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Original Article

Genetic variants at 6p21, 10q23, 16q21 and 22q12 are associated with esophageal cancer risk in a Chinese Han population

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Abstract: Objective: A number of recently published genome-wide association studies (GWAS) identified several genetic loci at 6p21, 10q23, 16q12 and 22q12 that were associated with digestive tract tumors, including esophageal cancer (EC). We conducted a case-control study in a Chinese Han population including 360 EC cases and 310 controls to evaluate whether these variants are related to EC susceptibility. Methods: All these SNPs were genotyped using Sequenom Mass-ARRAY technology. For each SNP, genotypic frequencies in controls were tested for departure from Hardy-Weinberg Equilibrium (HWE) using an exact test. A *P*-value of 0.05 was considered the threshold for statistical significance. We compared the allele frequencies of cases and controls using the chi-squared (χ^2) test. Associations between the gene and the risk of esophagus cancer were tested using various genetic models (co-dominant, dominant, recessive, and log-additive) and analysis by SNP stats. Odds ratios and 95% confidence intervals (CIs) were calculated by unconditional logistic regression with adjustments for age and gender. Results: We found significant association with risk of EC for five reported SNPs, including rs2274223 in PLCE1 at 10q23 [odds ratio (OR) = 1.390; 95% confidence interval (CI) = 1.075-1.798], rs10484761 near UNC5CL at 6p21 (OR = 1.422, 95% CI = 1.014-1.994), rs4785204 in HEATR3 at 16q12 (OR = 1.427; 95% CI = 1.116-1.824), rs4822983 in CHEK2 at 22q12 (OR = 1.361, 95% CI = 1.052-1.762), and rs738722 in CHEK2 at 22q12 (OR = 1.343, 95% CI = 1.053-1.713). Conclusion: Our findings, combined with previous studies, indicated that rs10484761 at 6p21, rs2274223 at 10q23, rs4785204 at 16q12, rs4822983 and rs738722 at 22q12 may be used as genetic biomarkers for EC susceptibility in Chinese Han population.

Keywords: Esophageal cancer, single nucleotide polymorphism, susceptibility

Introduction

Esophageal cancer is one of the most common cancers worldwide and, because of its high fatality rate, ranks sixth among all cancers in mortality [1, 2]. It affects more than 450000 people worldwide and the incidence is rapidly increasing [3]. In contrast to most Western countries, cancer of the esophagus is a common disease in many areas of China, especially in the North [4]. Survival rates for esophageal carcinoma are poor; 75% of patients die within 1 year after diagnosis, and the 5-year survival rate is only 5-10% [5].

EC can be histologically classified into two main forms: esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EA). The development of esophageal cancer is a multifactorial process associated with a variety of risk factors. Tobacco smoking and alcohol drinking have been repeatedly shown to be contributed to the etiology of EC; other risk factors include nutritional deficiencies, low intake of fruits and vegetables, dietary carcinogen exposure, and drinking beverages at high temperatures [6, 7]. In addition, genetic factors as well as environmental factors play a crucial role in the development of EC [8].

Recent studies have suggested that genetic polymorphisms may clarify the causes and events involved in esophageal carcinogenesis [5]. Over past few years, four genome-wide

Table 1. Basic characteristics of cases and controls in this study

Variables	Case N (%)	Control N (%)	*P*-value
Age (years)	60.7 ± 8.9	49.4 ± 7.9	< 0.001[a]
Sex			< 0.001[b]
Male	288 (80.0)	197 (63.5)	
Female	72 (20.0)	113 (36.5)	
Total	360	310	

[a]*P* values were calculated by Student t tests; [b]*P* values were calculated from two-sided chi-square tests.

association studies (GWAS) of ESCC have been conducted in Asian populations [6, 9-11], which were also well confirmed in Chinese populations [12, 13]. By far, several genetic variants associated with esophageal cancer have been identified by GWAS in Asians or Europeans, including rs10484761 at 6p21 [6], rs2274223 at 10q23 [10, 11], rs738722 and rs4822983 at 22q12 [11, 14], and rs4785204 at 16q12 [14]. These findings have greatly improved our understanding of genetic basis of EC, especially in Asian populations. Here, we performed a case-control study involving 360 esophageal cancer cases and 310 controls to investigate the association between the genetic variants identified above and the risk of esophageal cancer in a Chinese Han population.

