Meta-Analysis of MMP2, MMP3, and MMP9 Promoter Polymorphisms and Head and Neck Cancer Risk

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Abstract

Background: The 1306 C>T, 1171 5A>6A, and 1562C>T polymorphisms of matrix metalloproteinase (MMP) 2, MMP3, and MMP9 genes, respectively, have been found to be functional and may contribute to head and neck carcinogenesis. However, the results of case-control studies examining associations between MMP polymorphisms and head and neck cancer (HNC) risk remain inconclusive. Therefore, we performed a meta-analysis to further evaluate the role of these polymorphisms in HNC development.

Methods: We searched PubMed, ISI Web of Knowledge, MEDLINE, Embase, and Google Scholar to identify all published case-control studies of MMP2-1306 C>T, MMP3-1171 5A>6A, and MMP9-1562 C>T polymorphisms and HNC risk in the meta-analysis. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to assess the association between these polymorphisms and HNC risk.

Results: Thirteen studies were included in this meta-analysis. For MMP2-1306 C>T polymorphism, significant associations were observed under three genetic models both in overall comparison and in a hospital-based subgroup, and in oral cavity cancer and nasopharyngeal cancer under dominant model as well. For MMP3-1171 5A>6A and MMP9-1562 C>T polymorphisms, no association was found in overall comparison; however, in subgroup analyses based on ethnicity and tumor site, significant associations were detected between the MMP3-1171 5A>6A polymorphism and HNC risk in a European population and pharyngeal/laryngeal cancer under two genetic contrasts.

Conclusion: This meta-analysis suggests that the MMP2-1306 C>T polymorphism is associated with HNC risk, as is the MMP3-1171 5A>6A polymorphism specifically in some subgroups. Further studies with larger sample sizes are warranted.

Introduction

Head and neck cancer (HNC), which includes cancers of the oral cavity, pharynx, hypopharynx, and larynx, is one of the most common cancers worldwide [1]. It accounts for nearly 3% of all incident malignancies in the United States with an estimated 52,610 new cases and 11,500 deaths from HNC in 2012 [2]. It is characterized by local tumor aggressiveness that could lead to a high recurrence rate and a low survival rate [3]. Many factors, such as tobacco use, alcohol consumption, viral infection, and genetic susceptibility, are associated with an increased risk of HNC [4–6]. Although tobacco smoking and alcohol consumption play a critical role in HNC carcinogenesis, only a small proportion of smokers and drinkers are ultimately diagnosed with HNC. This implies that genetic susceptibility to HNC varies among individuals in the general population [7].

Studies have demonstrated that Matrix metalloproteinases (MMPs) may play an important role in HNC development [8]. MMPs are a family of zinc-dependent proteinases that are capable of degrading essentially all extracellular matrix components, which is a key event in the invasion and metastasis of most malignancies [9–12]. Under normal conditions, MMPs are implicated in both tissue regeneration and wound repair, as well as reproduction [13–15]. MMPs may also contribute to carcinogenesis, as previous studies have indicated that MMPs are involved in several steps of cancer development, including cancer cell growth, differentiation, apoptosis, migration, invasion, and metastasis [12]. MMP2, MMP3, and MMP9 are three important members of the MMP family. MMP2 (gelatinase-A), located on chromosome 16q13–q21, digests gelatin (denatured collagen), type IV collagen, and some bioactive molecules, such as growth factor-binding
proteins and growth factor receptors [16–18]. *MMP3* (stromelysin-1), located on chromosome 11q22.2–22.3, can lyse the collagen present in the basal membrane and induces the synthesis of other MMPs such as *MMP1* and *MMP9* [19,20]. *MMP9* (gelatinase B) is the most complex member of the MMPs family in terms of domain structure. It is capable of degrading decorin, elastin, fibrillin, laminin, gelatin, and types IV, V, XI, and XVI collagen [21,22]. Overexpression of *MMP2*, *MMP3*, and *MMP9* has been found to associate with the development of cancer, including HNC [8], thereby indicating that these MMPs may also be implicated in HNC development.

