The *Interleukin-10* (–1082A/G) Polymorphism is Strongly Associated with Increased Risk for Oral Squamous Cell Carcinoma

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Abstract. *Background: Increased levels of interleukin-10 (IL-10) have been observed in patients with oral cancer, possibly as a result of suppression of the immune response. Based on this, the –1082A/G polymorphism, which influences IL-10 gene expression level, was investigated in regard to its possible association with risk for oral cancer. Patients and Methods: The polymorphism was examined in DNA samples of 144 patients with oral squamous cell carcinoma and 141 healthy controls of equivalent gender, age and ethnicity. Results: The detected allele frequencies for the high expression G allele were significantly higher in patients compared to controls (34.7% versus 21.3%, respectively, p=0.0004), as well as in patients that were smokers but not those that were heavy alcohol consumers. This highly significant difference in G allele frequency was mainly due to the increase of AG heterozygotes in patients compared to controls (OR 3.05, 95% CI 1.84-5.05). Conclusion: These findings suggest that the high expression G allele of the –1082A/G polymorphism of the inflammation and angiogenesis-related IL-10 is strongly associated with increased risk for oral cancer.*

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Oral carcinogenesis is a multistep process in which many factors, such as smoking, alcohol, pathogenic infections, nutrition deficiency, and genetic factors (alterations in oncogenes and tumor suppressor genes) are involved (1). Recently, a number of factors related to inflammation, angiogenesis and thrombosis have been correlated with increased risk for the development of malignant tumors in the oral cavity (2-9).

A factor known to be involved in inflammation, angiogenesis, thrombophilia autoimmune disease and several types of cancer (including oral carcinoma) is interleukin-10 (IL-10) (10-17). Produced by lymphoid cells, monocytes and macrophages, IL-10 is a multifunctional immunosuppressant cytokine that not only inhibits the activation and effective function of T-cells but also determines the termination of inflammatory responses (18). Furthermore, it regulates growth and differentiation of B-cells, NK, cytotoxic and T helper cells, mast cells, granulocytes, dendritic cells, keratinocytes and endothelial cells (19). Increased expression levels of IL-10 have been observed in patients with oral squamous cell carcinoma and were associated with poor prognosis (11, 12).

The level of *IL-10* gene expression is strongly influenced by genetic factors such as polymorphisms at positions –3375, –2763 and –1082 in the promoter region of the gene (20). Due to linkage disequilibrium of certain polymorphic alleles, high or low IL-10 gene expression may be determined by analysis of only one polymorphic site (20). Therefore, the presence of the G allele of the –1082A/G polymorphism is associated with higher IL-10 production (20, 21). The G-carrying genotypes have been found to be significantly more frequent in patients with

lung squamous cell carcinoma (22). On the contrary, the AA genotype was significantly more frequent in patients with prostate cancer and cutaneous malignant melanoma (23, 24). No association of the –1082A/G polymorphism was observed with either esophageal squamous cell carcinoma or gastroesophageal junction adenocarcinoma (25, 26). The frequency of the high expression G allele ranges between 20-52% in Caucasians and 21-84% in Oriental Asians (23-27).

The purpose of this study was to examine whether the *IL-10* –1082A/G polymorphism is associated with risk for oral squamous cell carcinoma. Therefore, the –1082A/G polymorphism was studied in patients with oral cancer and healthy controls representing the general population.

Patients and Methods

In this study, 285 Greeks and Germans participated, after informed consent had been obtained, including 144 patients with oral squamous cell carcinoma and 141 healthy blood donors of equivalent ethnicity, gender and age. The patients were mostly men (N=114, 79.2%) and their age ranged between 40-84 years (mean 59.5±10 years). The age (ranged 38-76 years; mean 54.5±11.1 years) and the sex ratio of the controls (N=105, 74.5% men) were both comparable to those of the patients.

