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Polymorphisms of Genes in the Lipid Metabolism Pathway and the Risk of Biliary Tract Cancers and Stones: A Population-based Case-Control Study in Shanghai, China

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Abstract

Biliary tract cancers, encompassing the gallbladder, extrahepatic bile duct, and ampulla of Vater, are uncommon, yet highly fatal malignancies. Gallstones, the primary risk factor for biliary cancers, are linked with hyperlipidemia. We examined the associations of 12 single nucleotide polymorphisms (SNPs) of five genes in the lipid metabolism pathway with the risks of biliary cancers and stones in a population-based case-control study in Shanghai, China. We included 235 gallbladder, 125 extrahepatic bile duct, and 46 ampulla of Vater cancer cases, 880 biliary stone cases, and 779 population controls. Subjects completed an in-person interview and gave blood. Genotyping was conducted by Taqman assay using DNA from buffy coats. The effects of *APOE* IVS1+69 (rs440446) and *APOB* IVS6+360C>T (rs520354) markers were limited to men. Men carrying the G allele of *APOE* IVS1+69 had a 1.7-fold risk of stones (95% confidence interval (CI) =1.2–2.4), a 1.8–fold risk of gallbladder cancer (95% CI=1.0–3.3), a 3.7–fold risk of bile duct cancer (95% CI=2.0–7.0), and a 4-fold risk of ampullary cancer (95% CI=1.4–12.4). Male carriers of the T allele of *APOB* IVS6 +360C>T had a 2-fold risk of bile duct cancer (95% CI=1.2–3.4). The *APOB* T-T haplotype

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(*APOB* IVS6+360C>T, EX4+56C>T) was associated with a 1.6-fold risk of bile duct cancer (95% CI=1.1–2.3). Male and female carriers of the T allele of *LDLR* IVS9-30C>T (rs1003723) had 1.5-fold risks of bile duct cancer. Our findings suggest that gene variants in the lipid metabolism pathway contribute to the risk of biliary tract stones and cancers, particularly of the bile duct.

Keywords

single nucleotide polymorphisms; lipid metabolism; gallstones; biliary tract cancer

Introduction

Biliary tract cancers, consisting of tumors of the gallbladder, extrahepatic bile ducts, and ampulla of Vater, are rare, but highly fatal malignancies (1,2). Cholesterol gallstones are the most important known risk factor for biliary tract cancers, especially gallbladder cancer (3,4). Hyperlipidemia, characterized by increased total cholesterol, triglycerides, and low-density lipoprotein (LDL), and decreased high-density lipoprotein (HDL), has been associated with gallstones (5,6) and with biliary tract cancer (2,7).

Serum lipids are affected by both genetic and lifestyle factors, such as diet, body weight, and physical activity. In addition to a familial tendency, hyperlipidemia has been associated with several genetic polymorphisms in the lipid metabolism pathway (8,9). For example, variants of the *LDLR* (low-density lipoprotein receptor) gene encoding the LDL receptor which regulates the uptake of LDL have been linked to familial hyperlipidemia (8,9). Variants of the *APOB* and *APOE* genes which encode the major carrier and binding proteins of LDL, apolipoprotein B and E (apo B and E), have been associated with higher serum levels of total cholesterol, LDL, apo B, and lower HDL (10,11). Also, variants of the *LPL* gene, which encodes the lipoprotein lipase enzyme, have been associated with higher serum LDL levels (12), and variants of *ALOX5*, which is involved in the synthesis of inflammatory lipid mediators, have been linked to cancer and heart disease (13,14).

The role of genetic factors in biliary tract cancers and stones has been suggested by reports of ethnic and familial predisposition, but the genetic pathways are unclear (1,15). Although some gene variants in the lipid metabolism pathway have been linked to gallstones (16,17), few studies have examined their role in biliary tract cancers. In this population-based case-control study conducted in Shanghai, China, we examined the association of 12 polymorphic variants of 5 genes (*APOB*, *APOE*, *LDLR*, *LPL*, and *ALOX5)* in the lipid metabolism pathway with the risks of biliary tract stones and cancers, and also assessed whether the variants were related with biliary tract cancers through their association with gallstones.

Materials and Methods

Study Subjects

Details of the study methods have been reported elsewhere (4,15,18). Briefly, cancer cases were identified by a rapid reporting system established by the Shanghai Cancer Institute (SCI) and 42 collaborating hospitals in Shanghai. Through this system we identified more than 95% of all incident biliary tract cancer cases (ICD9 code 156) diagnosed among Shanghai residents between June 1997 and May 2001. A total of 627 incident biliary tract cancer cases (368 gallbladder, 191 extrahepatic bile duct, and 68 ampulla of Vater cases) between 35–74 years of age, were included in this study. In order to evaluate the risk of biliary stones independently of biliary tract cancer, 1,037 patients with biliary stones, (774 gallstones and 263 extrahepatic bile duct stones) without a history of cancer, were included and were frequency-matched to the cancer cases on age, (5-year groups) gender, and diagnosing hospital. Population controls,

without a history of biliary tract cancer, were randomly selected from the Shanghai Resident Registry that includes records of approximately 6 million Shanghai residents. We included a total of 959 population controls who were frequency-matched to cancer cases on age (5-year groups) and gender. Participation rates among eligible cancer patients and control subjects were 95% and 82%, respectively.

Case Confirmation

Biliary tract cancer and biliary stone diagnoses were confirmed by a panel of clinicians, ultrasonographers, and pathologists. Seventy percent of biliary tract cancer cases were confirmed by histopathologic assesment, while the remaining 30% of cases, for whom we did not have histopathologic material, were confirmed using medical records, surgical reports, and imaging data, including magnetic resonance imaging (MRI), endoscopic retrograde cholangiopancreatography (ERCP), or computed tomography (CT). Biliary stone cases were confirmed by review of abdominal ultrasound, ERCP films, medical records, and surgical records, or by pathologic material for those who underwent a cholecystectomy.

Gallstone Assessment

Gallstone status was assessed in nearly all biliary tract cancer cases and population controls. Among cancer cases, gallstones were identified by self-reported history, surgical reports, or imaging results from MRI, ERCP, CT, or ultrasound. Among population controls, gallstones were identified by self-reported history or by abdominal ultrasound among those who gave consent for the procedure (85% of all population controls).

Interview

Information on demographic characteristics, medical histories, and lifestyle factors was obtained through in-person interviews conducted by trained interviewers, using a structured questionnaire. Cases were interviewed within 3 weeks of diagnosis. Weight and height were measured at the time of interview. The response rate for interviews was over 95% among cases and 82% among controls. Five percent of the study subjects were randomly selected for re-interview three months after the initial interview to assess the reproducibility of responses. The concordance of responses to key questions between the original and follow-up interviews was greater than 90%.

