Cholesterol metabolism gene polymorphisms and the risk of biliary tract cancers and stones: a population-based case-control study in Shanghai, China

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Biliary tract cancers are rare but fatal malignancies, with increasing incidence in Shanghai, China. Gallstones, the primary risk factor for biliary tract cancer, typically result from oversaturation of cholesterol in bile. We examined the association of five variants in three lipid metabolism-related genes (CEIP, ABCG8 and LRPAIP1) and biliary tract cancers and stones in a population-based case-control study in Shanghai, China. We included 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. Carriers of the CG genotype of ABCG8 rs11887534 had higher risk of biliary stones [odds ratio (OR) = 2.3, 95% confidence interval (CI) 0.82–6.5], gallbladder cancer (OR = 4.3, 95% CI 1.7–10.4) and bile duct cancer (OR = 1.94, 95% CI 0.64–5.91), compared with carriers of the GG genotype. Analysis stratified by gender showed both male and female carriers of CG rs11887534 had higher risks of biliary stones and gallbladder cancer, although the association was statistically significant only for women and gallbladder cancer (OR = 6.3, 95% CI 1.86–22.3). Carriers of the ABCG8 haplotype C-C (rs4148217, rs11887534) had a 4.16-fold (95% CI 1.71–10.1) risk of gallbladder cancer compared with those carrying the C-G haplotype. Our findings suggest that ABCG8 rs11887534, identified as a gallstone risk single-nucleotide polymorphism by whole genome scan, is also associated with an increased risk of biliary tract cancer.

Introduction

Biliary tract cancers include cancers of the gallbladder, extrahepatic bile duct and ampulla of Vater. They are rare but highly fatal malignancies with poor survival (1). Gallstones are the most important risk factor for biliary tract cancers, especially those of the gallbladder. In a previous report, we showed that gallstones are associated with a 24-fold risk of gallbladder cancer in Shanghai (2). With increasing westernization of diet in China, most gallstone cases diagnosed in Shanghai in the recent past are cholesterol in origin, which are related to dyslipidemia and oversaturation of cholesterol in bile (3). High serum lipid levels are a risk factor for gallstones and have been associated with biliary tract cancers (4). Serum lipid levels are affected by both genetic and lifestyle factors (5,6). Several genes in the cholesterol metabolism pathway play an important role in lipid biosynthesis, metabolism and transport, which in turn can affect the pathogenesis of gallstones and biliary tract cancer. For example, the ABCG5/BCG8 gene, identified to be associated with gallstones by a genome-wide association study (GWAS) (7), is expressed exclusively in the liver and intestine, can form heterodimers to regulate the efflux of sterols into intestinal lumen and control the hepatic secretion of sterols into the bile (8,9). Similarly, cholesteryl ester transfer protein (CEIP) is an enzyme involved in facilitating the removal of excess cholesterol from the body via low-density lipoprotein (LDL) receptor-mediated uptake in the liver (10). LRPAIP1 is involved with trafficking of certain members of the LDL receptor family including LDL receptor-related proteins (LRP1and LRP2) (11).

In an earlier report, we showed that variants in the lipid metabolism genes APOE (rs440446), APOB (rs520354) and LDLR (rs1003723) were associated with biliary tract cancers and stones (12). To further clarify the effect of lipid metabolizing genes on biliary tract cancer and stone risks, we investigate the associations of variants in ABCG8 (rs148217, rs11887534), CETP (rs708272, rs1800775) and LRPAIP1 (rs11267919) with the risk of biliary tract cancer and stones in a population-based case-control study in Shanghai, China.

Materials and methods

Study subjects

Details of the study methods have been reported elsewhere (2,13,14). Briefly, 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 >95% of all incident biliary tract cancer cases (International Classification of Diseases, Ninth Edition code 156) diagnosed among Shanghai residents between June 1997 and May 2001. A total of 439 incident biliary tract cancer cases (253 gallbladder, 133 extrahepatic bile duct and 53 ampulla of Vater cases), between 36 and 75 years, were included in this study. To evaluate the risk of biliary stones independently of biliary tract cancer, 430 patients with biliary stones without a history of cancer were included and were frequency matched to the cancer cases on age (5 years groups), gender and diagnosing hospital. Population controls, without a history of biliary tract cancer, were randomly selected from the Shanghai Resident Registry, which includes records for ~6 million Shanghai residents. We included a total of 443 population controls who were frequency matched to cancer cases on age (5 years groups) and gender.

