Effect of Thrombosis-related Gene Polymorphisms upon Oral Cancer: A Regression Analysis

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Abstract. *It is well-known that there is an interplay between hemostasis, thrombosis and cancer. Functional DNA polymorphisms in genes encoding factors related to thrombosis have been associated with increased risk for oral squamous cell carcinoma (OSCC). The present study investigated the possible combinatory effect of 10 such polymorphisms as primary risk predictors for OSCC in a European population. Two groups including 160 patients with OSCC and 168 healthy controls of Greek and German origin were studied. The patient and control groups were comparable regarding ethnicity, age and gender. For all studied individuals, 10 genotypes of functional polymorphisms were investigated: 5,10-methylene tetrahydrofolate reductase (MTHFR) C677T, coagulation factor V (F5) Leiden, coagulation factor II (F2, also known as prothrombin) G20210A, coagulation factor XII (F12) C46T, coagulation factor XIII A1 subunit (F13A1) Val34Leu, serpine1 (SERPINE1, also known as plasminogen activator inhibitor-1) 4G/5G, protein Z (PROZ) -A13G, angiotensin I-converting enzyme (ACE) I/D, angiotensinogen (AGT) Met325Thr, and carboxypeptidase B2 (CPB2, also known as thrombin-activatable fibrinolysis inhibitor) C1040T. Multivariate logistic regression models were used in order to evaluate the relation and contribution of homozygous and heterozygous variant polymorphisms upon overall, early and advanced stages of OSCC. Five out of the studied polymorphisms, influencing the expression of SERPINE1 and ACE genes, as well as the activity of CPB2, F12 and F13 proteins, were recognized as significant predictive factors for OSCC. The 'mode of inheritance' regression model, in particular, revealed the low expression I allele of ACE to be a primary predictor in overall, early and advanced stages of oral cancer. Comparing the present findings with previous knowledge, possible interactions of these factors and their relation to the risk for OSCC development are discussed.*

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It is well-known that there is an interplay between hemostasis, thrombosis and cancer (1-7). Patients with cancer have an increased risk for venous thromboembolism (1, 3, 5) and several coagulation factors have been associated with the development of different cancer types (1, 3, 7).

Oral squamous cell carcinoma (OSCC) is the seventh most common human malignancy, while its incidence is increasing worldwide, especially among young people (8-10). Oral carcinogenesis is a multistep process involving gene alterations in oncogenes and tumor suppressor genes due to stochastic events, lifestyle factors such as smoking and alcohol abuse, viral infection and genetic predisposition, such as common DNA polymorphisms (11-26).

It is imperative to early diagnose oral cancer in order to achieve the best possible treatment and favorable prognosis. The established routine check-up includes medical history, extra-oral and intra-oral examination, and in case of clinical findings, a biopsy of the potentially malignant lesion (27). This protocol is used in everyday clinical practice of dentists and other medical professionals, but it seems to be inadequate for the prevention of the disease, as epidemiological data of recent decades indicate (11, 27). On the other hand, detection of individuals at risk for OSCC based on their genetic background might be a promising pre-

symptomatic method in the future. Such a protocol addressing genetic predisposition may result in greater intervention and follow-up, even before the appearance of oral lesions.

Although the majority of single nucleotide polymorphisms (SNPs) are silent, they may influence gene expression, protein function and cancer susceptibility in certain individuals (13-26). A number of SNPs related to thrombosis have been associated with an increased risk for developing oral malignancy, by our group and others (13-21, 27, 28). In addition, several studies have correlated tissue or serum levels of such factors with oral carcinogenesis, metastasis and, in some cases, with clinical prognosis and/or relapse-free survival in OSCC (29-32). These factors may also serve as genetic markers for cancer prognosis and prevention when genetic variations of certain individuals are taken into account. The concurrent analysis of all existing genetic data, as well as their possible combinatory effects can be used for the assessment of the polymorphic alleles which are of primary importance for cancer susceptibility.

The present multivariate logistic regression analysis is based on a retrospective case control study that investigated 10 common functional SNPs affecting the serum and tissue level or the activity of factors related to thrombosis. These factors include 5,10-methylene tetrahydrofolate reductase (MTHFR), coagulation factor V (F5), coagulation factor II (F2, also known as prothrombin), coagulation factor XII (F12), coagulation factor XIII A1 subunit (F13A1), serpine1 (SERPINE1, also known as plasminogen activator inhibitor-1), protein Z (PROZ), angiotensin I-converting enzyme (ACE), angiotensinogen (AGT), and carboxypeptidase B2 (CPB2, also known as thrombin-activatable fibrinolysis inhibitor). A primary objective of this study was the assessment of combinatory effects and possible interactions between the various examined genotypes, which might allow better intervention and follow-up in high-risk individuals. A secondary objective was the evaluation of various predictive models involving the studied factors in relation to occurrence of OSCC.