Genotyping

Five candidate genes were selected for their role in lipid metabolism, their effects on serum lipid levels, and their potential effects on biliary disease pathogenesis. SNPs were selected based on their putative functional significance, having a variant allele frequency of at least 5% among Asians as reported by NCI's SNP500Cancer Database (http://snp500cancer.nci.nih.gov) (19), and a validated assay at the NCI Core Genotyping Facility (CGF) (http://cgf.nci.nih.gov/home.cfm). The following 12 SNPs in 5 genes were selected: *APOB* [EX26–3573T>C (rs676210), IVS23–79T>C (rs673548), IVS6+360C>T (rs520354), EX4+56C>T (rs1367117)], *APOE* [IVS1+69C>G (rs440446)], *LDLR* [IVS9–30C>T (rs1003723), EX10+55G>A (rs5930), EX15–80G>A (rs5927), IVS17–42A>G (rs6413504), EX18+88G>A (rs14158)] and *LPL* [IVS5–540C>T (rs263)], and *ALOX5* [IVS3 +100G>A (rs2029253)]. In total, we included 7 SNPs in introns and 5 in exons (2 non-synonymous, 2 synonymous, and 1 non-coding 3'UTR). Overnight fasting blood samples were collected from all subjects who gave consent (over 80%). Genomic DNA was extracted from buffy coat by the standard phenol chloroform method at the NCI laboratory. Genotyping was conducted at the CGF using the TaqMan assay (Applied Biosystems, Foster City, CA, http://snp500cancer.nci.nih.gov). The genotyping failure rate among all samples was less than 2%. The quality and potential misclassification of the genotyping results were assessed by

evaluating 20 duplicate DNA samples from 4 quality control subjects (80 total samples) that were randomly placed within the same reaction plates used for study subjects. The concordance between duplicate samples was over 99%.

Statistical Analysis

The final analysis included subjects who completed the interview and for whom we had DNA, which totaled 406 incident biliary tract cancer cases (235 gallbladder, 125 extrahepatic bile duct, 46 ampulla of Vater), 880 biliary stone cases (663 gallstone, 217 bile duct), and 779 healthy controls. In order to make the appropriate case-control comparisons, for all analyses, gallbladder cancer cases were compared to controls without a history of cholecystectomy (n=730); bile duct cancer cases and ampulla of Vater cancer cases were compared to all controls (n=779); and biliary stone cases were compared to controls without biliary stones (n=586). Distributions of selected characteristics (gender, age group, education status, cigarette smoking status, alcohol drinking status, body mass index (BMI), hypertension, diabetes, and gallstone status (among cancer cases) were evaluated among cases and controls; characteristics with statistically significant different distributions between cases and controls (Fishers exact $p<0.05$) were examined for their associations with SNPs among controls.

The associations between SNPs and biliary tract stone and cancer risk were estimated by odds ratios (ORs) and 95% confidence intervals (95% CIs), using unconditional logistic regression. Risk estimates were calculated for the heterozygous and homozygous variant genotypes relative to the common homozygous genotype, as well as for the presence and absence of the variant allele, using indicator variables in the regression model. An initial model was adjusted for age, and additional models were further adjusted for gender, BMI, waist-to-hip ratio (WHR), cigarette smoking, alcohol drinking, hypertension, diabetes and gallstone status (cancer risk only) in order to evaluate the potential confounding by these factors. Associations between SNPs and biliary stones and cancers were assessed using the linear test of trend for the number of copies of the variant allele (0, 1, 2) assuming an additive model, or with the Wald chi-square test 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 the above mentioned characteristics and the SNPs on stone and cancer risk. Although gallstones may be an intermediate step in the causal pathway between genetics and biliary tract cancer, it is also possible that genetic variants may affect cancer risk independent of stones, especially for bile duct and ampulla of Vater cancers, which have a much lower prevalence of stones than gallbladder cancer. Thus, in subsequent analysis, we examined the association between SNPs and biliary tract cancer after adjustment for biliary stones.

In order to assess the overall gene effects on biliary tract cancer and stone risk, the Simes global test was used to calculate a summary p-value for each gene for which we examined multiple SNPs (*APOB* and *LDLR*). The Simes global test utilizes the p trend values of each SNP and accounts for multiple SNP comparisons within each gene by controlling for the gene-wide type I error rate (20,21). Similarly, the Simes global test was used to obtain an overall pathway p-value adjusting for all 12 SNPs and controlling for the familywise error rate.

We also examined the associations between the haplotypes of the *APOB* and *LDLR* genes and the risks of biliary stones and cancers. Among population controls, linkage disequilibrium (LD) between markers was assessed by calculating pariwise Lewontin's D` and r^2 using Haploview version 3.11 (22). Haplotypes were reconstructed for cases and controls separately from genotype data using Haplostats in R version 2.0.1 (23), which uses an expectation maximization algorithm to calculate maximum likelihood estimates of haplotype frequencies while taking into account phase ambiguity (24). Associations between haplotypes (>1% frequency) and the risks of biliary tract stones and cancer were evaluated by computing ORs and 95% CI, using Haplostats, assuming an additive model, and using the most common haplotype as the referent

category. Global differences in haplotype frequencies between cases and controls were assessed for each gene using the Score test in Haplostats (23).

Results

Selected characteristics of the study subjects are shown in Table 1. The frequency of selected characteristics did not differ considerably between the 3 subgroups of controls (all controls, controls without cholecystectomy, and controls without biliary stones). Biliary stone cases were more likely to be women, younger, overweight, and diabetic, but less likely to drink alcohol or have hypertension, compared to controls without stones. Gallbladder cancer cases were predominantly women (72.3%), while bile duct cancer cases were more common in men (60%). In general, biliary cancer cases were more likely to have a higher WHR (>0.92) and gallstones than controls. Bile duct and ampulla of Vater cancer cases were more likely to ever smoke cigarettes and drink alcohol, but less likely to have hypertension, while gallbladder cancer cases were more likely to be overweight (41.3%) and diabetic (13.2%) compared to controls.

The risk of biliary stones and cancers in relation to the 12 SNPs in the lipid metabolism pathway are shown in Table 2. Among population controls, the minor allele frequencies of the 12 SNPs ranged from 5% to 39%, and the genotype frequencies showed no deviation from the expected Hardy-Weinberg equilibrium proportions ($p > 0.05$). Of the 12 SNPs examined, only the *APOE* IVS1+69C>G marker was significantly associated with biliary stones. Specifically, carriers of the G allele had a 1.3-fold risk of biliary stones (95% CI=1.03–1.60, p_{trend}=0.02), compared with those having the CC genotype. Variants in the *APOE*, *APOB*, and *LDLR* genes were associated with either bile duct or ampulla of Vater cancers, while none of the markers examined was significantly associated with gallbladder cancer. Carriers of the G allele of the *APOE* IVS1+69C>G marker had a 2.0-fold risk of bile duct cancer (95% CI=1.32–3.11, p_{trend}=0.003) and a 2.5-fold risk of ampulla of Vater cancer (95% CI=1.21–5.08, p_{trend}=0.002), compared with those having the CC genotype. We also found that carriers of the T allele of the *APOB* IVS6+360C>T marker and the T allele of the *LDLR* IVS9–30C>T marker each had a 1.5-fold risk of bile duct cancer, compared with those having the CC genotype of each marker. In addition, carriers of the GA genotype of the *LDLR* EX18+88G>A marker had a 2.7-fold risk of ampulla of Vater cancer, relative to those having the GG genotype. The associations between the *APOE*, *APOB*, and *LDLR* gene variants with stone and cancer risks were independent of gender, BMI, WHR, hypertension, diabetes, cigarette smoking, and alcohol drinking, while the associations with cancer were independent of gallstones.

