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Aspirin Use and the Risk of Cholangiocarcinoma‡

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Abstract

Whether aspirin use is protective against cholangiocarcinoma (CCA) remains unclear. We determined the association between aspirin use and other risk factors for each CCA subtype individually. In a hospital-based case-control study, 2395 CCA cases (1169 intrahepatic, 995 perihilar, and 231 distal) seen at the Mayo Clinic, Rochester, MN, from 2000 through 2014 were enrolled. Controls selected from the Mayo Clinic Biobank were matched two to one with cases by age, sex, race, and residence (n 5 4769). Associations between aspirin use, other risk factors, and CCA risk were determined. Aspirin was used by 591 (24.7%) CCA cases and 2129 (44.6%) controls. There was a significant inverse association of aspirin use with all CCA subtypes, with adjusted odds ratios (AORs) of 0.35 (95% confidence interval [CI], 0.29-0.42), 0.34 (95% CI 0.27-0.42), and 0.29 (95% CI 0.19-0.44) for intrahepatic, perihilar, and distal CCA, respectively ($P < 0.001$ for all). Primary sclerosing cholangitis was more strongly associated with perihilar (AOR 5 453, 95% CI 104-999) than intrahepatic (AOR 5 93.4, 95% CI 27.1-322) or distal (AOR 5 34.0, 95% CI 3.6-323) CCA, whereas diabetes was more associated with distal (AOR 5 4.2, 95% CI 2.5-7.0) than perihilar (AOR 5 2.9, 95% CI 2.2-3.8) or intrahepatic (AOR 5 2.5, 95% CI 2.0-3.2) CCA. Cirrhosis not related to primary sclerosing cholangitis was associated with both intrahepatic

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Supporting Information

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and perihilar CCA, with similar AORs of 14. Isolated inflammatory bowel disease without primary sclerosing cholangitis was not associated with any CCA subtype.

Conclusions—: Aspirin use was significantly associated with a 2.7-fold to 3.6-fold decreased risk for the three CCA subtypes; our study demonstrates that individual risk factors confer risk of different CCA subtypes to different extents.

Cholangiocarcinoma (CCA), a cancer arising from the bile duct epithelium, is the second most common primary liver cancer. CCA is currently classified into three subtypes by anatomic location: intrahepatic (iCCA), perihilar (pCCA), and distal (dCCA). Although these three subtypes share some commonalities, they are considered to be separate diseases due to the differences in their genetics, presentations, management, and outcomes.

The incidence of CCA has been increasing in the United States.[1] CCA is one of the most fatal cancers, with 5-year survival rates for iCCA and pCCA of 30%-40% after complete resection and a median survival of patients with unresectable disease of only 12-15 months.[2,3] Due to limited treatment options and the dismal prognosis, the early detection of CCA and recognition of risk and protective factors are crucial for improving patient outcomes.

Parasitic infection of the biliary tract, primary sclerosing cholangitis (PSC), bile duct cysts, hepatolithiasis, and toxins (e.g., Thorotrast) are established risk factors for CCA.[4–6] Inflammatory bowel disease (IBD), hepatitis B virus (HBV), hepatitis C virus (HCV), cirrhosis, diabetes, obesity, and smoking have been proposed to be risk factors for CCA; but the available evidence remains conflicting.[4,6,7] This is in part due to the relatively low CCA incidence in Western populations, making it difficult to assemble large enough cohorts to achieve adequate power for statistical analysis. Accordingly, most previous studies on CCA risk factors combined pCCA and dCCA as extrahepatic CCA or combined all three subtypes together. Furthermore, Klatskin tumors, which are pCCA, may have been misclassified as iCCA under prior versions of the *International Classification of Disease* coding system, and this may have affected the attribution of risk factors for each CCA subtype.[8] Whether the identified risk factors confer risk to all CCA subtypes to a similar magnitude is not yet known. Because the three CCA subtypes are distinct molecular and clinical entities, it is important to determine the risk factors for each subtype separately.

Aspirin (acetylsalicylic acid), an antiplatelet and anti-inflammatory agent, has been widely used to reduce occlusive vascular events and inflammation. Aspirin inhibits the cyclooxygenase (COX) enzyme, and its antiplatelet effect acts through inhibition of the COX-1 isozyme. Aspirin also inhibits the COX-2 isozyme, which is known to trigger inflammation and to contribute to the development of many cancers. Long-term follow-up of multiple randomized trials has revealed that aspirin reduced the risk of colorectal cancer after a delay of several years.[9] Also, daily aspirin significantly reduced the risk of death from gastrointestinal tract cancers both during and after the trials.[10] The putative chemopreventive effect of aspirin has been attributed to a number of pathways, including inhibition of COX-2, inhibition of nuclear factor jB activation, and regulation of DNA mismatch repair proteins.[11,12]

Because overexpression of COX-2 was shown to promote growth and invasion of CCA cells, we hypothesized that aspirin use reduces the risk of CCA development. We therefore conducted a case-control study in a large CCA cohort to determine whether there is an association between aspirin use and CCA development. Secondarily, we comprehensively evaluated risk factors for CCA, including analyses stratified by the three CCA subtypes.

Patients and Methods

STUDY POPULATION

All CCA patients seen at the Mayo Clinic from January 2000 through December 2014 were included. All potential CCA cases were identified by searching the Mayo Clinic Life Sciences System database with the *International Classification of Diseases-9 Clinical Modification* codes of 155.1, 156.1, 156.2, 156.8, and 156.9 and the keywords "cholangiocarcinoma," "bile duct cancer," "intrahepatic cholangiocarcinoma," "extrahepatic cholangiocarcinoma," "hilar cholangiocarcinoma," "perihilar cholangiocarcinoma," "Klatskin tumor," and "distal cholangiocarcinoma" (n = 2865). CCA was unequivocally diagnosed by histopathology. Patients with mixed cholangiocarcinoma-hepatocellular carcinoma (n = 16), gallbladder cancer (n = 49), pancreatic cancer (n = 63), hepatocellular carcinoma (n = 63), ampulla of Vater cancer (n = 19), neuroendocrine tumor (n = 4), cancer of unknown primary origin (n = 37), and metastatic cancer from other primary sites to the liver (n = 24) were excluded. Fifteen patients diagnosed with CCA before the study period who had recurrence between 2000 and 2014 and another 180 patients with inadequate information to confirm the diagnosis of CCA were excluded. The final cohort comprised 2395 CCA patients. Radiologic images (computerized tomography, magnetic resonance imaging, and/or endoscopic retrograde cholangiopancreatography) were reviewed to delineate the anatomic tumor location. Classification of the tumor location was as described.[13]

