphisms in thymidylate synthase enhancer region (TSER), that competes with MTHFR for 5-methyltetrahydrofolate as substrate for thymidylate synthesis. An OR=5.38 (95% CI 1.23-23.56) has been indeed reported in subjects harbouring a combination of MTHFR 677CC with the TSER 2R(+) genotype, compared to MTHFR 677CC with TSER 2R(-).[119]

In patients with PSC, two polymorphisms of the natural killer cell receptor G2D (NKG2D) were found to associate with increased CCA risk: the rs11053781 (OR=2.08, 95% CI 1.31-3.29) and the rs2617167 (OR=2.32, 95% CI 1.47-3.66).[120] However, the functional role of these polymorphisms on CCA susceptibility still remains to be fully elucidated.

The multidrug resistance-associated protein 2 (MRP2/ABCC2) is one of the ATP-binding cassette (ABC) transporters expressed on the apical membrane of hepatocytes and cholangiocytes, and it is involved in the excretion of the conjugates of carcinogens into bile. The ABCC2 c.3972T allele has been found to be more frequent in patients with CCA (32%), compared to healthy subjects (26.0%), resulting in an OR=1.83 (95% CI 1.09-3.08).[121]

Polymorphisms in human oxoguanine glycosylase 1 (hOGG1) and MutY homolog (MUTYH, MYH) genes, that codify key proteins in DNA base excision repair pathway, have been also linked to CAA. Individuals with A/A genotype in MYHrs3219472 gene have been reported to have an increased risk for CCA (OR=2.816, 95% CI 0.992-7.999); conversely, T/G genotype in MYH rs3219476 was found to associate with a reduced risk (OR=0.478, 95% CI 0.17-0.758).[122] Another study reported a significant association between hOGG1 and GSTM1 polymorphisms for the risk for CCA. Indeed, when GSTM1 polymorphism was considered, the hOGG1 326 polymorphism was related to the decreased risk for CCA: OR=0.06 (95% CI 0.01-0.53) for subjects with hOGG1 Ser/Ser and GSTM1 null, OR=0.06 (95% CI 0.01-0.54) for subjects with hOGG1 Ser/Cys or Cys/Cys and GSTM1 wild-type and OR=0.14 (95% CI 0.02-1.08) for subjects with hOGG1 Ser/Cysor Cys/Cys and GSTM1 null, respectively.[123]

The aryl-hydrocarbon hydroxylase, a phase I enzyme encoded by the *CYP1A1* gene, metabolizes exogenous compounds (drugs, tobacco, polycyclic aromatic hydrocarbons, nitrosamines and aromatic amines) to carcinogenic intermediates. Among smoker male subjects, the CYP1A2*1A/*1A genotype was found to associate with a decreased CCA risk (OR=0.28, 95% CI 0.08-0.94), when compared to CYP1A2*1F/1*F.[124] Similarly, subjects harbouring the alleles NAT2*13, *6B and *7A of the arylamine N-acetyltransferases (involved in detoxification of xenobiotics and carcinogens) were associated with a decreased CCA risk (OR=0.26, 95%CI 0.15-0.44).[124]

Overall these studies suggest that polymorphisms of genes encoding enzymes involved in xenobiotic detoxification, DNA repair, multidrug resistance, immune response and folate metabolism may be involved in CCA development. However, because of some of these studies also included gallbladder and ampullary cancers in their analysis and because of the lack of replication in independent cohorts, no definitive conclusions can be drawn.

COMBINED HCC-iCCA

Combined hepatocellular-cholangiocarcinoma (CHC) account for between 0.5 to 14% of primary liver cancers. [125] They have a mixture of parent phenotypic characteristics and are typically even more aggressive than HCC or iCCA.[125] Although less well studied, CHCs are postulated to arise from hepatic progenitor cells in the canals of Hering. It is perhaps not surprising that HCC and iCCA share several chronic risk factors with respect to chronic liver disease and its causes.

CONCLUSIONS

Multiple risk factors have been associated with CCA, several of which have increased globally over the past few decades and may be contributing to rising CCA rates. However, most cases develop with any known risk factor and are sporadic. iCCA incidence appears to be increasing, although the impact of peri-hilar CCA is unclear, due to lack of clear data on sub-types. Asbestos, metabolic syndrome and other emerging risk factors for iCCA may be contributing to its increase worldwide. Greater surveillance in subjects exposed to these risk factors and thus at higher risk of disease should be considered in the future. Moreover, the contribution of host genetic factors to cholangiocarcinogenesis is also currently relatively basic compared to several other cancers. There is therefore an ongoing need for further studies of the mechanisms underlying malignant transformation in the biliary tree, including genetic and basic science studies in addition to epidemiological data to be recorded uniformly and accurately.

