Review Article

Biliary tract cancers: epidemiology, molecular pathogenesis and genetic risk associations

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Abstract: Biliary tract cancers (BTC) are malignancies that arise from the epithelium of the biliary system and comprise the second most common type of hepatobiliary cancer worldwide. BTC are sub-classified as intrahepatic cholangiocarcinoma (iCCA), perhilar/hilar cholangiocarcinoma (pCCA), distal cholangiocarcinoma (dCCA), and gallbladder carcinoma. Due to the differences in their etiologic risk factors, pathogenesis, and molecular and genetic characteristics, each of these subtypes is considered a separate biological entity. The geographic diversity of risk factors for the subtypes of biliary cancers results in profound differences in the worldwide incidence of each. In this article we provide a review of the current epidemiology of BTC and their associated risk factors. Further, we discuss the available evidence for genetic predisposition to BTC and anticipate the results of planned large-scale, genome-wide association studies (GWAS) exploring the inherited sequence variants conferring risk of BTC. These studies may also potentially of reveal important pathogenic mechanisms of the biliary tract cancer subtypes.

Keywords: Biliary tract neoplasms; cholangiocarcinoma (CCA); gallbladder neoplasms; genetic predisposition to disease; molecular pathology

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Introduction

Biliary tract cancers (BTC) represent the second most common type of hepatobiliary cancer worldwide, and are typically classified as intrahepatic cholangiocarcinoma (iCCA), perhilar/hilar cholangiocarcinoma (pCCA), distal cholangiocarcinoma (dCCA) and gallbladder cancer (GBC). It is difficult to obtain accurate worldwide incidence estimates for these cancers due to challenges with diagnosis, particularly in low- and middle-income countries, and discrepancies in classification methods worldwide. The most recent estimates from the Global Burden of Disease study are of 139,500 deaths from BTC in 2013, a 22% increase from the estimated 115,400 deaths in 1990, equivalent to age-standardized death rates of 2.3 per 100,000 per year and 3.4 per 100,000 per year, respectively (1). Worldwide, GBC is more common in females, while eCCA has a male predominance (2). In the United States (US) approximately 23,000 cases of BTC are diagnosed annually (3,4). Only 10% of these patients present at an early stage when they would be candidates for surgical resection. The vast majority present with locally advanced or metastatic BTC, for which there are very few therapeutic options (5). The lack of therapeutic options stems in part from our limited understanding of the etiology, risk factors, and pathogenesis

of CCA. The risk factors most strongly associated with BTC are those characterized by chronic inflammatory states, such as primary sclerosing cholangitis (PSC), chronic biliary tract infection [i.e., *Salmonella typhi* (*S. typhi*), *Opisthorchis viverrini* (*O.viverrini*), *Clonorchis sinensis* (*C. sinensis*), hepatitis B virus (HBV), and hepatitis C virus (HCV)], asymptomatic stone disease, and diabetes mellitus, among others. Hereditary factors may also play a role, and gene-environment interactions might be important in the pathogenesis of BTC. Thus, BTC, similar to most other cancers, is a complex disease resulting from the combination of familial genetic predisposition and certain environmental factors. Consequently, identifying the genetic variants and modifiers that influence the pathophysiological processes involved in BTC is crucial to understanding the gene-environment interactions important for BTC development. Recent advances using genome-wide association studies (GWAS) have facilitated the identification of multiple replicable common genetic variations associated with cancer, including colorectal cancer, breast, lung, pancreas, melanoma and brain cancers (6,7). Over the last few years, a number of single nucleotide polymorphism (SNP) association studies have identified genetic variants as risk-conferring or protective in the development of BTC. However, the mechanisms by which such SNP variations influence the pathogenesis of BTC are poorly understood. Additionally, some genetic variants are specific to certain populations, which limit the generalizability of recent discoveries. Thus, further epidemiological and functional investigations of these variants are needed to elucidate the etiology and pathologic process of this devastating disease. The results obtained could also potentially lead to the discovery of novel, cancer-specific biomarkers useful for assessing the risk for BTC, the prognosis of BTC patients, and predicting their response to specific treatments (8). In this review we provide an epidemiological overview of the BTCs and discuss the types of results anticipated from GWAS and their potential benefits.

Classification

BTC are highly lethal cancers that comprise a spectrum of carcinomas originating in the bile ducts, the gallbladder or the ampulla of Vater. Cancers of the bile duct are classified into three types based on their location (9). Intrahepatic bile duct cancers develop in the branches of the biliary system located within the liver; those that arise from the ducts outside the liver are referred to as hilar, also called perihilar cholangiocarcinoma (CCA), and dCCAs (10). The latter belong to the extrahepatic subtype of CCAs and are separated by the insertion of the cystic duct (10). Most extrahepatic bile duct tumors arise in the hilar region (60–70%), whereas those arising distally account for about 20–30% of extrahepatic biliary malignancies (11,12). Previously, tumors arising from the gallbladder and from the ampulla of Vater along with these two subtypes of CCA were all classified as extrahepatic biliary cancers. Under the previous classification, iCCA would be designated a primary liver cancer (13). Differences in the methods of classification of BTC can lead to misleading or erroneous epidemiological data (14). Changes in the classification codes over time also confound the interpretation of the available epidemiological data. Thus, epidemiologic studies on the BTC must be interpreted with caution (14). Overall, considering all three types of bile duct cancer, iCCA and pCCA each appear to comprise about 40% of CCA, while dCCA comprise the remaining 20%.

Epidemiology

The global epidemiological trends in incidence of BTC vary according to geographic regions, which in turn relate to the distribution of the risk factors associated with BTC (14). We will consider the epidemiology of GBC and CCA separately.

The incidence rate of GBC is highest in South America, specifically in the Andean region as discussed in further details in this issue by Arroyo *et al.* (15). Other populations with high rates are North American Indians and Mexican Americans (16). Cancer of the gallbladder is the most common gastrointestinal malignancy among Southwestern Native Americans and Mexican Americans (17). In South America, the main risk factor linked to GBC is symptomatic gallstone disease, particularly in Chile, Bolivia, and Ecuador. Of these countries, Chile has the highest mortality worldwide (18). In Europe, the highest incidence rates are found in Poland, the Czech Republic, and Slovakia (16). India, Pakistan, Korea and Japan are also high-incidence countries (19).

In the US the incidence of GBC is relatively low and actually decreased 0.5% per year between 1999–2011 among women (from 1.5 to 1.38 cases per 100,000) while remaining stable among men (20). Despite the decrease, GBC is still the most common BTC (4). GBC has an up to three times higher incidence in women than in men (16,20,21). The incidence of GBC in adults younger than

45 years of age increased by 2.4% per year from 0.04 to 0.06 cases per 100,000 between 1999 and 2011, while the incidence among patients aged 45 to 84 years remained stable. These data must be interpreted cautiously because the increased incidence in young patients occurred simultaneously with an improvement in diagnostic technologies. Thus, the apparent increased incidence may be related to more accurate diagnostic tools. In those older than 85 years, the incidence decreased 0.9% per year, from 11.74 to 9.93 cases per 100,000 (20).

Among the racial categories, American Indians and Alaskan Natives have higher incidence of GBC than non-Hispanic Whites (20). Relative to Whites, American Indians/Alaska Natives have a racial/ethnic incidence rate ratio (IRR) of 4.5 for women and 5.4 for men, while Hispanics had ratios of 3.1 and 1.8, respectively. Analysis of the temporal trends between 1992 and 2009 demonstrates that the incidence rates among the different races decreased among all except Blacks (21).

Regarding the global epidemiology of CCA, the incidence of iCCA has been increasing in Europe, Asia, Japan, Australia, and North America. In the US, the incidence of iCCA is significantly higher than eCCA, 1.6 *vs.* 1.3 per 100,000 years, P<0.01, with Asians having the highest incidence for both subtypes, however, this report excluded Alaskan Natives due to small numbers precluding the development of precise incidence estimates (22). Overall, CCA incidence is higher among men than women. In Caucasian populations, this may reflect the higher prevalence of PSC in males (13,18,22). When the incidence of CCA in men is compared among different races in the US, Alaskan Natives have a significantly higher incidence rate (3.4 per 100,000) compared to Whites, (1.6 per 100,000) and Blacks (1.4 per 100,000) (23). Another study compared the incidence rates of eCCA by race/ethnicity between 1992 and 2009 and found that the rates increased throughout the time period for all races and sex groups except for Hispanic males. This study also reported that Alaskan Natives and American Indians had the highest rates among all racial/ethnic groups (21). Secular trends in the incidence of risk factors associated with iCCA, such as HCV, alcoholic liver disease, and cirrhosis, may contribute, at least partially, to the rising trend.

Worldwide, the highest incidence rates of CCA are found in Thailand, which has age-standardized rates of 113 per 100,000 person years in males, and 50 per 100,000 person years in females (14,24). Infection with the liver fluke *O. viverrini*, which is acquired by eating raw fish, is endemic to northeast Thailand, and is the risk factor most strongly associated with development of CCA. CCA comprises 89% of all primary liver cancers in Thailand (25-27).

Other key factors influencing the risk of BTC are age, gender, race, and presence of comorbidities. In general, the incidence of GBC and CCA increases progressively with age. The reported average age of patients with iCCA is 70 years (9). For those individuals with PSC or choledochal cysts, the average age falls to 30–50 years (13).

Risk factors for biliary tract cancer

The increasing number of BTC worldwide is linked to several important risk factors. Gallstone disease is one of the strongest risk factors for GBC. Even though gallstones are present in 70% to 90% of GBC cases, the overall incidence of GBC in patients with cholelithiasis is 0.5% to 3% (28-32). The exact mechanism by which gallstones predispose to GBC is still unknown, but chronic mucosal damage and constant epithelial irritation may be involved (33). Stones >3 cm carry a 9.2 to 10.1 times greater risk of GBC than of stones <1 cm (33,34). The association of gallstones with CCA is less well established than the association of gallstones with GBC, however, the association of bile duct stones or hepatolithiasis with CCA is quite strong (13). Compared to the West (1–2%), hepatolithiasis is far more prevalent in Southeast Asia, particularly in Taiwan (20% in adults) (35,36). Fifty to seventy percent of patients that undergo resection for CCA in Taiwan have associated hepatolithiasis (13). A Korean study found that patients with hepatolithiasis had a 50-fold increase in the risk of CCA (37).

