Increased risk for oral cancer is associated with coagulation factor XIII but not with factor XII

ELEFTHERIOS VAIRAKTARIS[1], STAVROS VASSILIOU[1], CHRISTOS YAPIJAKIS[1], SOFIA SPYRIDONIDOU[1], ANTONIS VYLLIOTIS[1], SPYRIDOULA DERKA[1], EMEKA NKENKE[3], GREGORY FOURTOUNIS[1], FRIEDRICH W. NEUKAM[3] and EFSTRATIOS PATSOURIS[2]

[1]Department of Oral and Maxillofacial Surgery, University of Athens Medical School, Vas. Sofias 93 and Dim. Soutsou 1, Athens 11521; [2]Department of Pathology, University of Athens Medical School, Mikras Asias 75, Athens 11527, Greece; [3]Department of Oral and Maxillofacial Surgery, Universität Erlangen, Klinik und Poliklinik für Mund-, Kiefer-, Gesischtschirurgie, Glueckstrasse 11, Erlangen, D-91054 Nürnberg, Germany

Received March 28, 2007; Accepted July 2, 2007

Abstract. In light of recently found contribution of factors associated with thrombosis and inflammation to carcinogenesis, we investigated the possible association of coagulation factors XII and XIII with increased risk for oral cancer. In DNA samples of patients with oral squamous cell carcinoma and healthy controls of comparable ethnicity, age and sex, we studied the C46T polymorphism in *FXII* gene which affects gene transcription and the V34L polymorphism in *FXIII* gene which affects enzyme activity resulting in alteration of the fibrin network structure. No significant differences were observed in genotype and mutant allele frequencies of the *FXII* C46T polymorphism between patients and healthy controls. On the contrary, the obtained data for *FXIII* V34L polymorphism revealed a significant frequency increase of the L allele, which results in thinner fibrin network, in the whole group of patients compared to controls (33.1 versus 22.2% respectively, Fischer value P=0.006). In addition, LL homozygotes had a 3-fold greater risk for developing oral cancer (OR 2.893, 95% CI 1.056-7.890), while in VL heterozygotes a 2-fold grater risk was observed (OR 1.868, 95% CI 1.126-3.101). Significantly increased frequency of L allele was also observed in sub-groups of patients without family history of thrombophilia or cancer, with and without tobacco abuse and with alcohol abuse (P<0.05). Interestingly, in comparison to controls only patients with early cancer stages I and II had significantly increased L alleles and not patients with advanced stages III and IV. These findings suggest that the presence of L allele is strongly associated with oral cancer generation but not with its progression and metastasis. In the presence of L allele, the fibrin network is composed of thinner fibers, is less porous and facilitates tumor stroma formation and therefore tumor cell proliferation. Nevertheless, this thinner and less porous fibrin network inhibits cell migration.

Correspondence to: Dr Eleftherios Vairaktaris, Department of Oral and Maxillofacial Surgery, University of Athens Medical School, Vas. Sofias 93 and Dim. Soutsou 1, Athens 11521, Greece
E-mail: lvairakt@med.uoa.gr

Key words: oral cancer, angiogenesis, thrombophilia, polymorphism

Introduction

Oral squamous cell carcinoma (OSCC) is a common human malignancy, characterised by short survival time and poor prognosis, which make this disease a serious public health problem (1). The multistep process of oral carcinogenesis is affected by environmental factors, such as tobacco and alcohol abuse, and by multiple genetic events such as mutations in oncogenes and tumor suppressor genes (1,2). Recently, common polymorphisms in genes related to thrombosis, inflammation and angiogenesis have been also implicated in the predisposition for the development and advancement of oral cancer (3-10). Therefore, factors of the coagulation cascade appear to be candidate contributors in the process of oral oncogenesis.

Plasma coagulation factor XII (FXII) is a serine protease precursor, primarily produced by hepatocytes (11). FXII is mainly involved in fibrinolysis and in the initiation of FXII-mediated fibrin formation *in vitro*, while it has long been considered dispensable for normal blood clotting *in vivo* (12,13). Activated FXII (FXIIa) initiates the coagulation cascade by cleaving prekallikrein. Kallikrein cleaves the inactive zymogen factor XII to yield FXIIa, which in turn induces activation of FXI resulting in formation of fibrin. Covalent cross-links between the fibrin strands by the action of FXIIIa stabilize the network (14). To outbalance this system, fibrin has to be dissolved and FXII is also involved in fibrinolysis. After FXIIa-mediated cleavage of prekallikrein to kallikrein, the latter then cleaves pro-urokinase to form urokinase that in turn converts plasminogen to plasmin, which dissolves the fibrin clot (14).