Materials and methods

Study subjects

All patients and controls were members of the genetically unrelated Chinese Han population living in Xi'an City or nearby. The patients were recruited from the First Affiliated Hospital of the Medical College of Xi'an Jiao tong University between October 2011 and September 2013. All patients were newly diagnosed with esophageal cancer and were characterized histologically. None of them had a history of any cancers or had undergone radiotherapy or chemotherapy. Control subjects were randomly selected from the medical examination center of the Tang Du Hospital based on standard recruitment and exclusion criteria during the same period, and their medical history and a physical examination showed that they were in good health. As a result, a total of 360 cases and 310 controls were included in the study.

Blood sample was drawn from each individual and sent to the laboratory for immediate molecular analyses. All of the participants signed an informed consent agreement. The Human Research Committee for Approval of Research Involving Human Subjects, First Affiliated Hospital of the Medical College of Xi'an Jiao tong University, approved the use of human tissue in this study.

Polymorphisms selection and genotyping assays

All the SNPs were previously published to be associated with esophageal cancer, with minor allele frequencies >5% in the HapMap Chinese Han Beijing (CHB) population. Finally, 20 SNPs were selected for genotyping. DNA was extracted from whole blood samples by GoldMag-Mini Whole Blood Genomic DNA Purification Kit (GoldMag Co. Ltd. Xi'an City, China) and DNA concentration was measured by NanoDrop 2000 (Thermo Scientific, Waltham, MA, USA). The multiplexed SNP MassEXTENDED assay was designed using Sequenom MassARRAY Assay Design 3.0 Software [15]. Genotyping was done with the Sequenom MassARRAY RS1000 system using the standard protocol recommended by the manufacturer. Data management and analysis was done using Sequenom Typer 4.0 Software [15, 16].

Statistical analysis

The SPSS18.0 statistical software (SPSS, Chicago, IL, USA) and Microsoft Excel were used for statistical analysis. All *P* values obtained in this study were two-sided and we used $P \leq 0.05$ as the threshold of statistical significance. An exact test was used to assess the variation in each SNP frequency from Hardy-Weinberg equilibrium (HWE) in the control subjects. Differences in SNP genotype distribution between cases and controls were compared by χ^2 test [17]. We tested odds ratios (ORs) and constructed 95% confidence intervals (CIs) using unconditional logistic regression analysis with adjustments for age and gender [18].

The associations between the SNPs and the risk of esophageal cancer were tested using four different genetic models (co-dominant, dominant, recessive and log-additive) analysis by SNP-tats, website software from http://bioinfo.iconcologia.net. We calculated ORs and 95% CIs by unconditional logistic regression analysis adjusted for age and gender [18].

Table 2. Allele frequencies in cases and controls and odds ratio estimates for esophageal cancer