Control subjects were recruited from the Mayo Clinic Biobank. The Mayo Clinic Biobank comprises a collection of blood samples and health information provided with informed consent by Mayo Clinic patients and other community volunteers. Biobank participants allow access to all data from their Mayo Clinic medical records for health research. Fifty-six percent of Mayo Clinic Biobank participants had at least 15 years of electronic medical record (EMR) data, and 77% had at least two clinic visits per year and length of follow-up available in the EMR of greater than 1 year.[14] With the availability and continuity of data, the Mayo Clinic Biobank data set provides a good control group for epidemiologic studies of Mayo Clinic patients. The Mayo Clinic Biobank is not population-based, so it may not completely represent the general population; however, because the case group for this study was drawn from Mayo Clinic patients and not from the general population, the selection of matched controls from the Mayo Clinic Biobank helped to minimize selection bias. At the time of this study, 35,000 subjects were available for selection as controls. Cases were frequency-matched one to two with controls who participated in the Mayo Clinic Biobank from April 2009 through March 2015 for age (±5 years), sex, race, and residence (Olmsted County, Minnesota; Southeast Minnesota; Other Minnesota; Iowa; Wisconsin; North and South Dakota; and other regions of the United States [Northeast, Southeast, Southwest,

Northwest, and Midwest]). Controls with a history of cancers other than nonmelanoma skin cancer were excluded. The study was approved by the Mayo Clinic institutional review board.

CLINICAL INFORMATION

Clinical data were abstracted from the EMR. Data on risk factors of CCA cases were abstracted from the general health information form that is routinely completed by patients receiving care at the Mayo Clinic and added to the medical record. Self-reported data on risk factors of controls were gathered from the Mayo Clinic Biobank questionnaire. The EMRs of all study patients were then manually reviewed to confirm the accuracy of abstracted data and to maintain consistency of the data abstraction methods between cases and controls.

PSC was diagnosed by histopathology or clinical criteria as recommended by the PSC practice guidelines of the American Association for the Study of Liver Diseases. Cirrhosis was diagnosed by histopathology or radiographic evidence (nodular liver, caudate lobe hypertrophy, or portal hypertension). We also categorized subjects with cirrhosis but without PSC as non-PSC-related cirrhosis in order to evaluate cirrhosis as a risk factor for CCA. Biliary tract diseases included choledochal cyst and hepatolithiasis. IBD including ulcerative colitis and Crohn's disease was diagnosed by histopathology or typical endoscopic findings. HBV and HCV infections were defined as hepatitis B surface antigen positivity and positive HCV RNA, respectively. Because outside laboratory test results were not always abstracted into the medical record, we accepted as proof of viral infection a physician's note reporting a diagnosis of HBV or HCV infection. Nonalcoholic fatty liver disease or nonalcoholic steatohepatitis was diagnosed when a subject had histopathologic or radiologic evidence of fatty infiltration in the liver with elevation of liver enzymes after exclusion of other liver diseases and history of excessive alcohol intake (>140 and >70 g/week in men and women, respectively). Obesity was defined as body mass index 30 kg/m^2. *Ever-smoker* was defined as any person having a history of smoking.

A history of aspirin use was determined from medication lists and physicians' notes, which were manually reviewed at least 1 year before the index date (i.e., date of CCA diagnosis in CCA cases or date of Mayo Clinic Biobank enrollment in controls). Subjects were classified as current aspirin users if they were taking aspirin at least once per week at the index date. This definition was not altered if the medical record data indicated that aspirin was held during the investigation for CCA diagnosis or patients were advised by health care providers to avoid taking aspirin due to CCA-related symptoms. This definition prevents us from inflating the effect size of aspirin by underestimating the frequency of aspirin use in the case group. Patients who had ever taken aspirin but stopped taking aspirin 1 year or more before the index date were not coded as current aspirin users.

To determine the impact of aspirin dosage on CCA risk, aspirin dose was classified into two groups: low-dose aspirin (81-162 mg/day) and high-dose aspirin (≥325 mg/day). If the dose of aspirin had been changed during different time periods, the dose was determined by the dose at the time of study enrollment (i.e., at the time of CCA diagnosis for cases or at the time of Mayo Clinic Biobank enrollment for controls). Relevant factors influencing aspirin

use including hypertension, coronary artery disease, peripheral vascular disease, atrial fibrillation, and cerebrovascular accident (CVA) were also abstracted.

STATISTICAL ANALYSIS

Comparisons were performed using the Student *t* test for continuous variables and the chi-squared or Fisher exact test for categorized variables. Logistic regression analysis was used to identify factors associated with CCA development and to calculate odds ratios (ORs), adjusted odds ratios (AORs), and 95% confidence intervals (CIs). To avoid selection bias and potential confounding factors that might influence the chance of being prescribed aspirin, a multivariate analysis with propensity score adjustment was used to confirm the findings of the initial analyses. Propensity scores were computed by a binary logistic regression model consisting of the following input variables: age, sex, race, obesity, hypertension, diabetes, CVA, coronary artery disease, peripheral vascular disease, atrial fibrillation, nonalcoholic fatty liver disease/nonalcoholic steatohepatitis, PSC, cirrhosis, IBD, and smoking status. In addition to the main analysis, ORs of aspirin use stratified by tumor location were calculated. $P < 0.05$ was considered statistically significant. All analyses were performed using SAS statistical software (SAS Institute, Cary, NC) and R 3.1.3. (R Foundation for Statistical Computing; http://www.r-project.org).