The patients under study had been operated on for oral cancer recently or up to a decade ago. In addition to clinical presentation, a biopsy with pathological diagnosis of tumor stages I-IV and a family history regarding any type of cancer or thrombophilia were available for each patient. Fifty-two patients (36.1%) had one or two first degree relatives with cancer and their age range (mean=59.9 years) did not differ significantly from the whole group of patients. Furthermore, 28 patients (19.4%) had one or two first-degree relatives with idiopathic thrombosis (mean=58.9 years) but again with no statistical difference compared to the whole group. Twelve patients (8.3%) had a positive family history for both cancer and thrombophilia (mean age=57.8 years).

Nearly all patients (94.4%) were smokers and about a third of them (31.9%) were alcohol abusers, consuming >3 drinks per day. Two thirds of the controls (62.7%) reported abuse of tobacco and about one third (26%) abuse of alcohol. Most of the participants in the two groups generally worked in a low-risk environment (with the exception of one patient and three controls who worked in chemical factories).

Blood samples were collected from all individuals under study and DNA was isolated with the use of a Nucleon™ kit (Macherey-Nagel GmbH & Co, Düren, Germany). Molecular detection of the –1082A/G polymorphism in the *IL-10* gene was performed by restriction fragment length polymorphism typing. This involved a combination of PCR amplification and digestion with restriction endonuclease *Mnl I* followed by gel electrophoretic analysis. The PCR conditions consisted of an initial denaturation step at 94˚C, followed by 35 cycles of 94˚C for 50 sec, 54˚C for 1 min, and 72˚C for 55 s, as well as a final elongation step at 72˚C for 5 min. The pair of primers used was: 5'-ATCCAAGACAACATCTACTAA-3' and 5'-TAAATATCCTCAAAGTTCC-3'. The generated PCR product of 587 bp was cleaved by restriction enzyme *Mnl I* into two constant fragments of 271 bp and 237 bp, as well into one additional fragment of 113 bp when the A allele was present, or two additional fragments of 80 bp and 33 bp when the G allele was present. For verification of molecular analysis results, some of the samples were tested twice.

Statistical analyses were 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 age-adjusted Fisher's exact test and odds ratios. The Maentel-Haenzel 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

The detected *IL-10* genotypes in healthy controls (representing the general population) and patients with oral cancer are shown in Table I. The data for the two populations under study (Greek and German healthy controls) were analyzed together, since there were no significant differences in genotype and allele frequencies of the *IL-10* polymorphism among the two populations.

In the control group the observed high expression G allele frequency was 21.3% and the carrier frequency was 42.6% (Table I). The detected carrier and allele frequencies for the high expression G allele in the patient group were significantly higher in comparison with that of the control group ($p<0.0001$ and $p=0.0004$, respectively Table I). The same pattern of significance was observed between controls and the subgroups of patients: a) in initial (I and II) stages of cancer ($p=0.002$ and $p=0.0121$, respectively, Table I), b) in advanced (III and IV) stages of cancer ($p=0.0001$ and $p=0.0007$, respectively, Table I), c) with positive family history of cancer ($p<0.0001$ and $p=0.0001$, respectively, Table II), d) without positive family history of cancer ($p=0.0074$ and $p=0.0285$, respectively, Table II), e) with positive family history of thrombophilia ($p=0.0007$ and $p=0.0061$, respectively, Table II), f) without positive family history of thrombophilia ($p=0.0003$ and $p=0.0019$, respectively, Table II), g) with tobacco abuse ($p<0.0001$ and $p=0.0006$, respectively, Table III), and h) without alcohol abuse ($p<0.0001$ in both cases, Table III).

Since GG homozygotes are very rare in the studied populations, the observed statistical difference of high expression G allele frequencies was due to the increase of AG heterozygotes in patients, in comparison with controls (Tables I-III). Compared to individuals with the AA genotype, the relative risk (OR) for AG heterozygotes to develop oral cancer was 3.05 (95% CI 1.84-5.05, Table I). Interestingly, AG individuals with a positive family history of cancer or thrombophilia have an even higher relative risk of developing oral cancer (OR 6.12, 95% CI 2.7-14.01 and OR 4.74, 95% CI 1.8-12.60, respectively, Table II).

Table I. Prevalence of IL-10 (–1802A/G) polymorphism in healthy controls and patients in regard to initial (I, II) and advanced (III, IV) cancer stages.

