RESEARCH ARTICLE

# Association of TNF-α-308 and -238 Polymorphisms with Risk of Cervical Cancer: A Meta-analysis

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## Abstract

**Published data on the associations between tumor necrosis factor-alpha (TNF-α) promoter -308G>A and -238G>A polymorphisms and cervical cancer risk are inconclusive. To derive a more precise estimation of the relationship, a meta-analysis was performed. Data were collected from MEDLINE and PubMed databases. Crude odds ratios (ORs) with 95% confidence intervals (CIs) were calculated in a fixed/random effect model. 13 separate studies including 3294 cases and 3468 controls were involved in the meta-analysis. We found no association between TNF-α-308G>A polymorphism and cervical cancer in overall population. In subgroup analysis, significantly elevated risks were found in Caucasian population (A vs. G: OR = 1.43, 95% CI = 1.00-2.03; AA vs. GG: OR = 2.09, 95% CI = 1.34-3.25; Recessive model: OR = 2.09, 95% CI = 1.35- 3.25) and African population (GA vs. GG: OR = 1.53, 95% CI = 1.02-2.30). An association of TNF-α-238G>A polymorphism with cervical cancer was found (A vs. G: OR = 0.61, 95% CI = 0.47-0.78; GA vs. GG: OR = 0.59, 95% CI = 0.45-0.77; Dominant model: OR = 0.59, 95% CI = 0.46-0.77). When stratified by ethnicity, similar association was observed in Caucasian population (A vs. G: OR = 0.62, 95% CI = 0.46-0.84; GA vs. GG: OR = 0.59, 95% CI = 0.43-0.82; Dominant model: OR = 0.60, 95% CI = 0.44-0.83). In summary, this meta-analysis suggests that TNF-α-238A allele significantly decreased the cervical cancer risk, and the TNF-α-308G>A polymorphism is associated with the susceptibility to cervical cancer in Caucasian and African population.**

**Keywords:** Cervical cancer - genetic polymorphisms - TNF-α - susceptibility - meta-analysis

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## Introduction

Cervical cancer remains a major cause of illness and all cancer-related deaths among women in developing countries (Jemal et al., 2010). Etiologically, carcinogenesis of cervical cancer was regarded as a complex multi-step, multi-factor process and a result of multiple gene-environment interactions. A number of epidemiologic and molecular biologic data revealed that high-risk human papillomavirus (HPV) infection is the most well established environmental risk factor for cervical cancer (zur Hausen, 2002). Nonetheless, the virus could be naturally cleared in 70-90% of individuals with HPV-infection, while a small proportion of patients with persistent HPV infection ultimately develop cervical cancer, indicating that HPV infection is necessary but not sufficient risk factor for the development and progression of cervical cancer (Walboomers et al., 1999). Consequently, host genetic differences in effective host immune response may influence the risk for cervical cancer among those infected with HPV.

Several cytokines that mediate the immune response have been implicated in the development of cancer (Dranoff, 2004). Tumor necrosis factor-alpha (TNF-α) is a potential pro-inflammatory cytokine which plays a critical role in a wide range of inflammatory, autoimmune, and malignant diseases (Bazzoni and Beutler, 1996; Beutler and Bazzoni, 1998). TNF has been shown to be involved in the course of mediation of carcinogenesis through induction of proliferation, invasion, and metastasis of tumor cells (Shishodia et al., 2003). Previous study has confirmed the evidence supporting a pivotal role of TNF-α in tumor promotion (Moore et al., 1999). In addition, high serum TNF-α level in cancer patients have been detected (Abrahamsson et al., 1993) and were associated with a poor disease outcome (Nakashima et al., 1998). Hence, TNF-α expression levels may contribute to the pathogenesis and promoting malignant progression of cervical cancer.

Single-nucleotide polymorphisms (SNPs) have been identified in the promoter region of TNF-α, and have been related to the regulation of TNF-α transcription. TNF-α

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gene lies in the major histo-compatibility complex class III region on the short arm of chromosome 6 (6p21.3) (Hajeer and Hutchinson, 2000). As for two commonest polymorphisms, G to A substitution at position -308 in the promoter region of TNF-α have an effect on the expression of TNF-α cytokine production, and TNF-α -308 A allele produces higher level of TNF-α than the -308 G allele (Kroeger et al., 1997). Meanwhile, because a putative repressor site is located in a 25-bp stretch including the position -238, the G to A substitution at position -238 in the TNF-α promoter might also affect TNF-α expression (Fong et al., 1994). Accordingly, it is rational to speculate that TNF-α -308G>A and -238G>A polymorphisms may have an impact on the development of some types of cancers. In recent years, numerous case-control studies have been conducted to investigate the associations of TNF-α -308G>A and -238G>A promoter region polymorphisms with cancer risk, suggesting that these polymorphisms may be associated with cancer susceptibility, such as breast cancer, lung cancer, nasopharyngeal cancer and cervical cancer (Deshpande et al., 2005; Shih et al., 2006; Pooja et al., 2011; Sousa et al., 2011).

