

Original Article

Association between tumor necrosis factor α -308 G/A polymorphism and bladder cancer risk: a meta-analysis

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Abstract: Previous studies have investigated the association between *TNF- α* -308 G/A polymorphism and bladder cancer risk in various populations. However, these findings remain inconclusive. Therefore, we performed a meta-analysis to explore the relationship between *TNF- α* -308 G/A polymorphism and bladder cancer risk. A literature search in PubMed was performed to select eligible studies regarding the association between *TNF- α* -308 G/A polymorphism and bladder cancer risk. The strength of risk under fixed- and random-effects models were estimated using the odds ratios (ORs) with 95% confidence intervals (CIs). We collected 7 case-control studies including 1153 cases and 1587 controls were included in the present meta-analysis. Compared with subjects carrying the G/G genotype of *TNF- α* -308 G/A polymorphism, those with the A/A and G/A genotypes had non-significant bladder cancer risks under the fixed effects model (OR=1.037) and the random effects model (OR=1.012). In the recessive model, subjects with the A/A genotype had an increased bladder cancer risk (OR=1.875) compared with those carrying the G/A and G/G genotypes of *TNF- α* -308 G/A polymorphism. The major finding of this meta-analysis suggests that *TNF- α* -308 G/A polymorphism is correlated with the risk of bladder cancer in Asian population under the recessive model. Further investigate of the joint effects of environmental risk factors and *TNF- α* -308 G/A polymorphism on bladder cancer risk should be considered.

Keywords: Bladder cancer, meta-analysis, polymorphism, tumor necrosis factor

Introduction

Bladder cancer is a multi-factorial disease generally developed by chronic exposure of environmental risk factors and genomic instability caused by genetic variations [1]. Smoking is a dominant risk factor for bladder cancer [2, 3]. Other factors including arsenic exposure, exposure to carcinogenic chemicals, and inflammation are known risk factors for bladder cancer [4-6].

Cigarette smoking, secondhand smoke and arsenic exposure have been found to generate oxidative stress and trigger inflammation [7-9]. Chronic inflammation is a key mechanism for the development of bladder cancer [10, 11].

Reactive oxidative stress (ROS) can induce pro-inflammatory cytokines such as tumor necrosis factor alpha (*TNF- α*) which played a major role in the progression of several cancers [12, 13]. Cigarette smoking can increase *TNF- α* expression through up-regulating an activator protein, AP-1 [9]. In addition, *TNF- α* also involved in systemic immune response to intravesical instillation of Bacille Calmette-Guerin [14].

TNF- α is an inflammatory cytokine and is associated with bladder cancer [15, 16]. Several polymorphisms have been identified in the promoter region of the *TNF- α* gene [12]. Many previous studies commonly focused on the -308 G/A polymorphism (rs1800629) of the *TNF- α* gene and it is a G to A transition in the promoter

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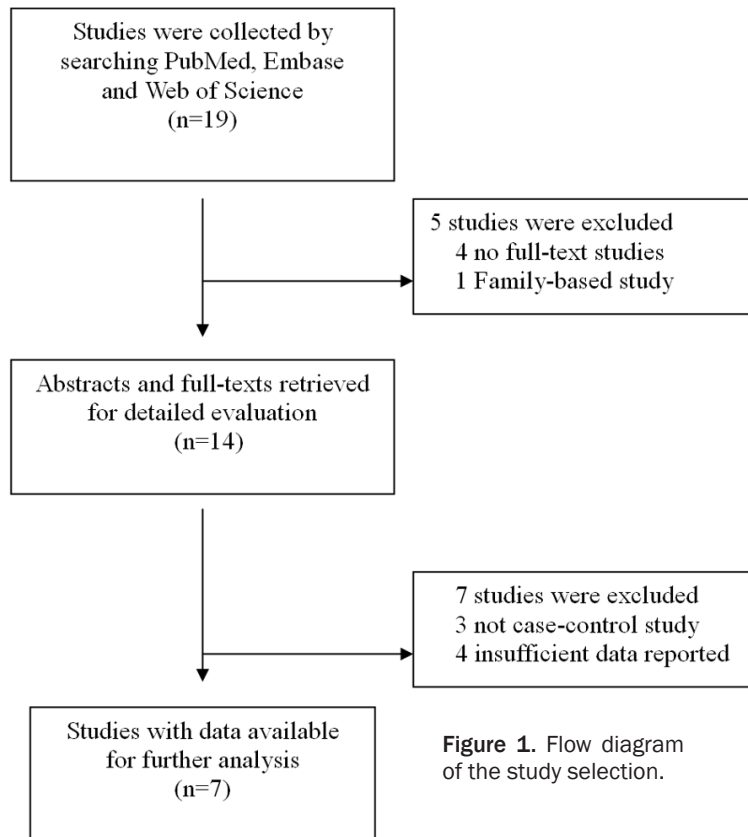