SNP ID	Gene(s)	Band	Alleles	MAF		HWE	ORs	95% CI		*P*-value
			A[a]/B	Case	Control	*P*-value				
rs4072037	MUC1	1q22	G/A	0.025	0.044	2.65E-16#	0.563	0.307	1.032	0.06
rs9288520	-	2q35	A/G	0.289	0.268	0.3097	0.111	0.873	1.414	0.39
rs9841504	ZBTB20	3q13.31	G/C	0.143	0.135	0.4716	1.069	0.783	1.458	0.675
rs2239612	ST6GAL1	3q27.3	T/C	0.186	0.193	0.5814	0.956	0.725	1.259	0.746
rs13361707	PRKAA1	5p13.1	C/T	0.477	0.495	1	0.93	0.749	1.155	0.512
rs2494938	LRFN1	6p21.1	A/G	0.246	0.252	0.653	0.969	0.756	1.243	0.806
rs10484761	-	6p21.1	G/A	0.137	0.100	1	1.422	1.014	1.994	0.04*
rs2285947	DNAH11	7p15.3	A/G	0.304	0.297	0.4159	1.036	0.819	1.309	0.769
rs2274223	PLCE1	10q23.33	G/A	0.263	0.204	0.6016	1.39	1.075	1.798	0.012
rs671	ALDH2	12q24.12	A/G	0.169	0.142	0.8146	1.234	0.915	1.663	0.168
rs4767364	NAA25	12q24.12	G/A	0.103	0.097	0.7521	1.069	0.747	1.531	0.751
rs11066280	C12orf51	12q24.12	A/T	0.190	0.166	1	1.18	0.89	1.563	0.25
rs4785204	HEATR3	16q12.1	T/C	0.301	0.232	0.749	1.427	1.116	1.824	0.004
rs1776184	SMG6	17p13.3	A/C	0.189	0.170	0.5472	1.142	0.862	1.512	0.355
rs6503659	HAP1	17q21.2	A/T	0.148	0.142	0.1641	1.047	0.771	1.421	0.768
rs2847281	PTPN2	18p11.21	C/T	0.182	0.179	0.4388	1.02	0.771	1.348	0.89
rs2014300	RUNX1	21q22.12	A/G	0.116	0.119	1	0.967	0.693	1.351	0.846
rs4822983	CHEK2	22q12.1	T/C	0.258	0.203	0.6021	1.361	1.052	1.762	0.019
rs738722	CHEK2	22q12.1	T/C	0.309	0.250	0.1296	1.343	1.053	1.713	0.017
rs2239815	XBP1	22q12.1	T/C	0.426	0.416	0.3494	1.039	0.836	1.293	0.728

[a]Minor allele; *P value ≤ 0.05 indicates statistical significance; #Site with HWE P ≤ 0.05 is excluded; Abbreviations: HWE, Hardy-Weinberg Equilibrium; MAF, minor allele frequency; SNP, single nucleotide polymorphism; ORs, odds ratios; CI, confidence interval.

Akaike's Information Criterion (AIC) and Bayesian Information Criterion (BIC) were applied to estimate the best-fit model for each SNP.

Results

We included a total of 670 subjects, with 360 cases (288 males, 72 females; mean age 60.7 ± 8.9) and 310 controls (197 males, 113 females; mean age 49.4 ± 7.9) for our analysis. There were significant differences in age and gender distribution between the case and control groups (P < 0.05). The basic characteristics of the participants, e.g., gender and age are listed in **Table 1**.

A total of twenty SNPs were genotyped in esophageal patients and healthy controls. **Table 2** summarizes the basic characteristics of the tested SNPs and their estimated association with esophageal cancer risk in crude analysis. The allelic frequency of other SNPs in the controls group was similar to those of the HapMap CHB population. One SNP (rs4072037) was excluded at the 5% Hardy-Weinberg equilibrium (HWE) *P*-level. Through the χ^2 test, we found five SNPs had an increased risk of esophageal cancer, including rs2274223 (OR = 1.390; 95% CI = 1.075-1.798, *P* = 0.012), rs10484761 (OR = 1.422; 95% CI = 1.014-1.994, *P* = 0.040), rs4785204 (OR = 1.427; 95% CI = 1.116-1.824, *P* = 0.004), rs4822983 (OR = 1.361; 95% CI = 1.052-1.762, *P* = 0.019) and rs738722 (OR = 1.343; 95% CI = 1.053-1.713, *P* = 0.017) (**Table 2**).

