Abstract. The present study was performed in order to investigate the possible association of the -418 G/C polymorphism in the tissue inhibitor of metalloproteinase-2 (TIMP-2) gene, which affects its expression, with the risk of developing oral cancer. PCR-based restriction analysis was performed in DNA samples from 158 patients with oral squamous cell carcinoma (OSCC) and 168 healthy controls of equivalent sex, age and ethnicity (Greeks and Germans). Statistical analyses were performed with Fisher's exact test and the calculation of odds ratios with a 95% confidence interval (CI). The frequency of the low C allele expression was ten times greater in the patients than the controls (31% vs 2.7%, respectively; P<0.001). The C/C and G/C genotypes were associated with an increased risk of developing OSCC (P<0.001, OR=40.88, 95% CI=2.24-744.40, and P<0.001, OR=21.31, 95%=9.82-46.21, respectively). The same pattern of significant differences with the controls was also observed in the subgroups of patients in regard to the initial or advanced stages of oral cancer, family history of any type of cancer or thrombosis, and smoking habits or alcohol abuse. These findings are consistent with the reduced levels of TIMP-2 in the presence of the low expression C allele, which are insufficient to inhibit the matrix metalloproteinase-driven degradation of the extracellular matrix (leading to cancer invasion) and mitogen-driven neoangiogenesis (leading to tumor growth and metastasis). In conclusion, the studied TIMP-2 polymorphism is strongly associated with an increased risk of OSCC in Europeans carrying the low C allele expression. These results indicate that this polymorphism could serve as a genetic marker for the susceptibility of cancer in the oral cavity.

Introduction

Oral squamous cell carcinoma (OSCC) is the sixth most common malignancy and a major cause of cancer morbidity and mortality worldwide, with more than 300,000 new cases being diagnosed annually all over the world (1,2). This malignancy arises as a result of a multistep process in which several factors, such as environmental agents (smoking, alcohol, dietary habits and certain viruses) and genetic events (alterations in oncogenes and tumor suppressor genes) play an accumulating role (3). Recently, common inherited polymorphisms that are related to inflammation, angiogenesis and thrombosis have also been correlated with an increased risk of OSCC in Europeans carrying the low C allele expression. These results indicate that this polymorphism could serve as a genetic marker for the susceptibility of cancer in the oral cavity.
addition, TIMP-2 is implicated in cell growth and apoptosis (13,14). Taking all these characteristics into account, several recent studies have reported a relationship between the increased TIMP-2 expression and the progression of certain malignancies including OSCC, serous ovarian carcinomas and urothelial bladder carcinomas (12,14-16).

A single nucleotide G/C polymorphism has been identified at position -418 in the promoter region of the TIMP-2 gene (17,18). The presence of the G allele has been shown to be associated with increased gene expression, possibly because it favours the binding of the Sp1 transcription factor on a consensus sequence in the promoter region of the TIMP-2 gene (19,20). The frequency of the C allele ranges between 10-20% in East Asians while in Caucasians it is considered to be much rarer. (18,20-24).

This polymorphism has been studied in Thai patients with head and neck squamous cell carcinoma. However, the results were inconclusive (20). Therefore, we investigated the possible association of the TIMP-2 -418 G/C gene polymorphism with an increased risk of oral cancer in European patients in comparison to healthy controls.

Materials and methods

The individuals under study were 326 Greeks and Germans, who participated after informed consent. The Departments of Oral and Maxillofacial Surgery of the Universities of Athens and Erlangen considered all the ethical aspects of this study and approved the protocol used. The studied individuals included 158 patients with oral squamous cell carcinoma and 168 healthy blood donors of equivalent ethnicity, age and gender. The age of the patients ranged between 40-80 years (mean age 58±9.9), again with no statistical difference when compared to the whole group. Sixteen patients (10.1%) had a positive family history for both cancer and thrombosis with a mean age of 58±9.9, again with no statistical difference when compared to the whole group. Sixteen patients (10.1%) had a positive family history regarding any type of cancer or thrombosis, nicotine or alcohol abuse. Thus, odds ratios are most likely expected to overestimate the true likelihood of the TIMP-2 genotypes and these variables. The age criterion for the adjustment of odds ratios has been set at 60 years. Similar frequency distributions regarding age are found in the respective genotypes between the controls and patients. The Mantel-Haenszel method was used for the calculation of all odds ratios with a 95% confidence interval (CI). A P-value <0.05 was considered statistically significant.

