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Variants in Inflammation Genes and the Risk of Biliary Tract Cancers and Stones: A Population-based Study in China

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Abstract

To evaluate the role of chronic inflammation in the development of gallstones and biliary tract cancer, we examined the risk associated with 62 single nucleotide polymorphisms (SNPs), including 22 inflammation-related genes, based on a population-based case-control study conducted in Shanghai, China, where the incidence of biliary tract cancer has been increasing in recent decades. The study included 411 cases with biliary tract cancer (237 gallbladder, 127 extrahepatic bile duct, and 47 ampulla of Vater), 895 with biliary stones, and 786 controls randomly selected from the population. Unconditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for the association of individual single nucleotide polymorphisms (SNPs) and haplotypes with biliary stones and biliary tract cancer. Of the 62 SNPs examined, 14 were related to the risk of biliary cancer and stones. Specifically, variants in the *IL8, IL8RB, RNASEL*, and *NOS2* genes were associated with biliary stones, while *VEGF* variants were associated with gallbladder cancer. Of the 10 genes with multiple SNPs from which we inferred haplotypes, only one *IL8RB* haplotype, consisting of 3 SNPs (rs2230054,

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rs1126579, rs1126580), was associated with the risk of bile duct cancer (p=0.003) and biliary stones (p=0.02), relative to the most frequent haplotype. In summary, common variants in genes that influence inflammatory responses may predispose to gallstones and biliary tract cancer, suggesting the need for future studies into the immunologic and inflammatory pathways that contribute to biliary diseases, including cancer.

Keywords

gallstones; biliary tract cancer; inflammation; genetic susceptibility

Introduction

Biliary tract cancers, encompassing tumors of the gallbladder, extrahepatic bile ducts, and ampulla of Vater, are rare but highly fatal malignancies (1). High incidence rates are reported for Native Americans and Hispanics living in the United States and among certain populations in Central and South America, Eastern Europe, and some parts of Asia, including China, Korea, Japan, and India (1,2). Apart from ethnicity and gallstones, the causes of biliary tract cancer are unclear (1,4). However, the large geographic and racial variation in incidence suggests that both genetic and lifestyle factors are important. In previous clinical and population-based studies, inflammatory processes associated with gallstones and cholecystitis have been linked to the development of gallbladder cancer, while primary sclerosing cholangitis predispose to bile duct cancer (1,3,4). In previous analyses from our case-control study in Shanghai, we reported that: a) gallstones are associated with an 18-fold risk of gallbladder cancer; b) the combination of gallstones and cholecystitis increases the risk of gallbladder cancer by 34-fold (3); c) use of aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) reduced the risk of biliary tract cancer (5); d) chronic infection with hepatitis B virus doubled the risk of extrahepatic bile duct cancer (6); and e) variants in the inflammatory gene, *PTGS2* (commonly called *COX2*), were associated with an increased risk of extrahepatic bile duct cancer (7).

Common variants in inflammation-related genes may alter the expression of inflammatory cytokines and chemokines, thereby predisposing to gallstones and/or biliary tract cancer (8). To further clarify the role of inflammation in biliary diseases, we examined the risks of biliary stones and cancer associated with 62 single nucleotide polymorphisms (SNPs) in 22 inflammation genes in a population-based study conducted in Shanghai, China, where the incidence of biliary tract cancer is increasing rapidly in recent years (9).

Material and Methods

Study Population

Details of the study have been reported elsewhere (3,5–7,10–12). Briefly, primary biliary tract cancer cases (ICD-9 156) diagnosed between 1997 and 2000 were identified through a rapid-reporting system established by the Shanghai Cancer Institute (SCI) with 42 collaborating hospitals in urban Shanghai. This system captured more than 95% of all biliary tract cancers diagnosed in Shanghai. Case patients were permanent residents of urban Shanghai between 40 to 75 years of age. A total of 411 patients with biliary tract cancer (237 gallbladder, 127 bile duct, and 47 ampulla of Vater) were included. In addition, we selected a total of 1,037 biliary stone cases (774 gallstone and 263 bile duct stone patients) from the same hospitals from which the cancer cases were selected. Biliary stone cases had no history of cancer and were matched to index cancer cases on gender, age (within 5 years), and hospital. A total of 959 healthy subjects who were randomly selected from the urban Shanghai population (6.5 million permanent residents), using the Shanghai Resident

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Registry records, were included in this study as population controls. Controls were free of non-skin cancer and were frequency-matched to cancer cases in a 1-to-1 ratio by age (within 5 years) and gender distributions. All study subjects provided written informed consent. The Institutional Review Boards of the National Cancer Institute and SCI approved the study protocol.

Clinical and Pathology Review

Review of pathology slides, imaging data, medical records, and surgical reports were carried out to confirm the diagnosis of both biliary tract cancer and stone cases. All cancer cases underwent magnetic resonance imaging (MRI), endoscopic retrograde cholangiopancreatography (ERCP), or computed tomography (CT). Pathology slides were obtained for 70% of cancer cases who underwent surgery and were reviewed by pathologists from Shanghai and US. Imaging studies, pathology and surgical reports, and medical records were reviewed by a panel of clinicians, ultrasonographers, and pathologists for the presence of cancer. Biliary stone cases were confirmed by abdominal ultrasound and ERCP films, and pathology slides for those who underwent a cholecystectomy.

Interviews

Study subjects were interviewed by trained interviewers, using a structured questionnaire to obtain information on demographic, lifestyle, and dietary factors. Cases were interviewed within 2 weeks of diagnosis. At interview, weight and height were measured. The response rate for interviews was over 95% for cases and 82% for controls. For quality-control purposes, all interviews were recorded and reviewed to ensure adherence to the study protocol. In addition, 5% of the subjects were randomly re-interviewed within three months to assess reproducibility; the concordance between the two interviews on responses to key questions was greater than 90%.

Assessment of Biliary Stones

Biliary stones status was assessed for all study subjects. Among cancer cases, biliary stone disease was identified by self-report from interview data and clinically from medical, surgical, and radiology records, including MRI, ERCP, CT, and ultrasound results. Among population controls, biliary stones were assessed by self-reported history and by abdominal ultrasound among those who gave consent for the procedure, which was 85% of population controls.

Blood Collection and Genotyping

Blood collection—Over 80% of the participants donated an overnight fasting blood sample for the study. Buffy coat samples were processed within four hours of collection at a laboratory in Shanghai Cancer Institute, stored at −70°C, and shipped to the U.S. on dry ice.