Table 1: Global incidence rates of CCA, per 100,000 (100):

in descending order (adapted from reference 1)

REGION	Age-standardised Incidence Rate/100,000 population
Thailand - North East	85
Thailand - North and Central	14.5
Thailand - South	5.7
China, Shanghai	7.6
Hong Kong	2.3
Taiwan	4.7
South Korea, Gwangju	8.8
South Korea, Busan	7.1
Japan, Osaka	3.5
Japan, Hiroshima	3.1
Italy	3.4
Germany	3
Austria	2.7
United Kingdom	2.2
United States	1.6
Singapore	1.5
Denmark	1.3
France	1.3
Philippines	1.2
Finland	1.1
Poland	0.7
Spain	0.5
Switzerland	0.5
Australia	0.4
Canada	0.4
New Zealand	0.4
Puerto Rico	0.4
Costa Rica	0.3
Israel	0.3

Table 2: Risk factors for iCCA and eCCA.

Risk factor	Strength of the association in iCCA	Strength of the association in eCCA
Bile duct cysts	++++	++++
Caroli's disease	++++	++++
PSC/ Cholangitis	++++[a]	++++[a]
Hepatolithiasis	+++/++++	No association
Cholelithiasis/ choledocholithiasis	++/+++	++++
Cirrhosis	+++/++++	++/+++
HBV	++/+++	+
HCV	++/+++	+/++
Hemochromatosis	++	No association
Wilson's disease	No association	No association
IBD	++	+/++
Chronic pancreatitis	++	+++
Duodenal/gastric ulcer	+	+
O. viverrini	+++[a]	+++[a]
C. sinensis	+++[a]	+++[a]
Diabete type II	+	+
Obesity	+[a]	+[a]
NAFLD/NASH	+++	++
Alcohol	++	No association
Cigarette smoking	+	+
Thorotrast	++++[a]	++++[a]
1,2-dichloropropane	++++[a]	++++[a]
Asbestos	+++	+/++

+ = weak/modest association (OR: 1-1.7); ++ = moderate association (OR: 1.7-3); +++ = strong association (OR: 3-8); ++++ = very strong association (OR> 8)
[a] Available studies did not distinguish between iCCA and eCCA

Table 3: Classification of Choledochal Cysts[23]

Type I	The most common variety (80-90%), involving saccular or fusiform dilatation of a portion or entire common bile duct (CBD) with normal intrahepatic ducts
Type II	Present as an isolated diverticulum protruding from the CBD
Type III	(or Choledochocele): arise from dilatation of duodenal portion of CBD or where pancreatic duct meets
Type IVa	Characterized by multiple dilatations of the intra- and extrahepatic biliary tree
Type IVb	Multiple dilatations involving only the extrahepatic bile ducts
Type V	Cystic dilatation of intrahepatic biliary ducts without extrahepatic duct disease. Multiple saccular or cystic dilations of the intrahepatic ducts is also known as Caroli's disease

REFERENCES

1. Banales JM, Cardinale V, Carpino G, et al. Expert consensus document: Cholangiocarcinoma: current knowledge and future perspectives consensus statement from the European Network for the Study of Cholangiocarcinoma (ENS-CCA). *Nat Rev Gastroenterol Hepatol.* 2016;13(5):261-280.

2. Khan SA, Taylor-Robinson SD, Toledano MB, Beck A, Elliott P, Thomas HC. Changing international trends in mortality rates for liver, biliary and pancreatic tumours. *J Hepatol.* 2002;37(6):806-813.

3. Rizvi S, Khan SA, Hallemeier CL, Kelley RK, Gores GJ. Cholangiocarcinoma - evolving concepts and therapeutic strategies. *Nat Rev Clin Oncol.* 2018;15(2):95-111.

4. Rizvi S, Gores GJ. Pathogenesis, diagnosis, and management of cholangiocarcinoma. *Gastroenterology.* 2013;145(6):1215-1229.

5. DeOliveira ML, Cunningham SC, Cameron JL, et al. Cholangiocarcinoma: thirty-one-year experience with 564 patients at a single institution. *Ann Surg.* 2007;245(5):755-762.

