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# Variants in motilin, somatostatin and their receptor genes and risk of biliary tract cancers and stones in Shanghai, China

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ABSTRACT

Altered motility of the gallbladder can result in gallstone and cholecystitis, which are important risk factors for biliary tract cancer. Motilin (MLN) and somatostatin (SST) are known important modulators of gallbladder motility. To determine whether genetic variants in motilin, somatostatin, and their receptor genes are associated with the risk of biliary tract cancers and stones, nine tag-SNPs were determined in 439 biliary tract cancer cases (253 gallbladder, 133 extrahepatic bile duct and 53 ampulla of Vater cancer cases), 429 biliary stone cases, and 447 population controls in a population-based case–control study in Shanghai, China. We found that subjects with the *MLNR* rs9568169 AA genotype and *SSTR5* rs169068 CC genotype were significantly associated with risk of extrahepatic bile duct cancer (OR = 0.49, 95% CI: 0.27–0.89; OR = 2.40, 95% CI: 1.13–5.13) compared to the major genotypes. *MLN* rs2281820 CT and rs3793079 AT genotypes had significantly increased risks of gallstones (OR = 1.52, 95% CI: 1.06–2.18; OR = 1.64, 95% CI: 1.20–2.25) compared to TT genotypes. Besides, haplotype analysis showed that *MLN* T-T-T haplotype (rs2281820–rs3793079–rs2281819) had a non-significantly elevated risk of gallstone (OR = 1.30, 95% CI: 0.91–1.86) compared with C-A-A haplotype. To the best of our knowledge, this is the first study to report an

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association between genetic polymorphisms in *MLN*, *MLNR* and their receptor genes and risk of biliary tract cancers and stones.


## Introduction

Biliary tract cancers, which include cancers of the gallbladder, extrahepatic bile duct, and ampulla of Vater, are relatively rare but highly fatal malignancies, with age-adjusted incidence and mortality rates of 1.6 and 1.2 (per 100,000), respectively, for gallbladder cancer in China in 2008 (Ferlay et al., 2010). We previously showed that in urban Shanghai, the age-adjusted incidence rates of biliary tract cancers were 1.7 and 1.2 for females and males, respectively, during the period 1972 to 1974, and increased to 4.6 and 3.1 between 1996 and 1999 (Liu et al., 2004). These rates were much higher than the average incidence rate for biliary tract cancer in China. Reasons for this regional difference, as well as the rapid rise in incidence in urban Shanghai, are unclear.

Gallstones are one of the most important risk factors for biliary tract cancers. The prevalence rate of gallstone in Shanghai was increasing during the past decades in Shanghai, which was 4.4% in 1987, but rose to 10.7 during 2002 to 2003 in adults (Zhang et al., 2011; Zhu et al., 2010). In Shanghai, ever having gallstones was associated with significantly increased risks of gallbladder cancer (23.8-fold), extrahepatic bile duct cancer (8.0-fold) and ampulla of Vater cancer (4.2-fold) (Hsing et al., 2007a). Lipid metabolism is an important risk factor for the formation of gallstones, and variants in the lipid metabolism pathway genes have been identified in association with biliary tract cancer and stone risk in the Shanghai population (Andreotti et al., 2008; Xu et al., 2011). Another important risk factor for gallstone formation is impaired gallbladder motility since hypomotility of the gallbladder leads to stagnant bile, which creates an environment for cholesterol supersaturation and subsequently gallstone formation (O'Donnell and Fairclough, 1993). On the other hand, impaired gallbladder contractile also has relation with other diseases, such as chronic acalculous cholecystitis which is also important in carcinogenesis of biliary tract cancers (Merg et al., 2002). Gallbladder motility is mainly regulated by many neural and hormonal factors and their interactions (Montet et al., 2005). We previously showed that a mutation in the *CCKAR* (rs1800855) gene, which codes for the receptor for cholecystokinin, a gastrointestinal peptide that mediates gallbladder emptying, was associated with gallbladder cancer risk in females (Xu et al., 2013).

