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Variants in hormone-related genes and the risk of biliary tract cancers and stones: a population-based study in China

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Biliary tract cancers, encompassing gallbladder, extrahepatic bile duct and ampulla of Vater cancers, are uncommon but often fatal malignancies. Hormone-related factors, including parity, oral contraceptive use, obesity, and gallstones, have been implicated in the etiology of these cancers. To further clarify the role of hormones in biliary tract cancers and biliary stones, we genotyped 18 single-nucleotide polymorphisms (SNPs) in nine genes involved in steroid hormone biosynthesis, metabolism and transport in a population-based case-control study in Shanghai, China. This study included subjects who completed an interview and provided blood, which totaled 411 biliary tract cancer and 893 biliary stone patients and 786 healthy Shanghai residents. The *CYP1A1* IVS1 + 606 (rs2606345) T allele was associated with gallbladder [odds ratio (OR) = 2.0, 95% confidence interval (CI), 1.3–3.0] and bile duct cancers (OR = 1.8, 95% CI = 1.1–3.1), whereas the *CYP1A1* Ex7 + 131 (rs1048943) G allele was associated with ampulla of Vater cancer (OR = 2.9, 95% CI = 1.5–5.4). After taking into account multiple comparisons for SNPs within each gene, *CYP1A1* was significantly associated with gallbladder ($P = 0.004$) and ampulla of Vater cancers ($P = 0.01$), but borderline with bile duct cancer ($P = 0.06$). The effect of *CYP1A1* IVS1 + 606 on gallbladder cancer was more pronounced among non-obese (body mass index < 23) (OR = 3.3, 95% CI = 1.8–6.1; P interaction = 0.001). Among women taking oral contraceptives, the effect of *SHBG* Ex8 + 6 (rs6259) on gallbladder cancer (OR = 6.7, 95% CI = 2.2–20.5; P interaction = 0.001) and stones (OR = 2.3, 95% CI = 1.1–4.9; P-interaction = 0.05) was statistically significant. Our findings suggest that common variants in hormone-related genes contribute to the risk of biliary tract cancers and stones, possibly by modulating hormone metabolism.

Abbreviations: BMI, body mass index; CI, confidence interval; 2-OH-E_2, 2-hydroxy-estradiol; OC, oral contraceptives; OR, odds ratio; SHBG, sex hormone-binding globulin; SNP, single-nucleotide polymorphisms.

Introduction

Biliary tract cancers encompass tumors originating in the gallbladder, extrahepatic bile duct and ampulla of Vater. They are relatively uncommon in most parts of the world (1,2); yet the highest rates are seen in regions of Asia, including Korea, Japan, China and India, as well as Eastern Europe and the USA among American Indians (1,2). In Shanghai, China, a rapid rise in incidence has been reported in the past few decades (119% in men and 124% in women from 1972 to 1994) (3).

Gallstones are the predominant risk factor for all three anatomic subsites of biliary tract cancer (4–7). Apart from gallstones, the etiology of biliary tract cancer is poorly understood. Gallbladder cancer is one of the few cancers with a female excess and therefore has been hypothesized to be related to steroid hormone metabolism. In further support of this hypothesis, several hormonal factors, including high parity (5,8–10), early age at first pregnancy (5,8–10), oral contraceptive use (10–12) and obesity (13,14), have been reported to increase the risk of gallbladder cancer in women.

Due to the ethnic and familial predisposition to biliary tract cancers, it is thought that genetics may play a role in the etiology of these cancers, although the genomic regions have not been delineated (2). We hypothesize that genetic variants in genes encoding for enzymes involved in steroidogenesis may influence biliary tract cancer pathogenesis, particularly gallbladder cancer. In this population-based case–control study conducted in Shanghai, China, we assessed the association between 18 genetic variants in nine genes (*CYP1A1*, *CYP1B1*, *COMT*, *HSD3B2*, *HSD17B1*, *HSD17B3*, *SRD5A2*, *CYP19A1* and *SHBG*) involved in steroid hormone biosynthesis, transport and metabolism and the risks of biliary tract cancer and stones. We also assessed whether the genetic variants were related to biliary tract cancers through their association with gallstones and how various hormonal factors, such as parity and oral contraceptive use, impacted these associations.

Materials and methods

Study population

The Institutional Review Boards of the USA National Cancer Institute (NCI) and the Shanghai Cancer Institute approved the study protocol, and all participants provided written informed consent for the study. Details of the study have been reported previously (7,15–19). A total of 2092 permanent residents of urban Shanghai between 35 and 74 years of age, including 627 biliary tract cancer patients (368 gallbladder, 191 bile duct and 68 ampulla of Vater), 1037 biliary stone patients (774 gallbladder and 263 bile duct) and 959 control subjects were enrolled into this study. Cancer cases were newly diagnosed with biliary tract cancer between June 1997 and May 2001 and were identified by a rapid reporting system established by the Shanghai Cancer Institute and 42 collaborating hospitals in Shanghai. This system captured over 95% of the incident cases diagnosed in the Shanghai population during the study period. Biliary tract cancer diagnosis was confirmed for all cases by expert review of histology slides and clinical data from computed tomography scan, magnetic resonance imaging, abdominal ultrasound or endoscopic retrograde cholangiopancretography. Biliary stone cases were selected from the same hospitals as cancer cases and were frequency matched to cancer cases on age (5-year intervals), sex and hospital. Biliary stone cases were confirmed by review of abdominal ultrasound, endoscopic retrograde cholangiopancretography films, medical records and surgical records or by pathologic material for those who underwent a cholecystectomy. Control subjects were healthy adults without a history of cancer, who were randomly selected from permanent residents listed in the Shanghai Resident Registry, and were frequency-matched to cancer cases on age (5-year intervals) and sex. Of the eligible subjects, 95% of the cancer and stone cases and 82% of the controls participated in the study.

Data collection

Trained interviewers conducted in-person interviews with each subject using a structured questionnaire to collect information on demographic and

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epidemiological factors. Cases were interviewed within 3 weeks of diagnosis. All interviews were tape-recorded and reviewed to ensure that they were conducted uniformly among participants and that the data were recorded accurately. Five percent of the study subjects were randomly selected for re-interview within 3 months to assess reproducibility of the interview data. Concordance between the two interviews on responses to key questions was 90%. Weight and height were measured at the time of interview.

Medical records of cancer and stone cases were abstracted to obtain information on clinical and pathological characteristics. Among cancer cases, status for biliary stones was determined using questionnaire data, medical record review or clinical diagnostic examinations (abdominal ultrasound, computed tomography scan, magnetic resonance imaging or endoscopic retrograde cholangiopancretography). Among controls, biliary stone status was based on questionnaire data and abdominal ultrasonography. Of the participating controls, 85% consented to ultrasound screening for the detection of asymptomatic stones.