Patients and Methods

Study population. The present regression analysis was based on a retrospective case control study design. The studied cohort (N=328) included Caucasians of Greek and German origin, who participated after informed consent. Studied individuals were divided in two groups: a case group (160 patients with OSCC) and a control group (168 healthy blood donors). All patients were diagnosed with OSCC based on histopathological confirmation of a biopsy. Controls were matched to patients in regard to ethnicity, gender, age, and a low-risk working environment.

Patients and controls with a positive history of any other cancer type were excluded from this study. The protocol was approved by the Ethical Review Boards of the Departments of Oral and Maxillofacial Surgery of the Universities of Athens and Erlangen.

Genotyping. Blood samples were collected from all participating individuals, and provided the source for genomic DNA isolation. Restriction fragment length polymorphism (RFLP) typing and gel electrophoretic analysis of DNA samples was performed blindly for detection of studied SNPs. PCR conditions and respective restriction enzymes have been previously described (13-21). Many samples were analyzed twice for verification of the molecular results. The following SNPs were studied: *MTHFR* C677T, *F2* G20210A, *F5* Leiden, *F12* C46T, *F13* V34L, *PZ* -13A/G, *SERPINE1* 4G/5G, *CPB2* 1040C/T, *ACE* intron16 D/I, *AGT* M235T. The rarer alleles in the healthy control groups are referred to as 'variant' alleles in the subsequent text.

Statistical analysis. Statistical comparisons were performed using SAS 9.0 version (SAS Institute Inc., Cary, North Carolina, USA). A p-value of ≤ 0.05 was considered as statistical significant. Fisher's exact test was used in order to detect any significant difference between frequency distribution of selected characteristics (age, gender) of controls and OSCC cases. The genotyping data were analyzed with the two-tailed Fisher's exact test and the Mantel–Hänszel method for the calculation of age-and gender-adjusted odds ratios (OR) and their respective 95% confidence intervals (CI). All genotypic frequencies in the combined control group were tested for compatibility with the Hardy–Weinberg equilibrium.

Ten multivariate logistic regression analyses were used in order to compare the variant homozygous/heterozygous genotypes *versus* the homozygous wild-type genotypes for each studied SNP. Univariate and stepwise backwards multivariate logistic regression analyses were performed in order to identify the most significant contribution of SNPs to OSCC risk in various models, including the matching binomial terms 'age' (<55 years *versus* ≥55 years) and 'gender' (female *versus* male). The cancer stage outcome (overall, early and advanced stages) was analyzed for each of the three following models: (A) a 'homozygous variant model' using homozygous variant genotypes; (B) a 'carrier model' using heterozygous variant/wild-type genotypes; and (C) a 'biological model' using either homozygous variant/variant or a combination of homozygous variant/variant and heterozygous variant/wild-type genotypes, according to the recessive or dominant character of the variant allele of each studied SNP, as determined by the observed significant difference between patients and controls in regard to either only homozygous variant genotypes (recessive) or both homozygous variant and heterozygous genotypes (dominant). The risks for development of OSCC, early-stage OSCC and advanced-stage OSCC were separately calculated. In order to quantify the significance of the constructed regression models, the Hosmer-Lemeshow goodness of fit test (HLGFT) was applied.

Results

Compatibility of patients and controls. The frequency distribution of selected characteristics of controls and OSCC cases was analyzed first. The age and gender distributions between controls and cases were not significantly different (p=0.08 and p=0.29, respectively). The mean age was 54.7 years (±11.9 years; range=31-83 years) for controls and 58.5 years (±10.1 years; range=40-84 years) for OSCC cases. Moreover, the genotypic distributions and allelic frequencies of all

polymorphisms were not significantly different in Greek and German controls when they were compared according to the two-tailed Fisher's exact test. Therefore, a single control group consisting of both ethnicities was used for all further analyses. All genotypic frequencies in the combined control group were compatible with the Hardy–Weinberg equilibrium.