Genotyping—Genomic DNA was extracted from buffy coat using the phenol-chloroform extraction method. All genotyping was conducted at the National Cancer Institute Core Genotyping Facility (CGF, Advanced Technology Corporation, Gaithersburg, MD) (http://cgf.nci.nih.gov/home.cfm) using the TaqMan assay (Applied Biosystems, Foster City, CA). The sequence information and validated assays are provided at http://snp500cancer.nci.nih.gov (13).

Gene and SNP Selection—The variants included in the study were chosen on the basis of *a priori* evidence suggesting possible functional consequences or previous association studies showing a link between inflammation or cancer. In addition, certain SNPs were selected for additional gene coverage for haplotype analysis, although the inclusion of these

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SNPs was limited by the availability of validated assays. A total of 62 SNPs in 22 genes, including *IL1A*, *IL1B*, *IL4*, *IL5*, *IL6*, *IL8*, *IL8RA*, *IL8RB*, *IL10*, *IL13*, *IL16*, *PPARD*, *PPARG*, *RNASEL*, *SOD2*, *MPO*, *NOS2*, *NOS3*, *TGFB1*, *TNF*, *VCAM1*, and *VEGF* were typed (Table 1).

Quality Control—For quality control (QC), 20 replicate samples from each of four blood donors and duplicate samples from 100 study subjects processed in an identical fashion were interspersed for all genotyping assays and blinded from the laboratory personnel. Concordance of genotyping on 80 samples from 4 QC subjects was >99%. Genotyping failure rate was less than 2% for each SNP.

Statistical Analysis

Analysis was performed on 411 incident cases with biliary tract cancer, 895 biliary stones, and 786 healthy controls. Differences in selected characteristics between cases and controls were tested using Fisher's exact test for categorical variables and the t-test for continuous variables. In order to make appropriate case-control comparisons, gallbladder cancer cases were compared with controls without a history of cholecystectomy; bile duct cancer cases and ampulla of Vater cancer cases were compared with all controls; and biliary stone cases were compared with population controls without biliary stones.

Among control subjects, genotype frequencies for each marker were examined for deviation from Hardy-Weinberg equilibrium (HWE), using the asymptotic chi-squared test. Differences in genotype frequencies between controls and cancer or stone cases were assessed with Fisher's exact test. Only SNPs whose genotype distribution was in HWE among controls were included in the analysis. Unconditional logistic regression was used to assess the relationship of each SNP with the risk of biliary stones and biliary tract cancer at each anatomic subsite, adjusting for age and gender. For each marker, odds ratios (ORs) and 95% confidence intervals (CIs) for the homozygous and heterozygous genotypes were calculated in reference to the most frequent homozygous genotype. Additional logistic regression models were run with further adjustment for biliary stone status to evaluate potential confounding by this factor, since individuals diagnosed with biliary tract cancer and stones may have similar susceptibility profiles. The risk of biliary stones associated with each marker was also estimated, controlling for age and gender, by comparing gallbladder or bile duct stone cases to the subset of population controls without stones. Our aim was to identify single-marker genetic associations with effects consistent with an additive model, a dominant model, or a codominant model with a monotonic relationship between the risk of disease and the number of copies of the variant allele. For this reason, we used the Cochran–Armitage Trend Test (with genotype scores of 0, 1, and 2) to screen for association, because it is optimal for the additive model but is also sensitive to associations with dominant and monotonic effects. Statistical associations between SNPs and biliary stones and cancers were also assessed using the linear test of trend (p-trend) for the number of copies of the variant allele (0,1,2) and for the presence or absence of the variant allele (0, 1). In addition, the likelihood ratio test was used to formally test for multiplicative interactions between lifestyle factors and SNPs on stone and cancer risk. The risk estimate was not calculated for a genetic variable if the frequency in either the case or control group was less than 5. To assess the overall gene effects on biliary tract cancer and stone risk while accounting for multiple comparisons, the Simes global test was used to calculate a summary p-value for each of the 10 genes for which we examined multiple SNPs (14,15). This test is based on the adjusted p-value for the minimum of the p-trend values of all SNPs within each gene; thus it effectively accounts for multiple SNP testing by controlling the familywise error rate (i.e., the chance that any marker is erroneously declared to be associated with disease will be <5%, if in fact no polymorphism is truly associated) (14).

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We also examined the association between the haplotypes of the 10 genes with multiple SNPs and the risk of biliary stones and cancers. Among population controls, linkage disequilibrium (LD) between these loci was assessed by calculating pariwise Lewontin's D' and r^2 using Haploview version 3.11 (16). The logistic regression with haplotypes was similar to that with single SNPs. We used the most common haplotype as the referent and estimated the OR and 95% CI for other haplotypes relevant to this referent. To circumvent the challenge of phase ambiguity, which is a special missing data issue in that the haplotype phase is missing, we employed the method described by Schaid et al (17) implemented in the haplo.stat package in R. This approach uses an Expectation-Maximization algorithm to account for the phase ambiguity and permits modeling of the association of haplotypes, as well as haplotype-environment interactions, with continuous and discrete outcomes (17). It also allows testing of global differences in haplotype frequencies between cases and controls. Only those haplotypes with frequencies above 1% were included in our analysis.

Results

Selected characteristics of the study subjects are shown in Table 2. As expected, the majority of gallbladder cancer (72.6%) and biliary stone (62.1%) cases were women, while slightly more than half of bile duct (59.8%) and ampulla of Vater (51.1%) cancers were men. Compared with controls, biliary stone cases were younger. Compared to controls, smoking was more common in gallstone cases, while more bile duct and ampulla of Vater cancer cases were smokers. Gallbladder cancer and biliary stone cases were less likely to drink alcohol but had a higher BMI and were more likely to be diabetic than controls. For all three cancer types, cases were significantly more likely to have gallstones than controls.

