Int J Clin Exp Pathol 2017;10(1):730-735
www.ijcep.com /ISSN:1936-2625/IJCEP0033723

Original Article

Impact of single nucleotide polymorphisms in ERCC2 gene and their interaction with smoking on esophageal squamous cell carcinoma risk in Chinese Han population

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Received June 14, 2016; Accepted August 26, 2016; Epub January 1, 2017; Published January 15, 2017

Abstract: Aims: To investigate the association between ERCC2 polymorphisms and ESCC risk, and impact of additional gene-smoking interaction on ESCC risk, based on a Chinese population. Methods: A total of 1162 subjects (493 men, 669 women), with a mean age of 65.0±12.8 years old, were selected, including 580 ESCC patients and 582 normal controls. Logistic regression model was used to examine the association between 3 single nucleotide polymorphisms (SNPs) in ERCC2 and ESCC risk. Odds ratio (OR) and 95% confident interval (95% CI) were calculated. Generalized multifactor dimensionality reduction (GMDR) was employed to analyze the gene-smoking interaction. Results: The carriers of homozygous mutant of two SNP have higher ESCC risk than those with wild-type homozygotes, OR (95% CI) were 1.72 (1.28-2.35) and 1.58 (1.29-2.06), respectively. We employed the GMDR analysis to assess the impact of the gene-smoking interactions among three SNPs and smoking. There was a significant two-locus model (P=0.0010) involving rs13181 and smoking, indicating a potential gene-smoking interaction between rs13181 and smoking. Overall, the two-locus model had a cross-validation consistency of 10/10, and had the testing accuracy of 62.17%. We also found that smokers with AC or CC genotype have the highest ESCC risk, compared to non-smokers with AA genotype, OR (95% CI) was 3.16 (2.01-4.32). Conclusions: Our results support an important association between rs13181, rs238406 minor allele of ERCC2 and increased risk of ESCC, and additional interaction between rs13181 and smoking.

Keywords: ERCC, ESCC, GMDR, SNP, interaction

Introduction

Esophageal squamous cell carcinoma (ESCC) was the sixth leading cause of cancer death worldwide, and was also the predominant histological subtype of esophageal cancer, which was characterized by high mortality and with regional variation [1, 2]. China is one of the countries with the highest incidence and mortality of esophageal cancer in the world, and ESCC account for 90% of all the cases in China [3]. The mechanism of ESCC pathogenesis, however, is not yet well known. Some studies have revealed that tobacco smoking, alcohol intake, nutritional deficiencies and dietary carcinogen exposure may contribute to the etiology of ESCC, but only a small proportion of exposed individuals actually develop esophageal cancer, suggesting that genetic factors may also play a vital role in susceptibility to ESCC [4-6]. Recent genetic association studies on cancer risk have focused on assessing effects of single nucleotide polymorphisms (SNPs) in candidate genes, among which DNA repair genes are increasingly studied because of their pivotal role in maintaining genome integrity. Sequence variants in DNA repair genes are thought to modulate DNA repair capacity and consequently are suggested to be associated with altered cancer risk [7].

Several Studies have reported that ERCC2 was associated with several types of cancer, including gastric cancer [8], lung adenocarcinoma [9],

Table 1. Description and Probe sequence used for Taqman fluorescence probe analysis for 3 SNPs

SNP ID	Chromosome	Functional Consequence	Nucleotide substitution	Probe sequence
rs238406	19:45365051	Nc transcript variant, synonymous codon	G>T	5'-CCTGCCCTCCAGTAACCTCATAGAA[G/T] CGGCAGTGGGGCAGGCTGGTGTCAT-3'
rs13181	19:45351661	Downstream variant 500 B, missense	A>C	5'-TGCTGAGCAATCTGCTCTATCCTCT[A/C] CAGCGTCTCCTCTGATTCTAGCTGC-3'
rs3916874	19:45353668	Intron variant	G>C	5'-TTGGCTCCACACTGTCTCTATTGTA[G/C] TGTCATATGGGAAGTCTCAGGGCAG-3'

Table 2. General characteristics of study participants in ESCC cases and controls