Because of gender differences in the incidence of biliary stones and biliary tract cancers, we evaluated the gender specific effects of the *APOE*, *APOB*, and *LDLR* markers for which we found significant main effects (Table 3). The effects of the *APOE* IVS1+69C>G marker were limited to men, with male carriers of the G allele having a 1.7-fold risk of biliary stones (95% CI=1.19–2.36), 1.8-fold risk of gallbladder cancer (95% CI=1.04–3.26), a 3.7-fold risk of bile duct cancer (95% CI=1.99–6.96), and a 4.1-fold risk of ampulla of Vater cancer (95% CI=1.36–12.38). A statistically significant interaction between gender and the *APOE* IVS1+69C>G marker was seen for biliary stones ($P_{interaction}$ =0.03) and bile duct cancer ($P_{interaction}$ =0.01). The effect of the *APOB* IVS6+360C>T marker was also limited to men, with male carriers of the T allele having a 2-fold risk of bile duct cancer (95% CI=1.21–3.40), although the interaction between gender and the *APOB* IVS6+360C>T marker was not statistically significant ($P_{interaction}$=0.09). The effects of these two SNPs among men were independent of BMI, WHR, hypertension, diabetes, cigarette smoking, alcohol drinking, and gallstones (for biliary cancer). No gender differences were seen in the association between the *LDLR* markers, IVS9–30C>T and EX18+88G>A, and the risks of biliary stones or cancers.

Using the Simes test to adjust for multiple SNP comparisons within the *APOB* and *LDLR* genes, we found that bile duct cancer had a borderline association with *APOB* (P_{Simes}=0.08), and a non-significant association with *LDLR* (P_{Simes} =0.20). Also, adjusting for all 12 SNPs, the combined effect of these gene variants was significantly associated with bile duct cancer (P_{Simes} =0.04). Among men, but not women, the associations of bile duct cancer with the *APOB* gene (P_{Simes} =0.01) and all variants examined in the lipid metabolism pathway (P_{Simes} =0.001) remained significant.

Of the 4 *APOB* SNPs examined in the study, strong linkage disequilibrium was present among the *APOB* EX26–3573T>C and IVS23–79T>C markers (D'=1.0) and the *APOB* IVS6 +360C>T and EX4+56C>T markers (D'=0.81). Thus, we inferred common haplotypes separately for these two sets of *APOB* SNPs. Two major haplotypes (frequency ≥ 1.0%) were inferred from the *APOB* EX26–3573T>C and IVS23–79T>C markers (TT=75.7%, CC=24.3%) and three from the *APOB* IVS6+360C>T and EX4+56C>T markers (CC=80.3%, TT=11.8%, TC=5.8%) (Table 4). Consistent with our single marker results, the *APOB* TT haplotype, which contains the risk-conferring alleles of the IVS6+360C>T and EX4+56C>T markers, was associated with a 1.6-fold risk of bile duct cancer (95% CI=1.07–2.33), compared with the most frequent CC haplotype. The CT haplotype was associated with a significant risk of ampulla of Vater cancer (OR=3.10, 95% CI=1.12–8.61); however, this estimate was based on very small number of cases (n=5). Although the Omnibus score test was significant for the *APOB* (EX26–3573T>C - IVS23–79T>C) and *LDLR* haplotypes, these p-values may reflect the small numbers of the uncommon haplotypes since none of the common haplotypes was associated with biliary tract cancers.

Discussion

In this population-based study, variants in lipid metabolism genes, including *APOE, APOB,* and *LDLR*, were associated with excess risks of biliary stones and cancers, although most of the effects were limited to men. The *APOB* TT haplotype, containing the risk-conferring alleles of the *APOB* IVS6+360C>T and EX4+56C>T markers, was associated with an excess risk of bile duct cancer, supporting further a relationship between *APOB* variants and bile duct cancer. These results, although needing confirmation, suggest that polymorphic variants in genes involved in lipid metabolism may play a role in the etiology of biliary stones and biliary tract cancers.

Our finding of an association between the *APOE* IVS1+69 marker and biliary stones corroborates previous studies relating gallstones to *APOE* gene polymorphisms. Several population-based studies of *APOE* have reported higher risks of gallstones among carriers of the e4 allele, a variant with a single amino acid change, compared to non-e4 carriers (25,26). The *APOE* e4 allele has been associated with high cholesterol content in gallstones, faster crystallization, and frequent stone recurrence (26). Another study reported an excess risk of gallstones mainly among *APOE* e2 allele carriers who also had lower serum LDL levels (27). Since apo E is a major component of lipoproteins and plays a critical role in the transport of cholesterol to the liver, our findings, together with previous studies, suggest that the *APOE* gene may predispose to biliary stones through effects on serum lipid levels (28).

The observed effects of the *APOE IVS1+69* marker on bile duct and ampulla of Vater cancer risks were independent of gallstones, suggesting that mechanisms in addition to stones may be involved in biliary carcinogenesis. Apart from its role in lipid metabolism, *APOE* has important effects on the inflammatory response. Specifically, serum apo E acts as a modulator of proinflammatory cytokines, including TNF-α and IL-1-β (29,30), which are expressed in biliary tract tumor tissue (31,32). In addition, apo E's inhibitory effect on endothelial and tumor cell proliferation suggests its possible involvement in angiogenesis, tumor cell growth, and

metastasis (33,34). Although the *APOE* gene has not been investigated previously in biliary tract cancer, it has been related to increased risk of other malignancies, including breast, colon/rectum, and prostate (35–37).

In our study, the effects of the *APOE* IVS1+69C>G marker on biliary stones and cancer were limited to men, suggesting a gender-specific role of *APOE*. These findings are noteworthy since previous studies have noted stronger positive associations between the *APOE* e4 allele and triglyceride levels among men than women (38,39), raising the possibility that the metabolic effect of *APOE* may contribute to the higher incidence of bile duct and ampullary cancers reported among men than women (1).

