Cancer Science
Japanese Cancer Association JCA
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Genetic variants at 8q24 are associated with risk of esophageal squamous cell carcinoma in a Chinese population

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Key words

8q24, esophageal cancer, gastric cancer, genome-wide association studies, PSCA

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Funding information

National Basic Research Program (973) (2013CB910304). National Natural Science Foundation of China (81230067, 81373090, 81202267, 81101889). Jiangsu Natural Science Foundation (BK2012443, BK2012841). Science Foundation for Distinguished Young Scholars in Jiangsu (BK20130042). Doctoral Fund of Ministry of Education of China (20123234120003). Jiangsu Province Clinical Science and Technology Projects (Clinical Research Center, BL2012008). Priority Academic Program for the Development of Jiangsu Higher Education Institutions (Public Health and Preventive Medicine).

Received December 9, 2013; Revised March 6, 2014; Accepted March 17, 2014

Cancer Sci **105** (2014) 731–735

doi: 10.1111/cas.12399

Esophageal cancer and gastric cancer have shared risk factors and inherited susceptibility. Recent genome-wide association studies have identified multiple genetic loci associated with gastric cancer risk, which may also involve in the development of esophageal cancer. Herein, we evaluated the relationship of gastric cancer risk-related variants at 1q22, 3q13.3, 5p13.1, and 8q24 with the risk of esophageal squamous cell carcinoma (ESCC) in a Chinese population with a case–control study (2139 cases and 2273 controls). We found that the T allele of rs2294008, an intronic variant of the *PSCA* gene at 8q24 that was previously associated with an increased risk of gastric cancer, was inversely associated with a decreased risk of ESCC (odds ratio = 0.90; 95% confidence interval, 0.81–0.99; *P* = 0.034). Of interest, the association of rs2294008 with ESCC was consistent with that observed in esophageal adenocarcinoma and ESCC in Caucasian populations. However, no significant associations were observed for the other three variants at 1q22 (rs4072037), 3q13.31 (rs9841504), and 5p13.1 (rs13361707). Our findings suggest that the susceptibility locus of *PSCA* at 8q24 may be a double-edged sword, as modulator between the carcinogenesis processes of stomach and esophagus.

Esophageal cancer is the eighth most common cancer and the sixth leading cancer death around the world, with an estimated 482 300 new cases and 406 800 deaths from EC in 2008.[1] Notably, more than half of the global cases and deaths occur in China (259 000 new cases and 211 000 deaths).[2] As one of the major upper gastrointestinal tract cancers, EC shares several known risk factors with GC, including alcohol consumption, high temperature of food and beverage, tobacco smoking, poor nutrition, family history[3] and even *Helicobacter pylori* infection,[4,5] suggesting these two types of cancer may share, at least in part, a common carcinogenesis mechanism. This may explain the coherent distribution of

EC and GC, such as the high incidence of both cancers in China.

In addition to these common risk factors, EC and GC may have shared genetic determinants as exposed to these risk factors. This has been supported by recent findings from genome-wide association studies (GWAS) in Chinese populations.[6,7] In a three-stage GWAS, Wang *et al.*[6] identified two new loci at chromosomes 10q23 and 20p13 associated with the risk of ESCC in Chinese populations. Interestingly, both of these two loci were also associated with the risk of GCA with similar effects.[6] In the same period, Abnet *et al.*[7] carried out a GWAS of ESCC and GC in Chinese populations, and also found that the susceptibility locus at 10q23 was shared for both ESCC and GC.

In the past several years, several loci associated with GC have been identified by GWAS in Asian populations, including those at 1q22, 3q13.31, 5p13.1, 8q24, 10q23, and 20p13.[6–9] Except for 10q23 and 20p13, the potential roles of the other four loci are largely unknown in the development of EC. Therefore, we carried out a case–control study including 2139 ESCC cases and 2273 cancer-free controls to investigate the associations of genetic variants at 1q22, 3q13.31, 5p13.1, and 8q24 with the risk of ESCC in a Chinese population.

