RESEARCH ARTICLE

Tumor Necrosis Factor-α Gene Polymorphisms and Risk of Oral Cancer: Evidence from a Meta-analysis

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Abstract

Numerous studies have been conducted regarding association between TNF-α and oral cancer risk, but the results remain controversial. The present meta-analysis is performed to acquire a more precise estimation of relationships. Databases of Pubmed, the Cochrane library and the China National Knowledge Internet (CNKI) were retrieved until August 10, 2013. Pooled odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated with fixed- or random-effect models. The heterogeneity assumption was assessed by I-squared test. Among the eight included case-control studies, all were focused on TNF-α-308G>A and four also concerned the TNF-α-238G>A polymorphism. It was found that oral cancer risk were significant decreased with the TNF-α-308G>A polymorphism in the additive genetic model (GG vs. AA, OR=0.19, 95% CI: [0.04, 1.00], *P*=0.05, I^2=68.9%) and the dominant genetic model (GG+GA vs. AA, OR=0.22, 95% CI: [0.06, 0.82], *P*=0.03, I^2=52.4%); however, no significant association was observed in allele contrast (G vs. A, OR=0.70, 95% CI: [0.23, 2.16], *P*=0.54, I^2=95.9%) and recessive genetic models (GG vs. GA+AA, OR=0.72, 95% CI: [0.33, 1.57], *P*=0.41, I^2=93.1%). For the TNF-α-238G>A polymorphism, significant associations with oral cancer risk were found in the allele contrast (G vs. A, OR=2.75, 95% CI: [1.25, 6.04], *P*=0.01, I^2=0.0%) and recessive genetic models (GG vs. GA+AA, OR=2.23, 95%CI: [1.18, 4.23], *P*=0.01, I^2=0.0%). Conclusively, this meta-analysis indicates that TNF-α polymorphisms may contribute to the risk of oral cancer. Allele G and the GG+GA genotype of TNF-α-308G>A may decrease the risk of oral cancer, while allele G and the GG genotype of TNF-α-238G>A may cause an increase.

Keywords: Tumor necrosis factor-α - oral cancer - meta-analysis - polymorphism

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Introduction

Oral cancer is one of the ten most common cancers in the world and occurs more often to males in developing countries than developed countries (Petersen, 2005; Petersen, 2009; Jemal et al., 2010; Jemal et al., 2011). According to the GLOBOCAN 2008 estimates, the incidence of oral cancer for males was listed at ninth worldwide. An estimated 263,900 new cases and 128,000 deaths from oral cavity cancer (including lip cancer) occurred in 2008 worldwide (Jemal et al., 2010). Generally, it is accepted that cigarette smoking, alcohol consumption, betel quid chewing and HPV infections are the possible factors increasing risks of developing oral carcinoma (Silverman, 2001; Petersen, 2009; Jemal et al., 2010). Although many individuals are exposed to these risk factors, oral cancer develops only in a small group of exposed people, implying that genetic factors may contribute to the carcinogenic mechanisms, and complex interactions between many genetic and environmental factors may be the major cause of oral cancer (Tsigris et al., 2007; Zhuo et al., 2009; Liu et al., 2012a; Miriam et al., 2012).

In recent years, genetic association studies have estimated the risk of developing a certain malignancy by comparing the frequency of polymorphic genotypes and allele frequencies in cancer patients and health controls. An allele or a genotype is associated with increased risk for neoplasia when its detected frequency is significantly higher in cases than controls (Tsigris et al., 2007; Serefoglou et al., 2008). Until now, several meta-analyses have demonstrated that the Arg194Trp polymorphism in x-ray repair cross-complementing group 1 (XRCC1) gene (Zhou et al., 2009), CYP2E1 RsaI/PstI gene polymorphism (Lu et al., 2011), GSTM1 gene polymorphism (Shukla et al., 2012), and TP53 codon 72 polymorphisms (Zhuo et al., 2009) are related to the susceptibility of oral cancer.

