Polymorphisms of estrogen receptors and risk of biliary tract cancers and gallstones: a population-based study in Shanghai, China

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Biliary tract cancer encompasses tumors of the gallbladder, bile duct and ampulla of Vater. Gallbladder cancer is more common in women, whereas bile duct cancer is more common in men, suggesting that sex hormones may play a role in the etiology of these cancers. The intracellular action of estrogens is regulated by the estrogen receptor (ESR); thus, we examined the role of common genetic variants in *ESR* genes on the risk of biliary tract cancers and stones in a population-based case-control study in Shanghai, China (411 cancer cases, 895 stone cases and 786 controls). We genotyped six single-nucleotide polymorphisms (SNPs), four in *ESR1* (rs2234693, rs3841686, rs2228480 and rs1801132) and two in *ESR2* (rs1256049 and rs4986938). In all participants, the *ESR1* rs1801132 (P325P) G allele was associated with excess risks of bile duct [odds ratio (OR) = 1.7, 95% confidence interval (CI) 1.1–2.8] and ampulla of Vater cancers (OR = 2.1, 95% CI 0.9–4.9) compared with the CC genotype. The association with bile duct cancer was apparent among men (OR = 2.8, 95% CI 1.4–5.7) but not among women (*P*-heterogeneity = 0.01). Also, the *ESR2* rs4986938 (38 bp 3' of STP) GG genotype was associated with a higher risk of bile duct cancer (OR = 3.3, 95% CI 1.3–8.7) compared with the AA genotype, although this estimate was based on a small number of subjects. None of the other SNPs examined was associated with biliary tract cancers or stones. False discovery rate-adjusted *P*-values were not significant (*P* > 0.1). No association was found for *ESR1* haplotype based on four SNPs. These preliminary results suggest that variants in *ESR* genes could play a role in the etiology of biliary tract cancers, especially bile duct cancer in men.

Introduction

Biliary tract cancers are composed of cancers of the gallbladder, bile duct and ampulla of Vater. They are rare malignancies with a poor prognosis (1). The most important risk factor for biliary tract cancers, particularly of the gallbladder, is gallstones (1,2). Although gallstone disease is common, <1% of persons with gallstones develop biliary tract cancer, indicating that additional factors play an important role in the pathogenesis of this cancer (1,2).

Gallbladder cancer is more common in women, whereas bile duct cancer is more common in men, suggesting that sex hormones may play a role in etiology of biliary tract tumors (3–5). The higher rate of gallbladder cancer in women has been attributed to such hormonal factors as high parity, early age at first full-term birth, oral contraceptive use, hormone replacement therapy and obesity via increased estrogen levels (1). The reason for higher rate of bile duct cancer in men is less clear but could be linked to smoking, infection or estrogen-testosterone imbalance (1,6). One mechanism by which estrogen acts on target tissues is by binding to estrogen receptors (ESR), *ESR1* and *ESR2*, which have been detected in different tissues, including the biliary tract (5,7).

Given the potential hormonal role in biliary tract cancers, we hypothesize that genetic variants in *ESR1* and *ESR2* genes may have an impact on the risks of biliary tract cancers and/or stones through their effect on estrogenic activity. To clarify further the role of genetic susceptibility in relation to estrogen, as well as sex differences in biliary tract cancer subtypes, we examined the associations of four *ESR1* and two *ESR2* genetic variants with the risks of biliary tract cancers and stones in a population-based study conducted in Shanghai, China.

Materials and methods

Study population

Details of the methods of this population-based case-control study have been described previously (4,8–11). This study included 411 incident cases of biliary tract cancer (237 gallbladder, 127 bile duct and 47 ampulla of Vater), 895 biliary stone cases and 786 healthy controls randomly selected from the general population. With the use of a rapid reporting system at 42 collaborating hospitals in Shanghai, we captured >95% of the biliary tract cancer cases diagnosed in Shanghai between 1997 and 2001. Biliary tract cancer diagnosis was confirmed by pathology for 70% of the subjects and by medical records, surgical reports and imaging data for the remaining 30%. Biliary stone cases were selected from the same hospitals as cancer cases and were confirmed by abdominal ultrasound, medical and/or surgical records or pathologic material for those who underwent a cholecystectomy. Stone cases were frequency matched to cancer cases on age (5 year intervals), sex and hospital. Population controls with no history of cancer were randomly chosen from the Shanghai Resident Registry, which includes records of ~6 million Shanghai residents. Controls were frequency matched to cancer cases on gender and age (5 year intervals).