Of the 4 *APOB* SNPs examined, the *APOB* IVS6+360C>T marker was associated with an excess risk of bile duct cancer, independently of gallstones and the *APOE* IVS1+69C>G marker. Supporting this observation, we also found a relation between bile duct cancer and the *APOB* TT haplotype containing the at-risk allele of the *APOB* IVS6+360C>T marker. The mechanism by which *APOB* confers risk to bile duct cancer through a pathway independent of gallstones is unclear, but it may be linked to its relationship with serum apo B, the major carrier and binding protein of LDL (40,41). Variants of *APOB* have been linked to higher levels of serum apo B (27,42), which are correlated with higher levels of serum LDL. The resulting elevation in oxidized LDL may trigger an increase in reactive oxygen species (43,44), DNA damage, activation of proto-oncogenes, or inactivation of tumor suppressor genes (45), all of which may play a role in biliary tract carcinogenesis (46).

Independent of gallstones and the *APOE* and *APOB* markers, two of the five *LDLR* markers, *LDLR* IVS9–30C>T and *LDLR* EX18+88G>A were associated with biliary cancers. The mechanisms are unclear, but may relate to the role of the LDL receptor in the uptake of LDL by the liver and extrahepatic tissue. Decreased activity of the LDL receptor has been associated with increased circulating levels of LDL, triglycerides, and total cholesterol (47), as well as proinflammatory cytokines, including TNF-α, IL-1, and IL-6, which are expressed in biliary tract cancer tissue (31,32).

Our observation that the effect of lipid metabolism genes varied by biliary cancer subsite is consistent with the etiologic heterogeneity suggested by subsite differences in the epidemiologic (1,2,48) and molecular characteristics of biliary tumors (46,49,50). In our study, the effects of lipid metabolism genes on biliary tumors were most consistently associated with bile duct cancer, despite the fact that gallstones are most closely linked to gallbladder cancer. Reasons for this apparent anomaly are unclear, but it is possible that in addition to gallstones and lipid metabolism, the etiology of gallbladder cancer involves a number of major risk factors, including obesity and reproductive variables, which may have obscured detection of a modest genetic effect.

As with most epidemiologic studies measuring the effect of many candidate genes, false positive findings due to multiple comparisons is a concern. To minimize this potential bias, we implemented rigorous procedures for statistical adjustment. For each gene we used the Simes global test to adjust for multiple SNPs, and found that the associations of *APOB* and *LDLR* with bile duct cancer persisted. The significant association between the TT *APOB* haplotype and bile duct cancer also persisted after adjustments for multiple comparisons. Even though the Simes p-value obtained for all 12 SNPs suggests an effect of lipid metabolism genes on bile duct cancer, it is possible that the observed associations may be due to linkage disequilibrium with causative SNPs that were not evaluated in this study, particularly since the functional significance of the SNPs examined is unknown and since the three SNPs with significant associations were in non-coding regions. For example, moderate to high linkage disequilibrium has been previously reported between *APOE* IVS1+69C>G and the markers

responsible for the e2 allele (51,52), suggesting that the observed effects of *APOE* IVS1 +69C>G may be due to the e2 allele. Nevertheless, the totality of our data suggests that *APOE*, *APOB*, and *LDLR* genes, and possibly other genes in the lipid metabolism pathway may play an important etiologic role in biliary tract cancers, especially of the bile duct.

Several strengths of this study should be noted: 1) Selection bias was minimal due to the population-based design and very high case ascertainment (>95%) and high response rates (>85%) ; 2) Misclassification of cancer and stone cases was also minimal due to the detailed review of pathology and clinical data; 3) The nearly complete assessment of gallstone status among cancer cases and controls made it possible to evaluate cancer risk while controlling for gallstones; 4) Misclassification of genotypes was unlikely given the good quality and purity of the extracted DNA, the 99% concordance between duplicate samples, and the 99% reproducibility of genotyping results among quality control samples; and 5) We were able to adjust for multiple comparisons, and provide confirmation of our single-locus results.

However, our study had some limitations: 1) We may have missed the effect of some important markers, since gene coverage was limited and SNP selection was not based on complete sequencing data; 2) The functional effect of the SNPs examined was unknown, and may actually represent markers for other functional SNPs that are in LD; 3) Although our study of biliary tract cancer is the largest to date, some small sample sizes limited the statistical power to evaluate certain main effects, especially of ampullary cancer, as well as gene-gene and gene-environment interactions; and 4) Our findings may have limited generalizability due to the homogeneous Chinese study population, although an effect of population stratification would be minimal.

In conclusion, this population-based study in Shanghai showed that among men, *APOE* was associated with the risk of biliary stones and biliary tract cancers, while *APOB* was associated with bile duct cancer. In addition, *LDLR* was related to the risk of bile duct cancer in both sexes. Further studies with greater statistical power and broader coverage of genes in the lipid metabolism pathway and other pathways are needed to identify the causal gene variants and mechanisms involved in the formation of biliary stones and cancers.

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Table 1

Selected characteristics of subjects by case-control status

Selected Characteristics	Controls		Biliary Stones[1]		Biliary Tract Cancers					
					Gallbladder[2]		Bile duct[3]		Ampulla of Vater[3]	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
All subjects	779	(100.0)	880	(100.0)	235	(100.0)	125	(100.0)	46	(100.0)
Sex										
Male	302	(38.8)	323	(36.7)	65	(27.6)	75	(60.0)	23	(50.0)
Female	477	(61.2)	557	(63.3)*	170	(72.3)*	50	(40.0)*	23	(50.0)
Age at interview (yrs)										
34–54	105	(13.5)	263	(29.9)	32	(13.6)	17	(13.6)	4	(8.7)
55–64	222	(28.5)	251	(28.5)	61	(25.9)	32	(25.6)	9	(19.6)
65–75	452	(58.0)	366	(41.6)*	142	(60.4)	76	(60.8)	33	(71.7)
Education										
Elementary	313	(40.2)	267	(30.3)	125	(53.2)	52	(41.6)	22	(47.8)
High school	347	(44.5)	463	(52.6)	88	(37.4)	55	(44.0)	19	(41.3)
Above high school	119	(15.3)	150	(17.0)*	22	(9.4)*	18	(14.4)	5	(10.8)
Ever Smoked Cigarettes [4]										
No	545	(69.9)	644	(73.2)	170	(72.3)	70	(56.0)	26	(56.5)
Yes	234	(30.0)	236	(26.8)	64	(27.2)	55	(44.0)*	20	(43.5)
Ever Drank Alcohol										
No	620	(79.6)	741	(84.3)	199	(84.7)	84	(67.2)	34	(73.9)
Yes	159	(20.4)	138	(15.7)*	36	(15.3)	41	(32.8)*	12	(26.1)
Body Mass Index [5] (kg/m^2)										
<18.5	65	(8.3)	40	(4.6)	11	(4.7)	6	(4.8)	1	(2.2)
18.5–22.9	319	(40.9)	295	(33.6)	79	(33.6)	57	(45.6)	20	(43.5)
23.0–24.9	166	(21.3)	213	(24.2)	47	(20.0)	33	(26.4)	10	(21.7)
≥25	228	(29.3)	331	(37.7)*	97	(41.3)*	29	(23.2)	15	(32.6)