Genotyping

Over 80% of study participants provided an overnight fasting blood sample. Genomic DNA was isolated from buffy coat using the phenol–chloroform method. We selected 18 single-nucleotide polymorphisms (SNPs) in nine candidate genes involved in steroid hormone biosynthesis, metabolism and transport (Table I). SNPs were chosen based on prior reports of possible functional significance and/or evidence of an association with biliary tract cancers or stones; selected SNPs had an expected variant allele frequency of at least 5% in Asians and a validated Taqman assay at the time of analysis at the NCI Core Genotyping Facility (Advanced Technology Corporation, Gaithersburg, MD). Sequence data and assay conditions are provided at http://snp500cancer.nci.nih.gov (20). Genotyping was conducted at the Core Genotyping Facility using the TaqMan assay (Applied Biosystems, Foster City, CA, http://snp500cancer.nci.nih.gov). The laboratory personnel were blinded to case–control status. Successful genotyping was achieved for 96–100% of DNA samples for all SNPs. External blinded quality controls (i.e., 80 samples from 20 individuals) were included to assess reproducibility of genotyping. Concordance for genotyping in duplicate samples was >97% for all assays.

Statistical analysis

We used unconditional logistic regression analysis to calculate odds ratios (ORs) and 95% confidence intervals (CIs) adjusted for age and sex to estimate the risk of each anatomic subsite of biliary tract cancer and biliary stones associated with each SNP. Additional models were run for cancer risk with further adjustment for biliary stone status to evaluate the risk of cancer independent of stones, since individuals diagnosed with biliary tract cancers and biliary stones potentially share similar genetic susceptibility profiles. Gallbladder cancer cases were compared with control subjects without a history of cholecystectomy, whereas bile duct and ampulla of Vater cancer cases were compared with all control subjects. Biliary stone cases were compared with control subjects who did not have biliary stones. Risk estimates were calculated for a codominant genetic model using the most common homozygous genotype as the referent category. Tests of linear trend using an ordinal variable for the number of copies of the variant allele (0, 1 or 2) were conducted to assess potential dose–response effects of genetic variants on biliary tract cancer and stone risk (21). Other putative risk factors for biliary tract cancer or stones, including education, body mass index (BMI), diabetes, cigarette smoking, alcohol drinking, history of other gallbladder diseases, use of oral contraceptives (OC), parity, age at menarche, age at menopause, age at first birth and breast feeding, were evaluated as potential effect modifiers by using the likelihood ratio test to assess multiplicative interactions between these characteristics and each SNP on stone and cancer risk, as well as their potential confounding effect by further adjusting for these factors and assessing their effect on risk estimates. The risk estimates of each SNP with or without these characteristics in relation to biliary tract cancer and stones were also compared.

When there were at least two SNPs in a gene, we computed gene-level *P*-values using the Simes' test and Bonferonni test, which uses the *P*-trend, or the *P*-value from the dominant model for markers with sparse genotype data, for each SNP to adjust for multiple SNP comparisons within each gene (22). We also inferred haplotypes for the *CYP19A1* gene for which we evaluated six SNPs. Among population controls, the presence of linkage disequilibrium between loci in the *CYP19A1* gene was assessed by calculating pairwise Lewontin's D' and r^2 values using Haploview version 3.11 (23). The risks for biliary tract cancers and stones in relation to *CYP19A1* haplotypes were assessed using the haplo.stats package (24) in R, version 2.0.1, which employs the expectation–maximization algorithm to estimate haplotypes and a global score test to assess overall differences in haplotype frequencies between cases and controls (25), adjusting for age and sex. Haplotype-associated risks were assessed by the generalized linear model implemented in the haplo.stats package using the most common haplotype as the referent category. Associations for *CYP19A1* haplotypes with observed frequencies >5% were evaluated.

Results

Selected characteristics of study subjects are shown in Table II. There were more women with gallbladder cancer (72.6%) and biliary stones (63.2%), but more men with bile duct cancer (59.8%). Gallbladder cancer cases and biliary stone cases had a higher BMI than controls. Bile duct cancer cases were more likely to be ex-smokers or former alcohol drinkers, whereas biliary stone cases were less likely to be former or current alcohol drinkers compared with controls. Gallbladder cancer and biliary stone cases were also more likely to have diabetes than controls. All three biliary tract cancer subsites, particularly the gallbladder, were more likely to have biliary stones compared with controls. Among women, gallbladder cancer cases tended to have higher parity, whereas biliary stone cases had lower parity than controls.

Table III shows the risk of biliary tract cancers and biliary stones in relation to each of the 18 SNPs. Among population controls, the

Table I. SNPs of steroid hormone biosynthesis, metabolism and transport genes examined in relation to risk of biliary tract cancers and stones

Gene	Name	Chromosome location	SNP rs #	Nucleotide change	Amino acid change
COMT	Catechol-*O*-methyltransferase	22q11.21	rs4633	Ex3-104C>T	H62H
			rs4818	Ex4-76C>G	L136L
CYP1A1	Cytochrome P450, family I, subfamily A, polypeptide 1	15q22–q24	rs2606345	IVS1+606G>T	
			rs1048943	Ex7+131A>G	I462V
CYP1B1	Cytochrome P450, family I, subfamily B, polypeptide 1	2p21	rs10012	Ex2+143C>G	R48G
			rs1056836	Ex3+251G>C	V432L
CYP19A1	Cytochrome P450, family 19, subfamily A, polypeptide 1	15q21.1	rs1065778	IVS4-76A>G	
			rs700518	Ex4-57A>G	V80V
			rs2304463	IVS7-106T>G	
			rs700519	Ex8+47C>T	R264C
			rs1065779	IVS9-53G>T	
			rs4646	Ex11+410G>T	
HSD3B2	Hydroxy-delta-5-steroid dehydrogenase, 3-beta-(steroid delta-isomerase 2)	1p13.1	rs1819698	Ex4-133C>T	
			rs1361530	Ex4-88C>G	
HSD17B3	Hydroxysteroid (17-beta) dehydrogenase 3	9q22	rs2066479	Ex11+43G>A	G289S
HSD17B1	Hydroxysteroid (17-beta) dehydrogenase 1	17q11–q21	rs2830	Ex1-486G>A	
SHBG	Sex hormone-binding globulin	17p13–p12	rs6259	Ex8+6G>A	D356N
SRD5A2	Steroid-5-alpha-reductase, alpha polypeptide 2	2p23	rs523349	Ex1-17G>C	V89L

Table II. Selected characteristics of biliary tract cancer cases, biliary stone cases and controls