SENSITIVITY ANALYSIS

Because the OR of PSC was dominant compared to other risk factors, sensitivity analysis excluding PSC patients was performed. Due to an imbalance between enrollment periods of cases (years 2000-2014) and controls (years 2009-2015), we performed sensitivity analyses categorizing the groups by CCA diagnosis year: group 1, 2000-2003; group 2, 2004-2007; group 3, 2008-2011; and group 4, 2012-2014. Changes in frequency of aspirin use and other variables over time in CCA cases were assessed by analysis of variance.

Information on the duration of aspirin use was available in the medical records only for 45.1% of current aspirin users. To minimize the proportion of patients with missing duration-of-aspirin use data, we repeated the analyses restricting to the 1321 subjects (165 cases/1156 controls, 18.4% of the entire cohort) living in the 11 counties in southeastern Minnesota that participate in the Rochester Epidemiology Project (REP). The REP is described in detail in the Supporting Information. Data on the duration-of-aspirin use were available for 97% of current aspirin users in this subset. In addition to the REP subset, we performed a second analysis restricted to the 1968 subjects (613 cases/1355 controls, 27.5% of the entire cohort) resident in the state of Minnesota. Data on the duration-of-aspirin use were available for 90.4% of current aspirin users who were Minnesota residents. We also performed conditional logistic regression analysis for assessing risk factors for CCA in 2172 cases and 4286 perfectly matched controls.

Results

PATIENT CHARACTERISTICS

There were 2395 CCA cases, including 1169 (48.8%) iCCAs, 995 (41.5%) pCCAs, and 231 (9.6%) dCCAs, as well as 4769 controls (Table 1). Male sex constituted 1094 (45.7%) of

cases and 2170 (45.5%) of controls. The mean ages were 61.5 and 61.6 years for cases and controls, respectively. The majority of the study population (95.5%) was white. The prevalences of PSC, biliary tract diseases, cirrhosis, HBV infection, HCV infection, IBD, diabetes, and smoking were higher in cases than in controls ($P = 0.006$ for HBV; $P < 0.001$ for all others).

RISK FACTORS FOR CCA

By multivariate analysis, PSC was the most significant risk factor for CCA, with an AOR of 171 (95% CI 72.6-404; $P < 0.001$) (Table 2). Biliary tract diseases (AOR = 12.1, 95% CI 4.0-36.9; $P < 0.001$), non-PSC-related cirrhosis (AOR = 10.8, 95% CI 6.5-18.0; $P < 0.001$), HBV infection (AOR = 2.8, 95% CI 1.0-7.4; $P = 0.04$), diabetes (AOR = 2.8, 95% CI 2.3-3.3; $P < 0.001$), and smoking (AOR = 1.3, 95% CI 1.1-1.5; $P < 0.001$) were also associated with increased risk for CCA. Regardless of PSC, IBD was not significantly associated with CCA development (AOR = 1.4, $P = 0.08$).

Given the very high OR for PSC, the true magnitude of effect of other risk variables might be masked. After exclusion of PSC patients, biliary tract diseases had the highest OR (AOR = 59.4, 95% CI 7.9-449.7; $P < 0.001$), followed by cirrhosis (AOR = 10.8, 95% CI 6.5-18.0; $P < 0.001$), HBV infection (AOR = 2.8, 95% CI 1.0-7.5; $P = 0.04$), diabetes (AOR = 2.8, 95% CI 2.3-3.3; $P < 0.001$), and smoking (AOR = 1.3, 95% CI 1.1-1.4; $P < 0.001$) (Supporting Table S1). These risk factor variables remained significant when the analysis was performed in perfect matching case-control pairs (Supporting Tables S4 and S5).

RISK FACTORS FOR EACH CCA SUBTYPE

The three subtypes of CCA cases were analyzed separately against their matching controls to determine the risk factors for each CCA subtype. PSC remained the most significant risk factor for all three subtypes, with AORs of 93.4 (95% CI 27.1-322), 453 (104-999), and 34.0 (3.6-323) for iCCA, pCCA, and dCCA, respectively ($P < 0.001$ for iCCA and pCCA, $P = 0.002$ for dCCA; Table 3). Non-PSC-related cirrhosis was associated with an increased risk for iCCA (AOR = 13.8, 95% CI 6.6-28.6; $P < 0.001$) and pCCA (AOR = 14.1, 95% CI 5.9-33.7; $P < 0.001$) but not for dCCA (AOR = 3.4, 95% CI 0.6-21.2; $P = 0.19$). Diabetes also conferred a risk for all CCA subtypes, with the largest magnitude for dCCA (AOR = 4.2, 95% CI 2.5-7.0), followed by pCCA (AOR = 2.9, 95% CI 2.2-3.8) and iCCA (AOR = 2.5, 95% CI 2.0-3.2) ($P < 0.001$ for all). Obesity, which was not associated with CCA development when the three CCA subtypes were combined, was inversely associated with the iCCA subtype only (AOR = 0.77, 95% CI 0.64-0.92; $P = 0.005$). The AOR of smoking was highest for dCCA (1.9, 95% CI 1.3-2.7; $P = 0.001$), followed by pCCA (1.3, 95% CI 1.0-1.5; $P = 0.02$) and iCCA (1.2, 95% CI 1.0-1.4; $P = 0.03$). These findings suggested that each risk factor conferred risk for each CCA subtype in different magnitudes.

CHARACTERISTICS OF ASPIRIN USERS

Five hundred ninety-one (24.7%) cases and 2129 (44.6%) controls were categorized as current aspirin users. Table 4 displays baseline characteristics of current aspirin users. The mean ages for aspirin users and nonusers were 69.3 and 59.0 years for cases and 66.8 and 57.4 years for controls, respectively. The prevalence of factors potentially influencing the

chance of taking aspirin, e.g., hypertension or CVA, was higher in current aspirin users than in nonusers in both cases and controls.

ASSOCIATION BETWEEN ASPIRIN USE AND CCA RISK

CCA cases were significantly less likely to report use of aspirin than controls (OR = 0.41, 95% CI 0.36-0.45; $P < 0.001$; Table 5). The result remained the same after adjusting for other potential risk factors for CCA (AOR = 0.34, 95% CI 0.30-0.39; $P < 0.001$). Multivariate analysis with propensity score adjustment did not substantially alter the associations (AOR = 0.38, 95% CI 0.33-0.44; $P < 0.001$). When stratified by CCA subtypes, the AORs were consistent for all three CCA subtypes, with AORs of 0.35 (95% CI 0.29-0.42), 0.34 (0.27-0.42), and 0.29 (0.19-0.44) for iCCA, pCCA, and dCCA, respectively ($P < 0.001$ for all; Table 3).