PSC, which is characterized by cholestasis with chronic inflammation of the biliary duct that ultimately results in fibrosis and stricturing of the bile ducts, is also a risk factor for CCA (38). The annual incidence of CCA in patients with PSC has been estimated to be between 0.5–1.5%, with a lifetime risk ranging from 5% to 15% (39-44). The increased prevalence of PSC may be due either to increased awareness of the disease or to as yet unidentified environmental factors (45,46). PSC patients develop CCA earlier than those without, typically in their 40s as opposed to 70s, and the incidence is higher in men (45,47-49). Thirty to fifty percent of CCA cases occurring in persons with PSC are diagnosed at the time of PSC diagnosis (44,48,50). The diagnosis of CCA in patients with PSC is extremely challenging because the inflammation-related changes in PSC may mimic CCA (47,51). Numerous genetic association studies have examined genetic variants

associated with PSC, and a strong association has been found with HLA complex polymorphisms, with the most robust findings localizing to chromosome 6p21 (52,53). Other susceptibility loci have been identified in chromosomes 2q35, 3p21 and 13q31 (53). A recent genetic study identified four new genome-wide significant susceptibility loci for PSC: rs7556897, rs17032705, rs12369214, rs11649613 (54).

The association of diabetes with BTC is difficult to assess due to the close associations of diabetes with obesity and gallstones, but many studies support the concept that obesity increases the risk of BTC (55-59). As BMI increases, the risk of BTC, especially iCCA, increases as well (56,60,61). A meta-analysis of 21 studies showed an increased risk of BTC in persons with diabetes compared to non-diabetics [relative risk (RR): 1.4; 95% CI: 1.2–1.7] (62). Another meta-analysis, assessed the risk by subtype: the RR for all CCA was 1.6 (95% CI: 1.6–2.5), for eCCA 1.6 (95% CI: 1.3–2.1), and for iCCA 2.0 (95% CI: 1.6–2.5) (63). Yet another meta-analysis focused on the risk of eCCA reported very similar risk [odds ratio (OR): 1.6, 95% CI: 1.1–2.5] (64). In a case-control study from Shanghai, the association of diabetes and GBC was strong and independent of obesity (OR: 2.6; 95% CI: 1.5–4.7), but the effect was mediated in part by biliary stones and in part by low serum HDL (57). Metformin, an oral anti-hyperglycemic agent of the biguanide family, may function as a protective agent against cancer in diabetic patients (65,66). A recent study concluded that metformin use significantly reduced the risk of iCCA in diabetic patients by 60% (67). Metformin inhibits the growth of iCCA cells by activating the AMPK/mTOR complex 1 (mTORC1) pathway, which in turn activates the p53 protein, inducing cell cycle arrest and preventing tumor cell proliferation (68).

Patients with choledochal cysts and Caroli's disease, a rare congenital fibropolycystic liver disease characterized by cystic dilations of the large intrahepatic bile ducts, appear to be at risk for iCCA (69,70). The overall lifetime incidence of CCA in persons with choledochal cysts is 10%, while an incidence as high as 28% has been reported in those that do not receive treatment (71-73). Patients with Caroli's disease have a 100-fold increase in risk of CCA when compared to the general population (71). Up to 90% of cases of congenital choledochal cysts present with an abnormal pancreaticobiliary junction (APBJ), which is a rare anatomic variation in which the pancreatic duct drains into the common bile duct (18,74). Prolonged exposure of the biliary epithelium to the refluxed pancreatic juice promotes carcinogenesis (75). Even though bile duct cancer is observed, GBC is much more common in patients with APBJ (18,75).

Infectious diseases, including parasites, bacteria and viruses, cause inflammation of the biliary tract, and a number of these diseases have been linked to the development of biliary tract malignancies. A strong association exists between liver fluke infestation and the development of biliary tract malignancies, notable by the high incidence of CCA in areas in Asia and Eastern Europe where liver fluke infestation is prevalent (76-80). Endemic areas for *C. sinensis* infestation include eastern Russia and Manchuria, South Korea, mainland China (except the northwest), Taiwan, and northern Vietnam, while *O. viverrini* is endemic in Laos and Northeast Thailand (80). In particular, northeast Thailand has very high overall CCA incidence rates (85/100,000 population) (78). The traditional consumption of raw or undercooked wild-caught cyprinoid fish and fish-based dishes causes infestation of humans with the adult forms of liver flukes, most commonly *O. viverrini* and *C. sinenesis* (81-83). The liver flukes occupy the gallbladder and biliary tree of the human host, causing desquamation of the biliary epithelium, inducing chronic inflammation with development of adenomatous hyperplasia and periductal fibrosis, and eventually promoting the malignant transformation of the biliary epithelium (84-86). The secretion of parasite proteins with mitogenic properties into the bile creates a tumorigenic environment that may drive this transformation (83). Potential cofactors in the carcinogenic process include carcinogens such as nitrosamines produced by bacteria in fermented fish and other foods, smoking, alcohol, and HBV infection (13).

Chronic infection with HBV and HCV also contribute to the unique geographical distribution of BTC. The prevalence of viral infection is higher in low- and middle-income Eastern countries, particularly in Southeast Asia (HBV 9.1%; HCV 3.6%), China (HBV 12%; HCV 3%), and Korea (HBV 12%; HCV 2%), with relatively low rates in Western countries (87,88). A cohort study found that HCV conferred more than two-fold elevated risk of iCCA (HR: 2.6; 95% CI: 1.3–5.0), while the risk of eCCA, on the other hand, was not significantly increased (HR: 1.5; 95% CI: 0.6–1.9) (89). Another study analyzed the risk factors of patients seen at the Mayo Clinic in Rochester, MN, and found that HCV infection is associated with an OR of 6.4 for iCCA (95% CI: 1.4–28.5; P<0.001) (67).

Regarding the association of iCCA with HBV infection, a meta-analysis found that persons with HBV infection

had an increased risk of iCCA (RR: 3.4; 95% CI: 2.5–43.7) compared to those without HBV (90). Another meta-analysis that included studies performed in regions of both high and low prevalence of hepatobiliary cancers, concluded that the presence of HBV was associated with a combined OR of 5.5 (95% CI: 3.2–9.6) for iCCA (91). The association between HBV and CCA is stronger in Asian countries (OR: 6.0) than in Western countries (OR: 4.0) (92,93). Furthermore, the presence of cirrhosis increases the risk of iCCA even more, by 2.5-fold (95% CI: 1.2–5.1; P=0.02) in HBV, and 3.2-fold (95% CI: 1.231–8.148, P=0.017) in HCV patients (94). Co-infection with HBV and HCV with concomitant cirrhosis increased the risk of iCCA by 12.6-fold (95% CI: 2.5–62.9; P=0.002) (94). A case-control study of patients with and without CCA found nonspecific cirrhosis to be significantly more prevalent among cases (OR, 27.2; P<0.0001) (58). In addition, a meta-analysis of persons with unspecified liver cirrhosis found that the overall OR for iCCA was 22.9 (95% CI: 18.2–28.8) (58,92). In a separate study, cirrhosis was associated with an increased OR of 8.0 (95% CI: 1.8–36.5; P<0.007) for iCCA (67). Thus, there is a clear and strong association between viral hepatitis and CCA.

Bacterial infection occurs through bacterial invasion of the mucosal surface of the intestine, with spread into organs such as the liver, spleen, and bone marrow after phagocytosis by macrophages (95). Chronic infection with *S. typhi* is one such example (96,97). *S. typhi* is a gram-negative serovar of the *Salmonella enterica* subspecies that spreads to the gallbladder via the vasculature or through the bile ducts from the liver during enterohepatic circulation (98,99). The primary reservoirs for *S. typhi* are chronic carriers who shed bacteria through their feces and urine (100-102). Annually, this bacterium causes 21 million newly diagnosed cases of typhoid fever and about 200,000 deaths (95,103). In areas endemic for *S. typhi* approximately <5% of all individuals acutely infected will become chronic asymptomatic carriers (104,105). Although individuals with chronic *S. typhi* infections are contagious with infection persisting for decades, it is difficult to identify chronic carriers due to its asymptomatic nature (106,107). Several studies have shown that chronic carriers of *S. typhi* have an increased risk of GBC (108-110). Chronic carriage of *S. typhi* is twice as common in women compared to men (111).

Helicobacters, spiral gram-negative bacteria, have also been identified as potential infectious carcinogens. Various species of helicobacter have been identified in the bile, gallbladder and liver tissue of patients with hepatobiliary diseases (112-114). *Helicobacter pylori* (*H. pylori*) colonize the gastric epithelium, living in the mucus layer that coats the internal lining of the stomach. There have been associations between BTC and *H. pylori*; however, a direct cause-and-effect relationship has not been established (115-118). Some studies have suggested that *H. pylori* are involved in the development of biliary neoplasms through enhancement of inflammation and proliferation of biliary cells. Most studies have had small sample sizes of CCA patients, and this relationship is still controversial (119).

Helicobacter bilis (*H. bilis*) is an opportunistic helicobacter associated with chronic liver disease, BTC and GBC, and chronic diarrhea (120-123). The isolation of Helicobacter species from the biliary system has stimulated interest in the role of these species in carcinogenesis. A meta-analysis found the infection rate of Helicobacter species to be higher in persons with biliary tract cancer compared to unaffected individuals, but did not reach statistical significance. *H. pylori*, *H. hepaticus*, and *H. bilis* but not *H. ganmani*, were significantly more frequent in the malignant group than in the benign biliary disease group (124). Mouse studies have linked *H. bilis* to the pathogenesis of chronic hepatitis and hepatocellular carcinoma; further, *H. bilis* has been implicated in cholesterol gallstone formation (125-128). Recent studies have shown that *H. bilis* infections activate NF-κB signaling, thus increasing the cellular expression of pro-angiogenic VEGF in BTC (129,130). However, a direct link between *H. bilis* and BTC has not been confirmed (131).

A variety of biological and chemical toxins have also been implicated in biliary tract carcinogenesis. Aflatoxin is a mycotoxin produced by *Aspergillus fungi*, mainly Aspergillus flavus and Aspergillus parasiticus, which are abundant in warm, humid areas (132). Aflatoxins are naturally occurring food contaminants and can be found in a wide range of produce including cereals, oilseeds, nuts, spices, milk, and meat (133). The contamination of Chilean red chili peppers from Santiago with aflatoxins was confirmed in a study aimed at identifying mutagens present in this produce, but the aflatoxin concentrations were relatively low (62,134). Aflatoxins were first recognized as carcinogenic in 1976, but their role in gallbladder carcinogenesis had not been assessed until recently (133,135). The proposed mechanism is exposure of the gallbladder to the carcinogenic metabolites of aflatoxin when these are stored for excretion in the bile (134). A case-control study found significantly more circulating aflatoxin-albumin adducts in patients with GBC compared to population controls (OR: 13.0; 95% CI: 3.0–52.5) (136).

The concentrations of aflatoxins B1, B2, G1, and G2, were found to be low in some preparations of red chili peppers from Chile (Santiago), Peru (Trujillo, Cusco and Lima) and Bolivia (La Paz) (137). Therefore, other mycotoxins, such as ochratoxin A (OTA), may also be associated with GBC development (137). OTA is produced by Penicillium and Aspergillus species and, similar to aflatoxins, is found in spices, cereals, and nuts as well as in cocoa, beer, and coffee (137,138). With the objective of assessing the association between the mycotoxins (both aflatoxin and ochratoxin) and the incidence of GBC, the authors measured the concentration of the mycotoxins in dried red chili peppers from these countries. They found that red chili peppers from Peru have higher levels of OTA than aflatoxins. Furthermore, since Chile and Bolivia have a higher GBC incidence than Peru and the mean OTA concentrations in the dried red chili peppers from these two countries were higher than in peppers from Peru, the authors suggest a stronger association between OTA contamination of red chili peppers and the development of GBC (16,137).