Materials and Methods

Study subjects. Patients with EC were consecutively recruited from January 2007 to June 2010 from the Jiangsu Tumor Hospital (Nanjing, China) and the First People's Hospital of Huai'an (Huai'an, China); both hospitals are located in Jiangsu province, a high incidence area of EC. Those cases histopathologically diagnosed as ESCC were included in this study. The cases were excluded if they had a history of any cancer before ESCC diagnosis or had undergone radiotherapy or chemotherapy prior to recruitment. As a result, a total of 2139 incident ESCC cases were included in this study. Controls were randomly selected from a pool of more than 40 000 cancer-free individuals who participated in the community-based screening program for non-infectious diseases carried out in Jiangsu province. After being frequency-matched to the cases on age (5-year interval) and gender, a total of 2273 controls were included in this study. All of the subjects were genetically unrelated ethnic Han Chinese. After signing informed consent, each subject was interviewed face-to-face with a standard questionnaire including information about demographic information and relevant risk factors, such as tobacco smoking and alcohol drinking. Smokers were defined as those who smoked at least once a day for more than 1 year, and drinkers were those who drank two or more times per week over 1 year. The characteristics of cases and controls are shown in Table S1. Cases and controls were adequately matched on age and gender. The cases had higher proportions of smokers and drinkers as compared with controls. Approximately 5 mL venous blood was obtained from each subject. This study was approved by the institutional review board of Nanjing Medical University (Nanjing, China).

Polymorphism selection and genotyping assays. Based on reported GWAS on GC, rs4072037 at 1q22, rs9841504 at 3q13.31, rs13361707 at 5p13.1, rs2294008 and rs2976392 at 8q24, rs2274223 at 10q23, and rs13042395 at 20p13 are associated with GC risk at genome-wide significance level or well replicated in independent studies.[6–10] The two loci of 10q23 and 20p13 had been associated with ESCC risk,[6,7] and were not subject to further evaluation in this study. As the two SNPs rs2294008 and rs2976392 at 8q24 are in strong linkage disequilibrium (LD) in Chinese populations,[11] we only selected rs2294008 in the current study. Finally, we genotyped four SNPs in this study, including rs4072037 at 1q22 associated with the *MUC1* gene, rs9841504 at 3q13.31 associated with the *ZBTB20* gene, rs13361707 at 5p13.1 associated with the *PRKAA1* gene, and rs2294008 at 8q24 associated with the *PSCA* gene (Table S2).

Genotyping was carried out using the TaqMan allelic discrimination assay on the ABI 7900 system (Applied Biosystems, Foster City, CA, USA). Two negative controls were included in each 384-well plate for quality control. Detailed information regarding the primers and probes is shown in Table S3. Technicians were blinded to the status of subjects (case or control) when carrying out the genotyping. The genotypes were called using the SDS 2.3 Allelic Discrimination Software (Applied Biosystems). The accordance rate of each SNP was 100% for the duplicates of 5% of randomly selected samples. The genotyping call rates were more than 95% for all four SNPs, and the observed genotype distributions among controls were all consistent with the Hardy–Weinberg equilibrium (Table S2).

Statistical analysis. The χ^2-test was used to analyze the distributions of demographic characteristics and relevant factors between cases and controls. The Hardy–Weinberg equilibrium was tested using a goodness-of-fit χ^2-test among the control subjects. Logistic regression analysis was used to test the associations between SNPs and ESCC risk, and also to estimate the ORs and 95% CIs with an adjustment for age, gender, smoking, and drinking status. The χ^2-based Q-test was used to test the heterogeneity of effect sizes (ORs and 95% CIs) between subgroups.[12] Multiplicative interaction was tested using a general logistic regression model by applying the equation:

$$Y = b_0 + b_1 \times A + b_2 \times B + b_3 \times (A \times B) + e \quad (1)$$

in which Y is the logit of case or control status, b_0 is the constant, A and B are the SNP and environment factors, b_1 and b_2 are the main effects of factor A and B respectively, and b_3 is the multiplicative interaction term. All the statistical analyses were carried out using STATA Version 12.0 software (Stata, College Station, TX, USA).

Results

The genotype distributions of the four SNPs between the cases and the controls are summarized in Table 1. In the additive model, the T allele of rs2294008 at 8q24 was significantly associated with a decreased risk of ESCC with a per-allele OR of 0.90 (95% CI, 0.81–0.99; $P = 0.034$). In the codominant model, individuals carrying either CT or TT genotype showed similar reduced ESCC risk (OR = 0.86 and 0.88, respectively) compared with those with CC genotype. However, no significant associations were observed for the other three SNPs with ESCC risk ($P = 0.181$, 0.908, and 0.666 for rs4072037 at 1q22, rs9841504 at 3q13.31, and rs13361707 at 5p13.1, respectively).