	Controls			Biliary tract cancers			Biliary stones[a]
	All	With gallbladder	Without biliary stones	Gallbladder[b]	Bile duct[c]	Ampulla of Vater[c]	
	n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
All subjects	786 (100.0)	737 (100.0)	592 (100.0)	237 (100.0)	127 (100.0)	47 (100.0)	895 (100.0)
Sex							
Male	305 (38.8)	290 (39.3)	252 (42.6)	65 (27.4)***	76 (59.8)***	24 (51.1)	329 (36.8)*
Female	481 (61.2)	447 (60.7)	340 (57.4)	172 (72.6)***	51 (40.2)***	23 (48.9)	566 (63.2)*
Age (years)							
34–54	107 (13.6)	109 (14.4)	97 (16.4)	32 (13.5)	18 (14.2)	4 (8.5)	269 (30.0)***
55–59	224 (28.5)	216 (29.3)	169 (28.5)	62 (26.2)	32 (25.2)	9 (19.1)	252 (28.2)***
60–64	239 (30.4)	220 (29.8)	177 (29.9)	68 (28.7)	50 (39.4)	17 (36.2)	212 (23.7)***
65–75	216 (27.5)	195 (26.5)	149 (25.2)	75 (31.6)	27 (21.2)	17 (36.2)	162 (18.1)***
BMI[d] (Kg/m^2)							
<23	392 (49.9)	379 (51.4)	324 (54.7)	91 (38.4)***	63 (49.6)	21 (44.7)	344 (38.4)***
23–25	229 (29.1)	214 (29.0)	169 (28.6)	73 (30.8)***	45 (35.4)	17 (36.2)	325 (36.3)***
26–29	133 (16.9)	114 (15.5)	78 (13.2)	63 (26.6)***	16 (12.6)	9 (19.2)	193 (21.6)***
>29	32 (4.1)	30 (4.1)	21 (3.6)	10 (4.2) ***	3 (2.4)	0 (0.0)	33 (3.7) ***
Cigarette smoking							
Never	549 (69.8)	514 (69.7)	405 (68.4)	172 (72.9)	71 (55.9)**	27 (57.4)	654 (73.1)
Ex-smokers	65 (8.3)	61 (8.3)	49 (8.3)	31 (13.1)	28 (22.1)**	12 (25.5)	79 (8.8)
Current	172 (21.9)	162 (22.0)	138 (23.3)	33 (14.0)	28 (22.1)**	8 (17.0)	162 (18.1)
Alcohol use							
Never	624 (79.4)	586 (79.5)	458 (77.4)	201 (84.8)	85 (66.9)**	35 (74.5)	752 (84.1)**
Former drinkers	27 (3.4)	22 (3.0)	18 (3.0)	17 (7.2)	20 (15.8)**	5 (10.6)	50 (5.6)**
Current drinkers	13 (17.2)	129 (17.5)	116 (19.6)	19 (8.0)	22 (17.3) **	7 (14.9)	92 (10.3) **
Gallstones							
No	592 (80.3)	592 (80.3)	592 (100.0)	36 (15.2)***	41 (32.3)***	19 (40.4)***	—
Yes	194 (24.7)	145 (19.7)	—	201 (84.8)***	86 (67.7)***	28 (59.6)***	895 (100)
Female subjects	481 (100.0)	447 (100.0)	592 (100.0)	172 (100.0)	51 (100.0)	23 (100.0)	895 (100.0)
Age at menarche							
<13	91 (19.0)	87 (19.5)	58 (17.1)	24 (14.2)	10 (19.6)	0 (0.0)	118 (20.9)
13–15	168 (35.0)	158 (35.4)	120 (35.4)	59 (34.9)	22 (43.1)	9 (40.9)	219 (38.8)
>15	221 (46.0)	201 (45.1)	191 (47.5)	86 (50.9)	19 (37.3)	13 (59.1)	228 (40.3)
Menopausal status							
No	35 (7.3)	35 (7.8)	31 (9.1)	16 (9.3)	1 (2.0)	0 (0.0)	117 (20.7)***
Yes	446 (92.7)	412 (92.2)	309 (90.9)	156 (90.7)	50 (98.0)	23 (100.0)	449 (79.3)***
Age at menopause							
<48	124 (27.9)	113 (27.5)	87 (28.2)	45 (29.0)	12 (24.5)	9 (40.9)	149 (33.4)
48–50	161 (36.2)	146 (35.5)	101 (32.8)	56 (36.1)	16 (32.6)	9 (40.9)	140 (31.2)
>50	160 (35.9)	152 (37.0)	120 (39.0)	54 (34.9)	21 (42.9)	4 (18.2)	158 (35.4)
Parity							
0	16 (3.3)	14 (3.1)	9 (2.6)	4 (2.3)*	2 (3.9)	0 (0.0)	25 (4.4)**
1–2	174 (36.2)	167 (37.4)	137 (40.3)	47 (27.3)*	18 (35.3)	5 (21.7)	288 (50.9)**
3–5	232 (48.2)	214 (47.9)	150 (44.1)	97 (56.4)*	24 (47.1)	14 (60.9)	204 (36.0)**
>5	59 (12.3)	52 (11.6)	44 (12.9)	24 (14.0)	7 (13.7)	4 (17.4)	49 (8.7) **
Use of oral contraceptives							
No	400 (83.5)	372 (83.6)	281 (82.7)	150 (87.7)	42 (82.4)	20 (87.0)	465 (82.2)
Yes	79 (16.5)	73 (16.4)	59 (17.3)	21 (12.3)	9 (17.6)	3 (13.0)	101 (17.8)

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
[a]Biliary stone cases include gallstone and bile duct stone cases, compared with population controls who did not have biliary stones ($n = 592$).
[b]Gallbladder cancer cases compared with population controls who did not have a cholecystectomy ($n = 737$).
[c]Bile duct and ampulla of Vater cancer cases compared with all population controls ($n = 786$).
[d]BMI 5 years prior to interview. Distribution based on World Health Organization classification of obesity among Asians.

genotype frequencies of each marker showed no deviation from Hardy–Weinberg equilibrium ($P > 0.05$). Of the 18 SNPs examined, two markers of *CYP1A1*, IVS1+606 (rs2606345) and EX7+131 (rs1048943), and one marker of *CYP19A1*, IVS7-106 (rs2304463), were associated with biliary tract cancer. Also, one marker, *COMT* Ex4-76 (rs4818), was associated with biliary stones. As shown in the table, carriers of the T allele (versus the GG genotype) of the *CYP1A1* IVS1+606 marker had a 2-fold risk of gallbladder cancer (95% CI 1.3–3.1) and a 1.8-fold risk of bile duct cancer (95% CI 1.1–3.2). Carriers of the G allele (versus the AA genotype) of the *CYP1A1* Ex7 + 131 marker had an excess risk of ampulla of Vater cancer (OR = 2.9, 95% CI 1.5–5.4). In contrast, carriers of the T allele (versus the GG genotype) of the *CYP19A1* IVS7-106 marker had a reduced risk of bile duct cancer (OR = 0.7, 95% CI 0.5–0.99). After adjustment for gallstone status and other potential confounding factors, the magnitude of the risk estimates was slightly attenuated, yet the associations remained statistically significant (data not shown). Biliary stone risk was associated with the *COMT* Ex4-76 marker, with carriers of the G allele (versus CC genotype) having a small excess risk (OR = 1.3, 95% CI 1.0–1.6; *P*-trend = 0.10).