Variables	ESCC cases (n=580)	Control (n=582)	*p-values*
Age (years)	65.1±13.3	64.9±13.8	0.801
Males N (%)	243 (41.9)	250 (43.0)	0.715
Drinking N (%)	211 (36.4)	224 (38.5)	0.458
Smoke N (%)	262 (42.2)	210 (33.4)	0.002
High fat diet N (%)	120 (20.7)	102 (17.5)	0.170
Low fiber diet N (%)	108 (18.6)	106 (18.2)	0.858
WC (cm)	83.3±15.3	84.7±15.8	0.125
BMI (kg/m^2)	23.6±10.7	23.2±10.2	0.514

Means ± standard deviation for age, WC, BMI; WC, waist circumference; BMI, body mass index.

cutaneous melanoma [10]. Studies [8, 11-13] regarding the association of ERCC2 gene with esophageal cancer in Chinese had been explored, and the results were inconsistent. But, whether more common ERCC2 variants are also associated with ESCC risk has not been well investigated. In addition, ESCC risk was influenced by both multiple genes and environmental factors, such as smoking, so it was necessary to detect the synergistic effect in multiple-SNPs and smoking on ESCC risk, and some genes like GSTM1, GSTT1, etc., have been found to modify the effect of tobacco exposure thereby increasing the susceptibility for developing ESCC [11-13]. However, no analysis on impact of ERCC gene-smoking interaction on ESCC risk was conducted in Chinese population. In this study, we aimed to investigate the association between ERCC2 polymorphisms and ESCC risk, and impact of additional gene-smoking interaction on ESCC risk, based on a Chinese population.

Materials and methods

Subjects

This was a hospital based case-control study. Participants were consecutively recruited between Jun 2011 and November 2014 from the First Affiliated Hospital of Medical College of Xi'an Jiaotong University. All cases were confirmed by clinical and histopathological diagnosis. Subjects who received chemotherapy or radiotherapy before surgery were excluded from this study. Controls without the others types of cancer were randomly selected from a population screening program for risk factors of ESCC in the same regions and 1:1 matched to cases on the basis of age (±3 years) and sex. Blood samples were collected from each participant. A total of 1162 subjects (493 men, 669 women), with a mean age of 65.0±12.8 years old, were selected, including 580 ESCC patients and 582 normal controls. Data on demographic information, diet, smoking and drinking information for all participants were obtained using a questionnaire administered by trained staffs. We defined current alcohol consumption as more than 1 drink of any type per month or not currently drinking as less than 1 drink of any type per month [14]; Current smokers were defined as those who have smoked for at least 100 cigarettes and still smoked at the time of the interview, individuals with no history of cigarette smoking were considered as never smokers [15, 16]. At recruitment, written informed consent was obtained from each participant in the study. The protocol of this study was approved by the Ethics Committee of Xi'an Jiaotong University.

Body measurements

Body weight, height, and waist circumference (WC) were also measured according to standardized procedures. Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters. Blood samples were collected in the morning after at least 8 hours of fasting. All plasma and serum samples were frozen at -80°C until laboratory testing.

Table 3. Genotype and allele frequencies of 3 SNP between case and control group

SNPs	Genotypes and Alleles	Frequencies N (%)		OR (95% CI)*	H-W test
		Cases (n=580)	Controls (n=582)		
rs3916874					
	GG	316 (54.5)	342 (58.8)	1.00	0.302
	GC	224 (38.6)	214 (36.8)	1.12 (0.91-1.49)	
	CC	40 (6.9)	26 (4.4)	1.24 (0.87-1.74)	
	GC+CC	264 (45.5)	240 (41.2)	1.18 (0.89-1.56)	
	G	856 (73.8)	898 (77.2)		
	C	304 (26.2)	266 (22.8)		
rs13181					
	AA	302 (52.1)	367 (63.1)	1.00	0.338
	AC	235 (40.5)	195 (33.5)	1.57 (1.23-1.98)	
	CC	43 (7.4)	30 (3.4)	2.06 (1.32-2.94)	
	AC+CC	278 (47.9)	215 (36.9)	1.72 (1.28-2.35)	
	A	839 (72.3)	929 (79.8)		
	C	321 (27.7)	235 (20.1)		
rs238406					
	GG	299 (51.6)	369 (63.4)	1.00	0.567
	GT	225 (38.7)	186 (32.0)	1.42 (1.19-1.77)	
	TT	56 (9.7)	27 (4.6)	2.01 (1.36-2.87)	
	GT+TT	281 (48.4)	213 (36.6)	1.58 (1.29-2.06)	
	G	823 (70.9)	924 (79.4)		
	T	337 (29.1)	240 (20.6)		

*Adjusted for gender, age, smoking, alcohol consumption, high fat diet, low fiber diet, BMI and WC.