The stabilization of fibrin clot requires the presence of blood coagulation factor XIII. FXIII is a tetrameric zymogen containing two activatable A subunits (FXIII-A) and two

carrier B subunits (FXIII-B) (15,16). In the final phase of the coagulation cascade, plasma FXIII loses the two FXIII-B subunits and is converted into FXIIIa, which is an active transglutaminase (17-19). FXIIIa catalyzes the formation of covalent bonds between the α- and γ-chains of adjacent fibrin monomers, thereby stabilizing the fibrin clot and protecting it from the prompt elimination by the fibrinolytic system (19).

Besides their role in hemostasis, both FXII and FXIII exert other physiological effects. FXII may also function as a mitogenic growth factor, since it plays a role in localized wound healing, in systemic response to trauma, as well as in fetal tissue development (21-24). On the other hand, FXIII participates in tissue remodeling, wound healing and recurrent miscarriages as can be inferred from defects in these processes in patients with inherited FXIII deficiency (25,26). It has also been shown that FXIIIa exhibits proangiogenic activity and stimulation of cell proliferation, migration and inhibition of apoptosis in endothelia (27).

Despite these functions that are possibly tumor-related, very little is known about the role of FXII and FXIII in human cancers. To our knowledge, there are only two separate reports investigating FXII and FXIII levels in patients with gastrointestinal cancer and one study of FXIII in breast cancer patients (28,29). In all three reports the levels of both coagulation factors were reduced in patients compared to the respective healthy control subjects. Surprisingly, even though cancer patients possess reduced FXIII levels, an accumulation of $FXIIIa^+$ cells in several types of tumor tissues has been reported (30-32).

The study of functional polymorphisms in *FXII* and *FXIII* genes which affect their plasma level or activity may provide useful evidence on their respective role in pathogenesis. A frequent C46T polymorphism in the 5'-untranslated region of the *FXII* gene located 4 bases upstream from the ATG translation initiation codon affects translation efficiency (33). The presence of T allele creates a new start codon resulting in lower translation efficiency and decrease in FXII plasma levels (33). Decreased levels of FXII have been associated with thrombosis and possibly with recurrent miscarriage (34-36).

On the other hand, several polymorphisms have been identified in the gene of FXIII-A subunit, the most functionally relevant of which encodes for a valine-to-leucine substitution at amino-acid position 34 (37). Located only 3 amino acids from the thrombin cleavage site, residue 34 plays a critical role in the interaction between FXIII and thrombin. The less common L allele of the V34L polymorphism confers a 2.5-fold higher FXIII catalytic efficiency resulting in shortened clot formation time when compared to its V counterpart (38). As a result, in the presence of the 34L isoform, fibrin formation and molecular structure of the clot are influenced and generated clots consist of thinner fibers, smaller pores, and ultimately, a finer meshwork with altered permeation characteristics and increased resistance to plasmin degradation is produced (38). Most of the recent studies reported that carriers of the 34L allele had a decreased risk for coronary artery disease, myocardial infraction and cerebrovascular disease (39). The L allele is relatively common in Caucasians (20-35%), but rare (1%) in East Asians (36).

Even though there is an established association between several factors of the coagulation system and cancer, FXIII and FXII have hardly been explored in cancer patients. Therefore, we studied the frequency of the two above-mentioned functional polymorphisms in the *FXII* and *FXIII* genes in patients with oral cancer in comparison to healthy controls representing the general population.

Materials and methods

The individuals under study were 265 Greeks and Germans, recruited by the participating departments. They included 130 patients with oral squamous cell carcinoma and 135 healthy blood donors of similar age, ethnicity and sex. All participating controls and patients gave their informed consent before collection of blood samples.

The patients, who were included in this study, had developed oral cancer and were operated 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 cancer and thrombophilia were available. Forty-six patients (35.4%) had one or two first degree relatives with any type of cancer and their age range (41-83 years; 58.7±/10.8) did not differ significantly from the whole group of patients. Furthermore, 26 patients (20%) had one or two first-degree relatives with idiopathic thrombosis and an earlier age range (44-75 years; 57.3±/9.7) but again with no statistical difference compared to the whole group. Ten patients (7.7%) had a positive family history for both cancer and thrombophilia (48-59 years; 53.5±/4.1).