Data and specimen collection

In-person interviews were conducted with each participant at the time of enrollment by trained interviewers using a structured questionnaire to collect information on factors potentially related to biliary tract cancers and stones. Cases were interviewed within 3 weeks of diagnosis. The response rate for interviews was >95% among cases and 82% among controls. Interview concordance on responses to key questions administered twice to 5% of randomly selected subjects was >90%. Weight and height were measured at the time of interview. Biliary stone status was determined in nearly all cancer cases by self-reported history, surgical reports, imaging or ultrasound results and in controls using self-reported history or abdominal ultrasound (85% of controls).

Blood processing and genotyping

Overnight fasting blood samples were collected from >80% of the participants who gave consent for blood collection. DNA was extracted from buffy coat by the phenol-chloroform method at the National Cancer Institute laboratory. Genotyping was conducted using optimized TaqMan assays (Applied Biosystems, Foster

City, CA, <http://snp500cancer.nci.nih.gov>) at the National Cancer Institute's Core Genotyping Facility (<http://cgf.nci.nih.gov/home.cfm>). For *ESR1*, we genotyped two intronic (rs2234693 and rs3841686) and two exonic single-nucleotide polymorphisms (SNPs) (rs1801132 and rs2228480), whereas in *ESR2*, we genotyped one exonic SNP (rs1256049) and one in the 3' untranslated region (rs4986938). These SNPs were selected *in silico* based on possible functional significance and a minor allele frequency of at least 10% in Asians in the National Cancer Institute SNP500Cancer Database (<http://snp500cancer.nci.nih.gov>) (12). Successful genotyping was achieved for 98–100% of DNA samples for all SNPs. A quality control process was undertaken to assess potential misclassification of genotyping results where 80 replicate samples from four quality control subjects were genotyped. The concordance between replicate samples was >99%. Among control subjects, genotype frequencies for each SNP were examined for deviation from Hardy–Weinberg equilibrium using the χ^2 test.

Statistical analysis

Gallbladder cancer cases were compared with control subjects without a history of cholecystectomy; bile duct and ampulla of Vater cancer cases were compared with all control subjects; and biliary stone cases were compared with control subjects who did not have biliary stones. Risk estimates were calculated for the additive and dominant genetic models using the most common homozygous genotype as the referent category. We used unconditional logistic regression analysis to calculate odds ratios (ORs) and 95% confidence intervals (CIs) of each biliary tract cancer and stone subsite in relation with each SNP and adjusted for age and sex. Additional models were run with further adjustment for biliary stone status (for cancer risk), body mass index (BMI), cigarette smoking, alcohol drinking and females' reproductive and menstrual factors such as use of oral contraceptives, parity, age at menarche, age at menopause, age at first birth and breast feeding to evaluate whether the risk of cancer was confounded by these factors. Factors that changed the risk estimate by >10% were retained in the model. 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 a potential dose–response effect of genetic variants on biliary tract cancer and stone risk (13). The Breslow–Day test was conducted to test the homogeneity between ORs in stratified analyses.

We computed the gene-level global *P*-values for *ESR1* and *ESR2* with the Simes' test which uses the *P*-value from the additive or dominant genetic models to adjust for multiple SNP comparisons within each gene (14). The significance of a gene is evaluated by comparing the false discovery rate-adjusted *P*-value to the threshold level of 0.05 (14,15).

Among population controls, the presence of linkage disequilibrium between loci in the *ESR1* gene was assessed by calculating pairwise Lewontin's *D'* values using Haploview version 3.11 (16). *ESR1* haplotypes were constructed using Haplo.stats in R version 2.0.1 (17), which uses an expectation–maximization algorithm to calculate maximum likelihood estimates of haplotype frequencies while taking into account phase ambiguity (18). The risks for biliary tract cancers and stones in relation to common haplotypes (>5%) with the most common haplotype as the referent category were assessed using Haplo.stats adjusting for age and sex.

