No association of TNF-α-308G/A polymorphisms with head and neck cancer risk
A PRISMA-compliant meta-analysis

Cheng Yuan, MMa, Xin-Hua Xu, MMb,*, Lu Xu, MMb, Yang Liu, MMb, Min Sun, MD, PhDc, Li-Hua Ni, MD, PhDb, Xiao-Long Wang, MD, PhDe, Zhuo Chen, MMb, Kun Zhang, MMb, Guang Zeng, PhDf

Abstract
Background: A number of studies had reported the association between tumor necrosis factor-alpha (TNF-α) gene polymorphisms and head and neck cancer (HNC) risk. However, the results remained controversial. Therefore, we performed a meta-analysis to derive a more precise evaluation of the association between TNF-α-308G/A polymorphism and overall HNC risk and evaluated influence of cancer types and ethnicities.

Methods: A systematic literature search was performed using Pubmed, Embase, Cochrane Library, and Web of science. In total, we identified 15 studies including 2005 cancer cases and 2876 controls to evaluate the association of TNF-α-308G/A polymorphism with risk for HNC.

Results: Overall, there was no significant association between TNF-α-308G/A polymorphism and the risk of HNC. Furthermore, subgroup analyses were performed according to the types of tumor and the ethnicities, we also found there was no significant association between TNF-α-308G/A polymorphism and the risk of NPC and OC, and European and Asian populations had no statistically significant difference in the relationship of TNF-α-308G/A polymorphism and HNC susceptibility.

Conclusion: This meta-analysis indicates that the TNF-α-308G/A polymorphism is not associated with HNC risk. In the future, large and well-designed case-control studies are needed to validate our findings.

Abbreviations: BCC = basal cell carcinoma of head and neck, CIs = confidence intervals, HNC = head and neck cancer, HWE = Hardy–Weinberg equilibrium, NOS = Newcastle-Ottawa scale, NPC = nasopharyngeal carcinoma, OC = oral cancer, OPSCC = oral and pharyngeal squamous cell carcinoma, OR = odds ratio, TC = thyroid carcinoma, TNF-α = tumor necrosis factor alpha.

Keywords: head and neck cancer, meta-analysis, polymorphisms, TNF-α

1. Introduction
Head and neck cancer (HNC) has ranked the sixth most frequent malignancy worldwide, which comprises a number of epithelial cancers originated from oral, nasal cavity, pharynx, and larynx.[1] The occurrence of HNC shows a decreasing trend and its mobility is still high in spite of receiving comprehensive treatment involving radiation, chemotherapy, and surgical treatment modalities. HNC contributes nearly 600,000 new cases diagnosed, and over 300,000 deaths each year.[2] Alongside traditional risk factors such as smoking and excessive alcohol consumption,[3,4] human papillomavirus type 16 (HPV16) infection has been recognized to cause a subset of these cancers.[5] However, its pathogenesis is not yet fully understood, more evidences demonstrated that HNC was a disease involving multiple genetic factors, and further studies confirmed that the genetic susceptibility of HNC was closely related to the mutation of several genes.[6]

Tumor necrosis factor alpha (TNF-α) is a kind of proinflammatory cytokines mainly secreted by tumor cells and macrophages. It is a cytokine ligand that is capable of interacting with different receptors in the tumor necrosis factor receptor family.[7,8] TNF-α can not only take part in the mechanisms of apoptosis induction, but also accelerate tumor growth in the process of tumor progression. And increasing evidence showed that TNF-α participated in many key processes of tumor progression including activation of cancer gene, DNA damage, and tumor metastasis.[9,10] High expression of TNF-α is closely related to tumor recurrence and lymph node metastasis.[11,12] But another study suggested there was no significant increase in the incidence of tumors exposed to TNF-α antagonists.[13] TNF-α has a wide range of biological activities including cell apoptosis, inflammation, cell proliferation, and differentiation, but the underlying mechanisms are not fully elucidated.[14] In 1992, Wilson[15] found the TNF-α gene was located on chromosome...
6p21.3 MHC III region, and single-gene polymorphism was present from G to A in the promoter region of 308. TNF-α, especially the polymorphism of 308G/A, is thought to be related to the genetic susceptibility of many tumors, such as esophageal cancer,[16] osteosarcoma,[17,18] and lung cancer.[19] Recently, a variety of molecular epidemiological studies have been conducted to examine the association between TNF-α -308G/A polymorphism and HNC susceptibility, but the results remain inconclusive. Therefore, the association between TNF-α -308G/A polymorphism and HNC risk requires further investigation.