Next, we determined the impact of dose, frequency, and duration of aspirin use on CCA risk. Low-dose aspirin (81-162 mg/day) was used by 384 (16.0%) cases and 1697 (35.6%) controls. Only 13 patients were administered 162 mg/day of aspirin. High-dose aspirin (≥325 mg/day) was used by 142 (5.9%) cases and 429 (9.0%) controls. The AORs were 0.29 (95% CI 0.25-0.33) and 0.39 (0.31-0.49) for the doses of 81-162 and ≥325 mg/day, respectively ($P < 0.001$ for both). When considering the frequency of aspirin use, 560 (23.4%) cases and 2008 (42.1%) controls took aspirin daily, resulting in an AOR of 0.34 (95% CI 0.30-0.39) for the daily use of aspirin ($P < 0.001$). When stratified by duration of aspirin use as >3 years or 3 years, the AORs were 0.12 (95% CI 0.10-0.15) and 0.30 (0.23-0.38) for aspirin use of >3 years and 3 years, respectively ($P < 0.001$ for both).

Sensitivity analyses, for which the data on duration of aspirin use were complete for 95.9% and 90.4% of the REP and Minnesota subsets, respectively, confirmed that aspirin use was statistically significantly associated with decreased CCA risk, with AORs of 0.41 (95% CI 0.28-0.61) and 0.23 (0.18-0.30) for the REP and Minnesota subsets, respectively ($P < 0.001$ for both).

Because the frequency of aspirin use in cases increased over time during the study period, i.e., 14.7%, 23.4%, 27.0%, and 31.1% in groups 1-4, respectively ($P < 0.001$ for trend; Supporting Table S2), subgroup analyses were performed to adjust for the differences in frequencies of aspirin current users. Current aspirin use still had an inverse association with CCA development when the analyses were restricted to case groups 2-4 (AOR = 0.37, 95% CI 0.33-0.43; $P < 0.001$), groups 3 and 4 (AOR = 0.40, 95% CI 0.34-0.47; $P < 0.001$), or group 4 (AOR = 0.45, 95% CI 0.36-0.58; $P < 0.001$; Supporting Table S3). These findings suggest that the protective effect of aspirin observed in this study is less likely to be due to a time-related bias.

Discussion

This is one of the largest hospital-based case-control studies evaluating risk factors for CCA in Western populations. We found that aspirin use had a significant inverse association with CCA development. Additionally, PSC, biliary tract diseases, non-PSC-related cirrhosis, HBV infection, diabetes, and smoking conferred risks of different magnitudes for the

different CCA subtypes. This supports the hypothesis that the three CCA subtypes are distinct diseases and that each subtype thus has its own susceptibility to risk factors.

As anticipated, cirrhosis was significantly associated with CCA. Unlike in other studies where the effect of PSC was not taken into account, we investigated the association between liver cirrhosis (in the absence of PSC) and CCA risk to determine whether cirrhosis *per se* is an independent risk factor for CCA. We observed a strong relationship between non-PSC-related cirrhosis and CCA, with a 14-fold increased risk of iCCA and pCCA (but not dCCA). Consistent with our previous study, diabetes was associated with risk for CCA, conferring the greatest risk for dCCA, followed by pCCA and then iCCA, suggesting that the role of diabetes in cholangiocarcinogenesis may differ in different CCA subtypes.[13]

It remains unclear whether IBD occurring in isolation from PSC is associated with CCA. Because IBD is usually accompanied by PSC, its impact on CCA risk may be confounded by the effect of the PSC.[4] In this study all analysis models, particularly when PSC patients were excluded, consistently demonstrated that isolated IBD was not related to CCA, suggesting that IBD by itself does not contribute to CCA risk. Similarly, obesity was not an independent risk factor for CCA when all subtypes were combined. Previous reports showed inconsistent findings for obesity and CCA risk.[4,6,13] Here, obesity was associated with a decreased risk for iCCA. This may be partially explained by the retrospective study design as the body mass index data were abstracted at the time of CCA diagnosis and the potential weight-loss due to cancer or cancer-related symptoms was not taken into account. The causal association between obesity and iCCA should be investigated in further studies, which ideally should include body mass index obtained when patients were in a healthy state.

Aspirin use was significantly associated with an approximately 3-fold reduction in CCA risk. Risk reductions by aspirin use were consistent across all three subtypes: 2.9-fold for iCCA, 2.9-fold for pCCA, and 3.4-fold for dCCA. This observation is biologically plausible as supported by preclinical studies of CCA and other cancers. *In vitro* studies have suggested that COX-2 is implicated in cholangiocarcino-genesis.[15–17] Overexpression of COX-2 promoted tumor growth and invasion in human CCA cells.[15–17] Additionally, selective COX-2 inhibitors and aspirin were shown to inhibit vascular endothelial cell proliferation in CCA cell or CCA-conditioned medium and partially prevented CCA cell growth in rats.[16] Thus, inhibition of COX-2 by aspirin may prevent CCA development through inhibition of inflammatory processes.[18] Aspirin-mediated inhibition of nuclear factor jB activation has been proposed as another mechanism of cancer prevention.[12] The anticancer effect of aspirin has been reported to be dose-dependent in studies of colon adenomas and colon cancer, suggesting that higher doses achieve more complete inhibition of the COX-2 isoenzyme.[19] However, in the present study, 79% of aspirin users in the Control group took low-dose (81-162 mg/day) aspirin; thus, the protective effect shown might be achieved even at low doses that do not completely inhibit COX-2. Recently, Wnt signaling was reported to enhance CCA growth and aspirin was shown to modulate Wnt signaling by both COX-dependent and COX-independent pathways.[20,21] Furthermore, aspirin may up-regulate tumor suppressor genes and stabilize DNA mismatch-repair

proteins.[22] Altogether, these mechanisms may potentially contribute to the chemopreventive effect of aspirin.