6. Nakeeb A, Pitt HA, Sohn TA, et al. Cholangiocarcinoma. A spectrum of intrahepatic, perihilar, and distal tumors. *Ann Surg.* 1996;224(4):463-473; discussion 473-465.

7. Taylor-Robinson SD, Toledano MB, Arora S, et al. Increase in mortality rates from intrahepatic cholangiocarcinoma in England and Wales 1968-1998. *Gut.* 2001;48(6):816-820.

8. Patel T. Worldwide trends in mortality from biliary tract malignancies. *BMC Cancer.* 2002;2:10.

9. Bertuccio P, Bosetti C, Levi F, Decarli A, Negri E, La Vecchia C. A comparison of trends in mortality from primary liver cancer and intrahepatic cholangiocarcinoma in Europe. *Ann Oncol.* 2013;24(6):1667-1674.

10. Saha SK, Zhu AX, Fuchs CS, Brooks GA. Forty-Year Trends in Cholangiocarcinoma Incidence in the U.S.: Intrahepatic Disease on the Rise. *Oncologist.* 2016;21(5):594-599.

11. Hainsworth JD, Rubin MS, Spigel DR, et al. Molecular gene expression profiling to predict the tissue of origin and direct site-specific therapy in patients with carcinoma of unknown primary site: a prospective trial of the Sarah Cannon research institute. *J Clin Oncol.* 2013;31(2):217-223.

12. Lepage C, Cottet V, Chauvenet M, et al. Trends in the incidence and management of biliary tract cancer: a French population-based study. *J Hepatol.* 2011;54(2):306-310.

13. Jepsen P, Vilstrup H, Tarone RE, Friis S, Sorensen HT. Incidence rates of intra- and extrahepatic cholangiocarcinomas in Denmark from 1978 through 2002. *J Natl Cancer Inst.* 2007;99(11):895-897.

14. Altekruse SF, Petrick JL, Rolin AI, et al. Geographic variation of intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, and hepatocellular carcinoma in the United States. *PLoS One.* 2015;10(3):e0120574.

15. Shaib YH, Davila JA, McGlynn K, El-Serag HB. Rising incidence of intrahepatic cholangiocarcinoma in the United States: a true increase? *J Hepatol.* 2004;40(3):472-477.

16. Bridgewater J, Galle PR, Khan SA, et al. Guidelines for the diagnosis and management of intrahepatic cholangiocarcinoma. *J Hepatol.* 2014;60(6):1268-1289.

17. Khan SA, Emadossadaty S, Ladep NG, et al. Rising trends in cholangiocarcinoma: is the ICD classification system misleading us? *J Hepatol.* 2012;56(4):848-854.

18. Welzel TM, McGlynn KA, Hsing AW, O'Brien TR, Pfeiffer RM. Impact of classification of hilar cholangiocarcinomas (Klatskin tumors) on the incidence of intra- and extrahepatic cholangiocarcinoma in the United States. *J Natl Cancer Inst.* 2006;98(12):873-875.

19. Walter D, Ferstl P, Waidmann O, et al. Cholangiocarcinoma in Germany: Epidemiologic trends and impact of misclassification. *Liver Int.* 2018.

20. Kilander C, Mattsson F, Ljung R, Lagergren J, Sadr-Azodi O. Systematic underreporting of the population-based incidence of pancreatic and biliary tract cancers. *Acta Oncol.* 2014;53(6):822-829.

21. Tyson GL, El-Serag HB. Risk factors for cholangiocarcinoma. *Hepatology.* 2011;54(1):173-184.

22. Bragazzi MC, Ridola L, Safarikia S, et al. New insights into cholangiocarcinoma: multiple stems and related cell lineages of origin. *Ann Gastroenterol.* 2018;31(1):42-55.

23. Todani T, Watanabe Y, Narusue M, Tabuchi K, Okajima K. Congenital bile duct cysts: Classification, operative procedures, and review of thirty-seven cases including cancer arising from choledochal cyst. *Am J Surg.* 1977;134(2):263-269.

24. Sastry AV, Abbadessa B, Wayne MG, Steele JG, Cooperman AM. What is the incidence of biliary carcinoma in choledochal cysts, when do they develop, and how should it affect management? *World J Surg.* 2015;39(2):487-492.

25. Abdalla EK, Forsmark CE, Lauwers GY, Vauthey JN. Monolobar Caroli's Disease and cholangiocarcinoma. *HPB Surg.* 1999;11(4):271-276; discussion 276-277.