Most of the participants in the two groups worked in a low-risk environment (with the exception of one patient and three controls who worked in chemical factories). No data were available on controls regarding their family history or smoking and alcohol consumption habits.

DNA was isolated from blood with the use of Nucleon™ kit (Amersham). Molecular detection of the two polymorphisms in *FXII* and *FXIII* genes was performed by restriction fragment length polymorphism typing of PCR products.

Molecular analysis of C46T polymorphism. The PCR conditions consisted of an initial denaturation step at 94˚C, followed by 36 cycles of 94˚C for 60 sec, 57˚C for 60 sec, and 72˚C for 60 sec, as well as a final elongation step at 72˚C for 5 min. The primers used were: forward, 5'-ACTTCCAGG ACCGCCTTTGGAGGC-3' and reverse, 5'-GTTGACGCCC CGGGGCACCG-3'. After treatment with restriction enzyme *Hga*I the PCR product of 369 bp remains intact in the presence of C allele, while it is cleaved into two fragments of 247 and 122 bp in the presence of T allele (Fig. 1).

Molecular analysis of the V34L polymorphism. The PCR conditions consisted of an initial denaturation step at 95˚C, followed by 36 cycles of 94˚C for 55 sec, 55˚C for 1 min, and 72˚C for 50 sec, as well as a final elongation step at 72˚C for 5 min. The primers used were: forward, 5'-ACTTCCAGG ACCGCCTTTGGAGGC-3' and reverse, 5'-GTTGACGCCC CGGGGCACCG-3'. The generated PCR product of 114 bp after application of the restriction enzyme *Hha*I stays intact in the presence of L, while it is cleaved into two fragments of 94 and 20 bp in the presence of Val (Fig. 2). The above

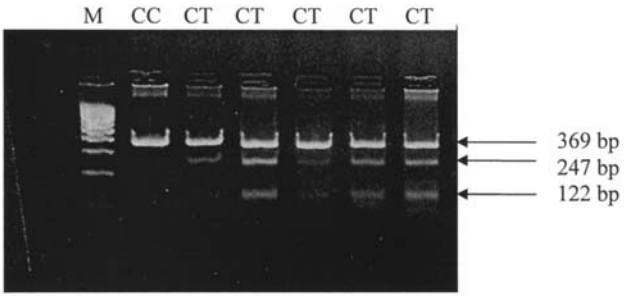

Figure 1. *Hga*I restriction pattern of the FXII C46T polymorphism, observed in six patients. CC, homozygote; CT, heterozygotes; M, molecular weight marker.

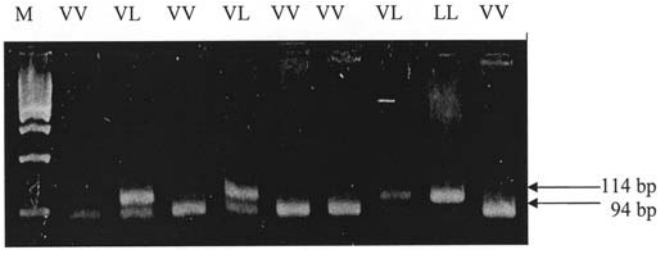

Figure 2. *Hha*I restriction pattern of the FXIII V34L polymorphism, observed in nine patients. VV, homozygotes; VL, heterozygotes; LL, homozygote; M, molecular weight marker.

mentioned digestion patterns were confirmed by sequencing analysis performed in representative samples for each gene.

The statistical analyses were performed using SAS® software (version 9.0; SAS Institute Inc.). 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. All statistical analyses concerning: number of relatives with a history of cancer, number of relatives with a history of thrombosis, nicotine use, alcohol use, have assumed that all controls have nil values for the above variables (i.e., all controls do not have a family history of cancer, all controls do not have a 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 FXII and FXIII genotypes and these variables. The Maentel-Haenzel method was used for the calculation of all odds ratios with a 95% confidence interval (CI). A $P<0.05$ was considered statistically significant. All observed genotype and allele frequencies were tested for compliance with Hardy-Weinberg equilibrium.

