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Polymorphism of genes related to insulin sensitivity and the risk of biliary tract cancer and biliary stone: a population-based case–control study in Shanghai, China

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Biliary tract cancer, encompassing tumors of the gallbladder, extrahepatic bile ducts and ampulla of Vater, is a rare but highly fatal malignancy. Obesity and gallstones, both related to insulin resistance, are linked to an elevated risk of biliary cancer. The peroxisome proliferator-activated receptors (PPARs) and the retinoid X receptors (RXRs), expressed in adipose tissue, play a key role in the regulation of obesity-related insulin sensitivity, thus genetic variants of these two receptor genes may be related to biliary cancer and stones. We examined the associations of seven single-nucleotide polymorphisms in the *PPAR*-γ, *PPAR*-δ, *RXR*-α, *RXR*-β and *INS* genes with biliary cancer and stones in a population-based case–control study in Shanghai, China. We included 237 gallbladder, 127 extrahepatic bile duct and 47 ampulla of Vater cancer cases, 895 stone cases and 786 population controls. Relative to individuals with the *RXR*-β C51T (rs2076310) CC genotype, those having the TT genotype had a 1.6-fold risk for bile duct cancer [odds ratio (OR) = 1.67; 95% confidence interval (CI) = 0.99–2.84], with a more pronounced association among men (OR = 2.30; 95% CI = 1.14–4.65; *P* interaction = 0.07). This marker was also associated with a higher risk of gallstones among subjects with a higher body mass index (BMI) (≥23 kg/m^2) (OR = 1.80; 95% CI = 1.09–2.94), although the interaction with BMI was not statistically significant (*P* interaction = 0.28). No association was found between other variants and biliary cancers and stones. Results from this population-based study suggest that certain genetic variants involved in the regulation of obesity-related insulin sensitivity may increase susceptibility to bile duct cancer and gallstones.

Introduction

Cancer of the biliary tract, comprising tumors of the gallbladder, extrahepatic bile ducts and ampulla of Vater, is a rare but often fatal cancer with incidence rates ranging from 1 to 10 per 100 000 globally (1). The prognosis of the disease is poor; most patients do not survive longer than a year, and 5-year relative survival rates range from 10 to 20% (2). Large geographic variations and ethnic differences in incidence have been reported (1), with Europeans having a high risk of gallbladder cancer and Asians having a high risk of bile duct cancer. Geographic and ethnic variation in rates suggest a possible role of genetic susceptibility, whereas the recent increase in biliary tract cancer in certain Eastern populations, including Shanghai, China (2,3) also suggests the importance of environmental factors. Apart from a close association between gallstones and gallbladder cancer, putative risk factors such as obesity (3,4), diabetes (5,6), smoking (7) alcohol consumption (8) and reproductive and hormonal factors (1,9), have been implicated.

Obesity has been linked to gallbladder cancer, particularly in women (10,11), and extrahepatic bile duct cancer in both men and women (12). Although the risk of biliary tract cancers associated with obesity may reflect the well-established association with antecedent gallstones, the mechanism underlying obesity and biliary tract cancer is unclear. Because obesity often leads to insulin resistance, we speculate that insulin resistance may mediate the risk of biliary tract cancer through obesity.

One of the potential biological pathways mediating obesity and insulin resistance in biliary tract cancer pathogenesis is through the functional heterodimers of peroxisome proliferator-activated receptors (PPARs) and retinoid X receptors (RXRs) (13,14). The PPARs are members of a nuclear hormone receptor subfamily of transcription factors that is expressed predominantly in the adipose tissue and the immune system and has been implicated to play a role in the regulation of obesity-related insulin sensitivity (15). PPARs form heterodimers with RXRs, which regulate the transcription of various genes implicated in promoting insulin sensitization in the context of obesity and the control of lipid metabolism, carcinogenesis and inflammation (15,16). Polymorphisms in the *PPAR* gene have been associated with insulin resistance (17), obesity (17,18) and colorectal cancer (19,20); variants of *RXR* have been linked to hyperlipidemia (21) and renal cell carcinoma (22) and *INS* gene variants have been associated with prostate cancer (23). Despite the associations of diabetes and obesity with gallstones and biliary tract cancers, the role of insulin-related genes has not been examined in biliary tract stones or cancer. In this report, we examined the associations of polymorphisms in the *PPAR*, *RXR* and *INS* genes with biliary tract cancer and stone risk in a population-based case–control study in Shanghai, China.