TNF-α gene is located on chromosome 6p21.231 in the polymorphic region of MHC III, and its promoter polymorphisms have been intensively studied as a potential determinant of disease susceptibility (Kaluza et al., 2000; Gupta et al., 2008). There is also an increasing

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evidence that TNF-α may promote the development and spread of cancer (Liu et al., 2005; Hohberger et al., 2008; Liu et al., 2012b). Commonly described variants of TNF-α gene polymorphisms consist of G to A transitions in the promoter region at positions _238 and _308 (Kaluza et al., 2000). So far, TNF-α promoter polymorphism has been related to numerous cancers, such as bladder cancer (Marsh et al., 2003), renal cell carcinoma (Nakajima et al., 2001), non-small cell lung carcinoma (Shih et al., 2006) cervical cancer (Govan et al., 2006; Liu et al., 2012b) and breast carcinoma (Mestiri et al., 2001).

Several studies have been conducted to try to confirm the association between TNF-α and the susceptibility of oral cancer (Chiu et al., 2001; Chen et al., 2005; Liu et al., 2005; Gupta et al., 2008; Yapijakis et al., 2009; Kietthubthew et al., 2010; Yang et al., 2011; Jin et al., 2013), but these have produced controversial or inconclusive results. We therefore performed a meta-analysis of all eligible studies to derive a more precise estimation of the association between TNF-α gene polymorphisms and oral cancer.

Materials and Methods

Search Strategy

We carried out a search in Pubmed, Cochrane library, and China National Knowledge Internet (CNKI) databases for studies published up to August 10, 2013 with a combination of the following keywords: "tumor necrosis factor" or "TNF", and "gene" or "genetic" or "polymorphism" or "genotype" or "variant", and "oral cancer" or "oral carcinoma" or "head and neck cancer" or "mouth neoplasm" or "oral squamous cell carcinoma" or "oral leukoplakia" or "oral submucous fibrosis". References from recent review articles were also checked for relevant articles. If there were duplicated publications from the same study, the most complete or most recent publication was given precedence. We evaluated potentially associated publications by checking their titles and abstracts and then procured the most relevant publications for a closer examination.

Inclusion and Exclusion Criteria

Two reviewers (Chen F, Zhang Z) screened all the retrieved literatures and citations by title/abstract and then full texts. Inclusion criteria were: (1) case-control studies concerning the association of TNF-α polymorphism with oral carcinoma; (2) studies offering the size of the samples, source of cases and controls, distribution of alleles, genotypes, or other information that could help us infer the results; (3) genetic distribution of the pooled control group was consistent with Hardy-Weinberg equilibrium (HWE). Exclusion criteria were: (1) duplication studies; (2) family-based study design with linkage considerations; (3) concerned with oral lichen planus, not carcinoma; (4) a number of cases and controls and other essential information were not offered; (5) publication types as news, letter, editorial, comment, review, conference summary or bibliography; (6) publication language was not in English or Chinese.

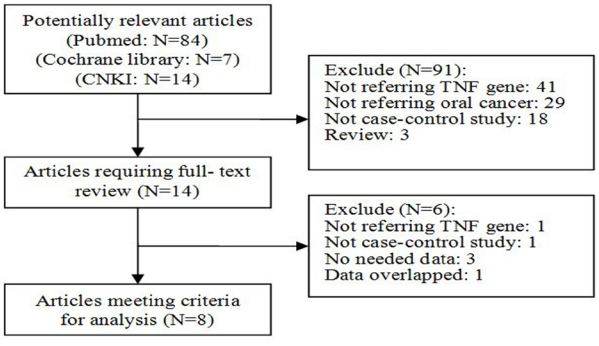

Figure 1. Flow Chart of Study Selection. Flow chart shows literature search for relevant studies about the association between TNF-α gene and risk of oral cancer

Data Extraction

The extraction was performed by two reviewers (Zhang Z, Chen F) independently. The following data were collected: the first author's name, publication year, ethnicity of subjects, source of case or control group(population-based or hospital-based), environmental status, studied polymorphisms, deviation from Hardy-Weinberg equilibrium (HWE) in controls, and distribution of alleles and genotypes in case and control groups. If a consensus could not be reached, a third party was consulted to resolve the dispute. The extracted information was entered into a database.