Genotypic frequencies of patients and controls. The genotypic frequencies of all studied polymorphisms are summarized in Table I. *SERPINE1* (-675 4G/4G and 5G/4G), *CPB2* (1040T/T), *ACE* (Intron 16I/I), *F12* (C46T T/T) and *F13* (V34L) genotypes were found to be strongly associated with OSCC overall ($p<0.05$). Similarly, a significant association with early-stage OSCC was also detected for the *SERPINE1* (-675 4G/4G and 5G/4G), *ACE* (intron 16I/I) and *F13* (V34L L/L) genotypes ($p<0.05$). Regarding advanced-stage OSCC, *CPB2* (1040T/T), *ACE* (intron 16I/I), *F13* (V34L) and *F12* (C46T T/T) genotypes had a significant association ($p<0.05$).

Multivariate analysis of homozygous variant genotypes and OSCC risk (model A). Multivariate logistic regression analysis for homozygous variant genotypes and overall OSCC stages indicated that gender, as well as *SERPINE1* and *ACE* homozygous variant genotypes, met the $p<0.05$ significance criterion (Table II) and could therefore be mentioned as independent predictors of OSCC in the final regression model (HLGFT χ^2 value=4.81; $p=0.68$). This initial model varied according to early-and late-stage OSCC, respectively: Only gender and *ACE* homozygous variant genotype were independent predictors of early-stage cancer in the final regression model (HLGFT χ^2 value=7.10; $p=0.13$), while age and *ACE* homozygous variant genotype were independent predictors of advanced-stage OSCC (HLGFT χ^2 value=2.37; $p=0.50$).

Multivariate analysis of carrier genotypes and OSCC risk (model B). The multivariate logistic regression analysis for carrier genotype and overall OSCC stage resulted in *SERPINE1* carrier genotype (Table II) being found as an independent predictor (HLGFT $\chi 2$ value=5.5; $p=0.48$). Moreover, independent predictors for early-stage OSCC were age, gender and *MTHFR* carrier genotype (HLGFT χ^2 value=3.17; $p=0.67$). Analogous multivariate logistic regression analysis for late OSCC stage indicated *F12* and *F13* carrier genotypes as remarkable prognostic factors for this stage (HLGFT χ^2 value=7.9; $p=0.25$).

Multivariate analysis of the 'mode of inheritance' polymorphic genotypes and OSCC risk (model C). A biological model (based on 'mode of inheritance') predicting the risk for OSCC was constructed according to the multivariate logistic regression procedure. In this regression model (HLGFT χ^2 value=6.56; $p=0.16$), age, gender and *ACE* dominant genotype were shown to independently predict the risk for developing OSCC (Table II). In another biological model constructed for predicting the risk for early-stage OSCC (HLGFT χ^2 value=7.1; $p=0.131$), gender and *ACE* homozygous variant were proven significant. Finally, a biological model for predicting the risk for developing advanced-stage OSCC was constructed (HLGFT χ^2 value=2.37; $p=0.50$), indicating age and *ACE* genotype as being significant predictors for these stages of malignancy.

Discussion

Functional SNPs that affect gene expression or activity of factors related to thrombosis may confer susceptibility to oral cancer and its progression (13-26). Regression analysis of combined effects of SNPs in genes encoding factors that participate in interconnected biological mechanisms may reveal the relative significance of each polymorphism to the final clinical outcome. In light of the above, the present study utilised multivariate logistic regression analysis in order to assess the combinatory effect of 10 common DNA polymorphisms related to thrombosis in OSCC risk.

Each of the three studied regression models (A, B and C) was analyzed according to the cancer overall, and early and advanced cancer stage. Despite the modest sample size, interesting evidence emerged that associated OSCC development with half of the studied SNPs (*SERPINE1* 5G/4G, *ACE* intron 16 I/D, *MTHFR* C677T, *F12* C46T and *F13* V34L). The findings of multivariate logistic regression for each model highlight some possible molecular mechanisms underlying the development of OSCC.

Model A reveals significant independent factors for OSCC risk to be age, gender, *ACE* I/I genotype and 4G/4G genotype of the *SERPINE1* polymorphism. Consequently, this model suggests that increased risk of OSCC involves mainly proteins ACE and SERPINE1. It is known that ACE de-activates bradykinin, which in turn is implicated in the production of SERPINE1 (28). It seems that in oral cancer, the decreased production of ACE due to the low expression genotype I/I results in reduced degradation of bradykinin and increased SERPINE1 level. This notion is underlined by the fact that the high expression *SERPINE1* genotype 4G/4G is also associated with increased OSCC risk. The increased amount of SERPINE1 affects carcinogenesis in the oral cavity primarily through the regulation of cell detachment and migration rather than by layer proteolysis. It must be noted, though, that according to the same model, only the *ACE* polymorphism is significant for both early and advanced stages of OSCC.

