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Association between biliary tract cancers and family history of cancers in the Biliary Tract Cancers Pooling Project (BiTCaPP)

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Bachelor of Science
Villanova University
2015

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ABSTRACT

Association between biliary tract cancers and family history of cancers in the Biliary Tract Cancers Pooling Project (BiTCaPP)

By Margaret Langhamer

Background: Biliary tract cancer (BTC) has a varied geographic and racial distribution, which differs by sex and site within the biliary tract. The established association of family history of gallstones with BTC, and the increased incidence of BTC among those with hereditary cancer syndromes, suggests a genetic component to BTC risk. We examined associations of family history of cancers with site-specific BTC risk and investigated whether these associations differ by sex and age.

Methods: This pooled analysis of seven prospective cohorts participating in the Biliary Tract Cancers Pooling Project (BiTCaPP) included individual-level data on 1,619 BTC cases and 1,185,000 non-cases from the United States and Finland. Hazard ratios (HR) and 95% confidence intervals (CI) were estimated using Cox proportional hazards regression. Multivariable models were adjusted for sex, self-reported race, and calendar year at baseline. Study-specific estimates were pooled using a fixed effects model.

Results: A family history of cancer was not statistically significantly associated with independent risk of cancers of the gallbladder (HR = 0.88, 95% CI = 0.71-1.08), intrahepatic bile duct (HR = 0.99, 95% CI = 0.81-1.18), extrahepatic bile duct (HR = 0.99, 95% CI = 0.79-1.24), or ampulla of Vater (HR = 0.89, 95% CI = 0.72-1.00).

Conclusion: In the largest study to date of BTC risk, we found that family history of cancer may not be associated with risk of developing BTC.

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Table of Contents

CHAPTER I

Background

1.1 Epidemiology of biliary tract cancers

Biliary tract cancers (BTCs) include cancers at four organ sites: gallbladder (GBC), extrahepatic bile duct (EHBDC), intrahepatic bile duct (IHBDC), and ampulla of Vater (AVC)(1). While BTCs have a uniformly poor prognosis as a result of non-specific symptoms and late stage at diagnosis, the incidence of these cancers varies widely by site within the biliary tract, sex, geographic location, and ethnicity (2). GBC is the most common biliary tract malignancy with a global age-standardized incidence rate of 2.2 per 100,000 (3); however, this rate is as high as 20 per 100,000 among women in southern-central Chile (4) and 7.8 per 100,000 among men in South Korea (3). Both GBC and overall BTC rates are even higher when incidence rates are restricted to indigenous populations (5, 6). For example, in the United States (US) the BTC incidence rate is highest among American Indians and Alaska Natives at 8.5 per 100,000, followed by Hispanics at a rate of 5.9 per 100,000 (5, 6). Other notable patterns include an increased incidence of GBC among women relative to men and an increased incidence of EHBDC among men relative to women (6). Because the epidemiologic associations differ by site within the biliary tract, examination of associations by BTC site is crucial.

1.2 Risk factor epidemiology of BTCs

As a group, BTCs share a number of common risk factors including personal history of gallstones (7) or diabetes (8-11) and family history of gallstones (12, 13), as well as lower education level, increased body mass index, and high parity (13); however, the magnitude of the association between these risk factors and BTC differs across the

four organ sites (Supplemental Table 1). To date, the majority of the literature focuses on GBC and is methodologically limited by the small number of total cases – a problem that becomes even more apparent when trying to identify site-specific risk factors for the remaining types of BTC.

1.2.1 Gallstones

Gallbladder cancer. The most well-studied risk factor for GBC is gallstones (2, 7-9, 14-17). Hsing *et al.* (2007) compared biliary cancer cases and biliary stone cases to population controls and found that 19.3% of GBC cases had a family history of gallstones compared to only 9.5% of controls (12). A personal history of gallstones was associated with a 21-fold increased risk of GBC (95% CI=14.8-330.1) while a combined personal and family history was associated with a 57-fold increase (95% CI=30.2-110.5). It remains unknown whether the familial association suggests a genetic factor that increases predisposition to gallbladder disease or if a shared environmental factor plays an etiologic role.

Other BTC sites. The association of gallstones with the remaining sites is not as well understood. However, Hsing *et al.*(2007) found that 15.3% of cholangiocarcinoma (either IHBDC or EHBDC) and 14.7% of AVC cases have a family history of gallstones relative to 9.5% of population-based controls (12).

1.2.2 Lifestyle and Environment

Gallbladder cancer. The existing literature on environmental risk factors for GBC has examined exposures such as tobacco use, chronic typhoid infection, and a range of dietary products (7, 8, 14, 15). These findings support the growing number of hypotheses suggesting that inflammatory and lithogenic pathways are involved in gallstone

development and the subsequent risk of GBC. High parity and obesity have also been independently and jointly associated with GBC (8, 15-17). Women who have given birth four or more times are at an increased risk of gallstones relative to women with fewer births (RR=2.04, 95% CI=0.51-8.20), and it is believed that changing estrogen levels during and between pregnancies alter the composition of bile to be more lithogenic (7). Under this hypothesis and given that obesity also affects hormone levels, it is of particular interest to consider hormonal risk factors in future analyses(18). This includes potential clustering of GBC with hormonally-driven cancers including those of the breast, endometrium, ovary, and prostate.

Intra- and Extrahepatic bile duct cancer. At least three case-control studies in the US and China examined associations between lifestyle and environmental risk factors and cholangiocarcinomas (9, 10, 19). Independently, IHBDC is more likely to be associated with infectious agents including liver flukes, HCV, and HIV (19). Shared risk factors for the two sites of cholangiocarcinomas include cholecystectomy, cirrhosis, and alcoholic liver disease. In a population-based US case-control study, cholecystectomy was associated with a 12-fold (OR=12.0, 95% CI=9.5-15.3) and 5-fold (OR=5.4, 95% CI=3.9-7.5) increased risk of EHBDC and IHBDC, respectively (9). However, the link between these cancers and cholecystectomy is speculative given that the majority of cholecystectomy surgeries occurred in the year prior to cancer diagnosis and may have been an effect of disease and not a true risk factor. However, gallbladder removal changes the flow of bile through the biliary system exposing the biliary tree and small intestine to caustic bile, and thus alters the risk of these cancers (20, 21).

Ampulla of Vater cancer. Similar to the limited data on EHBDC and IHBDC risk factors, few studies have evaluated AVC. One case-control study conducted in China specifically examined environmental and lifestyle risk factors for AVC (11). The most highly associated risk factors share links to inflammatory pathways, such as diabetes wherein patients generate more reactive oxygen species or increased total cholesterol, which is related to an increase in pro-inflammatory cytokines (11).

1.2.3 Family History

As with the medical history and lifestyle- and environmental-related risk factors, associations between BTCs and family history of cancers are difficult to delineate due to sparse amount of data. It is especially challenging to find a link between site-specific family histories and BTCs; therefore many studies examine BTCs as a single class to increase precision of association estimates (Supplemental Table 2). One such study reported an increased risk of BTCs among cases with a family history of any cancer (OR=1.52, 95% CI=1.02-2.24) (13). Despite finding a statistically significant association, it is important to note that this analysis included only 59 cases. Furthermore, the results from the remaining literature have been inconsistent. While most studies have found positive associations, a recent case-control study in Utah observed statistically nonsignificant decreased risks of BTCs among first-degree (OR=0.95, 95% CI=0.28-3.09) and second-degree (OR=0.25, 95% CI=0.06-1.03) relatives of BTC cases (22). Due to the etiologic diversity among cancers of the biliary tract, it is difficult to interpret associations between family history of cancers and BTCs as a class, thus underlying the importance of site-specific studies.