Statistical Analysis

The association between TNF-α polymorphisms and oral cancer was estimated by calculating summary odds ratios (ORs) with 95% confidence intervals (CIs). The pooled ORs were calculated for the allele contrasts, additive genetic model, dominant genetic model, and recessive genetic model, respectively. The heterogeneity assumption was assessed by an I-squared test. The heterogeneity was not considered as significant when I^2<50%. When significant heterogeneity was not found, the pooled OR estimated of each study was calculated by the fixed effects model using the Mantel-Haenszel method (Overton, 1998). Otherwise, the random effects model was used. Sensitivity analyses were performed by sequential removal of individual studies. Begg's and Egger's linear regression tests were used to assess the possibility of publication bias.

The software STATA was used for analysis. We calculate HWE through the online resource (http://www.oege.org/software/hwe-mr-calc.shtml). A p value less than 0.05 for any test or model was considered to be statistically significant.

Results

Eligible Studies

One hundred and five citations were searched for retrieval. After title and abstract screening, 91 citations were excluded. The remaining fourteen publications were subjected to further examination. Finally, after the full text screening, six studies were excluded by followed reasons: one study was not referred gene TNF-α (Hung et al., 2012), one study was not case-control designed

Table 1. Characteristics of Studies Included in the Meta-analysis

First author	Year	Country	Ethnicity	Number of cases (male/female)	Number of controls (male/female)	Source of cases	Source of controls	Matched factors
Chiu CJ	2001	Taiwan (China)	Asian	60(60/0)	284(284/0)	OC (areca-chewers)	Hospital-based and Population-based	Age, ethnicity, areca-chewing
Liu CJ	2005	Taiwan(China)	Asian	192(173/19)	146(130/16)	OSCC	Hospital-based	Age and gender
Chen WC	2005	Taiwan(China)	Asian	137(125/12)	102(60/42)	OC (BQ chewers and (or) smokers)	Population-based	NA
Gupta R	2007	India	Asian	94(76/18)	133(NA)	OSCC (at least six months' tobacco use)	Population-based	Age, gender and ethnicity
Yapijakis C	2009	Greece, Germany	Caucasian	160(128/32)	153(115/38)	OSCC	Hospital-based	Age, gender and ethnicity
Kietthubthew S	2010	Thailand	Asian	107(79/28)	157(87/70)	OSCC	Population-based	Gender, 5-year age group, cigarette smoking and alcohol drinking
Yang CM	2011	Taiwan(China)	Asian	205(205/0)	198(198/0)	OPSCC (BQ-related, most BQ chewers had a habit of cigarette smoking)	Hospital-based	Age, ethnicity of Band years Q chewing
Jin L	2013	USA	Caucasian	325(241/84)	335(269/66)	OSCC	Hospital-based	Age, sex, and smoking and drinking status

Table 2. Distribution of TNF-α Genotype among Oral Cancer Cases and Controls Included in the Meta-analysis

SNP	First author	Cases					Controls					HWE	X^2
		GG	GA	AA	G	A	GG	GA	AA	G	A		
TNF-α-308	Chiu CJ	47	12	1	106	14	232	50	2	514	54	0.69	0.15
	Liu CJ	175	16	1	366	18	120	24	2	264	28	0.53	0.39
	Chen WC	125	12	0	262	12	88	14	0	190	14	0.46	0.55
	Gupta R	61	23	10	145	43	114	19	0	247	19	0.37	0.79
	Yapijakis C	36	49	75	121	199	121	19	13	261	45	0	39.02
	Kietthubthew S	83	14		NA	NA	133	19		NA	NA	NA	
	Yang CM	180	23	2	383	27	155	43	0	353	43	0.09	2.94
	Jin L	101	224		NA	NA	138	207		NA	NA	NA	
TNF-α-238	Liu CJ	188	4	0	380	4	136	10	0	282	10	0.67	0.18
	Gupta R	94	0	0	188	0	133	0	0	266	0	NA	NA
	Kietthubthew S	92	5		NA	NA	141	11		NA	NA	NA	
	Yang CM	200	5	0	405	5	187	11	0	385	11	0.69	0.16

