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Variants in *CCK* and *CCKAR* genes to susceptibility to biliary tract cancers and stones: a population-based study in Shanghai, China

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Abstract

Background and Aim—Altered motility of the gallbladder is associated with an increased risk of gallstones and can result in biliary tract cancers. Cholecystokinin (CCK) is an important modulator of gallbladder motility which functions by activating CCK type-A receptor (CCKAR). The aim of this study was to determine whether genetic variants in *CCK* and *CCKAR* are associated with the risk of biliary tract cancers and stones.

Methods—We investigated the associations between 9 single nucleotide polymorphisms (SNPs) in *CCK* and *CCKAR* in a population-based case-control study, including 439 biliary tract cancer cases (253 gallbladder, 133 extrahepatic bile duct and 53 ampulla of Vater cancer cases), 429 biliary stone cases, and 447 population controls in Shanghai, China.

Results—We found that women with the *CCKAR* rs1800855 AA genotype had an increased risk of gallbladder cancer (odd ratio (OR) =2.37, 95% confidence interval (CI): 1.36–4.14) compared to subjects with the TT genotype, and remained significant after Bonferroni correction (P=0.0056). Additionally, female carriers of the *CCKAR* haplotype C-T-C-T (rs2071011-rs915889-rs3822222-rs1800855) had a reduced risk of gallbladder cancer (OR=0.61, 95%CI: 0.43–0.86) compared to those with the G-C-C-A haplotype, the association also remained significant after Bonferroni correction.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Conclusions—These findings suggest that variants in the *CCKAR* gene may influence the risk of gallbladder cancer in women. Additional studies are needed to confirm our findings.

Keywords

biliary tract cancer; gallstone; *CCK* and *CCKAR*; variant; haplotype; susceptibility

Introduction

Biliary tract cancers, which includes cancers of the gallbladder, extrahepatic bile duct, and ampulla of Vater, are relatively rare but highly fatal malignancies, with age-adjusted incidence and mortality rates of 1.6 and 1.2 (per 100,000), respectively, for gallbladder cancer in China in 2008 (1). In urban Shanghai, age-adjusted incidence rates of biliary tract cancers were 1.7 and 1.2 for females and males, respectively during the period 1972 to 1974, but increased to 4.6 and 3.1 between 1996 to 1999 (2). These incidence rates were higher than the average incidence rate in China. Reasons for this regional difference as well as the rapid rise in incidence in urban Shanghai are unclear.

Gallstones are one of the most important risk factors for biliary tract cancers. In Shanghai, ever having gallstones were associated with a 23.8-fold increased risk of gallbladder cancer, an 8.0-fold increased risk of extrahepatic bile duct cancer and a 4.2-fold increased risk of ampulla of Vater cancer (3). Cholesterol supersaturation of bile is an important risk factor for the formation of cholesterol stones, and a variant in the cholesterol metabolism gene (*ABCG8* D19H), the same risk variant identified in GWAS (Genome-wide association studies) studies of gallstones, was significantly associated with biliary tract cancer risk in the Shanghai population (4). Another important risk factor for gallstone formation is impaired gallbladder motility since hypomotility of the gallbladder leads to stagnant bile which creates an environment for cholesterol supersaturation and subsequently gallstone formation (5). Gallbladder motility is in part controlled by *CCK* (Cholecystokinin), which is a gastrointestinal peptide released from the upper small intestine that mediates gallbladder emptying through *CCK* type-A receptor (*CCKAR*) (6).

To help clarify whether the effects of *CCK* and *CCKAR* genes are related to biliary tract cancer risk, we examined the associations of five SNPs in *CCK* (rs747455, rs8192473, rs1799923, rs3774396 and rs10865918) and four SNPs in *CCKAR* (rs2071011, rs915889, rs3822222 and rs1800855) with the risk of biliary tract cancers in a population-based case-control study conducted in Shanghai, China.