Of the 22 genes, 5 (*IL8, IL8RB,RNASEL*, *TNF*, and *NOS2*) showed some association with biliary stone risk. Table 3 shows the ORs of biliary stones in relation to SNPs of these 5 genes. As shown, all three *IL8* SNPs (rs4073, rs2227307, rs2227306), in close LD with each other (r^2=0.99), were associated with reduced risk of bile duct stones (global p <0.04): −351A>T (also called *IL8*-251, rs4073): $OR_{TA/AA}$=0.55 (95% CI 0.40–0.76), p_{trend}=0.04; IVS1+230 T>G (rs2227307): $OR_{TG/GG}$=0.55 (95% CI 0.40–0.76), p_{trend}=0.03; IVS1–204C>T (rs2227306): $OR_{TC/CC}$=0.57 (95% CI 0.42–0.78), p_{trend}=0.03. In contrast, two of the three *IL8RB* variants were associated with an increased risk of biliary stones (gallstones and/or bile duct stones) (global p=0.0006): Ex3+811C>T (rs2230054): $OR_{CT/TT}$=1.40 (95% CI 1.13–1.74), p_{trend}=0.002; Ex3+1235T>C (rs1126579): $OR_{TC/CC}$=1.25 (95% CI 1.00–1.55), p_{trend}=0.01. One (Ex1–96A>G, rs486907) of the two *RNASEL* markers was associated with increased risk of gallbladder stones (95% CI 1.08–1.71) (p_{trend}=0.001), and the global p value for the gene was 0.002. Of the 7 *TNF* markers, only one (*TNF*-1042C>A, rs1800630) was associated with a reduced risk of gallstones (95% CI 0.56–0.93; p_{trend}=0.008) but the global p value for the *TNF* gene was not significant. One of the two *NOS2* SNPs, Ex16+14C>T (rs2297518), was associated with gallstones (p_{trend}=0.01, global p=0.02). Gender did not modify these risk patterns and adjustment for other covariates, including smoking, alcohol drinking, BMI, and gallstones, as well as adjustment for multiple comparisons did not materially change the results.

Table 4 shows the risks of biliary tract cancer in relation to *IL10* and *VEGF* variants. Three (rs1800871, rs1800872, rs1800896) of the five variants in the *IL10* gene were associated with a reduced risk of gallbladder cancer. Relative to subjects with the most common genotype, those with the C alleles of the *IL10*-626A>C (also called *IL10*-627, rs1800872) and *IL10*-853 C>T (also called *IL10*-854, rs1800871) markers and the G allele of the −1116A>G (also called *IL10*-1117, rs1800896) marker had a reduced risk of gallbladder cancer. For *VEGF*, the T allele (CT and TT genotype) of the 236 bp3' of STP C>T marker (rs3025039) conferred reduced risk of gallbladder cancer (OR=1.30 95% CI 0.50–0.97,

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P_{rend}=0.04). These associations persisted after further adjustment for covariates, including smoking, drinking, BMI, and gallstones, as well as adjustment for multiple comparisons, although the global p for the *IL10* gene was not statistically significant. No significant associations were seen for bile duct or ampulla of Vater cancers perhaps due to smaller numbers of cases.

The associations between *IL8RB* haplotypes and the risk of biliary stones and cancers are shown in Table 5. Based on the three *IL8RB* SNPs (in the order of Ex3+811C>T, Ex3+1235T>C, Ex3–1010G>A), we inferred five haplotypes among our population controls, with three common haplotypes, C-T-G (64.4%), T-C-A (23%), and T-C-G (8.3%), accounting for greater than 95% of the haplotype variation. The haplotype frequencies were signficnatly different in relation to bile duct cancer and gallstones, with global p vlues of 0.003 and 0.02, respectively. When specific haplotypes were examined, the *IL8RB* T-C-G haplotye was associated with an increased risk of gallstones (95% CI 1.14–2.07), relative to the most frequent haplotype (C-T-G).

We found no association between variants of the *IL1A*, *IL1B*, *IL4*, *IL5*, *IL6*, *IL13*, *IL16*, *PPARD*, *PPARG*, *MnSOD2*, *MPO*, *TGFB*, *VCAM1*, and *NOS3* genes and risk of biliary tract cancer or stones. Results of single locus and haplotype analyses of these variants are presented in the supplementary Table 1 (gallstones) and 2 (cancer). Although the main effect of *IL10* was not significant, there was suggestive interaction between *IL10* and *TNF* variants, with subjects having the *IL10* −627C allele and the *TNF* IVS1+123A allele having reduced risk of gallbladder cancer (OR=0.55, 95% CI 0.33–0.90, p interaction=0.03), relative to those with the *IL10* TT and *TNF* GG genotypes (supplementary Table 2).

Joint effects of gallstones and several inflammation genes on the risk of gallbladder and bile duct cancers are shown in supplementary Table 3. We observed significant interactions between gallstones and variants of *IL8RA* and *TGFB1* on the risk of gallbladder cancer. For example, among subjects with gallstones, carriers of the C allele of the *IL8RA* Ex2+860G>C (rs2234671) marker had a 26-fold risk (95% CI 14.0–48.4; p interaction=0.04), and carriers of the T allele of the *TGFB1* marker (rs2241718) had a 20-fold risk (95% CI 12.2–35.5; p interaction=0.008), compared with those with the corresponding genotype who did not have gallstones. In addition, significant interactions between gallstones and *SOD2*, *TNF*, and *VCAM1* variants on the risk of bile duct cancer were seen.

Discussion

In this population-based case-control study, we found that variants of the *IL8*, *IL8RB*, *RNASEL*, and *NOS2* genes were associated with biliary stone risk, while polymorphisms of the *IL10* and *VEGF* genes were associated with gallbladder cancer risk. Consistent with our single locus results, the T-C-G *IL8RB* haplotype containing the risk-conferring allele of *IL8RB* Ex3+811 C>T was significantly associated with gallstones. Although the magnitude of these risk estimates was generally modest, the findings provide support for the hypothesis that common gene variants in the inflammatory pathway contribute to the etiology of both gallstones and biliary tract cancer.

The findings for gallstones are consistent with epidemiologic and experimental evidence indicating that prior use of aspirin and other NSAIDs have a protective effect (18,19). Recent data also show that the human lithogenic gene (*LITH*), which is associated with gallstone susceptibility, encodes inflammatory molecules, their receptors, and other mediators, suggesting a close relationship between gallstones and inflammation (18). In addition, circulating inflammatory cytokines, including IL-8, IL-10, and TNF, are associated

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with risk factors for gallstones, including obesity, hyperlipidemia, and insulin resistance (19).

In our study, the three *IL8* variants, in strong LD with each other, provided evidence of a locus associated with bile duct stones. Interestingly, two of the three *IL8RB* variants, in high LD with each other (pairwise values of r^2 between 0.93 and 0.99), were also associated with gallstone risk. These associations are biologically plausible given the role of *IL8* and *IL8RB* in inflammation, but require further epidemiologic and laboratory studies. IL-8, encoded by the *IL8* gene, is an important pro-inflammatory cytokine involved not only in the initiation and amplification of inflammatory processes but also in tumorigenesis (20). Although the function of most of the SNPs we examined is unclear, rs4073 in the *IL8* promoter region has been related to increased *IL8* expression (21). Biological function of IL-8 is mediated through its two receptors: IL8RA and IL8RB. IL8RA binds exclusively to IL-8, while IL-8RB binds to IL-8 and other alpha-chemokines. Despite the close relationship between IL-8 and IL-8RB, we did not find a significant interaction between *IL8* and *IL8RB* SNPs on gallstone risk.