Using the Simes test to adjust for multiple SNP comparisons within each gene, we found statistically significant associations for *CYP1A1* with gallbladder cancer (*P*-Simes = 0.004) and ampulla of Vater cancer (*P*-Simes = 0.01) and a borderline statistically significant

Table III. ORs and 95% CIs for biliary tract cancers and stones in relation to polymorphisms of hormone biosynthesis, metabolism and transport genes

Genotype	All controls	Biliary tract cancer						Biliary stones[a]	
		Gallbladder[b]		Bile duct[c]		Ampulla of Vater[c]			
	n (%)	*n* (%)	OR (95% CI)[d]	*n* (%)	OR (95% CI)[d]	*n* (%)	OR (95% CI)[d]	*n* (%)	OR (95% CI)[d]
CYP1A1									
IVS1+606G>T (rs2606345)									
GG	705 (90.4)	196 (83.4)	1.0	105 (84.0)	1.0	43 (91.5)	1.0	784 (88.7)	1.0
GT	74 (9.5)	37 (15.7)	**2.0 (1.3–3.1)****	20 (16.0)	**1.8 (1.1–3.2)***	4 (8.5)	0.9 (0.3–2.6)	95 (10.8)	1.1 (0.8–1.5)
TT	1 (0.1)	2 (0.9)	—	0 (0.0)	—	0 (0.0)	—	5 (0.6)	—
P-trend			—		—		—		—
GT + TT	75 (9.6)	39 (16.6)	**2.0 (1.3–3.0)****	20 (16.0)	**1.8 (1.1–3.1)***	4 (8.5)	0.9 (0.3–2.6)	90 (11.3)	1.2 (0.8–1.6)
Ex7+131A>G (rs1048943)									
AA	461 (59.6)	144 (61.3)	1.0	78 (62.4)	1.0	16 (34.8)	1.0	520 (59.5)	1.0
AG	274 (35.5)	81 (34.5)	0.9 (0.7–1.2)	40 (3.2)	0.9 (0.6–1.4)	28 (60.9)	**3.1 (1.6–5.8)****	310 (35.5)	1.0 (0.8–1.2)
GG	38 (4.9)	10 (4.3)	0.8 (0.4–1.7)	7 (5.6)	1.1 (0.5–2.6)	2 (4.3)	—	44 (5.0)	0.9 (0.6–1.5)
P-trend			*0.44*		*0.85*		—		*0.73*
AG + GG	312 (40.4)	91 (38.7)	0.9 (0.7–1.2)	47 (37.6)	0.9 (0.6–1.4)	30 (65.2)	**2.9 (1.5–5.4)****	354 (40.5)	1.0 (0.8–1.2)
P-Simes CYP1A1			***0.004***		*0.06*		***0.01***		*0.54*
CYP19A1									
Ex4-57A>G (rs700518)									
AA	229 (29.3)	74 (31.2)	1.0	35 (27.6)	1.0	17 (36.2)	1.0	269 (30.2)	1.0
AG	395 (59.5)	113 (47.7)	0.9 (0.6–1.3)	58 (45.7)	1.0 (0.6–1.5)	21 (44.7)	0.8 (0.4–1.4)	465 (52.3)	1.0 (0.8–1.3)
GG	158 (20.2)	50 (21.1)	1.0 (0.7–1.5)	34 (26.7)	1.5 (0.9–2.5)	9 (19.1)	0.8 (0.3–1.8)	156 (17.5)	0.8 (0.6–1.1)
P-trend			*0.86*		*0.18*		*0.44*		*0.30*
AG + GG	553 (70.7)	163 (68.8)	0.9 (0.7–1.3)	92 (72.4)	1.1 (0.7–1.7)	30 (63.8)	0.7 (0.4–1.4)	621 (69.8)	1.0 (0.8–1.2)
IVS4-76A>G (rs1065778)									
AA	224 (29.2)	73 (31.5)	1.0	34 (27.2)	1.0	15 (33.3)	1.0	263 (30.3)	1.0
AG	388 (50.5)	110 (47.4)	0.9 (0.6–1.2)	58 (46.4)	1.0 (0.6–1.6)	21 (46.7)	0.8 (0.4–1.7)	451 (52.0)	1.0 (0.8–1.3)
GG	156 (20.3)	49 (21.1)	1.0 (0.6–1.5)	33 (26.4)	1.4 (0.9–2.5)	9 (20.0)	0.9 (0.4–2.0)	153 (17.7)	0.8 (0.6–1.1)
P-trend			*0.82*		*0.20*		*0.67*		*0.26*
AG + GG	544 (70.8)	159 (68.5)	0.9 (0.7–1.3)	91 (72.8)	1.1 (0.7–1.7)	30 (66.7)	0.8 (0.4–1.6)	605 (69.7)	1.0 (0.8–1.2)
IVS7-106G>T (rs2304463)									
GG	237 (30.4)	82 (34.6)	1.0	49 (38.9)	1.0	14 (29.8)	1.0	258 (29.3)	1.0
GT	390 (49.9)	106 (44.7)	0.8 (0.6–1.1)	57 (45.2)	0.7 (0.5–1.1)	24 (51.1)	1.0 (0.5–2.1)	447 (50.7)	1.1 (0.9–1.3)
TT	154 (19.7)	49 (20.7)	0.9 (0.6–1.4)	20 (15.9)	0.6 (0.4–1.1)	9 (19.1)	1.0 (0.4–2.3)	176 (20.0)	1.1 (0.8–1.4)
P-trend			*0.47*		*0.06*		*0.98*		*0.64*
GT + TT	544 (69.6)	155 (65.4)	0.8 (0.6–1.1)	77 (61.1)	**0.7 (0.5–0.99)***	33 (70.2)	1.0 (0.5–2.0)	623 (70.7)	1.1 (0.9–1.3)
Ex8+47C>T (rs700519)									
CC	559 (72.0)	173 (74.6)	1.0	96 (76.8)	1.0	33 (71.7)	1.0	654 (74.6)	1.0
CT	196 (25.3)	51 (22.0)	0.8 (0.6–1.2)	26 (20.8)	0.7 (0.5–1.2)	12 (26.1)	1.0 (0.5–2.0)	206 (23.5)	0.9 (0.7–1.1)
TT	21 (2.7)	8 (3.4)	1.1 (0.5–2.6)	3 (2.4)	—	1 (2.2)	—	17 (1.9)	0.7 (0.4–1.4)
P-trend			*0.55*		—		—		*0.16*
CT + TT	217 (28.0)	59 (25.4)	0.9 (0.6–1.2)	29 (23.2)	0.7 (0.5–1.2)	13 (28.3)	1.0 (0.5–2.0)	223 (25.4)	0.9 (0.7–1.1)
IVS9-53G>T (rs1065779)									
GG	224 (28.6)	70 (29.5)	1.0	44 (34.7)	1.0	12 (25.5)	1.0	253 (27.3)	1.0
GT	392 (50.0)	115 (48.5)	0.9 (0.7–1.3)	60 (47.2)	0.8 (0.5–1.2)	25 (53.2)	1.2 (0.6–2.4)	449 (50.5)	1.1 (0.9–1.4)
TT	168 (21.4)	52 (22.0)	1.0 (0.6–1.5)	23 (18.1)	0.7 (0.4–1.2)	10 (21.3)	1.1 (0.5–2.6)	197 (22.2)	1.1 (0.8–1.5)
P-trend			*0.85*		*0.14*		*0.81*		*0.45*
GT + TT	560 (71.4)	167 (70.5)	0.9 (0.7–1.3)	83 (63.3)	0.7 (0.5–1.1)	35 (74.5)	1.2 (0.6–2.3)	646 (72.7)	1.1 (0.9–1.4)
Ex11+410G>T (rs4646)									
GG	394 (50.5)	122 (51.9)	1.0	66 (53.7)	1.0	23 (48.9)	1.0	411 (46.6)	1.0
GT	314 (40.8)	93 (39.6)	0.9 (0.7–1.3)	51 (41.4)	0.9 (0.6–1.4)	22 (46.8)	1.2 (0.6–1.3)	389 (44.1)	1.2 (1.0–1.5)
TT	68 (8.7)	20 (8.5)	1.0 (0.6–1.7)	6 (4.9)	0.6 (0.2–1.3)	2 (4.3)	—	82 (9.3)	1.2 (0.8–1.7)
P-trend			*0.78*		*0.28*		—		*0.12*
GT + TT	544 (70.8)	159 (68.5)	1.0 (0.7–1.3)	91 (72.8)	0.9 (0.6–1.3)	30 (66.7)	1.1 (0.6–1.9)	471 (53.4)	1.2 (1.0–1.5)
P-Simes CYP19A1			*0.86*		*0.26*		*0.98*		*0.45*
COMT									
Ex3-104C>T (rs4633)									
CC	414 (53.5)	132 (56.9)	1.0	72 (57.6)	1.0	23 (50.0)	1.0	482 (55.3)	1.0
CT	302 (39.1)	83 (35.8)	0.9 (0.7–1.3)	43 (34.4)	0.8 (0.5–1.2)	16 (34.8)	0.9 (0.5–1.8)	327 (37.6)	0.9 (0.7–1.1)
TT	57 (7.4)	17 (7.3)	0.9 (0.5–1.7)	10 (8.0)	0.9 (0.5–2.0)	7 (15.2)	2.2 (0.9–5.3)§	62 (7.1)	1.0 (0.6–1.4)