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 assessment, whereas 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, endoscopic retrograde cholangiopancreatography or computed tomography. Biliary stone cases were confirmed by review of abdominal ultrasound, endoscopic retrograde cholangiopancreatography 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 magnetic resonance imaging, endoscopic retrograde cholangiopancreatography, computed tomography or ultrasound. Among population controls, gallstones were identified by self-reported history or by abdominal ultrasound for 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 >95% among cases and 82%

Abbreviations: BMI, body mass index; CETP, cholesteryl ester transfer protein; CI, confidence interval; GWAS, genome-wide association study; LDL, low-density lipoprotein; OR, odds ratio; WHR, waist to hip ratio.

These authors contributed equally to this work.

Published by Oxford University Press 2010.

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among controls. Five percent of the study subjects were randomly selected for
re-interview 3 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 >90%.

Genotyping

Three candidate genes were selected based on their role in cholesterol metab-
olism and potential effects on biliary disease pathogenesis. Single-nucleotide
polymorphisms (SNPs) were selected based on their putative functional sig-
nificance (ABCG8 rs148217, CETP rs708272 and rs1800775 and LRPAP1
rs11267919) or by association with gallstone risk from whole genome scan
(ABCG8 rs11887534). Overnight fasting blood samples were collected from
all subjects who gave consent (>80%). Genomic DNA was extracted from
buffy coat using Promega DNA Extraction Kit. Genotyping of ABCGS
(rs148217, rs11887534) and CETP (rs708272, rs1800775) were performed by
the TaqMan assay, using the ABI PRISM 7900HT Sequence Detection
System (Applied Biosystems, Foster City, CA), in 384-well format, with dual
fluorescent reporter probes VIC and FAM. Genotyping of LRPAP1
(rs11267919), an insertion/deletion variation, was conducted by polymerase
chain reaction and visualized by agarose gel electrophoresis. The genotyping
failure rate among all samples was <1.5%. The quality and potential
misclassification of the genotyping results 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 replicates were 100% concordant.

Statistical analysis

The final analysis included subjects who completed the interview and for
whom we had genotyping results, which totaled 439 incident biliary tract
cancer cases (253 gallbladder, 133 extrahepatic bile duct and 53 ampulla of
Vater cases), 430 biliary stone cases and 447 healthy controls. To make the
appropriate case–control comparisons, for all analyses, gallbladder cancer
cases were compared with controls without a history of cholecystectomy
(n = 422), bile duct cancer cases and ampulla of Vater cancer cases were
compared with all controls (n = 447) and biliary stone cases were compared
with controls without biliary stones (n = 341). Distributions of selected char-
acteristics, including gender, age, education, cigarette smoking, alcohol drink-
ing, gallstone status (among cancer cases), hypertension, diabetes, body mass
index (BMI) and waist to hip ratio (WHR), were evaluated among cases and
controls; characteristics with statistically significant different distributions be-
tween cases and controls (Fisher’s exact P < 0.05) were examined for their
associations with SNPs among controls.

The associations between SNPs and biliary tract cancer and stone risk
were estimated by odds ratios (OR) and 95% confidence intervals (CI) using
unconditional logistic regression. Risk estimates were calculated for the
homozygous and homozygous variant genotypes relative to the common
homozygous genotype (codominant model), as well as for the presence and
absence of the variant allele (dominant model), using indicator variables in the
regression model. An initial model was adjusted for age, and additional models
were further adjusted for gender, education, cigarette smoking, alcohol drink-
ing, gallstone status (cancer risk only), hypertension, diabetes, BMI and WHR.
Associations between SNPs and biliary diseases were also evaluated separately
by gender and biliary stone status, and statistical interaction was evaluated
using the likelihood ratio test.