Genotype	Controls	Patients			Cancer stages					
					I & II			III & IV		
	n (%)	n (%)	*P*-value	OR (CI)	n (%)	*P*-value	OR (CI)	n (%)	*P*-value	OR (CI)
G/G	0 (0.0%)	2 (1.4%)	N.S.	7.13 (0.33-155.4)	0 (0.0%)			2 (3.13%)	**0.0376**	15.95 (0.71-358.3)
A/A	81 (57.45%)	46 (31.94%)		1 (referent)	28 (35.0%)		1 (referent)	28 (43.8%)		1 (referent)
A/G	60 (42.55%)	96 (66.67%)	**<0.0001**	3.05 (1.84-5.05)	52 (65.0%)	**0.002**	2.53 (1.40-4.55)	44 (68.75%)	**0.0002**	3.48 (1.71-7.10)
Total	141 (100%)	144 (100%)			80 (100%)			64 (100%)		
Prevalence of G allele										
G allele frequency	21.3%	34.7%	**0.0004**	2.10 (1.42-3.08)	32.5%	**0.0121**	1.86 (1.18-2.94)	37.5%	**0.0007**	2.46 (1.52-3.97)
Carrier frequency of G allele	42.6%	68.1%	**<0.0001**	3.12 (1.88-5.15)	65.0%	**0.002**	2.53 (1.40-4.55)	71.9%	**0.0001**	3.71 (1.84-7.50)

Fischer'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.

Discussion

Increased levels of IL-10 have been observed in patients with solid tumors, including oral squamous cell carcinoma, suggesting that this pleiotropic cytokine may have an important role in malignancy (10-12). IL-10 has been found to promote tumor proliferation by suppressing the immune and inflammatory responses (10, 14, 17).

A single nucleotide polymorphism (–1082A/G) in the promoter region of the *IL-10* gene affects its expression, and as a consequence the levels of IL-10 (21). The purpose of this study was to examine whether this polymorphism is associated with risk for oral cancer. The individuals under study were patients with oral squamous cell carcinoma whose genotypes were compared to those of matched healthy controls.

Despite the modest number of studied individuals, a significant difference was observed in the total group and several subgroups of patients. The AG heterozygotes were significantly more frequent in patients with oral cancer, regardless of their family history of either cancer or thrombophilia, and regardless of their cancer stage. Interestingly, the high expression G allele behaves as a true dominant trait. The same pattern was also observed in subgroups of patients with smoking habits and without alcohol abuse. While the effect of tobacco may not be evaluated because almost all studied patients were smokers, it seems that the effect of the high expression G allele is more pronounced in individuals without heavy drinking habits. Based on these results, constitutive high levels of *IL-10* gene expression may have an important role in increasing susceptibility for development of oral cancer, taking into account its multifunctional effects.

The present findings are in accordance with the previously observed increase of IL-10 expression in oral carcinomas (11, 12). In an immunohistochemical study of oral and oropharyngeal carcinomas, the intensity of positive staining for IL-10 was inversely correlated with the overall survival rate (12). Increased plasma levels of IL-10 were detected in patients with head and neck squamous cell carcinoma, especially in those with metastasizing tumors (11).

IL-10 may increase susceptibility to oral cancer in a synergistic manner with other cytokines. For example, although IL-10 inhibits some cytokines, including IL-6, in lymphocyte cultures, it may act as a cooperative growth factor with IL-6 for non-Hodgkin's lymphoma (28). Such a cooperative role of IL-10 and IL-6 in oral malignancies might be extremely potent. In accordance with this notion, we have previously found that a polymorphic allele, which confers higher gene expression of *IL-6* after an inflammatory stimulus, is significantly associated with the aggressive progress of oral oncogenesis (5).