Table III. *Continued*

Genotype	All controls	Biliary tract cancer						Biliary stones[a]	
		Gallbladder[b]		Bile duct[c]		Ampulla of Vater[c]			
	n (%)	*n* (%)	OR (95% CI)[d]	*n* (%)	OR (95% CI)[d]	*n* (%)	OR (95% CI)[d]	*n* (%)	OR (95% CI)[d]
P-trend			*0.62*		*0.39*		*0.27*		*0.27*
CT + TT	359 (46.4)	100 (42.1)	0.9 (0.7–1.2)	53 (42.4)	0.8 (0.5–1.2)	23 (50.0)	1.1 (0.6–2.1)	399 (44.7)	0.9 (0.8–1.1)
Ex4-76C>G (rs4818)									
CC	369 (47.3)	98 (41.7)	1.00	50 (40.6)	1.0	19 (40.4)	1.0	354 (40.2)	1.0
CG	315 (40.3)	105 (44.7)	1.2 (0.9–1.7)	58 (47.2)	1.4 (0.9–2.1)	23 (49.0)	1.5 (0.8–2.8)	411 (46.6)	**1.3 (1.1–1.7)***
GG	97 (12.4)	32 (13.6)	1.2 (0.7–1.9)	15 (12.2)	1.3 (0.7–2.4)	5 (10.6)	—	116 (13.2)	1.2 (0.8–1.6)
P-trend			*0.28*		*0.20*		—		*0.10*
CG + GG	412 (52.7)	137 (58.3)	1.2 (0.9–1.7)	73 (59.4)	1.4 (0.9–2.1)	28 (59.6)	1.4 (0.8–2.6)	527 (59.8)	**1.3 (1.0–1.6)***
P-Simes COMT			*0.56*		*0.40*		*0.54*		*0.20*
HSD17B3									
Ex11+43G>A (rs2066479)[e]									
GG	438 (56.0)	135 (57.2)	1.0	71 (56.8)	1.0	29 (61.7)	1.0	534 (60.1)	1.0
AG	282 (36.1)	90 (38.1)	1.0 (0.7–1.4)	48 (38.4)	1.1 (0.7–1.6)	16 (34.0)	0.9 (0.5–1.6)	317 (35.7)	0.9 (0.7–1.5)
AA	62 (7.9)	11 (5.7)	0.6 (0.3–1.1)	6 (4.8)	0.6 (0.2–1.4)	2 (4.3)	—	37 (4.2)	0.5 (0.7–1.3)
P-trend			*0.11*		*0.21*		—		*0.94*
AG + AA	344 (44.0)	101 (42.8)	0.9 (0.7–1.3)	54 (43.2)	1.0 (0.7–1.4)	18 (38.3)	0.8 (0.4–1.4)	354 (39.9)	1.1 (0.9–1.4)
HSD17B1									
Ex1-486G>A (rs2830)									
GG	249 (33.0)	71 (30.7)	1.0	38 (30.6)	1.0	17 (37.8)	1.0	277 (32.1)	1.0
GA	362 (48.0)	116 (50.2)	1.1 (0.8–1.6)	61 (49.2)	1.1 (0.7–1.7)	16 (35.5)	0.7 (0.3–1.3)	432 (50.0)	1.1 (0.9–1.4)
AA	143 (19.0)	44 (19.1)	1.0 (0.7–1.6)	25 (20.2)	1.2 (0.7–2.0)	12 (26.7)	1.2 (0.6–2.7)	155 (17.9)	1.0 (0.7–1.3)
P-trend			*0.81*		*0.56*		*0.79*		*0.98*
GA + AA	344 (44.0)	101 (42.8)	1.1 (0.8–1.5)	54 (43.2)	1.1 (0.8–1.7)	18 (38.3)	0.8 (0.4–1.5)	587 (67.9)	1.1 (0.9–1.3)
HSD3B2									
Ex4-133C>T (rs1819698)									
CC	439 (55.9)	133 (56.1)	1.0	82 (63.0)	1.0	28 (59.6)	1.0	492 (55.6)	1.0
CT	303 (38.6)	85 (35.9)	0.9 (0.7–1.2)	42 (33.1)	0.8 (0.5–1.2)	16 (34.0)	0.8 (0.5–1.6)	337 (38.1)	1.0 (0.8–1.2)
TT	43 (5.5)	19 (8.0)	1.4 (0.8–2.5)	5 (3.9)	—	3 (6.4)	—	56 (6.3)	1.1 (0.7–1.7)
P-trend			*0.74*		—		—		*0.96*
CT + TT	346 (44.1)	104 (43.9)	1.0 (0.8–1.4)	47 (37.0)	0.8 (0.5–1.1)	19 (40.4)	0.9 (0.5–1.6)	393 (44.4)	1.0 (0.8–1.2)
Ex4-88C>G (rs1361530)									
CC	436 (57.3)	133 (57.1)	1.0	74 (61.2)	1.0	26 (57.8)	1.0	482 (56.1)	1.0
CG	286 (37.6)	82 (35.2)	0.9 (0.7–1.3)	42 (34.7)	0.9 (0.6–1.3)	17 (37.8)	1.0 (0.5–1.9)	322 (37.9)	1.0 (0.8–1.2)
GG	39 (5.1)	18 (7.7)	1.5 (0.8–2.7)	5 (4.1)	—	2 (4.4)	—	55 (6.0)	1.3 (0.8–1.9)
P-trend			*0.94*		—		—		*0.60*
CG + GG	325 (42.7)	100 (42.9)	1.0 (0.7–1.3)	47 (38.8)	0.9 (0.6–1.3)	19 (42.2)	1.0 (0.5–1.9)	377 (43.9)	1.0 (0.8–1.2)
P-Simes HSD3B2			*0.94*		*0.32*		*0.99*		*0.96*
CYP1B1									
Ex2+143C>G (rs10012)									
CC	498 (66.3)	155 (69.9)	1.0	76 (64.4)	1.0	31 (70.5)	1.0	539 (65.6)	1.0
CG	218 (29.0)	61 (27.1)	0.9 (0.6–1.3)	35 (29.7)	1.0 (0.6–1.5)	11 (25.0)	0.8 (0.4–1.6)	261 (30.8)	1.1 (0.9–1.4)
GG	35 (4.7)	9 (4.0)	0.9 (0.4–1.9)	7 (5.9)	1.2 (0.5–2.9)	2 (4.5)	—	48 (5.6)	1.2 (0.8–1.9)
P-trend			*0.53*		*0.72*		—		*0.30*
CG + GG	253 (33.7)	70 (31.1)	0.9 (0.6–1.2)	42 (35.6)	1.0 (0.7–1.6)	13 (29.5)	0.8 (0.4–1.6)	309 (36.4)	1.1 (0.9–1.4)
Ex3+251C>G (rs1056836)									
CC	597 (76.6)	183 (78.2)	1.0	92 (73.6)	1.0	36 (78.2)	1.0	688 (77.8)	1.0
CG	172 (22.1)	46 (19.7)	0.8 (0.6–1.2)	30 (24.0)	1.2 (0.7–1.8)	9 (19.6)	0.9 (0.4–1.9)	188 (21.3)	1.0 (0.8–1.2)
GG	10 (1.3)	5 (2.1)	—	3 (2.4)	—	1 (2.2)	—	8 (0.9)	0.8 (0.3–2.1)
P-trend			—		—		—		*0.60*
CG + CG	182 (23.4)	51 (21.8)	0.9 (0.6–1.3)	33 (26.4)	1.2 (0.8–1.9)	10 (21.7)	0.9 (0.4–1.9)	196 (22.2)	1.0 (0.8–1.2)
P-Simes CYP1B1			*0.66*		*0.54*		*0.94*		*0.60*
SRD5A2									
Ex1-17G>C (rs523349)									
CC	232 (29.8)	76 (32.3)	1.0	71 (56.8)	1.0	29 (61.7)	1.0	254 (28.9)	1.0
CG	402 (51.6)	111 (47.2)	0.8 (0.5–1.2)	48 (38.4)	0.8 (0.5–1.2)	16 (34.0)	1.0 (0.5–2.0)	435 (49.4)	1.0 (0.8–1.2)
GG	144 (18.5)	48 (20.4)	1.0 (0.7–1.5)	6 (4.8)	1.2 (0.7–2.0)	2 (4.3)	—	191 (21.7)	1.2 (0.9–1.7)
P-trend			*0.86*		*0.68*		—		*0.76*
GC + CC	344 (44.0)	101 (42.8)	0.9 (0.6–1.3)	54 (43.2)	0.9 (0.6–1.3)	18 (38.3)	1.2 (0.6–2.3)	689 (78.3)	1.1 (0.9–1.3)