Hardy–Weinberg equilibrium for genotypic distribution and linkage
disequilibrium between loci in ABCGS and CETP genes were assessed
by HaploView version 4.0 (15). Associations between haplotypes (>1% fre-
quency) and the risks of biliary tract stones and cancer were evaluated by
computing OR and 95% CI, using HAPSTAT, assuming an additive model,
and using the most common haplotype as the referent category (16). Global
differences in haplotype frequencies between cases and controls were
assessed for each gene using the Score test in HAPSTAT.

Table I. Selected characteristics of subjects by case–control status

<table>
<thead>
<tr>
<th>Selected characteristics</th>
<th>Controls, n (%)</th>
<th>Biliary stonesa, n (%)</th>
<th>Biliary tract cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gallbladderb, n (%)</td>
<td>Bile ductc, n (%)</td>
<td>A.V.d, n (%)</td>
</tr>
<tr>
<td>All subjects</td>
<td>447 (92.3)</td>
<td>430 (93.2)</td>
<td>253 (94.4)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>178 (39.8)</td>
<td>159 (37.1)</td>
<td>68 (26.9)</td>
</tr>
<tr>
<td>Female</td>
<td>269 (60.2)</td>
<td>270 (62.9)</td>
<td>185 (73.1)*</td>
</tr>
<tr>
<td>Age at interview</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34–54</td>
<td>58 (13.0)</td>
<td>124 (28.9)</td>
<td>39 (15.4)</td>
</tr>
<tr>
<td>55–64</td>
<td>123 (27.5)</td>
<td>118 (27.5)</td>
<td>61 (24.1)</td>
</tr>
<tr>
<td>65–75</td>
<td>266 (59.5)</td>
<td>187 (43.6)*</td>
<td>163 (60.5)</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>47 (10.5)</td>
<td>44 (10.3)</td>
<td>61 (24.1)</td>
</tr>
<tr>
<td>Elementary</td>
<td>137 (30.7)</td>
<td>91 (21.2)</td>
<td>74 (29.5)</td>
</tr>
<tr>
<td>Middle school</td>
<td>109 (24.4)</td>
<td>124 (28.9)</td>
<td>63 (24.9)</td>
</tr>
<tr>
<td>High school</td>
<td>86 (19.2)</td>
<td>97 (22.6)</td>
<td>33 (13.0)</td>
</tr>
<tr>
<td>College and above</td>
<td>68 (15.2)</td>
<td>73 (17.0)</td>
<td>22 (8.7)*</td>
</tr>
<tr>
<td>Ever smoke</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>145 (32.4)</td>
<td>121 (28.2)</td>
<td>66 (26.2)</td>
</tr>
<tr>
<td>No</td>
<td>302 (67.6)</td>
<td>308 (71.8)</td>
<td>186 (73.8)</td>
</tr>
<tr>
<td>Ever drink alcohol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>91 (20.4)</td>
<td>70 (16.3)</td>
<td>40 (15.8)</td>
</tr>
<tr>
<td>No</td>
<td>356 (79.6)</td>
<td>359 (83.7)*</td>
<td>213 (84.2)</td>
</tr>
<tr>
<td>Gallstone status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>106 (23.7)</td>
<td>429 (100.0)</td>
<td>214 (84.6)</td>
</tr>
<tr>
<td>No</td>
<td>341 (76.3)</td>
<td>—</td>
<td>39 (15.4)*</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>65 (14.6)</td>
<td>69 (16.1)</td>
<td>26 (10.3)</td>
</tr>
<tr>
<td>No</td>
<td>381 (85.4)</td>
<td>356 (83.2)</td>
<td>227 (89.7)</td>
</tr>
<tr>
<td>BMI&lt;18.5</td>
<td>123 (30.7)</td>
<td>94 (24.7)</td>
<td>46 (21.5)</td>
</tr>
<tr>
<td>18.5–22.9</td>
<td>239 (59.6)</td>
<td>238 (62.6)</td>
<td>131 (61.2)</td>
</tr>
<tr>
<td>23.0–24.9</td>
<td>29 (7.2)</td>
<td>34 (9.0)</td>
<td>24 (11.2)</td>
</tr>
<tr>
<td>≥25.0</td>
<td>10 (2.5)</td>
<td>14 (3.7)</td>
<td>13 (6.1)*</td>
</tr>
</tbody>
</table>