Materials and methods

Study subjects

The details of the study methods have been described in detail elsewhere (3,7,8). Briefly, incident cancer cases were identified by a rapid reporting system established by the Shanghai Cancer Institute and 42 collaborating hospitals in Shanghai. Through this system, we identified more than 95% of all incident biliary tract cancer cases (International Classification of Diseases, Ninth Edition code 156) diagnosed among urban Shanghai residents between June 1997 and May 2001. A total of 627 incident biliary tract cancer cases were identified. For this study, we included cases who were between 35 and 74 years old, completed the in-person interview and provided a blood sample which left 439 (70.0%) incident biliary tract cancer cases (253 gallbladder, 133 extrahepatic bile duct and 53 ampulla of Vater cancer cases). Population controls, without a history of cancer, were randomly selected from the Shanghai Resident Registry, which includes the records for approximately 6 million Shanghai residents. The controls were frequency matched to the

cancer cases by age (by 5 year age groups) and gender. Patients with biliary stones, without a history of cancer and undergoing cholecystectomy or medical treatment, were also included and were frequency matched to the cancer cases by age (5 year age groups), gender and hospital of diagnosis. A total of 429 biliary stone patients and 447 population controls were included. The study was approved by IRBs of the Shanghai Cancer Institute, and all participants gave written informed consent.

Gallstone assessment

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

Interview

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

Genotyping

Tagging single nucleotide polymorphisms (SNPs) were selected for this study by searching data from Han Chinese from the HapMap project using the Tagger program (9), using the following criteria: (a) a minor allele frequency ≥ 0.1 , since the sample size in the subgroups was relatively small; and (b) using an r^2 threshold of 0.90. A total of 9 tagging SNPs from the two genes were identified (*CCK* rs747455, rs8192473, rs1799923, rs3774396 and rs10865918; *CCKAR* rs2071011, rs915889, rs3822222 and rs1800855).

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

Statistical analysis

For all analyses, gallbladder cancer cases were compared with population controls without a history of cholecystectomy ($n = 422$), bile duct cancer cases and ampulla of Vater cancer cases were compared with all population controls ($n = 447$) and biliary stone cases were compared with population controls without biliary stones ($n = 341$). Distributions of selected characteristics including age, gender, cigarette smoking, prevalent gallstones, alcohol

drinking, and diabetes were compared between cases and controls by χ^2 test. The odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between the SNPs and biliary tract cancer and stone risk were estimated by unconditional logistic regression. P values (from the additive model) of less than 0.0056 before adjustment were deemed to be statistically significant after Bonferroni correction. Additional multiple logistic regression models, adjusted for potential confounding factors, were created to test the independence of the candidate SNPs on cancer and stone risk. All statistical analyses were conducted using SAS 9.0. Hardy–Weinberg equilibrium for genotypic distribution and linkage disequilibrium between loci were assessed by HaploView version 4.0 (Daly Lab at the Board Institute, Cambridge, MA, USA) (10). Associations between haplotypes (>1% frequency) and the risks of biliary tract cancers and stones were evaluated by computing OR and 95% CI using HAPSTAT, assuming an additive model, using the most common haplotype as the referent category (11). Global differences in haplotype frequencies between cases and controls were assessed for each gene using the Score test in HAPSTAT.

Results

Selected characteristics of the study subjects are shown in Table 1. Gallbladder cancer cases were more likely to be female and have prevalent gallstones than controls without a history of cholecystectomy. Bile duct and ampulla of Vater cancer cases were more likely to be male, smoke cigarettes, and have prevalent gallstones than all population controls. Bile duct cancer cases were also more likely to drink alcohol than all population controls. Biliary stone cases were more likely to be younger, but less likely to drink alcohol compared with controls without stones.

The associations of the *CCK* and *CCKAR* variants with biliary tract cancers and stones are presented in Table 2. The genotype distributions of these nine SNPs showed no deviation from the expected Hardy–Weinberg equilibrium among controls ($P > 0.05$). Of these SNPs, the *CCKAR* rs1800855 AA genotype conferred increased risk of gallbladder cancer (OR = 1.68, 95% CI: 1.07–2.64), although this association did not remain significant after adjusting for age, gender and gallstone status in multivariate models. TT or TT/CT genotype of *CCKAR* rs915889 had a higher risk of ampulla of Vater cancer (OR = 2.72, 95% CI: 1.18–6.25 and OR = 1.99, 95% CI: 1.04–3.80) compared with the CC genotype, but were not significant after Bonferroni correction ($P > 0.0056$). No significant association was found between the other SNPs and risk for biliary tract cancers or stones.