Results

Characteristics of the study subjects are shown in Table I. Subjects with biliary stones were younger, whereas subjects with ampulla of

Vater cancer were older than controls. There were more women with gallbladder cancer and biliary stones but more men with bile duct cancer. Gallbladder cancer and biliary stone cases had a higher BMI and were more likely to have diabetes than controls. Bile duct cancer cases were more likely to be smokers and alcohol drinkers, whereas biliary stone cases were less likely to drink alcohol compared with controls. All biliary tract cancer cases were more likely to have biliary stones compared with controls.

Table II shows the risk of biliary tract cancers and stones in relation to each of the SNPs of *ESR1* and *ESR2*. Among population controls, the genotype frequencies of each marker showed no deviation from Hardy–Weinberg equilibrium ($P > 0.05$). The *ESR1* rs1801132 (P325P) marker was associated with an excess risk of bile duct cancer (CG/GG versus CC: OR = 1.7, 95% CI 1.1–2.8), although the *P*-trend was not statistically significant (*p*-trend = 0.07). This marker was also associated with ampulla of Vater cancer (CG: OR = 1.9, 95% CI 0.8–4.4 and GG: OR = 2.8, 95% CI 1.1–6.9), with a statistically significant test of trend (*p*-trend = 0.03). One *ESR2* marker, rs4986938 (38 bp 3' of STP), was also associated with an excess risk for bile duct cancer (GG versus AA: OR = 3.3, 95% CI 1.3–8.7); however, this estimate was based on seven cases and did not show a significant linear trend (*p*-trend = 0.25). For bile duct cancer, the global *P*-values for the *ESR1* gene were 0.07 using an additive model and 0.02 using a dominant model, whereas global *P*-values for the *ESR2* gene was not significant ($P > 0.1$) for either model. False discovery rate-adjusted *P*-values for the six SNPs and each cancer and stone risk were not significant ($P > 0.1$).

Table III shows the risks of biliary cancer and stones for *ESR1* rs1801132 (P325P) stratified by gender, BMI and stone status. Men carrying the CG/GG genotype of this marker had a 2.8-fold risk of bile duct cancer (95% CI: 1.4–5.7; *p*-trend = 0.02) compared with those with the CC genotype, which was a stronger association than observed among all subjects. There was no association between this marker and any of the biliary tract cancers or stones among women, with a significant gender interaction for bile duct cancer risk (*p*-heterogeneity = 0.01). Among all participants with a low BMI (<23 kg/m²), carriers of the CG/GG genotype, compared with those having the CC genotype, had a significant 2.2-fold risk of bile duct cancer (95% CI: 1.0–4.7), whereas subjects who had a high BMI (≥23 kg/m²) did not have a significant excess risk (OR = 1.4, 95% CI: 0.8–2.8); however, the test for interaction was not statistically significant (*p*-heterogeneity = 0.45), and the linear test of trend for those with a low BMI was not significant (*p*-trend = 0.57) but borderline significant for those with a high BMI ($P = 0.05$). Among all participants who did not have stones, we also found that carriers of the CG/GG genotype compared with those having the CC genotype had a significant 3.4-fold risk of bile duct cancer (95% CI: 1.2–9.9; *p*-trend = 0.08), whereas subjects who had stones did not have a significant excess risk (OR = 1.7, 95% CI: 0.9–3.1; *p*-trend = 0.35); the test for interaction and the linear test of trend were not statistically significant.