Note. Total number of subjects may vary because of missing values. A.V., ampulla of Vater.

aBiliary stone (gallstone and bile duct stone) cases compared with controls without biliary stones (n = 341).
bGallbladder cancer cases compared with population controls who had a gallbladder (n = 422).
cBile duct and A.V. cancer cases compared with all population controls (n = 447).
dP < 0.05 for Fisher’s exact test for difference between cases and controls.
Results

Selected characteristics of the study subjects are shown in Table I. The frequency of selected characteristics did not differ considerably between the three subgroups of controls (all controls, controls without cholecystectomy and controls without biliary stones). Biliary stone cases were more probably to be younger, have a higher WHR but less probably to drink alcohol compared with controls without stones. Gallbladder cancer cases were more probably to be female (73.1%), illiterate, have gallstone (84.6%), have higher BMI and WHR than the controls with gallbladder. Bile duct cancer and ampulla of Vater cancer cases were more common in men, more probably to smoke cigarettes, have gallstone, have higher WHR but less probably to have hypertension. Furthermore, bile duct cancer cases were more probably to drink alcohol, and ampulla of Vater cancer cases were more probably to have a moderate BMI (18.5–22.9 kg/m²).

The associations of the \textit{ABCG8}, \textit{CETP}, and \textit{LRPAP1} variants with biliary stones and cancers are presented in Table II. The genotype distributions of these five variants showed no deviation from the expected Hardy–Weinberg equilibrium among controls ($P > 0.1$). Of these SNPs, carriers of the GC genotype of \textit{ABCG8} rs11887534 had a higher risk of biliary stones (OR = 2.3, 95% CI 0.82–6.5), gallbladder cancer (OR = 4.3, 95% CI 1.7–10.4) and bile duct cancer (OR = 1.9, 95% CI 0.64–5.91) compared with those carrying the GG genotype. The association for gallbladder cancer remained statistically significant after further adjustment for gender, education, BMI and WHR (OR = 4.9, 95% CI 1.9–13.0) but was no longer significant after adjustment for gallstone status (OR = 2.6, 95% CI 0.80–8.5). Analysis stratified by gender showed that both male and female carriers of CG rs11887534 also had higher risks for biliary stones and gallbladder cancer, although the association was statistically significant only for women and gallbladder cancer (OR = 6.3, 95% CI 1.86–22.3). We were not able to evaluate the effects of rs11887534 by biliary stone status due to the small number of subjects in these strata. None of the other SNPs examined were associated with biliary disease.

The two \textit{CETP} SNPs, rs708272 and rs1800775, are in strong linkage disequilibrium ($D^* = 1.0$), whereas the linkage disequilibrium between \textit{ABCG8} rs4148217 and rs11887534 is modest ($D^* = 0.56$) (17). Table III lists the associations between \textit{ABCG8} and \textit{CETP} haplotypes and biliary stones and cancers. Carriers of the \textit{ABCG8} (rs4148217-rs11887534) C–C haplotype had excess risks of biliary stones (OR = 1.9, 95% CI, 0.83–4.32), gallbladder cancer (OR = 4.16, 95% CI, 1.71–10.1) and bile duct cancer (OR = 1.87, 95% CI, 0.62–5.65) relative to carriers of the C-G haplotype. The \textit{CETP} haplotypes were not significantly associated with biliary stones or cancers. These haplotype results are consistent with the individual SNP results.