To further clarify whether other gallbladder motility-related genes are related to biliary tract cancer risk, we examined the associations of nine SNPs in gallbladder motility-related genes (MLN, MLNR, SSTR2, and SSTR5) with the risk of biliary tract cancers in a population-based case–control study conducted in Shanghai, China.

## Materials and methods

### *Study subjects*

The details of the study design and methods have been described in detail elsewhere (Hsing et al., 2007a, 2007b, 2008). Briefly, incident cancer cases were identified by a rapid reporting system established by the Shanghai Cancer Institute and 42 collaborating hospitals in Shanghai. Through this system, we identified more than 95% of all incident biliary tract cancer cases (International Classification of Diseases, Ninth Edition code 156) diagnosed among urban Shanghai residents between June 1997 and May 2001. A total of 627 incident biliary tract cancer cases were identified. For this study, we included 439 (70.0%) incident biliary tract cancer cases (253 gallbladder, 133 extrahepatic bile duct and 53 ampulla of Vater cancer cases) who were between 35 and 74 years old, completed the in-person interview and provided a blood sample. Population controls without a history of cancer were randomly selected from the Shanghai Resident Registry, which includes the records for approximately 6 million Shanghai urban residents. The controls were frequency matched to the cancer cases by age (by 5 year age groups) and gender. Patients with biliary stones without a history of cancer and undergoing cholecystectomy were also included and were frequency matched to the cancer cases by age (5 year age groups), gender and hospital of diagnosis.

A total of 429 biliary stone patients and 447 population controls were included. The study was approved by the Institutional Review Boards of the Shanghai Cancer Institute and the U.S. National Cancer Institute, and all participants gave written informed consent.

*Gallstone assessment*

Prevalent gallstones were assessed in nearly all biliary tract cancer cases and population controls. Among cancer cases, gallstones were identified by self-reported medical history, surgical reports or imaging results from magnetic resonance imaging, endoscopic retrograde cholangiopancreatography, computed tomography or ultrasound. Among population controls, gallstones were identified mainly by organization of abdominal ultrasound examination for those who gave consent (85% of population controls). For the rest, gallstone status was determined by self-report.

*Interview*

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

*Genotyping*

Tagging single nucleotide polymorphisms (SNPs) were selected for this study by searching data from Han Chinese from the HapMap project using the Tagger program (de Bakker et al., 2006), using the following criteria: (a) a minor allele frequency $\geq 0.1$, since the sample size in the subgroups was relatively small; and (b) using an $r^2$ threshold of 0.90. A total of 9 tagging SNPs from four genes were identified (*MLN* rs2280820, rs3793079 and rs2281819; *MLNR* rs9568169; *SSTR2* rs7210080, rs1466113, rs2236748 and rs2236750; *SSTR5* rs169068). We also evaluated SNPs in the *SST* gene, but no SNPs met the criteria for this gene.

Blood samples were collected at the time of interview, and then separated into plasma, red blood cells and buffy coat. Genomic DNA was extracted from buffy coat using Promega DNA Extraction Kit at the Shanghai Cancer Institute. Genotyping was performed by using the TaqMan assay on the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA), in a 384-well format, with dual fluorescent reporter probes VIC and FAM. The genotyping call rate was >95% and the completion rate was >99%. The quality and potential misclassification of the genotyping were assessed by evaluating 5% of duplicate DNA samples from four quality-control subjects (63 total samples) that were randomly placed within the same reaction plates used for the study subjects. The concordance rate for the quality control samples was 100%.

