The Interleukin-1 Beta Gene Polymorphism +3953 C/T is not Associated with Risk for Oral Cancer

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Abstract. *Background: Elevated serum levels of interleukin-1 beta (IL-1β) have been previously observed in patients with oral cancer. Considering the demonstrated effect of other interleukins to the development of oral cancer, this study investigated whether the +3953 C/T polymorphism in the IL-1β gene is associated with this malignancy. Patients and Methods: The +3953 C/T polymorphism was examined in DNA samples of 108 patients with oral squamous cell carcinoma and 156 healthy controls. Results: The detected allele and carrier frequencies of the high expression T allele in the control group were 28.8% and 48.1%, respectively. In the patient group there was a slight decrease both in allele and carrier frequencies (24.1% and 38.9%, respectively), but these findings were not statistically significant. The same pattern was observed in subgroups of patients regarding cancer stage, family history of cancer or thrombosis, as well as smoking or heavy drinking habits. Conclusion: The +3953 C/T polymorphism, was not found to be associated with risk for oral cancer. It seems that IL-1β does not play a primary role in oral oncogenesis, since other interleukins, already associated with this malignancy, appear to exert a more prominent effect.*

Oral squamous cell carcinoma (OSCC) is the most common malignancy in the oral cavity and the eighth most common type of cancer worldwide (1). Oral carcinogenesis is a multistep process in which genetic alterations in oncogenes and tumor suppressor genes, as well as other factors such as smoking and alcohol abuse are involved (2). Recently, factors related to inflammation, angiogenesis and thrombosis have been implicated in the development of oral cancer (3-9). One such factor known to be involved in some types of cancer is the pro-inflammatory cytokine interleukin-1 beta (1L-1β) (10-11).

IL-1β has many pleiotropic biological functions, including mediation of inflammation by promoting the movement of inflammatory cells from the blood to inflamed tissues, regulation of the extracellular matrix and induction of endothelial cell production of cytokines such as tumor necrosis factor-alpha (TNF-α), IL-8, IL-6 and multiple cytokine receptors (12-17). Moreover, IL-1β increases VEGF gene expression in human colon cancer cells (18). Finally, IL-1β controls coagulant activity by increasing the expression of fibrinogen, tissue factor, type 1 and 2 plasminogen activator inhibitor genes and production of prostaglandin (19). Since IL-1β regulates many different functions, any alteration in its blood concentration may significantly affect some of these functions. In accordance with that assumption, serum levels have been found to be higher in patients with oral, gastro-esophageal, gastric and breast cancer than healthy controls (20-23).

The *IL-1β* gene has three biallelic polymorphisms at positions –511, –31, and +3953 from the transcriptional start site, which show total linkage disequilibrium (24, 25). We decided to study the +3953 C/T polymorphism located in exon 5 which has been found to affect *IL-1β* gene expression (26-27). Increased levels of IL-1β in serum have been associated with the less common T allele of the *IL-1β* +3953 C/T polymorphism (26-28). The frequency of the high expression T allele is 22-30% in Caucasians and 1-6%

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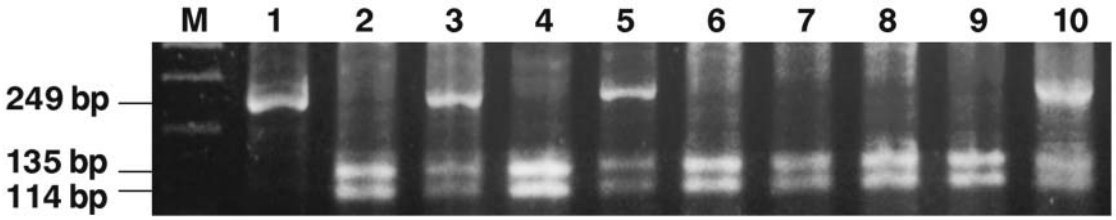

Figure 1. *Illustrative example of observed IL-1β genotypes. Agarose gel electrophoresis of Taq-digested PCR products for genotyping the +3953 IL-1β C/T polymorphism in 10 patients with oral cancer. The C allele corresponds to two fragments of 135 bp and 114 bp, while the T allele to a single fragment of 249 bp. M: molecular weight marker; 1: T/T homozygote; 2, 4, 6, 7, 8, 9: C/C homozygotes; 3, 5, 10: C/T heterozygotes.*

