Original Article

The association of *TNF*-308 (G/A) gene polymorphisms and hepatocellular carcinoma risk: a meta-analysis

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Background: Many studies have examined the association between the *TNF*-308 G/A polymorphism gene polymorphisms and hepatocellular carcinoma risk in various populations, but their results have been inconsistent. To assess this relationship more precisely, a meta-analysis was performed.

Methods: The PubMed and CNKI (China National Knowledge Infrastructure) database was searched for case-control studies. Odds ratios (OR) with 95% CIs were used to determine the strength of association between the *TNF*-308 G/A polymorphisms and HCC risk. The pooled ORs for the risk associated with the *TNF*-308 G/A genotype, the A carriers (A/G + A/A) *vs.* the wild-type homozygotes (G/G), A/A *vs.* G/G were calculated, respectively. Subgroup analyses were done by ethnicity and smoking status. Heterogeneity assumptions were assessed by chi-square-based *Q*-test.

Results: Ultimately, 21 studies, comprising 2,923 hepatocellular carcinoma cases and 4,323 controls were included. Overall, the A carriers (G/A + A/A) *vs.* the wild-type homozygotes (G/G), the pooled OR was 1.05 (95% CI, 0.93-1.19; P=0.000 for heterogeneity), for A/A *vs.* G/G the pooled OR was 1.07 (95% CI, 0.95-1.21; P=0.007 for heterogeneity). In the stratified analysis by ethnicity, the significantly risks were found among non-Asians. However, for Asians, significantly risks were not found.

Conclusions: The *TNF*-308 G/A polymorphisms are not associated with hepatocellular carcinoma risk among Asians, but for non-Asians.

Keywords: *TNF*-308; polymorphism; hepatocellular carcinoma; susceptibility

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Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide (748,300 new cases per year) and the third most common cause of cancer-related deaths causes (695,900 deaths per year) (1). Moreover, hepatocellular carcinoma is particularly burdensome in the Asia-Pacific region, and control policy or preventive interventions are urgently needed in this region (2,3). Though several major risk factors of hepatocellular carcinoma have been identified, including chronic infection of hepatitis B virus and hepatitis C virus, the etiology of hepatocellular carcinoma is still unclear (4). Current studies have shown genetic factors may also contribute to the etiology of hepatocellular carcinoma, and several genetic polymorphisms have been proven to be associated with increased risk of hepatocellular carcinoma (5).

Tumor necrosis factor (TNF) is one of the cytokines principally secreted by monocyte-macrophage lineage cells, and is a ubiquitous cytokine involved in various physiologic as well as pathologic processes such as inflammation, immune-regulation, proliferation, apoptosis and oncology. In 1992, the genomic polymorphism resulting in

substitution of the nucleotide adenine (A) for guanine (G) at position *TNF*-308 was discovered within a regulatory region of the TNF-α locus (6). The predominant homozygous allele, the heterozygous allele and the homozygous rare allele of the *TNF*-308 polymorphism are known as the homozygous wild-type genotype (G/G), the heterozygote (G/A) and the homozygote (A/A), respectively. Presence of the A substitution has been shown to result in increased binding of nuclear factors and enhanced transcription of the gene (7,8).

Recently, many studies have investigated the role of the *TNF*-308 G/A polymorphisms in HCC. However, the results of these studies remain inconclusive. A single study might not be powered sufficiently to detect a small effect of the polymorphisms on HCC, particularly in relatively small sample sizes. Various types of study populations and study designs might also have contributed to these disparate findings. To clarify the effect of the TNF-308 G/A polymorphism on the risk for HCC, we performed a meta-analysis of all eligible case-control studies that have been published.

Materials and methods

Publication search

We searched for studies in the PubMed and CNKI (China National Knowledge Infrastructure) electronic databases to include in this meta-analysis, using the terms "liver cancer" or "hepatocellular carcinoma" "TNF-α" or "TNF" and "polymorphism". An upper date limit of March 01, 2015 was applied; no lower date limit was used. The search was performed without any restrictions on language and was focused on studies that had been conducted in humans. We also reviewed the Cochrane Library for relevant articles. Concurrently, the reference lists of reviews and retrieved articles were searched manually. Only full-text articles were included. When the same patient population appeared in several publications, only the most recent or completes study was included in this meta-analysis.