*Statistical analysis*

For all analyses, gallbladder cancer cases were compared with population controls without a history of cholecystectomy ($n = 422$), extrahepatic bile duct cancer cases and ampulla of Vater cancer cases were compared with all population controls ($n = 447$) and biliary stone cases were compared with population controls without biliary stones ($n = 341$). Distributions of selected characteristics including age, gender, cigarette smoking, prevalent gallstones, alcohol drinking, and diabetes were compared between cases and controls by $\chi^2$ test. The odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between the SNPs and biliary tract cancer and stone risk were estimated by unconditional logistic regression. An initial model was adjusted for age and gender. Additional models were created to test the independence of the candidate SNPs on cancer and stone risk by adjusting for additional potential confounding factors with significantly different distributions between cases and controls. Interactions between SNPs and other risk

factors such as gender, smoking, drinking and diabetes were also analyzed to determine its contribution to stone and cancer risk. All statistical analyses were conducted using SAS 9.3. Hardy–Weinberg equilibrium for genotypic distribution and linkage disequilibrium between loci were assessed by HaploView version 4.0 (Barrett et al., 2005). Associations between haplotypes (>1% frequency) and the risks of biliary tract cancers and stones were evaluated by computing OR and 95% CI using HAPSTAT, assuming an additive model, using the most common haplotype as the reference category (Lin et al., 2005). Global differences in haplotype frequencies between cases and controls were assessed for each gene using the score test in HAPSTAT.

## Results

Selected characteristics of the study subjects are shown in Table 1. Gallbladder cancer cases were more likely to be female (73.1% vs. 59.6%), and have prevalent gallstones (84.6% vs. 18.4%) than controls. Extrahepatic bile duct and ampulla of Vater cancer cases were more likely to be male (59.4% and 54.7% vs. 39.8%), smoke cigarettes (45.1% and 47.2% vs. 32.4%), and have prevalent gallstones (66.9% and 54.7% vs. 23.7%) than controls. Extrahepatic bile duct cancer cases were also more likely to drink alcohol than population controls (30.1% vs. 20.4%). Biliary stone cases were more likely to be younger, but less likely to drink alcohol (16.3% vs. 22.3%) compared with controls without stones.

The age- and gender-adjusted associations of the *MLN*, *MLNR*, *SSTR2* and *SSTR5* variants with biliary tract cancers and stones are presented in Table 2. The genotype distributions of these nine SNPs showed no deviation from the expected Hardy–Weinberg equilibrium among controls ($P > 0.05$). Of these SNPs, the *MLN* rs3793079 AT genotype and *SSTR5* rs169068 CC genotype had significant higher extrahepatic bile duct cancer risk compared with their common allele homozygotes, and the *MLNR* rs9568169 AA genotype had a reduced extrahepatic bile duct cancer risk compared with the CC genotype. For gallstones, both the *MLN* rs2281820 CT genotype and rs3793079 AT genotype conferred increased disease risk compared with CC and AA genotypes, respectively. In addition, the SSTR2 rs2236750 GA genotype had reduced risk of