Results

The prevalence of detected *FXII* and *FXIII* genotypes in healthy controls and patients with oral cancer are shown in Tables I-IV. The *FXII* allele frequencies in the control group and in the group of patients were in Hardy-Weinberg equilibrium, but the genotype distributions were not. The *FXIII* genotype distributions and allele frequencies in the control group and in the group of patients were as expected for a sample in Hardy-Weinberg equilibrium.

Association study of the FXII C46T polymorphism. The observed frequencies of genotypes and low expression T allele were quite different in the two studied populations (Table I). There was a significant difference regarding the relative prevalence of CT heterozygotes and CC homozygotes in German compared to Greek controls ($P<0.001$). The T allele frequency was lower in German than in Greek controls (26.7 and 36.7%, respectively; Table I). Therefore, for this specific polymorphism, the data for the two tested populations (Greeks and Germans) were analyzed separately.

Table I. Prevalence of *FXII* C46T polymorphism in patients and healthy controls of Greek and German origin.

	Germans				Greeks			
Genotypes	Controls (%)	Patients (%)	Fisher's p-value	OR CI	Controls (%)	Patients (%)	Fisher's p-value	OR CI
Mutant: TT	4 (8.9)	2 (4.4)	0.861	0.568 (0.111-2.972)	4 (4.4)	1 (1.2)	0.396	0.241 (0.035-1.749)
Normal: CC	25 (55.5)	22 (48.9)		1 referent	28 (31.2)	29 (34.1)		1 referent
Carrier: CT	16 (35.7)	21 (46.7)	0.493	1.491 (0.631-3.523)	58 (64.4)	55 (64.7)	0.916	0.916 (0.486-1.725)
Total	45 (100)	45 (100)			90 (100)	85 (100)		
Prevalence of T allele								
T allele frequency	26.7	27.8	1.000	1.058 (0.551-2.030)	36.7	33.5	0.616	0.871 (0.562-1.351)

Frequencies of genotypes and C alleles are significantly different among the two studied populations.

Table II. Prevalence of *FXIII* (V34L) polymorphism in healthy controls and total group of patients and their subgroups regarding cancer stage.

Genotypes	Controls (%)	Patients (%)	Fisher's p-value	OR CI	Patients with cancer stages I&I (%)	Fisher's p-value	OR CI	Patients with cancer stages III&IV (%)	Fisher's p-value	OR CI
Mutant: LL	6 (4.4)	12 (9.2)	0.045	2.893 (1.056-7.890)	10 (13.2)	0.01	4.219 (1.462-12.134)	2 (3.7)	NS	1.125 (0.245-5.273)
Normal: VV	81 (60)	56 (43.1)		1 referent	32 (42.1)		1 referent	24 (44.4)		1 referent
Carrier: VL	48 (35.6)	62 (47.7)	0.021	1.868 (1.126-3.101)	34 (44.7)	NS	1.793 (0.986-3.252)	28 (51.9)	0.047	1.969 (1.030-3.726)
Total	135 (100)	130 (100)			76 (100)			54 (100)		
Prevalence of L allele										
L allele frequency	22.2	33.1	0.006	1.730 (1.117-2.542)	35.5	0.004	1.929 (1.245-2.987)	29.6	NS	1.474 (0.894-2.431)
Carrier frequency of L allele	40	56.9	0.007	1.982 (1.217-3.228)	57.9	0.015	2.063 (1.168-3.642)	55.6	NS	1.875 (0.995-3.533)

NS, non-significant p-value.

No significant differences of genotypes and allele frequencies of the C46T polymorphism among controls and patients for both populations were found (Table I). Additionally, the same pattern was observed in both populations for all subgroups of patients regardless tumor stage, family history of thrombophilia or cancer and smoking or alcohol consumption (data not shown).

FXIII association study of the V34L polymorphism. Lifestyle characteristics along with the prevalence of detected *FXIII*

Table III. Prevalence of *FXIII* (V34L) polymorphism in healthy controls and patients with oral cancer regarding family history of either cancer or thrombosis.