However, the results from previous studies that were conducted to assess the association of these two polymorphisms (-308 G>A and -238 G>A) with cervical cancer are still inconclusive or contradictory (Jang et al., 2001; Calhoun et al., 2002; Gostout et al., 2003; Stanczuk et al., 2003; Deshpande et al., 2005; Duarte et al., 2005; Govan et al., 2006; Kohaar et al., 2007; Ivansson et al., 2008; Singh et al., 2009; Wang et al., 2009; Ivansson et al., 2010; Wang et al., 2011). This inconsistency may be partially attributed to studies with relative small sample size, inadequate statistical power, racial and ethnic differences, publication bias, or uncorrected multiple hypothesis testing. Therefore, we performed this meta-analysis of the published studies to derive a more accurate estimation of the relationship between TNF-α -308G>A and -238G>A polymorphisms and susceptibility to cervical cancer.

## Materials and Methods

*Literature search strategy*

MEDLINE and PubMed databases were applied to a comprehensive search of the literature for all publications on the association between TNF-α-308G>A and -238 G>A polymorphisms and cervical cancer with the last report up to 1 October 2011. The Keywords were as follows: tumor necrosis factor/TNF-α, polymorphism/polymorphisms/variant, cervical, cancer/carcinoma/tumor. No restrictions were placed on language. All eligible studies were retrieved, and their references were checked for other relevant publications. In addition, the reference lists of reviews and bibliographies were hand searched simultaneously. Unpublished or abstracts reports were not included. For overlapping studies, only the one with the largest sample numbers was included in this meta-analysis. For studies including subjects of different ethnic groups, each study should be treated as a separate comparison whenever possible.

*Inclusion and exclusion criteria*

The included studies must meet the following criteria: (1) a case-control study; (2) a study of the TNF-α-308G>A or -238G>A polymorphism and cervical cancer risk; (3) a study provided available genotypes frequency of TNF-α-308G>A or -238G>A. The exclusion criterions were as follows: (1) the study was conducted on animals; (2) the study was not case-control studies; (3) the study did not have available data.

*Data extraction*

All data were extracted carefully and independently by two reviewers (Pan and Tian) according to the selection criteria listed above, and any controversy was discussed and reached a consensus. The following characteristics were collected from all eligible studies: the first author's name, year of publication, the country of origin, ethnicity, the number of cases and controls, source of control groups (population- or hospital-based controls), genotyping method, control matching method, and studying period. Different ethnic descents were categorized as Caucasian, Asian, African, or Mixed which included more than one ethnic descent.

*Statistical methods*

Crude odds ratios (ORs) with 95% confidence intervals (95% CI) were used to evaluate the strength of associations between the TNF-α-308G>A and -238G>A polymorphisms and cervical cancer risk. The pooled ORs were performed for additive model (A vs. G), codominant model (AA vs. GG; GA vs. GG), dominant model (AA + GA vs. GG), and recessive model (AA vs. GG + GA), respectively. Heterogeneity assumption was assessed by the Chi-square test based *Q*-statistic (Cochran, 1954) and $I^2$ statistics ($I^2 = 100\% \times (Q–df)/Q$) (Higgins and Thompson, 2002). A *P* value greater than 0.10 for the Q-test and $I^2 \leq 50\%$ indicate the absence of heterogeneity among studies, so the pooled OR estimate of each study was calculated by the fixed-effects model (Mantel and Haenszel, 1959). Otherwise, the random-effects model was explored (DerSimonian and Laird, 1986). The significance of the pooled OR was determined by the Z-test. Potential publication bias was observed by the Begg's funnel plot. An asymmetric plot suggests a possible publication bias. Funnel plot asymmetry was further assessed by the method of Egger's linear regression test (Egger et al., 1997).

All the statistical analyses were done using software Stata version 7.0 (Stata Corporation, College Station, TX). A goodness-of-fit Chi-square test with one degree of freedom test was used to test whether observed frequencies of genotypes conformed to Hardy-Weinberg equilibrium (HWE) expectations in the controls. A *P* value of less than 0.05 was considered statistically significant, and all the *P* values were two tailed.