Selected Characteristics	Controls		Biliary Stones[1]		Biliary Tract Cancers					
					Gallbladder[2]		Bile duct[3]		Ampulla of Vater[3]	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Waist to Hip Ratio [6]										
<0.80	145	(18.6)	105	(11.9)	19	(8.1)	8	(6.4)	7	(15.2)
0.80–0.83	134	(17.2)	96	(10.9)	14	(5.9)	9	(7.2)	3	(6.5)
0.84–0.87	175	(22.5)	195	(22.2)	48	(20.4)	20	(16.0)	8	(17.4)
0.88–0.91	176	(22.6)	212	(24.1)	57	(24.3)	31	(24.8)	10	(21.7)
≥0.92	149	(19.1)	272	(30.9)*	97	(41.3)	57	(45.6)	18	(39.1)
Hypertension										
No	444	(57.0)	591	(67.2)	149	(63.4)	88	(70.4)	34	(73.9)
Yes	335	(43.0)	289	(32.8)*	86	(36.6)	37	(29.6)*	12	(26.1)*
Diabetes										
No	714	(91.7)	780	(88.7)	204	(86.8)	113	(90.4)	43	(93.5)
Yes	65	(8.3)	99	(11.3)*	31	(13.2)*	12	(9.6)	3	(6.5)
Gallstone status										
No	586	(75.2)	-	-	36	(15.3)	40	(32.0)	18	(39.1)
Yes	193	(24.8)	880	(100.0)	199	(84.7)*	85	(68.0)*	28	(60.9)*

[1] Biliary stone cases include gallstone and bile duct stones. Biliary stone cases compared with controls without biliary stones (n=586)

[2] Gallbadder cancer cases compared with population controls who had a gallbladder (n=730)

[3] Bile duct and ampulla of Vater cancer cases compared to all population controls (n=779)

[4] Ever smoked cigarettes for at least 6 consecutive months

[5] Body mass index = weight/(height^2); Distribution based on WHO classification for obesity among Asians

[6] Cutoffs for waist-to-hip-ratio based on quintiles among population controls

* $p<0.05$ for Fisher's exact test for difference between cases and controls

Note: Total number of subjects may vary because of missing values

Table 2

Odds Ratios (ORs) and 95% Confidence Intervals (CIs) for Biliary Stones and Cancer in Relation to Polymorphisms of Lipid Metabolism Genes

Genotype	Controls		Biliary Stones [1]				Biliary Tract Cancers											
							Gallbladder[2]				Bile duct[3]				Ampulla of Vater[3]			
	n	(%)	n	(%)	OR[4]	(95% CI)[4]	n	(%)	OR[4]	(95% CI)[4]	n	(%)	OR[4]	(95% CI)[4]	n	(%)	OR[4]	(95% CI)[4]
TOTAL	779	(100.0)	880	(100.0)	-	-	235	(100.0)	-	-	125	(100.0)	-	-	46	(100.0)	-	-
APOE																		
IVS1+69C>G (rs440446)																		
CC	319	(41.4)	326	(37.4)	1.00	-	85	(36.8)	1.00	-	32	(25.8)	1.00	-	10	(21.7)	1.00	-
GC	355	(46.1)	425	(48.7)	1.25	(0.99–1.57)	113	(48.9)	1.21	(0.87–1.67)	72	(58.1)	2.01	(1.29–3.14)	24	(52.2)	2.09	(0.98–4.44)
GG	96	(12.5)	121	(13.9)	1.43	(1.01–2.02)	33	(14.3)	1.32	(0.83–2.10)	20	(16.1)	2.07	(1.13–3.79)	12	(26.1)	3.96	(1.66–9.46)
GC+GG	451	(58.6)	546	(62.6)	1.29	(1.03–1.60)	146	(63.2)	1.23	(0.91–1.67)	92	(74.2)	2.03	(1.32–3.11)	36	(78.3)	2.48	(1.21–5.08)
P [5]						*0.02*				*0.18*				*0.003*				*0.002*
APOB																		
EX26-3573T>C (rs676210)																		
TT	439	(56.7)	474	(53.9)	1.00	-	137	(58.5)	1.00	-	63	(50.4)	1.00	-	26	(57.8)	1.00	-
TC	288	(37.2)	348	(39.6)	1.12	(0.89–1.40)	80	(34.2)	0.91	(0.66–1.25)	57	(45.6)	1.38	(0.94–2.04)	14	(31.1)	0.84	(0.43–1.64)
CC	47	(6.1)	57	(6.5)	1.02	(0.66–1.58)	17	(7.3)	1.15	(0.64–2.08)	5	(4.0)	0.74	(0.29–1.94)	5	(11.1)	1.89	(0.69–5.18)
TC+CC	335	(43.3)	405	(46.1)	1.10	(0.89–1.37)	97	(41.5)	0.94	(0.69–1.27)	62	(49.6)	1.30	(0.89–1.89)	19	(42.2)	0.99	(0.54–1.81)
P [5]						*0.50*				*0.94*				*0.47*				*0.67*
IVS23-79T>C (rs673548)																		
TT	407	(56.4)	446	(54.5)	1.00	-	129	(58.6)	1.00	-	59	(50.9)	1.00	-	26	(60.5)	1.00	-
TC	266	(36.9)	320	(39.1)	1.09	(0.86–1.37)	76	(34.5)	0.92	(0.66–1.27)	52	(44.8)	1.35	(0.90–2.02)	13	(30.2)	0.78	(0.40–1.56)
CC	48	(6.7)	53	(6.5)	0.92	(0.59–1.42)	15	(6.8)	0.97	(0.52–1.80)	5	(4.3)	0.72	(0.27–1.87)	4	(9.3)	-	-
TC+CC	314	(40.6)	373	(45.5)	1.06	(0.85–1.32)	91	(41.4)	0.93	(0.68–1.26)	57	(49.1)	1.25	(0.85–1.86)	17	(39.5)	0.87	(0.46–1.63)
P [5]						*0.86*				*0.69*				*0.60*				*0.62*
IVS6+360C>T (rs520354)																		
CC	515	(67.2)	602	(69.6)	1.00	-	157	(67.1)	1.00	-	71	(57.7)	1.00	-	32	(69.6)	1.00	-
TC	234	(30.6)	242	(28.0)	0.82	(0.65–1.04)	69	(29.5)	0.93	(0.67–1.29)	46	(37.4)	1.41	(0.94–2.10)	14	(30.4)	0.96	(0.50–1.84)
TT	17	(2.2)	21	(2.4)	0.97	(0.48–1.95)	8	(3.4)	1.51	(0.63–3.60)	6	(4.9)	2.51	(0.96–6.57)	0	0	-	-