Genotypes	Controls (%)	Patients with family history of cancer (%)	Fisher's p-value	OR CI	Patients without family history of cancer (%)	Fisher's p-value	OR CI	Patients with family history of thrombosis (%)	Fisher's p-value	OR CI	Patients without family history of thrombosis (%)	Fisher's p-value	OR CI
Mutant: LL	6 (4.4)	0 (0)	NS	0 (0.000-2.264)	12 (14.3)	0.002	5.063 (1.801-14.170)	2 (7.7)	NS	1.929 (0.408-9.419)	10 (9.6)	NS	3.214 (1.129-9.116)
Normal: VV	81 (60)	24 (52.2)		1 referent	32 (38.1)		1 referent	14 (53.8)		1 referent	42 (40.4)		1 referent
Carrier: VL	48 (35.6)	22 (47.8)	NS	1.547 (0.788-3.037)	40 (47.6)	0.017	2.109 (1.177-3.781)	10 (38.5)	NS	1.205 (0.506-2.878)	52 (500	0.009	2.089 (1.218-3.583)
Total	135 (100)	46 (100)			84 (100)			26 (100)			104 (100)		
Prevalence of L allele													
L allele frequency	22.2	23.9	NS	1.100 (0.632-1.915)	38.1	0.000	2.154 (1.412-3.286)	26.9	NS	1.289 (0.661-2.518)	34.6	0.003	1.853 (1.237-2.775)
Carrier frequency of L allele	40	47.8	NS	1.375 (0.705-2.682)	61.9	0.002	2.438 (1.397-4.254)	46.2	NS	1.286 (0.561-2.950)	59.6	0.003	2.214 (1.316-3.724)

NS, non-significant p-value.

Table IV. Prevalence of *FXIII* (V34L) polymorphism in healthy controls and patients with oral cancer in regard to either alcohol consumption or smoking habits.

Genotypes	Controls (%)	Patients with tobacco abuse(%)	Fisher's p-value	OR CI	Patients without tobacco abuse (%)	Fisher's p-value	OR CI	Patients with alcohol abuse (%)	Fisher's p-value	OR CI	Patients without alcohol abuse (%)	Fisher's p-value	OR CI
Mutant: LL	6 (4.4)	12 (9.7)	0.045	2.893 (1.056-7.890)	0 (0)	NS	-	2 (5.6)	NS	1.227 (0.266-5.783)	10 (10.6)	0.021	3.971 (1.380-11.381)
Normal: VV	81 (60)	56 (45.2)		1 referent	0 (0)		1 referent	22 (61.1)		1 referent	34 (36.2)		1 referent
Carrier: VL	48 (35.6)	56 (45.2)	NS	1.688 (1.010-2.819)	6 (100)	0.003	Inf (2.558-inf)	12 (33.3)	NS	0.920 (0.423-2.006)	50 (53.2)	0.002	2.482 (1.416-4.350)
Total	135 (100)	124 (100)			6 (100)			36 (100)			94 (100)		
Prevalence of L allele													
L allele frequency	22.2	32.3	0.013	1.667 (1.128-2.463)	50	0.037	3.500 (1.145-10.700)	22.2	NS	1.000 (0.539-1.858)	37.2	0.001	2.076 (1.377-3.131)
Carrier frequency of L allele	40	54.8	0.018	1.821 (1.113-2.980)	100	0.005	Inf (2.279-inf)	38.9	NS	0.955 (0.454-2.010)	63.8	0.000	2.647 (1.540-4.550)

NS, non-significant p-value.

genotypes in healthy controls, patients with oral cancer and subgroups of patients are shown in Tables II-IV. The data for the two tested populations (Greek and German healthy controls) were analyzed together, since there were no significant differences of allele frequencies of the (V34L) polymorphism among the two populations. The observed mutant allele (L) frequency in the control group was 22.2% (similar to other European populations) and the carrier frequency was 40%. All V34L genotype distributions were as expected in Hardy-Weinberg equilibrium in the control group, as well as in the whole group and subgroups of patients.

The detected mutant allele (L) and carrier frequencies in the patient group had significantly increased values compared to the equivalent ones in the control group (33.1 and 56.9% respectively, P<0.05 in both cases). Significant increase of both frequencies was also detected in certain subgroups of patients in comparison to controls. Statistical difference in mutant allele and carrier frequencies was observed in the subgroups of patients: i) in early (I and II) stages of cancer (P<0.05 and <0.05, respectively), ii) without positive family history of cancer (P<0.001 and <0.05, respectively), iii) without positive history of thrombophilia (P<0.05 and <0.05, respectively), iv) with tobacco abuse (P<0.05 and <0.05, respectively), v) without tobacco abuse (P<0.05 and <0.05, respectively), and vi) without alcohol abuse (P<0.001 and <0.001, respectively). Finally, there were no major differences due to categorizations of sex, age and age at onset of oral cancer.