Abbreviations: BMI, body mass index; CI, confidence interval; INS, insulin; LD, linkage disequilibrium; OR, odds ratio; PPAR, peroxisome proliferator-activated receptor; RXR, retinoid X receptor; SNP, single-nucleotide polymorphism.

Materials and methods

Study population

The study protocol was approved by the Institutional Review Boards of the USA National Cancer Institute and Shanghai Cancer Institute. All participants provided written informed consent. Details of the study have been reported elsewhere (24–26). The Shanghai biliary tract cancer study is a population-based case–control study that recruited permanent Shanghai residents between the ages of 40 and 74 years who were newly diagnosed with primary biliary cancer (International Classification of Diseases 9, code 156) between June 1997 and May 2001 through a rapid reporting system established by the Shanghai Cancer Institute and 42 collaborating hospitals in urban Shanghai. This reporting system captured >95% of biliary tract cancer patients diagnosed in Shanghai during this study period. A total of 2092 permanent residents of urban Shanghai, including 627 biliary tract cancer cases (368 gallbladder, 191 bile duct and 68 ampulla of Vater), 1037 biliary stone cases (774 gallbladder and 263 bile duct) and 959 population controls were enrolled

into the study. All cancer diagnoses were confirmed by Shanghai pathologists who reviewed histology slides and clinical data from computed tomography scan, magnetic resonance imaging, abdominal ultrasound or endoscopic retrograde cholangiopancretography. An independent review was carried out by a USA pathologist to further confirm the cancer diagnosis. Biliary stone cases were selected by frequency matching to cancer cases on age (5-year intervals), gender and hospital. Population controls were recruited from all permanent residents listed in the Shanghai Resident Registry. Healthy subjects who did not have a history of cancer were selected randomly and frequency matched to the expected age distribution (5-year category) of biliary tract cancer cases. The participation rates were 95 and 82% among the eligible cancer cases and controls, respectively.

All participants were assessed for their biliary stone status. For biliary cancer cases, presence of biliary stones was determined using data from clinical diagnostic workup (abdominal ultrasound, computed tomography scan, endoscopic retrograde cholangiopancretography and magnetic resonance imaging), medical record review and in-person interview. Cases were interviewed within 3 months of their initial cancer diagnosis. Biliary stone status among population controls was determined based on their self-reported history of stones or cholecystectomy and abdominal ultrasound results. About 85% of the controls agreed to ultrasound screening for the detection of stones. The 15% of controls who did not agree to an ultrasound were also included in the study since it was estimated that about only 1% of them would have had silent gallstones.

Data collection

Information on potential risk factors was elicited through an in-person interview by trained interviewers, using a structured questionnaire. The interview included information on demographic characteristics, occupation, dietary history, smoking history, consumption of alcohol and other beverages, reproductive factors, medical history, family history of cancer, physical activity and body size. Subjects were measured for current weight, waist and hip circumferences and height at the interview. All measurements were taken by trained interviewers, using a standard protocol. The response rate for interviews was >95% among cases and 82% among controls. Five percent of the study subjects were randomly selected for reinterview 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%.