Table 3. Subgroup Analyses about the Association Between TNF-α -308G>A Polymorphism and Risk of Oral Cancer

Subgroup Genetic model	OR	95%CI	*P* value	I^2	Effect model
Asian					
G vs. A	1.04	[0.47, 2.28]	0.93	88.00%	Random
GG vs. AA	0.32	[0.05, 2.22]	0.25	52.20%	Random
GG+GA vs. AA	0.33	[0.05, 2.03]	0.23	47.30%	Fixed
GG vs. GA+AA	1.08	[0.58, 2.02]	0.18	81.10%	Random
Hospital-based					
G vs. A	0.73	[0.09, 5.81]	0.76	98.10%	Random
GG vs. AA	0.28	[0.02, 3.96]	0.34	80.90%	Random
GG+GA vs. AA	0.32	[0.04, 2.53]	0.28	69.20%	Random
GG vs. GA+AA	0.69	[0.18, 2.67]	0.59	96.70%	Random
Population-based					
G vs. A	0.63	[0.11, 3.78]	0.62	92.50%	Random
GG vs. AA	0.03	[0.001, 0.44]	0.01	NA	Random
GG+GA vs. AA	0.03	[0.002, 0.52]	0.02	NA	Random
GG vs. GA+AA	0.74	[0.28, 1.96]	0.54	81.30%	Random
OSCC					
G vs. A	0.38	[0.07, 2.18]	0.28	97.00%	Random
GG vs. AA	0.15	[0.01, 1.95]	0.15	80.70%	Random
GG+GA vs. AA	0.2	[0.02, 1.71]	0.14	73.00%	Random
GG vs. GA+AA	0.49	[0.17, 1.41]	0.19	94.60%	Random

(Li et al., 2001), three studies were not supplied the data we needed (Matthias et al., 1998; de Lius et al., 2007; Wenghoefer et al., 2009), and one study was using the overlapped data (Vairaktaris et al., 2008). Among the included eight case-control studies (Chiu et al., 2001; Chen et al., 2005; Liu et al., 2005; Gupta et al., 2008; Yapijakis et al., 2009; Kietthubthew et al., 2010; Yang et al., 2011; Jin et al., 2013), all of which were concerned with the association between SNP TNF-α-308G>A and the risk of oral cancer, and four studies were in regard to the SNP TNF-α-238G>A (Liu et al., 2005; Gupta et al., 2008; Kietthubthew et al., 2010; Yang et al., 2011). The whole process is shown in Figure 1.

Meta-analysis Database

A database was established according to the extracted information from each article. All the included eight studies were published in English. One study (Yapijakis et al., 2009) was conducted in Europe, one study was in USA (Jin et al., 2013), and the remaining six studies were in Asia. Of which, four studies were conducted in Taiwan of China (Chiu et al., 2001; Chen et al., 2005; Liu et al., 2005; Yang et al., 2011), one study was conducted in India (Gupta et al., 2008;), and another was in Thailand (Kietthubthew et al., 2010). The subjects investigated were most males or all males. The relevant information is listed in Table 1. Distribution of TNF genotype for cases and controls included were listed in Table 2. HWE of each study were calculated and it was found that the distribution of genotypes in the controls was consistent with HWE except for the study conducted by Yapijakis et al. (2009) for TNF-α-308G>A and the study conducted by Gupta et al. (2008) for TNF-α-238G>A.