Interestingly, both homozygotes and heterozygotes for the mutant (L) allele were significantly increased in the patients group compared to the controls group. The odds ratio (relative risk) for OSCC of homozygotes is 2.893 (95% CI 1.056-7.890), while for heterozygotes is 1.868 (95% CI 1.126-3.101). In the subgroup of patients in oral cancer stages I and II only the mutant homozygotes were significantly increased in comparison to the control group (P<0.05), revealing an even greater relative risk for oral cancer development 4.219 (95% CI 1.462-12.134). On the contrary, in the subgroup of patients in oral cancer stages III and IV only the mutant heterozygotes were significantly increased as compared to the control group (P<0.05, relative risk 1.969, 95% CI 1.030-3.726).

Discussion

Based on recent findings that implicate thrombosis-related factors to oral oncogenesis and oral cancer progression (3-10) we studied the impact of two coagulation factors (XII and XIII) with mitogenic properties in the pathogenesis of this disease. Therefore, we studied the C46T and V34L functional polymorphisms of *FXII* gene and *FXIII* gene respectively, in a cohort of 130 patients with oral squamous cell carcinoma and 135 healthy controls of similar age, ethnicity and sex.

The overall obtained data for the C46T polymorphism of the *FXII* gene revealed no significant difference in genotype and allele frequencies between patients and controls, regardless their ethnicity (Greeks or Germans). Therefore, it can be speculated that the mitogenic activity of FXII, due to its EGF-like domains, is not significantly exerted in oral oncogenesis.

On the contrary, the functional V34L polymorphism of the *FXIII* gene seems to affect oral cancer pathogenesis. Despite the relatively small sample of studied individuals, the overall obtained data for this specific polymorphism revealed a definite association of the L allele with an increased risk for oral squamous cell carcinoma. Compared to the controls,

a significant increase in L allele frequency was observed not only in the whole group of patients but also in subgroups of patients with oral cancer at the initial stages, without family history of thrombophilia or cancer, with and without tobacco abuse and with alcohol abuse.

Additionally, the statistical analysis showed that LL homozygotes have a greater likelihood for developing oral cancer than VL heterozygotes (about three times versus two, respectively). Therefore, the L allele of this functional polymorphism is not only associated with oral cancer development but its effect is also quantitative.

The finding of this study that the V34L polymorphism is not associated with patients with family history of thrombophilia is in accordance with previous report suggesting a protective role of the L allele against thrombosis-related diseases (39). This notion is further underlined by the observed increase in the L allele frequency in the subgroup of patients without family history of thrombophilia. Additionally, the fact that the L allele frequency was not significantly different in the subgroup of patients with cancer in stages III and IV, compared to controls, indicates that there is no association of this allele with cancer progression and metastasis. This suggestion is reinforced by the fact that the presence of L allele generates a fibrin network consisting of thinner fibers and smaller pores, which inhibits cell migration (38,40).

In contrast, the overall increased L allele frequency observed in the whole group of patients and in their subgroup with cancer stages I and II probably implies that the L allele-resulting thinner fibrin network supports initial tumor cell proliferation (38). It is widely accepted that solid human tumors contain considerable amounts of cross-linked fibrin, indicating that is important for tumor stroma formation (41,42). Two of the properties of this network are the more rapid fibrin formation and the resistance to plasmin degradation, which could obviously result in fibrin accumulation that in turn serves as tumor stroma (38).

In conclusion, the studied C46T FXII polymorphism revealed no association with increased risk for oral cancer, in contrast to V34L FXIII polymorphism which is strongly associated with the generation of the disease but not with its progression and metastasis. As a consequence, it is of great importance to perform further genetic association studies regarding the contribution of additional factors related to thrombosis, angiogenesis, and inflammation to oncogenesis in the oral region. Any positive findings could ultimately result in the undertaking of preventive measures, safeguarding the health of at risk individuals in the general population.

Acknowledgements

This study was co-funded by the European Social Fund and National Resources (EPEAEK II 'Pythagoras' 70/3/7391) grant to E.V.

