Abstract. Background: We have previously found an association of platelet glycoprotein Ia polymorphism with increased risk for oral cancer. The purpose of this study was to investigate the possible relation of another platelet glycoprotein, Ib- (GPIb-), with oral oncogenesis. Patients and Methods: The variable number of tandem repeats (VNTR) polymorphism of the GPIb- gene, which affects the protein’s structure and function, was examined in 162 Greek and German patients with oral squamous cell carcinoma and 225 healthy controls of equivalent age, gender and ethnicity. Results: The B allele frequency detected, representing higher platelet activation, in the patient group and in the subgroups of patients without family history either of cancer or thrombophilia were significantly elevated in comparison with that of the control group (p=0.03, p=0.016 and p=0.036, respectively). The D allele frequency (lower platelet activation) was significantly lower in comparison with controls only in patients with family history of thrombophilia. The frequency of B/B homozygotes was significantly increased in the total group of patients and the subgroup of patients with a family history of thrombophilia, in comparison with the control group (p=0.042 and p=0.043, respectively), while the frequency of heterozygotes for the C/B alleles was significantly lower in the subgroups of patients with a family history of cancer and thrombophilia (p=0.036 and p=0.027, respectively) compared to the control group. Conclusion: The VNTR polymorphism of the GPIb- gene, which affects the structure and function of this platelet glycoprotein, seems to be associated with risk for oral cancer, especially in patients without a family history of cancer.

Oral squamous cell carcinoma (OSCC) is a common malignancy, characterized by a fairly poor prognosis (1). Carcinogenesis in the oral region is known to involve genetic alterations and exposure to other factors, such as tobacco and alcohol (2). Recently, common polymorphisms in genes involved in angiogenesis, inflammation and thrombosis have also been associated with increased risk for oral cancer (3-9). One such factor, previously correlated with cerebrovascular disease and cancer, is platelet glycoprotein Ib- (GPIb-) (10-12).

GPIb- is the largest polypeptide of a major platelet complex called the membrane glycoproteins (GP) Iba-IX-V complex (13, 14). GPIb- is disulfide-linked to GPIbβ, and non-convalently complexed with GPIX and GPV in the ratio 2:2:1 (14). GPIb-IX complex is a platelet receptor for another large multimeric glycoprotein, von Willebrand factor (vWF), and plays a major role in mediating platelet adhesion to the subendothelium (15, 16). The GPIb-IX-V complex binding to subendothelial vWF at high shear rates is an initial step in the cascade of events which lead to thrombosis (17, 18). GPIbα is not only pivotal to the process of arterial thrombosis but is also highly expressed in epithelial and tumor cells (12, 19). The levels of GPIbα expression in tumor cells are associated with motility, invasiveness and cell differentiation (12).

A functional polymorphism has been located in the polymorphic site to the heavily O-glycosylated region of the GPIbα, affecting its structure (20). This polymorphism was found to result from a variable number of tandem repeats (VNTR) of 39 bp of GPIbα: either one repeat (D allele),

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two (C allele), three (B allele), or four repeats (A allele) (21, 22). At the protein level, each repeat leads to addition of 13 amino acids moving the vWF-binding domain out of the platelet membrane (22, 23). This may result in the exposure of the molecule to greater shear forces and thus lower the threshold for shear-induced interaction with vWF and subsequent platelet activation (23). A previous study did not detect any association between the GPIbα VNTR polymorphism and breast cancer, while another found a significant correlation of GPIbα expression with breast cancer aggressiveness (24, 25). Some studies of large patient cohorts have detected a strong association between the GPIbα VNTR polymorphism and venous or arterial thrombosis, while other studies with smaller patient numbers have not found any such association (26-31).

We have previously detected an association of another platelet glycoprotein polymorphism with risk for oral cancer, namely GPIa C807T (6). In order to determine whether the GPIbα VNTR polymorphism is also associated with oral cancer, we investigated this polymorphism in patients with oral squamous cell carcinoma and matched healthy controls.