Genomic DNA extraction and genotyping

We selected SNPs within the ERCC2 gene according to the following standards: 1) SNP, which have been reported associations with ESCC risk; 2) SNP, the minor allele frequency (MAF) of which was more than 3%. A total of three SNPs of ERCC2 gene were selected for genotyping in the study: rs13181, rs238406 and rs3916874. Genomic DNA from participants was extracted from EDTA-treated whole blood, using the DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. All SNPs were detected by Taqman fluorescence probe. Probe sequences of all SNPs were shown in **Table 1**. ABI Prism7000 software and allelic discrimination procedure were used for genotyping of forementioned 3 SNPs. A 25 μl reaction mixture including 1.25 ul SNP Genotyping Assays (20×), 12.5 μl Genotying Master Mix (2×), 20 ng DNA, and the conditions were as follows: initial denaturation for 10 min and 95°C, denaturation for 15 s and 92°C, annealing and extension for 90 s and 60°C, 50 cycles.

Statistical analysis

The mean and SD were calculated for normally distributed continuous variables, and percentages were calculated for categorical variable. The categorical data were compared using χ^2 test. Further, continuous variables were compared using Student's t test between groups. Hardy-Weinberg equilibrium (HWE) was performed by using SNPStats (available online at http://bioinfo.iconcologia.net/SNPstats). Logistic regression was performed to investigate association between SNP and ESCC using gender, age, high fat diet, low fiber diet, smoking and alcohol status, BMI and WC as covariates in the model. Generalized MDR (GMDR) [17] was used to investigate the impact of gene-smoking interaction on ESCC risk, and some parameters were calculated, including cross-validation consistency, the testing balanced accuracy and the sign test.

Results

A total of 1162 subjects (493 men, 669 women), with a mean age of 65.0±12.8 years old, were selected, including 580 ESCC patients and 582 normal controls. Participants characteristics stratified by cases and controls are shown in **Table 2**. The distribution of smoking was significantly different between cases and controls, and the smoking rate was higher in ESCC cases than that in controls. The mean of BMI, WC and age and the distribution of gender, drinking, low-fiber and high-fat diet were not significantly different between cases and controls.

Table 4. Best gene-smoking interaction models, as identified by GMDR

Locus no.	Best combination	Cross-validation consistency	Testing accuracy	*p-values*[a]
2	rs13181 Smoking	10/10	0.6217	0.0010
3	rs13181 rs238406 Smoking	9/10	0.6011	0.0547
4	rs13181 rs238406 rs3916874 Smoking	8/10	0.5399	0.3770

[a]Adjusted for gender, age, alcohol consumption, high fat diet, low fiber diet, BMI and WC.

Table 5. Logistic regression on interaction between rs13181 and smoking on ESCC risk

rs13181	Smoking	OR (95% CI)[a]	*P*-values
AA	Never	1.00	-
AA	Always	1.56 (1.18-2.04)	0.001
AC or CC	Never	1.37 (1.09-1.83)	0.030
AC or CC	Always	3.16 (2.01-4.32)	<0.001

[a]Adjusted for gender, age, alcohol consumption, high fat diet, low fiber diet, BMI and WC.

There were significant differences in rs13181 and rs238406 alleles and genotypes distributions between cases and controls (**Table 3**). The frequencies for the two SNP minor alleles were higher in cases than that in controls, C allele of rs13181 was 27.7% in ESCC cases and 20.1% in controls subjects, and the T allele of rs238406 was 29.1% in ESCC cases and 20.6% in controls. Logistic analysis showed the significant association between genotypes of variants in two SNP and increased ESCC risk, after adjustment for gender, age, high fat diet, low fiber diet, smoking and alcohol status, BMI and WC. The carriers of homozygous mutant of two SNP polymorphism revealed higher ESCC risk than those with wild-type homozygotes, OR (95% CI) were 1.72 (1.28-2.35) and 1.58 (1.29-2.06), respectively.