Blood collection and DNA extraction

Overnight fasting blood samples were collected from subjects who gave consent. The blood samples were collected in Vacutainer tubes containing ethylenediaminetetraacetic acid or heparin and processed within 4 hours of collection at a central laboratory at Shanghai Cancer Institute and stored at −70°C. The frozen buffy coat samples (separated from 5 ml of blood) were shipped to USA on dry ice. Genomic DNA was extracted from buffy coat using phenol–chloroform with a standard protocol. Genotyping for all seven single-nucleotide polymorphisms (SNPs) [*PPAR*-γ (exon 6, C161T, rs3856806), *PPAR*-δ (exon 4, T15C, rs2016520), *RXR*-α (intron 7, G70A, rs1536475 and intron 8, A-27G, rs1805343), *RXR*-β (exon 10, G392T, rs2744537 and intron 3, C51T, rs2076310) and *INS* (intron 1, A-6T, rs689)] were performed using TaqMan assay (Applied Biosystems, Foster City, CA) at the National Cancer Institute Core Genotyping Facility (Gaithersburg, MD). These polymorphisms were selected *a priori* based on two criteria: (i) having either a reported prevalence of at least 5% for the variant allele among Asians and reported evidence of an association with disease (particularly cancer) and (ii) availability of a validated assay at the Core Genotyping Facility. All assays were validated and optimized at the Core Genotyping Facility laboratory. Specific primers, probes and methods used to detect polymorphisms are publicly available at http://snp500cancer.nci.nih.gov/ (27). Coriell DNA samples containing homozygous major allele, heterozygous and homozygous minor allele genotype were used as internal laboratory quality control for each polymorphism studied, including four of each type and four no-template controls per plate (384 samples). DNA samples for cases and controls were grouped into pairs to minimize the effect of day-to-day laboratory variation. Laboratory personnel were blinded to the identity of subjects. A total of 80 quality control samples from four individuals were placed along with study samples to estimate the reproducibility of genotyping assays. The concordance was >99%.

Statistical analysis

This study included all subjects who consented to provide blood and for whom we had genotype data: 411 biliary tract cancer cases (237 gallbladder, 127 bile duct and 47 ampulla of Vater), 895 biliary stone cases (673 gallbladder and 222 bile duct) and 786 population controls. All statistical analyses were conducted using SAS version 8.2 (SAS Institute, Cary, NC). Baseline characteristics of cases and controls were compared using the chi-square test. Asymptotic Pearson's chi-square test was used to test Hardy–Weinberg equilibrium for genotype frequencies for all the SNPs among population controls in this study. Odds ratios (ORs) for biliary tract cancer and biliary stone risk and 95% confidence intervals (CIs) were calculated using unconditional logistic regression. For the entire analyses, gallbladder cancer cases were compared with population controls who did not have a cholecystectomy ($n = 737$), bile duct and ampullary cancer cases were compared with all population controls ($n = 786$) and biliary stone cases (gallstone or bile duct stone) were compared with the subset of population controls without any type of stone ($n = 592$). An initial model was adjusted for age and additional models were further adjusted for gender, body

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

Selected characteristics	Population control[a]			Biliary tract cancers			Biliary stones	
	N1 ($n = 786$)	N2 ($n = 737$)	N3 ($n = 592$)	Gallbladder ($n = 237$)	Bile duct ($n = 127$)	Ampulla of Vater ($n = 47$)	Gallbladder ($n = 673$)	Bile duct ($n = 222$)
Gender (%)								
Male	38.8	39.4	42.6	27.4[b]	59.8[b]	51.1	33.3[b]	47.3
Female	61.2	60.6	57.4	72.6	40.2	48.9	66.7	52.7
Age at interview (%)								
<55	14.3	15.1	17.1	14.0	15.1	8.7	33.8[b]	24.5[b]
55–64	24.8	25.7	25.4	23.6	20.2	17.4	23.9	27.8
≥65	60.9	59.2	57.5	62.4	64.7	73.9	42.3	47.7
Education (%)								
None/primary	40.2	39.1	37.7	53.2[b]	41.7	46.8	28.7[b]	35.1
Middle/high school	44.7	45.9	46.5	37.5	44.1	42.6	53.6	49.1
College or above	15.1	15.1	15.9	9.3	14.2	10.6	17.7	15.8
BMI (kg/m^2) (%)								
<18.5	8.4	8.8	9.3	4.7[b]	4.7	2.1	3.7[b]	6.8[b]
18.5–22.9	41.4	42.5	45.4	33.5	44.9	42.6	34.1	33.3
23–24.9	21.2	21.1	20.8	19.9	26.0	53.4	24.4	23.0
≥25	29.0	27.6	24.5	42.0	24.4	31.9	37.8	36.9
History of hypertension (%)	42.8	41.9	40.0	36.7	29.1[b]	27.7[b]	34.0[b]	28.8[b]
History of diabetes (%)	8.3	7.6	6.4	13.1[b]	9.5	6.4	11.1[b]	11.3[b]
Cigarette use (%)	30.1	30.3	31.6	27.1	44.1[b]	42.5	23.9[b]	36.0
Alcohol use (%)	20.6	20.5	22.6	15.2	33.1[b]	25.5	15.0[b]	18.5