Moreover, in an effort to investigate the association between rs2294008 and ESCC risk in subgroups, we carried out stratification analyses on age, sex, and smoking and drinking status. Of interest, a stronger strength of association of rs2294008-T allele with ESCC risk was observed among non-drinkers (OR = 0.81; 95% CI, 0.70–0.93; $P = 2.61 \times 10^{-3}$) compared with that of drinkers (OR = 1.00; 95% CI, 0.86–1.17; $P = 0.998$) ($P = 0.045$ for the heterogeneity test). There were no significant differences of association for other groups (Table 2).

We carried out a further interactive analysis for rs2294008 genotypes and alcohol drinking on ESCC risk. As shown in Table 3, compared with non-drinkers with CC genotype, a

Table 1. Summary of associations between genetic variants and esophageal squamous cell carcinoma risk

Location (gene)	SNP	Cases, *n* (%)	Controls, *n* (%)	OR (95% CI)	*P*-value†
1q22 (*MUC1*)	rs4072037	*n* = 2072	*n* = 2204		
	AA	1415 (68.3)	1553 (70.5)	1.00	
	AG	602 (29.1)	596 (27.0)	1.11 (0.96–1.27)	0.152
	GG	55 (2.7)	55 (2.5)	1.07 (0.73–1.58)	0.718
	G allele	712 (17.2)	706 (16.0)	1.08 (0.96–1.22)	0.181
3q13.31 (*ZBTB20*)	rs9841504	*n* = 2075	*n* = 2232		
	CC	1513 (72.9)	1629 (73.0)	1.00	
	CG	515 (24.8)	560 (25.1)	0.96 (0.84–1.11)	0.613
	GG	47 (2.3)	43 (1.9)	1.14 (0.74–1.75)	0.550
	G allele	609 (14.7)	646 (14.5)	0.99 (0.88–1.12)	0.908
5p13.1 (*PRKAA1*)	rs13361707	*n* = 2072	*n* = 2254		
	TT	558 (26.9)	603 (26.7)	1.00	
	TC	1054 (50.9)	1144 (50.8)	0.98 (0.85–1.13)	0.760
	CC	460 (22.2)	507 (22.5)	0.96 (0.81–1.15)	0.669
	C allele	1974 (47.6)	2158 (47.9)	0.98 (0.90–1.07)	0.666
8q24 (*PSCA*)	rs2294008	*n* = 2083	*n* = 2220		
	CC	1232 (59.1)	1222 (55.0)	1.00	
	CT	724 (34.8)	851 (38.3)	0.86 (0.76–0.98)	0.023
	TT	127 (6.1)	147 (6.6)	0.88 (0.68–1.13)	0.305
	T allele	978 (23.5)	1145 (25.8)	0.90 (0.81–0.99)	0.034

†Derived from logistic regression with an adjustment for age, sex, and smoking and drinking status. OR, odds ratio; SNP, single nucleotide polymorphism.

Table 2. Stratified analyses of association between rs2294008 at 8q24 and esophageal squamous cell carcinoma risk

Variables	rs2294008 (CC/CT/TT)		OR (95% CI)	*P*-value†	*P*-value‡
	Cases	Controls			
Age, years					
<60	539/322/55	533/389/67	0.87 (0.75–1.01)	0.078	0.647
≥60	693/402/72	689/462/80	0.92 (0.80–1.05)	0.205	
Sex					
Male	896/531/102	896/643/113	0.90 (0.80–1.01)	0.061	0.772
Female	336/193/25	326/208/34	0.86 (0.69–1.09)	0.212	
Smoking status					
Never	572/338/46	597/427/72	0.83 (0.71–0.97)	0.022	0.238
Ever	660/386/81	625/424/75	0.94 (0.82–1.07)	0.358	
Drinking status					
Never	569/331/55	760/561/99	0.81 (0.70–0.93)	2.61×10^{-3}	0.045
Ever	662/393/72	462/290/48	1.00 (0.86–1.17)	0.998	

†Derived from the additive model (minor homozygote *vs*. heterozygote *vs*. major homozygote) using logistic regression analysis with an adjustment for age, sex, and smoking or drinking status where appropriate. ‡*P*-values were from the heterogeneity test based on the χ^2-based Q-test. CI, confidence interval; OR, odds ratio.