We employed the GMDR analysis to assess the impact of the multiple gene-smoking interactions among three SNP and smoking. **Table 4** summarizes the results obtained from GMDR analysis for two- to four-locus models. There was a significant two-locus model (P=0.0010) involving rs13181 and smoking, indicating a potential gene-smoking interaction between rs13181 and smoking. Overall, the two-locus model had a cross-validation consistency of 10/10, and had the testing accuracy of 62.17%. We also found that smokers with AC or CC genotype have the highest ESCC risk, compared to non-smokers with AA genotype, OR (95% CI) was 3.16 (2.01-4.32), after adjustment for gender, age, alcohol status, high fat diet, low fiber diet, BMI and WC. (**Table 5**).

Discussion

In current study, we found a significant association between variants of rs238406 and rs13181 in ERCC2 and increased ESCC risk, after covariates adjustment. Several studies have reported the association between ERCC2 SNPs and ESCC risk in different populations. rs13181 in ERCC2 gene has been more studied, and previous studies suggested that rs13181 may play a role in the development of head and neck cancer, lung cancer, and bladder cancer [19, 20]. Meta-analysis also showed that rs13181 was not associated with the others types of cancer risk [21, 22]. In a recent meta-analysis [23], evaluating the association between rs13181 and EC risk, in which a total of 12 studies were included, rs13181 was associated with increased risk of ESCC in two models (CC vs. AA and CC+AC VS. AA) in Chinese population, and rs13181 CC genotype could also increase the risk of EADC (esophageal adenocarcinoma). However, Zhu et al [24] conducted a study and indicated that rs13181 in ERCC2 gene was not associated with ESCC risk. Zhang et al [25] suggested that genetic variations in the ERCC2 gene (rs13181) were associated with increased risk of ESCC in a Chinese population. Zhai et al [26] conducted a study for Sichuan population and also suggested that ERCC2 codon 751Gln allele was associated with a borderline decrease of ESCC. Yu et al [6] reported that ERCC2 SNP was associated with an increased risk of ESCC in a Chinese population. In another study, small associations of the XPD Lys751Gln polymorphism with cancer risk for esophageal cancer are revealed [27]. In contrast to rs13181, rs238406 was less studied previously. A Chinese study conducted by Zhu et al [24] indicated that rs238406 was associated with increased ESCC risk, which was consistent with results in current study.

The ESCC susceptibility was a result of many gene polymorphism and gene-environment interaction, furthermore, the association between smoking and incident ESCC have been reported in some previous studies [28, 29] and

showed that tobacco smoking was the risk factors for esophageal cancer in general population. However, till now no study focused on impact of ERCC gene-smoking interaction on ESCC risk was conducted in Chinese population. This study investigated the impact of additional gene-smoking interaction on ESCC risk, based on a Chinese population using GMDR model, and we found a significant interaction between rs13181 and smoking, and this interaction was associated with increased ESCC risk, smokers with AC or CC genotype have the highest ESCC risk, compared to non-smokers with AA genotype. This is the first study focused on ERCC gene-smoking interaction. However, some studies have reported the interaction between others gene and smoking and the interaction between ERCC gene and family history of cancer. The interaction of tobacco related carcinogens and carcinogen metabolizing genes like GSTM1, GSTT1, etc., were found to modify the effect of tobacco exposure thereby increasing the susceptibility for developing ESCC [11, 12]. Talukdar et al [13] conducted a gene-environment interaction analysis, and found tobacco chewing and smoking showed the highest individual effects as well as strongest synergistic effects among each other in ESCC with promoter hypermethylation, supporting the role of tobacco carcinogens in promoting DNA methylation in ESCC.

The limitations of this study should be considered. Firstly, more SNPs in ERCC gene should be included in the analysis. Secondly, gene-gene interaction should be conducted for different gene. Thirdly, some others environmental factors should be included in the gene-environment interaction analysis, such as alcohol consumption. The last, the results obtained in this study should be verified in different populations or different nationality of China.

In conclusion, we found a significant association between rs238406 and rs13181 in ERCC2 with increased ESCC risk. We also found a potential gene-smoking interaction between rs13181 and smoking, and smokers with AC or CC genotype have the highest ESCC risk, compared to non-smokers with AA genotype, which was 3.16 times compared to non-smokers with AA genotype.

Acknowledgements

The writing of this paper was supported by the First Affiliated Hospital of Medical College of Xi'an Jiaotong University. We thank all the partners and staffs who help us in the process of this study.

Disclosure of conflict of interest

None.

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