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Study on the correlation between CD14 gene polymorphism and susceptibility to laryngeal cancer

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Abstract. – OBJECTIVE: CD14 is the cell surface glycoprotein, which plays an important role in the occurrence and development of tumors. This study was designed to assess the association between CD14 SNPs and laryngeal cancer risk.

PATIENTS AND METHODS: This case-control study including 406 cases of laryngeal cancer and 893 healthy controls. The relationship between the genetic variation of CD14, rs2569190 and rs5744455, and the onset risk of laryngeal cancer were investigated. Logistic regression analysis was used to calculate the odds ratio (OR) and 95% confidence interval (CI) to study the relationship between CD14 gene polymorphism and pathogenesis of laryngeal cancer.

RESULTS: The results showed that rs5744455 mutation could increase the onset risk of laryngeal cancer (TT *vs.* CC: OR = 1.20, 95% CI = 1.01-1.41; additive model: OR = 1.20, 95% CI = 1.01-1.42). The results of stratified analysis showed that rs5744455 was associated with the susceptibility to laryngeal cancer in the elderly, females, non-smokers and non-drinkers (OR = 1.32, 95% CI = 1.04-1.66; OR = 1.58, 95% CI = 1.21-2.06; OR = 1.35, 95% CI = 1.08-1.69; OR = 1.31, 95% CI = 1.05-1.65). The analysis of combined effect of rs2569190 and rs5744455 showed that there was a combined effect between the two mutant loci (ptrend = 0.011).

CONCLUSIONS: This study suggested that the genetic variation of CD14, rs5744455, is related to the susceptibility to laryngeal cancer, providing a theoretical basis for the study of the pathogenesis of laryngeal cancer.

Key Words:
CD14, Polymorphism, Laryngeal cancer, Association.

Introduction

Laryngeal cancer is one of the most common malignant tumors, accounting for 13.9%, in the head and neck, whose incidence rate is only second to nasopharyngeal cancer. In the tumors in respiratory tract, the incidence rate of laryngeal cancer ranks second only to lung cancer[1]. According to the statistical data of global cancer epidemiology in 2012 (GLOBOCAN2012), the incidence and mortality rates of laryngeal cancer in the world were up to 2.1/100,000 and 1.1/100,000, respectively. As China has a huge population base, 12.76% of new cases and 14.65% of deaths of laryngeal cancer in the world are in China[2]. Despite of the continuous improvement in the treatment means of laryngeal cancer and the continuous development of medical detection techniques, the overall survival rate of patients with laryngeal cancer is not improved significantly, and the survival rate of advanced laryngeal cancer is lower[3]. At present, the exact causes of laryngeal cancer are still unclear. Many scholars in China and foreign countries studied the ontogeny, genetics, and molecular biology of laryngeal cancer and the results showed that the occurrence of laryngeal cancer may be closely related to the combined effects of the environmental and genetic risk factors[4,5]. Epidemiological studies have confirmed that smoking and drinking are important risk factors for the occurrence of laryngeal cancer[5]. However, only a very few smokers and drinkers living in the similar or the same environment suffer from laryngeal cancer. This fact shows that although environmental factors can lead to the occurrence of tumor, the sensitivity degrees of different individuals to environmental carcinogens are different, suggesting that the occurrence of laryngeal cancer is related to its genetic variation[6]. The human CD14 gene is located on the long arm of chromosome 5 (5q23-31) and plays an important role in mediating the immune response and inflammatory response of

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the body[7]. CD14 can mediate the immune escape and promote the growth of tumor[8,9]. Many studies have hypothesized that CD14 genetic variation is closely related to the occurrence and development of malignant tumors. A recent study has shown that CD14 is highly expressed in leukemic cells and peripheral vascular endothelial cells, and its migration capacity is significantly enhanced, indicating that CD14 also plays a role in tumor angiogenesis[10]. In order to investigate the relationship between CD14 genetic variation and onset risk of laryngeal cancer, the correlation between rs2569190 and rs5744455 and susceptibility to laryngeal cancer was studied using a case-control study with 406 patients with laryngeal cancer and 893 healthy subjects as the objects of study.