The statistical analyses were performed using SAS® software (version 9.0; SAS Institute Inc.). The genotype distribution and allelic frequencies were analyzed with Fisher's exact test using the exact table P-values. The genotype frequencies of the whole group or subgroups of patients were compared to the respective genotypes of the control group. In all the statistical analyses, it was assumed that there were no patients in the control group with a family history of cancer, or thrombosis, nicotine or alcohol abuse. Thus, odds ratios are most likely expected to overestimate the true likelihood of the TIMP-2 genotypes and these variables. The age criterion for the adjustment of odds ratios has been set at 60 years. Similar frequency distributions regarding age are found in the respective genotypes between the controls and patients. The Mantel-Haenszel method was used for the calculation of all odds ratios with a 95% confidence interval (CI). A P-value <0.05 was considered statistically significant.

The obtained data of the detected TIMP-2 genotypes in the healthy controls and patients with OSCC are shown in Tables I-IV. The two studied European populations (Greeks and Germans) displayed no significant differences in the genotype and allele frequencies of the -418 G/C polymorphism, either among the controls or the patients (Table I). Therefore, the data for the two populations under study were analyzed together (Tables II-IV). All the observed genotype and allele frequencies did not decline from the Hardy-Weinberg equilibrium.

A highly significant difference in the G/C heterozygotes was observed between the oral cancer patients and the controls (54.4% vs 5.4%, respectively, OR=21.31, 95% CI=9.82-46.21, P<0.001). In comparison to the controls, who had a very low C allele frequency (2.7%) in accordance to previously studied Caucasian populations, the patients had a C allele frequency which was about ten times higher (31%, P<0.001).

This pattern of highly significant differences in the C allele and carrier frequencies in comparison to the controls was observed in all the subgroups of patients in regard to i) early or advanced cancer stages, ii) with or without positive family history of cancer, iii) with or without positive family
The frequencies of the genotypes and C alleles are not significantly different among the two studied populations, either among the controls or the patients. TIMP-2, tissue inhibitor of metalloproteinase-2.

All odds ratios are age-adjusted. TIMP-2, tissue inhibitor of metalloproteinase-2; CI, confidence interval.

Table II. Prevalence of the TIMP-2 (-418G/C) polymorphism in the healthy controls and the total group of patients and their subgroups, in regard to the oral cancer stage.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Controls</th>
<th>Patients</th>
<th>Fisher's P-value</th>
<th>OR (CI)</th>
<th>Patients with cancer stages I &amp; II</th>
<th>Fisher's P-value</th>
<th>OR (CI)</th>
<th>Patients with cancer stages III &amp; IV</th>
<th>Fisher's P-value</th>
<th>OR (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C</td>
<td>0 (0%)</td>
<td>6 (3.8%)</td>
<td>&lt;0.001</td>
<td>40.88</td>
<td>4 (4.5%)</td>
<td>0.0014</td>
<td>78.12</td>
<td>2 (9.0%)</td>
<td>0.0273</td>
<td>24.11</td>
</tr>
<tr>
<td></td>
<td>(0%)</td>
<td>(3.8%)</td>
<td></td>
<td>(2.24-744.40)</td>
<td>(4.5%)</td>
<td>(3.97-1537.73)</td>
<td>(2.9%)</td>
<td>(1.12-519.46)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>159 (94.6%)</td>
<td>66 (41.8%)</td>
<td>1 (referent)</td>
<td>36 (40.9%)</td>
<td>1 (referent)</td>
<td>1 (referent)</td>
<td>30 (42.9%)</td>
<td>1 (referent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/C</td>
<td>9 (5.4%)</td>
<td>86 (54.4%)</td>
<td>&lt;0.001</td>
<td>21.31</td>
<td>48 (54.5%)</td>
<td>&lt;0.001</td>
<td>33.81</td>
<td>38 (54.3%)</td>
<td>&lt;0.001</td>
<td>15.36</td>
</tr>
<tr>
<td></td>
<td>(5.4%)</td>
<td>(54.4%)</td>
<td></td>
<td>(9.82-46.21)</td>
<td>(54.5%)</td>
<td>(13.70-83.41)</td>
<td>(54.3%)</td>
<td>(6.50-36.31)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>168 (100%)</td>
<td>158 (100%)</td>
<td>88 (100%)</td>
<td>70 (100%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Prevalence of C allele