Figure 1. Flow diagram of the study selection.

at position -308 [17, 18]. The -308 G/A nucleotide transition has been shown to influence the expression of TNF- α [19]. In addition, the TNF- α -308 G/A polymorphism has been investigated to explore its effect on several cancers such as breast, gastric and bladder cancers [12, 17, 20, 21]. However, results from previous studies were inconsistent.

Because these inconclusive findings may be caused by the smaller sample size or the potential heterogeneity of individual study, we conducted a meta-analysis from the published case-control studies to assess the association between the TNF- α -308 G/A polymorphism and the risk of bladder cancer.

Materials and methods

Search strategy and study selection

Eligible studies regarding the association of TNF- α -308 G/A polymorphism and bladder cancer were chosen from a literature search on PubMed, Embase, and Web of Science published from 2001 through July 2013. There was no language restriction. Databases were

searched by the following keywords: “tumor necrosis factor alpha”, “TNF- α ” and “polymorphism” or “genotype” or “rs-1800629” or “promoter”; and “bladder cancer” or “urothelial carcinoma”. The inclusion criteria must meet the following rules: (1) studies on human subjects; (2) studies regarding the association between TNF- α -308 G/A polymorphism and bladder cancer; (3) case-control study design; (4) sufficient information for evaluating genotype frequency or odds ratio (OR) with 95% confidence interval (CI). Following above searching strategy, we collected 19 studies, of which 7 were eligible for following evaluation in this meta-analysis (**Figure 1**).

Data extraction

The data extraction were performed independently by two of the authors (C.H. Shen and W.T. Kao), and discrepancies were initially resolved by discussion. If they can get a consensus, another independent investigator (C.C. Wu) was consulted to make a final decision. Information recorded from each study included first author’s name, year of publication, country where this study was conducted, the ethnicity of participants, the sum of genotype frequency of TNF- α -308 G/A polymorphism for cases and controls, minor allele frequency, source of control groups, and genotyping method. For individual study, the examination of Hardy-Weinberg equilibrium (HWE) in controls was estimated using the chi-square goodness-of-fit test.

Statistical analysis

The strength of the association between the TNF- α -308 G/A polymorphism and risk of bladder cancer was calculated as a measure of the pooled OR and its corresponding 95% CI. We performed the Cochran Q-statistic test and an I^2 test to evaluate the heterogeneity across the different studies ($I^2 < 25\%$, low heterogeneity; $I^2 = 25\text{--}50\%$, moderate heterogeneity; $I^2 > 50\%$, obvious heterogeneity). Based on the heteroge-

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Table 1. The major characteristics of studies included in the meta-analysis

Author	Year	Country	Ethnicity	Cases	Controls	Genotyping method
Tsai et al.	2001	Taiwan	Asian	114	150	PCR-RFLP
Marsh et al.	2003	United Kingdom	British	196	208	ARMS-PCR*
Jeong et al.	2004	Korea	Asian	113	109	PCR-RFLP
Kim et al.	2005	Korea	Asian	153	153	PCR-RFLP
Nonomura et al.	2006	Japan	Asian	141	173	PCR-RFLP
Ahirwar et al.	2008	India	Asian	136	200	ARMS-PCR*
Wu et al.	2013	Taiwan	Asian	300	594	PCR-RFLP

*ARMS-PCR: Amplification refractory mutation system-polymerase chain reaction.