Patients and Methods

All participants in the study were informed of the purpose and significance of the study, signed the informed consent and provided the necessary biological samples for detection analysis. All personal information, disease history, family history and genetic information of the subjects were strictly confidential. This study was reviewed and approved by the Ethics Institution of The First Hospital of Hebei Medical University.

Objects of Study and Epidemiological Survey

All objects of study were Chinese Han population without blood relation from Eastern China, including Shanghai, Jiangsu and the surrounding areas. 406 patients diagnosed via histopathology as laryngeal cancer admitted and treated in the First Hospital of Hebei Medical University from March 2013 to September 2016 were selected. They did not receive the radiotherapy and/or chemotherapy medication before blood collection. 893 normal healthy people involved the First Hospital of Hebei Medical University in the same period were randomly selected as the control. They had no history of cancer or biological relevancy with the case group, matching with the case frequency according to gender and age (±5 years old). The unified epidemiological questionnaire was designed. The demographic data and environmental exposure data of each subject were investigated, including gender, age, race, weight, height, smoking, drinking, history of individual disease and family history of cancer. 5 mL venous blood was collected from each object using the vacuum anticoagulant tube containing EDTA, and placed at -70°C for standby application.

SNP Selection

Based on the English references and bioinformatics database (http://www.ncbi.nlm.nih.gov/snp/), two SNP loci, rs2569190 and rs5744455, in the CD14 promoter region were selected in this study.

Genotyping

DNA was extracted from all samples using the traditional ammonia-chloroform method, and the genotype was identified via ABI 7900 (Thermo Fisher Scientific, Waltham, MA, USA). Real-time fluorescent quantitative PCR instrument (Foster City, CA, USA) was done by using TaqMan genotyping method. Two blank controls and two duplicate specimens were randomly selected in each 384-well detection unit as the quality control.

Statistical Analysis

Statistical analysis system (SAS) (version 9.1; SAS Institute, Gary, NC, USA) was used for data collation and statistical analysis. $p < 0.05$ suggested that the difference was statistically significant. All statistical tests were two-sided probability tests. x^2 test was used for the differences of distribution frequencies in demographic characteristics, environmental exposure parameters (gender, age, smoking and drinking) and each genotype between case group and control group. Whether the genotype frequency of normal control group met the Hardy-Weinberg equilibrium law, it was calculated to confirm the randomness of selecting subjects. The odds ratio (OR) and 95% confidence interval (CI) were calculated using the univariate and multivariate Logistic multiple regression model to present the relative risk, and all OR values were corrected by gender, age, smoking status and drinking status. Stratified analysis was performed using Logistic regression according to the gender, age, smoking and drinking. The combined effects of the two selected loci were analyzed. $p < 0.05$ was considered statistically significant.

Results

General Characteristics of Objects

There were 406 cases of laryngeal cancer and 893 cases of healthy controls; there was no significant difference in the age constituent ratio ($p = 0.130$). There were 177 females (43.6%) and 229

males (56.4%) in case group, 360 females (40.3%) and 533 males (59.7%) in control group; there was no significant difference in the gender constituent ratio (p = 0.265). There were 172 smokers (42.4%) in patients with laryngeal cancer, which was significantly higher than that in control group (299 smokers, 33.5%) (p = 0.002). There were 224 drinkers (44.8%) in patients with esophageal cancer, which was significantly higher than that in control group (332 drinkers, 37.2%) (p = 0.009) (Table I).

Table I. Selected characteristics in laryngeal cancer cases and controls.

Variables	Case N (%)	Control N (%)	p^a
All subjects	406 (100)	893 (100)	
Age			0.130
< 60	193 (47.5	465 (52.1)	
≥ 60	213 (52.5)	428 (47.9)	
Gender			0.265
Females	177 (43.6)	360 (40.3)	
Males	229 (56.4)	533 (59.7)	
Smoking status			0.002
Never	234 (57.6)	594 (66.5)	
Ever	172 (42.4)	299 (33.5)	
Drinking status			0.009
Ever	182 (44.8)	332 (37.2)	

[a]Two-sided χ^2-test.