Although TNF-alpha, a potent inflammatory cytokine, promotes hyperlipidemia by increasing hepatic triglyceride production and decreasing clearance, only one (rs#1800630) of the seven variants we examined was associated with reduced risk of gallstones. However, the A allele of this SNP has a higher transactivating effect than that of the dominant C allele (22) and is associated with periodontitis (23). We did not find an association with the more widely studied *TNF*-308 G>A (rs1800629) and *TNF*-238 A>G (rs361525) variants of the promoter region, possibly due to the much lower frequency (7%) of the variant allele in these two SNPs in our study population. It is noteworthy that *TNF*-308A allele has been linked to primary sclerosing cholangitis (24), a strong risk factor for bile duct cancer. However, we did not find a clear association between any *TNF* variants and bile duct cancer.

Our finding that *RNASEL* and *NOS2* variants are associated with gallstones is novel and requires confirmation. The excess risk associated with the *RNASEL* Ex1–96 A>G variant is of interest, since *RNASEL*, which encodes an interferon-inducible ribonuclease, has been linked to several cancers for which inflammatory processes appears to be important, including cancers of the prostate, pancreas, and colon (25–27). *NOS2A* Leu/Leu homozygotes at amino acid position 608 is reported to confer higher enzymatic activity and gene expression (28), resulting in increased NOS2 expression and inflammation.

In our study, three *IL10* promoter polymorphisms were associated with a modest increase in the risk of gallbladder cancer. These SNPs (*IL10* −672, −854, and −1082) have been previously associated with several cancers, including the stomach, breast, cervix, and liver as well as non-Hodgkin lymphoma and melanoma (29–34). IL-10 is a multifunctional cytokine with both anti-inflammatory and pro-inflammatory properties. Because *IL10* variants have been shown to alter circulating IL-10 levels, with the −627A allele correlated with low IL10 concentrations (35), and because much of the inter-individual variation in IL10 expression (75%) may result from genetic variation (36), the role of *IL10* variants in biliary tract cancer warrants further investigation. IL-10 is known to suppress expression of inflammatory cytokines such as TNF-alpha, IL-6, and IL-1 by activated macrophages (37).

We also found a modest association between gallbladder cancer and *VEGF* variants, which have been linked to several cancers, including prostate, bladder, colon, and breast (38,39). However, since the association was observed for only one variant of *VEGF*, its role in biliary tract cancer needs further study.

Given the strong link between gallstones and biliary tract cancer and the effects of inflammation on both gallstones and biliary tract cancers, it is unclear why certain

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inflammation-related genes are associated with gallstones but not with biliary tract cancer. Several factors may contribute to the discrepancy, including the smaller sample size for biliary tract cancers than for gallstones, and the likely importance of etiologic co-factors in the development of biliary tract cancer. In addition, some of the observed associations could be in LD with one or more causal variants not tested, and some false-positive associations may have arisen by chance, especially in view of the multiple comparisons made in our study. The statistical power would be equally limited in detecting associations for subsites of biliary tract cancer and additional studies will be needed to confirm our results. Despite these concerns, the overall results suggest that genetically related inflammatory processes attribute to the development of gallstones and biliary tract cancer,

Several strengths of our study should be noted, especially the population-based design with nearly complete case ascertainment for cancer, a high participation rate, and confirmation of case status by comprehensive pathologic and clinical review, which minimized the potential for selection, survival and misclassification bias. In addition, the relatively homogenous study population minimizes the potential for bias related to population stratification. Furthermore, the inclusion of two separate case groups, one for biliary tract cancer and one for biliary stones, produced a unique opportunity to determine the effects of specific risk factors, including susceptibility or modifier genes, on these two closely related conditions. However, like most candidate gene studies, our coverage of the inflammation-related gene pathways was limited, since SNP selection was not based on complete sequencing data for our target population and only validated assays could be applied to the study. In addition, due to low minor allele frequency and the small number of bile duct and ampullary cancer cases, there was limited power to evaluate the main effects of SNPs with low minor allele frequencies, or to test for interaction.

In summary, our population-based study in Shanghai revealed that common variants in the *IL8*, *IL8RB*, and *RNASEL* genes were associated with biliary stones, and variants in the *IL1A*, *IL10*, and *VEGF* genes were associated with biliary tract cancer. Further studies are needed to dissect the immunologic and inflammatory pathways that contribute to risk of biliary stones and cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Selected inflammatory genes and SNPs included in the study

Gene	Name	Major Function Related to Inflammation	Location	No. of SNPs[a]	SNP rs #	Nucleotide Change (Amino Acid Change)	Putatively Functional Location in Gene[b]	Also called
22				**62**				
Interleukins								
IL1A	Interleukin 1-alpha	Stimulates proliferation and activation of T, B, and other immune cells.	2q13	4	rs17561 rs2856841 rs2071374 rs1800587	Ex5+21G>T (A114S) IVS4-96T>C IVS4-109A>C Ex1+12C>T	Exon 5 Intron Intron Exon 1/899, 5'UTR	
IL1B	Interleukin 1-beta	Stimulates proliferation and activation of T, B, and other immune cells.	2q14	3	rs16944 rs1143634 rs1143627	−1060T>C Ex5+14C>T (F105F) −580C>T	Intron Exon 5 Intron	
IL4	Interleukin 4	Simulates proliferation of T and endothelium cells	5q31.1	6	rs2243250 rs2243248 rs2070874 rs2243267 rs2243268 rs2243290	−588C>T −1098G>T Ex1-168C>T IVS2-1520G>C IVS2-1443A>C IVS3-9C>A	Intron Intron Exon 1/5'UTR Intron Intron Intron	
IL5	Interleukin 5	Induces differentiation of eosinophils and B cells	5q31.1	3	rs2069812 rs2069807 rs2069818	−745C>T −1551C>T Ex4+78C>A (T128T)	Intron Intron Exon 4	
IL6	Interleukin 6	Induces differentiation of hematopoietic stem and other immune cells	7p15.3	3	rs1800795 rs1800796 rs1800797	−236C>G −635C>G −660A>G	Intron Intron Intron	
IL8	Interleukin 8	Involved in neutrophil chemotaxis	4q12-q13	3	rs4073 rs2227307 rs2227306	−351A>T IVS1+230T>G IVS1-204C>T	Intron Intron Intron	*IL8-251* *IL8+396* *IL8-781*
IL10	Interleukin 10	Inhibits cytokine production of T helper 1 cells while stimulates T helper 2 cells	1q31-q32	5	rs3024496 rs3024491 rs1800871 rs1800872 rs1800896	Ex5+210T>C IVS1-286G>T −7334T>C −853C>T −6653A>C −1116A>G	3'UTR Intron Intron Intron Intron	*IL10-819* *IL10-824* *IL10-854* *IL10-592* *IL10-595* *IL10-627* *IL10-1082* *IL10-1087* *IL10-1117*