## Results

*Characteristics of eligible studies*

Characteristics of studies investigating the associations of TNF-α-308G>A and -238G>A polymorphisms with

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

| First author | Year | Country | Ethnicity | Sample size case | control | Source of controls | Genotyping method | Control matching method | Polymorphisms | Studying period |
|---|---|---|---|---|---|---|---|---|---|---|
| Jang WH | 2001 | South Korea | Asian | 51 | 92 | PB | PCR-RFLP | Ethnical and regional | TNF-α-308,238 | NA |
| Stanczuk GA | 2003 | Zimbabwe | African | 103 | 101 | PB | ARMS-PCR | Age, ethnical | TNF-α-308 | NA |
| Gostout BS | 2003 | USA | Caucasian | 127 | 175 | HB | Direct sequencing | NM | TNF-α-308, 238 | 1991 to 1996 |
| Duarte I | 2005 | Portugal | Caucasian | 195 | 244 | PB | PCR-RFLP | NA | TNF-α-308 | NA |
| Deshpande A | 2005 | USA | Caucasian | 341 | 446 | HB | PCR-based sequencing | NA | TNF-α-308, 238 | 1996 to 2000 |
| Govan VA[a] | 2006 | South Africa | African | 161 | 169 | HB | ARMS-PCR | Age, ethnical and regional | TNF-α-308 | 1998 to 2001 |
| Govan VA[b] | 2006 | South Africa | Mixed | 83 | 59 | HB | ARMS-PCR | Age, ethnical and regional | TNF-α-308 | 1998 to 2001 |
| Kohaar I | 2007 | India | Caucasian | 120 | 165 | HB | PCR-RFLP | Age, ethnical | TNF-α-308, 238 | NA |
| Ivansson EL | 2008 | Sweden | Mixed | 1306 | 288 | PB | Inflastrip | NA | TNF-α-238 | NA |
| Singh H | 2009 | India | Caucasian | 150 | 162 | HB | PCR-RFLP | Age, ethnical | TNF-α-308 | 2005 to 2007 |
| Wang SS | 2009 | Costa Rica | Caucasian | 471 | 844 | PB | Taqman | NA | TNF-α-308, 238 | 1993 to 2001 |
| Ivansson EL | 2010 | Sweden | Caucasian | 1306 | 841 | PB | Taqman, Inflastrip | NA | TNF-α-308 | NA |
| Wang Q | 2011 | China | Asian | 186 | 200 | PB | PCR | NA | TNF-α-308 | NA |

[a]Participants were black African; [b]Participants were mixed-ancestry; N, number; HB, hospital-based case-control study; PB, population-based case-control study; PCR-RFLP, polymerase chain reaction restriction fragment length polymorphism; ARMS-PCR, amplification refractory mutation system-polymerase chain reaction; PCR, polymerase chain reaction; NA, not available; NM, not matched

**Table 2. Distributions of TNF-α Gene Genotypes and Alleles among Patients and Controls**

| First author | Year | Case | | | Control | | | Case | | Control | | HWE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | GG | GA | AA | GG | GA | AA | G | A | G | A | |
| TNF-α-308 | | | | | | | | | | | | |
| Jang WH | 2001 | 46 | 3 | 2 | 85 | 7 | 0 | 95 | 7 | 177 | 7 | 0.704 |
| Stanczuk GA | 2003 | 74 | 28 | 1 | 81 | 18 | 2 | 176 | 30 | 180 | 22 | 0.411 |
| Gostout BS | 2003 | 91 | 27 | 9 | 117 | 53 | 5 | 209 | 45 | 287 | 63 | 0.731 |
| Duarte I | 2005 | 138 | 50 | 7 | 200 | 40 | 4 | 326 | 64 | 440 | 48 | 0.237 |
| Deshpande A | 2005 | 188 | 54 | 16 | 297 | 100 | 14 | 430 | 86 | 694 | 128 | 0.13 |
| Govan VAa | 2006 | 110 | 45 | 6 | 127 | 36 | 6 | 265 | 57 | 290 | 48 | 0.102 |
| Govan VAb | 2006 | 64 | 17 | 2 | 45 | 10 | 4 | 145 | 21 | 100 | 18 | 0.008 |
| Kohaar I | 2007 | 94 | 22 | 4 | 150 | 15 | 0 | 210 | 30 | 315 | 15 | 0.541 |
| Singh H | 2009 | 122 | 17 | 11 | 147 | 11 | 4 | 261 | 39 | 305 | 19 | 0 |
| Wang SS | 2009 | 386 | 67 | 3 | 666 | 126 | 8 | 839 | 73 | 1458 | 142 | 0.458 |
| Ivansson EL | 2010 | 891 | 340 | 32 | 589 | 188 | 27 | 2122 | 404 | 1366 | 242 | 0.015 |
| Wang Q | 2011 | 149 | 30 | 7 | 144 | 46 | 10 | 328 | 44 | 334 | 66 | 0.019 |
| TNF-α-238 | | | | | | | | | | | | |
| Jang WH | 2001 | 50 | 1 | 0 | 80 | 11 | 1 | 101 | 1 | 171 | 13 | 0.391 |
| Gostout BS | 2003 | 121 | 5 | 1 | 154 | 19 | 2 | 247 | 7 | 327 | 23 | 0.126 |
| Deshpande A | 2005 | 286 | 20 | 2 | 393 | 40 | 0 | 592 | 24 | 826 | 40 | 0.314 |
| Kohaar I | 2007 | 119 | 1 | 0 | 159 | 6 | 0 | 239 | 1 | 324 | 6 | 0.812 |
| Ivansson EL | 2008 | 1202 | 77 | 2 | 261 | 25 | 1 | 2481 | 81 | 547 | 27 | 0.631 |
| Wang SS | 2009 | 427 | 30 | 0 | 730 | 79 | 3 | 884 | 30 | 1539 | 85 | 0.583 |