Gallbladder cancer. At least four case-control studies have examined associations between family history of cancer and GBC and found that GBC tends to aggregate in families (16, 22-24). While the Utah case-control study of relatives of BTC cases previously mentioned found a decreased risk of cancer when all BTC sites were combined, an increased risk was observed when the analysis was restricted to GBC cases and their relatives (First-cousin OR=1.20, 95% CI=0.51-2.8) (22). This finding is consistent with the remaining literature and underlines differences between the four BTC sites. It may suggest that certain BTC sites may have a greater tendency for familial clustering than others. Hemminki *et al.* (2003) conducted a case-control study of the Swedish Family Cancer Database to assess the degree of familial clustering of BTCs and other cancers (24). Cases with a parental history of liver/biliary cancer (SIR=3.13, 95% CI=1.70-5.26) or pancreatic cancer (SIR=2.39, 95% CI=1.23-4.18) were found to have an increased incidence of GBC. Other studies reported an increased risk of GBC among those with any family history of GBC (16, 23). A case-control study by Fernandez et al. (1994) found that individuals with family history of GBC had a 14-fold increased risk of GBC (95% CI=1.2-163.9); however, the estimate was based on only one case and was therefore very imprecise (23). While these results suggest a positive association of GBC with a family history of cancer, further examination is needed to improve the precision of these estimates.

Intra- and Extrahepatic bile duct cancer. The association between family history of any cancer and cholangiocarcinoma was evaluated in two case-control studies with opposite findings. One study in Northeast Thailand found an increased risk of cholangiocarcincoma among cases with a family history of any cancer (OR=4.34, 95%

CI=1.80-10.43) (20). In contrast, the Utah Cancer Registry study found a decreased risk of cholangiocarcinoma among first-degree relatives of cholangiocarcinoma cases (OR=0.68, 95% CI=0.09-5.14). Only one study has attempted to take this study question a step further to investigate the association of site-specific family histories with either IHBDC or EHBDC. This study of Swedish women sought to identify site-specific cancer family histories that may be associated with risk of EHBDC and observed an increased incidence of EHBDC (SIR=3.83, 95% CI=1.00-9.91) among individuals with a parental history of ovarian cancer (24).

Ampulla of Vater cancer. The only study to examine family history of cancer among those with AVC reported an increased incidence of AVC (SIR=3.40, 95% CI=1.07-7.99) among individuals with a parental history of thyroid cancer (24). This finding, as well as the aforementioned association of parental ovarian cancer with EHBDC, suggests that hormonal factors may contribute to BTC risk.

1.3 Genetic associations

Further support for the hypothesis that having a family history of cancer increases the risk of BTCs comes from genetic association studies that have identified mutations common to familial cancer syndromes in BTC patients (23-28). One example of a germline mutation of potential importance in the development of BTC occurs in the *STK11/LKB1* gene, which is mutated in Peutz-Jegher Syndrome (PJS), a disease of autosomal dominant inheritance that leads to the development of gastrointestinal (GI) polyps (29). The wild-type *STK11/LKB1* gene may have a tumor suppressive role, and mutations occur in both sporadic and familial (PJS) cancers of the biliary tract and pancreas. Further, individuals with the PJS mutation have as high as a 30-fold increased

risk of death from GI tract cancers relative to those without the syndrome. If *STK11/LKB1* mutations observed in BTC cases are found to be familial, as opposed to sporadic, this could provide support for the hypothesis that family history of cancer is an important risk factor for BTC. BTCs also occur in Lynch syndrome, also referred to as hereditary nonpolyposis colorectal cancer (HNPCC), families. A nearly 10-fold increased risk of BTC is seen in carriers of mutations associated with HNPCC, which include germline mutations of the *MSH2* or *MLH1* genes that function in DNA mismatch repair (25, 27).

Gallbladder cancer. Until recently, no studies had investigated common genetic variants and their association with GBC specifically. Mhatre at al. (2017) conducted a case-control study of GBC cases and controls in India to identify single nucleotide polymorphisms associated with an increased risk of GBC (30). Two genetic variants located in the *ABCB1* and *ABCB4* genes had a statistically significant relationship with GBC risk. These genes have been previously associated with inflammatory bowel disease and altered phospholipid transport, which may play an important role in gallstone development and, therefore, an increased risk of GBC (31).

Intra- and Extrahepatic bile duct cancer. Despite the number of genetic association studies that have identified sporadic and germline mutations that may be involved in overall BTC carcinogenesis (28, 29, 32, 33), only one study specifically examined IHBDC. This Japanese study found that 11 of 18 patients with IHBDC demonstrated loss of heterozygosity at a locus on chromosome 8 near the locus of tumor-suppressor gene *TP53* (28), which is common in familial breast and colorectal cancers (34). The authors

hypothesized that similar mutations may also give rise to IHBDC, and potentially other BTCs, in families.

Ampulla of Vater Cancer. The *KRAS* oncogenes are implicated at a much higher rate in AVC than in other BTC sites (32). In a study of Chinese BTC cases, Rashid *et al.* (2002) detected a *KRAS* mutation in 18 of 126 BTC cases with 11 of those cases being AVC. This finding is especially striking considering AVC made up only 18 of the total BTC cases and is the least common of the four BTCs.

While certain risk factors are common to all BTCs and many previous studies have pooled together data from the four BTC sites in their analyses, including those of family history, the literature does suggest site-specific variations, emphasizing the necessity of studying BTCs independently by site within the biliary tract.

1.4 Significance of Thesis

Future evaluation of site-specific risk factors for BTC will require the collection of large amounts of data on these rare, etiologically distinct cancers. Such a resource can be created by pooling existing studies to conduct a pooled analysis of the associations between family history of cancers and BTCs overall and by BTC site.

Results from these larger studies can have profound clinical implications in promoting BTC screening practices for individuals with familial cancer syndromes or with a family history of certain cancers as no such practices are currently in place (25). It is easy to model family history data; and, when ascertained in detail, family history of cancers has a potential in driving targeted screening methods. Since the poor prognosis of BTCs is due in large part to the lack of clinical presentation of symptoms and late stage at

diagnosis, (2, 13) such screening measures could improve detection and provide added years of life for those at greatest risk.

1.4.1 Aims of Thesis

Primary Specific Aim: We will examine the associations between family history of cancers and risk for cancers of the biliary tract including GBC, IHBDC, AVC, and EHBDC.

i. We will examine family history of any cancer.
ii. We will examine family history of GI tract cancers.
iii. We will examine family history of hormonally-driven cancers.

Overarching Hypothesis: Individuals with a family history of cancer are at an increased risk for developing BTC compared to those with no family history of cancer.

CHAPTER II

2.1 Introduction

Biliary tract cancer (BTC), including cancers of the gallbladder (GBC), extrahepatic bile duct (EHBDC), intrahepatic bile duct (IHBDC), and ampulla of Vater (AVC), are rare in developed countries but are common in certain populations (1, 3). Because the symptoms from these cancers tend to be non-specific until the cancer is advanced, the cancers tend to be diagnosed at late stages, resulting in a poor prognosis (2). The incidence rate of the most common of these neoplasms, GBC, is greater than 20 per 100,000 among women in southern-central Chile (4) and nearly 8 per 100,000 among men in South Korea (3). GBC incidence is even higher among indigenous populations (5). In the United States, the incidence rates of BTC are highest among American Indians and Alaska Natives at 8.5 per 100,000, followed by Hispanics at a rate of 5.9 per 100,000 (6).