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Gene	Name	Major Function Related to Inflammation	Location	No. of SNPs[a]	SNP rs #	Nucleotide Change (Amino Acid Change)	Putatively Functional Location in Gene[b]	Also called
IL13	Interleukin 13	Stimulates growth and differentiation of B cells and inhibits T helper cells as well as inhibits production of macrophage inflammatory cytokines	5q31	3	rs1800925 rs1295686 rs20541	−1069C>T IVS3-24T>C Ex4+98A>G (Q144R)	Intron Intron Exon 4	
IL16	Interleukin 16	Influences CD4+ T cell function	15q25.1	2	rs859 rs11325	Ex22+871A>G Ex22+889G>T	3'UTR 3'UTR	
Interleukin Receptors								
IL8RA	Interleukin 8 receptor, alpha	Signals neutrophil chemotaxis after binding its ligand (i.e., IL8)	2q35	1	rs2234671	Ex2+860G>C (S276T)	Exon 2	
IL8RB	Interleukin 8 receptor, beta	Signals neutrophil chemotaxis after binding its ligand (i.e., IL8)	2q35	3	rs1126579 rs1126580 rs2230054	Ex3+1235T>C Ex3-1010G>A Ex3+811C>T (L262L)	Exon 3 3'UTR Exon 3	*IL8RB1440*
Other Inflammation-related Genes								
PPARD	Peroxisome Proliferative activated receptor, delta	May suppress macrophage activity that leads to reduction of atherosclerotic change	6p21.2-p21.1	1	rs2016520	Ex4+15C>T	5'UTR	
PPARG	Peroxisome Proliferative activated receptor, gamma	May be involved in anti-inflammatory processes through its ability to inhibit transcription factors of pro-inflammatory genes; can be activated by some non-steroidal anti-inflammatory drugs	3p25	2	rs2938392 rs3856806	IVS7+35G>A Ex10+161C>T (H477H)	Intron Exon 10	
RNASEL	Ribonuclease L	Encodes an interferon-inducible ribonuclease	1q25	2	rs486907 rs11072	Ex1-96G>A (R462Q) Ex6-560T>C	Exon 1 3'UTR	*R462Q*
SOD2	Superoxide dismutase 2	Regulates inflammation through reactions with reactive oxygen species	6q25.3	1	rs4880	Ex2+24T>C (V16A)	Exon 2	
MPO	Myeloperoxidase	Regulates inflammation through reactions with reactive oxygen species	17q23.1	2	rs2333227 rs2243828	−642G>A −764T>C	Intron Intron	
NOS2	Nitric oxide synthase2	Controlled by inflammatory mediators and cytokines resulting in production of unregulated quantities of nitric oxide.	17q11.2-q12	2	rs944722 rs2297518	IVs20+524G>A Ex16+14C>T (S608L)	Intron Exon 16	

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Gene	Name	Major Function Related to Inflammation	Location	No. of SNPs[a]	SNP rs #	Nucleotide Change (Amino Acid Change)	Putatively Functional Location in Gene[b]	Also called
NOS3	Nitric oxide synthase3	Produces nitric oxide in response to acute inflammation.	7q36	2	rs1799983 rs1007311	Ex8-63T>G (D298E) IVS7-26A>G	Exon 8 Intron	
TGFB1	Transforming growth factor, beta 1	Maintains T cell homeostasis	19q13.2	2	rs1800469 rs1982073	308bp 3` of STP C>T Ex1-327C>T (P10L)	Intron Exon 1	
TNF	Tumor necrosis factor	Inflammatory cytokine that promotes hyperlipidemia by increasing hepatic triglyceride production and decreasing clearance	6p21.3	7	rs1799964 rs1800630 rs1799724 rs1800750 rs1800629 rs361525 rs1800610	−1210T>C −1042C>A −1036T>C −555A>G −487A>G −427A>G IVS1+123G>A	Intron Intron Intron Intron Intron Intron Intron	*TNF-301* *TNF-863* *TNF-857* *TNF-367* *TNF-308* *TNF-238*
VCAM1	Vascular cell adhesion molecule 1	A cell surface glycoprotein of the Ig gene superfamily and may be responsible for recruiting immune cells to sites of active inflammation	1p32-p31	3	rs1041163 rs3176878 rs3176879	−1591C>T Ex9+20C>T (D601D) Ex9+149G>A (K644K)	Intron Exon 9 Exon 9	
VEGF	Vascular endothelial growth factor	Involved in inflammation-related angiogenesis	6p12	1	rs3025039	236bp 3' of STP C>T EX8+259C>T	Exon 8/3'UTR	*C936T*

[a] UNumber of SNPs typed in the study

[b] UTR= Untranslated region

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Table 2

Selected characteristics of biliary tract cancer cases, stone cases, and controls