Patients and Methods

A total of 387 individuals of Greek and German origin were enrolled after informed consent was obtained, consisting of 225 healthy controls and 162 patients with oral squamous cell carcinoma of similar gender, age and ethnicity. In addition to clinical presentation, a biopsy with pathological diagnosis of tumor stages I-IV and family history regarding cancer and thrombophilia were available. The age of patients ranged between 40-84 years, with a mean age of 58.5±10.1 years. The age of the controls varied between 38-92 years, while the mean age was 56.9±14.3 years. Sixty of the patients (37%) had one or two first degree relatives with cancer and their age range 41-83 years (58.7±10.2) did not differ significantly from the whole group of patients. Furthermore, 32 patients (19.8%) had one or two first-degree relatives with idiopathic thrombosis and an age range of 44-75 years (58.0±9.9), again with no statistical difference compared to the whole group. Sixteen patients (9.9%) had a positive family history for both cancer and thrombosis and an age range of 48-74 with a mean age of 56.3±8.0 years. Nearly all patients (95.0%) were smokers and about a third of them were alcohol abusers (32.5%). Most of the studied individuals worked in a low-risk environment (with the exception of one patient and three controls who worked in chemical factories).

DNA was isolated using the NucleoSpin™ kit (Macherey-Nagel GmbH & Co, Düren, Germany). Molecular detection of the GPIbα polymorphism in the GPIbα gene was performed by restriction fragment length polymorphism typing, as described elsewhere (24). An example of observed alleles is illustrated in Figure 1.

The 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 and subgroups of patients were compared to the respective frequencies of the controls using Fisher’s exact test. 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

The prevalence of detected GPIbα genotypes, allele and carrier frequencies are shown in Table I. The data for the two tested populations (Greek and German healthy controls) were analyzed together since there were no significant differences in genotype or allele frequency of the GPIbα polymorphism between the two populations.

In comparison with controls, the frequency of the B allele, representing higher platelet activation, was significantly elevated in the patient group and in the subgroups of patients without a family history either of cancer or thrombophilia (p=0.03, p=0.016, p=0.036, respectively; Table I). The D allele frequency (lower platelet activation) was significantly lower in comparison with controls only in patients with family history of thrombophilia (p=0.035). Finally, there were no major differences in the frequencies of the three GPIbα alleles due to categorizations of gender, age, age at onset of oral cancer, or cancer stage.

Compared to the control group, only the homozygotes for the higher activation B allele were significantly increased in the total group of patients (p=0.042), as well as in patients with a family history of thrombophilia (p=0.043; Table I).

In the subgroups of patients with a family history of cancer and thrombophilia, the frequency of heterozygotes for the C/B alleles was significantly lower (p=0.036 and p=0.027, respectively). No statistically significant difference in the frequency of genotypes or alleles of the controls was observed in the subgroups of patients with initial or advanced stages of cancer, or with and without tobacco or alcohol abuse (data not shown).

Interestingly, compared to individuals with the referent C/C genotype, B/B homozygotes have an almost double...
relative risk for oral squamous cell carcinoma (odds ratio (OR) 1.74, 95% confidence interval (CI) 0.7-4.4; Table I). Additionally, B/B homozygotes with family history of thrombophilia have an even greater risk for developing oral cancer (OR 3.29, 95% CI 0.9-11.8; Table I).

**Discussion**

Platelet glycoprotein Ibα is highly expressed in tumor cells and the level of its expression is associated with motility, invasiveness and distant metastasis (12, 19, 24, 25, 31). The GPIbα VNTR polymorphism, which affects the structure and function of GPIbα, was investigated in patients with oral cancer and matched healthy controls in order to detect whether it affects risk of cancer in the oral region.

Despite the modest number of studied individuals, the findings of this study seem to support the association of VNTR polymorphism with risk for oral cancer. In comparison with controls, the B allele (higher platelet activation) frequency was significantly higher in the total group of patients and their subgroup without positive family history of cancer or thrombophilia. Furthermore, the D allele frequency (lower platelet activation) was reduced in the total group and most subgroups of patients, while this trend reached a significant value in the subgroup of patients with a family history of thrombophilia.