Several polymorphisms in the promoter regions of the *MMP2*, *MMP3*, and *MMP9* genes have been well described. Previous researchers reported that these polymorphisms play critical roles in the regulation of *MMP* gene transcription. *MMP2*-1306 C>T (rs243865), which contains a C to T transition at −1306, is associated with high transcriptional activity of the *MMP2* gene [23]. *MMP3*-1171 5A>6A (rs3025058), which is characterized by the insertion or deletion of a single adenosine at position −1171, could alter *MMP3* transcription levels [24]. *MMP9*-1562 C>T (rs3918242), which includes a C>T transition at position −1562 near the upstream transcription initiation site, also influences the *MMP9* transcriptional levels [25]. Several epidemiologic studies of the association of these three polymorphisms with HNC risk have been carried out [26–37]; however, their results remain inconclusive. Thus, we conducted a meta-analysis of all eligible case-control studies published to date to further evaluate the associations between these three polymorphisms and HNC risk.

**Materials and Methods**

**Search Strategy**

Using key words search in the PubMed, Web of Knowledge, MEDLINE, Embase, and Google Scholar electronic databases and search engines, we identified all eligible case-control studies of the associations of *MMP2*, *MMP3*, and *MMP9* polymorphisms with HNC risk conducted between January 2000 and June 2012. We used the following key words:

“*MMP*”, “matrix metalloproteinase”, “collagenase”, “gelatinase”, “matrilysins” or “PUMP” and “head and neck cancer”, “oral cancer”, “pharyngeal cancer”, “hypopharyngeal cancer” or “laryngeal cancer” and “polymorphism”, “variant”, “genotype” or “SNP”. After performing the electronic key word searches, we manually reviewed the references of the search results to identify additional evaluable studies. We contacted authors directly for important data that were not reported in original articles. Abstracts, unpublished reports, and articles not written in English were not included.

**Data Extraction**

The following details were extracted from each article included in the meta-analysis: first author, publication year, ethnicity of the study population (categorized as Asian and European), the number of cases and controls, and genotype distribution, genotyping methods, allele frequency, and so on. To minimize bias and improve reliability, two investigators extracted the data independently and reached a consensus on all items (the details of each study) via discussion.

**Inclusion and Exclusion Criteria**

Studies were included if they: (1) were case-control studies, (2) assessed the associations between *MMP2*, *MMP3*, and *MMP9* polymorphisms and HNC risk, (3) had sufficient available data to calculate an odds ratio (OR) with a 95% confidence interval (CI) and P-value, and (4) were published in English.

Studies were excluded if they: (1) had insufficient information about genotype frequency or number, (2) if the same population was evaluated in two or more studies, only the most recent or the one with the largest study population was included in this meta-analysis.

**Statistical Analysis**

We evaluated the association of *MMP* polymorphisms and HNC risk using ORs and 95% CIs. The significance of pooled ORs was estimated via a Z test (P<0.05 was considered statistically significant). Heterogeneity between studies was assessed via Cochran’s chi-square Q statistic test. A random-effects model was used when the P value for heterogeneity was less than 0.05, which indicated obvious heterogeneity of the data; otherwise, a fixed-effects model was used. Heterogeneity across studies was also detected using an I² test. As a guide, I² values of <25% were considered low, I² values of 25 to 75% were considered moderate, and I² values of >75% were considered high [38]. The associations between *MMP2*, *MMP3*, and *MMP9* polymorphisms and the risk of HNC were evaluated using a recessive genetic model (BB versus AB+AA), dominant genetic model (BB+AB versus AA), and allele contrast model (B-allele versus A-allele), respectively (A represented major allele and B represented minor allele). In addition to overall comparison, subgroup analyses based on the ethnicity of each study population and the source of the control subjects were also performed using different genetic models. Furthermore, sensitivity analyses were performed to reflect the influence of the individual dataset on the pooled ORs by sequential removing each eligible study. Finally, we assessed the publication bias using Begg’s funnel plot and Egger’s test. Additionally, the Hardy–Weinberg equilibrium (HWE) was calculated via a chi-square test at a significance level of α <0.05. All P values were two-sided, and all statistical analyses were performed using STATA 12.0 software (Stata Corporation, College Station, TX, USA).