[a]Participants were black African; [b]Participants were mixed-ancestry; HWE, Hardy–Weinberg equilibrium

cervical cancer are presented in Table 1 and Table 2. One article (Calhoun et al., 2002) was excluded due to overlapping data with Gostout's study published in 2003 (Gostout et al., 2003), and we selected the study with larger sample size (Gostout et al., 2003). Govan's study (2006) contained data on two independent populations and thus was treated as two separate studies. Finally, a total of 13 separate studies were included in this meta-analysis based on the inclusion criteria (Jang et al., 2001 Calhoun et al., 2002; Gostout et al., 2003; Stanczuk et al., 2003; Deshpande et al., 2005; Duarte et al., 2005; Govan et al., 2006; Kohaar et al., 2007; Ivansson et al., 2008; Singh et al., 2009; Wang et al., 2009; Ivansson et al., 2010; Wang et al., 2011), which involved 3294 cervical cancer patients and 3468 controls. Of eligible studies, seven studies were conducted in Caucasian population, two studies for Asian, African and mixed-ancestry population, respectively. There were six hospital-based studies and seven population-based studies. The results of the HWE test for the genotypes distribution in controls were also listed in Table 2. All studies did not deviate from HWE except for four studies (Govan et al., 2006; Singh et al., 2009; Ivansson et al., 2010; Wang et al., 2011) for the TNF-α-308G>A polymorphism.

*Meta-analysis*

The summary of meta-analysis for TNF-α-308G>A and -238G>A polymorphisms with cervical cancer is

**Table 3. Main Results of Pooled ORs in the Meta-analysis**

| | N | A vs. G | | AA vs. GG | | GA vs. GG | | Dominant model | | Recessive model | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | OR(95%CI) | $P_h$ | OR(95%CI) | $P_h$ | OR(95%CI) | $P_h$ | OR(95%CI) | $P_h$ | OR(95%CI) | $P_h$ |
| TNF-α-308 | | | | | | | | | | | |
| Overall | 12 | 1.22(1.00-1.50) | 0.001 | 1.31(0.84-2.06) | 0.076 | 1.14(0.92-1.42) | 0.009 | 1.20(0.97-1.49) | 0.004 | 1.30(0.83-2.04) | 0.069 |
| Ethnicities | | | | | | | | | | | |
| African | 2 | 1.33(0.95-1.87) | 0.848 | 1.00(0.35-2.81) | 0.994 | 1.53(1.02-2.30) | 0.7 | 1.47(0.99-2.16) | 0.764 | 0.90(0.32-2.53) | 0.571 |
| Asian | 2 | 0.99(0.38-2.58) | 0.086 | 1.66(0.14-19.3) | 0.108 | 0.65(0.40-1.05) | 0.764 | 0.70(0.45-1.09) | 0.271 | 1.73(0.16-18.4) | 0.119 |
| Caucasian | 6 | 1.43(1.00-2.03) | 0 | 2.09(1.34-3.25) | 0.387 | 1.19(0.82-1.72) | 0.005 | 1.33(0.92-1.94) | 0.001 | 2.09(1.35-3.25) | 0.407 |
| Mixed | 2 | 1.06(0.89-1.25) | 0.418 | 0.73(0.44-1.20) | 0.358 | 1.20(0.98-1.46) | 1 | 1.13(0.93-1.37) | 0.662 | 0.70(0.43-1.14) | 0.392 |
| Control | | | | | | | | | | | |
| HB | 6 | 1.37(0.97-1.94) | 0.007 | 1.88(1.20-2.95) | 0.211 | 1.20(0.82-1.76) | 0.028 | 1.31(0.90-1.90) | 0.017 | 1.88(1.20-2.94) | 0.191 |
| PB | 6 | 1.11(0.85-1.45) | 0.013 | 0.90(0.61-1.34) | 0.32 | 1.12(0.84-1.50) | 0.029 | 1.12(0.84-1.50) | 0.019 | 0.88(0.59-1.30) | 0.374 |
| TNF-α-238 | | | | | | | | | | | |
| Overall | 6 | 0.61(0.47-0.78) | 0.358 | 0.79(0.27-2.32) | 0.579 | 0.59(0.45-0.77) | 0.482 | 0.59(0.46-0.77) | 0.43 | 0.83(0.28-2.45) | 0.584 |
| Ethnicities | | | | | | | | | | | |
| Caucasain | 4 | 0.62(0.46-0.84) | 0.375 | 0.97(0.27-3.52) | 0.281 | 0.59(0.43-0.82) | 0.481 | 0.60(0.44-0.83) | 0.449 | 1.02(0.28-3.69) | 0.285 |
| Others | 2 | 0.40(0.09-1.81) | 0.118 | 0.47(0.07-3.41) | 0.921 | 0.44(0.11-1.72) | 0.152 | 0.41(0.09-1.77) | 0.131 | 0.50(0.07-3.64) | 0.89 |
| Control | | | | | | | | | | | |
| HB | 3 | 0.64(0.41-0.97) | 0.215 | 1.85(0.38-9.35) | 0.226 | 0.54(0.34-0.88) | 0.326 | 0.58(0.37-0.91) | 0.267 | 1.95(0.38-9.93) | 0.235 |
| PB | 3 | 0.59(0.44-0.80) | 0.307 | 0.36(0.01-1.84) | 0.931 | 0.62(0.45-0.85) | 0.364 | 0.60(0.44-0.82) | 0.328 | 0.38(0.08-1.93) | 0.922 |