**Table 1**
Distributions of selected variables by case–control status.

| Selected characteristics | Controls, n (%) | Biliary stones[a], n (%) | Biliary tract cancers | | |
|---|---|---|---|---|---|
| | | | Gallbladder[b], n (%) | Bile duct[c], n (%) | Ampulla of Vater[c], n (%) |
| All subjects | 447 (100) | 429 (100) | 253 (100) | 133 (100) | 53 (100) |
| Age at interview | | | | | |
| Age at interview | | | | | |
| 34–54 | 58 (13.0) | 124 (28.9) | 39 (15.4) | 21 (15.8) | 5 (9.4) |
| 55–64 | 123 (27.5) | 118 (27.5) | 61 (24.1) | 36 (27.1) | 12 (22.7) |
| 65–75 | 266 (59.5) | 187 (43.6)* | 153 (60.5) | 76 (57.1) | 36 (67.9) |
| Gender | | | | | |
| Male | 178 (39.8) | 159 (37.1) | 68 (26.9) | 79 (59.4) | 29 (54.7) |
| Female | 269 (60.2) | 270 (62.9) | 185 (73.1)* | 54 (40.6)* | 24 (45.3)* |
| Ever smoke | | | | | |
| Yes | 145 (32.4) | 121 (28.2) | 66 (26.2) | 60 (45.1) | 25 (47.2) |
| No | 302 (67.6) | 308 (71.8) | 186 (73.8) | 73 (54.9)* | 28 (52.8)* |
| Gallstones | | | | | |
| Yes | 106 (23.7) | 429 (100.0) | 214 (84.6) | 89 (66.9) | 29 (54.7) |
| No | 341 (76.3) | – | 39 (15.4)* | 44 (33.1)* | 24 (45.3)* |
| Ever drink alcohol | | | | | |
| Yes | 91 (20.4) | 70 (16.3) | 40 (15.8) | 40 (30.1) | 14 (26.4) |
| No | 356 (79.6) | 359 (83.7)* | 213 (84.2) | 93 (69.9)* | 39 (73.6) |
| Diabetes | | | | | |
| Yes | 65 (14.6) | 69 (16.2) | 26 (10.3) | 25 (18.9) | 7 (13.2) |
| No | 381 (85.4) | 356 (83.8) | 227 (89.7) | 107 (81.1) | 46 (86.8) |

Note. Total number of subjects may vary due to missing values.
[a] Biliary stone (gallstone and bile duct stone) cases compared with controls without biliary stones (n = 341).
[b] Gallbladder cancer cases compared with population controls who had a gallbladder (n = 422).
[c] Bile duct and A.V. cancer cases compared with all population controls (n = 447).
* $P < 0.05$.

ampulla of Vater cancer compared with the GG allele. After further adjustment for other risk factors (age, gender, alcohol consumption, smoking cigarettes and gallstone for extrahepatic bile duct cancer; age, gender and alcohol consumption for gallstones), *MLNR* rs9568169 AA genotype and *SSTR5* rs169068 CC genotype were still significantly associated with risk of extrahepatic bile duct cancer (OR = 0.51, 95% CI: 0.28–0.92; OR = 3.15, 95% CI: 1.31–7.54), and *MLN* rs2281820 CT and rs3793079 AT genotypes were still significantly associated with risk of gallstones (OR = 1.55, 95% CI: 1.08–2.23; OR = 1.69, 95% CI: 1.23–2.32) compared to the major genotypes. No statistically significant associations were found for the other SNPs.

We also conducted gene–environment interaction analyses to determine if genetic susceptibility to biliary tract diseases differed by gender, smoking, drinking alcohol and diabetes. However, we did not find any significant interactions between SNPs and environment factors that influence the risk of biliary tract disease.

Mutations in the *MLN* gene were in linkage disequilibrium, with D′ ranging from 0.74 to 0.99 and $r^2$ ranging from 0.48 to 0.54. Given that the $r^2$ values were all below 0.8, it seemed possible that these three SNPs may not exactly represent each other and might provide additional information despite being in linkage disequilibrium. We found that *MLN* T-T-T (rs2281820–rs3793079–rs2281819) had a non-significant raised risk of gallstones compared with C-A-A haplotype (Table 3).

## Discussion

At present, the knowledge of the genetic background underlying pathogenesis of biliary tract cancers and stones are limited. In the present study, we investigated the associations between 9 SNPs in four genes (*MLN*, *MLNR*, *SSTR2* and *SSTR5*) and the risk of biliary tract cancers and stones. Our results suggest that variants in the *MLNR* gene (rs9568169) and *SSTR5* (rs169068) gene were potential genetic risk factors for extrahepatic bile duct cancer, and variants in the *MLN* gene (rs2281820 and rs3793079) were potential genetic risk factors for gallstone. To our knowledge, this is the first report of associations of *MLN*, *MLNR*, *SSTR2* and *SSTR5* tagging SNPs with susceptibility to biliary tract cancers and stones.

