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Original Article

Association between 8473T>C polymorphism in the cyclooxygenase-2 gene and the risk of nasopharyngeal carcinoma

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Abstract: Background: No studies have examined the relationship between COX-2 8473T>C polymorphism and the risk of nasopharyngeal carcinoma in Chinese population. Material and methods: 296 patients with nasopharyngeal carcinoma and 300 age and gender-matched healthy controls recruited were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Cancer risk associated with the genotypes was estimated as odds ratios (ORs) and 95% confidence intervals (95% CIs) using unconditioned logistic regression. Results: There was significant difference in the distribution of COX-2 8473T>C polymorphism genotype between nasopharyngeal carcinoma patients and healthy controls (*P*=0.027). When the TT genotype was used as the reference group, the CC genotype was associated with significantly decreased risk for nasopharyngeal carcinoma (adjusted OR=0.67, 95% CI=0.33-0.83; *P*=0.01). Under the recessive model of inheritance, the CC genotype was associated with significantly decreased risk for nasopharyngeal carcinoma (adjusted OR=0.43, 95% CI=0.37-0.81; *P*=0.007). Furthermore, the C allele was associated with significantly decreased risk for nasopharyngeal carcinoma (adjusted OR=0.48, 95% CI=0.39-0.85; *P*=0.009). Conclusion: These data suggested that COX-2 8473T>C polymorphism was associated with reduced risk of nasopharyngeal carcinoma.

Keywords: Nasopharyngeal carcinoma, Cox-2, polymorphism, risk

Introduction

Nasopharyngeal carcinoma is an epithelial malignancy with an unusual ethnic and geographic disparity. The average incidence increases to 30 per 100,000 people [1, 2]. Environmental factors, such as Epstein-Barr virus, tobacco use, dietary habits, and occupational exposure to poisonous chemicals accelerate the development of nasopharyngeal carcinoma, but only a small fraction of individuals who are exposed to these factors develop nasopharyngeal carcinoma, implicating an effective role of genetic susceptibility in this cancer [3].

Cyclooxygenases catalyze the first step in the synthesis of prostaglandins (PG) from arachidonic acid. There are two isoforms of COX, designated COX-1 and COX-2. COX-2 gene however is compact and contains a TATA box and several inducible enhancer elements including CEBP/NF-IL6, CRE and NFkB [4]. COX-2 is not detected in most normal tissues but is rapidly induced by a variety of mitogenic and inflammatory stimuli resulting in elevated levels of PGs in neoplastic and inflamed tissues [5]. A common SNP 8473T>C (rs5275) in the 3'-UTR of the COX-2 gene was shown to be associated with the alteration of mRNA level of the gene, because sequences within the 3'-UTR of the COX-2 gene are important for enhancing messager translation as well as for translational silencing [6].

Previously, Mamoghli et al have investigated the frequencies of COX-2 genotypes in Tunisian population to determine whether that polymorphism was associated with the risk of nasopharyngeal carcinoma in Tunisian population. They found that CC-genotype and C allele of COX-2 T8473C gene polymorphism are associated with decreased risk of nasopharyngeal carcinoma in Tunisian population [7]. However, no studies have examined the relationship between this COX-2 T8473C polymorphism and

Table 1. Characteristics of nasopharyngeal carcinoma patients and healthy controls

Characteristics	Patients (n=296)		Controls (n=300)		*P* value
	N	%	N	%	
Gender					
Female	136	45.95	148	49.33	0.413
Male	160	54.05	152	50.67	
Age (years)					
≥60	97	32.77	109	36.33	0.389
<60	199	67.23	191	63.67	
Smoking					
No	27	9.12	22	7.33	0.458
Yes	269	90.88	278	92.67	
Alcohol consumption					
No	49	16.55	58	19.33	0.395
Yes	247	83.45	242	80.67	
T stage					
T1-T2	102	34.46	-		
T3-T4	194	65.54			
N stage					
N0	121	40.88	-		
N1/N2/N3	175	59.12			
Metastasis					
No	52	17.57	-		
Yes	244	82.43			
Clinical stage					
I-II	85	28.72	-		
III-IV	211	71.28			

the risk of nasopharyngeal carcinoma in Chinese population.

Materials and methods

Study population

This study was approved by the ethical committee of General Hospital of TianJin Medical University, and informed consent was obtained from all healthy controls and patients before their enrolment. 296 patients diagnosed with nasopharyngeal carcinoma were recruited at Department of Otolaryngol Head Neck Surgery, General Hospital of Tianjin Medical University, TianJin, China between April 2005 and February 2014. All patients who voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. 300 healthy controls were selected by matching for age and gender after initial random sampling from the Health Examination Cohort of General Hospital of TianJin Medical University. Exclusion criteria for the control group included previous malignancy, metastasized cancer from other or unknown origin, and any familial or genetic diseases.