Table III. *Continued*

Genotype	All controls	Biliary tract cancer						Biliary stones[a]	
		Gallbladder[b]		Bile duct[c]		Ampulla of Vater[c]			
	n (%)	*n* (%)	OR (95% CI)[d]	*n* (%)	OR (95% CI)[d]	*n* (%)	OR (95% CI)[d]	*n* (%)	OR (95% CI)[d]
SHBG									
Ex8+6G>A (rs6259)									
GG	557 (71.7)	163 (69.1)	1.0	92 (72.4)	1.0	30 (65.2)	1.0	599 (66.8)	1.0
GA	203 (26.1)	64 (27.1)	1.1 (0.8–1.5)	32 (25.2)	1.0 (0.6–1.5)	15 (32.6)	1.4 (0.7–2.7)	265 (30.5)	0.8 (0.6–1.1)
AA	17 (2.2)	9 (3.8)	1.9 (0.8–4.5)	3 (2.4)	—	1 (2.2)	—	25 (2.7)	0.8 (0.6–1.1)
P-trend			*0.13*		—		—		*0.28*
GA + AA	220 (28.3)	73 (30.9)	1.1 (0.8–1.5)	35 (27.6)	1.0 (0.6–1.5)	16 (34.8)	1.4 (0.7–2.6)	290 (33.2)	0.8 (0.6–1.1)

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.
[a]Biliary stone cases include gallstone and bile duct stone cases, compared with population controls who did not have biliary stones ($n = 592$).
[b]Gallbladder cancer cases compared with population controls who did not have a cholecystectomy ($n = 737$).
[c]Bile duct and ampulla of Vater cancer cases compared with all population controls ($n = 786$).
[d]Adjusted for age and sex.
[e]In the recessive model, *HSD17B3* Ex11+43 was associated with biliary stones (OR = 0.6, 95% CI 0.4–0.9), especially gallstones (OR = 0.6, 95% CI 0.4–0.9), but not bile duct stone (OR = 0.5, 95% CI 0.2–1.1).

association with bile duct cancer (*P*-Simes = 0.06) (Table III). The Simes test was not statistically significant for any of the other genes examined. To consider multiple comparisons more stringently, we examined the associations for the significant variants using a Bonferonni correction and found that only the association between *CYP1A1* IVS1+606 and gallbladder cancer remained statistically significant (*P*-Bonferroni = 0.03).

Table IV shows the risk of biliary tract cancers and stones in relation to the *CYP1A1* IVS1+606 and Ex7+131 markers stratifying by BMI and biliary stone status. We found a statistically significant interaction between BMI and the *CYP1A1* IVS1+606 marker on gallbladder cancer risk (*P*-interaction = 0.03), with non-obese (BMI < 23) carriers of the T allele having a 3.3-fold risk (95% CI = 1.8–6.1) and obese subjects (BMI ≥ 23) not having a statistically significant excess risk (OR = 1.3, 95% CI 0.7–2.4). After adjustment for gallstone status and other potential confounders, the magnitude of the risk estimates was slightly attenuated, yet the associations remained (data not shown). We did not observe statistically significant interactions for BMI or stone status with any of the other markers. Also, we did not observe statistically significant interactions between any of the genetic markers and gender, cigarette smoking, alcohol use and the other risk factors among total subjects based on the statistical interaction tests using the likelihood ratio test (data not shown).