In addition to geographic and racial/ethnic variation, BTC incidence differs by sex and site within the biliary tract. Men are at higher risk for EHBDC while GBC is predominantly found in women (2). To date, most of the literature on BTCs is focused on GBC (8, 9, 14-16). Examples of independent risk factors for the development of GBC include personal history of gallstones (7-9, 13), family history of gallstones (8, 9, 15, 16), higher body-mass index (15), and history of diabetes (8). However, less is known about site-specific BTC risk factors for other sites within the biliary tract. While diabetes and family history of gallstones have also been implicated in other BTC sites (9, 11, 13), cholangiocarcinomas (EHBDC and IHBDC) are more notably associated with high alcohol consumption and a history of cigarette smoking (9, 19, 20, 35). There is evidence

to suggest that risk factors differ substantially across BTC sites; however, previous studies have been underpowered to evaluate site-specific differences because of their small sample size.

The geographic and racial/ethnic distributions of BTCs, as well as the associations reported between BTCs and family history of gallstones, suggest that there might be a genetic component underlying BTC development. Furthermore, the higher incidence of BTCs among those with hereditary cancer syndromes, such as hereditary nonpolyposis colorectal cancer, familial adenomatous polyposis patients, and Peutz-Jegher syndrome (24-26, 29), suggests that family history of cancer may be associated with a higher risk of BTC.

Previous studies were unable to address differences in BTC risk by site, sex, age, and race (9, 13, 16, 23, 24). Understanding whether family history may be associated with BTC risk across levels of these factors could inform clinical management in the future. For example, identifying groups at high-risk for developing BTC could encourage health professionals to monitor these patients more closely and thereby improve prognosis through earlier detection.

In the largest study of its kind, we analyzed data from seven cohorts participating in the Biliary Tract Cancers Pooling Project (BiTCaPP) to examine associations between family history of cancers and site-specific BTCs. We also investigated whether these associations differed by sex, age, and history of gallstones.

2.2 Methods

2.2.1 Study Populations

Prospective studies were invited to participate in the Biliary Tract Cancers Pooling Project (BiTCaPP), which was assembled to analyze associations of various exposures and other risk factors with BTCs. Twenty-eight prospective studies contributed data on over 2.8 million individuals with over 4,000 BTC cases. At the time of analysis, information about family history of cancer was available from the following seven studies: the AARP-NIH Diet and Health Study (AARP); the Agricultural Health Study (AgHealth); the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC); the Breast Cancer Detection Demonstration Project (BCDDP); Cancer Prevention Study-II (CPS-II); the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO); and the Women's Health Initiative (WHI).

The seven studies included in this analysis included four prospective cohort studies (AARP, AgHealth, BCDDP, CPS-II), two randomized controlled trials (ATBC, WHI), and one cancer screening trial (PLCO). Further information about each study can be found at the NCI Cancer Epidemiology Descriptive Cohort Database (https://cedcd.nci.nih.gov). The NCI Office of Human Subjects Research determined the BiTCaPP to be exempt from review by the Institutional Review Board (IRB). Each study that contributed data to the BiTCaPP was approved by its respective IRB. All data were received, processed, and harmonized through a third-party data broker (Information Management Services, Inc.; Calverton, MD), and statistical analyses were performed at NCI. This study was exempt from IRB review at Emory University.

Data from these seven studies included 1,202,900 participants, representing greater than 45% of the total BiTCaPP dataset. Individuals were excluded from the analysis if they were less than 18 years of age or missing age, or if they reported a

prevalent BTC at baseline and/or had negative or missing person-time (n=3,198). After exclusions, greater than 99% of the data from these seven studies was kept for analysis including 1,892 BTC cases and 1,197,810 non-cases.

2.2.2 Outcome assessment

Participants were followed and person-time was calculated from age at enrollment/randomization until age of cancer diagnosis, death, loss to follow-up, or study-specific end date, whichever occurred first. Cases included all individuals who developed incident tumors of the gallbladder (ICD-9 code 156.0 or ICD-O code C23.9), intrahepatic bile duct (155.1; C22.1), extrahepatic duct (156.1; C24.0), and ampulla of Vater (156.2; C24.1), as well as biliary tract not otherwise specified (156.9; C24.9) including overlapping sites (156.8; C24.8). Self-reported incident cases were confirmed by review of medical records and pathology reports by study physicians (ATBC, BCDDP, CPS-II, PLCO, WHI), or through linkage to cancer registries (AARP, AgHealth, ATBC, BCDDP, CPS-II), and/or National Death Index reports (AARP, BCDDP, CPS-II, PLCO). The primary analyses were conducted after excluding prevalent BTC, but included all incident BTC cases regardless of a previous history of other cancers (n=1,892).

2.2.3 Exposure assessment

Each study provided de-identified data on family history of cancers, which was collected by self-report in all seven studies and was supplemented by in-person or telephone interviews in two studies (AgHealth and WHI). Any family history of any cancer was defined as having at least one first- or second-degree relative with any cancer. All studies reported data on family history of cancer in first-degree relatives, and, except

for AgHealth, all provided relative-specific information on cancers in parents, siblings, and children (ATBC did not ask about children). AARP and BCDDP also reported family history of cancer in second-degree relatives, and AARP provided relative-specific information on cancers in grandparents, aunts, uncles, nieces, and nephews. The questionnaires for each study differed on the site-specific family history information that was requested (Supplemental Table 1). Questions on family history of breast and colorectal cancers were included in all seven studies. Questions on family history of ovarian, prostate, and pancreatic cancers were included in studies except for ATBC (ovarian), BCDDP (prostate and pancreatic), and WHI (pancreatic). Only two studies, CPS-II and PLCO, included data on family history of BTC.

2.2.4 Covariates

De-identified data were also provided on sex, age (years; continuous), race and ethnicity, level of education, smoking status[1], history of diabetes, and other characteristics. Race was collected by self-report and categorized as Caucasian, African American, or other. Education was categorized as less than high school, high school graduate or GED, some college. Sex (male/female), ethnicity (Hispanic/non-Hispanic), personal history of gallstones (yes or no/missing), history of cholecystectomy (yes or no/missing), diabetes (yes or no/missing), and smoking status (ever/never) were defined as binary variables. Data on personal history of gallstones was available from all studies except AgHealth, and cholecystectomy information was reported by four of the seven studies (AARP, ATBC, CPS-II, WHI).

2.2.5 Statistical analysis and Pooling

[1] At the time of analysis, smoking data was missing for all former smokers from the CPS-II study.

The characteristics of BTC cases and non-cases were compared using two-sample *t*-tests for continuous variables, χ^2 tests for two-level categorical variables, and Cochran-Armitage tests for trend (36) for ordinal variables. Results of these tests were considered statistically significant at $p < 0.05$. Cox proportional hazards regression models (36) with right censoring and left truncation were used to estimate hazard ratios (HR) and 95% confidence intervals (CI) for associations of the family history variables with BTCs. Time-to-event was defined as an individual's age at onset of BTC relative to their age at enrollment into the study. Individuals who reached the end of study-specific follow-up period (Table 1) without being diagnosed with BTC were censored. Any individuals who were lost to follow-up or died from other causes during the study-specific follow-up period were also censored.