Genotyping assay

Blood samples were drawn, following overnight fasting, into tubes containing EDTA and plasma was immediately separated by centrifugation. Genomic DNA was Extracted from peripheral blood leukocytes by the phenol/chloroform method. Genotyping the COX-2 8473T>C polymorphism was detected using a nested polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) assay. The 20 μL PCR mixture was composed by 50 ng of genomic DNA, 12.5 pmol of each primer, 0.1 mM each dNTP, 1×PCR buffer (50 Mm KCl, 10 mM Tris-HCl, and 0.1% Triton X-100), 1.8 mM $MgCl_2$, and 1.0 unit of Taq polymerase (Promega). The PCR consisted of an initial melting step of 96°C for 5 min; 35 cycles of 96°C for 30 s, 53°C for 40 s and 72°C for 45 s; and a final extension step of 72°C for 10 min. Five micro liters of the PCR products were digested at 50°C overnight with 3U of BclI (Fermentas). The digestion products were then separated on a 3% agarose gel at.

80V for 100 min and stained with ethidium bromide. Two fragments of 124 and 23 bp characterize the 8473C allele, when a single 147 bp fragment defines the 8473T allele.

Statistical analysis

The Hardy-Weinberg equilibrium was utilized to compare the observed with expected genotype frequencies in the control groups. Pearson's chi-square test or Fisher's exact test was used to compare the distribution of the genotypes between cases and controls. Cancer risk associated with the genotypes was estimated as odds ratios (ORs) and 95% confidence intervals (95% CIs) using unconditioned logistic regression. Data were deemed significant when

Table 2. Distribution of Cox-2 genotypes and allelic frequencies among nasopharyngeal carcinoma patients and control subjects

	Patients (n=296)	%	Controls (n=300)	%	*P* value
Genotype					
TT	139	46.96	110	36.67	0.027
TC	129	43.58	149	49.67	
CC	28	9.46	41	13.67	
Allele					
Allele T	407	68.75	369	61.50	0.009
Allele C	185	31.25	231	38.50	

P<0.05. Statistical analyses were performed with SPSS 18.0 software.

Results

Characteristics of patients with nasopharyngeal carcinoma and healthy controls

A total of 596 samples were included in this case-control study, including 296 patients with nasopharyngeal carcinoma and 300 healthy controls. The baseline clinical characteristics of patients with nasopharyngeal carcinoma and control subjects are summarized in **Table 1**. The characteristics of the patients and controls were all well-matched. None of the differences in these characteristics between both groups were statistically significant (*P*>0.05).

Distribution of Cox-2 genotypes and allelic frequencies

The genotype and allele distribution for the COX-2 8473T>C polymorphism by study group was shown in **Table 2**. Genotype frequency of COX-2 8473T>C polymorphism was in agreement with Hardy–Weinberg equilibrium in control group. Genotypes TT, TC and CC were detected in 139 (46.96%), 129 (16.8%) and 28 (9.46%) of 296 patients and in 110 (36.67%), 149 (49.67%) and 41 (13.67%) of 300 healthy control samples, respectively. There was significant difference in the distribution of COX-2 8473T>C polymorphism genotype between nasopharyngeal carcinoma patients and healthy controls (*P*=0.027). The frequency of variant allele T was 407 (68.75%) and allele C was 185 (31.25%) in the cases, and 369 (61.50%) and 231 (38.50%) in the controls, respectively. C allele frequency was significantly lower in cancer group as compared to control group (*P*=0.009).

COX-2 8473T>C polymorphism and the risk of nasopharyngeal carcinoma

When the TT genotype was used as the reference group, the CT genotype was not significantly associated with decreased risk (adjusted OR, 0.81, 95% CI, 0.62-4.19; *P*=0.39), but the CC genotype was associated with significantly decreased risk for nasopharyngeal carcinoma (adjusted OR=0.67, 95% CI=0.33-0.83; *P*=0.01). Under the recessive model of inheritance, the CC genotype was associated with significantly decreased risk for nasopharyngeal carcinoma (adjusted OR=0.43, 95% CI=0.37-0.81; *P*=0.007), compared with other genotypes, after adjustment for age, sex, smoking and alcohol use in the multivariate logistic regression analysis. Furthermore, the C allele was associated with significantly decreased risk for nasopharyngeal carcinoma (adjusted OR=0.48, 95% CI=0.39-0.85; *P*=0.009, shown in **Table 3**).