The association between the SNPs and esophageal cancer were tested under four different genetic models (co-dominant, dominant, recessive, and log-additive). The results showed that the rs2274223 was significantly associated with an increased risk of EC, based on the results from the co-dominant model (OR = 1.49; 95% CI = 1.01-2.20, P = 0.023 for the "G/A" genotype, and OR = 2.59; 95% CI = 1.07-6.27, P = 0.023 for the "G/G" genotype), dominant model (OR = 1.60; 95% CI = 1.10-2.32, P = 0.014 the "G/A-G/G" genotype) and log-addi-

Table 3. Logistic regression analysis of the associations between SNPs and esophageal cancer risk

Location (Gene)	SNP	Model	Genotype	Controls	Cases	OR (95% CI)[a]	*P*-value[a]	AIC	BIC
10q.23 (PLCE1)	rs2274223 (G>A)	Co-dominant	A/A	190 (62.3%)	194 (54.2%)	1	0.023*	696.8	719.3
			G/A	104 (34.1%)	140 (39.1%)	1.49 (1.01-2.20)			
			G/G	11 (3.6%)	24 (6.7%)	2.59 (1.07-6.27)			
		Dominant	A/A	190 (62.3%)	194 (54.2%)	1	0.014	696.3	714.3
			G/A-G/G	115 (37.7%)	164 (45.8%)	1.60 (1.10-2.32)			
		Recessive	A/A-G/A	294 (96.4%)	334 (93.3%)	1	0.067	699	717
			G/G	11 (3.6%)	24 (6.7%)	2.21 (0.93-5.28)			
		Log-additive	-	-	-	1.54 (1.13-2.11)	0.0063	694.9	712.9
16q.12 (HEATR3)	rs4785204 (T>C)	Co-dominant	C/C	179 (58.9%)	179 (49.7%)	1	0.0022	690.2	712.6
			C/T	110 (36.2%)	145 (40.3%)	1.27 (0.86-1.87)			
			T/T	15 (4.9%)	36 (10%)	3.69 (1.71-7.95)			
		Dominant	C/C	179 (58.9%)	179 (49.7%)	1	0.031	695.8	713.8
			C/T-T/T	125 (41.1%)	181 (50.3%)	1.50 (1.04-2.16)			
		Recessive	C/C-C/T	289 (95.1%)	324 (90%)	1	0.001	689.6	707.6
			T/T	15 (4.9%)	36 (10%)	3.33 (1.57-7.06)			
		Log-additive	-	-	-	1.58 (1.17-2.11)	0.0022	691	709
22q.12 (CHEK2)	rs4822983 (T>C)	Co-dominant	C/C	193 (63.1%)	196 (55.2%)	1	0.085	693.2	715.7
			C/T	102 (33.3%)	135 (38%)	1.48 (1.00-2.19)			
			T/T	11 (3.6%)	24 (6.8%)	1.81 (0.76-4.33)			
		Dominant	C/C	193 (63.1%)	196 (55.2%)	1	0.03	691.4	709.4
			C/T-T/T	113 (36.9%)	159 (44.8%)	1.52 (1.04-2.21)			
		Recessive	C/C-C/T	295 (96.4%)	331 (93.2%)	1	0.29	695	713
			T/T	11 (3.6%)	24 (6.8%)	1.57 (0.67-3.70)			
		Log-additive	-	-	-	1.42 (1.03-1.95)	0.028	691.3	709.3
22q.12 (CHEK2)	rs738722 (T>C)	Co-dominant	C/C	166 (54.6%)	163 (47.1%)	1	0.064	683.4	705.8
			T/C	124 (40.8%)	152 (43.9%)	1.48 (1.01-2.18)			
			T/T	14 (4.6%)	31 (9%)	1.90 (0.87-4.13)			
		Dominant	C/C	166 (54.6%)	163 (47.1%)	1	0.024	681.8	699.7
			T/C-T/T	138 (45.4%)	183 (52.9%)	1.53 (1.06-2.22)			
		Recessive	C/C-T/C	290 (95.4%)	315 (91%)	1	0.22	685.4	703.3
			T/T	14 (4.6%)	31 (9%)	1.59 (0.75-3.40)			
		Log-additive	-	-	-	1.43 (1.05-1.94)	0.02	681.5	699.4

[a]Adjusted for age and sex. *P-value ≤ 0.05 indicates statistical significance. Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; AIC, Akaike's Information Criterion; BIC, Bayesian Information Criterion.