Potential confounding variables considered for inclusion in the model were sex, age, calendar year at baseline (by decade), race, ethnicity, history of gallstones, history of cholecystectomy, diabetes, smoking status, and level of education. Variables meeting at least one of the following criteria were included in the final adjusted models: previous literature supporting association of the variable with exposure and outcome (Supplemental Table 1), adequate amount of individuals with data on the variable (no more than 20% of total individuals missing data), and whether or not including or excluding the variable in the model changed the adjusted hazard ratio estimate for the primary exposure variable by more than 10% (36). Proportional hazards assumptions were assessed by examining log-log survivor curves for each exposure variable and covariate over time.

The final adjusted models for study-specific estimates included sex, self-reported race, and calendar year at baseline as covariates. If a study population consisted of one race (ATBC) or only one sex (ATBC, BCDDP, and WHI), then that variable was dropped from the model. Models for each family history variable and BTC site were also conducted with and without adjustment for personal history of gallstones in addition to the afore mentioned covariates. If the study collected information on history of cholecystectomy (yes or no/missing), the initial multivariate GBC model was further restricted to those individuals who did not report a history of having a cholecystectomy. These individuals were later reintroduced to the GBC model for sensitivity analysis.

Hazard ratios pooled across the seven studies were estimated using a fixed effects Cox proportional hazards model adjusted for the aforementioned variables and stratified by study. A likelihood ratio test was used to assess the heterogeneity across studies by comparing a model that included a multiplicative interaction term between the study variable and the family history variable of interest to a model without the interaction term. If heterogeneity was found to be significant across studies, we reported study-specific hazard ratios.

Multiplicative interaction terms between overall family history and age group at baseline (years; <45, 45-65, >65), sex (male/female), and gallstones (yes or no/missing) were modeled to examine potential interactions. All analyses were conducted using SAS, version 9.3 (Cary, NC).

2.3 Results

The characteristics of the seven studies included in this analysis of the BiTCaPP are shown in **Table 1**. A total of 1,892 BTC cases were pooled across the seven studies,

with cases from each study comprising a similar relative percent (0.1%) of their respective total study population. AARP contributed nearly half of the participants in the dataset analyzed. Except for Finnish participants in the ATBC cohort, the contributing studies were from the United States.

Most of the total pooled study population was Caucasian (91%) and non-Hispanic (98%). The overall distributions of male and female participants were approximately equal although some studies were restricted to women (BCDDP, WHI) or men (ATBC) only (Supplemental Table 4). The proportion of participants with any family history of any cancer was similar among cases and non-cases in the pooled study population. However, the frequency of any family history was notably greater among non-cases from three studies: AgHealth (26.9% vs. 20.3% of cases); BCDDP (46.5% vs. 25.0% of cases); and PLCO (47.3% vs. 41.0% of cases) (Supplemental Table 5).

The overall characteristics of the study participants stratified by case/non-case status are shown in **Table 2**. On average, cases were older than non-cases and more likely to be male (54% vs. 48%, $p<0.0001$). There was no difference in the distribution of race or ethnicity between cases and non-cases. A personal history of gallstones was more common among cases than among non-cases (14% vs. 11%, $p<0.01$), although non-cases were more likely to have reported having had a cholecystectomy ($p>0.05$). Cases were more likely to be current or former smokers (64% vs. 58%, $p<0.0001$) and to have smoked at a higher intensity than did non-cases.

Twenty-nine percent of BTC cases had GBC (n=548), followed by 26% EHBDC (n=368), 20% IHBDC (n=488), and 18% AVC (n=344). The study-specific distribution of BTC case site was similar to the pooled distribution; GBC accounted for the largest

proportion of the cases in each study (Supplemental Table 6). One notable deviation from this distribution was that ATBC reported that 33% and 36% of BTC cases were IHBDC and EHBDC, respectively, compared to only 14% having GBC.

We observed inverse associations between any family history of cancer (HR=0.89, 95% CI=0.89-0.91), having a male relative with any cancer (HR=0.81, 95% CI=0.73-0.91), or any relative with a hormonally-driven cancer (HR=0.89, 95% CI=0.80-0.98) and all BTC sites combined when adjusting for sex, race, and calendar decade at study-specific baseline (**Table 3**). Additional adjustment for a personal history of gallstones did not substantially change these associations (**Table 3**). When all BTC sites were examined together, there was no difference in the association of any family history of cancer with BTC by sex or age (data not shown). There was some evidence of heterogeneity by BTC site (*p* for interaction=0.05).

For BTC site-specific associations, we observed near null or modest reductions in BTC risk among those with a family history of cancer. For example, we found that first- and second-degree family history of cancer was associated with a 25% risk reduction of GBC (HR=0.75, 95% CI=0.51-1.10) while the associations for EHBDC (HR=1.01, 95% CI=0.73-1.40), AVC (HR=0.93, 95% CI=0.63-1.37) and IHBDC (HR=0.95, 95% CI=0.64-1.41) were null. The site-specific risks for those individuals with a male relative with any cancer were also largely inverse, showing a near 20% risk reduction at three sites: EHBDC (HR=0.77, 95% CI=0.62-0.96), GBC (HR=0.80, 95% CI=0.64-1.02), IHBDC (HR=0.79, 95% CI=0.62-1.01). Notably, there was one positive association between family history of cancer and site-specific BTC risk, where having a female relative with any cancer was associated with a 17% increased risk of EHBDC (HR=1.17,

95% CI=0.96-1.42). Effect estimates for the remaining sites were at (GBC and AVC) or slightly below (IHBDC) the null. Results of the sensitivity analysis demonstrated that GBC estimates were only null when restricted to those individuals who did not report having a cholecystectomy, informing the importance of implementing this restriction. For the three studies included in our analysis that did not collect information on past cholecystectomy, we were unable to apply this restriction and observed strong inverse associations, which may have been due to informative censoring (Supplemental Table 7).

2.4 Discussion

The results from our pooled analysis of studies participating in the BiTCaPP suggest that there may be no association of family history of cancer with risk of developing BTC at any of the four BTC sites. The magnitude of the association estimates differed slightly depending on the family history variable being assessed, but most associations were slightly inverse or null for all cancer sites. Despite having greater power than studies previously conducted, our site-specific analyses still may have been limited because we saw significant and precise estimates when the four BTC sites were analyzed as one group.

The existing literature explaining the association of family history with site-specific GBC and cholangiocarcinoma risk is varied (20, 22, 23). In a case-control study (n=740 cases, 1408 controls) of digestive tract cancers from hospitals in Northern Italy, a family history of GBC was statistically significantly associated with a nearly 14-fold increased risk of GBC (OR=13.9, 95% CI=1.2-163.9) (23). Although more than 700 cases participated in this study, only 58 were pathology-confirmed GBC cases and only one case reported a family member with cancer. A similarly imprecise, yet positive,

association was found in a hospital-based case-control study in Northeast Thailand (n= 123 cases, 123 controls) where cases with a family history of cancer (n=38) had a 4-fold increased risk (OR=4.34, 95% CI=1.8-10.43) of cholangiocarcinoma (20).