Because women are more likely to be diagnosed with gallbladder cancer than men, we conducted stratified analysis to determine if genetic susceptibility to gallbladder cancer differ by gender. The results showed that three SNPs in the *CCKAR* gene had significant interactions with gender on the risk of gallbladder cancer (Table 3). Specifically, the mutations are *CCKAR* rs2071011 ($P_{\text{interaction}} = 0.004$ in codominant model), rs915889 ($P_{\text{interaction}} = 0.007$ in codominant model), and rs1800855 ($P_{\text{interaction}} = 0.04$ in recessive model). The rs207011 CC and rs915889 TT/CT genotypes were associated with decreased gallbladder cancer risk, while the rs1800855 AA and AA/AT genotypes were related to increased gallbladder cancer risk in females. For males, in the *CCKAR* gene, the rs2071011 GC and CC/GC and rs915889 TT and TT/CT genotypes were associated with increased gallbladder cancer risk. However, only the association between the rs1800855 AA genotype (OR = 2.37, 95% CI: 1.36–4.14) and gallbladder cancer risk in females remained statistically significant after Bonferroni correction ($P < 0.0056$) and after adjustment for other risk factors. No statistically significant associations were found for the other six SNPs (data not shown).

The four SNPs in the *CCKAR* gene were in linkage disequilibrium with D' ranging from 0.97 to 1.00 and r^2 ranging from 0.08 to 0.56. Table 4 presents the associations between *CCKAR* haplotypes and gallbladder cancer risk. Carriers of the *CCKAR* (rs2071011-rs915889-rs3822222-rs1800855) G-C-T-T haplotype had a reduced risk of gallbladder cancer (OR = 0.67, 95% CI, 0.49–0.92) relative to the G-C-C-A haplotype carriers. After stratification by gender, female carriers of the C-T-C-T haplotype had a reduced gallbladder cancer risk (OR = 0.61, 95% CI, 0.43–0.86), which was still significant after Bonferroni correction and adjustment for other risk factors. These results were consistent with the individual SNP results. No significant associations were found among males.

Discussion

In the present study, we investigated the associations between 9 SNPs in two genes (*CCK* and *CCKAR*) and the risk of biliary tract cancers. Our results suggest that one variant (rs1800855) and haplotype (C-T-C-T) in the *CCKAR* gene were significantly associated with gallbladder cancer risk in females. To our knowledge, this is the first report of associations of *CCK* and *CCKAR* tagging SNPs with susceptibility to biliary tract cancers and stones.

Our finding that variations in *CCKAR* are related to risk for biliary tract cancer is consistent with previous studies that have linked *CCKAR* with gallbladder disease. *CCKAR* is one of the G-protein-coupled receptors that mediates the CCK-controlled gallbladder emptying and release of digestive enzymes from the pancreas into the digestive tract (12). All these physiological actions are mediated by G-protein-coupled receptors, including *CCKAR* and *CCK* type-B receptor (*CCKBR*). *CCKARs* are predominantly distributed in the gallbladder, pancreas and brain, while *CCK-B/gastrin* receptors are present in the gut mucosa and brain (13). Studies have shown that lowered expression of *CCKAR* contributes to the hypomotility of the gallbladder in gallstone patients (14), and aberrant *CCKAR* expression is associated with disease progression from cholelithiasis to early carcinoma (15). In addition, a case-control study found that *CCKAR* expression in the gallbladder was significantly higher in gallbladder cancer patients compared to gallstone controls (76.6% versus 44.1%, respectively) (16), while another study found that expression of *CCKAR* was significantly decreased in gallbladders with gallstones compared to those without gallstones (17). Thus, our finding that genetic susceptibility to gallbladder cancer due to variations in *CCKAR* is biologically plausible.

Studies considering mutations in the *CCK* and *CCKAR* genes have mainly focused on diseases related to the central and peripheral nervous system, including schizophrenia, panic disorder, Parkinson's disease and smoking behavior (18–21). Studies on biliary tract-related diseases are rare, with only one study by Srivastava et al. (22) that reported an association of the *CCKAR* Pst I mutation with an increased risk of gallstones (OR = 2.25, 95% CI: 1.2–4.1).