**Results**

**Study Characteristics**

We identified 45 relevant articles using the aforementioned search strategy. However, 33 studies were excluded: 26 did not assess the association between *MMP2*, *MMP3*, and *MMP9* polymorphisms and HNC risk; 2 had insufficient data for further analysis; 4 were review articles; and one was a commentary. Zhou [28] evaluated *MMP2*, *MMP3*, and *MMP9* polymorphisms in a case–control study of two independent populations. Each population was regarded as a separate study. Consequently, 13 studies of the association of *MMP2*, *MMP3*, and *MMP9* polymorphisms with the risk of HNC were ultimately included in this meta-analysis (Figure 1). Table 1 illustrates the characteristics of all the included studies, such as their publication year, the ethnicity of the study population, tumor site, genotyping data, and sample size (case vs. controls). All the articles included in the meta-analysis were published in English. Polymersase chain reaction-restriction fragment length polymorphism was the most commonly used genotyping method in these studies. The results from chi-square tests showed that genotypic distribution of the controls was in agreement with the HWE except one study [36] at a statistical significance level of 0.05.
Quantitative Data Synthesis

**MMP2-1306 C>T**: Four studies evaluated the association of the **MMP2-1306 C>T** polymorphism with HNC risk [26–28] with 1163 cases and 1156 controls. In the overall comparison, significant associations between the **MMP2-1306 C>T** polymorphism and HNC risk were observed using three genetic models (OR, 0.12; 95% CI, 0.02–0.69; I^2, 0, $P_{\text{heterogeneity}} = 0.865$ for the recessive model; OR, 0.52; 95% CI, 0.40–0.66; I^2, 0, $P_{\text{heterogeneity}} = 0.97$ for the dominant model; and OR, 0.52; 95% CI, 0.41–0.65; I^2, 0, $P_{\text{heterogeneity}} = 0.963$ for the allele contrast model; Figure 2). Similarly, in subgroup analyses based on the source of control subjects and tumor site, the **MMP2-1306 C>T** polymorphism was significantly associated with HNC risk in the hospital-based subgroup (OR, 0.10; 95% CI, 0.01–0.78; I^2, 0, $P_{\text{heterogeneity}} = 0.907$ for the recessive model; OR, 0.52; 95% CI, 0.36–0.75; I^2, 0, $P_{\text{heterogeneity}} = 0.911$ for the dominant model; and OR, 0.50; 95% CI, 0.35–0.70; I^2, 0, $P_{\text{heterogeneity}} = 0.913$ for the allele contrast model); in the population-based subgroup (OR, 0.52; 95% CI, 0.37–0.71; I^2, 0, $P_{\text{heterogeneity}} = 0.628$ for the dominant model and OR, 0.53; 95% CI, 0.39–0.73; I^2, 0, $P_{\text{heterogeneity}} = 0.662$ for the allele contrast model); in the oral cavity cancer (OR, 0.47; 95% CI, 0.31–0.73; I^2, 0, $P_{\text{heterogeneity}} = 0.607$ for the dominant model); and in the nasopharyngeal cancer (OR, 0.52; 95% CI, 0.37–0.71; I^2, 0, $P_{\text{heterogeneity}} = 0.628$ for the dominant model; Table 2).