Our null results are more consistent with those of a larger case-control study in Utah (n=1302 cases, 13,020 controls), which sought to determine the site-specific risk of GBC and cholangiocarcinoma in relatives of GBC and cholangiocarcinoma cases (22). GBC cases were not statistically significantly more likely to have relatives diagnosed with GBC (OR=1.20, 95% CI=0.51-2.8). Similarly, cholangiocarcinoma cases were not statistically significantly more likely to have relatives diagnosed with cholangiocarcinoma (OR=0.68, 95% CI=0.09-5.14).

When we modeled all four BTC sites together as one outcome to compare our results to those from existing studies, we found a modest but statistically significant inverse association with any family history of cancer. The results were again consistent with the findings of the Utah Cancer Registry study, which suggested an inverse and near-null association of BTC risk among relatives of BTC cases (OR=0.96, 95% CI=0.61-1.51). These parallel results suggest that the size of the Utah Cancer Registry study and our own large study are improvements upon prior studies that evaluated the association of a family history of any cancer with BTC risk and found higher relative risks, ranging from 1.16 to 1.52 (13, 24). However, one of these studies did not differentiate across cancers of the liver and biliary tract, thus limiting the comparability of these results to our own. Of these two prior studies, the one that reported a higher risk (OR=1.52, 95% CI=1.03-2.24) pooled data from 59 BTC cases and 228 controls who participated in two Italian case-control studies (13). Despite finding a positive

association, the precision and modest effect size found in this study is more consistent with our own findings when compared to the study of GBC that had reported a 14-fold higher risk.

Additionally, when the above study was restricted to a family history of any digestive tract cancers, gallbladder cancer, or intestinal cancers, all associations were null (13). We observed similar results when we restricted the exposure variable to a family history of gastrointestinal tract cancers. These findings may suggest that previous studies, which reported large risk estimates for BTC development among those with a family history of cancer, may have been biased and likely overestimated BTC risk by relying on a small number of total cases.

A key strength of our study was its large size, which allowed for site-specific analysis of BTCs across a range of family history variables. Because of their small number of cases, prior studies were limited and underpowered (13, 16, 20, 23, 24). This exemplifies the difficulty of studying rare cancers, which is further challenged by the low prevalence of persons with a family history of certain types of cancers, including BTCs. For example, Hemminki and Li (2003) sought to identify associations between family histories of individual types of cancer and site-specific risk of EHBDC and AVC, but were restricted to four and five cases who reported a family history of ovarian and thyroid cancer, respectively (24). In contrast, our preliminary analysis had more than 300 cases at each of the four BTC sites, among whom approximately 58% reported at least one family member with a history of any cancer. The large case count and variety of site- and relative-specific family history data collected across the seven studies allowed us to estimate cancer risk with more precision than was possible in previous studies.

Our study further benefited from ascertainment of key variables associated with BTCs, including personal history of gallstones and cholecystectomy. Gallstones are a known risk factor for all BTCs (7, 9, 13), and cholecystectomy is an important factor to consider when modeling risk of GBC, which was not taken into account in previous studies of GBC and family history of cancer (16, 22-24). Additionally, there is growing literature to support the hypothesis that cholecystectomy may increase risk of AVC (37-39). The study-specific GBC estimates for the three studies included in our analysis that did not collect information on history of cholecystectomy were dramatically skewed towards inverse associations compared to the remaining studies for which we restricted our analysis to those without a history of cholecystectomy.

While the range of family history variables available serves as a strength for the number of possible hypotheses that may be generated from the BiTCaPP, it is also a potential limitation due to the various methods of collecting data on family history of cancer across studies. Family histories of several common cancers, such as breast, colorectal, ovarian, and prostate, were collected by most studies, but the numbers were few for less common cancers. For example, family history of thyroid cancer, which was previously associated with EHBDC risk (24), was only collected by AgHealth and could not be accurately modeled as a risk factor for BTC. Furthermore, ascertainment of family history by self-report in all studies is susceptible to misclassification bias. When the accuracy of reporting family history of cancer was evaluated in several studies (40-42), most studies found that accurate reporting was not differential by case versus non-case status and would thus lead to an underestimation of the association between family history of cancer and BTC. The sensitivity for self-reporting a family history of cancer

differed across familial cancer sites and was as low as 0.20 for rare cancers (42). This could be an important factor to consider in evaluating BTC risk in cases with a family history of BTC, such misclassification could create a strong bias toward the null.

In the future, we plan to expand this analysis to include data from the remaining studies participating in BiTCaPP with available data on family history of cancers. This expansion will lend greater strength and precision to our estimates, as a consequence of a larger total case count at all BTC sites and the number of individuals with specific family histories (i.e., family history of gastrointestinal tract or hormonally-driven cancers). Future cohort studies could benefit from validating self-reported family history of cancer and by establishing a uniform method of ascertaining family histories of specific cancers to guarantee comparability to existing and future studies.

In summary, the findings from our large pooled analysis do not support a positive association of family history of cancer with risk of BTC at any of the four BTC sites. Our results are similar to those of at least one prior study and in contrast with a few smaller studies that reported a higher risk of BTC among those with a family history of cancer. This work may provide evidence against those associations since we were able to examine a greater number of BTC cases overall. Despite the greater number of cases included in this study, the power to detect site-specific differences may still have been limited thus informing the need to expand this study in the future.

Chapter III

3.1 Summary

Our findings suggest that there may be no positive association of family history of cancer with risk of developing GBC, IHBDC, EHBDC, or AVC. We considered a number of different family history of cancer variables, including the degree of familial separation and the relative's sex and cancer site, which have been largely unexamined in their associations with site-specific BTC risk. The magnitude of association estimates differed slightly depending on the family history variable being assessed, but most associations were null for all cancer sites or slightly inverse when the four BTC sites were assessed together.

These results are inconsistent with those of a few small studies, which found an increased risk of any BTC, non-specific cholangiocarcinoma, and GBC among cases with a family history of cancer (13, 20, 23, 24). However, a larger study of the Utah Cancer Registry found null associations similar to our own (22). To our knowledge, BiTCaPP is the largest study of its kind to examine associations between family history of cancers and site-specific BTC risk. Our findings suggest that the existing literature likely overestimated BTC risk by relying on a small number of total cases.

Our study is an improvement on past efforts to quantify the risk of BTC among those with a family history of cancer. In the largest investigation known to date, we examined site-specific BTC risk across a variety of family history variables, which had not previously been assessed.

Despite having a larger sample size than any previous investigation of BTC risk, site-specific risk estimates remained imprecise, and future studies would benefit from

continued pooling efforts. Additionally, our study was largely homogeneous in terms of race and Hispanic ethnicity, factors that have been demonstrated to be associated with increased BTC risk (5, 6). While we considered important competing risks and factors that could bias the associations between family history of cancer and BTC, including personal history of cholecystectomy and gallstones, information on these variables was not collected uniformly across all studies resulting in a notable amount of data missing differentially by study.

3.2 Future Directions

To further ascertain the relationship between family history of cancers and BTC, this analysis will be expanded to include data from five additional prospective cohorts participating in the BiTCaPP that collected information on family history. These studies which include the Health Professionals Follow-Up Study, Iowa Women's Health Study, Melbourne Collaborative Cohort Study, Multiethnic Cohort Study of Diet and Cancer, and Nurse's Health Study II, will add a degree of demographic variety to our study and allow us to further explore differences by race and ethnicity. This expansion will increase our overall study population by more than 400,000 individuals and 700 BTC cases with the aim of obtaining more precise estimates for site-specific BTC risk.