Genotype	Controls		Biliary Tract Cancers															
			Biliary Stones [1]				Gallbladder[2]				Bile duct[3]				Ampulla of Vater[3]			
	n	(%)	n	(%)	OR[4]	(95% CI)[4]	n	(%)	OR[4]	(95% CI)[4]	n	(%)	OR[4]	(95% CI)[4]	n	(%)	OR[4]	(95% CI)[4]
TC+TT	251	(32.8)	263	(30.4)	0.83	(0.66–1.05)	77	(32.9)	0.97	(0.71–1.33)	52	(42.3)	1.48	(1.01–2.18)	14	(30.4)	0.90	(0.47–1.71)
P [5]						*0.15*				*0.95*				*0.02*				*0.74*
EX4+56C>T (rs1367117)																		
CC	564	(73.6)	650	(75.3)	1.00	-	182	(78.1)	1.00	-	81	(65.9)	1.00	-	32	(71.1)	1.00	-
TC	192	(25.1)	195	(22.6)	0.84	(0.66–1.08)	43	(18.5)	0.68	(0.47–0.99)	40	(32.5)	1.44	(0.95–2.17)	12	(26.7)	1.13	(0.57–2.24)
TT	10	(1.3)	18	(2.1)	1.39	(0.61–3.17)	8	(3.4)	2.54	(0.97–6.70)	2	(1.6)	-	-	1	(2.2)	-	-
TC+TT	202	(26.4)	213	(24.7)	0.87	(0.69–1.11)	51	(21.9)	0.77	(0.54–1.09)	42	(34.1)	1.44	(0.96–2.15)	13	(28.9)	0.85	(0.65–1.10)
P [5]						*0.44*				*0.44*				*0.08*				*0.67*
LDLR																		
IVS9-30C>T (rs1003723)																		
CC	565	(72.6)	636	(72.6)	1.00	-	170	(72.3)	1.00	-	80	(64.5)	1.00	-	39	(84.8)	1.00	-
TC	199	(25.6)	223	(25.5)	0.98	(0.77–1.25)	61	(26.0)	1.02	(0.73–1.44)	39	(31.5)	1.38	(0.91–2.10)	7	(15.2)	0.50	(0.22–1.14)
TT	14	(1.8)	17	(1.9)	1.02	(0.47–2.22)	4	(1.7)	-	-	5	(4.0)	2.53	(0.89–7.22)	0	0	-	-
TC+TT	213	(27.4)	240	(27.4)	0.98	(0.78–1.25)	65	(27.7)	1.02	(0.73–1.41)	44	(35.5)	1.46	(0.98–2.18)	7	(15.2)	0.47	(0.21–1.06)
P [5]						*0.92*				*0.93*				*0.04*				*0.07*
EX10+55G>A (rs5927)																		
GG	307	(39.9)	356	(41.0)	1.00	-	79	(33.9)	1.00	-	54	(43.2)	1.00	-	17	(37.0)	1.00	-
GA	348	(45.2)	382	(44.0)	0.94	(0.74–1.18)	120	(51.5)	1.34	(0.97–1.85)	52	(41.6)	0.85	(0.56–1.28)	17	(37.0)	0.88	(0.44–1.76)
AA	115	(14.9)	130	(15.0)	0.94	(0.68–1.30)	34	(14.6)	1.10	(0.70–1.74)	19	(15.2)	0.94	(0.53–1.65)	12	(26.0)	1.88	(0.87–4.07)
GA+AA	463	(60.1)	512	(59.0)	0.94	(0.75–1.17)	154	(66.1)	1.28	(0.95–1.75)	71	(56.8)	0.90	(0.61–1.32)	29	(63.0)	1.16	(0.63–2.15)
P [5]						*0.62*				*0.35*				*0.66*				*0.18*
EX15-80G>A (rs14158)																		
GG	696	(89.6)	794	(90.7)	1.00	-	204	(86.8)	1.00	-	114	(91.9)	1.00	-	42	(91.3)	1.00	-
GA	76	(9.8)	79	(9.0)	0.85	(0.59–1.22)	30	(12.8)	1.29	(0.82–2.02)	10	(8.1)	0.80	(0.40–1.60)	4	(8.7)	0.87	(0.30–2.49)
AA	5	(0.6)	2	(0.2)	0.30	(0.06–1.55)	1	(0.4)	-	-	0	0	-	-	0	0	-	-
GA+AA	81	(10.4)	81	(9.3)	0.81	(0.57–1.15)	31	(13.2)	1.24	(0.80–1.94)	10	(8.1)	0.75	(0.38–1.50)	4	(8.7)	0.81	(0.28–2.31)
P [5]						*0.16*				*0.33*				*0.42*				*0.70*

Genotype	Controls		Biliary Stones [1]				Biliary Tract Cancers											
							Gallbladder[2]				Bile duct[3]				Ampulla of Vater[3]			
	n	(%)	n	(%)	OR[4]	(95% CI)[4]	n	(%)	OR[4]	(95% CI)[4]	n	(%)	OR[4]	(95% CI)[4]	n	(%)	OR[4]	(95% CI)[4]
IVS17-42A>G (rs6413504)																		
AA	411	(53.4)	448	(51.2)	1.00	-	120	(51.3)	1.00	-	59	(47.2)	1.00	-	25	(54.3)	1.00	-
GA	291	(37.8)	359	(41.0)	1.19	(0.95–1.49)	98	(41.9)	1.19	(0.87–1.62)	56	(44.8)	1.34	(0.90–1.99)	15	(32.6)	0.84	(0.43–1.62)
GG	67	(8.8)	68	(7.8)	0.94	(0.63–1.39)	16	(6.8)	0.81	(0.45–1.46)	10	(8.0)	1.04	(0.51–2.13)	6	(13.0)	1.42	(0.56–3.60)
GA+GG	358	(46.6)	427	(48.8)	1.14	(0.92–1.41)	114	(48.7)	1.12	(0.83–1.50)	66	(52.8)	1.28	(0.88–1.87)	21	(45.7)	0.95	(0.52–1.72)
P [5]						*0.52*				*0.87*				*0.38*				*0.78*
EX18+88G>A (rs5930)																		
GG	290	(37.5)	320	(36.7)	1.00	-	86	(36.9)	1.00	-	49	(39.5)	1.00	-	12	(26.1)	1.00	-
GA	364	(47.2)	416	(47.7)	1.05	(0.83–1.33)	108	(46.4)	1.01	(0.73–1.40)	56	(45.2)	0.91	(0.60–1.38)	21	(45.7)	1.38	(0.67–2.86)
AA	118	(15.3)	136	(15.6)	1.04	(0.75–1.44)	39	(16.7)	1.12	(0.72–1.73)	19	(15.3)	0.95	(0.54–1.69)	13	(28.3)	2.71	(1.19–6.12)
GA+AA	482	(62.4)	552	(63.3)	1.05	(0.84–1.31)	147	(63.1)	1.04	(0.76–1.41)	75	(60.5)	0.92	(0.62–1.36)	34	(73.9)	1.70	(0.87–3.34)
P [5]						*0.74*				*0.66*				*0.78*				*0.02*
LPL																		
IVS5-540C>T (rs263)																		
CC	485	(62.6)	539	(61.3)	1.00	-	151	(64.3)	1.00	-	79	(63.7)	1.00	-	28	(60.9)	1.00	-
TC	254	(32.8)	308	(35.0)	1.17	(0.93–1.47)	76	(32.3)	0.97	(0.71–1.34)	39	(31.5)	0.94	(0.62–1.42)	18	(39.1)	1.20	(0.65–2.21)
TT	36	(4.6)	32	(3.6)	0.68	(0.41–1.13)	8	(3.4)	0.69	(0.31–1.52)	6	(4.8)	1.02	(0.42–2.51)	0	0	-	-
TC+TT	290	(37.4)	340	(38.7)	1.10	(0.88–1.36)	84	(35.7)	0.94	(0.69–1.27)	45	(36.3)	0.95	(0.64–1.41)	18	(39.1)	1.05	(0.57–1.94)
P [5]						*0.95*				*0.50*				*0.86*				*0.85*
ALOX5																		
IVS3+100G>A (rs2029253)																		
GG	286	(37.0)	319	(36.4)	1.00	-	84	(35.9)	1.00	-	48	(38.7)	1.00	-	15	(32.6)	1.00	-
GA	376	(48.5)	432	(49.3)	1.09	(0.86–1.37)	113	(48.3)	1.05	(0.76–1.44)	60	(48.4)	0.95	(0.63–1.43)	25	(54.3)	1.24	(0.64–2.39)
AA	112	(14.5)	125	(14.3)	1.12	(0.80–1.57)	37	(15.8)	1.16	(0.74–1.82)	16	(12.9)	0.85	(0.46–1.56)	6	(13.0)	0.98	(0.37–2.59)
GA+AA	488	(63.1)	557	(63.6)	1.09	(0.88–1.36)	150	(64.1)	1.07	(0.79–1.46)	76	(61.3)	0.93	(0.63–1.37)	31	(67.4)	1.17	(0.62–2.22)
P [5]						*0.43*				*0.53*				*0.60*				*0.85*