The AOR of 0.34 in aspirin users detected in the main analysis was very small, suggestive of a very strong protective effect of aspirin. This raises the concern that we may be overestimating the impact of aspirin use on CCA prevention. To obtain a more reliable estimate, we performed a multivariate analysis with propensity score adjustment. With the propensity score adjustment, the estimated AOR of aspirin use was 0.38, which was very similar to the AOR of 0.34 estimated by the multivariate analysis without propensity score adjustment. These consistent findings indicated that aspirin use was significantly associated with a reduction in the risk of CCA regardless of the reason for taking aspirin. Interestingly, the AOR of aspirin use in this study was close to the AOR of 0.45 which was reported in a relatively small UK study of 81 CCA patients.[23] Similarly, another study, conducted in China of 191 patients with extrahepatic bile duct cancer, demonstrated a comparable AOR of 0.48 (95% CI 0.19-1.19) for aspirin ever-users, although statistical significance was not reached.[24] However, as the authors described, the prevalence of aspirin use in the Chinese study was assessed by self-report, without verifying information on aspirin use by medical record review; and their prevalence of aspirin use was very low, i.e., six (3.1%) of the 191 cases. In our study, the prevalence of aspirin use was 44.6% in controls, which was close to the rates of 41% and 52% from two large US national surveys on aspirin use.[25,26] Therefore, our control group may be more representative of the general population, at least in terms of the frequency of aspirin use. Interestingly, aspirin users in the REP and Minnesota subsets of cases were 47.3% and 31.0%, respectively, which was higher than in the total population of cases (24.7%). When we compared baseline medical conditions that might influence aspirin use, the REP and Minnesota subsets had more CVA, cardiovascular disease, peripheral vascular disease, atrial fibrillation, and diabetes compared to the total population. However, similar trends were seen in the control group (Supporting Table S6).

Although our study of CCA, along with accumulating evidence from other cancers, has shown the benefit of aspirin use in cancer prevention, a consensus has not been reached regarding the optimal dose of aspirin for chemoprevention.[12] Nonetheless, 384/591 (65.0%) aspirin users in cases and 1697/2129 (79.7%) aspirin users in controls took low-dose aspirin. Thus, our finding implies that aspirin given at a dose of 81-162 mg/day may be enough to exert a protective effect against CCA. Intriguingly, the duration and a schedule of daily use of aspirin appear to be more important than the dose of aspirin in prevention of certain cancers. The Women's Health Study, a randomized trial of 100 mg aspirin taken on alternate days, did not show a reduction in the incidence of cancers except lung cancer during 10 years of follow-up.[27] However, Rothwell et al. reported that when taken daily aspirin doses of at least 75 mg reduced the long-term incidence of colorectal cancer.[10] Moreover, previous observational studies have confirmed the importance of daily aspirin use in reducing the incidence of cancer.[28] Although we defined subjects who took aspirin at least once a week as current users of aspirin, >95% of current aspirin users in both case and control groups took aspirin daily.

Unlike most studies of risk factors for CCA, to the best of our knowledge, this is the first comprehensive report of risk factors for all three CCA subtypes. We were therefore able to

show that each risk factor contributes to development of each CCA subtype in different magnitudes. Another strength of this study was the very large cohort size and the robust results, suggested by the fact that the estimated effect of aspirin in CCA prevention was consistent in all statistical analysis models.

There are some limitations to our study. First, we were not able to obtain detailed information on duration and dose of aspirin use in 442 (16.3%) current users, which limited our ability to determine possible time-response and dose-response associations between duration of aspirin use and CCA risk. Establishing a time-response and dose-response relationship will strengthen the associations in this type of study. In this study, the aspirin dosage was obtained at the time of study enrollment and could have varied before the patients were enrolled. Thus, we were not able to consider cumulative doses in the analysis. To overcome this limitation, we analyzed the data using two subsets, the REP and Minnesota subsets. The data on dose, frequency, and duration of aspirin use were complete in 97.0% and 90.4% of current users, respectively, in the REP and Minnesota subsets. The main finding was confirmed with comparable AORs of 0.34, 0.23, and 0.41 for the entire cohort, the Minnesota subset, and the REP subset, respectively. Interestingly, in the REP subset there was a significant association between aspirin use for >3 years and decreased risk of CCA development, with an AOR of 0.28 (95% CI 0.18-0.44; $P < 0.001$), compared to aspirin use ≤3 years, which was associated with a nonsignificant AOR of 0.80 (95% CI 0.46-1.41; $P = 0.8$). Because previous studies showed a greater benefit in terms of cancer prevention and death in persons taking aspirin for more than 5 years, we might have observed a more significant association between the duration of aspirin use and CCA development in long-term aspirin users if we had more complete data. The second limitation was the discrepancy in enrollment periods between cases (years 2000-2014) and controls (years 2009-2015). The 2000-2014 period was used for cases in order to maximize the number of CCA cases, given the relative rarity of this cancer. Recently, Suissa et al. described time-related biases in observational studies of drug effects, primarily a risk in cohort studies but also possibly in case-control studies.[29] They underscored the importance of ensuring an equal time window for comparison of the drug exposure variable for cases and controls in order not to overestimate the effect of the drug.[29] To avoid and adjust for this potential time-related bias, we performed sensitivity analyses including cases and controls from 2009 through 2014. We detected a similar effect of aspirin with an AOR of 0.45 (95% CI 0.36-0.58; $P < 0.001$), confirming the inverse relationship between aspirin use and CCA development observed in the main analysis. The third limitation is recall bias, which is always an inherent concern in case-control studies. However, recall bias is unlikely to apply to our study because all cases were asked whether they were taking aspirin and the self-reported questionnaires were completed as a routine part of the medical evaluation before seeing physicians at our institution. For controls, the Mayo Clinic Biobank participants were required to answer health-related questionnaires, which include their history of aspirin use. Lastly, we were not able to consider other possible chemopreventive drugs in our analysis. Our group has reported that metformin use was associated with a decreased risk of iCCA in diabetic patients.[13] For this study, we abstracted metformin use at the time of CCA diagnosis for cases or enrollment for controls but were not able to obtain detailed information on amount and duration of metformin use for our cohort. Thus,

although the proportions of diabetic case and control patients using metformin were similar to our previous report, we could not fully assess the combined effects of metformin and aspirin due to the lack of detailed information. Similarly, we were also not able to assess the possible influence of nonsteroidal anti-inflammatory drugs (NSAIDs) on CCA in the analysis because of the heterogeneity of information on NSAID use, such as the diverse types of drugs, frequent dose changes, and frequency of intermittent use. Although NSAIDs have shown chemopreventive effects in certain types of cancer, two previous studies did not find a significant association between NSAID use and CCA.[23,30] Also, due to the retrospective nature of this study, we were not able to obtain detailed smoking histories, which might have allowed a determination of the dose-response relationship between smoking and CCA development.