Model B for the OSCC overall also revealed significant independent factors in the development of neoplasia to be age, gender and 4G/5G genotype of the *SERPINE1*

Table I. *Genotypes of healthy controls and patients and their subgroups with early and advanced stages of oral squamous cell carcinoma (OSCC).*

Genotype	Controls (N=168)	Patients (N=160)								
		Overall (N=160)			Early-stage (N=88)			Advanced-stage (N=72)		
	N (%)	N (%)	*p*-Value*	OR (95% CI)	N (%)	*p*-Value*	OR (95% CI)	N (%)	*p*-Value*	OR (95% CI)
SERPINE1 -675										
4G/4G	31 (29.2)	45 (43.3)	0.001	3.09 (1.30-7.36)	32 (54.2)	<0.001	12.82 (2.71-60.59)	13 (28.9)	1.000	1.04 (0.37-2.87)
5G/5G	32 (30.2)	12 (11.5)		1.00 (reference)	0 (0.0)		1.00 (reference)	12 (26.7)		1.00 (reference)
5G/4G	43 (40.6)	47 (45.2)	0.009	2.41 (1.02-5.71)	27 (45.8)	<0.001	8.54 (1.79-40.77)	20 (44.4)	0.672	1.11 (0.44-2.79)
CPB2 1040										
T/T	18 (13.0)	8 (5.3)	0.018	0.29 (0.11-0.74)	6 (7.1)	0.164	0.53 (0.17-1.61)	2 (3.0)	0.017	0.22 (0.06-0.82)
C/C	54 (39.1)	72 (48.0)		1.00 (reference)	38 (45.2)		1.00 (reference)	34 (51.5)		1.00 (reference)
T/C	66 (47.8)	70 (46.7)	0.386	0.74 (0.45-1.23)	40 (47.6)	0.663	0.79 (0.43-1.43)	30 (45.5)	0.353	0.68 (0.36-1.29)
ACE intr. 16										
I/I	9 (5.9)	30 (18.8)	<0.001	5.92 (2.00-17.48)	20 (22.7)	<0.001	7.37 (2.19-24.75)	10 (13.9)	0.031	4.65 (1.34-16.09)
D/D	78 (51.0)	60 (37.5)		1.00 (reference)	32 (36.4)		1.00 (reference)	28 (38.9)		1.00 (reference)
D/I	66 (43.1)	70 (43.8)	0.226	1.39 (0.85-2.27)	36 (40.9)	0.378	1.29 (0.71-2.33)	34 (47.2)	0.288	1.57 (0.82-3.01)
AGT M235T										
T/T	15 (12.1)	23 (14.1)	0.448	1.71 (0.76-385)	14 (17.1)	0.384	1.79 (0.66-4.83)	8 (11.8)	0.596	1.59 (0.54-4.74)
M/M	48 (38.7)	53 (32.5)		1.00 (reference)	30 (36.6)		1.00 (reference)	18 (26.5)		1.00 (reference)
M/T	61 (49.2)	87 (53.4)	0.363	1.40 (0.82-2.39)	38 (46.3)	1.000	1.00 (0.52-1.92)	42 (61.8)	0.099	2.18 (1.06-4.47)