[1] Biliary stone cases include gallstone and bile duct stones. Biliary stone cases compared with controls without biliary stones (n=586)

[2]Gallbadder cancer cases compared with population controls who had a gallbladder (n=730)

[3]Bile duct and ampulla of Vater cancer cases compared to all population controls (n=779)

[4]Adjusted for age

[5]Test of trend for the number of copies of the variant allele (0,1,2);

Pearson chi-square for absence or presence of the variant allele if variant genotype has n < 5

Note: Total number of subjects per SNP may vary because of missing values

Table 3

Odds Ratios (ORs) and 95% Confidence Intervals (CIs) for Biliary Stones and Cancer in Relation to the*APOE*, *APOB* and *LDLR* markers by Gender

									Biliary Tract Cancers													
	Controls		Biliary Stones[1]						Gallbladder[2]						Bile Duct[3]						Ampulla of Vater[3]					
Genotype	Males	Females	Males			Females			Males			Females			Males			Females			Males			Females		
	n	n	n	OR[4]	(95% CI)[4]	n	OR[4]	(95% CI)[4]	n	OR[4]	(95% CI)[4]	n	OR[4]	(95% CI)[4]	n	OR[4]	(95% CI)[4]	n	OR[4]	(95% CI)[4]	n	OR[4]	(95% CI)[4]	n	OR[4]	(95% CI)[4]
TOTAL	302	477	323	-	-	557	-	-	65	-	-	170	-	-	75	-	-	50	-	-	23	-	-	23	-	-
APOE																										
IVS1+69C>G (rs440446)																										
CC	138	181	120	1.00	-	206	1.00	-	21	1.00	-	64	1.00	-	14	1.00	-	18	1.00	-	4	1.00	-	6	1.00	-
GC	128	227	156	1.57	(1.10-2.25)	269	1.04	(0.77-1.41)	36	1.84	(1.02-3.34)	77	0.98	(0.66-1.44)	48	3.67	(1.93-6.99)	24	1.06	(0.56-2.01)	12	3.18	(0.99-10.13)	12	1.53	(0.56-4.18)
GG	30	66	46	2.19	(1.23-3.91)	75	1.07	(0.69-1.66)	8	1.84	(0.74-4.59)	25	1.09	(0.63-1.88)	12	3.96	(1.67-9.43)	8	1.21	(0.50-2.93)	7	8.38	(2.29-30.64)	5	2.26	(0.67-7.70)
GC+GG	158	293	202	1.68	(1.19-2.36)	344	1.05	(0.79-1.39)	44	1.84	(1.04-3.26)	102	1.00	(0.69-1.44)	60	3.72	(1.99-6.96)	32	1.10	(0.60-2.01)	19	4.11	(1.36-12.38)	17	1.69	(0.65-4.39)
P [5]					*0.002*			*0.73*			*0.06*			*0.85*			*0.0001*			*0.70*			*0.001*			*0.22*
P gender interaction								*P interaction=0.03*						*P interaction=0.07*						*P interaction=0.01*						*P interaction=0.23*
APOB																										
IVS6+360C>T (rs520354)																										
CC	204	311	226	1.00	-	376	1.00	-	43	1.00	-	114	1.00	-	40	1.00	-	31	1.00	-	17	1.00	-	15	1.00	-
TC	83	151	89	0.94	(0.65-1.37)	153	0.75	(0.56-1.01)	20	1.16	(0.64-2.09)	49	0.83	(0.56-1.22)	29	1.83	(1.06-3.14)	17	1.07	(0.58-1.99)	6	0.88	(0.33-2.31)	8	1.10	(0.45-2.66)
TT	7	10	7	0.74	(0.25-2.18)	14	1.19	(0.47-3.04)	2	-	-	6	1.64	(0.57-4.72)	6	4.44	(1.42-13.93)	0	-	-	0	-	-	0	-	-
TC+TT	90	161	96	0.92	(0.64-1.33)	167	0.78	(0.58-1.04)	22	1.17	(0.66-2.07)	55	0.87	(0.59-1.27)	35	2.03	(1.21-3.40)	17	1.01	(0.54-1.86)	6	0.81	(0.31-2.13)	8	1.02	(0.42-2.47)
P [5]					*0.50*			*0.19*			*0.60*			*0.74*			*0.003*			*0.99*			*0.67*			*0.96*
P gender interaction								*P interaction=0.48*						*P interaction=0.40*						*P interaction=0.09*						*P interaction=0.70*
LDLR																										
IVS9-30C>T (rs1003723)																										
CC	217	348	238	1.00	-	398	1.00	-	45	1.00	-	125	1.00	-	50	1.00	-	30	1.00	-	19	1.00	-	20	1.00	-
TC	80	119	78	0.91	(0.62-1.34)	145	1.03	(0.75-1.41)	20	1.26	(0.70-2.28)	41	0.95	(0.63-1.44)	21	1.13	(0.64-2.01)	18	1.75	(0.94-3.26)	4	0.57	(0.19-1.74)	3	0.41	(0.12-1.41)
TT	5	9	5	1.02	(0.27-3.87)	12	0.98	(0.37-2.57)	0	-	-	4	-	-	4	-	-	1	-	-	0	-	-	0	-	-
TC+TT	85	128	83	0.92	(0.63-1.34)	157	1.02	(0.75-1.39)	20	1.18	(0.65-2.12)	45	0.97	(0.65-1.44)	25	1.27	(0.74-2.19)	19	1.72	(0.93-3.16)	4	0.54	(0.18-1.63)	3	0.39	(0.11-1.34)
P [5]					*0.70*			*0.91*			*0.59*			*0.85*			*0.38*			*0.09*			*0.27*			*0.13*
P gender interaction								*P interaction=0.65*						*P interaction=0.58*						*P interaction=0.48*						*P interaction=0.71*
EX18+88G>A (rs14158)																										
GG	109	219	110	1.00	-	210	1.00	-	23	1.00	-	63	1.00	-	29	1.00	-	20	1.00	-	7	1.00	-	5	1.00	-
GA	145	181	152	1.09	(0.75-1.57)	264	1.03	(0.76-1.40)	32	1.07	0.59-1.94	76	1.00	(0.68-1.48)	32	0.83	(0.47-1.45)	24	0.99	(0.53-1.85)	8	0.86	(0.30-2.45)	13	2.09	(0.73-5.99)
AA	46	72	59	1.49	(0.89- 2.48)	77	0.81	(0.53-1.24)	10	1.06	0.47-2.43	29	1.14	(0.68-1.92)	13	1.06	(0.51-2.22)	6	0.76	(0.29-1.96)	8	2.72	(0.93-7.97)	5	2.58	(0.72-9.23)
GA+AA	191	253	211	1.18	(0.83-1.67)	341	0.98	(0.73-1.30)	42	1.07	0.61-1.88	105	1.04	(0.72-1.50)	45	0.89	(0.53-1.49)	30	0.93	(0.51-1.69)	16	1.31	(0.52-3.28)	18	2.21	(0.80-6.06)
P [5]					*0.15*			*0.48*			*0.84*			*0.68*			*0.95*			*0.64*			*0.10*			*0.12*
P gender interaction								*P interaction=0.39*						*P interaction=0.94*						*P interaction=0.90*						*P interaction=0.46*