Generally, the strongest evidence of the beneficial effect of aspirin for cancer prevention is drawn from randomized double-blind controlled trial designs, which minimize biases and confounders.[9,10] Compared with randomized control trials, our retrospective case-control study design achieves a lower standard of evidence. Given the relatively low incidence of CCA, it may be challenging to conduct a randomized trial of aspirin for CCA prevention. However, a randomized study of aspirin for CCA prevention in a well-defined high-risk population, such as PSC patients, might be feasible and potentially practice-changing. As an initial step toward this goal, we plan to perform a case-control study comparing aspirin use in PSC without CCA controls to PSC with CCA cases, to determine whether the protective effect of aspirin can be validated in patients with PSC. Moreover, genetic variation may influence the chemopreventive effect of aspirin on cancer. The effect of aspirin on colonic adenoma risk was shown to modify by variants in the gene encoding the UGT1A6 enzyme, which is responsible for delayed aspirin metabolism.[31] A recent study showed that the association between aspirin use and colorectal cancer varied by polymorphisms of single-nucleotide polymorphism rs2965667 at chromosome 12 and rs16973225 at chromosome 15.[32] It will be of interest to study the relationship between genetic variations and the chemopreventive effect of aspirin in CCA.

In conclusion, aspirin use was significantly associated with a lower risk for all three CCA subtypes. Importantly, we found that individual risk factors confer risk for different CCA subtypes to different extents. Because over 90% of patients in this cohort were Caucasian, this finding needs to be further validated in other ethnicities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AOR	adjusted odds ratio
CCA	cholangiocarcinoma
CI	confidence interval
COX	cyclooxygenase
CVA	cerebrovascular accident
dCCA	distal cholangiocarcinoma
EMR	electronic medical record
HBV	hepatitis B virus
HCV	hepatitis C virus
IBD	inflammatory bowel disease
iCCA	intrahepatic cholangiocarcinoma
NSAID	nonsteroidal anti-inflammatory drug
OR	odds ratio
pCCA	perihilar cholangiocarcinoma
PSC	primary sclerosing cholangitis
REP	Rochester Epidemiology Project

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TABLE 1

Baseline Characteristics of CCA Cases and Controls

Characteristics	CCA Cases				Controls	
	Total (n = 2395)	Intrahepatic (n = 1169)	Perihilar (n = 995)	Distal (n = 231)	(n = 4769)	*P**
Age (years, mean ± SD)	61.5 ± 13.5	60.6 ± 13.1	61.6 ± 13.9	65.9 ± 13.0	61.6 ± 13.5	0.8
Sex, male	1094 (45.7%)	501 (42.9%)	471 (47.3%)	122 (52.8%)	2170 (45.5%)	0.9
Race†						0.09
White	2074 (95.0%)	1003 (94.9%)	868 (94.9%)	203 (95.8%)	4540 (95.8%)	
African American	34 (1.5%)	17 (1.5%)	15 (1.6%)	2 (0.9%)	88 (1.9%)	
Asian	58 (2.7%)	26 (2.5%)	27 (3.0%)	5 (2.4%)	88 (1.9%)	
American Indian	15 (0.7%)	8 (0.8%)	5 (0.5%)	2 (0.9%)	19 (0.4%)	
Others	3 (0.1%)	3 (0.3%)	0 (0.0%)	0 (0.0%)	5 (0.1%)	
PSC	411 (17.2%)	131 (11.2%)	253 (25.4%)	27 (11.7%)	6 (0.1%)	<0.001
Biliary tract diseases‡	37 (1.5%)	20 (1.7%)	16 (1.6%)	1 (0.4%)	4 (0.1%)	<0.001
Choledochal cyst	23 (1.0%)	15 (1.3%)	7 (0.7%)	1 (0.4%)	1 (0.0%)	<0.001
Hepatolithiasis	15 (0.6%)	6 (0.5%)	9 (0.9%)	0 (0.0%)	3 (0.1%)	<0.001
Cirrhosis	224 (9.4%)	114 (9.8%)	98 (9.8%)	12 (5.2%)	24 (0.5%)	<0.001
Non-PSC-related cirrhosis§	110 (4.6%)	72 (6.2%)	33 (3.3%)	5 (2.2%)	20 (0.4%)	<0.001
HBV infection	13 (0.5%)	10 (0.9%)	3 (0.3%)	0 (0.0%)	8 (0.2%)	0.006
HCV infection	36 (1.5%)	23 (2.0%)	13 (1.3%)	0 (0.0%)	17 (0.4%)	<0.001
IBD	346 (14.5%)	121 (10.3%)	196 (19.7%)	29 (12.6%)	79 (1.7%)	<0.001
Ulcerative colitis	294 (12.3%)	103 (8.8%)	165 (16.6%)	26 (11.3%)	42 (0.9%)	<0.001
Crohn's disease	52 (2.2%)	18 (1.5%)	31 (3.1%)	3 (1.3%)	37 (0.8%)	<0.001
Other comorbidities						
Diabetes	435 (18.2%)	208 (17.8%)	170 (17.1%)	57 (24.7%)	479 (10.0%)	<0.001
Obesity‖	660 (28.4%)	333 (29.6%)	264 (27.2%)	63 (27.9%)	1474 (31.3%)	0.01
NAFLD/NASH	113 (4.7%)	61 (5.2%)	37 (3.7%)	15 (6.5%)	181 (3.8%)	0.06
Smoking, ever-smoker	1030 (43.0%)	499 (42.7%)	414 (41.6%)	117 (50.6%)	1929 (40.4%)	0.04
Aspirin, current user	591 (24.7%)	276 (23.6%)	245 (24.6%)	70 (30.3%)	2129 (44.6%)	<0.001

* *P* value for difference between total CCA cases and controls.