F5 Leiden										
Mutants	0 (0.0)	0 (0.0)	NC	NC	0 (0.0)	NC	NC	0 (0.0)	NC	NC
Normals	114 (95.0)	133 (92.4)		1.00 (reference)	71 (92.2)		1.00 (reference)	62 (92.5)		1.00 (reference)
Heteroz.	6 (5.0)	11 (7.6)	0.456	1.49 (0.54-4.13)	6 (7.8)	0.544	1.58 (0.44-5.62)	5 (7.5)	0.527	1.67 (0.50-5.53)
F12 C46T										
T/T	6 (4.1)	0 (0.0)	0.008	0.20 (0.03-128)	0 (0.0)	0.167	0.33 (0.03-3.33)	0 (0.0)	0.039	0.28 (0.04-1.85)
C/C	42 (28.6)	56 (36.8)		1.00 (reference)	22 (25.0)		1.00 (reference)	34 (53.1)		1.00 (reference)
C/T	99 (67.4)	96 (63.2)	0.217	0.70 (0.42-1.17)	66 (75.0)	0.453	0.99 (0.52-1.87)	30 (46.9)	0.002	0.41 (0.21-0.77)
F13 Val34Leu										
L/L	6 (4.4)	12 (9.2)	0.046	2.63 (0.91-7.62)	10 (13.2)	0.010	3.73 (1.23-11.33)	2 (3.7)	1.000	1.96 (0.37-10.50)
V/V	81 (60.0)	56 (43.1)		1.00 (reference)	32 (42.1)		1.00 (reference)	24 (44.4)		1.00 (reference)
V/L	48 (35.6)	62 (47.7)	0.021	1.70 (0.98-2.95)	34 (44.7)	0.066	1.72 (0.91-3.26)	28 (51.9)	0.047	1.78 (0.84-3.75)
MTHFR C677T										
T/T	10 (8.3)	6 (5.5)	1.000	1.02 (0.30-3.48)	4 (6.6)	0.746	1.10 (0.25-4.93)	2 (4.1)	1.000	1.24 (022-7.10)
C/C	45 (37.5)	28 (25.5)		1.00 (reference)	15 (24.6)		1.00 (reference)	13 (26.5)		1.00 (reference)
C/T	65 (54.2)	76 (69.0)	0.043	1.93 (1.04-3.56)	42 (68.8)	0.088	2.15 (1.04-4.47)	34 (69.4)	0.149	1.60 (0.72-3.57)
F2 G20210A										
A/A	0 (0.0)	0 (0.0)	NC	NC	0 (0.0)	NC	NC	0 (0.0)	NC	NC
G/G	115 (95.8)	98 (96.1)		1.00 (reference)	53 (94.7)		1.00 (reference)	45 (97.8)		1.00 (reference)
G/A	5 (4.2)	4 (3.9)	1.000	0.94 (0.25-3.59)	3 (5.4)	0.711	1.68 (0.39-7.27)	1 (2.2)	1.000	1.42 (0.24-8.49)
PZ -13A/G										
G/G	0 (0.0)	0 (0.0)	NC	NC	0 (0.0)	NC	NC	0 (0.0)	NC	NC
A/A	147 (87.5)	134 (83.7)		1.00 (reference)	76 (86.4)		1.00 (reference)	58 (80.6)		1.00 (reference)
A/G	21 (12.5)	26 (16.3)	0.349	0.93 (0.44-2.00)	12 (13.6)	0.845	1.61 (0.5-5.4)	14 (19.4)	0.168	1.34 (0.60-3.01)