Table 2. The distribution of *TNF- α* -308 G/A polymorphism among studies included in the meta-analysis

Author	Year	Genotype frequencies for cases				Genotype frequencies for controls			
		G/G	G/A	A/A	A allele (%)	G/G	G/A	A/A	A allele (%)
Tsai et al.	2001	95	18	1	8.8	124	25	1	9.0
Marsh et al.	2003	102	79	15	28.0	93	92	23	33.0
Jeong et al.	2004	97	15	1	7.5	90	19	0	8.7
Kim et al.	2005	139	12	2	5.2	130	22	1	7.8
Nonomura et al.	2006	136	5	0	1.8	172	1	0	0.3
Ahirwar et al.	2008	112	19	5	11.0	171	22	7	9.0
Wu et al.	2013	249	44	7	9.7	507	83	4	7.7

neity, the pooled OR was estimated by a fixed-effect model (Mantel-Haenszel method) or a random-effect model (DerSimonian and Laird method).

The sensitivity analysis was conducted to assess the influence of each study on the pooled OR by omitting individual study. Publication bias was evaluated by funnel plot, and the asymmetric plot implied a publication bias. The asymmetry was tested by the Egger's linear regression, and a *P* value of < 0.05 was considered to be a significant publication bias. All statistical analyses were carried out using the Comprehensive Meta-Analysis version 2.0 (Biostat, Englewood, NJ, USA).

Results

Major characteristics of selected studies

After the literature search under the inclusion criteria, a total of seven eligible studies regarding the association between *TNF- α* -308 G/A polymorphism and risk of bladder cancer were included in the present meta-analysis. **Table 1** shows major characteristics of these selected studies. The eligible literatures were published

between 2001 and 2013. Among these selected studies, only one study (Marsh et al.) was conducted in the United Kingdom. The other six included studies were conducted in populations of Asian ethnicity. A total of 1,153 cases and 1,587 controls were included in the present study. The genotype frequency among cases and controls for *TNF- α* -308 G/A polymorphism were shown in **Table 2**. The distributions of genotype in the control group were in HWE for most of studies (*P* > 0.05).

Main findings of meta-analysis

Although no heterogeneity existed between these eligible studies under the dominant model ($I^2=29.4$) or the recessive model ($I^2=0.00$), the British study with a significant different allele (A) frequency was excluded in the subsequent analyses. In **Figure 2A** (the dominant model), compared with subjects carrying the G/G genotype of *TNF- α* -308 G/A polymorphism, those with the A/A and G/A genotypes had non-significant bladder cancer risks under the fixed effects model (OR=1.037, 95% CI=0.810-1.328, *P*=0.773) and the random effects model (OR=1.012, 95% CI=0.737-1.390, *P*=0.941). In **Figure 2B** (the recessive model),

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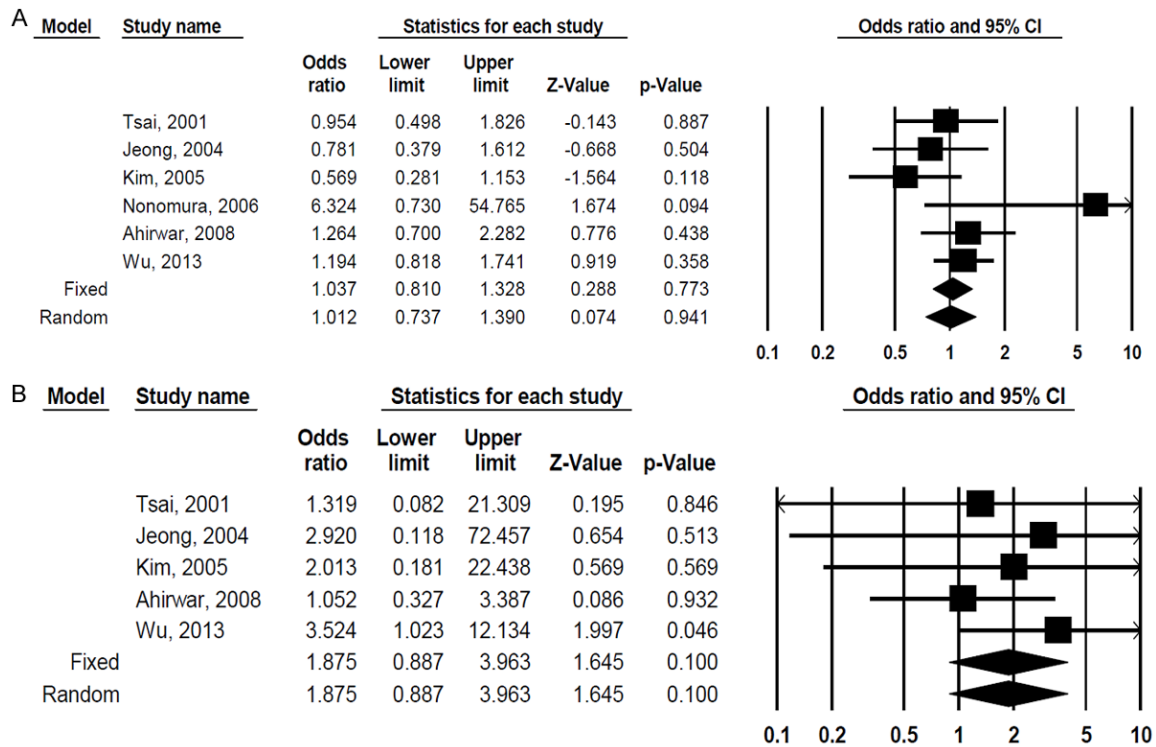