							Biliary Tract Cancer (n=411)						Biliary Stones (n=895)			
Characteristics	**Controls[a]**		**Controls[b]**		**Controls[c]**		**Gallbladder Cancer**		**Extrahepatic Bile Duct Cancer**		**Ampulla of Vater Cancer**		**Gallbladder Stones**		**Bile Duct Stones**	
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Total	786	100.0	737	100.0	592	100.0	237	100.0	127	100.0	47	100.0	673	100.0	222	100.0
Age																
<55	107	13.6	106	14.4	97	16.4	32	13.5	18	14.2	4	8.5	216	32.1*	53	23.9*
55–64	224	28.5	216	29.3	169	28.5	62	26.2	32	25.2	9	19.1	186	27.6	66	29.7
>=65	455	57.9	415	56.3	326	55.1	143	60.3	77	60.6	34	72.3	271	40.3	103	46.4
Gender																
Male	305	38.8	290	39.3	252	42.6	65	27.4	76	59.8	24	51.1	224	33.3*	105	47.3
Female	481	61.2	447	60.7	340	57.4	172	72.6	51	40.2	23	48.9	449	66.7	117	52.7
Education																
None/Primary	316	40.2	288	39.1	223	37.7	126	53.2	53	41.7	22	46.8	193	28.7*	78	35.1
Jr. Middle	196	24.9	188	25.5	148	25.0	59	24.9	34	26.8	12	25.5	193	28.7	59	26.6
Sr. Middle	155	19.7	150	20.4	127	21.5	30	12.7	22	17.3	8	17.0	168	25.0	50	22.5
Some college	119	15.1	111	15.1	94	15.9	22	9.3	18	14.2	5	10.6	119	17.7	35	15.8
Marital status																
Married	612	77.9	571	77.5	468	79.1	187	78.9	113	89.0	36	76.6	576	85.7*	189	85.1
Widowed	146	18.6	139	18.9	103	17.4	46	19.4	11	8.7	11	23.4	83	12.4	31	14.0
Divorced/separated	18	2.3	17	2.3	15	2.5	3	1.3	1	0.8	0	0.0	7	1.0	1	0.5
Never married	10	1.3	10	1.4	6	1.0	1	0.4	2	1.6	0	0.0	6	0.9	1	0.5
Smoking (double check)	237	30.2	223	30.3	187	31.6	64	27.1	56	44.1	20	42.6	161	23.9*	80	36.0
Drinking	162	20.6	151	20.5	134	22.6	36	15.2	42	33.1	12	25.5	101	15.0*	41	18.6
Diabetes	65	8.3	56	7.6	38	6.4	31	13.1	12	9.4	3	6.4	75	11.1*	25	11.3*
Hypertension	336	42.7	309	41.9	237	40.0	87	36.7	37	29.1	13	27.7	229	34.0*	64	28.8*
Body mass index																

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Characteristics	Controls[a]		Controls[b]		Controls[c]		Biliary Tract Cancer (n=411)						Biliary Stones (n=895)			
							Gallbladder Cancer		Extrahepatic Bile Duct Cancer		Ampulla of Vater Cancer		Gallbladder Stones		Bile Duct Stones	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
<18.5	66	8.4	65	8.8	55	9.3	11	4.7	6	4.7	1	2.1	25	3.7*	15	6.8*
18.5–22.9	325	41.4	313	42.5	268	45.3	79	33.5	57	44.9	20	42.6	229	34.1	74	33.3
23.0–24.9	166	21.1	155	21.1	123	20.8	47	19.9	33	26.0	11	23.4	164	24.4	51	23.0
>=25.0	228	29.0	203	27.6	145	24.5	99	41.9	31	24.4	15	31.9	254	37.8	82	36.9

*Chi-square test, p<0.05.

[a]All population controls. Bile duct cancer and ampulla of Vater cancer were compared to all population controls.

[b]Excluded population controls with a history of cholecystectomy (n=49).

[c]Excluded population controls with gallstones (n=145)

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Table 3

Odds ratios (ORs) and 95% confidence intervals (CIs) for biliary stones in relation to selected variants in inflammation genes

		Biliary Stones				Gallstones				Bile Duct Stones			
Gene/SNP (dbSNP ID)	**Controls**	**Cases**	**OR[a]**	**95% CI**	***P-rend***	**Cases**	**OR[a]**	**95% CI**	***P trend***	**Cases**	**OR[a]**	**95% CI**	***P trend*[b]**
Total	592	883				664				219			
IL8													
−351A>T (rs4073)													
TT	181	295	1.00	-		196	1.00	-		99	1.00	-	
TA	317	439	0.86	0.68–1.09		358	1.06	0.82–1.37		81	0.48	0.34–0.68	
AA	89	149	1.02	0.74–1.42		110	1.14	0.80–1.63		39	0.80	0.51–1.26	
					0.82				*0.45*				*0.04*
TA or AA	406	588	0.90	0.71–1.12		468	1.08	0.84–1.38		120	0.55	0.40–0.76	
IVS1+230 T>G (rs2227307)													
TT	180	296	1.00	-		196	1.00	-		100	1.00	-	
TG	317	437	0.85	0.67–1.08		355	1.04	0.81–1.35		82	0.48	0.34–0.68	
GG	87	148	1.03	0.75–1.44		110	1.17	0.82–1.66		38	0.79	0.50–1.25	
					0.83				*0.42*				*0.03*
TG/GG	404	585	0.89	0.71–1.12		465	1.07	0.83–1.37		120	0.55	0.40–0.76	
IVS1-204C>T(rs2227306)													
CC	213	351	1.00	-		239	1.00	-		112	1.00	-	
CT	305	423	0.84	0.67–1.06		345	1.01	0.79–1.30		78	0.50	0.35–0.70	
TT	70	121	1.03	0.73–1.46		89	1.11	0.77–1.61		32	0.87	0.54–1.41	
					0.65				*0.64*				*0.03*
CT or TT	375	544	0.88	0.71–1.09		434	1.03	0.82–1.31		110	0.57	0.42–0.78	
***Global P value*[b]**					*0.83*				*0.64*				*0.04*
IL8RB													
Ex3+811C<T (rs2230054)													
CC	282	357	1.00	-		270	1.00	-		87	1.00	-	