Table I. *Prevalence of IL-1β (+3953 C/T) polymorphism in healthy controls and patients with oral cancer (total group of patients and subgroups with regard to cancer stage).*

Genotype	Controls (%)	Patients			Patients with cancer stages I/II			Patients with cancer stages III&IV		
		(%)	*P*-value	OR (CI)	(%)	*P*-value	OR (CI)	(%)	*P*-value	OR (CI)
T/T	15 (9.6%)	10 (9.3%)	N.S.	0.82 (0.35-1.91)	6 (10%)	N.S.	0.85 (0.32-2.31)	4 (8.3%)	N.S	0.77 (0.25-2.42)
C/C	81 (51.9%)	66 (61.1%)			38 (63.3%)			28 (58.3%)		
C/T	60 (38.5%)	32 (29.6%)	N.S	0.65 (0.38-1.12)	16 (26.7%)	N.S.	0.57 (0.3-1.11)	16 (33.3%)	N.S.	0.77 (0.39-1.54)
Total	156 (100%)	108 (100%)			60 (100%)			48 (100%)		
Prevalence of T allele										
T allele frequency	28.8%	24.1%	N.S.	0.78 (0.53-1.16)	23.3%	N.S.	0.75 (0.46-1.22)	25%	N.S.	0.82 (0.49-1.4)
Carrier frequency of T allele	48.1%	38.9%	N.S.	0.69 (0.42-1.13)	36.7%	N.S.	0.62 (0.34-1.15)	41.7%	N.S.	0.77 (0.40-1.48)

Fisher's *p*-value corresponds to genotype comparisons and allele frequency comparisons; odds ratios (OR) are age-adjusted; N.S. not significant; CI: 95% confidence interval.

in East Asians (29-32). Two studies of *IL-1β* +3953 C/T polymorphism in Chinese patients with gastric carcinoma reported conflicting results. One suggested association of *IL-1β* +3953 C/T polymorphism with gastric cancer, while the other failed to detect any relationship (31, 33). A significant association was found between the +3953 T allele and the aggressive phenotype of breast carcinoma as defined by the high histological grade, axillary lymph node metastasis and large tumor size (34).

Additionally, no association of *IL-1β* +3953 C/T polymorphism with oral squamous cell carcinoma has been detected in Chinese patients, but as mentioned previously the high expression T allele is extremely low in the Chinese population (32). In light of the above, we examined whether the *IL-1β* +3953 C/T polymorphism is associated with increased risk for oral cancer in Europeans.

Patients and Methods

Study participants. DNA samples of 264 individuals of Greek and German origin were studied: 108 patients had oral squamous cell carcinoma and 156 healthy blood donors were recruited as controls with equivalent ethnicity, gender and age. The age of patients ranged between 40-83 years (52.1±7.2 years, median 58 years) and that of the controls ranged between 40-82 years (51.1±5.5 years, median 58 years). This study included patients operated on recently or up to 10 years ago and a pathological diagnosis of the tumor was available. From each patient, a family history was obtained for any type of cancer and thrombophilia. Forty-four patients (40%) had one or two first-degree relatives with some type of cancer, but their age range was similar to the whole group (median 58 years). Moreover, 26 individuals (23.6%) had one or two first-degree relatives with idiopathic thrombosis and an earlier age range (median 52 years). Twelve patients (9.2%) had a positive family history for both cancer and thrombophilia (median 48 years).

Table II. *Prevalence of IL-1β (+3953 C/T) polymorphism in healthy controls and subgroups of patients with regard to family history of any type of cancer or thrombosis.*