26. Petrick JL, Yang B, Altekruse SF, et al. Risk factors for intrahepatic and extrahepatic cholangiocarcinoma in the United States: A population-based study in SEER-Medicare. *PLoS One.* 2017;12(10):e0186643.

27. Soreide K, Korner H, Havnen J, Soreide JA. Bile duct cysts in adults. *Br J Surg.* 2004;91(12):1538-1548.

28. Kobayashi S, Asano T, Yamasaki M, Kenmochi T, Nakagohri T, Ochiai T. Risk of bile duct carcinogenesis after excision of extrahepatic bile ducts in pancreaticobiliary maljunction. *Surgery.* 1999;126(5):939-944.

29. Geller LT, Barzily-Rokni M, Danino T, et al. Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. *Science.* 2017;357(6356):1156-1160.

30. Jang MH, Lee YJ, Kim H. Intrahepatic cholangiocarcinoma arising in Caroli's disease. *Clin Mol Hepatol.* 2014;20(4):402-405.

31. T, Alpers DH. Atlas of Gastroenterology. 2009. 4th ed, Wiley-Blackwell, Oxford.

32. Boonstra K, Weersma RK, van Erpecum KJ, et al. Population-based epidemiology, malignancy risk, and outcome of primary sclerosing cholangitis. *Hepatology.* 2013;58(6):2045-2055.

33. Patel T. Cholangiocarcinoma. *Nat Clin Pract Gastroenterol Hepatol.* 2006;3(1):33-42.

34. Erichsen R, Jepsen P, Vilstrup H, Ekbom A, Sorensen HT. Incidence and prognosis of cholangiocarcinoma in Danish patients with and without inflammatory bowel disease: a national cohort study, 1978-2003. *Eur J Epidemiol.* 2009;24(9):513-520.

35. Sorensen JO, Nielsen OH, Andersson M, et al. Inflammatory bowel disease with primary sclerosing cholangitis: A Danish population-based cohort study 1977-2011. *Liver Int.* 2018;38(3):532-541.

36. Huai JP, Ding J, Ye XH, Chen YP. Inflammatory bowel disease and risk of cholangiocarcinoma: evidence from a meta-analysis of population-based studies. *Asian Pac J Cancer Prev.* 2014;15(8):3477-3482.

37. Kim HJ, Kim JS, Joo MK, et al. Hepatolithiasis and intrahepatic cholangiocarcinoma: A review. *World J Gastroenterol.* 2015;21(48):13418-13431.

38. Lin CC, Lin PY, Chen YL. Comparison of concomitant and subsequent cholangiocarcinomas associated with hepatolithiasis: Clinical implications. *World J Gastroenterol.* 2013;19(3):375-380.

39. Lee TY, Lee SS, Jung SW, et al. Hepatitis B virus infection and intrahepatic cholangiocarcinoma in Korea: a case-control study. *Am J Gastroenterol.* 2008;103(7):1716-1720.

40. Donato F, Gelatti U, Tagger A, et al. Intrahepatic cholangiocarcinoma and hepatitis C and B virus infection, alcohol intake, and hepatolithiasis: a case-control study in Italy. *Cancer Causes Control.* 2001;12(10):959-964.

41. Huang MH, Chen CH, Yen CM, et al. Relation of hepatolithiasis to helminthic infestation. *J Gastroenterol Hepatol.* 2005;20(1):141-146.

42. Liu ZY, Zhou YM, Shi LH, Yin ZF. Risk factors of intrahepatic cholangiocarcinoma in patients with hepatolithiasis: a case-control study. *Hepatobiliary Pancreat Dis Int.* 2011;10(6):626-631.

43. Schottenfeld D, Beebe-Dimmer J. Chronic inflammation: a common and important factor in the pathogenesis of neoplasia. *CA Cancer J Clin.* 2006;56(2):69-83.

44. Cai H, Kong WT, Chen CB, et al. Cholelithiasis and the risk of intrahepatic cholangiocarcinoma: a meta-analysis of observational studies. *BMC Cancer.* 2015;15:831.

45. Mittal S, El-Serag HB. Epidemiology of hepatocellular carcinoma: consider the population. *J Clin Gastroenterol.* 2013;47 Suppl:S2-6.

46. Palmer WC, Patel T. Are common factors involved in the pathogenesis of primary liver cancers? A meta-analysis of risk factors for intrahepatic cholangiocarcinoma. *J Hepatol.* 2012;57(1):69-76.