Table 3. Interaction between rs2294008 genotypes and alcohol drinking on esophageal squamous cell carcinoma risk

rs2294008	Drinking status	Cases	Controls	OR (95% CI)	*P*†
CC	Never	569	760	1.00	
CT	Never	331	561	0.79 (0.67–0.95)	0.010
TT	Never	55	99	0.75 (0.53–1.07)	0.112
CC	Ever	662	462	1.96 (1.66–2.31)	8.28×10^{-16}
CT	Ever	393	290	1.86 (1.54–2.25)	1.12×10^{-10}
TT	Ever	72	48	2.05 (1.40–3.01)	2.24×10^{-4}
Multiplicative interaction					0.091

†Derived from logistic regression with an adjustment for age, sex, and smoking status. CI, confidence interval; OR, odds ratio.

decreased risk of ESCC was observed for those with CT or TT genotypes, whereas a significant increased risk was observed in drinkers with similar ORs among different genotype carriers. However, no significant interaction between rs22940078 genotypes and alcohol drinking was detected on the risk of ESCC ($P = 0.091$).

Discussion

In the current study, in an effort to explore the potential roles of genetic loci associated with GC risk in the development of EC, we investigated the associations of genetic variants at 1q22, 3q13.31, 5p13.1, and 8q24 with the risk of ESCC in a Chinese case–control study with 2139 cases and 2273 controls.

We found that the T allele of rs2294008 was significantly associated with a reduced risk of ESCC, which indicated that genetic variants at 8q24 may be implicated with ESCC susceptibility. These findings may further improve our understanding on ESCC development.

In a two-stage GWAS, genetic variants at 8q24 were identified to be associated with GC risk in Japanese populations, especially for diffuse-type GC with an increased risk for the T allele of the lead SNP rs2294008 (OR = 1.67; 95%CI, 1.47–1.90).[8] This association was also replicated in a Korean case–control study of GC (OR = 1.91; 95%CI, 1.57–2.33).[8] Thereafter, the association between rs2294008 and GC risk was consistently reported in several independent studies in Asian or Caucasian populations and was further collectively confirmed in a meta-analysis.[13] Of interest, in a population-based case–control study in the USA with individuals of predominantly Caucasian ethnicity, those carrying the rs2294008-T allele were significantly associated with reduced risks of GCA (OR = 0.5; 95% CI, 0.3–0.9), EA (OR = 0.5; 95% CI, 0.3–0.9), and ESCC (OR = 0.4; 95% CI, 0.2–0.9) as compared with those with CC genotype, although the sample size was relatively small (123 GCA, 107 EA, 52 ESCC, and 211 matched controls). Consistent with those findings, we also observed a significantly decreased risk of ESCC for those with the rs2294008-T allele in a Chinese population with larger sample size (2139 cases and 2273 controls), which was clearly opposite to that observed in GC. This discrepancy may reflect the opposing effect of genetic variants at 8q24 in regulating pathogenesis between proximal and distal upper gastroesophageal cancers. Nevertheless, additional independent studies in diverse populations merit further investigation on the association of 8q24 locus and proximal gastroesophageal cancer risk.

We also conducted an interactive analysis for rs2294008 genotypes and alcohol drinking on ESCC risk. The *P*-value was 0.091 for the multiplicative interaction, which did not support an interaction in a statistical scale. However, a significant association between rs2294008 and ESCC risk was only observed among non-drinkers. Compared with CC genotype with non-drinkers, a significantly decreased risk of ESCC was observed for those with CT or TT genotypes of non-drinkers whereas similar elevated risks were shown in all three groups with different genotypes of drinkers. These findings may indicate that rs2294008 acts as an effect modifier of alcohol drinking in the development of ESCC. Of course, large well-designed studies are warranted to reevaluate these findings.