Discussion

The etiology of nasopharyngeal carcinoma is still incompletely understood [8]. In the past decade, much attention has been directed to the research of host genetic factors and initiation of nasopharyngeal carcinoma. A number of susceptibility genes, such as Interleukin-18, NFKB1, Cyclin D1, and HSP70-2, have been investigated across different populations and recognized as risk factors [9-12]. Therefore, identification of candidate genes may facilitate an extended understanding of pathogenesis of this malignancy.

Multiple lines of evidence suggest that COX-2 plays a significant role in carcinogenesis [13-15]. In transgenic mice, overexpression of COX-2 led to neoplastic changes in the breast, pancreas, and skin [16]. Tumor formation and growth are reduced in animals that are engineered to be COX-2-deficient or treated with a selective inhibitor of COX-2 [17]. Treatment with selective COX-2 inhibitors has proven efficacy in the prevention and treatment of malignancy in humans [18]. COX-2 derived PGs can stimulate cell proliferation, promote angiogenesis, and inhibit apoptosis and immune surveillance [19]. PGE2 secreted from the breast tumor cells stimulates aromatase gene expression in

Table 3. The association of COX-2 8473T>C polymorphism with nasopharyngeal carcinoma risk

COX-2 8473T>C polymorphism	Patients	Controls	OR (95% CI)[1]	*P* value
General genotype				
TT	139	110	1.00 (Reference)	
TC	129	149	0.81 (0.62-4.19)	0.39
CC	28	41	0.67 (0.33-0.83)	0.01
Dominant genotype				
TT	139	110	1.00 (Reference)	
CT+CC	157	190	0.58 (0.27-1.29)	0.43
Recessive genotype				
TT+CT	268	259	1.00 (Reference)	
CC	28	41	0.43 (0.37-0.81)	0.007
Allele frequency				
T	407	369	1.00 (Reference)	
C	185	231	0.48 (0.39-0.85)	0.009

[1]Adjusted for sex, age, smoking status, and drinking status.

the stromal cells of the surrounding adipose tissue. Aromatase is responsible for the biosynthesis of estrogen in adipose tissue, which is secreted out and serves as a mitogen for the epithelial cells including tumor cells. COX-2-derived PGs may also promote metastasis by stimulating cell invasion [20]. The gene for COX-2, designated as PTGS2, carries several polymorphisms, but few studies analyzed the relation between COX-2 gene polymorphisms and nasopharyngeal carcinoma. A common SNP 8473T>C (rs5275) in the 3'-UTR of the COX-2 gene was shown to be associated with the alteration of mRNA level of the gene, because sequences within the 3'-UTR of the COX-2 gene are important for enhancing messager translation as well as for translational silencing [6]. This COX-2 8473T>C variant locates at nt427 downstream from the stop codon, and this locus is within a functional region, nt373 to nt792, which could alter gene expression through both messager stability and translational efficiency in vitro [6]. Previously, Mamoghli et al have investigated the frequencies of COX-2 genotypes in Tunisian population to determine whether that polymorphism was associated with the risk of nasopharyngeal carcinoma in Tunisian population. They found that CC-genotype and C allele of COX-2 T8473C gene polymorphism are associated with decreased risk of nasopharyngeal carcinoma in Tunisian population [7]. However, no studies have examined the relationship between this COX-2 T8473C polymorphism and the risk of nasopharyngeal carcinoma in Chinese population. In the present study, a total of 596 samples were included, including 296 patients with nasopharyngeal carcinoma and 300 healthy controls. There was significant difference in the distribution of COX-2 8473T>C polymorphism genotype between nasopharyngeal carcinoma patients and healthy controls. When the TT genotype was used as the reference group, the CT genotype was not significantly associated with decreased risk, but the CC genotype was associated with significantly decreased risk for nasopharyngeal carcinoma. Under the recessive model of inheritance, the CC genotype was associated with significantly decreased risk for nasopharyngeal carcinoma, compared with other genotypes, after adjustment for age, sex, smoking and alcohol use in the multivariate logistic regression analysis. Furthermore, the C allele was associated with significantly decreased risk for nasopharyngeal carcinoma.

In conclusion, this is the first study to demonstrate that a genetic variation in Cox-2 may influence the risk of nasopharyngeal carcinoma in Chinese population. We have provided evidence for a potent biomarker for nasopharyngeal carcinoma early detection in Chinese population.

Disclosure of conflict of interest

None.

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