Correlation Analysis CD14 Gene Polymorphism and Laryngeal Cancer Risk

The basic information of the loci selected is shown in Table II. The distribution frequencies of loci in control group met the Hardy-Weinberg equilibrium law (p > 0.05). The minor allele frequency (MAF) of each locus in control group and case group was larger than 0.05. The Logistic regression analysis showed that there was a significantly positive correlation between rs5744455 and occurrence of laryngeal cancer. The frequencies of CC, CT and TT genotypes in case group and control group were 26.4%, 50.0% and 23.6%, 31.9%, 48.3% and 19.8%, respectively. Compared with that in CC genotype carriers, the risk of esophageal squamous carcinoma in TT genotype carriers was increased (adjusted OR = 1.20, 95% CI = 1.01-1.41, p = 0.037). The results of additive model also showed a significantly positive correlation between rs2296l47 and occurrence of esophageal squamous carcinoma (adjusted OR = 1.20, 95% CI = 1.01-1.42, p = 0.034). No association between rs2569190 and onset risk of laryngeal cancer was found (Table III). Stratified analysis was performed for the locus rs5744455 according

Table II. Primary information and minor allele frequencies (MAFs) of selected SNPs.

SNP	Chr	Position	Alleles	HWE[a]	MAF[b] Cases	MAF[b] Controls
rs2569190	5q23	Promoter	C > T	0.120	0.460	0.465
rs5744455	5q23	Promoter	C > T	0.542	0.486	0.487

[a]p-values for Hardy-Weiberger equilibrium (HWE) tests. [b]Minor allele frequency (MAF).

Table III. Summary association between selected SNPs and laryngeal cancer risk.

SNP	Controls	All cases	Adjusteda OR (95% CI)	p^a
rs2569190				
CC	288 (32.3)	118 (29.1)	1	
CT	458 (45.8)	202 (49.8)	1.05 (0.79-1.38)	0.751
TT	147 (16.5)	86 (21.2)	1.14 (0.96-1.36)	0.135
Additive model			1.14 (0.96-1.36)	0.139
rs5744455				
CC	285 (31.9)	107 (26.4)	1	
CT	431 (48.3)	203 (50.0)	1.25 (0.95 1.67)	0.117
TT	177 (19.8)	96 (23.6)	**1.20 (1.01-1.41)**	**0.037**
Additive model			**1.20 (1.01-1.42)**	**0.034**

[a]Logistic regression with adjustment for age, sex, smoking status and alcohol status in additive model. Significant values (p < 0.05) are in bold.

Table IV. Stratified analysis for associations between rs5744455variant genotype and laryngeal cancer risk.

Variables	rs5744455			
	Cases CC/CT/TT	Controls CC/CT/TT	Adjusteda OR (95% CI)	p^a
Age, yr.				
< 60	55/91/47	138/232/395	1.09 (0.86-1.39)	0.463
≥ 60	52/112/49	147/199/82	**1.32 (1.04-1.66)**	**0.022**
Sex				
Females	39/85/53	114/180/66	**1.58 (1.21-2.06)**	**0.001**
Males	68/118/43	171/251/111	1.01 (0.80-1.27)	0.941
Smoking				
Never	96/133/70	189/298/107	**1.35 (1.08-1.69)**	**0.007**
Ever	46/95/31	61/108/65	1.07 (0.80-1.42)	0.654
Drinking				
Never	109/156/67	176/275/110	**1.31 (1.05-1.65)**	**0.018**
Ever	51/93/38	56/110/58	1.18 (0.90-1.56)	0.238

[a]Adjusted by age, sex, smoking status and alcohol status.

to gender, age, smoking status and drinking status. Logistic regression analysis showed that the elderly, women, non-smokers and non-drinkers were more likely to suffer from laryngeal cancer (adjusted OR = 1.32, 95% CI = 1.04-1.66, p = 0.022; adjusted OR = 1.58, 95% CI = 1.21-2.06, p = 0.001; adjusted OR = 1.35, 95% CI = 1.08-1.69, p = 0.007; adjusted OR = 1.31, 95% CI = 1.05-1.65, p = 0.018). No statistically significant differences in the above results were found in the heterogeneity test (p > 0.05) (Table IV). Two SNPs mutant loci (OR > 1.0) of CD14 gene were combined to study the combined effect on susceptibility to laryngeal cancer. The risk base was rs5744455T + rs2569190T. With the increase of risk genotypes, the onset risk of laryngeal cancer showed a good dose-response effect (additive model: adjusted OR = 1.17, 95% CI = 1.04-1.32, p_{trend} = 0.011) (Table V).