As emphasized above, our preliminary analysis was limited by a high amount of missing data for variables important in BTC risk prediction including ethnicity (13% missing), personal history of gallstones (7% missing), and cholecystectomy (24% missing). For the expanded analysis and all other analyses that come out of the BiTCaPP we plan to implement multivariate imputation techniques to impute data for variables (i.e., race/ethnicity, gallstones, cholecystectomy, diabetes, smoking) with less than 40%

missingness within each study included in the BiTCaPP (43). We hope that these methods will allow for more accurate BTC risk predictions, especially where data are missing not at random.

This study is the first exploration of the BiTCaPP, which provides a wealth of information to both strengthen this preliminary analysis and to identify environmental and lifestyle risk factors that may have a greater influence on BTC risk. Planned analyses using this dataset will examine the association of BTCs with body mass index, smoking, diabetes, and non-steroidal anti-inflammatory drug use to further understand site-specific differences in BTC risk. These findings have the potential to help clinicians identify patients whose medical history and lifestyle factors may put them at an increased risk of developing BTC to encourage lifestyle changes to decrease cancer risk or increased monitoring to diagnose these fatal cancers at an earlier stage.

3.3 Public Health Implications

As a group, BTCs have an abysmal 5-year survival rate, similar to that of lung cancer, at less than 15% (44). While incidence of GBC in most of the world appears to be on the decline, the rates of other BTCs are increasing in some regions (3, 4). In addition, GBC mortality remains extremely high in certain areas, such as Chile, where it is the second leading cause of cancer death in women (45). In contrast, the number of new cholangiocarcinoma cases is rising (6). Therefore, the need to understand the etiology and risk factors behind these fatal malignancies is of utmost importance.

We sought to better understand the etiology of GBC, IHBDC, EHBDC, and AVC. While some of the established BTC risk factors, such as diabetes and gallstones, may be genetically influenced (7-9, 11-13, 16), our study suggests that there is not a familial

predisposition for developing BTC and that environmental/lifestyle factors may have a greater influence. Further exploration of the BiTCaPP and of the association between family history of cancer and BTC may identify a link between a genetic and lifestyle factors, which could help inform cancer prevention and treatment in the future.

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Table 1. Summary of Study Characteristics by Study Included in the Biliary Tract Cancer Pooling Project

Study Name (Abbreviation)	Study Design	Study Pop.	Follow-Up Period	Baseline Cohort Sample Size, N (% of Total of Pooled)	Follow-Up Time (years), Mean (SD)	BTC, N (% of Study)
NIH-AARP Diet & Health Study (AARP)	Cohort	U.S.A.	1995-2011	566,377 (47.1)	13.9 (3.6)	951 (0.2)
Agricultural Health Study (Ag Health)	Cohort	U.S.A.	1993-2013	89,655 (7.4)	16.8 (3.6)	79 (0.1)
Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC)	RCT	Finland	1985-2010	29,133 (2.4)	16.4 (7.3)	125 (0.4)
Breast Cancer Detection Demonstration Project (BCDDP)	Cohort	U.S.A.	1980-1998	50,942 (4.2)	7.9 (1.4)	40 (0.1)
Cancer Prevention Study-II (CPS-II)	Cohort	U.S.A.	1982-2011	155,092 (12.9)	14.9 (5.0)	264 (0.2)
Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial	Screening Trial	U.S.A.	1993-2009	149,893 (12.5)	11.1 (2.5)	171 (0.1)
Women's Health Initiative (WHI)	RCT	U.S.A.	1993-2014	161,808 (13.5)	13.7 (4.8)	262 (0.2)
Total				**1,202,900**	**13.7 (4.3)**	**1,892 (0.2)**

Abbreviations: BTC, Biliary Tract Cancer; SD, Standard Deviation; U.S.A., United States of America; RCT, Randomized controlled trial.

Table 2. Participant Characteristics by Biliary Tract Cancer Case-Non Case Status in the Biliary Tract Cancers Pooling Project – A Prospective Study from 1980 - 2014*

Characteristic	BTC Case	Non-Case	*P*-value
N (%)	1,892 (0.1)	1,197,810 (99.9)	N/A
Sex: %			
Male	53.5	47.6	
Female	46.5	52.4	**<0.0001**
Enrollment Age (years): mean (SD)	63.8 (6.4)	61.0 (8.0)	**<0.0001**
Race: *%*			
Caucasian	92.9	92.7	
African American	4.2	4.2	
Other	2.9	3.1	0.93
Ethnicity: %			
Non-Hispanic	98.0	98.1	
Hispanic	2.0	1.9	0.61
Gallstones at Baseline: %			
No	86.3	88.6	
Yes	13.7	11.4	**0.002**
Cholecystectomy: %			
No	87.2	86.5	
Yes	12.8	13.5	0.39
Diabetes at Baseline: %			
No	91.7	92.9	
Yes	8.4	7.1	**0.03**
Smoking Status[2]: %			
Never	35.8	42.2	
Former	43.5	40.3	
Current	20.7	17.5	**<0.0001**
Smoking Pack-year History among Smokers: mean (SD)	35.5 (25.7)	31.2 (25.6)	**<0.0001**
Education: %			
Less than High School	13.8	9.1	
High School Grad/GED	22.6	23.3	
College+	63.6	67.7	**<0.0001**
BTC Site: N (%)			
Gallbladder	548 (29.0)		
Intrahepatic Bile Duct	368 (19.5)		
Extrahepatic Bile Duct	488 (25.8)		
Ampulla of Vater	344 (18.2)		
Other	141 (7.5)		

Abbreviations: BTC, Biliary Tract Cancer; SD, Standard Deviation.

[1]Data are missing for the following variables from cohorts with data on any BTC: Race – 21,607; Ethnicity – 155,229; Gallstones at Baseline – 89,143; Cholecystectomy – 288,769; Smoking Status – 30,518; Education level – 26,782; BTC site – 3.

[2]Smoking-status was missing for all former smokers from the Cancer Prevention Study-II at the time of this preliminary analysis.

*Pooled studies include: AARP-NIH Diet and Health Study ; Agricultural Health Study ; Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study ; Breast Cancer Detection Demonstration Project; Cancer Prevention Study-II ; Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; and Women's Health Initiative

Table 3. Association of Family History of Cancer with Biliary Tract Cancers in the Biliary Tract Cancers Pooling Project – A Prospective Study from 1980 - 2014*