Inclusion criteria

For inclusion, the studies must have met the following criteria: they (I) evaluated *TNF*-308 G/A polymorphisms and HCC risk; (II) were case-control studies; (III) supplied the number of individual genotypes for the TNF-308 G/A polymorphisms in HCC cases and controls, respectively; and (IV) demonstrated that the distribution of genotypes among controls were in Hardy-Weinberg equilibrium.

Data extraction

Information was extracted carefully from all eligible publications independently by two authors, based on the inclusion criteria above. Disagreements were resolved through a discussion between the two authors.

The following data were collected from each study: first author's surname, year of publication, ethnicity, total numbers of cases and controls, and numbers of cases and controls who harbored the *TNF*-308 G/A genotypes, respectively. If data from any category were not reported in the primary study, the items were designated "not applicable". We did not contact the author of the primary study to request the information. Different ethnicity descents were categorized as Asian, Caucasian and mixed population. We did not require a minimum number of patients for a study to be included in our meta-analysis.

Statistical analysis

Odds ratios (OR) with 95% CIs were used to determine the strength of association between the *TNF*-308 G/A polymorphisms and HCC risk. The pooled ORs for the risk associated with the *TNF*-308 G/A genotype, the A carriers (A/G + A/A) *vs.* the wild-type homozygotes (G/G), A/A *vs.* G/G were calculated, respectively. Subgroup analyses were done by ethnicity and smoking status. Heterogeneity assumptions were assessed by chi-square-based *Q*-test (9). A P value greater than 0.10 for the *Q*-test indicated a lack of heterogeneity among the studies. Thus, the pooled OR estimate of each study was calculated using the fixed-effects model (the Mantel-Haenszel method) (10); otherwise, the random-effects model (the DerSimonian and Laird method) was used (11).

One-way sensitivity analyses were performed to determine the stability of the results-each individual study in the meta-analysis was omitted to reflect the influence of the individual dataset on the pooled OR (12). Potential publication biases were estimated by funnel plot, in which the standard error of log (OR) of each study was plotted against its log (OR). An asymmetrical plot suggests a publication bias. Funnel plot asymmetry was assessed by Egger's linear regression test, a linear regression approach that measures the funnel plot asymmetry on a

Table 1 Distribution of *TNF*-308 G/A genotypes among hepatocellular carcinoma cases and controls included in this meta-analysis

First author (ref)	Ethnicity (country of origin)	Total sample (case/control)	HCC			Controls		
			GG	GA	AA	GG	GA	AA
Teixeira *et al.* (14)	Non-Asian(Brazil)	111/202	81	30[#]	–	173	3	26
Radwan *et al.* (15)	Non-Asian (Egypt)	128/160	82	42	4	136	24	–
Shi *et al.* (16)	Asian (China)	73/116	51	20	2	84	30	2
Yang *et al.* (17)	Asian(China)	620/625	496	118	6	510	108	7
Talaat *et al.* (18)	Non-Asian (Egypt)	45/45	42	2	1	25	13	7
Shi *et al.* (19)	Asian (China)	88/88	30	43	15	45	35	8
Chen *et al.* (20)	Asian (China)	126/126	118	8	0	111	14	1
Wang *et al.* (21)	Asian (China)	70/96	62	8	0	85	11	0
Ognjanovic *et al.* (22)	Non-Asian (USA)	120/230	90	28[#]	–	225	176	49
Akkiz *et al.* (23)	Non-Asian (Turkey)	110/110	72	35	3	110	99	11
Jeng *et al.* (24)	Asian (China)	108/108	80	27	1	100	8	0
Sakamoto *et al.* (25)	Asian (Japan)	209/656	205	4	0	641	15	0
Jeng *et al.* (26)	Asian (China)	108/108	80	28[#]	–	108	100	8
Kummee *et al.* (27)	Asian (Thailand)	50/350	42	8	0	291	58	1
Migita *et al.* (28)	Asian (Japan)	48/188	47	1	0	183	5	0
Niro *et al.* (29)	Non-Asian (Italy)	30/96	24	6[#]	–	96	75	21
Chen *et al.* (30)	Asian (China)	572/381	468	95	9	311	67	3
Ho *et al.* (31)	Asian (China)	74/289	37	34	3	225	62	2
Wang *et al.* (32)	Asian (Japan)	125/204	109	15	1	179	23	2
Heneghan *et al.* (33)	Asian (China)	98/97	88	10	0	158	13	1
Ben *et al.* (34)	Non-Asian (USA)	10/48	9	1[#]	–	48	42	6