NC: Non countable; Odds ratio (OR) and 95% (CI) matched with gender and age; *Two-tailed Fisher's exact test.

Table II. *Multivariate logistic regression models adjusted for age and gender for overall, early and advanced stages of oral squamous cell carcinoma (OSCC) risk in association with polymorphism genotypes.*

	Overall OSCC			Early-stage OSCC			Advanced-stage OSCC		
	Estimate*	*p*	OR (95% CI)	Estimate*	*p*	OR (95% CI)	Estimate*	*p*	OR (95% CI)
Model A – Homozygous variant genotypes	HLGFT (χ^2:4.809, DF:7, p=0.683)			HLGFT (χ^2:7.095, DF:4, p=0.131)			HLGFT (χ^2:2.370, DF:3, p=0.499)		
Age (≥55 years)	1.351	0.057	3.86 (0.96-15.52)	0.703	0.089	2.02 (0.90-4.54)	1.004	0.025	2.73 (1.14-6.55)
Gender (female)	–1.859	0.009	0.16 (0.04-0.63)	–1.749	0.004	0.17 (0.05-0.58)	–0.342	0.465	0.71 (0.28-1.78)
SERPINE1 -675 4G/4G	1.609	0.018	5.00 (1.32-18.92)						
ACE intron 16 I/I	2.215	0.037	9.16 (1.14-73.50)	2.107	<0.001	8.22 (2.92-23.19)	1.532	0.007	4.63 (1.52-14.05)
Model B – Carrier genotypes	HLGFT (χ^2:5.498, DF:6, p=0.482)			HLGFT (χ^2:3.169, DF:5, p=0.674)			HLGFT (χ^2:7.901, DF:6, p=0.245)		
Age (≥55 years)	0.766	0.379	2.51 (1.04-4.43)	0.739	0.036	2.09 (1.05-4.18)	0.022	0.955	1.02 (0.47-2.20)
Gender (female)	–0.609	0.154	0.54 (0.24-1.26)	–1.460	0.002	0.23 (0.09-0.58)	0.608	0.168	1.84 (0.77-4.36)
SERPINE1 -675 5G/4G	0.869	0.037	2.39 (1.05-5.40)						
MTHFR C677T C/T				0.855	0.024	2.35 (1.12-4.94)			
F12 C46T C/T							–1.514	<0.001	0.22 (0.10-0.49)
F13 Val34Leu V/L							1.244	0.002	3.47 (1.60-7.51)
Model C – 'Mode of Inheritance' Genotypes	HLGFT (χ^2:6.559, DF:4, p=0.161)			HLGFT (χ^2:7.095, DF:4, p=0.131)			HLGFT (χ^2:2.370, DF:3, p=0.499)		
Age (≥55 years)	0.780	0.021	2.18 (1.13-4.23)	0.702	0.089	2.02 (0.90-4.54)	1.004	0.025	2.73 (1.14-6.55)
Gender (female)	–0.853	0.033	0.43 (0.20-0.93)	–1.749	0.004	0.17 (0.05-0.58)	–0.342	0.465	0.71 (0.28-1.78)
ACE intron 16 I/I	1.716	<0.001	5.56 (2.32-13.33)	2.107	<0.001	8.22 (2.92-23.19)	1.532	0.007	4.63 (1.52-14.05)

*Two-tailed Fisher's exact test, Odds ratio (OR) and 95% (CI) matched with gender and age.

polymorphism. The same model for early-stage OSCC includes the factors age and gender, and additionally the heterozygosity for the *MTHFR* polymorphism. Finally, for advanced-stage OSCC, model B includes the factors age, gender, heterozygosity for the *F12* polymorphism, and heterozygosity for the *F13* polymorphism. These regression analyses indicate that carcinogenesis in the oral cavity might be affected by various independent mechanisms.

The first mechanism is associated with the *SERPINE1* gene expression. In the case of heterozygotes, an intermediate amount of SERPINE1 protein is produced, which is probably sufficient to affect oral carcinogenesis in the same way as mentioned above, mainly through the regulation of cell detachment and migration rather than layer proteolysis. The second molecular mechanism is related to the *MTHFR* gene and the early stages of oral cancer. Most patients with oral cancer appear to be carriers of the T allele of the *MTHFR* C677T polymorphism, which results in reduced activity of the enzyme. MTHFR is an enzyme which plays an important role in the metabolism of folic acid, a compound that provides the methyl group required for intracellular methylation reactions and the *de novo* synthesis of triphosphate deoxynucleoside. Therefore, reduced MTHFR activity could have carcinogenic effects, mediated by the disruption of the synthesis and methylation of DNA (29). Model B results also revealed a possible protective action against advanced stages of oral cancer involving the studied polymorphisms in the *F12* and *F13* genes. In particular, the L allele of the *F13* V34L polymorphism results in production of a fibrous network with thinner fibres and smaller pores that favors initial tumor cell proliferation but not metastasis (30).

The 'mode of inheritance' model C revealed age, gender and the I/I *ACE* genotype to be significant independent factors of oral carcinogenesis. As mentioned above, an established role of ACE is the de-activation of bradykinin. Bradykinin is known to contribute to tumor formation through its ability to increase vascular permeability and stimulate tissue growth (31). In light of the above, it can be assumed that the observed increased frequency of low expression I allele of the *ACE* gene may lead to reduced deactivation of bradykinin and therefore, promotion of oncogenesis.

The important role of ACE in oral carcinogenesis has been revealed in a previous regression analysis of nine factors related to angiogenesis and inflammation, as well as thrombosis (32). In that study, ACE was implicated in the increased production of matrix metalloproteinase-9 and reduced levels of tissue metalloproteinase inhibitor-2, two important factors in tissue reconstruction that favour neoplasia in the oral region (32).

In conclusion, this regression analysis revealed the significant contributions of five out of ten studied factors related to thrombosis, which may serve as primary predictors for OSCC risk. Most importantly, the role of ACE stood out as the most significant contributor to oral carcinogenesis. Prospective studies in a larger population sample are needed to further evaluate the involvement of ACE and other potentially important factors in predisposing for oral cancer.