					BTC Sites			
Family History Variable	BTC Case[1], N (%)	BTC Non-Case, N (%)	**All BTC sites** Adjusted for Covariates[2†], HR (95% CI)	**All BTC sites** Adjusted for Covariates and Gallstones, HR (95% CI)	**GBC** Adjusted for Covariates, HR (95% CI)	**EHBDC** Adjusted for Covariates, HR (95% CI)	**AVC** Adjusted for Covariates, HR (95% CI)	**IHBDC** Adjusted for Covariates, HR (95% CI)
Any Family History of Any Cancer	1,102 (58)	701,848 (59)	0.89 (0.81 - 0.98)	0.90 (0.82 - 0.99)	0.88 (0.71 - 1.08)	0.98 (0.81 - 1.18)	0.99 (0.79 - 1.24)	0.89 (0.72 - 1.00)
First-Degree Family History of Cancer	962 (51)	606,284 (51)	0.91 (0.83 - 1.00)	0.92 (0.84 - 1.02)	0.85 (0.69 - 1.04)	1.02 (0.85 - 1.22)	1.03 (0.83 - 1.29)	0.91 (0.73 - 1.12)
Second-Degree Family History of Cancer	235 (24)	159,565 (27)	0.91 (0.78 - 1.05)	0.91 (0.78 - 1.05)	0.88 (0.64 - 1.20)	0.96 (0.72 - 1.28)	0.87 (0.62 - 1.22)	0.88 (0.62 - 1.25)
First- and Second-Degree Family History of Cancer	159 (8)	104,117 (9)	0.93 (0.78 - 1.11)	0.93 (0.78 - 1.10)	0.75 (0.51 - 1.10)	1.01 (0.73 - 1.40)	0.93 (0.63 - 1.37)	0.95 (0.64 - 1.41)
Female Relative with History of Any Cancer	675 (36)	398,000 (33)	1.01 (0.91 - 1.12)	1.01 (0.91 - 1.12)	0.98 (0.80 - 1.21)	1.17 (0.96 - 1.42)	0.98 (0.77 - 1.24)	0.89 (0.70 - 1.12)
Male Relative with History of Any Cancer	496 (26)	322,775 (27)	0.81 (0.73 - 0.91)	0.81 (0.73 - 0.91)	0.80 (0.64 - 1.02)	0.77 (0.62 - 0.96)	0.96 (0.75 - 1.24)	0.79 (0.62 - 1.01)
Family History of GI Tract Cancers	244 (13)	153,115 (13)	0.92 (0.80 - 1.05)	0.93 (0.81 - 1.06)	0.87 (0.65 - 1.16)	1.02 (0.79 - 1.33)	0.93 (0.68 - 1.28)	0.91 (0.67 - 1.24)
Family History of Hormonally-Driven Cancers	473 (25)	318,198 (27)	0.89 (0.80 - 0.98)	0.89 (0.80 - 0.99)	0.90 (0.72 - 1.12)	0.98 (0.79 - 1.20)	0.91 (0.71 - 1.16)	0.79 (0.62 - 1.02)

Abbreviations: BTC, Biliary Tract Cancer; HR, Hazard Ratio; CI, Confidence Interval; GBC, Gallbladder Cancer; EHBDC, Extrahepatic Bile Duct Cancer; AVC, Ampulla of Vater Cancer; Intrahepatic Bile Duct Cancer; GI, Gastrointestinal.

[1]Any BTC

[2]Covariates in adjusted models included: sex, race, calendar year at baseline

[†]Wald test for interaction by BTC site and any family history of cancer, p = 0.05

*Pooled studies include: AARP-NIH Diet and Health Study ; Agricultural Health Study ; Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study ; Breast Cancer Detection Demonstration Project; Cancer Prevention Study-II ; Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; and Women's Health Initiative

Supplemental Table 1. Previously Reported Risk Factors for Biliary Tract Cancers by Biliary Tract Cancer Site

Biliary Tract Cancer Site	Risk Factors
Gallbladder	Personal history of gallstones Family History of gallstones Lower education level Hot Hungarian pepper consumption Race Increased BMI Typhoid infection Age Parity Lower socio-economic status Rural residence Chemical Exposure Diabetes Smoking
Intrahepatic Bile Duct	Diabetes Cholelithiasis Cholecystolithiasis Previous cholecystectomy HBV infection High alcohol consumption Alcoholic liver disease Biliary and nonspecific cirrhosis Thyrotoxicosis Chronic Pancreatitis
Extrahepatic Bile Duct	Diabetes Cholelithiasis HBV infection Alcoholic liver disease Biliary and nonspecific cirrhosis Thyrotoxicosis Chronic pancreatitis
Ampulla of Vater	Diabetes Cholecystolithiasis Chronic pancreatitis Total cholesterol High density lipoprotein Apolipoprotein A

Supplemental Table 2. Characteristics and Findings of Epidemiologic Studies Investigating Family History of Cancer in Development of Biliary Tract Cancer

Study	Study Type	Population	Exposure	Endpoint	# cases	OR/SIR/HR[1] (95% CI)
Fernandez *et al.*, 1994	Case-Control	Northern Italy/Hospital controls	FamHx of GBC	GBC	1	13.9 (1.2-163.9)
Hemminki and Li, 2003	Case-Control	Sweden/Cancer Registry Data	Parental liver and biliary cancers	Offspring GBC	14	3.13 (1.70-5.26)
			Parental pancreatic cancer	Offspring GBC	12	2.39 (1.23-4.18)
			Offspring liver and biliary cancers	Parental GBC	14	1.93 (1.05-3.25)
			Offspring cervical cancer			
			Parental ovarian cancer	Parental GBC	40	1.63 (1.17-2.23)
			Parental thyroid cancer	Offspring EHBDC	4	3.83 (1.00-9.91)
				Offspring AVC	5	3.40 (1.07-7.99)
Kumar *et al.*, 2006	Case-Control	Varansi, India/Hospital controls with gallstone disease	FamHx of GBC	GBC	20	3.48 (1.38-8.79)
Manwong *et al.*, 2013	Case-Control	Northeast Thailand/Hospital controls	FamHx of any cancer	Cholangiocarcinoma	38	4.34 (1.8-10.43)
Rosato *et al.*, 2016	Pooled two Case-Control studies	Italy/Hospital controls	FamHx of any cancer	Any BTC	59	1.52 (1.03-2.24)
			FamHx of GBC	Any BTC	2	3.83 (0.59-24.75)
			FamHx of any digestive tract cancer	Any BTC	32	1.39 (0.89-2.17)
Samadder *et al.*, 2016	Case-control	Utah Cancer Registry	BTC case	FDR w/ BTC	5	0.95 (0.28-3.09)
			BTC case	SDR w/ BTC	2	0.25 (0.06-1.03)
			BTC case	FC w/ BTC	20	0.96 (0.61-1.51)
			GBC case	SDR w/ GBC	9	2.15 (0.46-9.94)
			GBC case	FC w/ GBC	48	1.20 (0.51-2.8)
			Cholangiocarcinoma case	FDR w/ cholangiocarcinoma	15	0.68 (0.09-5.14)
			Cholangiocarcinoma case	FC w/ cholangiocarcinoma	52	1.56 (0.74-3.29)

Abbreviations: GBC, Gallbladder Cancer; BTC, Biliary Tract Cancer; OR, Odds Ratio; CI, Confidence Interval; EHBDC, Extrahepatic Bile Duct Cancer; AVC, Ampulla of Vater Cancer; FDR, First Degree Relative; SDR, Second Degree Relative; FC, First Cousin; SIR, Standardized Incidence Ratio; HR, Hazard Ratio

[1]ORs calculated for all studies except Hemminki and Li (SIRs) and Samadder et al (HRs).

Supplemental Table 3. Family History Variables Collected by Study Included in the Biliary Tract Cancers Pooling Project – A Prospective Study from 1980 - 2014

Family History of Cancer Sites	AARP	AgHealth	ATBC	BCDDP	CPS-II	PLCO	WHI
Breast	X	X	X	X	X	X	X
Biliary Tract					X	X	
Colorectal	X	X	X	X	X	X	X
Esophageal		X			X	X	
Liver		X			X	X	
Ovarian	X	X		X	X	X	X
Prostate	X	X	X		X	X	X
Pancreatic	X	X	X		X	X	
Stomach		X	X				
Thyroid		X					

Abbreviations: AARP, AARP-NIH Diet and Health Study; AgHealth, Agricultural Health Study; ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; BCDDP, Breast Cancer Detection Demonstration Project; CPS-II, Cancer Prevention Study-II; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; WHI, Women's Health Initiative.