Gallbladder cancer is a highly aggressive disease which has a threefold higher incidence in females (23, 24). In our study, *CCKAR* rs2071011, rs915889 and rs1800855 had significant interactions with gender on the risk of gallbladder cancer. We found that rs1800855 was associated with gallbladder cancer risk only in females and was associated with a non-significant elevated risk of gallstones in females (OR = 1.51, CI: 0.84–2.72). The same interaction was found between the C-T-C-T (rs2071011, rs915889, rs3822222, rs1800855) haplotype and gender in gallbladder cancer ($P_{\text{interaction}} = 0.004$ in dominant model). Rs1800855 is located in intron 1 of the *CCKAR* gene and was previously reported to have an inverse association with panic disorder among women for the rs1800855/rs1800857

haplotype (25). No previous research has proposed an underlying mechanism for these associations.

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

Overall, our results suggest that genetic variation in the *CCKAR* gene may play a role in gallbladder cancer pathogenesis, in particular among women. Future epidemiologic studies with larger sample sizes as well as functional studies are needed to confirm our findings.

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Table 1

Distributions of selected variables by case-control status

Selected Characteristics	Controls, n(%)	Biliary Stones [†] , n(%)	Biliary tract cancers		
			Gallbladder [‡] , n(%)	Bile duct [§] , n(%)	Ampulla of Vater [§] , n(%)
All subjects	447 (100)	429 (100)	253 (100)	133 (100)	53 (100)
Age at interview					
34–54	58 (13.0)	124 (28.9)	39 (15.4)	21 (15.8)	5 (9.4)
55–64	123 (27.5)	118 (27.5)	61 (24.1)	36 (27.1)	12 (22.7)
65–75	266 (59.5)	187 (43.6)*	153 (60.5)	76 (57.1)	36 (67.9)
Gender					
Male	178 (39.8)	159 (37.1)	68 (26.9)	79 (59.4)	29 (54.7)
Female	269 (60.2)	270 (62.9)	185 (73.1)*	54 (40.6)*	24 (45.3)*
Ever smoke					
Yes	145 (32.4)	121 (28.2)	66 (26.2)	60 (45.1)	25 (47.2)
No	302 (67.6)	308 (71.8)	186 (73.8)	73 (54.9)*	28 (52.8)*
Prevalent gallstones					
Yes	106 (23.7)	429 (100.0)	214 (84.6)	89 (66.9)	29 (54.7)
No	341 (76.3)	none detected	39 (15.4)*	44 (33.1)*	24 (45.3)*
Ever drink alcohol					
Yes	91 (20.4)	70 (16.3)*	40 (15.8)	40 (30.1)	14 (26.4)
No	356 (79.6)	359 (83.7)	213 (84.2)	93 (69.9)*	39 (73.6)
Diabetes					
Yes	65 (14.6)	69 (16.2)	26 (10.3)	25 (18.9)	7 (13.2)
No	381 (85.4)	356 (83.8)	227 (89.7)	107 (81.1)	46 (86.8)

Note. Total number of subjects may vary due to missing values.

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

[‡] Gallbladder cancer cases compared with population controls who had a gallbladder (n = 422).

[§] Bile duct and A.V. cancer cases compared with all population controls (n = 447).

* P < 0.05.

Table 2

Associations between *CCK* and *CCKAR* genotypes with the risk of biliary tract cancers and stones.