On the other hand, there is a discrepancy between this study and reports for absence of association of the –1082A/G polymorphism with esophageal cancer in a

Table II. *Prevalence of IL-10 (–1802A/G) polymorphism in healthy controls and the subgroup of patients in regard to family history of cancer and thrombophilia.*

Genotype	Controls n (%)	Positive family history of cancer						Positive family history of thrombophilia					
		Patients with			Patients without			Patients with			Patients without		
		n (%)	*P*-value	OR (CI)	n (%)	*P*-value	OR (CI)	n (%)	*P*-value	OR (CI)	n (%)	*P*-value	OR (CI)
G/G	0 (0.0%)	2 (3.85%)	**0.0154**	67.0 (2.5-1811.3)	0 (0.0%)			0 (0.0%)			2 (1.72%)	N.S	7.8 (0.4-170.2)
A/A	81 (57.45%)	10 (19.23%)		1 (referent)	36 (39.13%)		1 (referent)	6 (21.4%)		1 (referent)	40 (34.5%)		1 (referent)
A/G	60 (42.55%)	40 (76.9%)	**<0.0001**	6.12 (2.7-14.01)	56 (60.87%)	**0.0074**	2.24 (1.29-3.90)	22 (78.6%)	**0.0007**	4.74 (1.8-12.6)	74 (63.79%)	**0.0004**	2.73 (1.6-4.7)
Total	141 (100%)	52 (100%)			92 (100%)			28 (100%)			116 (100%)		
Prevalence of G allele													
G allele frequency	21.28%	42.3%	**0.0001**	3.07 (1.83-5.16)	30.4%	**0.0285**	1.72 (1.11-2.65)	39.3%	**0.0061**	2.45 (1.3-4.5)	33.6%	**0.0019**	2.00 (1.3-3.03)
Carrier frequency of G allele	42.55%	80.8%	**<0.0001**	6.33 (2.77-14.47)	60.9%	**0.0074**	2.24 (1.3-3.9)	78.6%	**0.0007**	4.74 (1.8-12.6)	65.5%	**0.0003**	2.81 (1.6-4.8)

Fischer'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.

Table III. *Prevalence of IL-10 (–1802A/G) polymorphism in healthy controls and patients with oral cancer according to either smoking or heavy alcohol consumption habits.*

Genotype	Controls n (%)	Positive family history of cancer						Positive family history of thrombophilia					
		Patients with			Patients without			Patients with			Patients without		
		n (%)	*P*-value	OR (CI)	n (%)	*P*-value	OR (CI)	n (%)	*P*-value	OR (CI)	n (%)	*P*-value	OR (CI)
G/G	0 (0.0%)	2 (1.47%)	N.S.	7.79 (0.4-70.2)	0 (0.0%)			0 (0.0%)			2 (2.0%)	N.S.	15.95 (0.7-59.3)
A/A	81 (57.45%)	44 (32.35%)			2 (25%)		1 (referent)	22 (47.8%)		1 (referent)	24 (24.5%)		1 (referent)
A/G	60 (42.55%)	90 (66.2%)	**0.0001**	2.97 (1.11-3.66)	6 (75%)	N.S.	3.80 (0.83-17.50)	24 (52.2%)	N.S.	1.6 (0.8-3.2)	72 (73.5%)	**<0.0001**	4.43 (2.5-7.97)
Total	141 (100%)	136 (100%)			8 (100%)			46 (100%)			98 (100%)		
Prevalence of G allele													
G allele frequency	21.3%	34.6%	**0.0006**	2.07 (1.40-3.07)	37.5%	N.S.	2.43 (0.81-7.27)	26.1%	N.S.	1.42 (0.80-2.51)	38.8%	**<0.0001**	2.49 (1.6-3.8)
Carrier frequency of G allele	42.6%	67.7%	**<0.0001**	3.04 (1.83-5.06)	75.0%	N.S.	3.80 (0.8-17.5)	52.2%	N.S.	1.59 (0.79-3.20)	75.5%	**<0.0001**	4.55 (2.5-8.2)

Fischer'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.

Chinese population (25, 26). That discrepancy might be explained either by the diverse ethnic background of the two studied populations or by the different mechanisms possibly involved in the development of oral and esophageal cancer. The latter explanation is reinforced by findings of two other Chinese studies in which a matrix *metalloproteinase-1* gene polymorphism was found to be associated with oral cancer but not with esophageal cancer (29, 30).

In conclusion, the present study indicates that the –1082A/G polymorphism of the IL-10 gene is associated with increased risk for oral cancer.

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