Supplemental Table 4. Summary of Baseline Participant Characteristics[1] by Study Included in the Biliary Tract Cancers Pooling Project – A Prospective Study from 1980 – 2014

			Race: %				Education Level: %						
Study	**Women %**	**Age Mean (SD)**	**Caucasian**	**African American**	**Other**	**Hispanic Ethnicity %**	**Less Than High School**	**High School**	**Some College**	**Gallstones %**	**Cholecys-tectomy %**	**Diabetes %**	**Ever Smoker %**
AARP	40.0	62 (5)	91.2	3.9	1.6	1.9	6.4	19.7	70.9	9.7	15.3	9.3	61.5
AgHealth	37.6	47 (13)	95.0	1.7	0.6	1.0	7.8	41.8	42.9	N/A	N/A	3.0	38.9
ATBC	0	57 (5)	100.0	0.0	0.0	0.0	94.2	5.8	0.0	5.6	4.9	4.3	100.0
BCDDP	100	62 (8)	90.2	4.1	5.7	2.0	12.2	41.9	45.1	19.3	N/A	9.1	42.5
CPS-II	52.6	63 (6)	97.7	1.4	0.6	0.4	6.6	25.8	66.9	11.6	9.6	7.4	55.2
PLCO	50.8	63 (5)	88.4	5.1	6.4	1.9	7.4	22.9	69.4	11.4	N/A	7.7	53.8
WHI	100.0	63 (7)	82.5	9.0	8.2	4.0	5.3	17.1	76.8	16.2	12.8	0.8	48.9
Total	**52.4**	**61 (8)**	**91.0**	**4.2**	**3.0**	**1.9**	**8.9**	**22.8**	**66.1**	**10.6**	**10.3**	**7.1**	**56.5**

Abbreviations: SD, Standard Deviation; AARP, AARP-NIH Diet and Health Study; AgHealth, Agricultural Health Study; ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; BCDDP, Breast Cancer Detection Demonstration Project; CPS-II, Cancer Prevention Study-II; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; WHI, Women's Health Initiative.

[1]Data are missing for the following variables: Race – 21,655; Ethnicity – 182,206; Education – 26,872; Gallstones at Baseline – 89,655; Cholecystectomy – 290,490; Diabetes – 12,089; Smoking status – 28,890.

Supplemental Table 5. Frequency of Family History Variables by Biliary Tract Cancer Case-Non Case Status by Study Included in the Biliary Tract Cancers Pooling Project – A Prospective Study from 1980 - 2014

	Any Family History of Any Cancer		First-Degree Relative with Any Cancer		Second-Degree Relative with Any Cancer	
Study	**BTC Case N (%)**	**Non-Case N (%)**	**BTC Case N (%)**	**Non-Case N (%)**	**BTC Case N (%)**	**Non-Case N (%)**
AARP	625 (65.7)	380,402 (67.4)	553 (58.2)	331,464 (58.7)	229 (24.1)	148,441 (26.3)
AgHealth	16 (20.3)	23,926 (26.9)	16 (20.3)	23,926 (26.9)	N/A	N/A
ATBC	38 (30.4)	8,960 (30.9)	38 (30.4)	8,960 (30.9)	N/A	N/A
BCDDP	10 (25.0)	23,579 (46.5)	6 (15.0)	16,954 (33.4)	6 (25.0)	11,124 (32.9)
CPS-II	167 (63.3)	93,554 (60.4)	167 (63.3)	93,554 (60.4)	N/A	N/A
PLCO	71 (41.5)	70,247 (47.3)	71 (41.5)	70,247 (47.3)	N/A	N/A
WHI	175 (66.8)	102,161 (63.5)	111 (42.4)	62,160 (38.6)	N/A	N/A
Total	**1,102 (58.3)**	**701,848 (58.6)**	**962 (50.9)**	**606,284 (50.6)**	**235 (24.1)**	**159,565 (26.7)**

Abbreviations: BTC, Biliary Tract Cancer; AARP, AARP-NIH Diet and Health Study; AgHealth, Agricultural Health Study; ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; BCDDP, Breast Cancer Detection Demonstration Project; CPS-II, Cancer Prevention Study-II; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; WHI, Women's Health Initiative.

Supplemental Table 6. Any Biliary Tract Cancer Case-Non Case Status by Study Included in the Biliary Tract Cancers Pooling Project – A Prospective Study from 1980 - 2014

Study	Non-Case *N (%)*	BTC Case *N (%)*	GBC Case *N (%)*	IHBDC Case *N (%)*	EHBDC Case *N (%)*	AVC Case *N (%)*	Other Case *N* (%)
AARP	564,642 (99.8)	951 (0.2)	267 (28.1)	179 (18.8)	264 (27.8)	191 (20.0)	50 (5.3)
AgHealth	89,064 (99.9)	79 (0.1)	23 (29.1)	20 (25.3)	17 (21.5)	13 (16.5)	6 (7.6)
ATBC	29,008 (99.6)	125 (0.4)	17 (13.6)	41 (32.8)	45 (36.0)	16 (12.8)	6 (4.8)
BCDDP	50,748 (99.9)	40 (0.1)	12 (30.0)	8 (20.0)	8 (20.0)	9 (22.5)	3 (7.5)
CPS-II	154,827 (99.8)	264 (0.2)	81 (30.8)	67 (25.5)	58 (22.1)	42 (16.0)	15 (5.7)
PLCO	148,667 (99.9)	171 (0.1)	49 (28.7)	25 (14.6)	56 (32.8)	36 (21.1)	5 (2.9)
WHI	160,854 (99.8)	262 (0.2)	99 (38.1)	28 (10.8)	40 (15.4)	37 (14.2)	56 (21.5)
Total	**1,197,810**	**1,892**	**548 (29.0)**	**368 (19.5)**	**488 (25.8)**	**344 (18.2)**	**141 (7.5)**

Abbreviations: BTC, Biliary Tract Cancer; GBC, Gallbladder Cancer; EHBDC, Extrahepatic Bile Duct Cancer; AVC, Ampulla of Vater Cancer; Intrahepatic Bile Duct Cancer; AARP, AARP-NIH Diet and Health Study; AgHealth, Agricultural Health Study; ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; BCDDP, Breast Cancer Detection Demonstration Project; CPS-II, Cancer Prevention Study-II; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; WHI, Women's Health Initiative.

Supplemental Table 7. Associations of Family History of Cancer and Gallbladder Cancer by Cohort Included in the Biliary Tract Cancers Pooling Project – A Prospective Study from 1980 – 2014

Study	HR[1] (95% CI)
AARP[2]	0.78 (0.60-1.04)
AgHealth	0.29 (0.09-0.98)
ATBC[2]	1.13 (0.41-3.10)
BCDDP	0.40 (0.11-1.49)
CPS-II[2]	0.83 (0.52-1.33)
PLCO	0.55 (0.30-1.00)
WHI[2]	1.11 (0.74-1.72)
Pooled	0.89 (0.71-1.12)

Abbreviations: HR, Hazard Ratio; CI, Confidence Interval; AARP, AARP-NIH Diet and Health Study; AgHealth, Agricultural Health Study; ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; BCDDP, Breast Cancer Detection Demonstration Project; CPS-II, Cancer Prevention Study-II; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; WHI, Women's Health Initiative.

[1]Adjusted for sex, race, and calendar year at baseline

[2]Restricted to those with no personal history of cholecystectomy