Discussion

In this population-based study in Shanghai, we found that \textit{ABCG8} rs11887534, the SNP identified to be associated with risk for

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### Table II. ORs and 95% CIs for biliary stones and cancers in relation to polymorphisms of cholesterol metabolism genes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls</th>
<th>Biliary stones</th>
<th>Gallbladder cancer</th>
<th>Bile duct cancer</th>
<th>Ampulla of Vater cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>$n$</td>
<td>OR (95% CI)</td>
<td>$n$</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>All subjects</td>
<td>447</td>
<td>430</td>
<td>253</td>
<td>133</td>
<td>53</td>
</tr>
<tr>
<td>\textit{ABCG8}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs4148217</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>357</td>
<td>327</td>
<td>1.00</td>
<td>203</td>
<td>1.00</td>
</tr>
<tr>
<td>CA</td>
<td>81</td>
<td>97</td>
<td>1.22 (0.85–1.75)</td>
<td>48</td>
<td>1.05 (0.71–1.57)</td>
</tr>
<tr>
<td>AA</td>
<td>5</td>
<td>2</td>
<td>—</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>CA + AA</td>
<td>86</td>
<td>99</td>
<td>1.19 (0.83–1.70)</td>
<td>49</td>
<td>1.03 (0.70–1.54)</td>
</tr>
<tr>
<td>rs11887534</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>434</td>
<td>413</td>
<td>1.00</td>
<td>235</td>
<td>1.00</td>
</tr>
<tr>
<td>GC</td>
<td>9</td>
<td>16</td>
<td>2.3 (0.82–6.5)</td>
<td>17</td>
<td>4.3 (1.7–10.4)</td>
</tr>
<tr>
<td>CC</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>\textit{CETP}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs708272</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>151</td>
<td>152</td>
<td>2.3 (1.8–2.9)</td>
<td>129</td>
<td>1.09 (0.80–1.50)</td>
</tr>
<tr>
<td>CT</td>
<td>216</td>
<td>216</td>
<td>1.0 (0.80–1.30)</td>
<td>192</td>
<td>1.02 (0.80–1.33)</td>
</tr>
<tr>
<td>TT</td>
<td>76</td>
<td>77</td>
<td>1.04 (0.86–1.24)</td>
<td>65</td>
<td>1.07 (0.87–1.30)</td>
</tr>
<tr>
<td>CT + TT</td>
<td>292</td>
<td>277</td>
<td>0.98 (0.82–1.17)</td>
<td>166</td>
<td>1.04 (0.79–1.38)</td>
</tr>
<tr>
<td>rs1800775</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>125</td>
<td>122</td>
<td>1.00</td>
<td>67</td>
<td>1.00</td>
</tr>
<tr>
<td>AC</td>
<td>229</td>
<td>214</td>
<td>1.07 (0.80–1.43)</td>
<td>128</td>
<td>1.04 (0.82–1.31)</td>
</tr>
<tr>
<td>CC</td>
<td>88</td>
<td>84</td>
<td>1.05 (1.00–1.30)</td>
<td>56</td>
<td>1.05 (0.85–1.32)</td>
</tr>
<tr>
<td>AC + CC</td>
<td>354</td>
<td>308</td>
<td>1.07 (0.86–1.31)</td>
<td>184</td>
<td>1.08 (0.74–1.56)</td>
</tr>
<tr>
<td>\textit{LRPAP1}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs11267919</td>
<td></td>
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<td></td>
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<tr>
<td>Del</td>
<td>135</td>
<td>112</td>
<td>1.00</td>
<td>72</td>
<td>1.00</td>
</tr>
<tr>
<td>Ins/del</td>
<td>221</td>
<td>222</td>
<td>1.00 (0.80–1.29)</td>
<td>124</td>
<td>1.00 (0.86–1.16)</td>
</tr>
<tr>
<td>Ins</td>
<td>89</td>
<td>95</td>
<td>1.29 (0.85–1.95)</td>
<td>57</td>
<td>1.21 (0.83–1.79)</td>
</tr>
<tr>
<td>Ins/del+ Ins</td>
<td>310</td>
<td>317</td>
<td>1.25 (0.91–1.72)</td>
<td>181</td>
<td>1.13 (0.80–1.59)</td>
</tr>
</tbody>
</table>

Note. Total number of subjects per SNP may vary because of 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 ampulla of Vater cancer cases compared with all population controls ($n = 447$).

$^d$Adjusted for age.