**MMP3-1171 5A>6A**: We identified eight studies that evaluated the association of the **MMP3-1171 5A>6A** polymorphism with the risk of HNC [28–34] with 1672 cases and 1779 controls. In the overall comparison, the **MMP3-1171 5A>6A** polymorphism was not significantly associated with HNC risk using three different genetic models (OR, 0.87; 95% CI, 0.65–1.17; I^2, 66.4%, $P_{\text{heterogeneity}} = 0.004$ for the recessive model; OR, 0.85; 95% CI, 0.62–1.16; I^2, 0, $P_{\text{heterogeneity}} = 0.505$ for the dominant model; and OR, 0.92; 95% CI, 0.74–1.14; I^2, 60.4%, $P_{\text{heterogeneity}} = 0.013$ for the allele contrast model). However, in subgroup analyses based on ethnicity and tumor site, the **MMP3-1171 5A>6A** polymorphism was significantly associated with HNC risk in Europeans (OR, 0.59; 95% CI, 0.41–0.85; I^2, 0, $P_{\text{heterogeneity}} = 0.339$ for the recessive model and OR, 0.76; 95% CI, 0.61–0.94; I^2, 0, $P_{\text{heterogeneity}} = 0.6$ for the allele contrast model) and in pharyngeal/laryngeal cancers (OR, 0.45; 95% CI, 0.28–0.72; I^2, 0, $P_{\text{heterogeneity}} = 0.658$ for the recessive model and OR, 0.66; 95% CI, 0.49–0.88; I^2, 46.5%, $P_{\text{heterogeneity}} = 0.172$ for the allele contrast model), but the **MMP3-1171 5A>6A** polymorphism was not significantly associated with HNC risk in Asians, in oral cavity cancer and in nasopharyngeal cancer using three genetic models (Figure 3). In stratified analyses...
Table 1. Characteristics of 13 case-control studies included in this meta-analysis.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Year</th>
<th>Ethnicity</th>
<th>Tumor site</th>
<th>Source of control</th>
<th>Case</th>
<th>Control</th>
<th>Genotyping method</th>
<th>P_{HWE}</th>
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<tr>
<td>MMP2-1306 C&gt;T</td>
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<td>2006 Asian</td>
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<td>HB</td>
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<td>206</td>
<td>33</td>
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<tr>
<td></td>
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<td>Nasopharynx</td>
<td>PB²</td>
<td>570</td>
<td>520</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Zhou</td>
<td>2007 Asian</td>
<td>Nasopharynx</td>
<td>PB²</td>
<td>233</td>
<td>216</td>
<td>17</td>
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MMP3-1171 5A >6A

<table>
<thead>
<tr>
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<th>Year</th>
<th>Ethnicity</th>
<th>Tumor site</th>
<th>Source of control</th>
<th>Case</th>
<th>Control</th>
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<td>HB</td>
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<td>6</td>
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<td>HB</td>
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<td>36</td>
<td>84</td>
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<td>HB</td>
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<td>Nishizawa</td>
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<td>Asian</td>
<td>Oral cavity</td>
<td>PB</td>
<td>170</td>
<td>3</td>
<td>50</td>
<td>117</td>
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<tr>
<td>Tu</td>
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<td>Asian</td>
<td>Oral cavity</td>
<td>PB</td>
<td>150</td>
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<tr>
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<td>PB</td>
<td>231</td>
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MMP9-1562 C>T

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<th>Ethnicity</th>
<th>Tumor site</th>
<th>Source of control</th>
<th>Case</th>
<th>Control</th>
<th>Genotyping method</th>
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<td>Nasopharynx</td>
<td>PB</td>
<td>234</td>
<td>190</td>
<td>44</td>
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<tr>
<td>Vairaktaris</td>
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<td>European</td>
<td>Oral cavity</td>
<td>HB</td>
<td>152</td>
<td>84</td>
<td>68</td>
<td>0</td>
</tr>
</tbody>
</table>