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

	Controls	Biliary tract cancers			Biliary stones ^a
		Gallbladder ^b	Bile duct ^c	Ampulla of Vater ^c	
Total (N)	786	237	127	47	895
Age (years) [mean (SD)]	63.6 (8.4)	64.0 (8.9)	63.6 (8.1)	66.0 (6.9)*	62.7 (7.9)***
Female [n (%)]	481 (61.2)	172 (72.6)***	51 (40.2)***	23 (48.9)	62.9 (8.8)*
Ever smoked cigarettes [n (%)]	237 (30.2)	64 (27.1)	56 (44.1)**	20 (42.6)	241 (26.9)
Ever drank alcohol [n (%)]	162 (20.6)	36 (15.2)	42 (33.1)**	12 (25.5)	142 (15.9)**
Body mass index ^d (kg/m ²) [mean (SD)]	23.3 (3.6)	24.2 (3.5)***	23.0 (3.1)	23.4 (2.9)	24.0 (3.3)***
Gallstones [n (%)]	194 (24.7)	201 (84.8)***	86 (67.7)***	28 (59.6)***	895 (100)***
Diabetes [n (%)]	64 (8.3)	31 (13.1)*	12 (9.4)	3 (6.4)	100 (11.2)**

^aBiliary stone cases compared with controls without biliary stones ($n = 592$).

^bGallbladder cancer cases compared with controls without cholecystectomy ($n = 737$).

^cBile duct and ampulla of Vater cancer cases compared with all controls ($n = 786$).

^d5 years prior to interview.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table II. ORs and 95% CIs for biliary tract cancers and stones in relation to ESR variants

Genotype	Controls	Gallbladder cancer ^a		Bile duct cancer ^b		Ampulla of Vater cancer ^b		Biliary stones ^c	
	<i>n</i>	<i>n</i>	OR (95% CI) ^d	<i>n</i>	OR (95% CI) ^d	<i>n</i>	OR (95% CI) ^d	<i>n</i>	OR (95% CI) ^d
<i>ESR1</i>									
IVS1-397C>T (rs2234693)									
CC	314	94	1.0	48	1.0	18	1.0	341	1.0
CT	356	100	0.9 (0.7–1.3)	54	1.0 (0.7–1.9)	24	1.2 (0.6–2.3)	401	1.1 (0.9–1.4)
TT	108	41	1.2 (0.8–1.9)	21	1.3 (0.8–2.4)	5	—	138	1.3 (0.9–1.7)
<i>P</i> -trend			0.57		0.39		0.98		0.16
CT/TT	464	141	1.0 (0.7–1.3)	75	1.1 (0.8–1.6)	29	1.12 (0.61–2.05)	539	1.1 (0.9–1.4)
IVS5-34->T (rs3841686)									
—	357	95	1.0	67	1.0	17	1.0	399	1.0
T	317	115	1.4 (1.0–1.9)	43	0.7 (0.5–1.1)	22	1.4 (0.7–2.7)	377	1.1 (0.9–1.3)
TT	105	24	0.8 (0.5–1.3)	14	0.7 (0.4–1.3)	8	1.7 (0.7–4.0)	105	0.9 (0.6–1.2)
<i>P</i> -trend			0.90		0.12		0.20		0.64
T/TT	422	139	1.2 (0.9–1.6)	57	0.7 (0.5–1.0)	30	1.5 (0.8–2.7)	482	1.0 (0.8–1.3)
Ex4-122G>C (rs1801132) (P325P)									
CC	219	65	1.0	23	1.0	7	1.0	249	1.0
CG	377	116	1.0 (0.7–1.5)	69	1.8 (1.1–2.9)	23	1.9 (0.8–4.4)	435	1.0 (0.8–1.3)
GG	181	53	0.9 (0.6–1.4)	32	1.7 (1.0–3.1)	16	2.8 (1.1–6.9)	201	1.0 (0.7–1.3)
<i>P</i> -trend			0.70		0.07		0.03		0.94
CG + GG	558	169	1.0 (0.7–1.4)	101	1.7 (1.1–2.8)	39	2.1 (0.9–4.9)	636	1.0 (0.8–1.3)
Ex8+229A>G (rs2228480)									
AA	467	136	1.0	85	1.0	27	1.0	528	1.0
AG	271	90	1.2 (0.9–1.6)	33	0.7 (0.4–1.0)	15	1.0 (0.5–1.8)	301	1.0 (0.8–1.3)
GG	43	9	0.7 (0.3–1.5)	7	0.8 (0.3–1.8)	5	—	51	1.0 (0.6–1.6)
<i>P</i> -trend			0.94		0.09		0.54		0.88
AG/GG	314	99	1.1 (0.8–1.5)	40	0.7 (0.4–1.0)	20	1.1 (0.6–1.9)	352	1.0 (0.8–1.3)
<i>p</i> -global ^e			1.0		0.07		0.1		0.9
<i>P</i> -global ^f			0.8		0.02		0.3		0.8
<i>ESR2</i>									
Ex6+32G>A (rs1256049) (V328V)									
AA	332	101	1.0	54	1.0	19	1.0	381	1.0
AG	357	105	1.0 (0.7–1.3)	56	1.0 (0.6–1.4)	25	1.2 (0.7–2.3)	380	0.9 (0.7–1.1)
GG	90	27	1.0 (0.6–1.6)	14	1.0 (0.5–1.8)	3	—	120	1.2 (0.9–1.7)
<i>P</i> -trend			0.87		0.83		0.77		0.70
AG/GG	447	132	1.0 (0.7–1.3)	70	1.0 (0.6–1.4)	28	1.1 (0.6–2.0)	500	1.0 (0.8–1.2)
38 bp 3' of STP (rs4986938)									
AA	589	174	1.0	91	1.0	32	1.0	669	1.0
AG	170	56	1.2 (0.8–1.6)	24	0.9 (0.6–1.5)	14	1.5 (0.8–2.9)	187	1.0 (0.8–1.3)
GG	13	4	—	7	3.3 (1.3–8.7)	0	—	16	1.2 (0.5–2.9)
<i>P</i> -trend			0.76		0.25		0.77		0.72
AG/GG	183	60	1.1 (0.8–1.6)	31	1.1 (0.7–1.7)	14	1.4 (0.7–2.7)	203	1.0 (0.8–1.3)
<i>P</i> -global ^e			0.9		0.5		0.4		0.4
<i>P</i> -global ^f			0.8		0.9		0.5		0.9
<i>P</i> -FDR for all six SNPs			0.9		0.2		0.9		0.9