$P_h$, $P$ value of $Q$-test for heterogeneity test; HB, hospital-based case-control study; PB, population-based case-control study; HWE, Hardy-Weinberg equilibrium

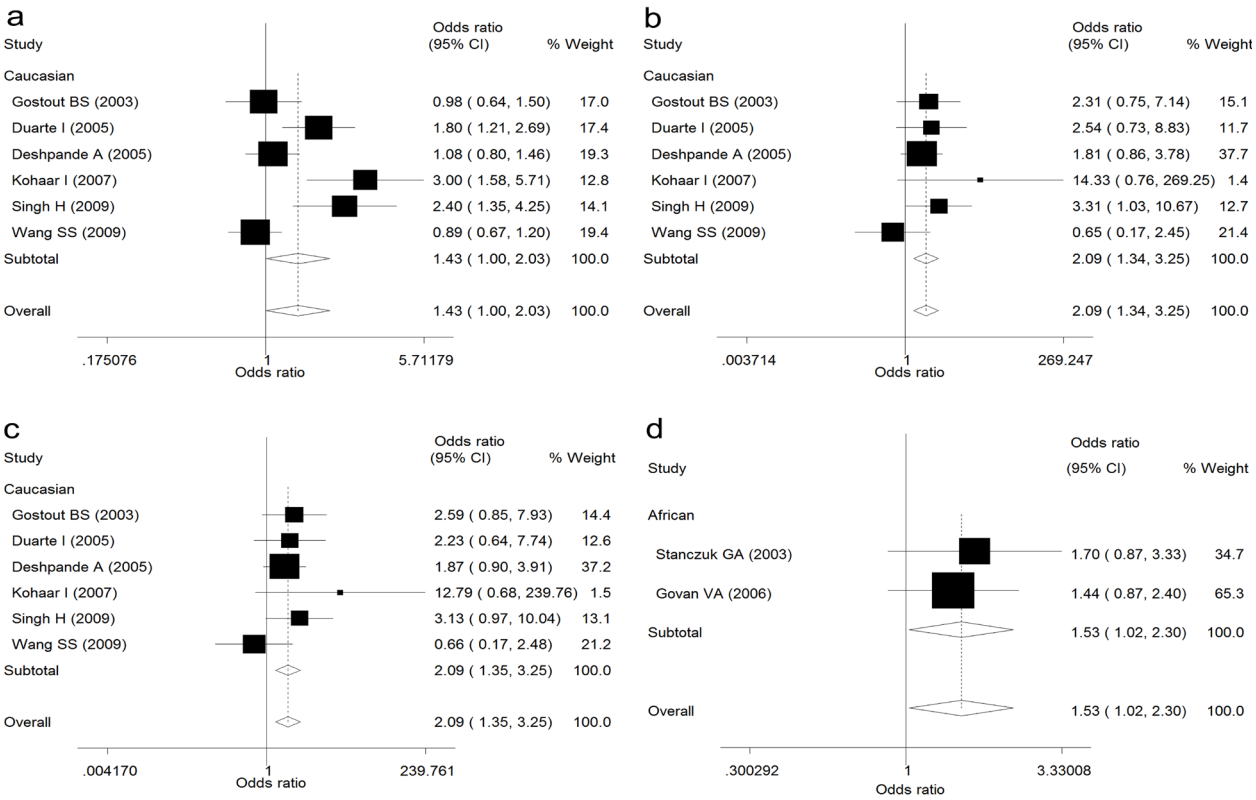


**Figure 1. Forest Plots of TNFA-308G>A Polymorphism and Cervical Cancer Risk** (a. A vs. G in Caucasian population; b. AA vs. GG in Caucasian population; c. Recessive model in Caucasian population; d. GA vs. GG in African population)

shown in Table 3.