¹HB: hospital-based, ²PB: population-based, ³HWE: Hardy-Weinberg equilibrium, ⁴PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism.
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MMP9-1562 C>T: We identified five studies that evaluated the association of the MMP9-1562 C>T polymorphism with the risk of HNC [28,35–37] with 1321 cases and 1280 controls. In the overall comparison, the MMP9-1562 C>T polymorphism was not significantly associated with HNC risk using three genetic models (OR, 1.87; 95% CI, 0.66–5.26; I² = 0, P_{heterogeneity} = 0.669 for the recessive model; OR, 1.06; 95% CI, 0.78–1.43; I², 60.7%, P_{heterogeneity} = 0.037 for the dominant model; and OR, 1.05; 95% CI, 0.89–1.25; I², 51.7%, P_{heterogeneity} = 0.082 for the allele contrast model; Figure 4). Similarly, in the subsequent analysis of HWE, studies, excluding the study by Vairaktaris and colleagues [35], did not reveal any significant associations between the MMP9-1562 C>T polymorphism and HNC risk (OR, 1.87; 95% CI, 0.66–5.26; I², 0, P_{heterogeneity} = 0.469 for the recessive model; OR, 0.93; 95% CI, 0.76–1.13; I², 0, P_{heterogeneity} = 0.527 for the dominant model; and OR, 0.96; 95% CI, 0.79–1.15; I², 0, P_{heterogeneity} = 0.465 for the allele contrast model). Furthermore, in subgroup analysis based on tumor site, no significant association was detected either in oral cavity cancer or in nasopharyngeal cancer.

Heterogeneity Analysis

In specific comparisons, the data from two of the three polymorphisms were hetergeneous. For the MMP2-1306 C>T polymorphism, no significant heterogeneity was found either in overall comparison (I² = 0, P_{heterogeneity} = 0.865 for the recessive model; I² = 0, P_{heterogeneity} = 0.97 for the dominant model; and I² = 0, P_{heterogeneity} = 0.963 for the allele contrast model) or in subgroup analyses using three genetic models (Table 2). For the MMP3-1171 5A>6A polymorphism, significant heterogeneity was observed in overall comparison using the recessive model (I² = 66.4%, P_{heterogeneity} = 0.004) and the allele contrast model (I² = 60.4%, P_{heterogeneity} = 0.013). However, heterogeneity was eliminated in the European population after stratifying by ethnicity (I² = 66.4%, P_{heterogeneity} = 0.004 for the recessive model and I² = 0, P_{heterogeneity} = 0.600 for the allele contrast model). Also, in subgroup analyses based on the source of control subjects, heterogeneity significantly decreased in the population-based subgroups (I² = 58.7%, P_{heterogeneity} = 0.064 for the recessive model and I² = 51.7%, P_{heterogeneity} = 0.102 for the allele contrast model). For the MMP9-1562 C>T significant heterogeneity was detected using the dominant model. However, the study by Vairaktaris and colleagues [35], in which genotypic distribution of the controls was not consistent with HWE, was excluded; heterogeneity was not detected, and the significance of pooled ORs using the dominant model was not influenced, thereby suggesting that this study was the major source of heterogeneity.

Sensitivity Analysis

Sensitivity analyses were performed to assess the influence of individual dataset on the pooled ORs by sequential removing each
eligible study. For MMP2-1306 C>T, the results demonstrated that the significance of pooled ORs was undetectable after excluding the studies [26,27] from a recessive model (data not shown). For MMP3-1171 5A>6A and MMP9-1562 C>T, the significance of the pooled ORs was not materially altered by exclusion of any individual study (Figure 5), thereby indicating that our results are statistically robust.

**Publication Bias**

For all the three polymorphisms, the shapes of the Begg’s funnel plots in all genetic models did not show any evidence of obvious asymmetry. Figure 6 shows the shape of the Begg’s funnel plots of MMP3-1171 5A>6A using allele contrast model. Moreover, Egger’s test did not reveal any significant evidence of publication bias of all the three polymorphisms (data not shown).

**Discussion**

In this meta-analysis, the MMP2-1306 C>T polymorphism was significantly associated with HNC risk both in overall comparison and in subgroup analyses based on the source of the controls and tumor sites. In contrast, no association was observed between either MMP3-1171 5A>6A or MMP9-1562 C>T polymorphism and HNC risk in overall comparison; however, in subgroup analyses based on ethnicity and tumor site, significant associations were found between the MMP3-1171 5A>6A polymorphism and HNC risk in Europeans and pharyngeal/laryngeal cancer under two genetic contrasts. Our findings indicate that MMP2-1306 C>T polymorphism might modulate risk of HNC, so does the MMP3-1171 5A>6A polymorphism in some subgroups.