[a]N1, all controls; N2, controls without cholecystectomy; N3, controls without biliary stone.
[b]P value <0.05 for comparison between cases and controls.

mass index (BMI), cigarette smoking, alcohol drinking, hypertension, diabetes and gallstone status (cancer risk only) in order to evaluate the potential confounding by these factors. To evaluate effect modification, stratum-specific risks were examined for each of the above-mentioned factors, and multiplicative interaction terms were added to the age-adjusted model. For the genes with more than one SNP examined in our study, a corrected *P* value of the overall association between polymorphisms and each outcome of interest was derived to further take into account the potential problem of multiple comparisons (28). In addition, we calculated the false positive reporting probability in order to assess the likelihood of having false-positive findings (29).

Results

Selected characteristics of cases and controls are shown in Table I. More women were diagnosed with gallstones and gallbladder cancer, whereas more men were diagnosed with bile duct cancer. Compared with controls, biliary stone and gallbladder cancer cases had a higher BMI and were more likely to have a history of diabetes. Bile duct cancer cases were more likely to smoke cigarettes and drink alcohol, whereas gallstone cases were less likely to smoke and drink alcohol than controls. Biliary tract cancer and biliary stone cases were less likely to have a history of hypertension than controls.

The age-adjusted ORs and 95% CI for biliary tract cancer and biliary stones in relation to seven variants are shown in Table II. Among controls, all SNPs were in Hardy–Weinberg equilibrium. Because two variants, *RXR*-β G392T and *INS* A-6T, had a low frequency of homozygous variant genotypes and *PPAR*-δ T15C did not have a homozygous variant genotype for ampulla of Vater cancer cases, we combined the homozygous and the heterozygous variant genotypes. Overall, there was no significant association between these seven variants and the risk of bilairy tract cancer and stones, although the homozygous genetic variant (TT) of the *RXR*-β C51T marker was marginally associated with an increased risk of bile duct cancer (OR = 1.67; 95% CI = 0.99–2.84). Among population controls, the frequency of the variant TT genotype of the *RXR*-β C51T marker was 12%, which is slightly less than the frequency of 17% among the 72 Asian/Pacific Rim subjects reported in SNP500 (http://snp500cancer.nci.nih.gov) (27).

The effect of the *RXR*-β C51T marker on bile duct cancer was limited to men (Table III). Among men, those with the TT genotype of the *RXR*-β C51T marker had a 2.3-fold risk of bile duct cancer (95% CI = 1.14–4.65; *P* adjusted for multiple comparisons = 0.04), whereas female carriers did not have an excess risk (*P* interaction = 0.07). Further adjustment for gallstones did not materially change the results (OR = 2.45; 95% CI = 1.11–5.38). In addition, among subjects with a higher BMI ($\geq$23 kg/m^2), those carrying the TT genotype of the *RXR*-β C51T marker had a 1.8-fold risk of gallstones (95% CI = 1.09–2.94; *P* adjusted for multiple comparisons = 0.04), but not among those with a lower BMI ($<$23 kg/m^2) (OR = 0.70; 95% CI = 0.38–1.31) (*P* interaction = 0.28). These findings for the *RXR*-β C51T marker among men and those with a high BMI were