Motilin is considered an endocrine regulator of the migrating motor complex (MMC), and cholesterol gallstone patients were proved to have an abnormal MMC and motilin release pattern (van Erpecum & van Berge Henegouwen, 2003). Luiking et al. (1998) first demonstrated that exogenous motilin reduced fasting gallbladder volume and increased antral contractions in humans. And it was also proved by other studies (Kamerling et al., 2004; Luiking et al., 2002). Two studies showed that motilin levels were higher in plasma and gallbladder tissues from gallstone patients than in the healthy control and gallbladder polyp groups (Wang and Wu, 2008; Zhang et al., 2008). But Zhang et al. (2001) found that both gallstone and cholecystitis were associated with delayed gallbladder emptying and decreased plasma MLN levels in older people. These studies demonstrated that MLN activity had relation with gallstone and cholecystitis. Thus, our finding that genetic susceptibility to biliary tract diseases due to variations in *MLN* and *MLNR* is biologically plausible.

Studies which focused on mutations in the *MLN* gene and disease risks are relatively rare. Annese et al. (1998) found a functional polymorphism in the second exon of the motilin gene that is significantly more frequent in the subset of anti-neutrophil cytoplasmatic antibody (ANCA)-positive Crohn's disease patients, who appear to share peculiar genetic and clinical features. Svenningsson et al. (2008) screened the *MLN* gene and found 3 novel mutations in 4 infantile hypertrophic pyloric stenosis patients. In our study, the gallstone risk mutation *MLN* rs2281820 was a missense mutation (Val15Ala), and rs3793079 was located in the intron region of *MLN* gene that has not been reported to be of clinical significance with any other diseases. And the *MLNR* rs9568169 was a synonymous mutation that conferred protective effect on extrahepatic bile duct cancer risk in our study that also has not been reported before.

SST is known to inhibit hepatic bile secretion and gallbladder emptying (Ahrendt et al., 1991). Study has shown that SSTR2 and SSTR5 may play a primary role in regulating gall bladder emptying in mice (Kaczmarek et al., 2010). The expression of SSTR5 was also found to be significantly increased in gallbladder cancer than in normal gallbladder tissue in human (Guo et al., 2013). And SST has a therapeutic effect on acute cholecystitis (Guo, 2012). Here, we found that SSTR5 rs169068 was significantly associated with extrahepatic bile duct cancer. Rs169068 was a missense mutation in exon 1

**Table 2**
Associations between *MLN*, MLNR, *SSTR2* and *SSTR5* genotypes with the risk of biliary tract cancers and stones.