GG indicates wild-type, GA indicates heterozygote, AA indicates variant homozygote. [#], the number of the combined GA and AA genotypes.

natural logarithm scale of the OR. The significance of the intercept was determined by *t*-test, as suggested by Egger *et al*. (P<0.05 was considered a statistically significant publication bias) (13). All calculations were performed using STATA, version 11.0 (Stata Corporation, College Station, TX, USA).

Results

Study characteristics

A total of 21 publications involving 2,923 HCC cases and 4,323 controls met the inclusion criteria and were ultimately analyzed (14-34). *Table 1* presents the main characteristics of these studies. Among the 21 publications, 20 were published in English. The sample sizes ranged from 58 to 1,245. The controls were primarily healthy populations and matched for age, ethnicity, and smoking status. There were 14 groups of Asians. All polymorphisms in the control subjects were in Hardy-Weinberg equilibrium.

Meta-analysis results

Table 2 listed the main results of this meta-analysis. Overall, the A carriers (G/A + A/A) *vs.* the wild-type homozygotes (G/G), the pooled OR for all studies combined 2,923 hepatocellular carcinoma cases and 4,323 controls was 1.05 (95% CI, 0.93-1.19; P=0.000 for heterogeneity), for A/A *vs.* G/G the pooled OR was 1.07 (95% CI, 0.95-1.21; P=0.007 for heterogeneity) (*Figure 1*). In the stratified analysis by ethnicity, significantly risks were not found among Asians for (G/A + A/A) *vs.* (G/G) (OR =0.90, 95% CI, 0.77-1.04; P=0.000 for heterogeneity) or A/A *vs.* G/G (OR =0.96; 95% CI, 0.78-1.13; P=0.003 for heterogeneity). However, for non-Asians, significantly risks were found for (G/A + A/A)

Table 2 Summary ORs for various contrasts of TNF-308 G/A polymorphisms in this meta-analysis

Variables	Number of studies	(G/A + A/A) *vs.* G/G		A/A *vs.* G/G	
		OR (95% CI)	P (*Q*-test)	OR (95%CI)	P (*Q*-test)
Total	21	1.05 (0.93-1.19)	0.000	1.07 (0.95-1.21)	0.007
Asian	14	0.90 (0.77-1.04)	0.000	0.96 (0.78-1.13)	0.003
Non-Asian	7	1.48 (1.19-1.85)	0.000	1.44 (1.13-1.74)	0.006

P (*Q*-test), P value of *Q*-test for heterogeneity test; CI, confidence interval; OR, odds ratio.