The recently established link between patients diagnosed with CCA at a young age and their employment in proof-printing plants has led the Japanese Ministry of Health, Labour and Welfare to categorize CCA as an occupational disease (139). Seventeen workers from a proof-printing plant in Osaka, Japan, were diagnosed with iCCA between November 1996 and November 2012. By February 2014, 83 patients had filed claims for workers compensation. Chronic exposure to organic solvents used in printing, such as 1,2-dicholoropropane (1,2-DCP) and dichloromethane (DCM), has been implicated as a causative factor (139). Studies have confirmed the exposure of some of these workers to very high levels of 1,2-DCP for prolonged periods of time (139-141). These patients were diagnosed between the ages of 25 to 45 years, were all exposed to organic solvents and presented with regional dilation of the bile ducts, high serum γ-glutamyl transpeptidase activity, and lesions arising from the large intrahepatic bile ducts (142). Similar results were found in another cohort of nine CCA patients from seven printing companies in Japan (143). For this reason, it is important to assess occupational history when evaluating a patient with suspected CCA (144).

Survival of patients with BTC

BTCs are highly lethal cancers. Due to substantial variation in the availability of complex medical care across the countries and regions with high prevalence of BTC, there is significant regional variation in patient outcomes. However, even in the most developed settings, BTCs are among the most aggressive, therapy-resistant, and recurrent of all cancers.

Data from the National Cancer Data Base of the American College of Surgeons report that the 5-year survival rate for patients with stage 0 GBC as 80%, for stage I 50% stage II 28% stage IIA 8%, stage IIB 7%, stage IVA 4%, and stage IVB 2% (145). Data from the US Surveillance Epidemiology and End Results (SEER) cancer registry on 5-year survival rates of CCA are also reported according to the stage of the disease. The 5-year overall survival rates for patients with localized, regional and distant metastatic iCCA are 15%, 6% and 2%, respectively. For the extrahepatic subtype, the rates are 30% for patients with localized disease, 24% for regional, and 2% for distant disease (9). A study performed in Thailand reported an overall 5-year survival rate for pCCA of 20.6% (95% CI: 13.8–28.4), with a median overall survival of 19.9 months (146).

Regarding patients receiving surgical therapy, a meta-analysis of 4,756 iCCA patients receiving surgical resection found that the 5-year overall survival following surgical management was 30% (range, 5–56%), with a median overall survival of approximately 28 months (range, 9–53 months) (147). Tumor recurrence was common following surgical resection, with 61% to 98% of patients experiencing recurrence 5 years after surgery (147). The median overall survival of 56 patients with dCCA who underwent pancreaticoduodenectomy was 36.9 months, and recurrence occurred in 67% (148). A Chinese study of 814 patients with pCCA found that patients who had surgery with curative intent had a median overall survival of 26.3 months (149).

The survival rates of patients with BTC vary with their underlying biliary or hepatic disease. For example, PSC patients with CCA who undergo surgical resection with negative tumor margins have a 3-year survival rate of <20% (44,48,50). A recent meta-analysis concluded that patients with iCCA and HBV infection had better overall and disease-free survival with a hazard ratio (HR) of 0.76 (95% CI: 0.70–0.83) and 0.78 (95% CI: 0.66–0.94), respectively. HCV infection, on the other hand, was associated with shortened overall survival, with a HR of 2.64 (95% CI: 1.77–3.93) (150).

Molecular pathogenesis

The molecular pathogenesis of BTC is poorly understood

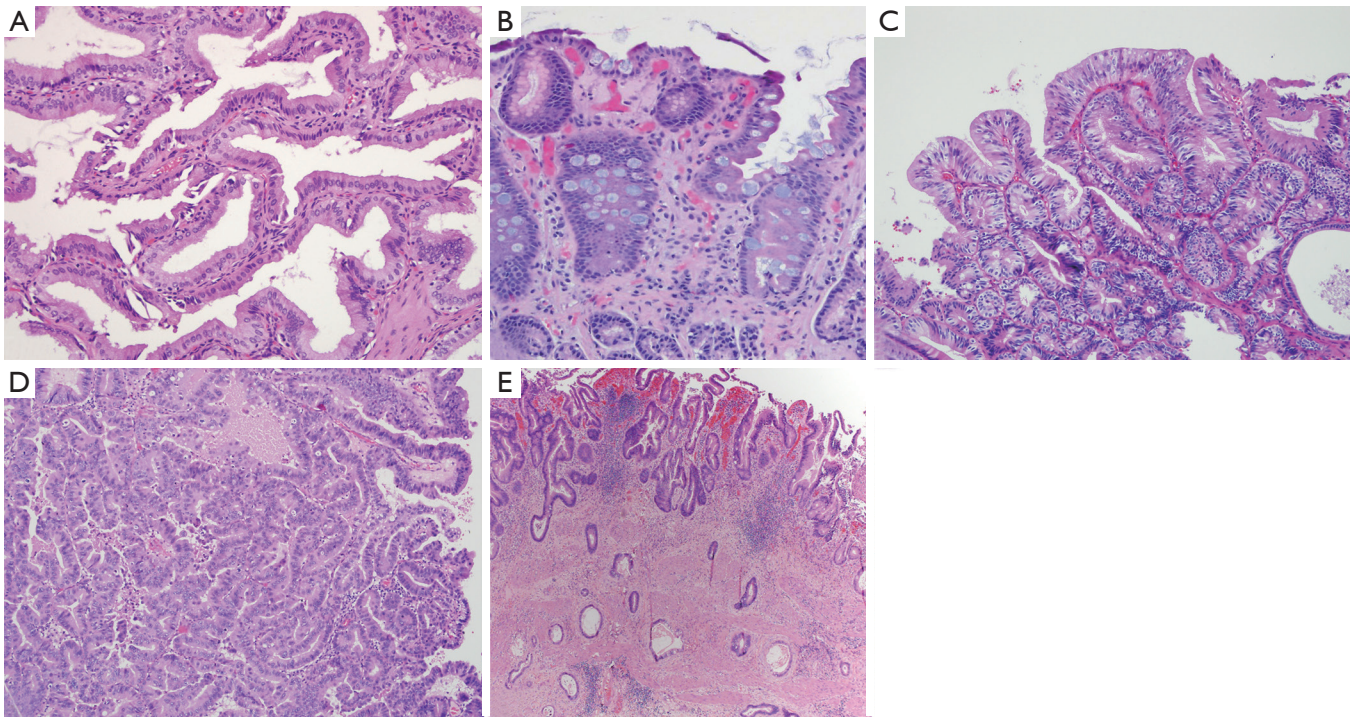

Figure 1 Pathologic progression of gallbladder adenocarcinoma. (A) Normal gallbladder mucosa. The normal biliary type mucosa features columnar epithelium with basally located nuclei and papillary projections (magnification ×100); (B) intestinal metaplasia involving gallbladder mucosa. Intestinal metaplasia, evidenced by the presence of multiple goblet cells within this biliary epithelium, is a consequence of chronic inflammatory injury (magnification ×100); (C) low-grade dysplasia in gallbladder mucosa. This abnormal glandular proliferation of crowded glands with enlarged, hyperchromatic, and pseudostratified nuclei, is consistent with low-grade columnar dysplasia (magnification ×100); (D) adenocarcinoma *in situ*. There is an abnormal glandular proliferation with extreme architectural complexity, breaching the basement membrane, consistent with adenocarcinoma *in situ* (magnification ×100); (E) invasive well-differentiated adenocarcinoma of the gallbladder. Infiltrating haphazardly arranged glands invading the gallbladder wall are consistent with adenocarcinoma. The surface epithelium shows intestinal metaplasia and dysplasia (magnification ×40).

Figure 1 (151). Thus far, two key pathogenic mechanisms have been identified in GBC (16,18,32). The main mechanism is through cholelithiasis, which leads to chronic cholecystitis and subsequent oncogenesis. GBC is strongly associated with race, female gender, age older than 65, and past medical history of symptomatic gallstone disease (16,18,32). Another mechanism implicated in GBC pathogenesis is the presence of an APBJ. GBC arising in the presence of an APBJ tends to occur in younger patients and have a lower incidence of associated cholelithiasis (16,18,32). GBC cases that develop in the context of an APBJ consistently demonstrate *KRAS* mutations, which activate inappropriate growth signals, while those with cholelithiasis rarely have KRAS mutations (5,32). Both pathogenic mechanisms induce mutations in the *p53* gene; however, the effects on the p53 pathway are induced during different stages of oncogenesis. Early-onset *p53* mutations are characteristic of cholelithiasis-induced GBC, while late onset mutations are more common in APBJ-associated cases (18). In general, the frequency of *p53* mutations in GBC ranges from 35% to 92%, with most studies showing a frequency >50% (16,32).

The progressive accumulation of oncogenic aberrations in the biliary epithelium induce malignant transformation (16). A definitive, stepwise model of the cellular and molecular events during malignant biliary transformation has not been established; yet current evidence supports the sequence of intestinal metaplasia to dysplasia, followed by carcinoma *in situ*, and finally, invasive carcinoma (5). The fact that about 60% of GBC have intestinal metaplasia and more than 90% have dysplasia in the adjacent mucosa supports this hypothesis (18,152,153). The spectrum and

temporal sequence of mutations occurring in the intestinal metaplasia to dysplasia to GBC sequence differ from the sequence seen in the adenoma to dysplasia to carcinoma sequence, such as is typical of colorectal cancer. Gallbladder adenomas appear distinct from non-adenomatous intestinal metaplasia/dysplasia, with a substantially lower propensity for transformation into GBC. Mutations in the *CTNNB1* gene are found at a high frequency in gallbladder adenomas, while they are virtually absent in metaplastic/dysplastic lesions, and rarely observed in GBC (154-156). In contrast, dysplastic lesions associated with progression to GBC, particularly those associated with an APBJ, frequently demonstrate *KRAS* mutations. Thus, there appears to be an inverse relationship between CTNNB1 and KRAS mutations observed in adenomas compared to pre-cancerous metaplastic/dysplastic lesions. The reciprocity of these molecular defects suggests that GBC preceded by metaplastic/dysplastic lesions is a different biological entity than those that develop from adenomas, with the former having a higher malignant potential (13). This concept is further supported by the fact that metaplasia/dysplasia is frequently found in association with GBC, while adenoma is a rare finding (5).

