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Effects of genetic variants of CCR5 chemokine receptors on oral squamous cell carcinoma

C.R. Tanyel[1], Z.B. Cincin[2], B. Gokcen-Rohlig[1], K. Bektas-Kayhan[3], M. Unur[3] and B. Cakmakoglu[2]

[1]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Istanbul University, Istanbul, Turkey
[2]Department of Molecular Medicine, Institute for Experimental Medicine Research, Istanbul University, Istanbul, Turkey
[3]Department of Maxillofacial Prosthodontics, Faculty of Dentistry, Istanbul University, Istanbul, Turkey

Corresponding author: B. Cakmakoglu
E-mail: bedia@istanbul.edu.tr

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ABSTRACT. We aimed to evaluate the effect of genetic variants of the chemokine C-C motif receptor (CCR5) in the pathogenesis of oral squamous cell carcinoma (OSCC). A total of 127 patients diagnosed with OSCC and 104 healthy individuals were included in the study. The polymorphisms CCR5 59029 and CCR5-delta32 were assessed with the polymerase chain reaction-restricted fragment length polymorphism (PCR-RFLP) method from peripheral blood samples of both groups. There was a statistically significant difference between the control and patient groups for CCR5 59029 A/G genotypes ($P < 0.01$). Individuals carrying the CCR5 59029 G allele (GG +AG genotypes) had a 2.84-fold increased risk for OSCC ($P < 0.0001$), and the CCR5 59029 AA genotype frequency was higher in the control group than in the patient group ($P < 0.0001$). The CCR5-delta 32 genotype frequencies did not differ significantly between controls and cases ($P > 0.05$). CCR5 59029 GG and CCR5-

delta32 DD + ID genotype frequencies were significantly increased in Grade II-III OSCC patients compared with Grade I-II OSCC patients. In conclusion, these results suggested that the G allele of the CCR5 59029 polymorphism might be a risk factor due to the loss of receptor function that might cause increased inflammation leading to the development of OSCC.

Key words: OSCC; Chemokine; Inflammation; Gene; Polymorphism

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is a multi-factorial disease that is mediated by both environmental and genetic factors (Warnakulasuriya, 2011). Experimental and clinical data have suggested that inflammatory mechanisms can contribute to OSCC tumor development (Gasche et al., 2011). Chemokines have been demonstrated as mediators of inflammation that play important roles, including regulation, leukocyte activation, and recruitment, in inflammation areas via their interactions with chemokine receptors (Lazennec and Richmond, 2010). Genetic variations in chemokine receptor genes can affect several chemokine functions, such as the control of leukocyte infiltration into tumors and the initiation of primary tumor growth and survival (Ghilardi et al., 2008).

The chemokine C-C motif receptor 5 (CCR5), which is a receptor for the pro-inflammatory macrophage inflammatory protein chemokines, plays an important role in cancer progression through immune cell recruitment (Srivastava et al., 2008). Several mutations in the CCR5 gene have been reported, and the most widely studied variants are a 32 base pair (bp) deletion mutation (CCR5-delta32) and a G to A polymorphism (CCR5 59029A) (Weng et al., 2010; Gurdol et al., 2012). The 32-bp deletion in the open reading frame of the CCR5 gene, which is referred to as the CCR5-delta32 mutation, influences the severity of several autoimmune and infectious disorders through modulating the inflammatory response (Gurdol et al., 2012). This polymorphism leads to a premature stop codon, and thus results in a shortened non-functional protein that fails to reach the cell surface (Khademi et al., 2008). The 59029 G/A polymorphism of the CCR5 gene, which is detected in the promoter region, causes increased transcriptional activity of the gene, thus increasing the protein level (Buraczynska et al., 2012).

Genetic variations in the CCR5 gene can increase susceptibility to cancer risk by modulating inflammation. The aim of this study was to evaluate the risk association between the CCR5 59029 and CCR5-delta32 polymorphisms and OSCC in a Turkish population.

MATERIAL AND METHODS

Subject selection

We investigated the CCR5 59029 and CCR5-delta32 polymorphisms in 127 OSCC patients (76 males, 51 females, age range: 23-83 years) who were followed by both the Istanbul Faculty of Medicine, Department of Otorhinolaryngology and Head and Neck Surgery and the Faculty of Dentistry, Department of Oral Surgery and Medicine of Istanbul University.

A further 104 non-tumoral, age-matched, healthy control subjects (38 males, 66 females, age range: 34-81 years) were also recruited, who were treated at the Faculty of Dentistry of Istanbul University for dental problems. Histopathological data of OSCC patients were recorded and classified according to the tumor-node-metastasis (TNM) classification system. The tumor stage was determined according to the TNM classification system and grouped into either I/II (early stage) or III/IV (advanced stage).