*Analysis for TNF-α-308G>A polymorphism*

The association between TNF-α-308G>A polymorphism and cervical cancer susceptibility was investigated in 12 separate studies with a total of 3294 cases and 3468 controls. Significant heterogeneity between studies was observed in overall comparisons and we conducted analyses using the random effect models. No obvious association was found for all genetic models when all studies were pooled into the meta-analysis (A vs. G: OR = 1.22, 95% CI = 1.00-1.50, $P_h = 0.001$, $I^2 = 66.2\%$; AA vs. GG: OR = 1.31, 95% CI = 0.84-2.06, $P_h = 0.076$, $I^2 = 39.8\%$; GA vs. GG: OR = 1.14, 95% CI = 0.92-1.42, $P_h = 0.009$, $I^2 = 56.0\%$; Dominant model: OR = 1.20, 95% CI = 0.97-1.49, $P_h = 0.004$, $I^2 = 60.3\%$; Recessive model: OR = 1.30, 95% CI = 0.83-2.04, $P_h = 0.069$, $I^2 = 40.8\%$ ).

When stratified by ethnicity, significantly elevated risks were found in Caucasian population (A vs. G: OR = 1.43, 95% CI = 1.00-2.03, $P_h = 0.000$, $I^2 = 77.6\%$; AA vs. GG: OR = 2.09, 95% CI = 1.34-3.25, $P_h = 0.387$, $I^2 = 0.0\%$; Recessive model: OR = 2.09, 95% CI = 1.35- 3.25, $P_h = 0.407$, $I^2 = 1.4\%$) and African population (GA vs. GG: OR = 1.53, 95% CI = 1.02-2.30, $P_h = 0.700$, $I^2 = 1.0\%$).

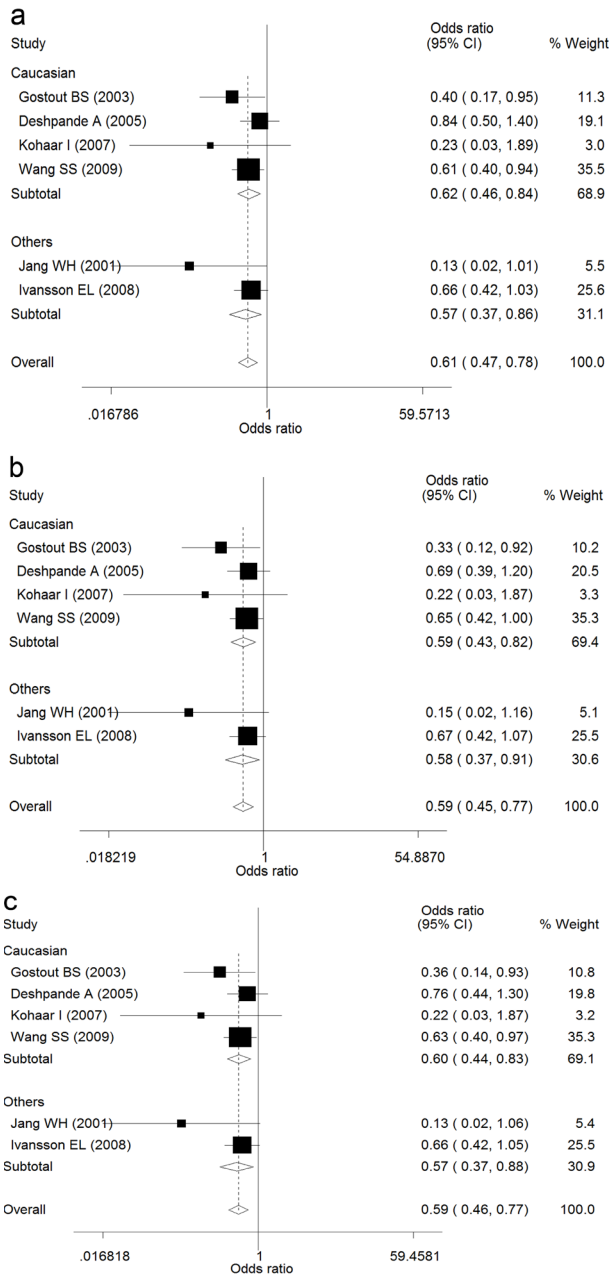


**Figure 2. Forest Plots of TNFA-238G>A Polymorphism and Cervical Cancer Risk** (a. A vs. G; b. GA vs. GG; c.

The forest plots of the distribution of the positive result are shown in Figure 1. In the subgroup analysis by source of controls, notably, we observed obviously increased risks among hospital-based case-control studies (AA vs. GG: OR = 1.88, 95% CI = 1.20-2.95, $P_h$ = 0.211, $I^2$ = 29.9%; Recessive model: OR = 1.88, 95% CI = 1.20-2.94, $P_h$ = 0.191, $I^2$ = 32.6%). However, similar association was not found in population -based case-control studies.