The pathogenic mechanisms leading to development of CCA are also unclear. There is a general consensus that the malignant transformation of the biliary epithelium is mediated through a chronic inflammatory state induced by the release of pro-inflammatory mediators. The resulting biliary damage generates cholestasis, which causes aberrant bile acid signaling. The subsequent activation of growth factors promotes cholangiocyte proliferation. These changes, occurring in an inflammatory milieu that promotes the accumulation of additional genetic and epigenetic alterations, lead to uncontrolled proliferation, survival, angiogenesis, invasion and metastasis (157,158). Recent evidence that aspirin use reduces the risk of all subtypes of CCA is consistent with this hypothesis (159).

Two key premalignant precursor lesions have been defined during the development of CCA: biliary intraepithelial neoplasia (BillN) and intraductal papillary neoplasm of bile ducts (IPNB) *Figure 2* (5,160). *Figure 3* grossly demonstrates an IPNB tumor in a dilated duct. *Figure 4* displays the papillary projections in low power magnification. The molecular profiles of these lesions have not yet been completely defined, although mutations in *p53* and *CDKN2A* have been described and loss of SMAD4 has also been shown by immunohistochemistry (5,160,161). CDKN2A encodes the cyclin-dependent kinase (CDK) inhibitor INK4, also known as p16, which inhibits CDK-4 and -6. Active CDK-4 and -6 form complexes with cyclin D1 to phosphorylate and inactivate the retinoblastoma (Rb) tumor suppressor protein, thus inducing progression of the cell cycle from the G1 to S phase (32,160). Consequently, inactivation of the p16 cell cycle inhibitor leads to checkpoint abrogation and abnormal progression through the cell cycle. Immunohistochemistry of IPMN lesions demonstrated cyclin D1 positivity in 65% of samples, less so in the BillN lineage (162). Although oncogenic molecular aberrations are observed in all CCA subtypes, the specific aberrant genes vary between iCCA, pCCA and dCCA and the genetic alterations reported thus far also vary in studies performed in different populations, suggesting population and/or etiologic variations in the carcinogenic mechanisms (163). We will now discuss the alterations most commonly found in the different CCA subtypes.

The most frequently identified mutations in iCCA include *TP53*, *KRAS/NRAS*, and *IDH1/2*, whereas *PIK3CA* mutations are rarely observed (164-170). KRAS and TP53 mutation and loss of PTEN by mutation or epigenetic silencing have been associated with worse survival in patients with iCCA (171,172). Whole exome sequencing has identified mutations in the chromatin remodeling genes *BAP1*, *ARID1A*, and *PBRM1* and these appear more frequently in iCCA than eCCA (164,165,173). FGFR2 fusions and *IDH1/2* mutations also are much more prevalent in the intrahepatic subtype, since they are rarely found in eCCA or HCC (174).

Two molecular subclasses of iCCA have been described in two independent studies: the proliferation molecular subclass or the poor prognosis subclass and the inflammation subclass or good prognosis subclass (175,176). The former is characterized by activation of oncogenic receptor tyrosine kinase signaling pathways, including MET, EGFR, HER2, ERBB3 and RAS-MAPK. The second subclass is distinguished by the activation of cytokine-related pathways and constitutive activation of STAT3 (174).

The mutational profiles of the eCCA subtypes have been studied less extensively than the intrahepatic subtype. Although *KRAS* and *TP53* mutations are relatively common in all *CCA*, *KRAS* and *TP53* mutations are notably more frequent in eCCA than iCCA (174,177,178). Genetic alterations in the chromatin remodeling genes *BAP1* and *PRBM1* have been associated with bone metastasis and worse survival in patients with eCCA (173).

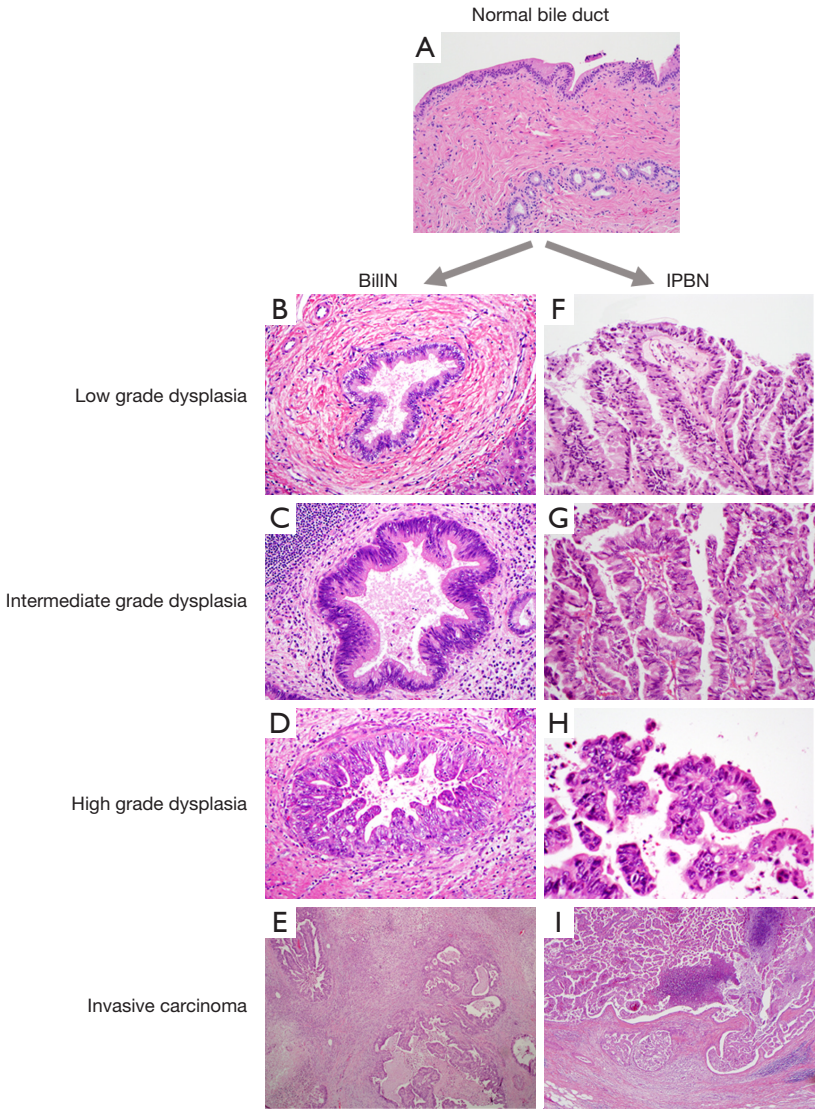

Figure 2 Pathological sequence of the progression of premalignant biliary lesions to invasive carcinoma. Two key premalignant precursor lesions have been defined during the development of cholangiocarcinoma: biliary intraepithelial neoplasia (BillN) and intraductal papillary neoplasm of bile ducts (IPNB). (A) Normal bile duct. Normal bile ducts are lined by columnar epithelium with eosinophilic cytoplasm and basally placed small nuclei (magnification ×100); (B) biliary intraepithelial neoplasia (BilIN-1), low grade dysplasia. The bile duct epithelium is composed of tall columnar cells with basally placed nuclei and mild atypia (magnification ×100); (C) biliary intraepithelial neoplasia (BilIN-2), intermediate grade dysplasia. There is micropapillary architecture and the columnar epithelial cells show nuclear crowding, with the nuclei reaching the luminal surface. There is mild to moderate nuclear atypia (magnification ×200); (D) biliary intraepithelial neoplasia (BilIN-3), high grade dysplasia. The lesion shows complex papillary architecture with loss of nuclear polarity, marked nuclear pleomorphism, and prominent nucleoli (magnification ×200); (E) BilIN with invasive carcinoma. BilIN-3 is identified in the left upper corner. Invasive adenocarcinoma, characterized by irregular infiltrating glands and desmoplastic stroma, is identified in the right and bottom of the image (magnification ×40); (F) intraductal papillary neoplasm of the bile duct, low grade dysplasia. This pancreaticobiliary-type epithelium shows tall columnar cells with mild atypia (magnification ×200); (G) intraductal papillary neoplasm of the bile duct, intermediate grade dysplasia. The epithelium shows nuclear pleomorphism, and nuclear crowding (magnification ×200); (H) intraductal papillary neoplasm of the bile duct, high grade dysplasia. The epithelium shows marked nuclear pleomorphism and loss of nuclear polarity (magnification ×400); (I) intraductal papillary neoplasm of the bile duct with associated invasive adenocarcinoma. The adenocarcinoma is of the ductal (tubular) type (magnification ×20).

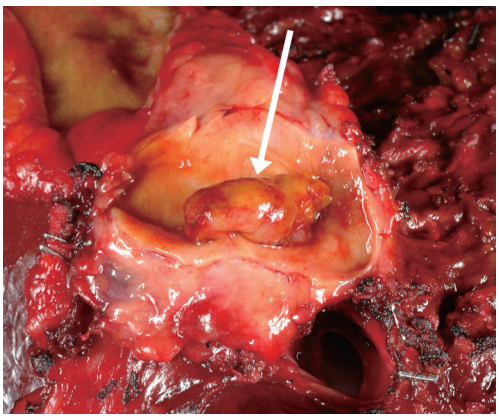

Figure 3 Intraductal papillary neoplasm of the bile duct. The tumor can be seen within a dilated bile duct lumen.

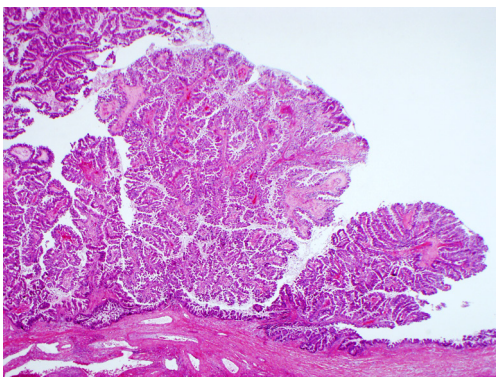

Figure 4 Intraductal papillary neoplasm of the bile duct. The papillary projections are easily seen on low power magnification (magnification ×20).

Genetic association studies in biliary tract cancer

Over the past three decades, technological advances have sparked substantial interest in the genetic basis of cancer. Cancers are complex diseases resulting from the combination of genetic, environmental, and lifestyle factors; yet, in BTC, the contributions of each of these factors to carcinogenesis and tumor progression are still poorly understood (179). In order to elucidate the inherited genetic variants related to the development of BTC, scientists have conducted genetic association studies that, analogous to traditional epidemiologic association studies, seek association between a risk variable, in this case genetic variants, and a disease outcome, BTC (180).