Genotype	Controls (%)	Positive family history of cancer						Positive family history of thrombophilia					
		Patients with			Patients without			Patients with			Patients without		
		(%)	*P*-value	OR (CI)	(%)	*P*-value	OR (CI)	(%)	*P*-value	OR (CI)	(%)	*P*-value	OR (CI)
T/T	15 (9.6%)	4 (8.7%)	N.S.	0.72 (0.23-2.25)	6 (9.7%)	N.S.	0.9 (0.33-2.44)	2 (7.7%)	N.S.	0.6 (0.14-2.6)	8 (9.8%)	N.S.	0.90 (0.36-2.24)
C/C	81 (51.9%)	30 (65.2%)			36 (58.1%)			18 (69.2%)			48 (58.5%)		
C/T	60 (38.5%)	12 (26.1%)	N.S.	0.54 (0.26-1.13)	20 (32.3%)	N.S.	0.75 (0.4-1.41)	6 (23.1%)	N.S.	0.45 (0.17-1.17)	26 (31.7%)	N.S.	0.73 (0.41-1.3)
Total	156 (100%)	46 (100%)			62 (100%)			26 (100%)			82 (100%)		
Prevalence of T allele													
T allele frequency	28.8%	21.7%	N.S.	0.68 (0.4-1.2)	25.8%	N.S	0.86 (0.54-1.4)	19.2%	N.S.	0.59 (0.3-1.21)	25.6%	N.S.	0.85 (0.55-1.3)
Carrier frequency of T allele	48.1%	34.8%	N.S.	0.58 (0.3-1.13)	41.9%	N.S.	0.71 (0.43-1.41)	30.8%	N.S.	0.48 (0.2-1.15)	41.5%	N.S.	0.76 (0.45-1.31)

Fisher's *p*-value corresponds to genotype comparisons and allele frequency comparisons; odds ratios (OR) are age-adjusted; N.S. not significant; CI: 95% confidence interval.

Molecular analysis. Blood samples were collected from both the patient and the control groups. All studied individuals were fully informed of the potential meaning of test results and willingly participated in the study. The patient samples were numbered and examined blindly.

DNA was extracted from blood with the use of NucleoSpin™ kit (Macherey-Nagel GmbH & Co, Dóren, Germany). Molecular analysis for the +3953 C/T polymorphism in the *IL-1β* gene was effected by restriction fragment length polymorphism analysis of polymerase chain reaction products (Figure 1). A genomic DNA fragment of 249 bp was amplified using primers 5'-GTTGTCATCAGACTTTGACC-3' and 5'-TTCAGTTCATATGG ACCAGA-3'. If the C allele is present at position +3953, the PCR product is digested by TaqI enzyme into two fragments of 135 bp and 114 bp. If the T allele is present, a single intact fragment of 249 bp is seen after electrophoretic analysis. For verification of genotyping, a number of DNA samples were analysed twice.

Statistical analysis. Statistical analysis was performed using SAS® software (version 9.0; SAS Worldwide Headquarters SAS Institute Inc., Cary, NC, USA). The frequencies of alleles and genotypes of the whole group or subgroups of patients were compared to the respective frequencies of the control group using the Fisher's exact test and odds ratios, while all genotype distributions were in accordance with Hardy-Weinberg estimates. All statistical analyses concerning: number of relatives with a history of cancer, number of relatives with a history of thrombosis, nicotine use, alcohol abuse have assumed that all controls have zero values for the above variables (*i.e.* all controls have no family history of cancer, all controls have no family history of thrombosis, all controls do not use tobacco, and all controls do not drink alcohol). Thus, odds ratios are most likely expected to overestimate the true likelihood of *IL-1β* genotypes and these variables. The Mantel-Haenszel method was used for the calculation of all odds ratios with a 95% confidence interval (CI). A *p*-value less than 0.05 was considered statistically significant.

Results

Table I shows the prevelance of the detected *IL-1β* genotypes in studied healthy controls and patients with oral cancer. There were no major differences in the allele frequencies of the two studied populations (Greeks and Germans), so all data for controls and patients were analysed together. The genotype distribution were compatible with Hardy-Weinberg equilibrium in the control group, as well as the total group and all subgroups of patients (Tables I-III).

The detected allele and carrier frequencies of the high expression T allele in the control group were 28.8% and 48.1% respectively. In patients there was a slight decrease both in allele and carrier frequencies (24.1% and 38.9%, respectively) but these findings were not statistically significant (Table I). The same pattern was observed in the

Table III. *Prevalence of IL-1β (+3953 C/T) polymorphism in healthy controls and subgroups of patients in regard to smoking or heavy alcohol drinking habits.*