Meta-analysis about TNF-α-308G>A polymorphism

TNF-α-308G>A polymorphism was significantly

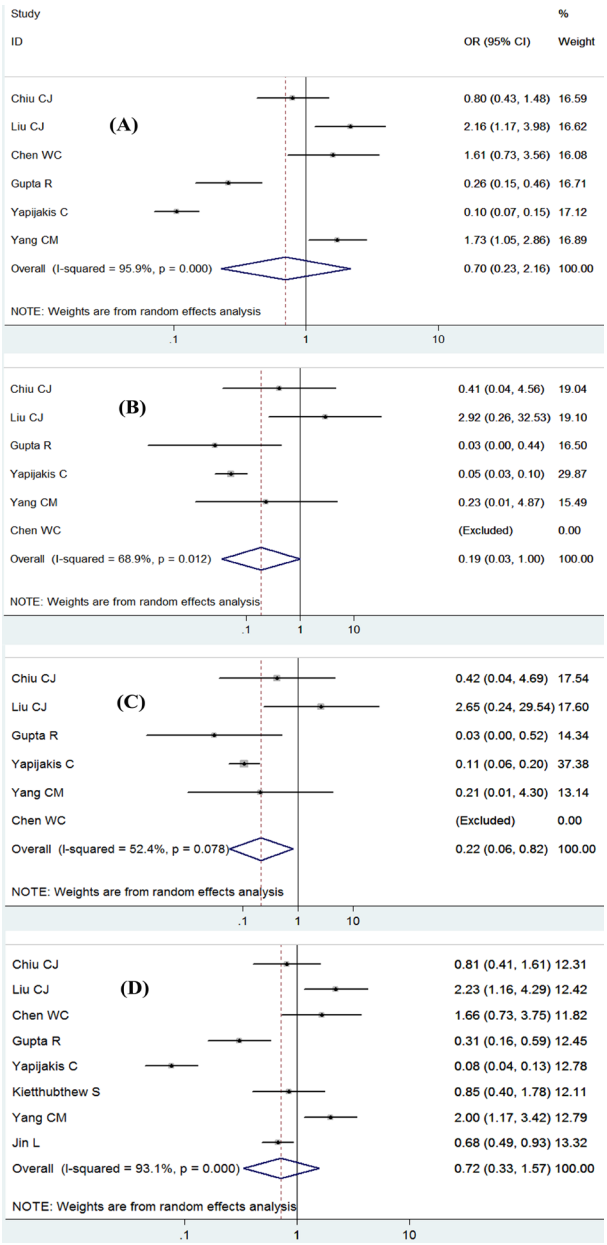

Figure 2. Forest Plots on Association Between TNF-α-308G>A Polymorphism and Oral Cancer Risk under Four Genetic Models. Results of individual and summary odds ratio (OR) estimates, 95% confidence intervals (CI) and weight of each study are shown. Horizontal lines represent 95%CI and dotted vertical lines represent the value of the summary OR. (A) Allele contrasts (G vs. A). (B) Additive genetic model (GG vs. AA). (C) Dominant genetic model (GG+GA vs. AA). (D) Recessive genetic model (GG vs. GA+AA)

associated with the risk of oral cancer in additive genetic model (GG vs. AA, OR=0.19, 95% CI: [0.04, 1.00], P = 0.05, I^2=68.9%) and dominant genetic model (GG+GA vs. AA, OR=0.22, 95% CI: [0.06, 0.82], P =0.03, I^2=52.4%); however, no significant associations with risk of oral cancer were found in allele contrast (G vs. A, OR=0.70, 95% CI: [0.23, 2.16], P=0.54, I^2=95.9%) and recessive genetic model (GG vs. GA+AA, OR=0.72, 95% CI =0.33-1.57, P=0.41, I^2=93.1%). All of the calculations were conducted under random-effect model. The details were shown in the Figure 2.

Subgroup analyses were conducted according to ethnicity, source of control, and type of oral cancer.

Table 4. The Publication Bias for the Genetic Model of TNF-α Polymorphisms

SNP	Begg's test		Egger's test	
Genetic model	Z	*P*	t	*P*
TNF-α-308G>A				
Allele contrast (G vs. A)	0.38	0.707	1.99	0.118
Additive genetic model (GG vs. AA)	-0.24	1	1.42	0.25
Recessive genetic model (GG vs. GA+AA)	0.62	0.536	0.34	0.744
Dominant genetic model (GG+GA vs. AA)	-0.24	1	0.94	0.418
TNF-α-238G>A				
Allele contrast (G vs. A)	0	1	NA	NA
Recessive genetic model (GG vs. GA+AA)	0	1	1.07	0.479

The details were shown in the Table 3. In brief, in the subgroups of Asian population, hospital-based case-control studies, and the studies concerned to OSCC patients, no significances were found in four genetic models. However, in the population-based case-control studies, it was found that TNF-α-308G>A polymorphism was significantly associated with the risk of oral cancer in additive genetic model and dominant genetic model.