There are two primary types of genetic association studies: candidate gene and genome wide. In the genome-wide approach, the entire genome of numerous patients with the disease of interest and disease-free controls are screened simultaneously for a large number of known genetic variants present in the genome (180,181). The most common genetic variant and the one most frequently studied is the SNP. SNPs of interest are identified by comparing the distribution of particular variants in cases and controls, under the premise of "common disease-common variant" (7). This premise assumes that common variants in many genes will each lead to a small rise, or fall, in the risk of disease and the sum of each, plus the effect of environmental exposures, account for the overall risk of disease (180). Only those SNPs reaching a certain threshold, that is, for which there is a statistically significant difference in the frequency between cases and control, are considered to be associated with the disease of interest. The associations between SNPs and cases do not necessarily imply that nearby genes are drivers of the disease, however, they may point to key mechanisms involved in carcinogenesis (52).

In the alternate approach of candidate gene studies, a variant is selected based on its hypothesized biological role in the disease and is genotyped in a case-control study (180). This type of study searches for a statistical correlation between the specific genetic variant(s) and the disease. Because these studies are based on the ability to predict functional candidate genes and variants, this approach has been subject to the criticism that current knowledge is insufficient to make accurate and reliable predictions of causative risk variants (182).

With the exception of one Japanese GWAS, which found that the SNP rs7504990 in the *deleted in colon cancer* (*DCC*) gene is associated with an increased risk of GBC in a small sample (OR: 7.0; 95% CI: 3.4–14.1, $P=7.46\times10^{-8}$), all reported genetic association studies on BTC thus far have used the candidate gene approach *Figure 5* (183). *Tables 1-3* summarize the genetic variants that have been associated with GBC, CCA, and BTC. In this section we will discuss the genetic variants most frequently studied in the context of BTC.

The cytochrome P450 enzymes play a role in the synthesis of steroid hormones, bile acids, and certain fats, as well as in the metabolism of medications and toxins (184). Aryl-hydrocarbon hydroxylase, a phase I enzyme encoded by the *CYP1A1* gene, forms part of the xenobiotic-metabolizing machinery, which is responsible for metabolizing exogenous compounds such as drugs, tobacco

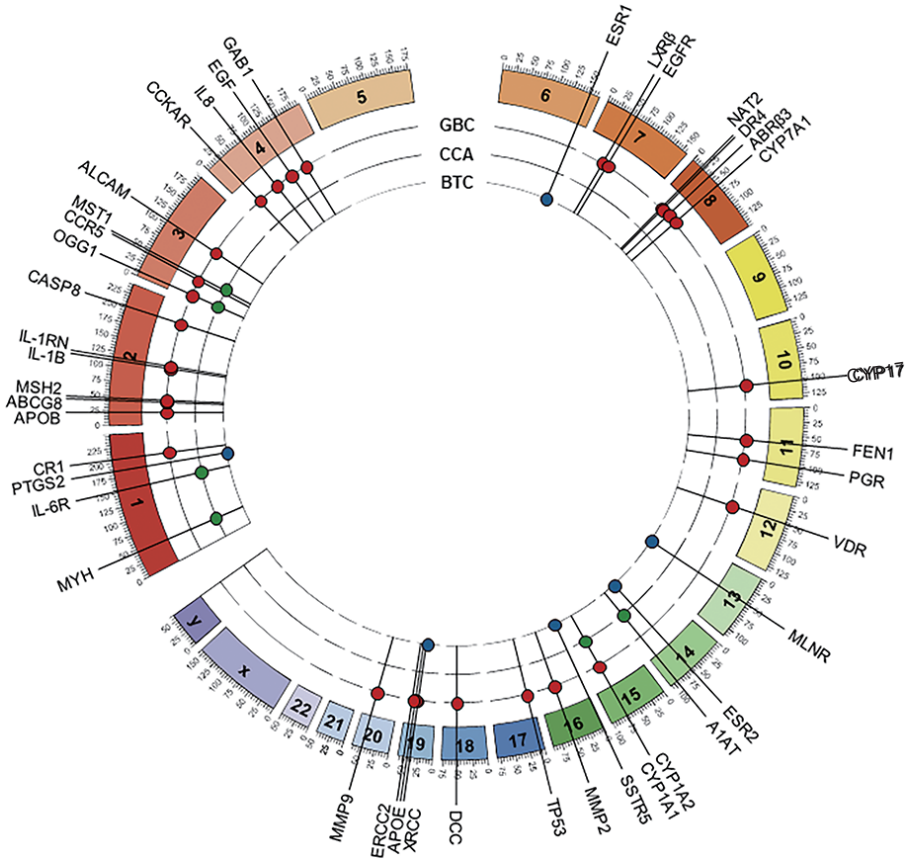

Figure 5 Circa plot. Circa plot of the gene variants associated with gallbladder cancer (GBC), cholangiocarcinoma (CCA), and biliary tract cancer (BTC).

and agricultural chemicals (185). Furthermore, this enzyme assists in metabolizing polycyclic aromatic hydrocarbons, nitrosamines, and aromatic amines to carcinogenic intermediates (186). The altered metabolism of xenobiotics may contribute to susceptibility to GBC.

The CC genotype resulting from the transition from T to C in position 6235 of the *CYP1A1* gene (rs4646903, also known as the CYP1A1 Msp1 polymorphism) is associated with a 2.3-fold increase in the risk of GBC (95% CI: 1.1–4.5, P=0.026) compared to the TT genotype (187). The C allele of this polymorphism has also been linked to smoking-related cancers (188). Another polymorphism in the CYP1A1 gene, rs1048943, with a transition from A to G at position 1506 of the mRNA, results in the substitution of isoleucine by valine at position 462 of the protein. Compared to those with the Ile/Ile genotype, patients with the Ile/Val genotype are 2.7 times more likely to develop GBC (95% CI: 1.1–6.4, P<0.05). Furthermore, carriers of the T allele of rs2606345 of CYP1A1 had an OR of 2.0 for GBC (95% CI: 1.3–3.0, P<0.01) (189). Regarding the rs4646903 polymorphism, the T allele of IVS1 + 606 was associated with twice the risk of GBC compared to the G allele (OR: 2.0; 95% CI: 1.3–3.0) (190).

Since there is a female predominance of GBC, endogenous estrogens and their metabolites may play an etiologic role in the development of this malignancy. Cytochrome P450C17a mediates steroid 17a-hydroxylase and 17,20-lyase activity and is, therefore, a key enzyme in estrogen metabolism (191). The rs743572 polymorphism at nucleotide 27 produces a transition from thymidine to cytosine. The T allele has been represented as the A1 allele, and the C allele has been denominated A2. A2 allele carriers may have higher levels of estrogens because the nucleotide substitution results in an additional Sp1-binding site with enhanced promoter activity and increased transcription rates. Subsequent studies demonstrated no difference in the promoter activity and mRNA expression between the two alleles (192). A case-control study from China found that carriers of the A1

Table 1 SNP variants associated with gallbladder cancer

Gene	Variant	Genotype/ allele	Number of cases	Number of controls	Crude OR	Adjusted OR	P value	Reference	Country/ ethnic group
ABCG8	rs11887534	DD	110 (64.3)	170 (76.9)	–	–	–	Srivastava, 2009 #1927	India
		DH	60 (35.1)	50 (22.6)	–	1.79 (1.1–2.8)	0.01		
		HH	1 (0.6)	1 (0.5)	–	1.63 (0.1–26.5)	0.70		
		D allele	281 (82.2)	391 (88.5)	–	1.0	–		
		H allele	61 (17.8)	51 (11.5)	–	1.6 (1.2–2.4)	0.02		
ABRB3	rs4994	TT	245 (61.2)	218 (81.3)	–	1.0	–	Rai, 2014 #2236	India
		TC	142 (35.5)	49 (18.3)	–	2.9 (1.9–4.3)	0.02		
		CC	13 (3.2)	1 (0.4)	–	10.33 (1.3–82.6)	0.04		
		T allele	632 (79.0)	485 (90.5)	–	1.0			
		C allele	168 (21.0)	51 (9.5)	–	2.7 (1.9–3.9)	0.01		
ADRB3	rs4994	TT	–	–	–	1.0		Rai, 2015 #8	North India
		TC	–	–	–	2.6 (1.8–3.8)			
		CC	–	–	–	10.6 (1.4–81.9)			
ALCAM	rs1157	GG	302 (49.5)	134 (53.6)	–	1.0		Yadav, 2016 #2234	North India
		GA	259 (42.5)	105 (42.0)	–	1.2 (0.9–1.8)	0.30		
		AA	49 (8.0)	11 (4.4)	–	2.4 (1.1–5.3)	0.03		
		G allele	863 (70.7)	373 (58.7)	–	1.0			
		A allele	357 (29.3)	263 (41.4)	–	1.4 (1.1–4.9)	0.02		
Apo B	rs520354	CC	40	204	–	1.0	P-trend 0.003	Andreotti, 2008 #1922	China
		CT	29	83	–	1.8 (1.1–3.1)			
		TT	6	7	–	4.4 (1.4–13.9)			
Apo B	rs693	CC	30 (52.6)	25 (35.7)	–	1.0		Báez, 2010 #2163	Chilean women
		CT	24 (42.1)	31 (44.3)	–	0.7 (0.3–1.5)	Non-significant		
		TT	3 (5.3)	14 (20.0)	–	0.1 (0.03–0.6)	0.01		
		C allele	84 (73.7)	81 (57.9)	–	1.0			
		T allele	30 (26.3)	59 (42.1)	–	0.5 (0.3–0.8)	0.01		

Table 1 (*continued*)

Table 1 (*continued*)

Gene	Variant	Genotype/ allele	Number of cases	Number of controls	Crude OR	Adjusted OR	P value	Reference	Country/ ethnic group
Apo E	rs440446	CC	14	138	–	1.0	P-trend 0.0001	Andreotti, 2008 #1922	China
		GC	48	128	–	3.7 (1.9–7.0)	–		
		GG	12	30	–	4.0 (1.7–9.4)	–		
CASP8	rs3834129	Ins/Ins	147 (64.5)	122 (53.0)	–	1.0	–	Srivastava, 2010 #2225	North India
		Ins/Del	69 (30.2)	84 (36.5)	–	0.7 (0.4–1.0)	0.04		
		Del/Del	12 (5.3)	24 (10.5)	–	0.4 (0.2–0.9)	0.02		
CCKAR	rs1800855	TT	49	91	1.0	–	P-trend 0.009	Xu, 2013 #2219	China, Shanghai, China women
		TA	90	125	1.3 (0.9–2.1)	–	–		
		AA	46	36	2.4 (1.4–4.1)	–	–		
CCR5	rs333	+/+	128 (88.9)	202 (96.2)	1.0	–	–	Srivastava, 2008 #2226	India
		+/Δ32	15 (10.4)	8 (3.8)	2.9 (1.1–7.2)	–	0.03		
		Δ32/Δ32	1 (0.7)	0 (0)	n/a	–	–		
		CCR5 +	271 (94.1)	412 (98.1)	1.0	–	–		
		CCR5 Δ32	17 (5.9)	8 (1.9)	3.1 (1.2–7.7)	–	0.01		
CR1	rs2274567	AA	56 (30.3)	65 (32.5)	1.0	–	–	Srivastava, 2009 #2229	North India
		AG	76 (41.1)	103 (51.5)	0.9 (0.5–1.3)	–	0.5		
		GG	53 (28.6)	32 (16.0)	1.9 (1.1–3.4)	–	0.02		
CYP1A1	rs1048943	Ile/Ile	15 (45.5)	59 (64.8)	–	1.0	<0.05	Tsuchiya, 2007 #196	Japan women
		Ile/Val	17 (51.5)	28 (30.8)	–	2.7 (1.1–6.4)	–		
		Val/Val	1 (3.0)	4 (4.4)	–	2.2 (0.2–25.0)	–		
CYP1A1	rs1048943	Ile/Ile	18 (48.6)	42 (87.5)	1.0	–	–	Kimura, 2008 #2240	Hungary
		Ile/Val	19 (51.4)	5 (10.4)	8.9 (2.9–27.4)	–	<0.001		
		Val/Val	0	1 (2.1)	“Infinite”	–	ns		
CYP1A1	rs2606345	GG	196 (83.4)	705 (90.4)	1.0	–	<0.01	Park, 2009 #2150	China
		GT	37 (15.7)	74 (9.5)	2.0 (1.3–3.1)	–	–		
		TT	2 (0.9)	1 (0.1)	n/a	–	–		