| Genotype | Controls | Biliary stones[a] | | Biliary tract cancer | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | n (%) | n (%) | OR[d] (95% CI) | Gallbladder[b] | | Bile duct[c] | | A.V.[c] | |
| | | | | n (%) | OR[d] (95% CI) | n (%) | OR[d] (95% CI) | n (%) | OR[d] (95% CI) |
| MLN | 477 | 439 | | 253 | | 133 | | 53 | |
| rs2281820 | | | | | | | | | |
| CC | 346 (77.6) | 317 (73.9) | 1.00 | 189 (74.7) | 1.00 | 94 (70.7) | 1.00 | 39 (75.0) | 1.00 |
| CT | 92 (20.6) | 107 (24.9) | 1.52 (1.06–2.18) | 62 (24.5) | 1.30 (0.89–1.90) | 37 (27.8) | 1.48 (0.94–2.33) | 13 (25.0) | 1.25 (0.63–2.45) |
| TT | 8 (1.8) | 5 (1.2) | 0.58 (0.18–1.82) | 2 (0.8) | – | 2 (1.5) | – | – | – |
| CT ± TT | 100 (22.4) | 112 (26.1) | 1.42 (1.00–2.00) | 64 (25.3) | 1.23 (0.85–1.79) | 39 (29.3) | 1.42 (0.91–2.21) | 13 (25.0) | 1.13 (0.57–2.20) |
| P trend | | | 0.03 | | 0.20 | | 0.09 | | 0.59 |
| Rs3793079 | | | | | | | | | |
| AA | 295 (66.1) | 246 (57.3) | 1.00 | 162 (64.0) | 1.00 | 75 (56.4) | 1.00 | 32 (61.5) | 1.00 |
| AT | 135 (30.3) | 166 (38.7) | 1.64 (1.20–2.25) | 80 (31.6) | 1.08 (0.77–1.53) | 54 (40.6) | 1.58 (1.04–2.38) | 18 (34.6) | 1.19 (0.64–2.21) |
| TT | 16 (3.6) | 17 (4.0) | 1.15 (0.55–2.38) | 11 (4.4) | 1.26 (0.56–2.80) | 4 (3.0) | – | 2 (3.9) | – |
| AT ± TT | 151 (33.9) | 183 (42.7) | 1.58 (1.17–2.13) | 91 (36.0) | 1.10 (0.79–1.54) | 58 (43.6) | 1.49 (1.00–2.23) | 20 (38.5) | 1.18 (0.65–2.14) |
| P trend | | | 0.002 | | 0.62 | | 0.03 | | 0.58 |
| Rs2281819 | | | | | | | | | |
| AA | 343 (76.9) | 315 (73.4) | 1.00 | 181 (71.5) | 1.00 | 95 (71.4) | 1.00 | 40 (76.9) | 1.00 |
| AT | 99 (22.2) | 108 (25.2) | 1.34 (0.95–1.90) | 71 (28.1) | 1.39 (0.97–2.01) | 36 (27.1) | 1.31 (0.83–2.05) | 11 (21.2) | 0.90 (0.44–1.83) |
| TT | 4 (0.9) | 6 (1.4) | – | 1 (0.4) | – | 2 (1.5) | – | 1 (1.9) | – |
| AT ± TT | 103 (23.1) | 114 (26.6) | 1.33 (0.95–1.87) | 72 (28.5) | 1.35 (0.94–1.94) | 38 (28.6) | 1.32 (0.85–2.06) | 12 (23.1) | 0.95 (0.48–1.88) |
| P trend | | | 0.10 | | 0.08 | | 0.23 | | 0.81 |
| *MLNR* | | | | | | | | | |
| Rs9568169 | | | | | | | | | |
| CC | 151 (33.9) | 129 (30.1) | 1.00 | 89 (35.2) | 1.00 | 54 (40.6) | 1.00 | 19 (36.5) | 1.00 |
| CA | 199 (44.6) | 212 (49.5) | 1.30 (0.93–1.81) | 115 (45.4) | 1.00 (0.70–1.42) | 61 (45.9) | 0.86 (056–1.32) | 25 (48.1) | 1.00 (0.53–1.90) |
| AA | 96 (21.5) | 87 (20.3) | 1.29 (0.85–1.95) | 49 (19.4) | 0.95 (0.61–1.48) | 18 (13.5) | 0.49 (0.27–0.89) | 8 (15.4) | 0.59 (0.25–1.41) |
| CA ± AA | 295 (66.1) | 299 (69.9) | 1.30 (0.95–1.77) | 164 (64.8) | 0.98 (0.71–1.37) | 79 (59.4) | 0.73 (0.49–1.10) | 33 (63.5) | 0.86 (0.47–1.57) |
| P trend | | | 0.13 | | 1.00 | | 0.56 | | 0.93 |
| *SSTR2* | | | | | | | | | |
| Rs7210080 | | | | | | | | | |
| TT | 230 (51.6) | 215 (50.1) | 1.00 | 117 (46.2) | 1.00 | 75 (56.4) | 1.00 | 28 (53.9) | 1.00 |
| TC | 176 (39.4) | 183 (42.7) | 1.04 (0.77–1.41) | 112 (44.3) | 1.25 (0.90–1.74) | 47 (35.3) | 0.82 (0.54–1.25) | 17 (32.7) | 0.78 (0.41–1.48) |