† Data on race were not available for 211 (8.8%) cases and 29 (0.6%) controls.

‡ One intrahepatic CCA case had both a choledochal cyst and hepatolithiasis.

§ Defined as a cirrhotic condition without existing PSC.

‖ Data on body mass index were not available for 72 (3.0%) cases and 64 (1.3%) controls.

Abbreviations: NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; SD, standard deviation.

TABLE 2

Risk Factors for CCA (All Subtypes Combined)

	Univariate Analysis			Multivariate Analysis		
Variables	**OR**	**95% CI**	***P***	**AOR**	**95% CI**	***P***
Age, per 10-year increase	1.00	0.96-1.03	0.83	1.25	1.19-1.31	<0.001
Gender, female	0.99	0.90-1.10	0.89	1.16	1.03-1.31	0.02
Race, white	0.83	0.65-1.05	0.13	0.82	0.62-1.07	0.1
PSC	164	73.3-369	<0.001	171	72.6-404	<0.001
Biliary tract diseases	18.7	6.66-52.5	<0.001	12.1	3.97-36.9	<0.001
Non-PSC-related cirrhosis *	11.4	7.08-18.5	<0.001	10.8	6.48-18.0	<0.001
HBV infection	3.25	1.34-7.85	0.009	2.78	1.04-7.43	0.04
HCV infection	4.27	2.39-7.61	<0.001	1.94	0.95-3.95	0.07
IBD	10.0	7.81-12.9	<0.001	1.43	0.96-2.15	0.08
Diabetes	1.99	1.73-2.29	<0.001	2.76	2.32-3.27	<0.001
Obesity	0.87	0.78-0.97	0.01	0.88	0.78-1.00	0.05
NAFLD/NASH	1.26	0.99-1.60	0.06	1.10	0.82-1.48	0.5
Smoking, ever-smoker	1.11	1.01-1.23	0.04	1.29	1.14-1.45	<0.001
Aspirin, current user	0.41	0.36-0.45	<0.001	0.34	0.30-0.39	<0.001

* Defined as a cirrhotic condition without existing PSC.

Abbreviations: NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

TABLE 3

Multivariate Logistic Regression Analysis of Risk Factors for Each CCA Subtype

	iCCA (n = 1169)		pCCA (n = 995)		dCCA (n = 231)	
Variables	**AOR (95% CI)**	***P***	**AOR (95% CI)**	***P***	**AOR (95% CI)**	***P***
Age, per 10-year increase	1.17 (1.09-1.25)	<0.001	1.36 (1.26-1.47)	<0.001	1.22 (1.04-1.43)	0.01
Gender, female	1.13 (0.95-1.33)	0.2	1.13 (0.94-1.37)	0.2	1.31 (0.89-1.91)	0.2
Race, white	0.93 (0.63-1.36)	0.7	0.68 (0.44-1.03)	0.07	0.89 (0.34-2.34)	0.8
PSC	93.4 (27.1-322.2)	<0.001	453 (104-999)	<0.001	34.0 (3.57-323.1)	0.002
Biliary tract diseases	7.60 (2.34-24.66)	0.001	42.3 (1.57-999)	0.03	*	*
Non-PSC-related cirrhosis †	13.8 (6.62-28.63)	<0.001	14.1 (5.87-33.7)	<0.001	3.40 (0.55-21.15)	0.2
HBV infection	12.9 (2.69-61.61)	0.001	0.17 (0.02-1.26)	0.08	1.32 (0.01-196.9)	0.9
HCV infection	1.95 (0.75-5.11)	0.2	3.51 (1.02-12.08)	0.047	0.17 (0.01-5.17)	0.3
IBD	1.32 (0.74-2.33)	0.4	1.31 (0.68-2.53)	0.4	4.32 (0.97-19.26)	0.06
Diabetes	2.50 (1.95-3.20)	<0.001	2.88 (2.19-3.78)	<0.001	4.22 (2.54-6.99)	<0.001
Obesity	0.77 (0.64-0.92)	0.005	1.13 (0.92-1.40)	0.2	0.69 (0.45-1.06)	0.09
NAFLD/NASH	1.40 (0.94-2.09)	0.1	0.61 (0.36-1.04)	0.07	2.33 (0.97-5.59)	0.06
Smoking, ever-smoker	1.21 (1.02-1.43)	0.03	1.25 (1.03-1.52)	0.02	1.85 (1.27-2.71)	0.001
Aspirin, current user	0.35 (0.29-0.42)	<0.001	0.34 (0.27-0.42)	<0.001	0.29 (0.19-0.44)	<0.001

*Not able to estimate.

†Defined as a cirrhotic condition without existing PSC.

Abbreviations: NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

TABLE 4

Comparison of Baseline Characteristics Between Current Aspirin Users and Nonusers