Disclosure Statement

All Authors declare that they have no conflict of interest regarding this study.

References

1 Baronzio G, Schwartz L, Kiselevsky M, Guais A, Sanders E, Milanesi G, Baronzio M and Freitas I: Tumor interstitial fluid as modulator of cancer inflammation, thrombosis, immunity and angiogenesis. Anticancer Res *32(2)*: 405-414, 2012.

2 Agnelli G and Verso M: Management of venous thromboembolism in patients with cancer. J Thromb Haemost *9(Suppl 1)*: 316-324, 2011.

3 Sutherland DE, Weitz IC and Liebman HA: Thromboembolic complications of cancer: epidemiology, pathogenesis, diagnosis, and treatment. Am J Hematol *72(1)*: 43-52, 2003.

4 Shaib W, Deng Y, Zilterman D, Lundberg B and Saif MW: Assessing risk and mortality of venous thromboembolism in pancreatic cancer patients. Anticancer Res *30(10)*: 4261-4264, 2010.

5 Khorana AA: Cancer and coagulation. Am J Hematol *87(Suppl 1)*: S82-87, 2012.

6 Heit JA: Predicting the risk of venous thromboembolism recurrence. Am J Hematol *87(Suppl 1)*: S63-67, 2012.

7 Yapijakis C, Bramos A, Nixon AM, Ragos V and Vairaktaris E: The interplay between hemostasis and malignancy: the oral cancer paradigm. Anticancer Res *32(5)*: 1791-1800, 2012.

8 Soerjomataram I, Lortet-Tieulent J, Parkin DM, Ferlay J, Mathers C, Forman D and Bray F: Global burden of cancer in 2008: a systematic analysis of disability-adjusted life-years in 12 world regions. Lancet *380(9856)*: 1840-1850, 2012.

9 Yako-Suketomo H and Matsuda T: Comparison of time trends in lip, oral cavity and pharynx cancer mortality (1990-2006) between countries based on the WHO mortality database. Jpn J Clin Oncol *40(11)*: 1118-1119, 2010.

10 Warnakulasuriya S: Global epidemiology of oral and oropharyngeal cancer. Oral Oncol *45(4-5)*: 309-316, 2009.

11 Gonzalez-Moles MA, Scully C and Ruiz-Avila I: Molecular findings in oral premalignant fields: update on their diagnostic and clinical implications. Oral Dis *18(1)*: 40-47, 2012.

12 McDowell JD: An overview of epidemiology and common risk factors for oral squamous cell carcinoma. Otolaryngol Clin North Am *39*: 277-294, 2006.

13 Vairaktaris E, Yapijakis C, Derka S, Vassiliou S, Serefoglou Z, Vylliotis A, Wiltfang J, Springer I, Nkenke E, Kessler P and Neukam FW: Association of platelet glycoprotein Ia polymorphism with minor increase of risk for oral cancer. Eur J Surg Oncol *32*: 455-457, 2006.

14 Vairaktaris E, Yapijakis C, Serefoglou Z, Vylliotis A, Ries J, Nkenke E, Wiltfang J, Derka S, Vassiliou S, Springer I, Kessler P and Neukam FW: Plasminogen activator inhibitor-1 polymorphism is associated with increased risk for oral cancer. Oral Oncol *42*: 888-892, 2006.

15 Vairaktaris E, Yapijakis C, Yiannopoulos A, Vassiliou S, Serefoglou Z, Vylliotis A, Nkenke E, Derka S, Critselis E, Avgoustidis D, Neukam FW and Patsouris E: Strong association of the tissue inhibitor of metalloproteinase-2 polymorphism with an increased risk of oral squamous cell carcinoma in Europeans. Oncol Rep *17*: 963-968, 2007.

16 Vairaktaris E, Yapijakis C, Nkenke E, Vassiliou S, Vylliotis A, Nixon AM, Derka S, Ragos V, Spyridonidou S, Tsigris C, Neukam FW and Patsouris E: The 1040 C/T polymorphism influencing thermal stability and activity of thrombin activatable fibrinolysis inhibitor (TAFI) is associated with risk for oral cancer. Am J Hematol *82*: 1010-1012, 2007.

17 Vairaktaris E, Yapijakis C, Tsigris C, Vassiliou S, Derka S, Nkenke E, Spyridonidou S, Vylliotis A, Vorris E, Ragos V, Neukam FW and Patsouris E: Association of angiotensin-converting enzyme gene insertion/deletion polymorphism with increased risk for oral cancer. Acta Oncol *46*: 1097-1102, 2007.