Figure 2. A. Forest plot for the association between *TNF- α* -308 G/A polymorphism and risk of bladder cancer in the dominant model (A/A+G/A vs. G/G). B. Forest plot for the association between *TNF- α* -308 G/A polymorphism and risk of bladder cancer in the recessive model (A/A vs. G/A+G/G).

compared with subjects carrying the G/A and G/G genotypes of *TNF- α* -308 G/A polymorphism, those with the A/A genotype had non-significant increased bladder cancer risks under the fixed effects model (OR=1.875, 95% CI=0.887-3.963, P=0.1) as well as the random effects model.

Sensitivity analysis and publication bias

Sensitivity analysis was generally conducted by omitting of individual study and cumulative statistics showed the pooled OR of *TNF- α* -308 G/A polymorphism were not significantly influenced by any individual study under the dominant or recessive model. Funnel plots and Egger's test were also performed to assess publication bias. There was no evidence of publication bias for the dominant model (P=0.796) and the recessive model (P=0.885) in *TNF- α* -308 G/A polymorphism (**Figure 3A** and **3B**).

Discussion

Chronic inflammation is an important pathogenesis of bladder cancer. *TNF- α* plays a pivot-

al role in the inflammation as a cytokine. Previous studies have investigated the association between *TNF- α* -308G/A polymorphism (rs1800629) and risk of bladder cancer, but their findings were still inconsistent [22-24]. In the present study, we performed a meta-analysis to evaluate the association between *TNF- α* -308G/A polymorphism and risk of bladder cancer in Asian populations. According to the inclusion criteria, seven eligible studies with 2,740 subjects were included in the meta-analysis.

Our results found that subjects carrying the A/A genotype had an increased risk of bladder cancer (OR=1.875) compared with those who carried the G/A and G/G genotypes of *TNF- α* -308 G/A polymorphism under the recessive model (**Figure 2B**). *TNF- α* -308 G/A polymorphism contributed to carcinogenesis as well as the variant A allele was associated with the expression of *TNF- α* [18]. A study found that *TNF- α* -308 G/A polymorphism is significantly associated with tumor stage and grade of bladder cancer [17]. Another study showed that the *TNF- α* -308 G/A polymorphism was not related with superficial bladder cancer [10, 18]. Their findings suggest