Discussion

In this experiment, a hospital-based case-control study with 406 patients of laryngeal cancer and 893 healthy controls was performed and it was found that rs5744455 was significantly associated with the occurrence of laryngeal cancer and the onset risk of laryngeal cancer was increased with the increase of mutant base, showing a good dose-response effect. CD14 is a member of cell surface glycoprotein family, which is a specific surface marker for monocytes, macrophages and neutrophils[11], involved in the body's phagocytosis and digestion of G-bacteria, playing an important role in mediating the immune response and inflammatory response[7]. The human CD14 gene is located on the long arm of chromosome 5 (5q23-31) and contains about 1338 nucleotide residues that can encode a polypeptide chain with 375 amino acids[12]. There are some SNPs in the promoter region sequence, including two loci, rs2569190 and rs5744455. The results showed that rs2569190 is close to the recognition site of Sp1 transcription factor, and the mutant allele T genotype is associated with the increased expression of CD14. It is speculated that the polymorphism of this locus is very important for CD14 gene expression[13,14]. This study investigated the relationship between rs2569190 polymorphism

Table V. Cumulative effect of rs2569190and rs5744455variants on the laryngeal cancer risk

No. of variants[a]	Cases (%)	Controls (%)	Adjusted[b] OR (95% CI)	p^b
0-1	147 (36.2)	380 (42.6)	1.00	
2	150 (6.9)	322 (36.1)	**1.16 (0.88-1.53)**	**0.290**
3-4	109 (26.8)	191 (21.4)	**1.40 (1.03-1.90)**	**0.032**
Trend	–	–	**1.17 (1.04-1.32)**	**0.011**

[a]Thers2569190T andrs5744455T allele were assumed as risk alleles based on main effect of individual locus. [b]Adjusted by age, sex, smoking status and alcohol status. Significant values (p < 0.05) are in bold

and genetic susceptibility to laryngeal cancer. The results showed that there was no correlation between rs2569190 polymorphism and susceptibility to laryngeal cancer. Results of a number of studies are consistent with this study. Zeljicet al[15] studied the relationship between CD14 rs2569190 and the onset risk of oral squamous cell carcinoma in Serbia, and the results showed no correlation between them. Masamune et al[16] found that rs2569190 polymorphism did not increase the onset risk of pancreatic cancer in Japanese. Hold et al[17] studied the relationship between rs2569190 and the genetic susceptibility to gastric cancer and esophageal cancer in white people. It was found that -159C > T had no correlation with the onset risks of gastric cancer and esophageal cancer. Due to the differences in race, numbers of subjects and tumors, some studies have shown that rs2569190 genetic variation is associated with the genetic susceptibility to tumors. Studies[18,19] have shown that rs2569190 is associated with the pathogenesis of colorectal cancer, gastric cancer and prostate cancer. Rs5744455 is also located in the CD14 gene promoter region. The functional significance of this locus polymorphism remains unclear. There are few studies on the relationship between the gene polymorphism of this locus and the tumor susceptibility, and the results are inconsistent. The results of this work showed that rs5744455 mutation increased the risk of laryngeal cancer, but the specific mechanism between them remains unclear. It is speculated that rs5744455 genetic variation may affect the protein expression level of CD14, thus controlling the downstream signal transduction and affecting the release of inflammatory factors and immune response, which further affect the occurrence and development of tumors. The relevant functions of this locus still need to be further studied. Some studies[16-19] have also confirmed that rs5744455 polymorphism is not associated with the occurrence of tumors.

Conclusions

The relationship between the genetic variation of CD14 gene promoter region and the genetic susceptibility to laryngeal cancer was investigated in this study for the first time. The reseearch showed that rs5744455 polymorphism was associated with the pathogenesis of laryngeal cancer, further confirming the important role of CD14 in the occurrence and development of laryngeal cancer. However, the functional mechanism of this polymorphic locus was not explored deeply, and the sample size in this study was relatively smaller, so large-sample experimental studies are further needed.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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