47. Welzel TM, Graubard BI, El-Serag HB, et al. Risk factors for intrahepatic and extrahepatic cholangiocarcinoma in the United States: a population-based case-control study. *Clin Gastroenterol Hepatol.* 2007;5(10):1221-1228.

48. Chang JS, Tsai CR, Chen LT. Medical risk factors associated with cholangiocarcinoma in Taiwan: a population-based case-control study. *PLoS One.* 2013;8(7):e69981.

49. Razumilava N, Gores GJ. Cholangiocarcinoma. *Lancet.* 2014;383(9935):2168-2179.

50. Matsumoto K, Onoyama T, Kawata S, et al. Hepatitis B and C virus infection is a risk factor for the development of cholangiocarcinoma. *Intern Med.* 2014;53(7):651-654.

51. El-Serag HB, Engels EA, Landgren O, et al. Risk of hepatobiliary and pancreatic cancers after hepatitis C virus infection: A population-based study of U.S. veterans. *Hepatology.* 2009;49(1):116-123.

52. Zhou Y, Zhao Y, Li B, et al. Hepatitis viruses infection and risk of intrahepatic cholangiocarcinoma: evidence from a meta-analysis. *BMC Cancer.* 2012;12:289.

53. Li M, Li J, Li P, et al. Hepatitis B virus infection increases the risk of cholangiocarcinoma: a meta-analysis and systematic review. *J Gastroenterol Hepatol.* 2012;27(10):1561-1568.

54. Zhang H, Zhu B, Zhang H, Liang J, Zeng W. HBV Infection Status and the Risk of Cholangiocarcinoma in Asia: A Meta-Analysis. *Biomed Res Int.* 2016;2016:3417976.

55. Li H, Hu B, Zhou ZQ, Guan J, Zhang ZY, Zhou GW. Hepatitis C virus infection and the risk of intrahepatic cholangiocarcinoma and extrahepatic cholangiocarcinoma: evidence from a systematic review and meta-analysis of 16 case-control studies. *World J Surg Oncol.* 2015;13:161.

56. Lee CH, Chang CJ, Lin YJ, Yeh CN, Chen MF, Hsieh SY. Viral hepatitis-associated intrahepatic cholangiocarcinoma shares common disease processes with hepatocellular carcinoma. *Br J Cancer.* 2009;100(11):1765-1770.

57. Ralphs S, Khan SA. The role of the hepatitis viruses in cholangiocarcinoma. *J Viral Hepat.* 2013;20(5):297-305.

58. Allen KJ, Gurrin LC, Constantine CC, et al. Iron-overload-related disease in HFE hereditary hemochromatosis. *N Engl J Med.* 2008;358(3):221-230.

59. Ellervik C, Tybjaerg-Hansen A, Nordestgaard BG. Risk of cancer by transferrin saturation levels and haemochromatosis genotype: population-based study and meta-analysis. *J Intern Med.* 2012;271(1):51-63.

60. Yaziji N, Martin L, Hillon P, Favre JP, Henninger JF, Piard F. [Cholangiocarcinoma arising from biliary micro-hamartomas in a man suffering from hemochromatosis]. *Ann Pathol.* 1997;17(5):346-349.

61. Fernandez Pelaez JM, Sanchez Martin E, Tirado Miranda R, Navarro Martinez A, Alamillo Sanz A. [Hemochromatosis and hilar cholangiocarcinoma: report of a case]. *Rev Esp Enferm Dig.* 2000;92(7):474-475.

62. Di Stefano F, Verna N, Balatsinou L, Schiavone C, Di Gioacchino M. Genetic hemochromatosis with normal transferrin saturation in a man with cholangiocarcinoma and yellow nail syndrome. *J Gastroenterol Hepatol.* 2003;18(10):1221-1222.

63. Sulpice L, Rayar M, Boucher E, et al. Intrahepatic cholangiocarcinoma: impact of genetic hemochromatosis on outcome and overall survival after surgical resection. *J Surg Res.* 2013;180(1):56-61.

64. Morcos M, Dubois S, Bralet MP, Belghiti J, Degott C, Terris B. Primary liver carcinoma in genetic hemochromatosis reveals a broad histologic spectrum. *Am J Clin Pathol.* 2001;116(5):738-743.

65. Nkontchou G, Tran Van Nhieu J, Ziol M, et al. Peripheral intrahepatic cholangiocarcinoma occurring in patients without cirrhosis or chronic bile duct diseases: epidemiology and histopathology of distant nontumoral liver in 57 White patients. *Eur J Gastroenterol Hepatol.* 2013;25(1):94-98.