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

Gene/variants	Controls[a]			Biliary tract cancers						Biliary stones			
				Gallbladder (*n* = 237)		Bile duct (*n* = 127)		Ampulla of Vater (*n* = 47)		Gallbladder (*n* = 673)		Bile duct (*n* = 222	
	N1	N2	N3	*n*	OR (95% CI)	*n*	OR (95% CI)	*n*	OR (95% CI)	*n*	OR (95% CI)	*n*	OR (95% CI)
RXR-α G70A (rs1536475)													
GG	443	414	334	135	1.00	74	1.00	27	1.00	385	1.00	123	1.00
GA	282	266	212	90	1.04 (0.76–1.42)	44	0.93 (0.62–1.39)	16	0.93 (0.49–1.76)	234	0.92 (0.72–1.17)	78	1.02 (0.73–1.43)
AA	51	47	37	10	0.65 (0.32–1.33)	6	0.71 (0.29–1.71)	3	—	43	0.91 (0.57–1.46)	14	0.99 (0.51–1.90)
P for trend					*0.55*		*0.46*		*0.89*		*0.49*		*0.95*
RXR-α A-27G (rs1805343)													
AA	317	298	239	98	1.00	51	1.00	21	1.00	268	1.00	94	1.00
GA	351	329	266	107	1.00 (0.73–1.37)	53	0.94 (0.62–1.43)	21	0.93 (0.50–1.73)	307	0.98 (0.77–1.26)	94	0.89 (0.64–1.25)
GG	105	98	76	26	0.80 (0.49–1.31)	21	1.25 (0.72–2.17)	4	—	84	0.91 (0.63–1.31)	26	0.87 (0.53–1.45)
P for trend					*0.50*		*0.60*		*0.39*		*0.66*		*0.48*
RXR-β G392T (rs2744537)													
GG	720	676	539	221	1.00	118	1.00	44	1.00	603	1.00	199	1.00
TG + TT[b]	56	52	45	14	0.82 (0.45–1.52)	7	0.76 (0.34–1.72)	2	—	59	1.21 (0.80–1.82)	17	1.01 (0.56–1.81)
P for trend					*0.53*		*0.51*		—		*0.37*		*0.98*
RXR-β C51T (rs2076310)													
CC	331	303	234	97	1.00	53	1.00	22	1.00	255	1.00	93	1.00
TC	353	339	284	101	0.94 (0.68–1.29)	47	0.84 (0.55–1.27)	21	0.92 (0.50–1.71)	313	0.98 (0.77–1.25)	92	0.82 (0.58–1.14)
TT	93	86	67	36	1.30 (0.83–2.04)	25	1.67 (0.99–2.84)	3	0.47 (0.14–1.60)	94	1.28 (0.89–1.85)	32	1.20 (0.74–1.95)
P for trend					*0.46*		*0.21*		*0.29*		*0.34*		*0.94*
INS A–6T (rs689)													
AA	698	658	528	214	1.00	116	1.00	43	1.00	603	1.00	200	1.00
TA + TT[b]	73	65	52	20	0.96 (0.57–1.62)	7	0.57 (0.26–1.99)	3	—	57	0.96 (0.64–1.44)	15	0.79(0.43–1.44)
P for trend					*0.88*		*0.18*		—		*0.85*		*0.44*
PPAR-γ C161T (rs3856806)													
CC	457	428	350	127	1.00	74	1.00	27	1.00	411	1.00	135	1.00
TC	284	264	205	95	1.22 (0.89–1.65)	44	0.96 (0.64–1.43)	18	1.10 (0.60–2.04)	226	0.92 (0.72–1.17)	69	0.86 (0.62–1.21)
TT	41	41	34	15	1.25 (0.67–2.33)	8	1.21 (0.55–2.69)	2	—	35	0.84 (0.51–1.39)	17	1.26 (0.68–2.34)
P for trend					*0.21*		*0.88*		*0.93*		*0.36*		*0.97*
PPAR-δ T15C (rs2016520)													
TT	388	362	286	119	1.00	65	1.00	26	1.00	311	1.00	114	1.00
TC	267	253	209	81	0.98 (0.71–1.36)	42	0.95 (0.63–1.45)	16	0.76 (0.40–1.44)[b]	224	0.96 (0.75–1.24)	77	0.89 (0.63–1.26)
CC	60	56	42	20	1.09 (0.63–1.89)	10	1.00 (0.49–2.06)			66	1.36 (0.89–2.08)	12	0.72 (0.36–1.42)
P for trend					*0.87*		*0.90*		*0.14*		*0.40*		*0.29*