*Analysis for TNF-α-238G>A polymorphism*

The association between TNF-α-238G>A polymorphism and cervical cancer susceptibility was investigated in 6 separate studies, which were consisted of three population-based studies and three hospital-based controls, with a total of 2416 cases and 2010 controls. Of eligible studies, four studies were conducted in Caucasian population, one study in Asian population and one study in mixed-ancestry, respectively, which were merged as other population.

There was no significant between-study heterogeneity in overall population and we conducted analyses using the fixed effect models. Overall, the -238A allele was associated with a significantly decreased risk when the six studies were pooled into the meta-analysis (A vs. G: OR = 0.61, 95% CI = 0.47-0.78, $P_h$ = 0.358, $I^2$ = 9.1%; GA vs. GG: OR = 0.59, 95% CI = 0.45-0.77, $P_h$ = 0.482, $I^2$ = 0.0%; Dominant model: OR = 0.59, 95% CI = 0.46-0.77, $P_h$ = 0.430, $I^2$ = 0.0%). When stratified by ethnicity, similar association was observed in Caucasian population (A vs. G: OR = 0.62, 95% CI = 0.46-0.84, $P_h$ = 0.375, $I^2$ = 3.6%; GA vs. GG: OR = 0.59, 95% CI = 0.43-0.82, $P_h$ = 0.481, $I^2$ = 0.0%; Dominant model: OR = 0.60, 95% CI = 0.44-0.83, $P_h$ = 0.449, $I^2$ = 0.0%), but not other population, and the forest plots of the distribution of the ORs are shown in Figure 2. In the subgroup analysis by study design, we also found significantly decreased risks both in population-based (A vs. G: OR = 0.59, 95% CI = 0.44-0.80, $P_h$ = 0.307, $I^2$ = 15.4%; GA vs. GG: OR = 0.62, 95% CI = 0.45-0.85, $P_h$ = 0.364, $I^2$ = 1.1%; Dominant model: OR = 0.60, 95% CI = 0.44-0.82, $P_h$ = 0.328, $I^2$ = 10.3%) and hospital-based (A vs. G: OR = 0.64, 95% CI = 0.41-0.97, $P_h$ = 0.215, $I^2$ = 35.0%; GA vs. GG: OR = 0.54, 95% CI = 0.34-0.88, $P_h$ = 0.326, $I^2$ = 10.7%; Dominant model: OR = 0.58, 95% CI = 0.37-0.91, $P_h$ = 0.267, $I^2$ = 24.3%) case-control studies.

*Publication bias*

Begg's funnel plot and Egger's linear regression test were used to assess the publication bias of literatures. If there is asymmetry, the regression line will not run through the origin. The shapes of the funnel plots did not reveal any evidence of obvious asymmetry in all comparison models. Then, the Egger's test was performed to provide statistical evidence of funnel plot asymmetry. For TNF-α-308G>A polymorphism, the results still suggested no obvious publication bias (A vs. G: $P$ = 0.181; AA vs. GG: $P$ = 0.242; GA vs. GG: $P$ = 0.683; Dominant model: $P$ = 0.351; Recessive model: $P$ = 0.259). For TNF-α-238G>A polymorphism, the Egger's linear regression test provided evidence of publication bias in some comparisons (A vs. G: $P$ = 0.040; GA vs. GG: $P$ = 0.005; Dominant model: $P$ = 0.016).

## Discussion

In recent decades, the associations between genes polymorphisms and cancer susceptibility have been extensively investigated, suggesting that genetic polymorphisms of host factors could contribute to individual differences of susceptibility to cancer. Inflammation developing through the action of various inflammatory mediators is considered as a cofactor in carcinogenesis (Coussens and Werb, 2002). Among inflammatory mediators, TNF-α, a multifunctional cytokine, has been implicated in the promotion of inflammatory responses and plays a critical role in the pathogenesis of inflammatory which may promote the development and progression of cancer (Moore et al.,

1999). The two most commonly studied polymorphisms were G to A substitutions in the promoter region at positions -308 and -238 which have been shown to influence the TNF-α expression (Fong et al., 1994; Kroeger et al., 1997). A large number of studies have been conducted to investigate the associations between these two variants and cervical cancer risk; however, results from these studies were still ambiguous due to small sample size, unified ethnicity, and low statistical power of individual studies (Jang et al., 2001; Calhoun et al., 2002; Gostout et al., 2003; Stanczuk et al., 2003; Deshpande et al., 2005; Duarte et al., 2005; Govan et al., 2006; Kohaar et al., 2007; Ivansson et al., 2008; Singh et al., 2009; Wang et al., 2009; Ivansson et al., 2010; Wang et al., 2011). Meta-analysis is a potent method through pooling of data from individual association studies, thereby increasing the sample size and enhancing the statistical power. To reach a more reliable and comprehensive conclusion, we present a more systematic review to further investigate the associations of TNF-α -308G>A and -238G>A polymorphisms with susceptibility to cervical cancer.