The present study indicates that the VNTR polymorphism B allele is associated with oral cancer risk, while the D allele plays a rather prophylactic role. The B allele has 3 tandem repeats of 39 bp, while the D allele has only one (21, 22). Therefore, the GPIbα protein with the longer amino acid sequence corresponding to that of the B allele may be exposed out of the platelet membrane, lowering the threshold for greater shear-induced interaction with vWF, resulting in easier as well as irreversible platelet activation (22, 23). It is well-known that in malignancies, tumor cells undergo complex interactions with vascular endothelium and platelets. In a human osteosarcoma study, it was shown that the initiation of the platelet aggregating activity of tumor cells required their interaction with platelet membrane GPIIb/IIa, but for irreversible platelet aggregating activity the platelet membrane glycoprotein GPIbα was responsible (19).

The fact that the B allele (higher platelet activation) frequency was not significantly increased in the subgroup of patients with a family history of either cancer or thrombophilia may suggest that the GPIbα polymorphism has a minor effect in oral carcinogenesis. Similar to the case of the minor contribution of GPIa in risk for oral cancer (6), it seems that many factors regulate cell/cell and cell substratum adhesion. Therefore, each one of them might slightly affect adhesion of platelets to the extracellular tumor cell and contribute to the risk for oral carcinogenesis.

**Table 1. Prevalence of GPIbα (VNTR) polymorphism in healthy controls and patients with oral cancer (total group of patients and subgroup with regard to family history of cancer and thrombophilia).**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls (%)</th>
<th>Patients (%)</th>
<th>Family history of cancer (%)</th>
<th>OR (CI)</th>
<th>P-value (OR, CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C</td>
<td>118 (52.4%)</td>
<td>88 (35.8%)</td>
<td>38 (40.0%)</td>
<td>1.00</td>
<td>N.S.</td>
</tr>
<tr>
<td>D/D</td>
<td>10 (4.4%)</td>
<td>18 (7.9%)</td>
<td>10 (10.0%)</td>
<td>0.80</td>
<td>N.S.</td>
</tr>
<tr>
<td>B/B</td>
<td>10 (4.4%)</td>
<td>32 (14.2%)</td>
<td>12 (12.5%)</td>
<td>5.95</td>
<td>p = 0.0042</td>
</tr>
<tr>
<td>C/D</td>
<td>38 (16.9%)</td>
<td>22 (9.9%)</td>
<td>8 (8.4%)</td>
<td>0.37</td>
<td>N.S.</td>
</tr>
<tr>
<td>D/B</td>
<td>2 (0.9%)</td>
<td>2 (0.9%)</td>
<td>0 (0.0%)</td>
<td>1.00</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Prevalence of variant alleles (%)</th>
<th>OR (CI)</th>
<th>P-value (OR, CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C</td>
<td>83 (36.3%)</td>
<td>1.00</td>
<td>N.S.</td>
</tr>
<tr>
<td>D/D</td>
<td>10 (4.4%)</td>
<td>0.28</td>
<td>N.S.</td>
</tr>
<tr>
<td>B/B</td>
<td>31 (13.7%)</td>
<td>2.82</td>
<td>p = 0.0330</td>
</tr>
<tr>
<td>C/D</td>
<td>13 (5.9%)</td>
<td>0.77</td>
<td>N.S.</td>
</tr>
<tr>
<td>D/B</td>
<td>0 (0.0%)</td>
<td>1.00</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Fisher's p-value was used for genotype comparisons; odds ratios (OR) are age-adjusted; CI: 95% confidence interval; N.S.: not significant.
In conclusion, the VNTR polymorphism of the GPIba gene, which affects the structure and function of this platelet glycoprotein, seems to be associated with risk for oral cancer, especially in patients without a family history of cancer.

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References