We performed sensitivity analysis by sequential removal of individual studies. We found that the results would be changed in the following situations: (1) In the additive genetic model, the I^2 was decreased to 18.6% when the study by Liu CJ et al. (2005) was excluded, however, the pooled OR and significance were not materially changed under fixed-effect model (OR=0.10, 95%CI:[0.06, 0.19], P=0.000); however, after the sequential removal of other individual studies, the pooled ORs were between 0.15 and 0.32, and no statistical significance was found. (2) In the dominant genetic model, the I^2 was decreased to 0.0% when the study by Liu CJ et al. (2005) was excluded, however, the pooled OR and significance were not materially changed under fixed-effect model (OR=0.10, 95%CI:[0.06, 0.19], P=0.000); however, after the sequential removal of other individual studies, the pooled ORs were between 0.22 and 0.32, and were not statistically significant.

Except for the above situations, the results were not materially altered by leaving out studies, though sometimes the I-square value for the heterogeneity was reduced or increased.

Meta-analysis about TNF-α-238G>A polymorphism

For SNP TNF-α-238G>A, significant associations with oral cancer risk were found in the allele contrast (G vs. A, OR=2.75, 95% CI: [1.25, 6.04], P = 0.01, I^2=0.0%) and recessive genetic model (GG vs. GA+AA, OR=2.23, 95%CI: [1.18, 4.23], P=0.01, I^2=0.0%). No heterogeneity was found between studies and the analyses were both conducted under fix-effect model. Additive and recessive genetic model were not presented because there was no distribution of genotype AA in the three available studies (Liu et al., 2005; Gupta et al., 2008; Yang et al., 2011). Subgroup and sensitivity analyses were not carried out due to the small number of studies.

Publication Bias

Begg's test or Egger's test, which was designed to indicate publication bias, proved to be no obvious publication bias for each type of genetic model for TNF-α-238G>A and TNF-α-308G>A (Table 4) .

Discussion

Here, we investigated the relationship between TNF-α promoter polymorphisms and oral cancer susceptibility. This study displayed a comprehensive and systematic evaluation of the risk of oral cancer related to possession of the alleles and genotypes in both the -238G>A and -308G>A polymorphisms of the TNF-α gene. To the best of our knowledge, this is the first meta-analysis of case-control study dealing with such a topic. The overall results of this meta-analysis suggested that TNF-α promoter polymorphisms are associated with the risk of oral cancer, moreover, the G allele and GG/GA variants in the locus of TNF-α-308G>A gene promoter caused an decreased susceptibility to oral cancer, however, the allele G and GG genotype of TNF-α-238G>A may increase the risk.

TNF-α is a potent proinflammatory and immunoregulatory cytokine and plays crucial roles in the initiation, up-regulation of the inflammatory response. It is secreted by macrophages infiltrating tissue in response to injury or infection (Dinarello and Wolff, 1993; Tracey and Cerami, 1993). It was initially identified as a serum factor inducing necrosis of transplanted tumors in mice (Carswell et al., 1975). Experiments tend to support that TNF-a may promote the development and metastasis of cancers (Leibovich et al., 1987).

Association of TNF-α-308G>A with different cancer has been inconclusive and controversial. Several studies reported that TNF-α-308G>A have been associated with greater risk of cervical (Liu et al. 2012b), hepatocellular (Cheng et al., 2013), gastric (Machado et al., 2003) and breast carcinomas, however, no association was reported in the case of prostate carcinoma (Wu et al., 2004). The studies conducted by Chiu et al. (2001) and Liu et al. (2005) both suggested that the 308G allele conferred a protective effect against oral cancer, possibly by increasing TNF-α production. But in the study by Chen et al. (2005), there were also no significant differences in the genotype distributions and allelic frequencies. The combined data revealed that the TNF-α-308G>A polymorphism can be serve as a candidate marker for screening the causes of oral cancer and the allele G and GG/GA variants may had protective effect on oral cancer.