66. Kowdley KV. Iron, hemochromatosis, and hepatocellular carcinoma. *Gastroenterology.* 2004;127(5 Suppl 1):S79-86.

67. Zhou H, Rogler LE, Teperman L, Morgan G, Rogler CE. Identification of hepatocytic and bile ductular cell lineages and candidate stem cells in bipolar ductular reactions in cirrhotic human liver. *Hepatology.* 2007;45(3):716-724.

68. Ala A, Walker AP, Ashkan K, Dooley JS, Schilsky ML. Wilson's disease. *Lancet.* 2007;369(9559):397-408.

69. Pfeiffenberger J, Mogler C, Gotthardt DN, et al. Hepatobiliary malignancies in Wilson disease. *Liver Int.* 2015;35(5):1615-1622.

70. Angele-Martinez C, Goodman C, Brumaghim J. Metal-mediated DNA damage and cell death: mechanisms, detection methods, and cellular consequences. *Metallomics.* 2014;6(8):1358-1381.

71. Kamamoto Y, Makiura S, Sugihara S, Hiasa Y, Arai M. The inhibitory effect of copper on DL-ethionine carcinogenesis in rats. *Cancer Res.* 1973;33(5):1129-1135.

72. Sternlieb I. Copper and the liver. *Gastroenterology.* 1980;78(6):1615-1628.

73. Wilkinson ML, Portmann B, Williams R. Wilson's disease and hepatocellular carcinoma: possible protective role of copper. *Gut.* 1983;24(8):767-771.

74. Axelrad JE, Lichtiger S, Yajnik V. Inflammatory bowel disease and cancer: The role of inflammation, immunosuppression, and cancer treatment. *World J Gastroenterol.* 2016;22(20):4794-4801.

75. Barral M, Dohan A, Allez M, et al. Gastrointestinal cancers in inflammatory bowel disease: An update with emphasis on imaging findings. *Crit Rev Oncol Hematol*. 2016;97:30-46.

76. Holmes E, Li JV, Athanasiou T, Ashrafian H, Nicholson JK. Understanding the role of gut microbiome-host metabolic signal disruption in health and disease. *Trends Microbiol*. 2011;19(7):349-359.

77. Saich R, Chapman R. Primary sclerosing cholangitis, autoimmune hepatitis and overlap syndromes in inflammatory bowel disease. *World J Gastroenterol*. 2008;14(3):331-337.

78. Vijungco JD, Prinz RA. Management of biliary and duodenal complications of chronic pancreatitis. *World J Surg*. 2003;27(11):1258-1270.

79. Kuroki T, Fukuda K, Yamanouchi K, et al. Helicobacter pylori accelerates the biliary epithelial cell proliferation activity in hepatolithiasis. *Hepatogastroenterology*. 2002;49(45):648-651.

80. Kaewpitoon SJ, Loyd RA, Rujirakul R, et al. Helicobacter Species are Possible Risk Factors of Cholangiocarcinoma. *Asian Pac J Cancer Prev*. 2016;17(1):37-44.

81. Sripa B, Kaewkes S, Sithithaworn P, et al. Liver fluke induces cholangiocarcinoma. *PLoS Med*. 2007;4(7):e201.

82. Dodson RM, Weiss MJ, Cosgrove D, et al. Intrahepatic cholangiocarcinoma: management options and emerging therapies. *J Am Coll Surg*. 2013;217(4):736-750 e734.

83. Shin HR, Oh JK, Masuyer E, et al. Epidemiology of cholangiocarcinoma: an update focusing on risk factors. *Cancer Sci*. 2010;101(3):579-585.

84. IARC. IARC monographs on the evaluation of carcinogenic risks to humans. IARC Monogr. 2012;100B:341–370.

85. Shin HR, Oh JK, Lim MK, et al. Descriptive epidemiology of cholangiocarcinoma and clonorchiasis in Korea. *J Korean Med Sci*. 2010;25(7):1011-1016.

86. Sithithaworn P, Yongvanit P, Duenngai K, Kiatsopit N, Pairojkul C. Roles of liver fluke infection as risk factor for cholangiocarcinoma. *J Hepatobiliary Pancreat Sci*. 2014;21(5):301-308.

87. Honjo S, Srivatanakul P, Sriplung H, et al. Genetic and environmental determinants of risk for cholangiocarcinoma via Opisthorchis viverrini in a densely infested area in Nakhon Phanom, northeast Thailand. *Int J Cancer*. 2005;117(5):854-860.