Table 1 (*continued*)

Table 1 (*continued*)

Gene	Variant	Genotype/ allele	Number of cases	Number of controls	Crude OR	Adjusted OR	P value	Reference	Country/ ethnic group
CYP1A1	rs4646903	TT	45 (31.7)	76 (44.4)	1.0	–	–	Pandey, 2008 #2228	North India
		TC	73 (51.4)	77 (45.0)	1.6 (1.0–2.6)	–	0.060		
		CC	24 (16.9)	18 (10.5)	2.3 (1.1–4.5)	–	0.030		
CYP1A1	rs4646903	TT	215 (52.4)	142 (61.7)	1.0	–	–	Sharma, 2014 #2237	India
		TC	160 (39.0)	77 (33.5)	1.5 (1.0–2.3)	–	0.090		
		CC	35 (8.5)	11 (4.8)	3.4 (1.4–8.2)	–	0.006		
		T allele	590 (72.0)	361 (78.0)	1.0	–	–		
		C allele	230 (28.0)	99 (22.0)	1.7 (1.2–2.4)	–	0.003		
CYP7A1	rs3808607	AA	36 (25.5)	70 (35.0)	1.0	–	–	Srivastava, 2008 #2153	North India
		AC	72 (51.1)	101 (50.5)	1.2 (0.7–2.2)	–	0.300		
		CC	33 (23.4)	29 (14.5)	2.8 (1.3–5.6)	–	0.005		
		A allele	144 (51.1)	241 (60.3)	1.0	–	–		
		C allele	138 (48.9)	159 (39.8)	1.6 (1.1–2.2)	–	0.008		
CYP7A1	rs3808607	AA	52 (28.1)	70 (30.5)	1.0	–	–	Srivastava, 2010 #1925	North India
		AC	89 (48.1)	101 (50.5)	1.2 (0.7–1.8)	–	0.500		
		CC	44 (23.8)	29 (14.5)	2.1 (1.1–3.7)	–	0.020		
		A allele	193 (52.2)	241 (60.2)	1.0	–	–		
		C allele	177 (47.8)	159 (39.8)	1.4 (1.1–1.8)	–	0.020		
CYP17	MspA1	A1A1	41 (42.7)	203 (79.3)	1.0	–	–	Dwivedi, 2015 #2162	North India
		A1A2	55 (57.3)	53 (20.7)	5.1 (3.1–8.5)	–	0.0001		
		Allele A1	137 (71.4)	459 (89.6)	1.0	–	–		
		Allele A2	55 (28.6)	53 (10.4)	3.5 (2.3–5.3)	–	0.0001		
DCC	rs174	AA	–	–	–	1.0	–	Rai, 2015 #8	North India
		AG	–	–	–	1.8 (1.3–2.6)	–		
		GG	–	–	–	1.7 (1.1–2.7)	–		

Table 1 (*continued*)

Table 1 (*continued*)

Gene	Variant	Genotype/ allele	Number of cases	Number of controls	Crude OR	Adjusted OR	P value	Reference	Country/ ethnic group
DCC	rs2229080	CC	–	–	–	1.0	–	Rai, 2015 #8	North India
		CG	–	–	–	0.6 (0.5–0.9)	–		
		GG	–	–	–	0.3 (0.2–0.7)	–		
DR4	rs20576	AA	–	–	–	1.0	<0.05	Rai, 2015 #8	North India
		AC	–	–	–	1.8 (1.2–2.8)	–		
		CC	–	–	–	3.3 (0.9–11.5)	–		
DR4	rs6557634	GG	–	–	–	1.0	–	Rai, 2015 #8	North India
		GA	–	–	–	1.6 (1.1–2.4)	–		
		AA	–	–	–	2.1 (0.9–4.7)	–		
EGF	rs4444903	AA	38 (30.2)	69 (36.3)	1.0	–	–	Vishnoi, 2008 #2164	India
		AG	48 (38.1)	87 (45.8)	1.0 (0.6–1.7)	–	0.900		
		GG	40 (31.7)	34 (17.9)	2.2 (1.2–4.2)	–	0.010		
		A allele	124 (49.2)	225 (59.2)	1.0	–			
		G allele	128 (50.2)	155 (40.8)	1.5 (1.1–2.1)	–	0.010		
EGFR	rs2017000	GG	89 (39.6)	102 (34.0)	1.0	–	–	Meng, 2014 #2238	Northeast China
		GA	110 (48.9)	134 (44.7)	1.1 (0.7–1.6)	–	0.800		
		AA	26 (11.5)	64 (21.3)	2.1 (1.3–3.7)	–	0.005		
		G allele	288 (64.0)	338 (56.3)	1.0	–	–		
		A allele	162 (36.0)	262 (43.7)	1.4 (1.1–1.8)	–	0.010		
ERCC2	rs1799793	GG	108 (47.0)	112 (48.7)	–	1.0	–	Srivastava, 2010 #2156	North India
		GA	87 (37.8)	100 (43.5)	–	2.1 (1.1–4.0)	0.700		
		AA	35 (15.2)	18 (7.8)	–	1.1 (0.7–1.6)	0.020		
FEN1	rs174538	AA	38 (11.1)	70 (20.6)	–	1.0	–	Jiao, 2015 #2212	China
		GA	164 (48.0)	160 (47.2)	–	1.7 (1.0–2.6)	–		
		GG	139 (40.8)	109 (32.2)	–	2.3 (1.3–4.0)	<0.001		

Table 1 (*continued*)

Table 1 (*continued*)

Gene	Variant	Genotype/ allele	Number of cases	Number of controls	Crude OR	Adjusted OR	P value	Reference	Country/ ethnic group
FEN1	rs4246215	TT	44 (12.9)	74 (21.8)	–	1.0	<0.001	Jiao, 2015 #2212	China
		GT	52 (48.1)	52 (49.1)	–	2.0 (1.1–2.9)	–		
		GG	31 (29.2)	42 (38.9)	–	2.6 (1.4–5.5)	–		
Gab1	rs3805246	GG	71 (31.6)	126 (42.0)	1.0	–	–	Meng, 2014 #2238	Northeast China
		GA	119 (52.9)	141 (47.0)	0.7 (0.5–1.0)	–	0.040		
		AA	35 (15.5)	33 (11.0)	0.5 (0.3–0.9)	–	0.040		
		G allele	261 (58.0)	393 (65.5)	1.0	–			
		A allele	189 (42.0)	207 (34.5)	0.7 (0.6–0.9)	–	0.010		
IL-1B	rs16944	TT	45 (36.3)	65 (29.2)	–	1.0	–	Vishnoi, 2008 #2168	North India
		CT	52 (41.9)	87 (52.4)	–	0.9 (0.5–1.6)	0.800		
		CC	27 (21.8)	14 (8.4)	–	3.4 (1.5–7.4)	0.030		
IL-1RN	VNTR	1/1	74 (59.7)	112 (67.5)	–	1.0	–	Vishnoi, 2008 #2168	North India
		1/2	29 (23.4)	43 (25.9)	–	1.0 (0.5–1.7)	0.800		
		2/2	15 (12.8)	9 (5.4)	–	3.3 (1.2–8.6)	0.020		
IL8	rs10805066	CC	187 (73.0)	647 (81.1)	1.0	–	P-trend 0.03	Castro, 2012 #2220	China
		CG	68 (26.6)	140 (17.5)	1.7 (1.2–2.3)	–	–		
		GG	1 (0.4)	11 (1.4)	n/a	–	–		
LXR-β	rs2695121	TT	174 (43.5)	108 (54.0)	1.0	–	–	Sharma, 2013 #2241	India
		TC	184 (46.0)	71 (35.5)	1.6 (1.1–2.3)	–	0.010		
		CC	42 (10.5)	21 (10.5)	1.2 (0.6–2.2)	–	0.460		
MMP2	rs2285053	CC	290 (70.7)	188 (81.7)	1.0	–	–	Sharma, 2012 #2221	India
		CT	112 (27.3)	40 (17.4)	1.8 (1.1–2.9)	–	0.020		
		TT	8 (2.0)	2 (0.9)	3.7 (0.6–21.8)	–	0.200		
		C allele	692 (84.0)	416 (90.0)	1.0	–	–		
		T allele	128 (16.0)	44 (10.0)	1.8 (1.2–2.8)	0.008	–		

Table 1 (*continued*)

Table 1 (*continued*)