Our results indicated that the variant genotypes of TNF-α-238G>A polymorphism were associated with significantly decreased risk of cervical cancer in overall population. In light of the biochemical properties of TNF-α in the development of inflammation and tumor, this finding might be biologically plausible. TNF-α expression is mostly regulated at the transcriptional level. A putative repressor site located in a 25-bp stretch including the position -238 is recognized to have influence on the expression of TNF-α. Previous study has demonstrated that G to A substitution at position -238 in the TNF-α promoter is associated with a decreased TNF-α production (D'Alfonso and Richiardi, 1994). Kaluza et al. (2000) showed that peripheral blood mononuclear cells carrying TNF-α-238A allele produced significantly less TNF-α after stimulation with T cell mitogens and streptococcal antigens in comparison to controls. In addition, other studies, which have analyzed the -238 polymorphism in some types of cancers, including renal cell cancer, gastric cancer, and colorectal cancer, have also showed this polymorphism may be correlated with a decreased susceptibility to cancer (Jang et al., 2001). Therefore, it is reliable to expect that TNF-α-238A allele plays a protective effect against cancer by its function of decreasing TNF-a production, in contrast, TNF-α-238G allele may be more susceptible to cervical cancer development.

In the subgroup analysis by ethnicity, we observed significantly increased risk in Caucasian with cervical cancer for TNF-α-308G>A polymorphism but not Asians and mixed-ancestry, and significantly decreased risk in cases of Caucasian for TNF-α-238G>A polymorphism rather than other population. To date, some possible reasons for these discrepancies should be considered despite it still have not been elucidated. First, different genetic backgrounds and the environment people lived in play a possible role in the mechanism of carcinogenesis. Another possible explanation for the ethnic discrepancy is that TNF-α-308G>A and -238G>A polymorphisms may be in linkage disequilibrium with the true causal polymorphism which is in a higher frequency in Caucasians. Third, there are only two studies on associations of TNF-α -308G>A and one study for -238G>A polymorphisms with cervical cancer susceptibility in Asian, mixed-ancestry population, respectively, which might not represent the general population, probably resulting in selective bias for this relationship. Remarkably, although an association was found between TNF-α-308G>A polymorphism and susceptibility to cervical cancer in African population, it seems insufficient statistical power to detect the association of the TNF-α-308G>A polymorphism with risk for cervical cancer due to only two studies including 264 cases and 270 controls conducted in African population, underpowered studies may contribute to false positive associations and misinterpretations. Hence, further studies based on larger sample size are still needed to re-evaluate the association in African population.

Significant between-study heterogeneity was observed in overall comparisons of TNF-α-308G>A. It is a potential problem when interpreting the results of the meta-analyses. To identify the source of heterogeneity, we stratified the studies according to ethnicity and source of controls, and found that the heterogeneity was effectively decreased or removed. These results suggested that subgroup analyses by different genetic backgrounds and sources of controls should be routinely conducted in meta-analyses of genetic association studies.

Some limitations of this meta-analysis should be acknowledged. First, cervical cancer is a multi-factorial disease resulting from complex interactions between environmental factors and genetic factors. Variants at TNF-α-308G>A and -238 G>A may play a crucial role in cancer progression, however, some environmental factors, such as HPV infection (zur Hausen, 2002) may predominate in cervical carcinogenesis development and mask the effects of the variants. Therefore, these factors should be taken into account to conclude a true effect. Second, there is limited sample size in some comparisons. For instance, concerning TNF-α-308G>A, only two studies involving 237 cases and 292 controls were conducted in Asians, and two studies including 264 cases and 270 controls in Africans. Thus, our results should be interpreted with caution. Further investigations with larger sample size are warranted. Third, publication bias was present in some comparisons of TNF-α-238G>A polymorphism, and may be distorting the meta-analysis. Fourth, four out of twelve studies for TNF-α-308G>A polymorphism were not in agreement with the HWE. Thus, the conclusion made for the polymorphic allele at the locus was not powerful. Finally, meta-analysis remains retrospective research that is subject to the methodological deficiencies of the included studies.

In conclusion, this meta-analysis suggests that TNF-α-238A allele significantly decreased the cervical cancer risk, and the TNF-α-308G>A polymorphism is associated with the susceptibility to cervical cancer in Caucasian and African population. To reach a definitive conclusion, gene-gene and gene-environment interactions studies based on larger sample size should be considered in future analysis.

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