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		Biliary Stones				Gallstones				Bile Duct Stones			
Gene/SNP (dbSNP ID)	**Controls**	**Cases**	**OR[a]**	**95% CI**	***P-rend***	**Cases**	**OR[a]**	**95% CI**	***P trend***	**Cases**	**OR[a]**	**95% CI**	***P trend[b]***
CT	223	373	1.34	1.06–1.69		277	1.33	1.03–1.70		96	1.41	1.00–1.98	
TT	54	107	1.65	1.14–2.38		82	1.70	1.15–2.52		25	1.50	0.88–2.56	
					0.002				*0.002*				*0.04*
CT or TT	277	480	1.40	1.13–1.74		359	1.40	1.10–1.77		121	1.42	1.03–1.97	
Ex3+1235T<C (rs1126579)													
TT	247	325	1.00	-		246	1.00	-		79	1.00	-	
TC	255	396	1.17	0.93–1.48		294	1.15	0.90–1.47		102	1.24	0.88–1.75	
CC	65	125	1.53	1.08–2.17		97	1.60	1.11–2.32		28	1.35	0.81–2.26	
					0.01				*0.02*				*0.16*
TC or CC	320	521	1.25	1.00–1.55		391	1.24	0.98–1.57		130	1.26	0.91–1.75	
Ex3-1010G<A (rs1126580)													
GG	339	463	1.00	-		354	1.00	-		109	1.00	-	
GA	196	336	1.27	1.01–1.59		248	1.22	0.95–1.56		88	1.41	1.01–1.97	
AA	30	44	1.09	0.67–1.78		35	1.17	0.69–1.97		9	0.89	0.41–1.95	
					0.11				*0.15*				*0.22*
GA/AA	226	380	1.24	1.00–1.55		283	1.21	0.96–1.53		97	1.34	0.97–1.85	
Global P value					*0.006*				*0.006*				*0.12*
RNASEL													
Ex1-96 A>G (rs486907)													
GG	357	488	1.00	-		355	1.00	-		133	1.00	-	
GA	204	348	1.22	0.98–1.53		270	1.34	1.05–1.70		78	1.01	0.72–1.40	
AA	28	53	1.37	0.84–2.23		43	1.52	0.91–2.53		10	0.93	0.44–1.97	
					0.05				*0.001*				*0.92*
GA/AA	232	401	1.24			313	1.36	1.08–1.71		88	1.00	0.72–1.37	
Ex6-560A>G (rs11072)													
TT	356	563	1.00	-		419	1.00	-		144	1.00	-	
TC	186	253	0.86	0.68–1.09		189	0.87	0.67–1.12		64	0.85	0.60–1.20	
CC	24	31	0.78	0.45–1.36		25	0.84	0.47–1.52		6	0.59	0.23–1.47	

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		Biliary Stones				Gallstones				Bile Duct Stones			
Gene/SNP (dbSNP ID)	**Controls**	**Cases**	**OR[a]**	**95% CI**	***P-rend***	**Cases**	**OR[a]**	**95% CI**	***P trend***	**Cases**	**OR[a]**	**95% CI**	***P trend[b]***
					0.16				*0.25*				*0.18*
TC/CC	210	284	0.85	0.68–1.07		214	0.86	0.68–1.10		70	0.82	0.59–1.15	
Global P value					*0.10*				*0.002*				*0.36*
TNF													
−1211C>T (rs1799964)													
TT	360	562	1.00	-		432	1.00	-		130	1.00	-	
TC	183	269	0.96	0.76–1.21		194	0.91	0.71–1.17		75	1.14	0.81–1.60	
CC	29	32	0.70	0.41–1.19		23	0.64	0.36–1.14		9	0.88	0.40–1.92	
					0.28				*0.15*				*0.74*
TC or CC	212	301	0.92	0.74–1.15		217	0.87	0.68–1.11		84	1.10	0.80–1.53	
−1042A>C (rs1800630)													
CC	382	621	1.00	-		479	1.00	-		142	1.00	-	
CA	163	210	0.81	0.64–1.04		146	0.74	0.57–0.97		64	1.05	0.74–1.49	
AA	20	19	0.59	0.31–1.13		14	0.54	0.27–1.10		5	0.68	0.25–1.86	
					0.03				*0.0008*				*0.83*
CA or AA	183	229	0.79	0.62–1.00		160	0.72	0.56–0.93		69	1.01	0.72–1.42	
−1036C>T (rs1799724)													
CC	439	659	1.00	-		491	1.00	-		168	1.00	-	
CT	139	208	0.97	0.76–1.25		161	1.00	0.76–1.30		47	0.90	0.62–1.31	
TT	9	19	1.23	0.54–2.78		13	1.10	0.46–2.65		6	1.60	0.56–4.60	
					0.94				*0.93*				*0.99*
CT/TT	148	227	0.99	0.78–1.26		174	1.01	0.77–1.30		53	0.94	0.66–1.36	
−487A>G (rs1800629)													
GG	510	772	1.00	-		582	1.00	-		190	1.00	-	
GA	66	102	1.05	0.75–1.46		78	1.06	0.74–1.51		24	0.97	0.59–1.60	
AA	6	3	-	-		2	-	-		1	-	-	
					0.67				*0.76*				*0.64*
GA or AA	72	105	0.99	0.71–1.37		80	1.00	0.71–1.42		25	0.93	0.57–1.51	

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		Biliary Stones				Gallstones				Bile Duct Stones			
Gene/SNP (dbSNP ID)	**Controls**	**Cases**	**OR[a]**	**95% CI**	***P-rend***	**Cases**	**OR[a]**	**95% CI**	***P trend***	**Cases**	**OR[a]**	**95% CI**	***P trend*[b]**
−417A>G (rs361525)													
GG	551	820	1.00	-		614	1.00	-		206	1.00	-	
GA	37	68	1.21	0.79–1.84		53	1.28	0.82–1.99		15	1.13	0.60–2.11	
AA	0	4	-	-		3	-	-		1	-	-	
					0.17				*0.11*				*0.46*
GA or AA	37	72	1.28	0.84–1.94		56	1.36	0.87–2.10		16	1.19	0.65–2.21	
IVS1+123G>A (rs1800610)													
GG	395	597	1.00	-		448	1.00	-		149	1.00	-	
GA	164	225	0.88	0.69–1.12		169	0.86	0.67–1.12		56	0.92	0.64–1.31	
AA	12	25	1.27	0.62–2.58		19	1.27	0.60–2.68		6	1.28	0.47–3.50	
					0.63				*0.58*				*0.89*
GA or AA	176	250	0.91	0.72–1.15		188	0.89	0.69–1.15		62	0.94	0.67–1.34	
Global P value					*0.18*				*0.30*				*0.99*
NOS2													
IVS20+524G>A (rs944722)													
AA	328	435	1.00	-		291	1.00	-		107	1.00	-	
AG	263	358	1.04	0.83–1.31		232	1.02	0.80–1.30		95	1.10	0.79–1.52	
GG	50	62	0.91	0.60–1.37		47	0.94	0.61–1.46		12	0.72	0.37–1.41	
					0.92				*0.91*				*0.77*
AG or GG	313	420	1.02	0.82–1.26		279	1.00	0.80–1.26		107	1.04	0.76–1.42	
Ex16+14C>T (rs2297518)													
CC	427	582	1.00	-		412	1.00	-		155	1.00	-	
CT	198	249	1.32	1.03–1.69		138	1.47	1.13–1.91		51	1.00	0.69–1.45	
TT	14	20	1.16	0.56–2.38		13	1.12	0.51–2.45		6	1.29	0.48–3.48	
					0.05				*0.01*				*0.80*
CT or TT	212	269	1.30	1.03–1.66		151	1.44	1.11–1.85		57	1.02	0.71–1.46	
Global P value					*0.10*				*0.02*				*0.80*

[a] Adjusted for age and gender.