The MMP2-1306 C>T polymorphism, which contains a C>T transition at the −1306 position upstream of the transcriptional site, can abolish Sp1-binding site and downregulate transcriptional activity. Previous studies have shown that MMP2 gene expression was significantly lower in individuals with the T allele than in individuals with the C allele [23]. Our meta-analysis indicates that individuals with variant genotypes (CT or TT genotype) are less susceptible to HNC than individuals with the wild genotype (CC genotype). However, our findings confirmed those of previous studies [8,23] which reported that MMP2 overexpression was associated with the development and aggressiveness of a variety of malignancies including HNC, as most patients in the studies included in our meta-analysis carried the C allele but not the T allele.

For example, O-Charoenrat and colleagues assessed the association of the MMP2-1306 C>T polymorphism and its expression level with the risk of HNC [27]. They found that the C and T allele frequencies were 93.1% and 6.9%, respectively, in patients, compared with 87.2% and 12.8%, respectively, in controls (P<0.05), and the CC genotype frequencies were significantly higher in patients than in controls (86.2% vs. 76%; P<0.05). Moreover, they also found that MMP2 expression in HNC cells containing the CC genotype was significantly higher than that in cells with the CT genotype. Similarly, in a study of the association of the MMP2-1306 C>T polymorphism with the risk of oral squamous cell carcinoma (OSCC) [26], Lin and colleagues reported that the CC genotype frequency was significantly higher in OSCC cases than in controls (P=0.04). However, because of the small samples and limited number of studies, our results should be interpreted with caution. Further studies with larger samples are needed to validate our findings.
For the MMP3-1171 5A>6A polymorphism, functional analysis in vitro showed that the 5A allele had approximately 2-fold higher promoter activity than the 6A allele. This finding implies that the 5A allele is responsible for increased MMP3 transcriptional levels and contributes to the carcinogenesis of most malignancies.

Several groups have evaluated the association between MMP3-1171 5A>6A polymorphism and the risk of HNC; however, the results of these studies remain inconsistent. Chaudhary and colleagues found that the 5A allele might play an important role in the susceptibility to HNC, as individuals with 5A/5A genotype had nearly two fold risk of HNC (OR = 1.94) when compared to controls [29]. However, Tu and colleagues found that the 5A/5A genotype was associated with the risk of oral submucous fibrosis but not OSCC [33]. Similarly, in studies by Nishizawa and Hashimoto, no significant association between the MMP3-1171 5A>6A polymorphism and HNC risk was found, which is consistent with the findings of this meta-analysis [31,32]. However, in our meta-analysis, the MMP3-1171 5A>6A polymorphism was significantly associated with risk of HNC in Europeans when the study population was stratified by ethnicity, thereby indicating that the discrepancies in the aforementioned results may be attributed to diverse genetic backgrounds and different environmental factors in different populations. Future studies with larger samples are warranted to further evaluate the role of the MMP3-1171 5A>6A polymorphism in HNC risk in different populations.

The MMP9-1562 C>T polymorphism, which is located at position 1562 bp upstream of the transcriptional start site and contains either C or T, has been shown to influence the transcriptional activity of the MMP9 gene. Zhang and colleagues performed transient transfection and DNA-protein interaction assays and found that T allele-associated promoter activity was higher than the C allele-associated promoter activity owing to the binding of a transcriptional repressor [25]. Although MMP9 plays an important role in head and neck carcinogenesis and MMP9 is frequently overexpressed in HNC, our meta-analysis indicated no significant association between the MMP9-1562 C>T polymorphism and HNC risk, suggesting that MMP9 expression might influence HNC progression via mechanisms other than regulation.
by the MMP9-1562 C>T polymorphism. Several other factors, such as interleukin-1, tumor necrosis factor α, and oncogenes, may also regulate MMP9 expression [21,39]. Further studies are needed to test these hypotheses.