Bold values are statistically significant at $P < 0.05$.

FDR, false discovery rate.

^aGallbladder cancer cases compared with controls without cholecystectomy ($n = 737$).

^bBile duct and ampulla of Vater cancer cases compared with all controls ($n = 786$).

^cBiliary stone cases compared with controls without biliary stones ($n = 592$).

^dORs (95% CI) were adjusted for age and sex. The ORs (95% CI) persisted after adjusting for gallstones.

^eGene-level global p -values by summarizing the likelihood ratio tests for the additive genetic effects.

^fGene-level global p -values by summarizing the likelihood ratio tests for the dominant genetic effects.

Based on the four SNPs examined in the *ESR1* gene, all pairwise Lewontin's D' values were <0.32 indicating low linkage disequilibrium between SNPs. We inferred six major haplotypes with corresponding frequencies ranging from 5.4 to 28.2% among controls. No associations for any of these haplotypes were found relative to the most common haplotype for each disease outcome (data not shown). Since we only examined two SNPs in *ESR2* and the global gene test was not significant, we did not examine haplotypes of this gene.

Discussion

This population-based study in China showed some evidence that the *ESR1* rs1801132 (P325P) marker may be associated with increased risks of bile duct and ampulla of Vater cancers. The association with bile duct cancer was more pronounced among men, those with a low

BMI or those without biliary stones. The *ESR2* 38 bp 3' of STP marker was also associated with an increased risk of bile duct cancer but was based on small numbers. Our results provide support for the hypothesis that variants in *ESR* genes, and perhaps estrogens, may play a role in the etiology of biliary tract cancers.