[a]Bile duct cancer and ampullary cancer cases were compared with all population controls (N1), gallbladder cancer cases were compared with controls without cholecytectomy (N2), and gallbladder and bile duct stone were compared with controls without biliary stones (N3).
[b]OR was calculated for homozygous and heterozygous variants combined due to rare homozygous variant carrier.

Table III. ORs and 95% CI for biliary tract cancers and stones in relation to RXR-β C/T polymorphism, a stratification of gender and BMI

RXR-β C/T	Population control[a]			Biliary tract cancers						Biliary stones			
				Gallbladder (n = 237)		Bile duct (n = 127)		Ampulla of Vater (n = 47)		Gallbladder stones (n = 673)		Bile duct stones (n = 222)	
	N1	N2	N3	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)
Male													
CC	126	115	96	30	1.0	27	1.0	10	1.0	83	1.0	42	1.0
CT	140	138	123	26	0.73 (0.41–1.32)	30	1.01 (0.57–1.80)	13	1.24 (0.52–2.96)	108	0.97 (0.65–1.45)	45	0.80 (0.48–1.32)
TT	36	34	31	9	0.99 (0.43–2.30)	18	2.30 (1.14–4.65)[b]	0	—	29	1.01 (0.56–1.83)	16	1.13 (0.55–2.30)
P for trend					*0.65*		*0.05*		*0.34*		*0.97*		*0.94*
Female													
CC	205	188	138	67	1.0	26	1.0	12	1.0	172	1.0	51	1.0
CT	213	201	161	75	1.06 (0.85–1.57)	17	0.62 (0.33–1.18)	8	0.63 (0.25–1.58)	205	1.01 (0.74–1.38)	47	0.82 (0.52–1.29)
TT	57	52	36	27	1.47 (0.45–2.54)	7	0.94 (0.39–2.29)	3	—	65	1.53 (0.95–2.46)	16	1.24 (0.63–2.44)
P for trend					*0.22*		*0.46*		*0.51*		*0.16*		*0.92*
P interaction					*0.30*		*0.07*		*0.80*		*0.91*		*0.34*
BMI $<$ 23 kg/m^2													
CC	145	139	113	37	1.0	26	1.0	14	1.0	100	1.0	37	1.0
CT	193	188	167	38	0.76 (0.46–1.25)	21	0.61 (0.33–1.13)	7	0.39 (0.15–0.99)[c]	124	0.81 (0.56–1.17)	39	0.72 (0.43–1.21)
TT	45	43	36	15	1.29 (0.65–2.58)	16	1.99 (0.97–4.05)	0	—	23	0.70 (0.38–1.31)	12	1.10 (0.51–2.38)
P for trend					*0.88*				*0.008*		*0.17*		*0.72*
BMI $\geq$ 23 kg/m^2													
CC	186	164	121	60	1.0	27	1.0	8	1.0	155	1.0	56	1.0
CT	160	151	117	62	1.14 (0.75–1.73)	26	1.13 (0.63–2.01)	14	2.07 (0.85–5.07)	188	1.22 (0.87–1.71)	53	0.98 (0.62–1.55)
TT	47	42	30	21	1.38 (0.75–2.51)	9	1.32 (0.58–3.00)	3	—	71	1.80 (1.09–2.94)[d]	20	1.40 (0.73–2.69)
P for trend					*0.29*		*0.50*		*0.28*		*0.02*		*0.45*
P interaction					*0.18*		*0.88*		*0.05*		*0.28*		*0.12*

[a]Bile duct cancer and ampullary cancer cases were compared with all population controls (N1), gallbladder cancer cases were compared with controls without cholecystectomy (N2) and gallbladder and bile duct stone were compared with controls without biliary stones (N3).
[b]P value corrected for multiple comparison = 0.04.
[c]P value corrected for multiple comparison = 0.10.
[d]P value corrected for multiple comparison = 0.04.

noteworthy at the 0.5 false positive reporting probability level, assuming a prior probability of association of 0.1.