Some heterogeneity factors between studies that could limit the strengths of the meta-analysis should be addressed. First, ethnicity was one of the most important factors that could lead to heterogeneity because of the diverse genetic backgrounds and environmental factors in different ethnicities. Second, tumor site was another reason for the heterogeneity between studies as HNC have quite different origins of organs, different histological subtypes, different etiology and different biological behavior. For example, tobacco use and alcohol consumption play important roles in oral cavity cancer, while viral infection is the major risk factor for oropharyngeal and nasopharyngeal cancer. Thus, different risk factors for different tumor sites may explain why the same polymorphism may play different roles in different subgroups of HNC. Furthermore, the source of the controls was another factor that could lead to heterogeneity. Population-based controls could be more reliable than hospital-based controls because the genotype distributions in hospital-based controls may be deviated from normal. Thus, population-based study design for individual subgroups of HNC is needed for future studies.

Since this is a pooled analysis, we thus have had relatively higher study power for the evaluation of such associations. In addition, we have performed stratified analysis by tumor sites in this meta-analysis, while our analysis by different tumor sites might minimize the issue of the confounding effect from mixed tumor sites. Although this analysis had such strengths, it also had some limitations. First, the number of eligible studies included in this meta-analysis was limited, and the sample size of each study was relatively small, especially in stratified analyses. For example, there were only two studies examined the association between the MMP3-1171 5A>6A polymorphism and HNC risk in Europeans. Although significant association was detected, the statistical power could have been limited. Second, if more detailed information about age, sex, alcohol consumption, tobacco smoking and/or HPV status had been available in the original studies, a more accurate OR would have been estimated after further stratification. Third, evaluating the association between MMP polymorphisms and HNC risk using linkage disequilibrium (LD) would have been more powerful. However, few studies performed haplotype analysis of these three MMPs. Additionally, publication bias may have occurred because we included only published studies in the meta-analysis, although it was not detected via a statistical test. Despite these limitations, however, the statistical power of the analysis could have been significantly increased as the cases and controls were pooled from different studies. Therefore, our results from this meta-analysis might be more reliable than those of individual studies.

In conclusion, this meta-analysis suggests that the MMP2-1306 C>T polymorphism is associated with the risk of HNC, as is

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**Figure 3. Forest plot for association between MMP3-1171 5A>6A and risk of head and neck cancer stratified by ethnicity under recessive model (6A/6A vs. 5A/5A+5A/6A).** A random effects model was used. The squares and horizontal lines represent the study-specific OR and 95% CI. The diamond corresponds to the summary OR and 95% CI. doi:10.1371/journal.pone.0062023.g003
Figure 4. Forest plot for association between MMP9-1562 C>T and risk of head and neck cancer under allele contrast (T-allele vs. C-allele). A fixed-effects model was used. The squares and horizontal lines represent the study-specific OR and 95% CI. The diamond corresponds to the summary OR and 95% CI.

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Figure 5. Sensitivity analysis of MMP3-1171 5A>6A via the deletion of one study at a time to reflect the influence of the individual dataset on the pooled ORs using dominant model.

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the MMP3-1171 5A>6A polymorphism specifically in some subgroups. However, the MMP9-1562 C>T polymorphism is not associated with HNC risk. Further studies with larger samples are warranted to further evaluate the association between MMP polymorphisms and HNC risk.

Supporting Information

Table S1 PRISMA 2009 Checklist.

Acknowledgments

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Author Contributions

Conceived and designed the experiments: CYZ, MHZ, HLZ. Performed the experiments: CYZ, MHZ, QZZ, ZHX, GN. Analyzed the data: CYZ, XCS, LJ, CL, GJL. Contributed reagents/materials/analysis tools: QZZ, ZHX, GN. Wrote the paper: CYZ, HLZ.

References