| C allele frequency | 2.7% | 31% | <0.001 | 31.8% | <0.001 | 30% | <0.001 |
| Carrier frequency of C allele | 5.4% | 58.2% | <0.001 | 59.1% | <0.001 | 57.1% | <0.001 |

Discussion

Higher levels of activated MMPs have been implicated in tumor development and metastasis (25). An important mechanism for the down-regulation of MMPs is via binding to the tissue inhibitors of metalloproteinases (TIMP-1 through TIMP-4), which belong to a family of homologous proteins (26). TIMP-2 is not only a natural inhibitor of...
Table III. Prevalence of the TIMP-2 (−418G/C) polymorphism in the healthy controls and patients with oral cancer in regard to family history of either cancer or thrombosis.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Controls Patients with family history of cancer</th>
<th>Fisher’s OR P-value (CI)</th>
<th>Patients without family history of cancer</th>
<th>Fisher’s OR P-value (CI)</th>
<th>Patients with family history of thrombosis</th>
<th>Fisher’s OR P-value (CI)</th>
<th>Patients without family history of thrombosis</th>
<th>Fisher’s OR P-value (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C</td>
<td>0 (0%)</td>
<td>4 (6.9%)</td>
<td>0.001 (3.97-1537.73)</td>
<td>2 (2%)</td>
<td>0.0495 (1.12-519.46)</td>
<td>6 (4.8%)</td>
<td>0.001 (2.71-904.76)</td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>159 (94.6%)</td>
<td>22 (37.9%)</td>
<td>1 (referent)</td>
<td>44 (44%)</td>
<td>1 (referent)</td>
<td>50 (39.7%)</td>
<td>1 (referent)</td>
<td></td>
</tr>
<tr>
<td>G/C</td>
<td>9 (5.4%)</td>
<td>32 (55.2%)</td>
<td>&lt;0.001 (10.50-66.69)</td>
<td>54 (54%)</td>
<td>&lt;0.001 (8.38-44.59)</td>
<td>70 (55.6%)</td>
<td>&lt;0.001 (8.91-44.24)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>168 (100%)</td>
<td>58 (100%)</td>
<td></td>
<td>100 (100%)</td>
<td></td>
<td>126 (100%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Prevalence of C allele

C allele frequency 2.7% 34.5% <0.001 29% <0.001 25% <0.001 32.5% <0.001

Carrier frequency of C allele 5.4% 62.1% <0.001 56% <0.001 50% <0.001 60.3% <0.001

All odds ratios are age-adjusted. TIMP-2, tissue inhibitor of metalloproteinase-2; CI, confidence interval; NC, non calculable P-value.

Table IV. Prevalence of the TIMP-2 (−418G/C) polymorphism in the healthy controls and patients with oral cancer in regard to either alcohol consumption or smoking habits.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Controls Patients with tobacco abuse</th>
<th>Fisher’s OR P-value (CI)</th>
<th>Patients without tobacco abuse</th>
<th>Fisher’s OR P-value (CI)</th>
<th>Patients with alcohol abuse</th>
<th>Fisher’s OR P-value (CI)</th>
<th>Patients without alcohol abuse</th>
<th>Fisher’s OR P-value (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C</td>
<td>0 (0%)</td>
<td>4 (2.7%)</td>
<td>0.0069 (1.68-611.23)</td>
<td>2 (20%)</td>
<td>0.0111 (5.02-2897.22)</td>
<td>2 (3.8%)</td>
<td>0.0119 (2.83-1438.99)</td>
<td>4 (3.8%)</td>
</tr>
<tr>
<td>G/G</td>
<td>159 (94.6%)</td>
<td>62 (41.9%)</td>
<td>1 (referent)</td>
<td>4 (40%)</td>
<td>1 (referent)</td>
<td>18 (34.6%)</td>
<td>1 (referent)</td>
<td>48 (45.3%)</td>
</tr>
<tr>
<td>G/C</td>
<td>9 (5.4%)</td>
<td>82 (55.4%)</td>
<td>&lt;0.001 (10.69-51.21)</td>
<td>4 (40%)</td>
<td>0.0011 (1.53-83.14)</td>
<td>32 (61.5%)</td>
<td>&lt;0.001 (13.51-99.13)</td>
<td>54 (50.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>168 (100%)</td>
<td>148 (100%)</td>
<td></td>
<td>10 (100%)</td>
<td></td>
<td>52 (100%)</td>
<td></td>
<td>106 (100%)</td>
</tr>
</tbody>
</table>