References

1. Silverman S Jr: Demographics and occurrence of oral and pharyngeal cancers. The outcomes, the trends, the challenge. J Am Dent Assoc 132: S7-S11, 2001.
2. Williams HK: Molecular pathogenesis of oral carcinoma. J Clin Pathol 53: 165-172, 2000.
3. Song C, Xing D, Tan W, Wei Q and Lin D: Methylenetetrahydrofolate reductase polymorphisms increase risk of esophageal squamous cell carcinoma in a Chinese population. Cancer Res 61: 3272-3275, 2001.
4. Vairaktaris E, Yapijakis C, Wiltfang J, *et al*: Are factor V and prothrombin mutations associated with increased risk of oral cancer? Anticancer Res 25: 2561-2566, 2005.
5. Vairaktaris E, Yapijakis C, Vylliotis A, *et al*: Methylenetetrahydrofolate reductase polymorphism and minor increase of risk for oral cancer. J Cancer Res Clin Oncol 132: 219-222, 2006.
6. Vairaktaris E, Yapijakis C, Serefoglou Z, *et al*: Plasminogen activator inhibitor-1 polymorphism is associated with increased risk for oral cancer. Oral Oncol 42: 888-892, 2006.
7. Vairaktaris E, Yapijakis C, Derka S, *et al*: Association of platelet Ia polymorphism with minor increase of risk for oral cancer. Eur J Surg Oncol 32: 455-457, 2006.
8. Vairaktaris E, Yiannopoulos A, Vylliotis A, *et al*: Strong association of interleukin-6 -174 G>C promoter polymorphism with increased risk of oral cancer. Int J Biol Markers 21: 246-250, 2006.
9. Vairaktaris E, Yapijakis C, Derka S, *et al*: Association of matrix metalloproteinase-1 (-1607 1G/2G) polymorphism with increased risk for oral squamous cell carcinoma. Anticancer Res 27: 459-464, 2007.
10. Vairaktaris E, Yapijakis C, Serefoglou Z, *et al*: The interleukin-8 (-251A/T) polymorphism is associated with increased risk for oral squamous cell carcinoma. Eur J Surg Oncol 33: 504-507, 2007.
11. Kitchens CS: The contact system. Arch Pathol Lab Med 126: 1382-1386, 2002.
12. Schloesser M, Zeerleder S, Lutze G, *et al*: Mutations in the human factor XII gene. Blood 90: 3967-3977, 1997.
13. Colman RW: Are hemostasis and thrombosis two sides of the same coin? JEM 203: 493-495, 2006.
14. Fuhrer G, Gallimore MJ, Heller W and Hoffmeister HE: FXII. Blut 61: 258-266, 1990.
15. Muszbek L, Ádány R and Mikkola H: Novel aspects of blood coagulation factor XIII. Structure, distribution, activation and function. Crit Rev Lab Sci 33: 357-421, 1996.
16. Muszbek L, Yee VC and Hevessy Z: Blood coagulation factor XIII: structure and function. Thromb Res 94: 271-305, 1999.
17. Siebenlist KR, Meh DA and Mosesson MW: Plasma factor XIII binds specifically to fibrinogen molecules containing γ-chains. Biochemistry 35: 10448-10453, 1996.
18. Bereczky Z, Katona É and Muszbek L: Fibrin stabilization (Factor XIII), fibrin structure and thrombosis. Pathophysiol Haemost Thromb 33: 430-437, 2004.
19. Lorand L, Losowsky MS and Miloszewski KJM: Human Factor XIII: Fibrin-stabilizing factor. Progr Haemost Thromb 5: 245-290, 1980.
20. Cool DE, Edgell CJ, Louie GV, Zoller MJ, Brayer GD and MacGillivray RT: Characterization of human blood coagulation factor XII cDNA. Prediction of the primary structure of factor XII and the tertiary structure of beta-factor XIIa J. Biol Chem 260: 13666-13676, 1985.
21. Saito H, Hamilton SM, Tavill AS, Goodnough LT, Louis L and Angell A: Synthesis and release of Hageman factor (Factor XII) by the isolated perfused rat liver. J Clin Invest 72: 948-954, 1983.
22. Warburton D, Seth R, Shum L, Horcher PG, Hall FL, Werb Z and Slavkin HC: Epigenetic role of epidermal growth factor expression and signalling in embryonic mouse lung morphogenesis. Dev Biol 149: 123-133, 1992.
23. Catterton WZ, Escobedo MB, Sexson WR, Gray ME, Sundell HW and Stahlman MT: Effect of epidermal growth factor on lung maturation in fetal rabbits. Pediatr Res 13: 104-108, 1979.
24. Gordon E, Venkatesan N, Salazar R, Tang H, Schmeidler-Sapiro K, Buckley S, Warburton D and Hall F: Factor XII-induced mitogenesis is mediated via a distinct signal transduction pathway that activates a mitogen-activated protein kinase. Proc Natl Acad Sci USA 93: 2174-2179, 1996.
25. Muszbek L, Adany R and Mikkola H: Novel aspects of blood coagulation factor XIII, I: structure, distribution, activation and function. Crit Rev Clin Lab Sci 33: 457-421, 1996.
26. Lorand L: Factor XIII: structure, activation and interactions with fibrinogen and fibrin. Ann NY Acad Sci 936: 291-311, 2001.