Considering the relatively small sample size in most studies and even controversial results in some studies, it is possible to perform a quantitative synthesis of the evidence with rigorous methods. Here, we performed a meta-analysis on 15 published case-control studies to derive a more precise evaluation of the association between TNF-α -308G/A polymorphism and overall HNC risk and evaluated influence of cancer types and ethnicities.

2. Materials and methods

2.1. Publication search

Systematic literature searches were conducted in the following electronic databases: Pubmed, Embase, Cochrane Library, and Web of Science, covering all articles published up to September 2016. We used the following terms: “tumor necrosis factor alpha,” “TNF-α,” “-308G/A,” “-308G>A,” “polymorphism,” “oral cancer,” “pharyngeal cancer,” “laryngeal cancer,” “nasopharyngeal carcinoma,” “head and neck cancer.” References of the retrieved publications were also screened. All eligible studies were retrieved, and their bibliographies were checked for other relevant publications. Only published studies with full-text articles were included. When overlapping articles were found, we only included the publications that reported the most extensive information.

2.2. Inclusion and exclusion criteria

The inclusion criteria were as follows: published in English; case-control studies of HNC with TNF-α -308G/A polymorphism; supply the available genotype frequencies in cancer cases and controls; sufficient published data for estimating an odds ratio (OR) with 95% confidence interval (CI).

The major reasons for exclusion of studies were reviews and letters; studies without detailed genotype frequencies; do not conform to Hardy–Weinberg equilibrium (HWE).

2.3. Data extraction

Two investigators independently (CY and ZC) reviewed the articles to exclude irrelevant and overlapping studies. The results were compared, and disagreements were resolved by discussion and consensus. We extracted the following information from each study: first author’s surname, publication year, ethnicity, tumor type, source of controls, and the number of cases and controls for each genotype. Different ethnicities were categorized as Asian and European. Cancer types were classified as nasopharyngeal carcinoma (NPC), oral cancer (OC), thyroid carcinoma (TC), basal cell carcinoma of head and neck (BCC), and oral and pharyngeal squamous cell carcinoma (OPSCC). HWE were calculated by $\chi^2$ test ($P<.01$ was considered significant disequilibrium) based on the 2 polymorphisms genotyping distribution in controls.

2.4. Evaluation of study quality

The methodological quality evaluation of the studies was conducted using the Newcastle–Ottawa Scale (NOS) for cohort study. The assessments were processed independently by 2 reviewers and the final decision was achieved by consensus.

2.5. Statistical analysis

Odds ratio (OR) with 95% confidence intervals (CIs) were used to assess the strength of association between TNF-α -308G/A polymorphism and risk of HNC, based on the genotype frequencies in cases and controls. The pooled OR were calculated for 5 models respectively: allelic model (A vs. G), homozygous model (AA vs. GG), dominant model (GA+AA vs. GG), and recessive model (AA vs. GA+GG). The between-study heterogeneity of studies was performed though $\chi^2$-based Q statistic test. If heterogeneity was considered to be not significant ($P>.1$), the fixed effects model was used; otherwise, the random effects model based on the Mantel–Haenszel method was applied. Subgroup analysis was conducted among variables, such as cancer types and ethnicities. Sensitivity analysis was conducted by removing 1 data set at a time to identify individual study’ effect on pooled results and test the reliability of results. Funnel plots were used to access the potential publication bias by the method of Egger linear regression test. All analyses were performed by Stata (version 12.0, Stata Corporation) and Review Manager (version 5.3, The Cochrane collaboration), using 2 side $P$ values.

3. Results

3.1. Characteristics of studies

Fifteen case-control studies[20–34] including 2005 cancer cases and 2876 controls met the including criteria. The characteristics of these studies and the evaluation results of each item with potential bias are listed in Table 1. There were 5 studies of NPC, 8 studies of OC, 1 study of TC, 1 study of BCC, and 1 study of OPSCC. In the subgroup of ethnicity, 10 were carried out in Asian population and 6 were in European population. The distribution of genotypes in the controls conformed to HWE.