*(continued on next page)*

**Table 2** (*continued*)

| Genotype | Controls | Biliary stones[a] | | Biliary tract cancer | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | n (%) | n (%) | OR[d] (95% CI) | Gallbladder[b] | | Bile duct[c] | | A.V.[c] | |
| | | | | n (%) | OR[d] (95% CI) | n (%) | OR[d] (95% CI) | n (%) | OR[d] (95% CI) |
| CC | 40 (9.0) | 31 (7.2) | 0.70 (0.41–1.20) | 24 (9.5) | 1.12 (0.64–1.97) | 11 (8.3) | 0.83 (0.40–1.71) | 7 (13.4) | 1.50 (0.61–3.70) |
| TC + CC | 216 (48.4) | 214 (49.9) | 0.97 (0.73–1.30) | 136 (53.8) | 1.23 (0.89–1.68) | 58 (43.6) | 0.82 (0.55–1.22) | 24 (46.1) | 0.91 (0.51–1.62) |
| P trend | | | 0.84 | | 0.19 | | 0.35 | | 0.49 |
| Rs1466113 | | | | | | | | | |
| GG | 121 (27.1) | 112 (26.1) | 1.00 | 76 (30.0) | 1.00 | 34 (25.6) | 1.00 | 16 (30.8) | 1.00 |
| GC | 240 (53.8) | 221 (51.5) | 1.08 (0.76–1.51) | 120 (47.5) | 0.83 (0.58–1.20) | 65 (48.9) | 0.96 (0.60–1.55) | 18 (34.6) | 0.53 (0.26–1.09) |
| CC | 85 (19.1) | 96 (22.4) | 1.31 (0.85–2.01) | 57 (22.5) | 1.12 (0.71–1.75) | 34 (25.5) | 1.46 (0.84–2.56) | 18 (34.6) | 1.63 (0.78–3.41) |
| GC + CC | 325 (72.9) | 317 (73.9) | 1.14 (0.82–1.57) | 177 (70.0) | 0.91 (0.64–1.28) | 99 (74.4) | 1.09 (0.70–1.71) | 36 (69.2) | 0.81 (0.43–1.52) |
| P trend | | | 0.82 | | 0.26 | | 0.69 | | 0.05 |
| Rs2236748 | | | | | | | | | |
| GG | 304 (68.2) | 315 (73.6) | 1.00 | 185 (73.1) | 1.00 | 92 (69.2) | 1.00 | 42 (80.8) | 1.00 |
| GA | 126 (28.2) | 108 (25.2) | 0.91 (0.65–1.27) | 64 (25.3) | 0.83 (0.58–1.20) | 38 (28.6) | 1.02 (0.66–1.59) | 10 (19.2) | 0.58 (0.28–1.20) |
| AA | 16 (3.6) | 5 (1.2) | – | 4 (1.6) | – | 3 (2.2) | – | – | – |
| GA + AA | 142 (31.8) | 114 (26.4) | 0.83 (0.61–1.15) | 68 (26.9) | 0.79 (0.55–1.12) | 41 (30.8) | 0.97 (0.63–1.48) | 10 (19.2) | 0.51 (0.25–1.06) |
| P trend | | | 0.45 | | 0.27 | | 0.98 | | |
| Rs2236750 | | | | | | | | | |
| GG | 122 (27.5) | 138 (32.2) | 1.00 | 73 (28.9) | 1.00 | 47 (35.3) | 1.00 | 21 (40.4) | 1.00 |
| GA | 231 (52.0) | 214 (49.9) | 0.78 (0.56–1.10) | 124 (49.0) | 0.92 (0.63–1.33) | 62 (46.6) | 0.67 (0.43–1.05) | 21 (40.4) | 0.49 (0.26–0.94) |
| AA | 91 (20.5) | 77 (17.9) | 0.70 (0.46–1.07) | 56 (22.1) | 1.00 (0.64–1.57) | 24 (18.1) | 0.68 (0.38–1.20) | 10 (19.2) | 0.65 (0.29–1.46) |
| GA + AA | 322 (72.5) | 291 (67.8) | 0.76 (0.55–1.05) | 180 (71.1) | 0.94 (0.66–1.34) | 86 (64.7) | 0.67 (0.44–1.02) | 31 (59.6) | 0.53 (0.29–0.97) |
| P trend | | | 0.20 | | 0.63 | | 0.09 | | 0.03 |
| *SSTR5* | | | | | | | | | |
| Rs169068 | | | | | | | | | |
| TT | 268 (60.2) | 259 (61.5) | 1.00 | 160 (63.5) | 1.00 | 79 (59.4) | 1.00 | 28 (53.9) | 1.00 |
| TC | 157 (35.3) | 145 (34.4) | 0.91 (0.67–1.24) | 80 (31.7) | 0.82 (0.59–1.15) | 41 (30.8) | 0.92 (0.60–1.42) | 21 (40.4) | 1.29 (0.71–2.37) |
| CC | 20 (4.5) | 17 (4.0) | 1.02 (0.49–2.13) | 12 (4.8) | 0.95 (0.44–2.05) | 13 (9.8) | 2.40 (1.13–5.13) | 3 (5.8) | – |
| TC + CC | 177 (39.8) | 162 (38.5) | 0.92 (0.69–1.24) | 92 (36.5) | 0.84 (0.60–1.16) | 54 (40.6) | 1.08 (0.72–1.62) | 24 (46.1) | 1.32 (0.74–2.37) |
| P trend | | | 0.57 | | 0.26 | | 0.84 | | 0.39 |