Prevalence of C allele

C allele frequency 2.7% 30.4% <0.001 40% <0.001 34.6% <0.001 29.2% <0.001

Carrier frequency of C allele 5.4% 58.1% <0.001 60% <0.001 65.4% <0.001 54.7% <0.001

All odds ratios are age-adjusted. TIMP-2, tissue inhibitor of metalloproteinase-2; CI, confidence interval.
MMP-2 but a suppressor of endothelial cell proliferation and angiogenesis as well (10,11). The complexity of the TIMP-2 functions indicates a possibly multiple role in cancer progression and metastasis (13,14,27). The levels of TIMP-2 have been correlated with the progression of OSCC, in addition to other carcinomas (12,14-16,27).

A single nucleotide polymorphism (-418G/C) in the promoter region of the TIMP-2 gene affects its transcription (20). Gene expression is lower when the less common C allele is present (18,20). In this light, the purpose of this study was to investigate the possible role of the -418G/C polymorphism in the risk of developing oral oncogenesis by comparing the TIMP-2 genotypes of patients with oral cancer and healthy controls of equivalent age, sex and ethnicity.

Despite the relatively small number of studied individuals, the overall obtained data revealed a strong association of the low expression C allele with an increased risk of developing oral cancer (P<0.001). Both the homozygous state C/C as well as the heterozygous G/C genotype were strongly associated with an increased risk of developing oral oncogenesis (P<0.001), indicating that the low expression C allele acts as a dominant genetic character. The association of the TIMP-2 polymorphism with oral cancer was so strong that no additive effect of the environmental factors that are known to be tumorigenic in the oral cavity (such as tobacco and alcohol) was observed.

These findings could be explained by the reduced amount of TIMP-2 in the presence of the C allele, which is insufficient to inhibit MMP-2 and mitogen-driven angiogenesis (10,11,20). MMP-2 is a protease involved in the degradation of the extracellular matrix and thus contributes to cancer invasion and metastasis, while neoangiogenesis is important for the viability, growth and metastatic potential of tumors (10,11,25).

The present study is not in accordance with another study conducted in a Thai population, concerning head and neck cancer (20). The distribution of the genotypes concerning the -418 G/C polymorphism in Thais was only borderline different (P=0.059) between the patients and the controls, while no significant difference was found among the C allele frequencies between the patients and the controls (P=0.320) (20). Nevertheless, only 46.4% of the Thai patients had OSCC (20). Therefore these results are not fully comparable with the findings of the present study. Diverse tumorigenic mechanisms possibly exist among tumors of the oral cavity and neck. This notion is reinforced by a number of studies, in which the polymorphisms in the MMP-1 or IL-8 genes have been associated with oral cancer but not esophageal cancer (9,28-30). Moreover, this discrepancy could be due to the fact that the frequency of the C allele is more common in the Thai controls (17.2%) and rare in Europeans (only 2.7%), indicating that there are differences in the distribution of genotypes among these ethnicities.

In conclusion, the studied TIMP-2 polymorphism is strongly associated with an increased risk of OSCC in Europeans carrying the low expression C allele. These results indicate that this polymorphism could serve as a genetic marker for the susceptibility to cancer in the oral cavity. Since other factors related to angiogenesis, inflammation and thrombosis have been also associated with this malignancy (7-9), further studies are necessary in order to clarify the role of all the potential genetic and environmental factors in the formation of OSCC, in an effort to safeguard the health status and lives of certain individuals who are at risk in the general population.

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References