The specimens were collected after obtaining informed consent from patients, and the study was conducted prospectively. Approval for the study was obtained from the Medical Ethics Committee of Istanbul Medical Faculty. The protocol followed was consistent with the World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects).

Polymorphism analysis

Genomic DNA was extracted from peripheral whole blood containing ethylenediaminetetraacetic acid (EDTA) according to the salting-out technique (Miller et al., 1988). Genotyping was performed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). The PCR-RFLP procedures are shown in Table 1 (Abdi et al., 2002). Appropriate primers were used to amplify the corresponding gene of the subjects by PCR, and the reaction products were digested using the appropriate enzyme at 37°C. The digested products were analyzed on 2% agarose gel stained with ethidium bromide and examined under transillumination.

Table 1. PCR and RFLP procedures and products of delta32 insertion/deletion (I/D) and 59029 A→G polymorphisms on the CCR5 gene.

CCR5 polymorphisms	Primers (forward and reverse)	PCR product	Restriction enzyme	Restriction products
delta32 I/D	5'-TGTTTGCGTCTCTCCCAG-3' 5'-CACAGCCCTGTGCCTCTT-3'	309 bp	-	II: 233 bp DD: 201 bp ID: 233 bp; 201 bp
59029 A/G	5'-CCCGTGAGCCCATAGTTAAAACTC-3' 5'-TCACAGGGCTTTTCAACAGTAAGG-3'	286 bp	*Bsp*1286I	AA: 258 bp GG: 130 bp AG: 258 bp; 130 bp

Statistical analysis

Statistical analyses were performed using the SPSS software package (revision 11.5 SPSS Inc., Chicago, IL, USA). Data are reported as means ± SD. Differences in the distribution of genotypes or alleles between cases and controls were tested using the chi-squared statistic. Relative risk at 95% confidence intervals (CI) was calculated as the odds ratio (OR). A multivariate logistic regression model was used to investigate possible risk factors for OSCC. Values of $P < 0.05$ were considered to be statistically significant.

RESULTS

Table 2 shows the clinical characteristics of the study groups. Gender, alcohol consumption, and smoking habits differed significantly between the patient and the control groups.

Table 3 summarizes the frequencies of the genotype and allele distributions of the CCR5 delta32 and 59029 A/G polymorphisms in OSCC patients and controls. CCR5 59029 differed significantly between the control and patient groups with respect to A/G genotypes ($P < 0.01$). Individuals carrying the G allele (GG + AG genotypes) had a 2.84-fold increased risk for OSCC ($P < 0.0001$; $\chi^2 = 12.90$; OR = 2.84; 95%CI = 1.59-5.09). The CCR5 59029 AA genotype frequency in the control group was higher than that of the patient group ($P < 0.0001$; $\chi^2 = 12.90$; OR = 0.35; 95%CI = 0.19-0.62). Conversely, CCR5-delta32 genotype frequencies were not significantly different between controls and cases ($P = 0.671$). However, both CCR5 59029 GG and CCR5-delta32 DD + ID genotype frequencies were significantly increased in Grade II-III OSCC patients compared with Grade I-II OSCC patients (Table 4).

Table 2. Clinical characteristics of study participants.

Parameters	Healthy controls (N = 104)	OSCC patients (N = 127)	P
Mean age	52.63 ± 11.58	55.84 ± 13.23	0.056
Gender (female/male)	63.5/36.5	40.2/59.8	0.000
Prosthesis (No/Yes, %)	28.3/71.7	32/68	0.643
Mechanical trauma (No/Yes, %)	69.6/30.4	58.2/41.8	0.177
Alcohol consumption (No/Yes, %)	97/7	95/32	0.000
Smoking (No/Yes, %)	58/46	52/75	0.025
Tumour size (%)			
<4 cm		67.8	
>4 cm		32.2	
Tumour localization			
Tongue		49.2	
Lip		10.2	
Floor of the mouth		11.9	
Gingiva/alveolar crest		11.9	
Retromolar trigone		8.5	
Hard palate		5.1	
Cheek		3.4	
Stages (%)			
Early		43.2	
Advanced		56.8	
Differentiation			
Well		33.3	
Moderate		50.4	
Poor		13.7	
No		2.6	

OSCC = oral squamous cell carcinoma.

Table 3. Frequencies of haplotypes of CCR5 59029 A/G and delta32 genotypes in patients and controls.

CCR5 59029	Control (N, %)	OSCC (N, %)	P
AA	44/42.3	26/20.5	
GG	25/24.0	38/29.9	
AG	35/33.7	63/49.6	0.001
Alleles			
A	123/59.13	115/45.27	
G	85/40.86	139/54.72	0.003
CCR5-delta 32			
II	88/84.6	112/88.2	
DD	3/2.9	2/1.6	
ID	13/12.5	13/10.2	0.671
Alleles			
I	189/90.86	237/93.3	
D	19/9.13	17/6.69	0.33

P value obtained by chi-square test ($P < 0.05$). OSCC = oral squamous cell carcinoma.