tive model (OR = 1.54; 95% CI = 1.13-2.11, P = 0.0063). We found that the minor allele "T" of rs4785204 was also increased the cancer risk in co-dominant (OR = 3.69; 95% CI = 1.71-7.95, P = 0.0022), recessive model (OR = 3.33; 95% CI = 1.57-7.06, P = 0.001) and log-additive model (OR = 1.58; 95% CI = 1.17-2.11, P = 0.0022). Additionally, we found the rs4822983 (OR = 1.42; 95% CI = 1.03-1.95, P = 0.028) and rs738722 (OR = 1.43; 95% CI = 1.05-1.94, P = 0.02) were significantly associated with increased esophageal risk under the log-additive model (**Table 3**).

Discussion

EC is one of the most prevalent cancers worldwide and occurs at a relatively high frequency in China. In the present case-control study, we investigated the association of 20 esophageal cancer risk-related variants obtained from previous GWAS with 360 cases and 310 controls in a Chinese Han population. We found that five tested SNPs rs2274223, rs10484761, rs4822983, rs738722, and rs4785204 were significantly associated with an increased risk of esophagus cancer.

The SNP rs2274223 at chromosome 10q23 in phospholipase C epsilon 1 (PLCE1), was identified as a novel susceptibility locus for esophageal and gastric cancers [10, 11]. Furthermore, PLCE1 is supposed to play a critical role in the development of many cancers, such as colorectal cancer [19], bladder cancer [20], and skin and 'head and neck' cancers [21, 22]. In addi-

tion, we found rs2274223 associated with increased risk of esophagus cancer in a Chinese Han population.

Rs10484761 loci lies in a ~200-kp region on 6p21, near *UNC5CL* gene, was discovered as a novel susceptible locus for esophageal squamous-cell carcinoma (ESCC) [23]. It is believed to play an important role in the development and prognosis of gastric cancer [24, 25]. We also found the increased risk in esophagus cancer. However, the mechanisms of functions of this polymorphism were not clearly, and further studies are warranted.

The marker rs4785204 at 16q12 in the HEATR3 gene, which was previously demonstrated to be associated with ESSC risk [14]. Additionally, it has been proved that the mutation of HEATR3 within the chromosome 16 region showed a genome-wide significance of Crohn's disease (CD) risk [26]. We also found the increased risk in esophagus cancer in present study.

The two remaining SNPs, rs4822983 and rs738722, maps on 22q12 were also identified to be relevant with risk of EC in Chinese Han population. This two SNPs are located in Checkpoint kinase 2 (*CHEK2*), which emerges as an important signal transducer of cellular responses to DNA damage and a candidate tumor suppressor whose defects can predispose to several types of cancer [27]. Previous study has demonstrated that the functional variant rs738722 in CHEK2 might contribute to susceptibility to esophageal cancer lymph node metastasis in Chinese population [28]. Rs4822983 was also associated with increased ESSC risk [14]. However, the mechanisms of functions of these two polymorphisms were not clearly.

Potential limitations of this study should be considered. First, the sample size of our study was relatively small. The statistical power may be limited because of the sample size. Second, the association between genetic polymorphism and clinicopathological type (ESCC or EA) was not evaluated in this study. Third, this was a hospital-based study; therefore, selection bias may be unavoidable. So larger well-designed studies combined with functional evaluations are needed to confirm the associations and clarify the potentially biological mechanisms of these polymorphisms in esophageal carcinogenesis.

In conclusion, this association study investigated 20 SNPs obtained from previous GWAS as genetic susceptibility factors for esophagus cancer risk in a Chinese Han population. We found that five genetic variants (rs10484761 at 6q21, rs2274223 at 10q23, rs4785204 at 16q12, rs4822983 and rs738722 at 22q12) were significantly associated with increased EC risk in a Chinese Han population. Our findings, combined with previous studies indicated that genetic variants at 6p21, 10q23, 16q21, 22q12 may influence esophageal cancer susceptibility. However, these SNPs require further investigation before definitive conclusions can be drawn.

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Disclosure of conflict of interest

None.

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