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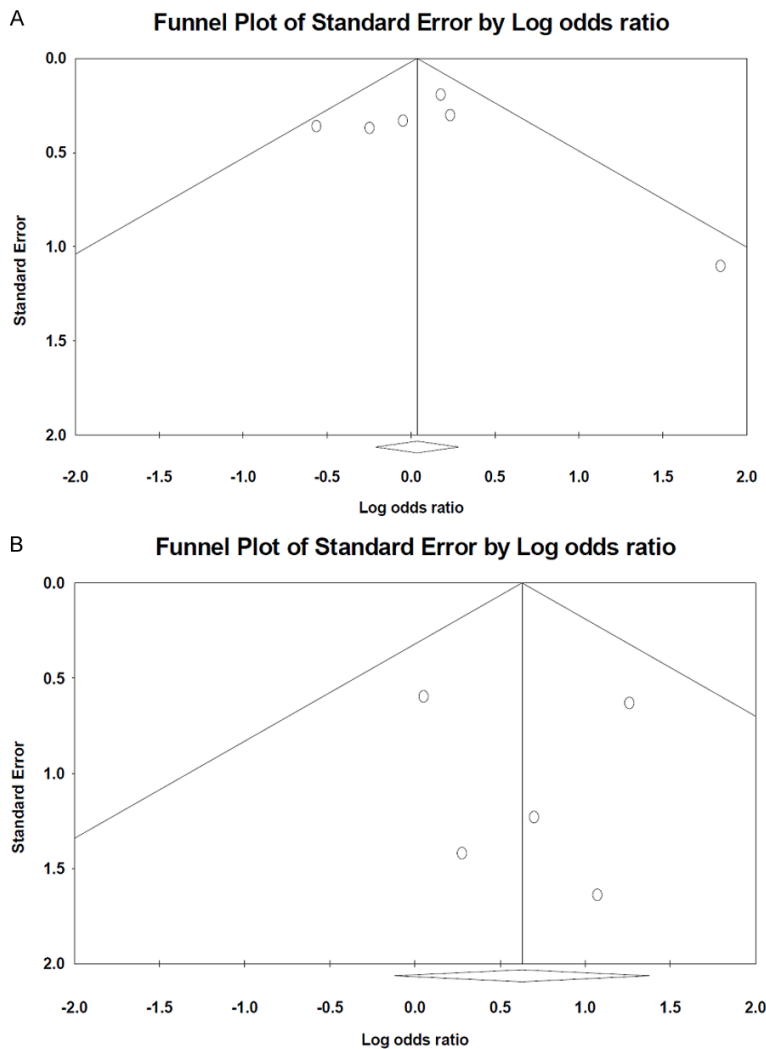


Figure 3. Funnel plots were used to visualize publication bias in the dominant (A) and recessive (B) models.

that *TNF- α* -308 G/A polymorphism may modulate angiogenesis, which plays an important role in invasion and metastasis of various malignancies. However, some studies did not have conclusive results [25].

In agreement with previous studies, the G/G genotype was predominated in cases and controls in the present meta-analysis [17, 26]. The ethnic variation might contribute to the differences in genotype frequencies. We omitted the British study which initially included as an eligible study with a higher minor allele (A) frequency of 0.28 and 0.33 for cases and controls, respectively. All the other six studies have similar geographic background as the Asian population. Although *TNF- α* -308 G/A polymorphism

was not significantly associated with an increased bladder cancer risk, some risk factors such as cigarette smoking or arsenic exposure might contribute to this non-significant finding. Some studies found that the *TNF- α* level of was higher in ever smokers compared to never smokers [20]. In addition, arsenic exposure was associated with pro-inflammatory cytokines such as *TNF- α* . Therefore, the different risk estimates associated with *TNF- α* -308 G/A polymorphism may be explained by the different genetic background and different carcinogen exposure in different populations. We should further investigate the joint effects of environmental risk factors and *TNF- α* -308 G/A polymorphism on bladder cancer risk.

This meta-analysis still has some limitations. First, we only investigated one polymorphism (rs1800629) located in promoter region which incompletely represent for the entire function of *TNF- α* . Second, all the included studies are hospital-based case-control study design. Third, the

overall effect size estimation was based on individual unadjusted OR, while a precise evaluation of bladder cancer risk should be adjusted by other risk factors. Therefore, more functional polymorphisms of *TNF- α* should be included to validate our findings in further studies. In addition, more cytokines should be taken into consideration to explore their combined effects on bladder cancer risk.

In conclusion, our meta-analysis suggested that *TNF- α* -308 G/A polymorphism is correlated with an increased risk of bladder cancer in Asian population. Further studies based on a population design are needed to identify the effects of *TNF- α* -308 G/A polymorphism on bladder cancer risk.

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Disclosure of conflict of interest

None.

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