Genotype	Controls (%)	Tobacco abuse						Alcohol abuse					
		Patients with			Patients without			Patients with			Patients without		
		(%)	*P*-value	OR	(%)	*P*-value	OR	(%)	*P*-value	OR	(%)	*P*-value	OR
T/T	15 (9.6%)	10 (9.8%)	N.S.	0.87 (0.37-2.04)	0 (0%)	N.S.	0 (0.0-5.57)	8 (23.5%)	N.S.	2.16 (0.82-5.7)	2 (2.7%)	N.S.	0.24 (0.06-0.97)
C/C	81 (51.9%)	62 (60.8%)			4 (66.7%)			20 (58.8%)			46 (62.2%)		
C/T	60 (38.5%)	30 (29.4%)	N.S.	0.65 (0.38-1.13)	2 (33.3%)	N.S.	0.67 (0.14-3.28)	6 (17.6%)	N.S.	0.41 (0.16-1.0)	26 (35.1%)	N.S.	0.76 (0.43-1.4)
Total	156 (100%)	102 (100%)			6 (100%)			34 (100%)			74 (100%)		
Prevalence of T allele													
T allele frequency	28.8%	24.5%	N.S.	0.8 (0.54-1.2)	16.7%	N.S.	0.49 (0.12-2.05)	32.4%	N.S.	1.18 (0.67-2.06)	20.3%	N.S.	0.63 (0.4-1.0)
Carrier frequency of T allele	48.1%	39.2%	N.S.	0.7 (0.42-1.15)	33.3%	N.S.	0.54 (0.11-2.6)	41.2%	N.S.	0.76 (0.36-1.6)	37.8%	N.S.	0.66 (0.37-1.15)

Fisher's *p*-value corresponds to genotype comparisons and allele frequency comparisons; odds ratios (OR) are age-adjusted; N.S. not significant; CI: 95% confidence interval.

subgroups of patients: a) with initial and advanced stages of cancer, b) with and without a positive family history of cancer, c) with and without a family history of thrombophilia, d) with and without tobacco or alcohol abuse (Tables I-III).

There was no statistical difference in genotypes between patients with oral cancer in comparison with controls (Table I). The same patern was observed in all subgroups of patients compared to control participants (Tables I-III).

Discussion

Cytokine IL-1β has been proven to be a multi-effect mediator of many physiological functions (12-17). As a result, any alteration in the IL-1β serum level may affect its biological activity. Due to the ability of IL-1β to regulate the degradation of the extracellular matrix and to induce the release of secondary cytokines, it is considered to play a crucial role in tumor progression, invasion and metastasis (10, 17).

Accordingly, increased serum levels of IL-1β have been detected in many malignancies, including oral squamous cell carcinoma (10, 20-23). A functional *IL-1β* +3953 C/T polymorphism in exon 5 has been associated with alterations in IL-1β production (26). In light of the above, the purpose of this study was to investigate whether the *IL-1β* +3953 C/T polymorphism had any association with oral squamous cell carcinoma in a cohort of 108 patients with oral cancer in comparison to 156 healthy controls of equivalent age, gender and ethnicity (Greeks and Germans).

There was no statistical difference in the T mutant allele and carrier frequencies between patients with oral cancer or their subgroups in comparison to controls. No significant difference with controls was observed in genotypes containing the mutant T allele in the total group and the subgroups of patients. As a result, it seems that the *IL-1β* +3953 C/T polymorphism is not associated with risk for oral cancer. Our results are in accordance with the findings of a study performed in a Chinese population in which no association of *IL-1β* +3953 C/T polymorphism with oral squamous cell carcinoma was detected, although in that population the T allele frequency is about twenty times lower than in Europeans (32).

The findings of the two genetic association studies in Europeans and Chinese are in discrepancy with the observed increase of IL-1β serum levels in patients with oral squamous cell carcinoma (10, 20). It seems that the elevation of IL-1β in oral cancer does not play a primary role due to its synergestic effect and interactions with other cytokines. IL-1β and TNF-α have the ability to regulate the immune response and simultaneously to induce the release of secondary cytokines, such as IL-6

and IL-8. Interestingly, increased levels of TNF-α, IL-6 and IL-8 have already been strongly associated with increased risk for oral squamous cell carcinoma (5, 8, 9). In addition, IL-6 and TNF-α stimulate oral cancer cells to increase secretion of matrix metalloproteinases (MMPs) and at least one of them, MMP-1, has also been associated with increased risk for oral cancer by promoting angiogenesis and invasion (9, 35). Moreover, increased levels of IL-6 may inhibit IL-1β (36), therefore the role of IL-1β in oral oncogenesis may be minimal in comparison with that of other factors.

As a consequence, it is of great importance to perform further genetic association studies regarding the contribution of additional cytokines or other factors related to angiogenesis, inflammation and thrombosis to oncogenesis in the oral region. Any positive findings could ultimately result in the undertaking of preventive measures safeguarding the health status and lives of certain at risk individuals in the general population.

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