Table V shows the risk of biliary tract cancers and stones in relation to the *SHBG* Ex8+6 marker among women, stratifying by OC use. Female carriers of the GG genotype of the *SHBG* Ex8+6 marker had a 3.2-fold risk of gallbladder cancer (95% CI = 1.1–9.1) compared with carriers of the AA genotype, although there was no statistically significant interaction between the *SHBG* Ex8+6 marker and sex. Among women, a statistically significant interaction between oral contraceptive use and the *SHBG* Ex8+6 marker was found for gallbladder cancer (*P*-interaction = 0.001) and biliary stone risk (*P*-interaction = 0.05). Carriers of the G allele (versus the AA genotype) of the *SHBG* Ex8+6 marker who had ever used oral contraceptives had excess risks of gallbladder cancer (OR = 6.7, 95% CI = 2.2–20.5) and biliary stones (OR = 2.3, 95% CI = 1.1–4.9), whereas those who never used oral contraceptives did not have excess risk of either disease. We did not see statistically significant interactions between parity and the *SHBG* marker (data not shown). Also, we did not observe significant statistical interactions between any other female reproductive factors and any genetic markers among female subjects based on the statistical interaction tests using the likelihood ratio test (data not shown).

Based on the six SNPs examined in the *CYP19A1* gene (Ex4-57A>G (rs700518), IVS4-76A>G (rs1065778), IVS7-106T>G (rs2304463), Ex8 + 47C>T (rs700519), IVS9-53A>G>T (rs1065779) and Ex11 + 410G>T (rs4646), we inferred four major haplotypes, A-A-G-C-T-T, G-G-G-C-T-T, G-G-T-C-G-G and G-G-T-T-G-T, with corresponding frequencies of 44.0, 9.2, 27.6 and 14.8% among all control subjects. No associations for any of these haplotypes were found relative to the most common haplotype for each disease outcome (data not shown).

Discussion

In this population-based study in China, common variants in genes involved in steroid hormone biosynthesis, metabolism and transport were associated with biliary tract cancers and biliary stones. Two *CYP1A1* markers (IVS1+606 and Ex7+131) and one *CYP19A1* marker (IVS7-106) were associated with biliary tract cancers. The effect of *CYP1A1* IVS1 + 606 on gallbladder cancer risk was more pronounced among non-obese subjects, whereas the effects of *SHBG* Ex8 + 6 on gallbladder cancer and gallstone risks were limited to women who used oral contraceptives. These results, although modest in effect size, support the hypothesis that variants in hormone-related genes could play a role in the etiology of biliary tract cancer and stones.

In our study, two markers in the *CYP1A1* gene conferred risk for biliary tract cancers, with the *CYP1A1* IVS1+606 marker linked to gallbladder and bile duct cancers and the *CYP1A1* Ex7+131 marker associated the ampulla of Vater cancer. The *CYP1A1* Ex7+131 marker was also associated with gallbladder cancer in women in a small study in Japan (32 cases and 64 controls) (26), although in our study this marker was linked to ampullary cancer only. The effects of *CYP1A1* IVS1+606 and Ex7+131 are independent of each other, although there is linkage disequilibrium ($D' = 0.83$) between the markers. Although our results took multiple SNP comparisons into account, we cannot rule out that the observed *CYP1A1* effect could have been due to other causative SNPs that were not examined, but are in linkage disequilibrium with the two markers examined in the study. The mechanisms by which *CYP1A1* variants may contribute to the risk of biliary tract cancer are unclear. The *CYP1A1* gene encodes the CYP1A1 enzyme, which catalyzes estradiol to 2-hydroxy-estradiol (2-OH-E_2) (27). Variants of the *CYP1A1* gene has been shown to affect the ratio of 2-OH-E_2 to 16-hydroxy-estradiols (28), resulting in higher estrogenic activity. Specifically, the *CYP1*A1 Ex7+131A>G (I462V) variant allele has been associated with increased

Table IV. ORs and 95% CIs for biliary tract diseases in relation to *CYP1A1* polymorphisms by BMI and biliary stone status

	Controls	Biliary tract cancers						Biliary stones[a]	
		Gallbladder[b]		Bile duct[c]		Ampulla of Vater[c]			
	n	*n*	OR (95% CI)[d]	*n*	OR (95% CI)[d]	*n*	OR (95% CI)[d]	*n*	OR (95% CI)[d]
BMI									
CYP1A1 IVS1 + 606T>G (rs2606345)									
BMI <23									
GG	350	68	1.0	54	1.0	20	1.0	295	1.0
GT + TT	36	22	**3.3 (1.8–6.1)****	9	1.6 (0.7–3.4)		—	42	1.4 (0.8–2.4)
BMI ≥23									
GG	354	127	1.0	51	1.0	23	1.0	488	1.0
GT + TT	39	17	1.3 (0.7–2.4)	11	**2.2 (1.0–4.7)***	3	1.2 (0.3–4.4)	58	1.1 (0.7–1.9)
P-interaction			***0.03***		*0.55*		*0.51*		*0.54*
CYP1A1 Ex7 + 131A>G (rs1048943)									
BMI <23									
AA	228	61	1.0	41	1.0	7	1.0	197	1.0
AG + GG	153	29	0.7 (0.4–1.1)	22	0.8 (0.5–1.5)	14	**3.3 (1.3–8.4)***	135	1.0 (0.7–1.4)
BMI ≥23									
AA	233	83	1.0	37	1.0	9	1.0	323	1.0
AG + GG	158	61	1.0 (0.7–1.6)	25	1.0 (0.6–1.8)	16	**2.5 (1.1–6.0)***	218	1.1 (0.8–1.4)
P-interaction			*0.11*		*0.62*		*0.73*		*0.94*
Biliary stones									
CYP1A1 IVS1 + 606T>G (rs2606345)									
No stones									
GG	534	31	1.0	33	1.0	17	1.0	—	—
GT + TT	54	5	1.5 (0.6–4.1)	7	2.2 (0.9–5.4)	2	—		
Stones									
GG	171	165	1.0	53	1.0	26	1.0	—	—
GT + TT	21	34	1.8 (0.9–3.6)	32	1.3 (0.6–3.1)	2	—		
P-interaction			*0.80*		*0.48*		*0.48*	—	—
CYP1A1 Ex7 + 131A>G (rs1048943)									
No stones									
AA	357	22	1.0	25	1.0	6	1.0	—	—
AG + GG	225	14	1.0 (0.5–2.0)	15	1.0 (0.5–2.0)	12	**3.3 (1.2–9.1)***		
Stones									
AA	73	122	1.0	53	1.0	10	1.0	—	—
AG + GG	69	77	0.7 (0.4–1.1)	32	0.7 (0.4–1.3)	18	2.2 (0.9–5.0)		
P-interaction			*0.35*		*0.47*		*0.52*	—	—

*$P < 0.05$, **$P < 0.01$.
[a]Biliary stone cases include gallstone and bile duct stone cases, compared with population controls who did not have biliary stones ($n = 592$).
[b]Gallbladder cancer cases compared with population controls who did not have a cholecystectomy ($n = 737$).
[c]Bile duct and ampulla of Vater cancer cases compared with all population controls ($n = 786$).
[d]Adjusted for age and sex.