$^e$Test of trend for the number of copies of the variant allele (0, 1 and 2).
We also found an association between the \textit{ABCG8} haplotype (rs1448217-rs11887534) and gallbladder cancer. These findings suggest that variants in lipid metabolism-related genes play a role in biliary stones and cancer.

Our finding of \textit{ABCG8} rs11887534 validates results from GWAS and is consistent with previous studies. In a GWAS study in Germany (1385 cases and 1233 controls), the rs11887534 GC carriers had 2.2-fold risk of gallstone disease (7). A study of monozygotic twins in Sweden also showed that that rs11887534 was significantly associated with the risk of gallstones (OR = 2.5; 95% CI, 1.33–4.82) (18). Among Chinese men, \textit{ABCG8} has been associated with gallstones in both Taiwan and China (19,20). For gallbladder cancer, the rs11887534 variant was found to be associated with a 1.8-fold risk (95% CI, 1.1–2.8), primarily in gallbladder cancer patients with stones (OR = 1.8; 95% CI, 1.0–3.1) in a study in India (21). In our study, rs11887534 variant was associated with both gallstones and gallbladder cancer, but we were not able to examine the joint effect of this variant and gallstones on the risk of gallbladder cancer due to small numbers. However, after adjustment for gallstones, the association between \textit{ABCG8} variant and gallbladder cancer was no longer significant, suggesting that the association between rs11887534 and gallbladder cancer is mediated by gallstones.

Our findings for \textit{ABCG8} rs11887534 are biologically plausible. \textit{ABCG8} rs11887534, also called D19H, is a missense variation that causes an amino acid change from an acidic amino acid (aspartic acid) to a basic amino acid (histidine). Although the biological effect of this amino acid change is still unclear, it has been shown that carriers of the rs11887534 variant have lower serum plant sterol levels (sitosterol and lathosterol) and higher cholesterol precursors (cholesterol and sterylamine) (18). Also, rs11887534 is associated with gallbladder cancer due to small numbers. However, after adjustment for gallstones, the association between \textit{ABCG8} variant and gallbladder cancer was no longer significant, suggesting that the association between rs11887534 and gallbladder cancer is mediated by gallstones.

In conclusion, our results suggest that \textit{ABCG8} rs11887534 has a potential role in biliary tract cancer pathogenesis. However, the relationship between the risk alleles and biliary tract disease need to be validated in a larger population. Furthermore, future studies are needed to determine if any of the risk SNPs are functional and if they directly contribute to biliary tract carcinogenesis and stone formation.

We did not find an association between \textit{CETP} variants and biliary tract stones or cancer. In previous studies, \textit{CETP} variants were associated with gallstones in Finnish and Chinese populations, but not in an Indian population (32–34).

Strengths and limitations of our study should be noted. Strengths of our study include the population-based design, thereby minimizing selection bias; detailed review of cancer diagnosis, thereby minimizing disease misclassification and use of good quality and purity of extracted DNA, thereby minimizing misclassification of genotypes. Although our study is the largest to date, the minor allele frequency of \textit{ABCG8} rs11887534 in our Chinese population was low (2%), which limits our ability to evaluate specific effects in subgroups, such as the joint effect of \textit{ABCG8} and gallstones on gallbladder cancer risk among women. We also cannot rule out chance findings due to low statistical power and multiple comparisons.

In conclusion, our results suggest that \textit{ABCG8} rs11887534 may play a role in biliary tract cancer pathogenesis. However, the relationship between the risk alleles and biliary tract disease need to be validated in a larger population. Furthermore, future studies are needed to determine if any of the risk SNPs are functional and if they directly contribute to biliary tract carcinogenesis and stone formation.

\section*{Funding}
Intramural Research Program of the National Institute of Health, National Cancer Institute, Division of Cancer Epidemiology and Genetics, USA.

\section*{Acknowledgements}
We thank the collaborating surgeons and pathologists in Shanghai for assistance in patient recruitment and pathology review; Chia-Rong Cheng, Lu Sun and Kai Wu of the Shanghai Cancer Institute for coordinating data and specimen collection.

\section*{Conflict of Interest Statement}
None declared.

\section*{References}


Received July 22, 2010; revised September 10, 2010; accepted September 13, 2010