Discussion

In this population-based case–control study, we found that the TT genotype of the *RXR*-β C51T marker was associated with a 2.3-fold risk of bile duct cancer in men and a 1.8-fold risk of gallstones among subjects with a BMI >23 kg/m^2. No association was found for the other SNPs examined in the study.

The observed effect of the *RXR*-β variant in bile duct cancer among men, independent of gallstones, suggests that RXRs may affect the risk of bile duct cancer through mechanisms other than gallstones. Since *RXR*-β gene variants may change the expression or function of PPAR/RXR heterodimers (13), which in turn can affect insulin sensitization (15,16), it is possible that *RXR*-β variants can affect the risk of bile duct cancer through insulin resistance or inflammation. It is unclear as to why the association between the *RXR*-β C51T marker and bile duct cancer was limited to men. Since this association persisted after further adjustment for additional variables, confounding by factors such as smoking and alcohol drinking, which are predominant among men in this study population, should be minimal. Thus, our finding suggests a gender-specific role of *RXR*-β through mechanisms other than smoking and alcohol drinking. However, it is important to note that we had considerably fewer women than men with bile duct cancer, which may have limited our statistical power to observe a genetic association among women.

The observation that carriers of the *RXR*-β TT genotype with a high BMI (≥23 kg/m^2) had an excess risk of gallstones is not surprising, since both obesity and diabetes, conditions closely related to insulin resistance, are important risk factors for gallstones (5,6,30–34). *RXR*-β can form heterodimers with PPARs to regulate the transcription of various genes implicated in insulin sensitization, thus it is possible that *RXR*-β can affect gallstone risk through regulation of insulin resistance (15,16). In some studies, the effect of insulin resistance on gallstones persisted after adjustment for obesity (32–34), suggesting that insulin resistance may play a role in the etiology of gallstones independent of obesity (35).

It is also possible that *RXR*-β may affect the risk of bile duct cancer or gallstones through other unknown causal genes that are in linkage disequilibrium (LD) with the *RXR*-β variant. For example, previous studies have reported that the *RXR*-β gene is localized in the major histocompatibility complex class II region between the *DPB1* and *RING2* genes (36). Significant LD between certain *RXR*-β alleles and *HLA-DPB1* alleles has been described (37); however, whether the *RXR*-β C51T has a LD with *HLA-DPB1* alleles warrants further study.

Strengths of our study should be noted. This is the first large population-based study to investigate genetic variants related to insulin sensitivity in relation to biliary tract cancer and biliary stones. The population-based design and high response rate of participants minimized the potential for selection bias. Because cancer diagnoses were confirmed by careful review of pathological records and case ascertainment was >95%, potential selection misclassification was also minimal. In addition, simultaneously investigating both biliary tract cancer and biliary stones allowed us to further examine whether risks associated with various exposures, including genetic susceptibility, are similar between these two closely related outcomes.

Despite being the largest case–control study of biliary tract cancer to date, we had limited statistical power to evaluate main effects of ampulla of Vater cancer and the effect of gene–environment or gene–gene interactions due to low allele frequency and the small number of bile duct and ampullary cancer cases. Because our study was conducted within a fairly homogeneous Chinese population, we had limited generalizability. A major limitation of our study is incomplete gene coverage since we included only seven SNPs in this pathway.

Thus, we may have missed the effect of some important markers, and our findings may actually represent markers for other functional SNPs that are in LD.

In summary, in this population-based study, we found that *RXR*-β gene variants are associated with bile duct cancer in men and to gallbladder stones among subjects with a higher BMI. Further studies with greater statistical power and broader coverage of the *RXR*-β gene are needed to confirm our findings and identify causal gene variants.

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