18 Vairaktaris E, Yapijakis C, Derka S, Serefoglou Z, Vassiliou S, Nkenke E, Ragos V, Vylliotis A, Spyridonidou S, Tsigris C, Yannopoulos A, Tesseromatis C, Neukam FW and Patsouris E: Association of matrix metalloproteinase-1 (_1607 1G/2G) polymorphism with increased risk for oral squamous cell carcinoma. Anticancer Res *27*: 459-464, 2007.

19 Yapijakis C, Vairaktaris E, Vassiliou S, Vylliotis A, Nkenke E, Nixon AM, Derka S, Spyridonidou S, Vorris E, Neukam F and Patsouris E: The low VEGF production allele of the +936C/T polymorphism is strongly associated with increased risk for oral cancer. J Cancer Res Clin Oncol *133*: 787-791, 2007.

20 Vairaktaris E, Vassiliou S, Nkenke E, Serefoglou Z, Derka S, Tsigris C, Vylliotis A, Yapijakis C, Neukam FW and Patsouris E: A metalloproteinase-9 polymorphism which affects its expression is associated with increased risk for oral squamous cell carcinoma. Eur J Surg Oncol *34(4)*: 450-455, 2008.

21 Vairaktaris E, Yapijakis C, Vassiliou S, Derka S, Nkenke E, Serefoglou Z, Vorris E, Vylliotis A, Ragos V, Neukam FW and Patsouris E: Association of -1171 promoter polymorphism of matrix metalloproteinase-3 with increased risk for oral cancer. Anticancer Res *27*: 4095-4100, 2007.

22 Masuda J, Nabika T and Notsu Y: Silent stroke: Pathogenesis, genetic factors and clinical implications as a risk factor. Curr Opin Neurol *14*: 77-82, 2001.

23 Beauchamp NJ, Daly ME, Hampton KK, Cooper PC, Preston FE and Peake IR: High prevalence of a mutation in the factor V gene within the U.K. population: relationship to activated protein C resistance and familial thrombosis. Br J Haematol *88*: 219-222, 1994.

24 Bertina RM: Factor V Leiden and other coagulation factor mutations affecting thrombotic risk. Clin Chem *43*: 1678-1684, 1997.

25 Caprini JA, Glase CJ, Anderson CB and Hathaway K: Laboratory markers in the diagnosis of venous thromboembolism. Circulation *109*: 4-8, 2004.

26 Gluba A, Banach M, Mikhailidis DP and Rysz J: Genetic determinants of cardiovascular disease: The rennin/angiotensin/aldosterone system, paraoxonases, endothelin-1, nitric oxide synthase and adrenergic receptors. In Vivo *23*: 797-812, 2009.

27 Kingsley K, O'Malley S, Ditmyer M and Chino M: Analysis of oral cancer epidemiology in the US reveals state-specific trends: Implications for oral cancer prevention. BMC Public Health *8*: 87, 2008.
28 Marney A, Ma J, Luther J, Ikizler TA and Brown NJ: Endogenous bradykinin contributes to increased plasminogen activator inhibitor 1 antigen following hemodialysis. J Am Soc Nephrol *20*: 2246-2252, 2009.
29 Choi SW and Mason JB: Folate and carcinogenesis: An integrated scheme. J Nutr *130*: 129-132, 2000.
30 Balogh I, Szoke G, Karpati L, Wartiovaara U, Katona E, Komáromi I, Haramura G, Pfliegler G, Mikkola H and Muszbek L: Val34Leu polymorphism of plasma factor XIII: biochemistry and epidemiology in familial thrombophilia. Blood *96*: 2479-2486, 2000.
31 Fujita M, Hayashi I, Yamashina S, Itoman M and Majima M: Blockade of angiotensin AT1a receptor signaling reduces tumor growth, angiogenesis, and metastasis. Biochem Biophys Res Commun *294*: 441-447, 2002.
32 Vairaktaris E, Serefoglou Z, Avgoustidis D, Yapijakis C, Critselis E, Vylliotis A, Spyridonidou S, Derka S, Vassiliou S, Nkenke E and Patsouris E: Gene polymorphisms related to angiogenesis, inflammation and thrombosis that influence risk for oral cancer. Oral Oncol *45(3)*: 247-253, 2009.

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