CYP1A1 enzyme activity (28,29), possibly leading to an increased conversion of estradiol to 2-OH-E_2, which has been detected in many tissues, including biliary epithelium (29). Increased levels of estrogen have been implicated in biliary tract cancers by causing decreased gallbladder motility, thereby increasing the formation of gallstones and the risk of infection and inflammation in the biliary tract (30,31). In addition to these hormonal effects, the *CYP1A1* enzyme has been shown to be involved in metabolizing polycyclic aromatic hydrocarbons to carcinogenic intermediates (32). It is unclear whether this mechanism is part of the uncommon biliary carcinogenesis that may not involve gallstones as an intermediate step since in our study, the association between *CYP1A1* and biliary tract cancers was independent of gallstone status, suggesting that mechanisms other than gallstones are involved in the effects of *CYP1A1* on biliary tract cancer risk. We also observed that the effect of *CYP1A1* IVS1+606 on gallbladder cancer risk was more pronounced among subjects with a BMI <23 kg/m^2. Reasons for this finding are unclear since obese subjects tend to have lower levels of sex hormone-binding globulin (SHBG) and thus higher levels of bioavailable estradiol and adipokines, which have been linked to gallbladder cancer (33,34). Future investigations should confirm these results and clarify the mechanisms involved.

We did not see main effects for either the *SHBG* Ex8+6 marker or OC use in our study; however, the *SHBG* Ex8+6 marker was associated with gallbladder cancer and biliary stones among women who used OC. Previous studies have reported increased risks of gallstones (35–40) and biliary tract cancers (8,41,42) among OC users, but have not examined a joint effect with *SHBG* variants. Women who used OCs tend to have higher serum SHBG levels, which have been associated with lower levels of bioavailable testosterone (43,44). The functional effect of the *SHBG* EX8 + 6 marker is unclear. Future research is needed to understand how these hormonal changes related to OC use influence gallbladder and biliary stone risk among carriers of the *SHBG* EX8+6 G allele.

The observation that the *CYP19A1* IVS7-106 T allele was associated with a reduced risk of bile duct cancer probably suggests a possible role of estrogen–androgen imbalance in biliary carcinogenesis. The CYP19 aromatase, which is encoded by the *CYP19A1* gene, converts testosterone to estradiol and androstenedione to estrone (45). Although gallstones and gallbladder cancer are more common in women and are closely linked to estrogen, we did not find an association between *CYP19A1* variants and these two conditions. Reasons for this are unclear, but may be related to the fact that gallstones and gallbladder cancer are more closely associated with estrogen-related lifestyle factors, including obesity and parity, making it difficult to detect a modest genetic effect.

The association between the C*OMT* Ex4-76 marker and biliary stones has not been reported previously. The *COMT* enzyme is

Table V. ORs and 95% CIs for gallbladder cancer and biliary stones in relation to SHBG Ex8 + 6A>G (rs6259) by oral contraceptive use, among female subjects

SHBG Ex8 + 6G>A (rs6259)	Controls	Gallbladder cancer[a]				Biliary stones[b]			
	n	*n*	OR[c]	95% CI[c]	*P* interaction	*n*	OR[c]	95% CI[c]	*P* interaction
All women									
GG	338	117	1.0	—		386	1.0	—	
GA	129	46	1.0	0.7–1.5		159	1.1	0.8–1.4	
AA	8	8	3.2	**1.1–9.1**		18	1.8	0.7–4.7	
AG + GG	137	54	1.1	0.8–1.7	*0.95*[d]	177	1.2	0.9–1.6	*0.65*[d]
OC use									
Never users									
GG	275	108	1.0	—		323	1.0	—	
GA + AA	119	41	0.9	0.6–1.3		139	1.0	0.7–1.4	
Ever users									
GG	62	8	1.0	—		63	1.0	—	
GA + AA	12	13	6.7	**2.2–20.5**	***0.001***[e]	38	2.3	**1.1–4.9**	***0.049***[e]

[a]Gallbladder cancer cases compared with population controls who did not have a cholecystectomy ($n = 737$).
[b]Biliary stone cases include gallstone and bile duct stone cases, compared with population controls who did not have biliary stones ($n = 592$).
[c]Adjusted for age.
[d]Interaction between SHBG Ex8 + 6G>A and gender among all subjects including males.
[e]Interaction between SHBG Ex8 + 6G>A and OC use.

responsible for the regulation of the level of catechol estrogens through the catalysis of *O*-methylation of catechol estrogens (2-OH-E_2 and 4-OH-E_2) to methoxy-catechol estrogens (46). Thus, the *COMT* enzyme may play a role in gallstone formation by modifying the responses of estrogens, but the exact function of the Ex4-76 variant needs to be further studied.

It is surprising that gender did not modify the observed associations between hormone gene variants and the risk of biliary tract cancers and stones, given the fact that both gallstones and gallbladder cancer are more common in women. Larger studies are needed to confirm the lack of any interactions.

Strengths of the study should be noted. This is the largest population-based study of biliary tract cancers to date. The population-based design, the nearly complete case ascertainment for cancer, a high participation rate and confirmation of case status by comprehensive pathologic and clinical review minimized the potential for selection, survival and misclassification bias. In addition, the inclusion of two case groups, namely the biliary tract cancer and biliary stone groups, offered the opportunity to assess whether risks associated with various exposures, including genetic susceptibility, are similar between these two closely related conditions.

Limitations of the study should also be mentioned. Gene coverage in our study was limited, since SNP selection was not based on complete sequencing data for our target population, nevertheless we were able to detect some positive signals. These findings are being incorporated in the next phase of the biliary study that will use a tag SNP approach to improve gene coverage. Despite being the largest population-based study of biliary tract cancer to date, the limited number of case subjects, especially with bile duct and ampullary cancers, precluded rigorous assessment of possible gene–environment interactions with sufficient statistical power. Lastly, generalizability of our results is limited due to the ethnic variations in genetic polymorphisms and lifestyle and anthropometric factors such as BMI between Chinese and Western populations. Since this study was conducted on a fairly homogenous Chinese population, the effect of population stratification was minimal.

In conclusion, in this population-based study, we showed that several variants in genes involved in steroid hormone biosynthesis, metabolism and transport are associated with the risk of biliary tract cancers and stones, providing support for the hypothesis that sex steroids, in particular estrogen, may play a role in biliary tract cancers and stones. Our subgroup analysis, specifically in women taking oral contraceptives, suggests a complex interplay between sex hormones and genetic susceptibility. Additional studies of biliary tract cancers and stones with a more comprehensive coverage of these genes are needed to confirm our results.

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