The associations between *ESR* polymorphisms and bile duct cancer are biologically plausible. Laboratory animal studies have shown that ESRs are present in the hepato-pancreatic-biliary tree, including bile duct epithelial cells, cholangiocytes and cholangiocarcinoma cell lines (19–21), suggesting that estrogens may play a role in bile duct carcinogenesis. Also, increased ESR expression has been observed in bile duct epithelial cells of primary sclerosing cholangitis (22,23), an inflammatory condition of the bile duct that is strongly associated with bile duct cancer (24). The functional effects of many *ESR* variants, including *ESR1* rs1801132 (P325P) and *ESR2* rs4986938 (38 bp

Table III. ORs and 95% CIs for biliary tract cancers and stones in relation to *ESR1* 1801132 (P325P) by gender, BMI and stone status

Genotype	Controls	Gallbladder cancer ^a		Bile duct cancer ^b		Ampulla of Vater cancer ^b		Biliary stones ^c	
	<i>n</i>	<i>n</i>	OR (95% CI) ^d	<i>n</i>	OR (95% CI) ^d	<i>n</i>	OR (95% CI) ^d	<i>n</i>	OR (95% CI) ^d
Gender									
Males									
CC	92	18	1.0	10	1.00	6	1.0	89	1.0
CG	142	37	1.3 (0.7–2.5)	44	2.8 (1.4–5.9)	11	1.1 (0.4–3.2)	163	1.1 (0.7–1.6)
GG	66	10	0.7 (0.3–1.7)	20	2.8 (1.2–6.3)	6	1.4 (0.4–4.6)	73	1.1 (0.7–1.8)
<i>P</i> -trend			0.60		0.02		0.58		0.65
CG/GG	208	47	1.1 (0.6–2.1)	64	2.8 (1.4–5.7)	17	1.2 (0.5–3.2)	236	1.1 (0.8–1.6)
Females									
CC	127	47	1.0	13	1.0	1	1.0	160	1.0
CG	235	79	0.9 (0.6–1.4)	25	1.0 (0.5–2.1)	12	—	272	0.9 (0.7–1.3)
GG	115	43	1.0 (0.6–1.6)	12	1.0 (0.4–2.3)	10	—	128	0.9 (0.6–1.3)
<i>P</i> -trend			0.92		0.98		—		0.62
CG/GG	350	122	0.9 (0.6–1.4)	37	1.0 (0.5–2.0)	22	—	400	0.9 (0.7–1.3)
<i>P</i> -heterogeneity ^e			0.6		0.01		—		0.4
BMI									
BMI < 23 kg/m²									
CC	100	30	1.0	9	1.00	5	1.0	83	1.0
CG	188	44	0.7 (0.4–1.3)	43	2.6 (1.2–5.7)	7	0.7 (0.2–2.2)	160	1.2 (0.8–1.8)
GG	95	16	0.5 (0.3–1.01)	11	1.4 (0.5–3.4)	9	1.7 (0.5–5.5)	95	1.6 (1.0–2.4)
<i>P</i> -trend			0.05		0.57		0.26		0.06
CG/GG	283	60	0.6 (0.4–1.1)	54	2.2 (1.0–4.7)	16	1.0 (0.4–2.9)	255	1.3 (0.9–1.9)
BMI ≥ 23 kg/m²									
CC	119	35	1.0	14	1.0	2	1.0	166	1.0
CG	188	72	1.3 (0.8–2.1)	26	1.2 (0.6–2.3)	16	—	275	0.9 (0.7–1.3)
GG	86	36	1.3 (0.8–2.3)	21	2.1 (0.9–4.4)	7	—	105	0.8 (0.5–1.2)
<i>P</i> -trend			0.28		0.05		—		0.26
CG/GG	274	108	1.3 (0.9–2.1)	47	1.4 (0.8–2.8)	23	—	380	0.9 (0.6–1.2)
<i>P</i> -heterogeneity ^e			0.06		0.45		—		0.39
Stone status									
No stones									
CC	158	14	1.0	4	1.00	3	1.0		
CG	293	16	0.7 (0.3–1.5)	26	3.5 (1.2–10.4)	11	—		
GG	134	6	0.6 (0.2–1.5)	10	3.1 (1.0–10.4)	4	—		
<i>P</i> -trend			0.23		0.08		—		
CG/GG	427	22	0.7 (0.3–1.4)	36	3.4 (1.2–9.9)	15	—		
Stones									
CC	61	51	1.0	19	1.0	4	1.0		
CG	84	100	1.7 (1.0–2.9)	43	1.8 (0.9–3.5)	12	—		
GG	47	47	1.0 (0.6–1.8)	22	1.4 (0.6–3.0)	12	—		
<i>P</i> -trend			0.92		0.35		—		
CG/GG	131	147	1.4 (0.9–2.2)	65	1.7 (0.9–3.1)	24	—		
<i>P</i> -heterogeneity ^e			0.05		0.22		—		

Bold values are statistically significant at $P < 0.05$.