88. Sripa B, Deenonpoe R, Brindley PJ. Co-infections with liver fluke and Helicobacter species: A paradigm change in pathogenesis of opisthorchiasis and cholangiocarcinoma? *Parasitol Int.* 2017;66(4):383-389

89. Welzel TM, Graubard BI, Zeuzem S, El-Serag HB, Davila JA, McGlynn KA. Metabolic syndrome increases the risk of primary liver cancer in the United States: a study in the SEER-Medicare database. *Hepatology.* 2011;54(2):463-471.

90. Jing W, Jin G, Zhou X, et al. Diabetes mellitus and increased risk of cholangiocarcinoma: a meta-analysis. *Eur J Cancer Prev.* 2012;21(1):24-31.

91. Chaiteerakij R, Yang JD, Harmsen WS, et al. Risk factors for intrahepatic cholangiocarcinoma: association between metformin use and reduced cancer risk. *Hepatology.* 2013;57(2):648-655.

92. Parsi MA. Obesity and cholangiocarcinoma. *World J Gastroenterol.* 2013;19(4):457-462.

93. Li JS, Han TJ, Jing N, et al. Obesity and the risk of cholangiocarcinoma: a meta-analysis. *Tumour Biol.* 2014;35(7):6831-6838.

94. Younossi ZM, Otgonsuren M, Henry L, et al. Association of nonalcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in the United States from 2004 to 2009. *Hepatology.* 2015;62(6):1723-1730.

95. Sanna C, Rosso C, Marietti M, Bugianesi E. Non-Alcoholic Fatty Liver Disease and Extra-Hepatic Cancers. *Int J Mol Sci.* 2016;17(5).

96. Wongjarupong N, Assavapongpaiboon B, Susantitaphong P, et al. Non-alcoholic fatty liver disease as a risk factor for cholangiocarcinoma: a systematic review and meta-analysis. *BMC Gastroenterol.* 2017;17(1):149.

97. Reddy SK, Hyder O, Marsh JW, et al. Prevalence of nonalcoholic steatohepatitis among patients with resectable intrahepatic cholangiocarcinoma. *J Gastrointest Surg.* 2013;17(4):748-755.

98. Kinoshita M, Kubo S, Tanaka S, et al. The association between non-alcoholic steatohepatitis and intrahepatic cholangiocarcinoma: A hospital based case-control study. *J Surg Oncol.* 2016;113(7):779-783.

99. Bosetti C, Turati F, La Vecchia C. Hepatocellular carcinoma epidemiology. *Best Pract Res Clin Gastroenterol.* 2014;28(5):753-770.

100. Petrick JL, Campbell PT, Koshiol J, et al. Tobacco, alcohol use and risk of hepatocellular carcinoma and intrahepatic cholangiocarcinoma: The Liver Cancer Pooling Project. *Br J Cancer.* 2018;118(7):1005-1012.

101. Makiuchi T, Sobue T, Kitamura T, et al. Smoking, alcohol consumption, and risks for biliary tract cancer and intrahepatic bile duct cancer. *J Epidemiol.* 2018

102. Zakhari S. Overview: how is alcohol metabolized by the body? *Alcohol Res Health.* 2006;29(4):245-254.

103. Ye XH, Huai JP, Ding J, Chen YP, Sun XC. Smoking, alcohol consumption, and the risk of extrahepatic cholangiocarcinoma: a meta-analysis. *World J Gastroenterol.* 2013;19(46):8780-8788

104. Kurtin WE, Schwesinger WH, Stewart RM. Effect of dietary ethanol on gallbladder absorption and cholesterol gallstone formation in the prairie dog. *Am J Surg.* 1991;161(4):470-474.

105. Staretz ME, Murphy SE, Patten CJ, et al. Comparative metabolism of the tobacco-related carcinogens benzo[a]pyrene, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, and N'- nitrosonornicotine in human hepatic microsomes. *Drug Metab Dispos.* 1997;25(2):154-162.

106. Weber RP, Coon JM, Triolo AJ. Nicotine inhibition of the metabolism of 3,4-benzopyrene, a carcinogen in tobacco smoke. *Science.* 1974;184(4141):1081-1083.

107. Ishikawa Y, Wada I, Fukumoto M. Alpha-particle carcinogenesis in Thorotrast patients: epidemiology, dosimetry, pathology, and molecular analysis. *J Environ Pathol Toxicol Oncol.* 2001;20(4):311-315.