Genotype	Controls		Biliary stones [†]		Gallbladder [‡]		Bile duct [§]		Ampulla of Vater [§]	
	n		n	OR(95%CI) ¶	n	OR(95%CI) ¶	n	OR(95%CI) ¶	n	OR(95%CI) ¶
<i>CCK</i>										
rs747455										
TT	131	116	1.00		61	1.00	35	1.00	17	1.00
TC	207	211	1.18 (0.84–1.67)		129	1.33 (0.91–1.95)	65	1.18 (0.74–1.90)	23	0.88 (0.45–1.71)
CC	105	102	1.16 (0.77–1.74)		63	1.27 (0.81–1.97)	31	1.16 (0.67–2.02)	13	1.00 (0.46–2.16)
TC+CC	312	313	1.18 (0.85–1.62)		192	1.31 (0.91–1.87)	96	1.18 (0.75–1.83)	36	0.92 (0.49–1.70)
<i>P</i> _{trend}			0.61			0.33		0.50		0.91
rs8192473										
GG	338	330	1.00		193	1.00	99	1.00	42	1.00
GA	104	98	1.04 (0.74–1.47)		57	0.97 (0.66–1.41)	34	1.16 (0.73–1.82)	10	0.79 (0.38–1.64)
AA	4	1	data not shown		3	data not shown	data not shown	data not shown	data not shown	data not shown
GA+AA	108	99	1.02 (0.72–1.43)		60	0.99 (0.68–1.44)	34	1.11 (0.71–1.75)	10	0.75 (0.36–1.56)
<i>P</i> _{trend}			0.53			0.75		data not shown		data not shown
rs1799923										
TT	221	204	1.00		112	1.00	63	1.00	29	1.00
TC	180	186	1.12 (0.82–1.52)		116	1.28 (0.92–1.78)	57	1.08 (0.71–1.63)	18	0.76 (0.41–1.42)
CC	45	39	0.92 (0.55–1.52)		25	1.08 (0.62–1.87)	13	1.11 (0.56–2.21)	5	0.91 (0.33–2.51)
TC+CC	225	225	1.08 (0.81–1.44)		141	1.24 (0.90–1.70)	70	1.08 (0.73–1.61)	23	0.79 (0.44–1.41)
<i>P</i> _{trend}			0.66			0.34		0.62		0.68
rs3774396										
GG	319	317	1.00		189	1.00	95	1.00	41	1.00
GA	121	110	0.99 (0.71–1.37)		60	0.85 (0.591.22)	37	1.07 (0.69–1.67)	11	0.73 (0.36–1.48)
AA	6	2	data not shown		4	data not shown	1	data not shown	data not shown	data not shown
GA+AA	127	112	0.97 (0.70–1.34)		64	0.87 (0.61–1.24)	38	1.05 (0.68–1.62)	11	0.69 (0.34–1.39)
<i>P</i> _{trend}			0.73			0.58		0.93		data not shown
rs10865918										
TT	347	323	1.00		188	1.00	100	1.00	43	1.00

Genotype	Controls		Biliary stones [†]		Gallbladder [‡]		Bile duct [§]		Ampulla of Vater [§]	
	n	n	OR(95%CI)	¶	n	OR(95%CI)	¶	n	OR(95%CI)	¶
TG	90	102	1.40 (0.98–2.01)	61	1.28 (0.87–1.87)	32	1.24 (0.78–1.98)	8	0.71 (0.32–1.58)	
GG	9	4	data not shown	4	data not shown	1	data not shown	1	data not shown	
TG+GG	99	106	1.31 (0.92–1.86)	65	1.24 (0.86–1.79)	33	1.16 (0.74–1.84)	9	0.72 (0.34–1.53)	
<i>P_{trend}</i>			0.087		0.43		0.42		0.68	
CCKAR										
rs2071011										
GG	256	260	1.00	145	1.00	76	1.00	24	1.00	
GC	155	146	0.96 (0.70–1.31)	94	1.09 (0.78–1.52)	49	1.16 (0.76–1.76)	22	1.58 (0.85–2.93)	
CC	35	23	0.60 (0.33–1.08)	14	0.68 (0.35–1.31)	8	0.86 (0.38–1.95)	6	2.09 (0.78–5.57)	
GC+CC	190	169	0.89 (0.66–1.19)	108	1.01 (0.73–1.38)	57	1.10 (0.74–1.64)	28	1.67 (0.93–2.99)	
<i>P_{trend}</i>			0.23		0.38		0.80		0.19	
rs915889										
CC	183	176	1.00	108	1.00	54	1.00	14	1.00	
CT	199	197	0.98 (0.71–1.33)	110	0.91 (0.65–1.28)	62	1.11 (0.73–1.70)	26	1.78 (0.90–3.52)	
TT	64	56	0.84 (0.53–1.32)	35	0.90 (0.55–1.46)	17	0.99 (0.53–1.85)	12	2.72 (1.18–6.25)	
CT+TT	263	253	0.94 (0.70–1.27)	145	0.91 (0.66–1.25)	79	1.08 (0.72–1.62)	38	1.99 (1.04–3.80)	
<i>P_{trend}</i>			0.74		0.83		0.80		0.052	
rs3822222										
CC	269	272	1.00	171	1.00	81	1.00	37	1.00	
CT	154	138	0.87 (0.64–1.19)	77	0.79 (0.56–1.10)	49	1.07 (0.71–1.62)	10	0.48 (0.23–1.00)	
TT	23	19	1.08 (0.52–2.22)	5	0.37 (0.14–1.01)	3	data not shown	5	1.58 (0.56–4.46)	
CT+TT	177	157	0.89 (0.66–1.21)	82	0.73 (0.53–1.02)	52	0.99 (0.66–1.48)	15	0.62 (0.33–1.18)	
<i>P_{trend}</i>			0.66		0.058		0.85		0.056	
rs1800855										
TT	151	133	1.00	71	1.00	39	1.00	21	1.00	
TA	221	215	1.08 (0.78–1.50)	124	1.21 (0.84–1.75)	73	1.22 (0.78–1.91)	28	0.87 (0.47–1.59)	
AA	74	81	1.25 (0.82–1.92)	58	1.68 (1.07–2.64)	21	0.98 (0.53–1.80)	3	data not shown	
TA+AA	295	296	1.12 (0.82–1.53)	182	1.33 (0.94–1.88)	94	1.16 (0.75–1.78)	31	0.71 (0.39–1.29)	
<i>P_{trend}</i>			0.59		0.08		0.27		0.048	