In the meta-analysis about the association between TNF-α polymorphisms and cervical cancer risk (Liu et al. 2012b) suggested that the TNF-α-238G genotype caused a increased susceptibility to cervical cancer. Kaluza et al (2000) reported that the TNF-α-238 G allele caused a significant increase in the transcription of the TNF-α gene in human T and B cells. Thus, the effect of the TNF-α-238G allele against cancer may be conferred by an increase in TNF-α production. Therefore, our results on the SNP may be biologically relevant.

There are some reasonable explanations for above controversial results. As oral cancer is a multifactor disease, overproduction of TNF-α is an important, but not an absolute factor in the pathogenesis of the disease (Farkas et al., 2007). In complex biologic systems, the effect of a single gene polymorphism in determining cytokine secretion may be affected by factors such as genetic and epigenetic determinants or may be minimized through interaction with environmental factors (Powell et al., 2001; Malleo et al., 2007). Most of all, both SNPs of the TNF-α gene may not be specific to oral cancer although they are associated with many inflammatory diseases (Lu et al., 2008; Pavy et al., 2010; Lee et al., 2012;).

And in the quantitative synthesis for TNF-α-308G>A, between-studies of heterogeneity may result in insufficient effect. Assessment of heterogeneity is necessary for most meta-analyses (Zintzaras et al., 2005; Moonesinghe et al., 2007). Meta-analyses might miss true effects in the presence of even modest between-study heterogeneity, because they are based on the assumption of etiologic homogeneity across studies (Moonesinghe et al., 2008). Moreover, we have limited knowledge about how much heterogeneity are resulted from errors and biases, or, on the other hand, what proportion of the heterogeneity represents a true difference in genetic effects across different populations (Zeggini et al., 2009). An inherent limitation of meta-analysis methodology is the possibility of heterogeneity of results due to dissimilarities between the individual studies. In the current study, the variability in the patient population, collection methodology, detection technique, diagnostic criteria, control selection, and anatomical location coding may have resulted in heterogeneity. In our eligible eight studies, the resources of cases such as anatomical location, pathological grading, treatment history, family history and tobacco/alcohol/betel quid use were not completely consistent or not be mentioned. And the resources of controls (hospital-based or population-based) were also inconsistent and the matching factors in the controls in the study by Chen et al. (2005) were not mentioned. These can result in heterogeneity. Further studies need to focus on exploring the sources of heterogeneity.

It is, however, important to consider the limitations of the current meta-analysis when interpreting these results. First, four studies among the eight eligible studies were conducted in Taiwan, one in India, another in Thailand. For Caucasians, one study conducted in the Europe and another one conducted in USA. A number of further studies are necessary to be carried out among other populations such as Europeans and Africans. Thus, a possible selection bias may exist. Second, the controls were hospital-based patients with other diseases but not cancer or population-based normal individuals, so the hospital-based studies have some biases because such controls may just represent a sample of ill-defined reference population, and may not be representative of the general population, particularly when the genotypes under investigation were associated with the disease conditions that the hospital-based controls may have. Therefore, using a proper and representative population-based control subjects is very important to reduce biases in such genetic association studies (Lu et al., 2011). Third, we did not carry out subgroup and sensitivity analyses such as ethnicity and environmental factors due to the small number for the SNP TNF-α-238G>A, which might lead to precluding the drawing of true conclusions.

In conclusion, our meta-analysis suggests that based on the published data, TNF-α polymorphism may contribute to the risk of oral cancer. Allele G and GG+GA genotype of TNF-α-308G>A may decrease the risk of oral cancer,

however, allele G and GG genotype of TNF-α-238G>A may increase the risk. Larger prospective or multicentric case-control studies on the TNF-α polymorphisms with the susceptibility to oral cancer are needed to get more consistent conclusions. For future relevant studies, more strict selection of patients, much larger sample size and well-matched controls will be required. Moreover, gene-gene and gene-environment interactions should also be considered in future studies.

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