108. Kato I, Kido C. Increased risk of death in thorotrast-exposed patients during the late follow-up period. *Jpn J Cancer Res.* 1987;78(11):1187-1192.

109. Kumagai S, Sobue T, Makiuchi T, et al. Relationship between cumulative exposure to 1,2-dichloropropane and incidence risk of cholangiocarcinoma among offset printing workers. *Occup Environ Med.* 2016;73(8):545-552.

110. Wu WT, Lin YJ, Li CY, et al. Cancer Attributable to Asbestos Exposure in Shipbreaking Workers: A Matched-Cohort Study. *PLoS One* 2015;10:e0133128.

111. Boulanger M, Morlais F, Bouvier V, et al. Digestive cancers and occupational asbestos exposure: incidence study in a cohort of asbestos plant workers. *Occup Environ Med* 2015;72:792-797.

112. Brandi G, Di Girolamo S, Farioli A, et al. Asbestos: a hidden player behind the cholangiocarcinoma increase? Findings from a case-control analysis. *Cancer Causes Control.* 2013;24(5):911-918.

113. Farioli A, Straif K, Brandi G, et al. Occupational exposure to asbestos and risk of cholangiocarcinoma: a population-based case-control study in four Nordic countries. *Occup Environ Med.* 2018;75(3):191-198.

114. Grosso F, Randi L, Croce A, et al. Asbestos fibers in the gallbladder of patients affected by benign biliary tract diseases. *Eur J Gastroenterol Hepatol.* 2015; 27:860-864.

115. Miserocchi G, Sancini G, Mantegazza F, et al. Translocation pathways for inhaled asbestos fibers. *Environmental Health* 2008;7:4.

116. Marahatta SB, Punyarit P, Bhudisawasdi V, Paupairoj A, Wongkham S, Petmitr S. Polymorphism of glutathione S-transferase omega gene and risk of cancer. *Cancer Lett.* 2006;236(2):276-281.

117. Honjo S, Srivatanakul P, Sriplung H, et al. Genetic and environmental determinants of risk for cholangiocarcinoma via Opisthorchis viverrini in a densely infested area in Nakhon Phanom, northeast Thailand. *Int J Cancer* 2005; 117: 854-860.

118. Songserm N, Promthet S, Sithithaworn P, et al. MTHFR polymorphisms and Opisthorchis viverrini infection: a relationship with increased susceptibility to cholangiocarcinoma in Thailand. *Asian Pac J Cancer Prev.* 2011;12(5):1341-1345.

119. Ko KH, Kim NK, Yim DJ, et al. Polymorphisms of 5,10-methylenetetrahydrofolate reductase (MTHFR C677T) and thymidylate synthase enhancer region (TSER) as a risk factor of cholangiocarcinoma in a Korean population. *Anticancer Res.* 2006;26(6B):4229-4233.

120. Melum E, Karlsen TH, Schrumpf E, et al. Cholangiocarcinoma in primary sclerosing cholangitis is associated with NKG2D polymorphisms. *Hepatology.* 2008;47(1):90-96.

121. Hoblinger A, Grunhage F, Sauerbruch T, Lammert F. Association of the c.3972C>T variant of the multidrug resistance-associated protein 2 Gene (MRP2/ABCC2) with susceptibility to bile duct cancer. *Digestion.* 2009;80(1):36-39.

122. You SH, Wang X, Huang S, et al. MYH rs3219476 and rs3219472 polymorphisms and risk of cholangiocarcinoma. *Mol Med Rep.* 2013;7(1):347-351.

123. Zeng L, You G, Tanaka H, et al. Combined effects of polymorphisms of DNA-repair protein genes and metabolic enzyme genes on the risk of cholangiocarcinoma. *Jpn J Clin Oncol.* 2013;43(12):1190-1194.

124. Prawan A, Kukongviriyapan V, Tassaneeyakul W, Pairojkul C, Bhudhisawasdi V. Association between genetic polymorphisms of CYP1A2, arylamine N-

acetyltransferase 1 and 2 and susceptibility to cholangiocarcinoma. *Eur J Cancer Prev.* 2005;14(3):245-250.

125. Wang AQ, Zheng YC, Du J, Zhu CP, Huang HC, Wang SS, Wu LC, Wan XS, Zhang HH, Miao RY, Sang XT, Zhao HT. Combined hepatocellular cholangiocarcinoma: Controversies to be addressed. *World J Gastroenterol.* 2016;22(18):4459-65.