Table 4. Association of CCR5 polymorphisms with grade of oral cancer.

Genotypes	Early grade (N, %)	Advanced grade (N, %)	OR	95%CI	P
CCR5 59029					
AA+AG	41/50.0	41/50.0	2.6	1.11-6.07	0.025
GG	10/27.8	26/72.2			
CCR5-delta32					
II	49/47.6	54/52.4	5.89	1.26-27.4	0.010
DD +ID	2/13.3	13/86.7			

Although CCR5 59029 G genotypes (GG + AG), gender, smoking, alcohol consumption, and age were all associated with OSCC in the univariate analysis, only CCR5 59029 G genotypes and alcohol consumption were found to be associated with the disease in the multivariate logistic regression analysis (Table 5).

Table 5. Results of multivariate logistic regression analysis.

Variables in the equation	B	S.E.	Wald	Sig.	Exp(B)
CCR5 59029 G+	0.926	0.316	8.610	0.003	2.526
Gender	0.598	0.316	3.579	0.059	1.819
Smoking	0.325	0.321	1.024	0.312	1.383
Alcohol consumption	1.049	0.487	4.638	0.031	2.854
Age	0.019	0.012	2.739	0.098	1.019

Variable(s) entered on step 1: CCR5 59029 G+ genotype, gender, smoking, alcohol consumption, and age.

DISCUSSION

There is growing interest in understanding the possible role of chemokines in cancer development. In the present study, we demonstrated, for the first time, the positive association of CCR5 gene variants, delta32 and 59029 A/G, with the grade of oral cancer development. We also determined that the CCR5 59029 G allele might affect both the development and prognosis of the disease.

Some previous studies have investigated the role of chemokines in cancer development. Degerli et al. (2005) studied the CCR5-delta32 allele distribution and its relationship with breast, laryngeal, thyroid, and brain carcinomas in a Turkish population, which did not reveal any statistically significant relationships. In our previous studies, we found that the MCP-1 A2518G and CCR2-V64I genotypes were risk factors for endometrial (Attar et al., 2010), bladder (Narter et al., 2010), and oral cancer (Bektas-Kayhan et al., 2012). To the best of our knowledge, only two studies have investigated CCR5 variants with respect to the risk of oral cancer development. Khademi et al. (2008) investigated the SDF1-3'A and CCR5-delta32 variants in patients with head and neck cancer in an Iranian population. This study did not find any significant differences in the frequencies of CCR5 genotypes and alleles between patients and controls. The other study (Weng et al., 2010) investigated the individual and synergistic effects of single nucleotide polymorphisms (SNPs) in CCL5 and CCR5 genes on the risk and clinicopathological characteristics of oral cancer. Again, no significant oral cancer risk was found between individuals with the CCR5-59029 G/A genotype and those with the CCR-559029 wild type gene. In the present study, we found a statistically significant difference between the control and patient groups for CCR5 59029 A/G genotypes ($P < 0.01$). Individu-

als with the G allele (GG + AG genotypes) had an increased risk for OSCC development. In the control group, the frequency of the CCR5 59029 AA genotype was higher than that of the patient group ($P < 0.0001$). Frequencies of CCR5 59029 GG and CCR5-delta32 DD + ID were higher in Grade II-III OSCC patients than in Grade I-II OSCC patients.

Epidemiological studies have suggested that OSCC is a multifactorial disease that can be mediated by both environmental factors, such as alcohol and tobacco consumption, and genetic factors (Weng et al., 2010; Bektas-Kayhan et al., 2012). In parallel with these studies, we found that CCR5 59029 G+ genotypes (GG + AG), gender, smoking, alcohol consumption, and age were all associated with OSCC risk in univariate analyses; however, only CCR5 59029 G+ genotypes and alcohol consumption were associated with the disease in multivariate logistic regression analysis.

Polymorphisms occurring in promoter or regulatory regions of genes may affect either mRNA expression or protein function, which may in turn cause receptor dysfunction. It has been suggested that the majority of CCR5-positive cells are macrophages (Weng et al., 2010). Dysfunction of the CCR5 receptor results in the recruitment of large amounts of macrophages to the inflammation area, and thus induces inflammation-derived carcinogenesis.

In conclusion, our findings suggested that the polymorphic allele frequency of CCR5 59029 might be a risk factor of OSCC due to the loss of receptor function, which might cause increased inflammation, leading to the development of the disease. Further studies with larger sample groups are necessary to clarify the role of CCR5 genetic variants and the development of OSCC.

Conflicts of interest

The authors declare no conflicts of interest.

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