Gene	Variant	Genotype/ allele	Number of cases	Number of controls	Crude OR	Adjusted OR	P value	Reference	Country/ ethnic group
MMP9	rs17577	RR	148 (36.1)	109 (47.4)	1.0	–	–	Sharma, 2012 #2221	India
		RQ	227 (55.4)	109 (47.4)	1.9 (1.2–2.8)	–	0.005		
		QQ	345 (8.5)	12 (5.2)	2.3 (1.0–5.2)	–	0.050		
		R allele	523 (64.0)	327 (71.0)	1.0	–			
		Q allele	262 (63.9)	133 (29.0)	1.9 (1.2–2.9)	–	0.007		
	rs8179090	G allele	699 (85.0)	410 (89.0)	1.0	–			
		C allele	121 (15.0)	50 (11.0)	1.8 (1.1–2.8)	–	0.010		
MSH2	rs2303426	GG	48 (20.9)	59 (25.7)	–	1.0	–	Srivastava, 2010 #2156	North India
		GC	107 (46.5)	119 (51.7)	–	1.1 (0.7–1.8)	0.600		
		CC	75 (32.6)	52 (22.6)	–	1.8 (1.1–3.1)	0.030		
NAT2		2*4 allele	87 (35.1)	142 (48.3)	1.0	–	–	Pandey, 2014 #2242	India
		2*5 allele	56 (22.6)	73 (24.8)	1.3 (0.8–1.9)	–	0.300		
		2*6 allele	65 (26.2)	57 (19.4)	1.9 (1.2–2.9)	–	0.006		
		2*7 allele	40 (16.1)	22 (7.5)	2.9 (1.6–5.2)	–	0.0001		
OGG1	rs2072668	CC	83 (36.1)	109 (47.4)	–	1.0	–	Srivastava, 2010 #2156	North India
		CG	103 (44.8)	92 (40.0)	–	1.5 (1.0–2.2)	–		
		GG	44 (19.1)	29 (12.6)	–	2.0 (1.2–3.5)	–		
	rs1052133	CC	117 (50.9)	137 (59.6)	–	1.0	–		
		CG	92 (40.0)	82 (35.7)	–	1.3 (0.9–1.9)	0.100		
		GG	21 (9.1)	11 (4.8)	–	2.5 (1.1–5.4)	0.030		
OGG1	rs1052133	Ser/Ser	88 (43.1)	78 (37.3)	–	1.0	<0.001	Jiao, 2007 #2158	China
		Ser/Cys	74 (36.3)	112 (53.6)	–	1.9 (1.0–3.7)	–		
		Cys/Cys	42 (20.6)	19 (9.1)	–	4.5 (1.1–22.4)	–		
OGG1	rs1052133	Ser/Ser	88	112	1.0	–	–	Srivastava, 2009 #1926	North India
		Ser/Cys	69	85	1.0 (0.7–1.5)	–	0.900		
		Cys/Cys	16	7	2.9 (1.1–7.5)	–	0.030		

Table 1 (*continued*)

Table 1 (*continued*)

Gene	Variant	Genotype/ allele	Number of cases	Number of controls	Crude OR	Adjusted OR	P value	Reference	Country/ ethnic group
PGR	rs1042838	Del/Del	368 (89.8)	181 (82.3)	–	1.0	–	Srivastava, 2012 #1929	North India
		Del/Ins	42 (10.2)	37 (16.8)	–	0.5 (0.3–0.8)	0.009		
		Ins/Ins	0	2 (0.9)	–	n/a	–		
		Del allele	778 (94.9)	399 (90.7)	–	1.0	–		
		Ins allele	42 (5.1)	41 (9.3)	–	0.4 (0.3–0.8)	0.002		
PLCE1	rs2274223	AA	174 (41.8)	111 (49.3)	1.0	–	–	Sharma, 2013 #2244	North India
		AG	229 (55.0)	98 (43.6)	2.0 (1.2–3.0)	–	0.002		
		GG	13 (3.1)	16 (7.1)	0.4 (0.1–1.1)	–	0.090		
TP53	rs1042522	Arg/Arg	5 (23.8)	36 (41.4)	–	1.0	<0.050	Tsuchiya, 2007 #2149	Japan men
		Arg/Pro	10 (47.6)	33 (37.9)	–	4.3 (1.1–17.2)	–		
		Pro/Pro	6 (28.6)	18 (20.7)	–	3.1 (0.8–12.2)	–		
VDR	rs10735810	TT	40 (13.8)	111 (28.0)	–	1.0		Li, 2014 #2213	China
		CT	140 (48.1)	195 (49.2)	–	2.0 (1.3–3.0)	0.003		
		CC	111 (38.1)	90 (22.7)	–	3.4 (2.2–5.4)	0.001		
XRCC1	rs1799782	Arg/Arg	80	65	1.0	–	–	Srivastava, 2009 #1926	North India
		Arg/Gln	74	99	0.6 (0.4–1.0)	–	0.040		
		Gln/Gln	19	40	0.4 (0.2–0.7)	–	0.003		

Table 2 SNP variants associated with cholangiocarcinoma

Gene	Variant	Genotype/ allele	Number of cases	Number of controls	Crude OR	Adjusted OR	P value	Reference	Ethnic group
α1AT	rs28929474	C allele	349 (96.0)	688 (98.0)	1.0	–	–	Mihalache, 2011 #2230	Caucasians
		T allele	15 (4.0)	12 (2.0)	2.5 (1.1–5.3)	–	0.020		
CYP1A2		1F/1F	85 (57.4)	88 (51.2)	–	1.0	–	Prawan, 2005 #2232	Thailand men
		1A/1F	59 (39.9)	69 (40.1)	–	0.9 (0.6–1.5)	0.700		
		1A/1A	4 (2.7)	15 (8.7)	–	0.3 (0.1–0.9)	0.040		
IL-6R	rs2228145	AA	60 (73.0) O-viverrini related CCA	37 (46.0)	1.0	–	–	Prayong, 2014 #2170	Thailand
		AC	17 (24.0)	37 (46.0)	0.3 (0.1–0.6)	–	0.0003		
		CC	2 (3.0)	6 (8.0)	0.2 (0.20–1.25)	–	0.040		
		A allele	137 (87.0)	111 (69.0)	1.0	–	–		
		C allele	21 (13.0)	49 (31.0)	0.4 (0.2–0.6)	–	0.0002		
MST1	rs3197999	GG	–	–	–	1.0	–	Krawczyk, 2013 #86	Caucasian
		AA	–	–	–	2.1 (1.1–3.8)	0.020		
MutY	rs3219472	TT	25 (42.4)	26 (26.0)	1.0	–	–	You, 2013 #2160	China
		TG	20 (33.9)	58 (58.0)	0.4 (0.2–0.8)	–	0.006		
		GG	14 (23.7)	16 (16.0)	0.9 (0.4–2.2)	–	0.838		
OGG1	rs1052133	Ser/Ser	19 (12.7) iCCA	37 (24.7)	1.0	–	–	Ding, 2015 #2159	China
		Ser/Cys	71 (47.3)	73 (48.6)	1.9 (1.0–3.6)	–	0.060		
		Cys/Cys	60 (40.0)	40 (26.7)	2.9 (1.5–5.8)	–	0.030		
		Ser allele	109 (36.3)	147 (49.0)	1.0	–	–		
		Cys allele	191 (63.7)	153 (51.0)	1.7 (1.2–2.3)	–	0.002		

Table 3 SNP variants associated with all biliary tract cancers

Gene	Variant	Genotype/ allele	Number of cases	Number of controls	Crude OR	Adjusted OR	P value	Reference	Country/ ethnic group
CYP1A1	rs2606345	GG	105 (84.0)	705 (90.4)	1.0	–	<0.05	Park, 2009 #2150	China
		GT	20 (16.0)	74 (9.5)	1.8 (1.1–3.2)	–	–		
		TT	0 (0)	1 (0.1)	n/a	–	–		
ESR1	rs1801132	CC	23	219	1.0	–	P-trend 0.07	Park, 2010 #1924	Shanghai, China
		CG	69	377	1.8 (1.1–2.9)	–	–		
		GG	32	181	1.7 (1.0–3.1)	–	–		
ESR2	rs4986938	AA	91	589	1.0	–	P-trend 0.30	Park, 2010 #1924	Shanghai, China
		AG	24	170	0.9 (0.6–1.5)	–	–		
		GG	7	13	3.3 (1.3–8.7)	–	–		
MLNR	rs9568169	CC	54 (40.6)	151 (33.9)	–	1.0	–	Xu, 2014 #2214	Shanghai, China
		CA	61 (45.9)	199 (44.6)	–	0.9 (0.6–1.3)	–		
		AA	18 (13.5)	96 (21.5)	–	0.5 (0.3–0.9)	–		
PTGS2	rs5275	TT	70 (55.6)	541 (69.5)	–	1.0	P-trend 0.004	Sakoda, 2006 #211	Shanghai, China
		TC	51 (40.5)	216 (27.8)	–	2.0 (1.2–2.7)	–		
		CC	5 (4.0)	21 (2.7)	–	1.0 (0.7–5.6)	–		
SSTR5	rs169068	TT	79 (59.4)	268 (60.2)	–	1.0	P-trend 0.84	Xu, 2014 #2214	Shanghai, China
		TC	41 (30.8)	157 (35.3)	–	0.9 (0.6–1.4)	–		
		CC	13 (9.8)	20 (4.5)	–	2.4 (1.1–5.1)	–		
XRCC1	rs1799782	RR	49	366	–	1.0	P-trend 0.03	Huang, 2008 #1889	China
		RW	57	344	–	1.2 (0.8–1.9)	–		
		WW	20	74	–	1.9 (1.1–3.5)	–		

allele of the CYP17 MspA1 polymorphism had an increased risk of GBC compared to those with the A2A2 genotype (OR: 1.5; 95% CI: 1.1–2.1) (193). A study from North India performed on considerably fewer subjects found the risk of GBC was 3.5 times greater in carriers of the A2 allele (95% CI: 2.3–5.3; P=0.0001) (192). The discrepancies between these two studies, both performed on the same polymorphism in different populations, attest to the fact that the frequency of variant alleles and their effects on phenotypes vary substantially by racial group. Therefore, it is imperative to reproduce association studies in different racial or ethnic groups (194,195).

Super-saturation of bile with cholesterol has been implicated in gallstone formation (196). Conversion of cholesterol into bile acids is a major pathway for eliminating cholesterol from the body (197). Because bile acids play a major role in cholesterol homeostasis, polymorphisms in the CYP7A1 gene, which encodes for the first and rate-limiting step in the classical bile acid synthesis pathway, may influence susceptibility to GBC. The CYP7A1 gene is located in chromosome 8q11-q12, and a SNP conferring an A>C transition is present at the 204 position of the gene. Subjects with the CC genotype are 2.8 times more likely to develop GBC than those with the AA genotype (95% CI: 1.3–5.6, P=0.005) (198). The mechanism by which GBC develops appears to be mediated by accumulation of toxic substances in the gallbladder, rather than gallstone formation. However, this must be confirmed with additional studies and in different populations (198).

The apolipoproteins are another class of key mediators of cholesterol homeostasis. Apolipoproteins are major components of lipoproteins and play a critical role in the transport of cholesterol to the liver. The APO E and APO B genes are located on chromosomes19q13.2 and 2p24-p23, respectively, and encode apolipoproteins, which are major carriers and binding proteins for low-density lipoproteins (LDL). Genetic polymorphisms in the apolipoprotein genes have been associated with gallstones and BTC, and some polymorphisms have been linked to higher serum levels of cholesterol and LDL and lower levels of HDL. Male carriers of the G allele of APOE IVS1 + 69C>G appear to have a 3.7-fold increased risk of bile duct cancer (95% CI: 2.0–7.0) (199). The same sex-specificity was found for the T allele of IVS6 + 360C>T (OR 2.0; 95% CI: 1.2–3.4) (199). The increased risk conferred by these variants was independent of the presence of gallstones. Rs693 is another polymorphism of the APO B gene specifically associated with GBC and is protective for carriers of the T allele (OR: 0.5; 95% CI: 0.3–0.8; P=0.01) (200). The TT genotype was demonstrated to be protective of GBC compared to CC (OR: 0.1; 95% CI: 0.03–0.6; P=0.01) (200).