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

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

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

[¶]Adjusted for age and gender.

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

Did not calculate OR when N was less than 5.

Table 3

Associations between selected *CCKAR* genotypes with the risk of gallbladder cancer stratified by gender.

Genotype	Males			Females		
	Controls, n	Cases, n	OR (95%CI) ^b	Controls, n	Cases, n	OR (95%CI) ^b
rs2071011						
GG	104	29	1.00	137	116	1.00
GC	55	33	2.17 (1.19–3.95) *	91	61	0.79 (0.52–1.19)
CC	10	6	2.16 (0.72–6.43)	25	8	0.39 (0.17–0.91) *
GC+CC	65	39	2.17 (1.22–3.85) **	115	69	0.71 (0.48–1.04)
<i>P</i> _{trend}			0.028			0.056
rs915889						
CC	75	20	1.00	94	88	1.00
CT	74	35	1.80 (0.95–3.42)	117	75	0.68 (0.45–1.03)
TT	20	13	2.47 (1.05–5.81) *	41	22	0.57 (0.32–1.04)
CT+TT	94	48	1.95 (1.06–3.57) *	158	97	0.65 (0.45–0.96) *
<i>P</i> _{trend}			0.067			0.082
rs1800855						
TT	52	22	1.00	91	49	1.00
TA	82	34	0.98 (0.52–1.86)	125	90	1.34 (0.86–2.08)
AA	35	12	0.81 (0.35–1.84)	36	46	2.37 (1.36–4.14) ***
TA+AA	117	46	0.93 (0.51–1.70)	161	136	1.57 (1.04–2.38) *
<i>P</i> _{trend}			0.86			0.009

Gallbladder cancer cases were compared with population controls who had never had a cholecystectomy (N =422).

[†] Adjusted for age.

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

* P < 0.05.

** P < 0.01.

*** P < 0.0056 (Bonferroni corrected threshold)

Table 4

Associations between the *CCKAR* haplotypes and gallbladder cancer risk

Haplotype [‡]	Whole population			Female population			Male population		
	Controls, %	Cases, %	OR (95%CI) [‡]	Controls, %	Cases, %	OR (95%CI) [§]	Controls, %	Cases, %	OR (95%CI) [§]
G-C-C-A	41.1	47.2	1.00	38.9	48.9	1.00	43.9	42.6	1.00
C-T-C-T	24.8	23.9	0.83 (0.63–1.10)	27.2	20.5	0.61(0.43–0.86) *	24.6	33.1	1.59(0.99–2.58)
G-C-T-T	21.4	17.0	0.67(0.49–0.92)	21.4	18.6	0.68(0.46–0.98)	18.7	12.5	0.60(0.32–1.11)
G-T-C-T	11.8	11.3	0.83(0.57–1.20)	11.9	11.1	0.75(0.48–1.16)	11.6	11.8	1.04(0.53–2.04)

[‡] In the order of rs2071011, rs915889, rs3822222, rs1800855.[‡] Adjusted for age and gender.[§] Adjusted for age.***
P < 0.0056 (Bonferroni corrected threshold).