The SNP rs2294008 is located on the 5′-UTR of *PSCA* at 8q24. Substitution of the C allele with the T allele at rs2294008 has been shown to reduce transcriptional activity of an upstream fragment of the *PSCA* gene. According to a web-based SNP analysis tool, SNPinfo (http://snpinfo.niehs.nih.gov/), the SNP rs2294008 may influence an exonic splicing enhancer or exonic splicing silencer and result in disequilibrium for different isoforms of *PSCA*. Moreover, analysis of the Encyclopedia of DNA Elements data as implemented in the online tool, RegulomeDB (http://regulomedb.org/), indicates that several SNPs in LD with rs2294008 ($r^2 > 0.8$) located in motifs may influence the binding of specified transcription factors (Table S4). Two SNPs rs1045574 and rs2976396 are both *cis* expression quantitative trait loci linked to the expression of *LYPD2*,[14] whereas the variants rs1045574, rs2976396, and rs2920282 fall into transcription factor binding sites or DNase I peaks. However, these data are still very preliminary, further experimental studies may clarify the potential functional variant(s) at 8q24 that modify the development of gastroesophageal cancer as causal variant (s).

Originally identified as a prostate-specific stem cell antigen,[15,16] PSCA was also reported to be expressed in the bladder, esophagus, and stomach.[17] The *PSCA* gene was found to be expressed in differentiating gastric epithelial cells, had cell-proliferation inhibition activity *in vitro*, and was frequently silenced in GC.[8] When stably transfecting *PSCA* cDNA into HSC57 (a *PSCA*-negative GC cell line), cells with *PSCA* showed fewer G418-resistant colonies than those without *PSCA*, indicating the cell proliferation inhibition and/or cell death induction activity of *PSCA*. Additionally, knockdown of *PSCA* in a bladder cancer cell line resulted in induction of inflammatory gene expression, accompanied by a reduction in cell growth.[18] The role of *PSCA* in tumorigenesis appears complex, involving protumorigenic and antitumorigenic effects in different contexts.[19] However, the evidence for the role of *PSCA* in esophageal carcinogenesis is still scarce, and biological studies in terms of *PSCA* function are warranted in further studies.

Our findings suggest that genetic variants at 1q22, 3q13.31, and 5p13.1 may not be important in the development of ESCC, though the obvious effects have been implicated in gastric carcinogenesis. Recently, an exploratory study reported that rs4072037 in *MUC1* at 1q22 was associated with a reduced risk of ESCC in Caucasian populations.[20] However, the small sample size (52 cases and 211 controls) may not represent sufficient statistical power. Moreover, there are still no other studies investigating the associations between ESCC risk and genetic variants at 3q13.31 and 5p13.1. Therefore, well-designed studies with large sample sizes may be required to further evaluate the potential effects of 1q22, 3q13.31, and 5p13.1 loci in EC development, especially for EA.

In summary, in a relatively large case–control study in a Chinese population, we reported an inverse association for genetic variants at 8q24 with ESCC risk compared with that for GC. The GC risk-related loci at 1q22, 3q13.31, and 5p13.1 were not involved in ESCC risk. Our findings may indicate the difference in terms of genetic determinants between proximal and distal upper gastroesophageal cancers.

Acknowledgments

This study was supported in part by the National Basic Research Program (973) (2013CB910304), the National Natural Science Foundation of China (81230067, 81373090, 81202267, 81101889), the Jiangsu Natural Science Foundation (BK2012443, BK2012841), the Science Foundation for Distinguished Young Scholars in Jiangsu (BK20130042), the Doctoral Fund of the Ministry of Education of China (20123234120003), Jiangsu Province Clinical Science and Technology Projects (Clinical Research Center, BL2012008), and the Priority Academic Program for the Development of Jiangsu Higher Education Institutions (Public Health and Preventive Medicine).

Disclosure Statement

The authors have no conflict of interest.

Abbreviations

CI	confidence interval
EA	esophageal adenocarcinoma
EC	esophageal cancer

ESCC	esophageal squamous cell carcinoma
GC	gastric cancer
GCA	gastric cardia adenocarcinoma
GWAS	genome-wide association studies
LD	linkage disequilibrium
OR	odds ratio
PSCA	prostate stem cell antigen
SNP	single nucleotide polymorphism

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Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1. Characteristics of esophageal squamous cell carcinoma cases and controls.

Table S2. Selected single nucleotide polymorphism (SNP) information and genotyping results.

Table S3. Primers and probes for genotyping.

Table S4. Functional annotation for single nucleotide polymorphisms (SNPs) in linkage disequilibrium ($r^2 > 0.8$) with the identified SNP rs2294008 based on Regulome DB.