[1] Biliary stone cases include gallstone and bile duct stones. Biliary stone cases compared with controls without biliary stones (n=586)

[2] Gallbadder cancer cases compared with population controls who had a gallbladder (n=730)

[3] Bile duct and ampulla of Vater cancer cases compared to all population controls (n=779)

[4] Adjusted for age

[5] Test of trend for the number of copies of the variant allele (0,1,2);

Pearson chi-square for absence or presence of the variant allele if variant genotype has n < 5

Note: Total number of subjects per SNP may vary because of missing values

Table 4

Odds Ratios (ORs) and 95% Confience Intervals (CIs) for Biliary Tract Cancer in relation to *APOB* and *LDLR* haplotypes

Gene	Haplotype	Controls		Biliary Tract Cancers											
				Gallbladder[1]				Bile Duct[2]				Ampulla of Vater[2]			
		n	(%)	n	(%)	OR[3]	(95%CI)[3]	n	(%)	OR[3]	(95%CI)[3]	n	(%)	OR[3]	(95%CI)[3]
Total #	**Haplotypes**	1558	(100.0)	470	(100.0)	-	-	250	(100.0)	-	-	92	(100.0)	-	-
APOB	**EX26-3573T>C - IVS23-79T>C**														
	T-T	1165	(74.8)	356	(75.7)	1.00	-	183	(73.2)	1.00	-	66	(71.7)	1.00	-
	C-C	385	(24.7)	114	(24.3)	0.98	(0.77–1.25)	67	(26.8)	1.11	(0.82–1.51)	25	(27.0)	1.13	(0.71–1.82)
	Other [4]	8	(0.5)	0	(0.0)	-	-	0	(0.0)	-	-	1	(1.3)	-	-
	P [5]						*0.40*				*0.50*				*<0.001*
APOB	**IVS6+360C>T - EX4+56C>T**														
	C-C	1251	(80.3)	376	(80.0)	1.00	-	189	(75.5)	1.00	-	73	(79.4)	1.00	-
	T-T	184	(11.8)	51	(10.9)	1.18	(0.77–1.82)	43	(17.0)	1.58	(1.07–2.33)	9	(9.8)	0.87	(0.42–1.81)
	T-C	90	(5.8)	34	(7.3)	0.91	(0.65–1.27)	17	(6.6)	1.21	(0.70–2.10)	5	(5.4)	0.84	(0.30–2.39)
	Other [4]	33	(2.1)	8	(1.8)	-	-	2	(0.9)	-	-	5	(5.4)	3.1	(1.12–8.61)
	P [5]						*0.76*				*0.05*				*0.12*
LDLR	**IVS9-30C>T - EX10+55G>A - EX15-80G>A - IVS17-42A>G - EX18+88G>A**														
	C-A-G-A-A	458	(29.4)	145	(30.8)	1.00	-	76	(30.5)	1.00	-	33	(35.8)	1.00	-
	C-G-G-A-G	430	(27.6)	116	(24.6)	0.86	(0.65–1.14)	70	(27.8)	0.96	(0.68–1.37)	10	(10.8)	0.38	(0.19–0.76)
	C-G-G-G-G	168	(10.8)	67	(14.2)	1.07	(0.76–1.51)	27	(10.8)	0.80	(0.50–1.26)	20	(21.7)	1.16	(0.65–2.06)
	T-G-G-G-G	185	(11.9)	54	(11.4)	0.89	(0.62–1.29)	45	(17.8)	1.43	(0.95–2.17)	7	(7.6)	0.46	(0.19–1.09)
	C-G-G-A-A	109	(7.0)	27	(5.8)	0.83	(0.51–1.35)	16	(6.3)	0.83	(0.45–1.55)	14	(15.2)	1.60	(0.79–3.22)
	C-A-A-A-G	50	(3.2)	28	(6.0)	1.06	(0.67–1.68)	8	(3.2)	0.64	(0.30–1.33)	4	(4.3)	-	-
	Other [4]	157	(10.1)	34	(7.2)	1.38	(0.85–2.24)	9	(3.6)	0.70	(0.31–1.57)	4	(4.6)	-	-
	P [5]						*0.04*				*1.00*				*0.001*

[1] Gallbadder cancer cases compared with population controls who had a gallbladder (n=730)

[2] Bile duct and ampulla of Vater cancer cases compared to all population controls (n=779)

[3] Adjusted for age

[4] Combined haplotypes having frequency <1% among population controls

[5] Omnibus score test