3.2. Main results

The evaluation of association between TNF-α -308G/A polymorphism and HNC risk is presented in Table 2. Overall, there was no significant association between TNF-α -308G/A polymorphism and the risk of HNC (A vs. G; OR =1.18, 95% CI: 0.77–1.82, $P=.45$; AA vs. GG; OR =1.94, 95% CI: 0.70–5.42, $P=.20$; GA vs. GG; OR =1.13, 95% CI: 0.79–1.60, $P=.50$; AA vs. GG; OR =1.19, 95% CI: 0.77–1.83, $P=.44$; AA vs. GA+GG; OR =1.77, 95% CI: 0.70–4.49, $P=.23$). Furthermore, subgroup analyses were performed according to the types of tumor and the ethnicities, and we found there was no significant association between TNF-α -308G/A polymorphism and the risk of NPC and OC. At the same time, we failed to find significant main effects for TNF-α -308G/A polymorphism on HNC risk in different genetic models when stratified according to ethnicity (Figs. 1 and 2).
### 3.3. Evaluation of Heterogeneity

There was significant heterogeneity in all gene models: allelic model (A vs. G: $I^2=91\%$, $P_h<.01$); homozygous model (AA vs. GG: $I^2=81\%$, $P_h<.01$); heterozygous model (GA vs. GG: $I^2=78\%$, $P_h<.01$); dominant genetic model (GA+AA vs. GG: $I^2=87\%$, $P_h<.01$); recessive model (AA vs. GA+GG: $I^2=81\%$, $P_h<.01$). Then, we assessed the source of heterogeneity for homozygote comparison by cancer type and ethnicity. As a result, cancer type and ethnicity were not found to contribute to substantial heterogeneity.

### 3.4. Sensitivity analysis

Sensitivity analysis was performed by sequential omission of individual studies in whole subjects and subgroups, respectively. In whole subjects, the study of Vairaktaris was the main originator of heterogeneity in Asian, and the study of Gupta was the main originator of heterogeneity in the NPC. When the study was excluded, heterogeneity was also decreased in OC (AA vs. GG: $I^2=46\%$, $P_h=.12$). Additionally, in the ethnicity subgroup analysis, sensitivity analysis suggested that the study of Gupta was the main originator of heterogeneity in Asian, and the study of Vairaktaris was the main originator of heterogeneity in European. After the exclusion of these studies, heterogeneity was significantly decreased (A vs. G: $I^2=41\%$, $P_h=.10$; AA vs. GA+GG: $I^2=0\%$, $P_h=.77$, respectively).

### 3.5. Publication bias

Funnel plots (Fig. 3) showed arrangement of data points did not reveal any evidence of obvious asymmetry. Formal evaluation using Egger regression asymmetry tests and the result still did not show any evidence of publication bias ($t=0.27$, $P=.793$).

## 4. Conclusion

We analyzed 15 case control studies to investigate the relationship between the polymorphism of TNF-α-308GA gene and the susceptibility of HNC. The results showed that there was no significant relationship between the TNF-α-308GA gene...
polymorphism and HNC susceptibility. In addition, the results also found no significant correlation through the subgroup analysis of the types of tumor and the ethnics.

The risk factors for HNC include genetic inheritance, viral infections, and environmental factors. Among these factors, the EBV has been recognized as a critical risk factor for NPC. In the process of carcinogenesis, several EBV latent proteins are highly expressed and then infected cells start to escape the cellular immune system and become resistant to the cytotoxic effect of TNF-α.

In addition, TNF-α is a proinflammatory cytokine, participating in the regulation of B cell differentiation and leading to cell death, nucleus fragmentation, and the proliferation and maturation of B lymphocytes. The above process is highly correlated with the pathogenesis and development of HNC. Even so, the results in several studies about the relationship between TNF-α-308G/A polymorphism and HNC risk remain controversial. The study of Yapijakis et al.[35] indicates a strong association of TNF-α high expression alleles with increased risk of OC. But another research[34] suggests that there is no association of TNF-α genotypes with clinical pathology or the survival of OPSCC patients. Thus, our meta-analysis from 15 papers is performed to precisely assess the possible association of TNF-α-308G/A polymorphisms with the susceptibility to develop HNC. A previous meta-analysis[36] has reported the relationship between NPC and TNF-α-308G/A polymorphisms; however, it cannot reflect all kinds of HNC.

In addition, this study has the following limitations: the sample of the research objects included in the study was not large enough, especially some special tumors (such as TC, BBC, and OPSCC); only English-language studies that were included in this meta-analysis might have led to publication bias, and the exclusion of unpublished data was generally associated with an overestimation of the true effect; the interaction between gene and gene, gene and environment, even the interaction between different polymorphic loci of the same gene can regulate cancer risk.

Our study suggested there is no significant relationship between TNF-α-308G/A gene polymorphism and HNC susceptibility, and there is also no significant relationship between tumor types and ethnics. Therefore, a further study with larger samples including different tumor types and ethnics is warranted, especially those researches involving interaction between gene—gene and gene—environment should be considered, which could contribute to fully understand the association of the TNF-α-308G/A polymorphism and HNC.

4.1. Ethical review

Ethical approval was not necessary, because this article is a meta-analysis and it does not involve the participation of ethics committee.
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