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[b] P value for global test to assess the overall gene effect.

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Table 4

Odds ratios (ORs) and 95% confidence intervals (CIs) for biliary tract cancers in relation to selected variants in inflammation genes

	Gallbladder Cancer					Extrahepatic Bile Duct Cancer					ampulla of Vater Cancer				
Gene/SNP (dbSNP ID)	**Cases**	**Controls**	**OR[a]**	**95% CI**	***P-trend***	**Cases**	**Controls**	**OR**	**95% CI**	***P-trend***	**Cases**	**Controls**	**OR[a]**	**95% CI**	***P-trend***
Total	237	737	-	-	-	127	786	-	-	-	47	786	-	-	-
IL10															
Ex5+210C>T (rs3024496)															
TT	223	678	1.00	-		116	720	1.00	-		40	720	1.00	-	
TC	12	51	0.74	0.38–1.41		9	57	0.95	0.45–1.98		7	57	2.12	0.90–5.00	
CC	0	1	-	-		0	1	-	-		0	1	-	-	
					0.30					*0.84*					*0.10*
TC or CC	12	52	0.72	0.38–1.38		9	58	0.94	0.45–1.96		7	58	2.10	0.89–4.96	
IVS1-286G>T (rs3024491)															
GG	225	683	1.00	-		118	726	1.00	-		40	726	1.00	-	
GT	11	50	0.69	0.35–1.35		9	56	0.95	0.46–2.00		7	56	2.16	0.92–5.11	
TT	0	1	-	-		0	1	-	-		0	1	-	-	
					0.23					*0.85*					*0.09*
GT or TT	11	51	0.67	0.34–1.32		9	57	0.94	0.45–1.98		7	57	2.14	0.91–5.06	
−6653A>C (rs1800872)															
AA	121	318	1.00	-		57	340	1.00	-		19	340	1.00	-	
AC	91	334	0.70	0.51–0.96		16	90	0.93	0.62–1.41		21	90	1.13	0.60–2.15	
CC	23	82	0.76	0.45–1.26		52	352	1.09	0.59–2.01		6	352	1.26	0.49–3.26	
					0.06					*0.94*					*0.60*
AC or CC	114	416	0.71	0.53–0.96		68	442	0.97	0.66–1.42		27	442	1.16	0.63–2.13	
−7334T>C (rs1800871)															
TT	122	311	1.00	-		55	334	1.00	-		20	334	1.00	-	
TC	92	335	0.69	0.50–0.94		52	353	0.94	0.62–1.42		6	353	1.06	0.56–2.00	
CC	23	82	0.74	0.44–1.23		17	90	1.19	0.65–2.16		21	90	1.19	0.46–3.07	
					0.04					*0.76*					*0.73*

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Gene/SNP (dbSNP ID)	Gallbladder Cancer					Extrahepatic Bile Duct Cancer					ampulla of Vater Cancer				
	Cases	Controls	OR[a]	95% CI	*P-trend*	Cases	Controls	OR	95% CI	*P-trend*	Cases	Controls	OR[a]	95% CI	*P-trend*
TC or CC	115	417	0.70	0.52–0.94		69	443	0.99	0.67–1.46		27	443	1.09	0.60–1.98	
−1116A>G (rs1800896)															
AA	231	624	1.00	-		107	664	1.00	-		38	664	1.00	-	
AG	23	99	0.65	0.40–1.06		18	108	1.11	0.65–1.93		9	108	1.50	0.70–3.21	
GG	1	7	-	-		0	7	-	-		0	7	-	-	
					0.06					*0.91*					*0.51*
AG or GG	24	106	0.63	0.40–1.03		18	115	1.04	0.60–1.80		9	115	1.41	0.66–3.00	
Global p value					*0.10*					*0.94*					0.25
VEGF															
236bp 3' of STP (rs3025039)															
CC	172	487	1.00	-		87	518	1.00	-		32	518	1.00		
CT	58	21	0.72	0.51–1.01		34	236	0.87	0.56–1.34		15	236	1.03	0.55–1.94	
TT	4	22	-	-		5	25	1.20	0.44–3.28		0	25	-		
					0.03					*0.78*					*0.55*
CT or TT	62	43	0.70	0.50–0.97		39	261	0.90	0.60–1.36		15	261	0.93	0.49–1.75	

[a] Adjusted for age and gender.

[b] P value for global test to assess the overall gene effect.

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Table 5

Odds ratios (OR)[a] and 95% confidence intervals (CI) for biliary tract cancer and biliary stones in relation to common haplotypes

Gene/Haplotype	Controls	Biliary Tract Cancer									Biliary Stones					
		Gallbladder			Bile Duct			ampulla of Vater			Gallstones			Bile Duct Stones		
	%	%	OR[a]	95% CI	%	OR[a]	95% CI	%	OR[a]	95% CI	%	OR[a]	95% CI	%	OR[a]	95% CI
IL8RB[b]																
C-T-G	64.4	62.4	1.00	-	61.0	1.00	-	62.9	1.00	-	61.5	1.00	-	61.0	1.00	-
T-C-A	23.0	23.7	1.09	(0.83–1.37)	24.9	1.05	(0.75–1.46)	22.2	0.94	(0.56–1.58)	24.2	1.20	(0.98–1.46)	25.9	1.25	(0.96–1.64)
T-C-G	8.3	9.6	1.16	(0.79–1.70)	6.0	0.75	(0.41–1.39)	11.4	1.42	(0.69–2.90)	10.4	1.54	(1.14–2.07)	8.7	1.25	(0.81–1.92)
C-C-G	2.9	3.8	1.36	(0.75–2.47)	3.8	1.27	(0.59–2.75)	--	--	--	3.1	1.04	(0.64–1.68)	2.3	0.79	(0.37–1.68)
C-C-A	1.2	0.5	--	--	1.5	1.42	(0.42–4.74)	--	--	--	0.7	--	--	1.4	1.29	(0.45–3.70)
Global p-value			*0.40*			*0.003*			*0.80*			*0.02*			*0.32*	

[a] Adjusted for age and gender.

[b] *IL8RB* SNPs: Ex3+811C>T, Ex3+1235T>C, Ex3-1010G>A.