	CCA Cases			Controls		
Characteristics	**Aspirin user (n = 591)**	**Nonuser (n = 1804)**	***P***	**Aspirin user (n = 2129)**	**Nonuser (n = 2640)**	***P***
Age (years, mean ± SD)	69.3 ± 10.7	59.0 ± 13.3	<0.001	66.8 ± 11.1	57.4 ± 13.8	<0.001
Sex, male	278 (47.0%)	816 (45.2%)	0.5	1101 (51.7%)	1069 (40.5%)	<0.001
PSC	59 (10.0%)	352 (19.5%)	<0.001	1 (0.0%)	5 (0.2%)	0.3
Biliary tract diseases	8 (1.4%)	29 (1.6%)	0.8	2 (0.1%)	2 (0.1%)	1.0
Non-PSC-related cirrhosis *	28 (4.7%)	82 (4.5%)	0.9	6 (0.3%)	14 (0.5%)	0.3
HBV infection	2 (0.3%)	11 (0.6%)	0.7	3 (0.1%)	5 (0.2%)	1.0
HCV infection	6 (1.0%)	30 (1.7%)	0.4	3 (0.1%)	14 (0.5%)	0.046
IBD	54 (9.1%)	293 (16.2%)	<0.001	28 (1.3%)	51 (1.9%)	0.1
Other comorbidities						
Diabetes	180 (30.5%)	255 (14.1%)	<0.001	365 (17.1%)	114 (4.3%)	<0.001
Obesity	200 (33.8%)	460 (25.5%)	<0.001	708 (33.3%)	766 (29.0%)	<0.001
NAFLD/NASH	38 (6.4%)	75 (4.2%)	0.3	117 (5.5%)	64 (2.4%)	<0.001
Hypertension	351 (59.4%)	590 (32.7%)	<0.001	1351 (63.5%)	699 (26.5%)	<0.001
Atrial fibrillation	65 (11.0%)	77 (4.3%)	<0.001	223 (10.5%)	69 (2.6%)	<0.001
PVD	20 (3.4%)	15 (0.8%)	<0.001	46 (2.2%)	9 (0.3%)	<0.001
CAD	92 (15.6%)	62 (3.4%)	<0.001	219 (10.3%)	54 (2.0%)	<0.001
CVA	41 (6.9%)	36 (2.0%)	<0.001	177 (8.3%)	48 (1.8%)	<0.001
Smoking, ever-smoker	293 (49.6%)	737 (40.9%)	<0.001	947 (44.5%)	982 (37.2%)	<0.001

* Defined as a cirrhotic condition without existing primary sclerosing cholangitis.

Abbreviations: CAD, coronary artery disease; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PVD, peripheral vascular disease.

TABLE 5

Association Between Duration, Dosage, and Frequency of Aspirin Use and Risk of CCA

Variables	Cases (%)	Controls (%)	Crude OR	*P*	AOR	*P*
All subjects (n = 7164)						
Nonuser	1804 (75.3)	2640 (55.4)	1 (ref)		1 (ref)	
Aspirin use						
Current user	591 (24.7)	2129 (44.6)	0.41 (0.36-0.45)	<0.001	0.34 (0.30-0.39)	<0.001
Duration of use *						
≤3 years	103 (4.3)	433 (9.1)	0.35 (0.28-0.43)	<0.001	0.30 (0.23-0.38)	<0.001
>3 years	161 (6.7)	1580 (33.1)	0.15 (0.13-0.18)	<0.001	0.12 (0.10-0.15)	<0.001
Dose †						
81-162 mg/day	384 (16.0)	1697 (35.6)	0.33 (0.29-0.38)	<0.001	0.29 (0.25-0.33)	<0.001
≥325 mg/day	142 (5.9)	429 (9.0)	0.48 (0.40-0.59)	<0.001	0.39 (0.31-0.49)	<0.001
Frequency						
Nondaily	31 (1.3)	121 (2.5)	0.38 (0.25-0.56)	0.002	0.35 (0.22-0.55)	<0.001
Daily	560 (23.4)	2008 (42.1)	0.41 (0.37-0.46)	<0.001	0.34 (0.30-0.39)	<0.001
REP subset (n = 1321)						
Nonuser	87 (52.7)	474 (41.0)	1 (ref)		1 (ref)	
Aspirin use						
Current user	78 (47.3)	682 (59.0)	0.62 (0.45-0.86)	0.005	0.41 (0.28-0.61)	<0.001
Duration of use ‡						
≤3 years	24 (14.5)	115 (9.9)	1.14 (0.69-1.87)	0.6	0.80 (0.46-1.41)	0.8
>3 years	45 (27.3)	553 (47.8)	0.44 (0.30-0.65)	<0.001	0.28 (0.18-0.44)	<0.001
Dose ‖						
81-162 mg/day	50 (30.3)	508 (43.9)	0.54 (0.37-0.78)	0.001	0.35 (0.23-0.54)	<0.001
≥325 mg/day	26 (15.8)	174 (15.1)	0.81 (0.51-1.30)	0.4	0.56 (0.33-0.96)	0.04
Frequency						
Nondaily	8 (4.8)	51 (4.4)	0.86 (0.39-1.86)	0.7	0.89 (0.42-2.13)	0.9
Daily	70 (42.4)	631 (54.6)	0.60 (0.43-0.85)	0.003	0.36 (0.24-0.55)	<0.001
Minnesota subset (n = 1968)						
Nonuser	423 (69.0)	563 (41.5)	1 (ref)		1 (ref)	

Variables	Cases (%)	Controls (%)	Crude OR	*P*	AOR	*P*
Aspirin use						
Current user	190 (31.0)	792 (58.5)	0.32 (0.26-0.39)	<0.001	0.23 (0.18-0.30)	<0.001
Duration of use[§]						
≤3 years	44 (7.2)	130 (9.6)	0.45 (0.31-0.64)	<0.001	0.33 (0.22-0.50)	<0.001
>3 years	73 (11.9)	641 (47.3)	0.15 (0.11-0.20)	<0.001	0.11 (0.08-0.15)	<0.001
Dose						
81-162 mg/day	117 (19.1)	599 (44.2)	0.26 (0.20-0.33)	<0.001	0.18 (0.14-0.24)	<0.001
≥325 mg/day	56 (9.1)	192 (14.2)	0.39 (0.28-0.53)	<0.001	0.29 (0.20-0.43)	<0.001
Frequency						
Nondaily	11 (1.8)	56 (4.2)	0.26 (0.14-0.51)	<0.001	0.28 (0.14-0.56)	0.004
Daily	179 (29.2)	736 (54.3)	0.32 (0.26-0.40)	<0.001	0.23 (0.18-0.29)	<0.001

All ORs estimated by nonuser as a reference. Model included age, sex, race, PSC, non-PSC-related cirrhosis, biliary tract diseases, diabetes, and ever-smoker.

[*] Unavailable for 327 (55.3%) current users in cases and 115 (5.4%) current users in controls.

[†] Unavailable for 65 (11.0%) current users in cases and 3 (0.1%) current users in controls.

[‡] Unavailable for 9 (11.5%) current users in cases and 14 (2.0%) current users in controls.

[¶] Unavailable for 2 (2.6%) current users in cases 1 (0.0%) current users in controls.

[§] Unavailable for 73 (38.4%) current users in cases 21 (2.7%) current users in controls.