^aGallbladder cancer cases compared with controls without cholecystectomy ($n = 737$).

^bBile duct and ampulla of Vater cancer cases compared with all controls ($n = 786$).

^cBiliary stone cases compared with controls without biliary stones ($n = 592$).

^dORs (95% CI) were adjusted for age and sex.

^e*P*-heterogeneity by Breslow–Day test.

3' of STP), are not well understood, but some other *ESR* variants have been shown to alter the function of the receptor affecting the tissue's response to estrogens (24). *ESR1* rs1801132 (P325P) is a synonymous SNP located in Exon 4 of *ESR1*. The variant allele (G) of this marker has been associated with decreased risks of breast cancer (25) as well as lower bone mineral density (26); both of these findings suggest that this variant has an impact on estrogen. It is unclear whether P325P's effects are due to its influence on messenger RNA stability or translation efficiency (27) or that it is in linkage disequilibrium with other nearby functional variants. In addition to impacting estrogen, *ESR* variants have been linked to variation in lipid levels including high-density lipoprotein, low-density lipoprotein and triglycerides (28,29). Increased low-density lipoprotein and triglycerides and decreased high-density lipoprotein levels have been associated with an increased risk of bile duct cancer in this study population (30).

It has been consistently reported that men have slightly higher rates of bile duct cancer than women (1); reasons for this are unclear but

may be due to differences in prevalence of smoking, infection, occupational exposure or hormone-related factors between men and women (1,6). While *ESRs* have been more studied in women, they have also been linked to certain diseases in men, including cancers of the prostate and colon (31–33). In addition, in a study of *ESR* polymorphisms and plasma lipid levels, *ESR1* variants, rs9322331 and rs9340799, were more strongly associated with apolipoprotein C in men but were more strongly associated with triglycerides in women, suggesting that *ESR1* variation has sex-dependent effects on circulating lipid levels (33). Although the functional effect of *ESR1* P325P is unclear, it is biologically plausible that its effect on hormonal action may impact men and women differently, possibly explaining why this variant was associated with bile duct cancer among men and not women.

We also found some evidence that the association between *ESR1* rs1801132 (P325P) and bile duct cancer was more pronounced among subjects with a low BMI or those without biliary stones. However, the

linear tests of trend and the tests for heterogeneity were not statistically significant for either BMI or biliary stones; thus, these associations should be interpreted with caution.

Our observation of different effects of *ESR* genes by biliary cancer subsite is consistent with the etiologic heterogeneity of these tumors (1,6). In our study, the effects of *ESR* genes were limited to bile duct and ampulla of Vater cancers and not gallbladder cancer or biliary stones. Reasons for the null findings for gallbladder and gallstones are unclear, especially since both of these diseases have been linked more to hormonal factors than bile duct and ampulla of Vater cancers (1,6).

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 the 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, biliary tract cancer and biliary stones, offered the opportunity to assess whether risks associated with various exposures, including genetic susceptibility, are similar between these two closely related conditions.

Limitations should also be noted. First, our coverage of the *ESR1* and *ESR2* genes was limited since SNP selection was not based on complete sequencing data for our target population. The possibility of a false-positive association cannot be ruled out entirely due to insignificant adjusted *P*-values for the false discovery rate. Second, due to a low minor allele frequency and the small number of ampullary cancer cases, there was limited statistical power to evaluate certain main effects.

In conclusion, our population-based study showed that the *ESR1* gene, particularly the rs1801132 (P325P) marker, is associated with bile duct cancer among men. This finding supports our hypothesis that sex steroids, in particular estrogen, may play a role in biliary tract cancers. Additional studies with a more comprehensive coverage of the *ESR* genes are needed to confirm our results.

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