OR, odds ratio; 95% CI, 95% confidence interval.

Test of trend for the number of copies of the variant allele (0, 1 and 2).

Did not calculate OR when N was less than 5.

[a] Biliary stone (gallstone and bile duct stone) cases were compared to controls without biliary stones (n = 341).

[b] Gallbadder cancer cases were compared with population controls who never had a cholecystectomy (n = 422).

[c] Bile duct and ampulla of Vater cancer cases were compared with all population controls (n = 447).

[d] Adjusted for age and gender.

**Table 3**
Associations between the MLN haplotypes and gallstone risk.

| Haplotype[a] | Controls, % | Cases, % | OR[b] (95% CI) |
|---|---|---|---|
| C-A-A | 80.7 | 76.0 | 1.00 |
| T-T-T | 9.0 | 10.5 | 1.30 (0.91–1.86) |
| C-T-A | 5.1 | 6.9 | 1.43 (0.92–2.23) |
| T-T-A | 2.6 | 3.0 | 1.31 (0.69–2.50) |
| C-T-T | 1.8 | 2.9 | 1.77 (0.85–3.70) |

[a] In the order of rs2281820, rs3793079 and rs2281819.
[b] Adjusted for age and gender.

resulting in a Pro → Leu change at amino acid 335, which has been reported to be associated with acromegaly in the population of Latvia (Ciganoka et al., 2011).

Strengths of this study should be noted. First, all cancer cases were histopathologically confirmed, thereby minimizing disease misclassification. Second, the assessment of gallstone status among cancer cases and controls made it possible to evaluate cancer risk while adequately controlling for the presence of gallstones. Third, misclassification of genotypes was minimal given the good quality and purity of the extracted DNA as well as the 100% concordance of genotyping results from replicated quality control samples. However, limitations of this study include the relatively small sample size, which limits the statistical power to detect some significant associations, especially in the stratified analyses.

## Conclusions

In conclusion, our results suggest that genetic variation in the *MLN*, *MLNR* and *SSTR5* genes may play roles in biliary tract diseases. Future epidemiologic studies with larger sample sizes as well as functional studies are needed to confirm our findings.

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