It is well known that impairment in DNA repair processes leads to cancer. Polymorphisms of DNA repair genes may affect the host's capacity to repair damaged DNA, leading to the accumulation of mutations and an increased risk of cancer. The 8-oxoguanine glycosylase 1 (OGG1) gene localized at chromosome 3p25 encodes a protein that initiates the base excision repair (BER) pathway and is responsible for the elimination of 8-oxoG, a byproduct of the attack of reactive oxygen species on DNA. A rs1052133 polymorphism GG (Cys/Cys) variant at position 1245 (Ser326Cys) reduces the capacity of OGG1 to repair oxidative DNA damage during conditions of intracellular oxidative stress. Cellular accumulation of Cys326-OGG1 protein under conditions of intracellular oxidative stress appears to contribute to the defect in DNA repair (201). The GG variant was associated with an OR of 2.9 (95% CI: 1.1–7.5; P=0.025) for development of GBC versus the CC (Ser/Ser) variant (202). Similarly, in a population from northern India, the GG genotype was associated with an OR of 2.5 (95% CI: 1.1–5.4, P=0.03) compared to the CC genotype (203). A Chinese population study demonstrated that individuals carrying the GG genotype had a higher risk of iCCA (OR: 2.9; 95% CI: 1.5–5.8) compared to individuals bearing the wild-type CC genotype (204). Another study conducted in China found that the heterozygous CG (Ser/Cys) genotype had an OR of 4.5 *vs.* CC (Ser/Ser) (95% CI: 1.1–22.4) (205). These studies have consistently shown that OGG1 is associated with BTC.

ERCC2 is also involved in the repair of damaged DNA, and a polymorphism in rs1799793 has been linked to GBC risk. Compared to the homozygous wild-type GG genotype, the AA genotype (Asp312Asn) was 2.1 more frequent in GBC cases than in controls (95% CI: 1.1–4.0; P=0.02) (203). The same study found an OR of 1.8 (95% CI: 1.1–3.1; P=0.03) for the IVS1 + 9G>C polymorphism in rs2303426 of MSH2 when comparing the CC genotype to the wild-type GG genotype.

The *X-ray repair cross-complementing group 1* gene (XRCC1) at chromosome 19q13.2 is involved in the single-strand break repair and BER pathways. Several variants of this gene have been implicated in the increased risk of bile duct cancer, including a substitution of arginine for tryptophan at position 194 (R194W), which was associated with an increased risk of bile duct cancer (OR: 1.9; 95% CI:

1.1–3.5, P=0.03), and a substitution of arginine for histidine (R280H) variant, which was associated with a reduced risk of bile duct cancer (166). Interestingly, the 399Gln allele of the Arg399Gln polymorphism conferred a significantly reduced risk of GBC but not bile duct cancer (OR: 0.37; 95% CI: 0.2–0.7, P=0.003) (202).

The *MutY* homolog is another key enzyme in the BER pathway. Mutations in this gene result in predisposition to colorectal and stomach cancer and its role in CCA tumorigenesis has not been established. One study found the TG genotype in the rs3219476 polymorphism to be associated with a decreased risk of CCA compared to the TT genotype (OR: 0.4; 95% CI: 0.2–0.8; P=0.006) (206).

Growth factors play important roles in carcinogenesis by promoting cell proliferation, inhibiting apoptosis, and enhancing invasion, metastasis, and angiogenesis. Polymorphisms of vascular endothelial factor (VEGF), epidermal growth factor (EGF) and transforming growth factor β 1 (TGFβ1) have been studied in the context of GBC. Results from a case-control study in India demonstrated that subjects with GG genotype at rs4444903 in the *EGF* gene are 2.2 times more likely to have GBC than those with the AA genotype (95% CI: 1.2–4.2; P=0.012) (207). The presence of the T allele in rs3025039 of the *VEGF* gene was associated with an OR of 0.7 compared to the CC genotype (95% CI: 0.5–1.0) (208). The G allele of rs1570360 (g.43737830A>G), also located within the *VEGF* gene region, was associated with a decreased risk of GBC (OR: 0.7; 95% CI: 0.5–0.9; P=0.008) (209). On the contrary, the T allele of rs112005313 (c.237C>T) is linked to an increased risk of GBC compared to those that carry the C allele (OR: 1.6; 95% CI: 1.1–2.4; P=0.02) (209).

A Japanese study found that DCC rs714 was associated with increased susceptibility to GBC. Another study assessed this association in a population in Northern India and failed to replicate the results (183,210).

The strongest risk factors for CCA are related to chronic inflammation, which is characterized by up-regulation of the inflammation-related genes, cyclooxygenase (COX-2), also known as prostaglandin-endoperoxidase synthase 2 (PTGS-2), and the cytokine interleukin-6 (IL-6) (211). Polymorphic variants in the *PTGS-2* gene located at 1q25.2-25.3 on chromosome 1 may affect cancer susceptibility. The C allele in Ex10 + 83T>C of the *PTGS-2* gene is associated with a 1.8-fold increased risk of bile duct cancer when compared to the T allele (95% CI: 1.2–2.7) (212). The SNPs rs2143417 and rs689466 in the *PTGS-2* gene are significantly associated with increased risk for CCA (OR: 1.4; 95% CI: 1.1–1.7; P=0.005 and OR: 1.5; 95% CI: 1.2–1.9; P=0.0003, respectively). To emphasize the high likelihood of false-positive results and the importance of validation of candidate gene SNP studies, of all the candidate gene studies discussed in this review, this study was the only one that conducted a replication study in a validation cohort but failed to replicate the original results found in the test cohort (211).

Since chronic inflammation predisposes to malignancy, cytokines that mediate inflammatory immune responses could be involved in the pathogenesis of such malignancies. IL-6 is a multifunctional cytokine that plays an important role in a wide range of biologic activities, mostly mediated by binding to the IL-6 receptor (IL-6R) (213). Association analyses identified that rs8192284 in exon 9 of IL-6R is associated with Opistorchiasis-related CCA in the Thai population. This polymorphism causes non-synonymous substitution from asparagine to alanine in position 358 (D358A). Allele C protects against CCA when compared to allele A (OR: 0.4; 95% CI: 0.2–0.6, P=0.0002) (214). Rs8192284 has also been linked to inflammatory diseases including diabetes, obesity, arthritis, and periodontitis and reportedly plays a pivotal role in the pathogenesis of pancreatic, gastric, and renal cell carcinoma (214).

The rs10805066 polymorphism of IL-8 is located outside the promoter region of the gene and the functional effects of the variant have not been reported. However, the CC genotype, compared to the CG (-13985C>G) resulted in an OR for BTC of 1.67 (95% CI: 1.2–2.3, P=0.03) (215). Interleukin-1 receptor antagonist (IL-1RA) is a naturally occurring anti-inflammatory cytokine, and an Indian study assessed the association between one of its genetic variants and GBC susceptibility. Specifically, the 2/2 genotype, when compared to 1/1, has an increased risk of 3.3-fold (95% CI: 1.2–8.6; P=0.02). *In vitro* and *in vivo* studies have shown allele 2 of the IL-1RN polymorphism increases the production of IL-1B (216).

The natural killer cell receptor G2D (NKG2D) is part of the innate immune system and plays an important role in tumor surveillance by modulating the activation of lymphocytes and promoting immunity to eliminate ligand-expressing cells (217). The rs11053781 and rs2617167 polymorphism of NKG2D conferred an increased risk of CCA in Scandinavian patients with PSC (OR: 2.1; 95% CI: 1.3–3.3, P=0.01; OR: 2.3; 95% CI: 1.5–3.7; P=0.002, respectively) (218). Clarifying the role of NKG2D in cholangiocarcinogenesis could stimulate the development of immunotherapies that target these molecules. However,

this association was not confirmed in a validation study performed in a US cohort (211).

The *MST1* gene, also referred to as MSP, codes for a protein involved in the MSP/RON signaling axis, which modifies cellular processes such as innate immunity, macrophage activation, and chemotaxis (219). MSP has been implicated in lung cancer, breast cancer, and pancreatic cancer (220). The rs3197999 variant in the MST1 gene has been associated with PSC in GWAS, and a study performed in a Caucasian population demonstrated a significant (P=0.02) association between the MST1 genotype AA and the extrahepatic subtype of BTC (OR: 2.0; 95% CI: 1.1–3.8) compared to carriers of the common GG allele (219). This missense coding variant results in a p.R689C amino acid substitution within the beta chain of the MSP protein.

Although each of the previously mentioned variants may produce a weak effect, when considered collectively a polygenic risk score (PRS) can be generated that incorporates the risk associated with each SNP; this creates a multiplicative model that allows an accurate prediction of individual risk given the number of risk alleles carried by the individual. Thus, in the future, clinicians may be able to stratify the population according to their risk level and implement appropriate surveillance strategies for early detection and even prevention of BTC. As opposed to broad knowledge of the average risk according to the population to which the patient belongs, personalized information can then be provided to each patient. More and larger GWAS are necessary to achieve this level of risk estimation for BTCs. Moreover, such GWAS must be performed in different racial groups, as the associations found in one racial group cannot be generalized to other groups.

Once common variants and the genes where they are located are identified, it is important to determine the effects of the genetic variation on individual gene expression and the pertinent cell-signaling pathways (221). This will provide insights into the molecular and cellular mechanisms affected by the genetic variation and their involvement in the pathogenesis of BTC. This knowledge may be critical to develop effective preventive and therapeutic strategies for BTC (222).

Summary, conclusions and future directions/ studies

The epidemiology of BTC demonstrates substantial geographical variability due to large differences in regional prevalence of the main environmental risk factors. The risk factors with the strongest links to the development of BTC are those that induce inflammation over prolonged periods of time. Among these are infectious agents such as *O. viverrini*, *C. sinensis*, HBV, and HCV, which are more common in the Asian continent, PSC, which is the most recognized risk factor in the Western countries, and biliary stone disease. Even though these factors have been linked to carcinogenesis, no risk factor is identified in the vast majority of cases. Therefore, there is an unmet need for better identification strategies for those patients at increased risk for BTC, with the aims of early detection or prevention. By identifying SNPs that confer increased risk, clinicians may be able to identify at-risk patients and to implement appropriate surveillance strategies to monitor BTCs at earlier stages, when more effective therapeutic interventions are feasible. Clearly, we are still a long way from this goal, and therefore, there is an urgent need for large GWAS to identify and validate known as well as novel candidate SNPs. Furthermore, since the associations vary according to population, these studies must be replicated in different at-risk populations. Functional studies performed on the variants identified through GWAS could eventually shed light into potential therapeutic targets for the development of targeted precision therapies for BTC.

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Footnote

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