27. Dardik R, Solomon A, Loscalzo J, Eskaraev R, Bialik A, Goldberg I, Schiby G and Inbal A: Novel proangiogenic effect of factor XIII associated with suppression of thrombospondin 1 expression. Arterioscler Thromb Vasc Biol 23: 1472-1477, 2003.
28. Jiang WG, Ablin R, Douglas-Jones A and Mansel RE: Expression of transglutaminases in human breast cancer and their possible clinical significance. Oncol Rep 10: 2039-2044, 2003.
29. Born P, Lippl F, Ulm K, Gerein P, Lersch C, Eckel F, Fischer G, Sandschin W, Dlaska U and Classen M: Reduced levels of coagulation factor XIII in patients with advanced tumor disease. Hepatogastroenterology 47: 194-198, 2000.
30. Silverman JS and Tamsen A: Interactive CD34-positive fibroblasts and factor XIIIa-positive histiocytes in cutaneous mesenchymal tumors. Am J Dermatopathol 20: 317-320, 1998.
31. Silverman JS and Tamsen A: CD34 and factor XIIIa-positive microvascular dendritic cells and the family of fibrohistiocytic mesenchymal tumors. Am J Dermatopathol 20: 533-536, 1998.
32. Ide F and Kusama K: Solitary fibrous tumor is rich in factor XIIIa+ dendrocytes. Am J Dermatopathol 24: 449-450, 2002.
33. Kanaji T, Okamura T, Osaki K, Kuroiwa M, Shimoda K, Hamasaki N and Niho Y: A common genetic polymorphism (46 C to T substitution) in the 5'-untranslated region of the coagulation factor XII gene is associated with low translation efficiency and decrease in plasma factor XII level. Blood 91: 2010-2014, 1998.
34. Santamarıa A, Mateo J, Tirado I, Oliver A, Belvıs R, Fabregas J, Felices R, Soria J, Souto J and Fontcuberta J: Homozygosity of the T allele of the 46 C3T polymorphism in the F12 gene is a risk factor for ischemic stroke in the spanish population. Stroke 35: 1795-1799, 2004.
35. Ogasawara MS, Aoki K, Katano K, Ozaki Y and Suzumori K: Factor XII but not protein C, protein S, antithrombin III or factor XIII is a predictor of recurrent miscarriage. Fertil Steril 75: 916-919, 2001.
36. Iinuma Y, Sugiura-Ogasawara M, Makino A, Ozaki Y, Suzumori N and Suzumori K: Coagulation factor XII activity, but not an associated common genetic polymorphism (46C/T), is linked to recurrent miscarriage. Fertil Steril 77: 353-356, 2002.
37. Ariens RA, Philippou H, Nagaswami C, *et al*: The factor XIII V34L polymorphism accelerates thrombin activation of factor XIII and affects cross-linked fibrin structure. Blood 96: 988-995, 2000.
38. Balogh I, Szoke G, Karpati L, *et al*: Val34Leu polymorphism of plasma factor XIII: biochemistry and epidemiology in familial thrombophilia. Blood 96: 2479-2486, 2000.
39. Wartiovaara U, Perola M, Mikkola H, *et al*: Association of FXIII Val34Leu with decreased risk of myocardial infarction in Finnish males. Atherosclerosis 142: 295-300, 1999.
40. Nehls V and Hermann R: The configuration of fibrin clots determines capillary morphogenesis and endothelial cell migration. Microvasc Research 51: 347-364, 1996.
41. Costantini V and Zacharski LR: Fibrin and cancer. Thromb Haemost 69: 406-414, 1993.
42. Bardos H, Molnar P, Csecsei G and Adany R: Fibrin deposition in primary and metastatic human brain tumors. Blood Coagul Fibrinolysis 7: 536-548, 1996.