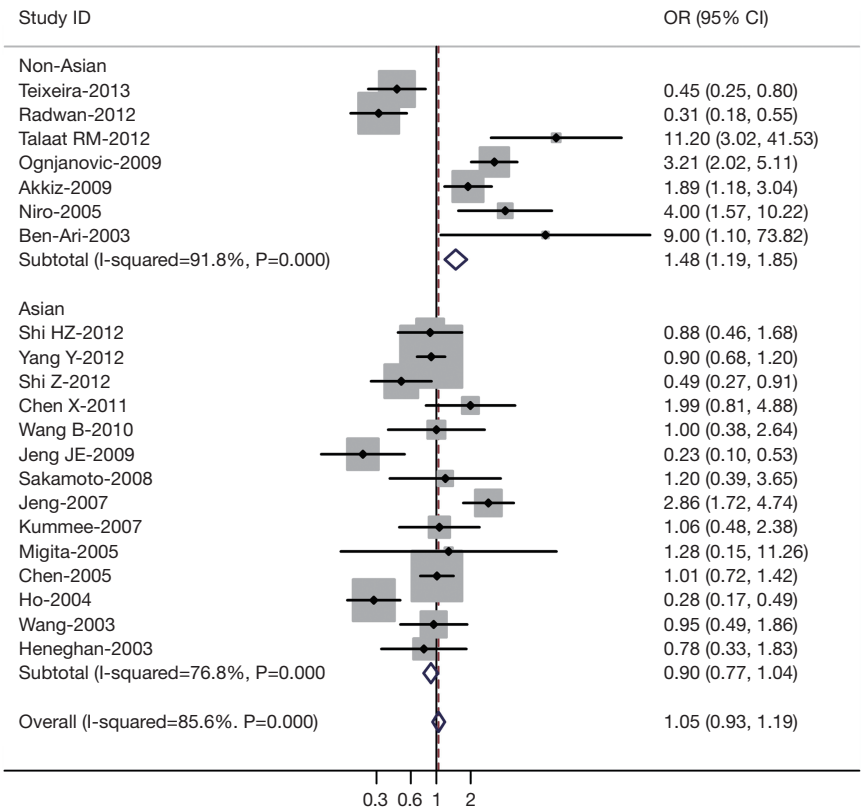

Figure 1 Forest plot (random-effects model) of HCC risk associated with *TNF*-308 G/A polymorphisms for the A carriers (G/A + A/A) *vs.* the wild-type homozygotes (G/G). Each box represents the OR point estimate, and its area is proportional to the weight of the study. The diamond (and broken line) represents the overall summary estimate, with CI represented by its width. The unbroken vertical line is set at the null value (OR =1.0).

vs. (G/G) (OR =1.48, 95% CI, 1.19-1.85; P=0.000 for heterogeneity) and A/A *vs.* G/G (OR =1.44; 95% CI, 1.13-1.74; P=0.006 for heterogeneity).

Sensitivity analyses

A single study involved in the meta-analysis was deleted each time to reflect the influence of the individual data set to the pooled ORs, and the corresponding pooled ORs were not materially altered (data not shown).

Publication bias

Begg's funnel plot and Egger's test were performed to access the publication bias of literatures. Evaluation of publication bias for (G/A + A/A) *vs.* (G/G) showed that

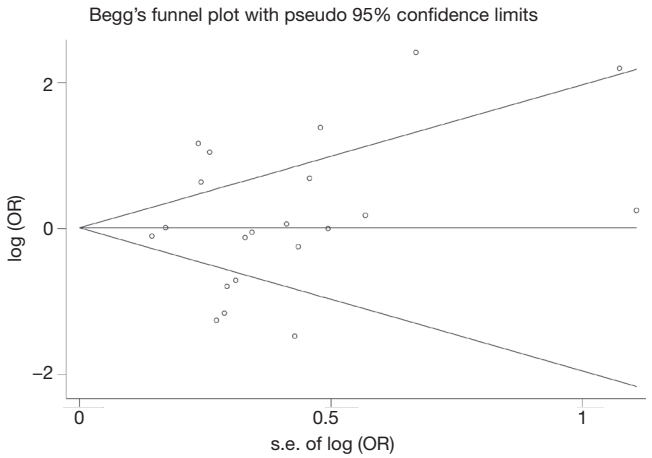

Figure 2 Begg's funnel plot of *TNF*-308 G/A polymorphism and HCC risk for the A carriers (G/A + A/A) versus the wild-type homozygotes (G/G).

the Egger test was significant (P=0.025). Meanwhile, for A/A *vs.* G/G the publication bias was found (P=0.018). The funnel plots for publication bias (*Figure 2*) also showed some asymmetry. These results indicated a potential for publication bias.

Discussion

Carcinogenesis of HCC is a complex, multistep and multi-factor processes, in which many factors are implicated. Although many environmental factors are found to correlate with the tumorigenesis of HCC (35,36), the risk factors still need to be further elucidated. As we know, chronic infection with HBV or HCV is the most well established environmental risk factor for HCC worldwide. However, only a fraction of HBsAg carriers eventually develop HCC and only 2.5% of HCV infected individuals develop HCC later in life (37). Moreover, there are a portion of patients without known risk factors who eventually developed HCC (38), suggesting the inter-individual differences in susceptibility. A previous study had shown an interaction of environmental factors and genetic predisposition in the development of HCC (39). Therefore, genetic predisposition may contribute to the process of tumorigenesis.

This meta-analysis explored the association between the *TNF*-308 G/A gene polymorphisms and HCC risk. Our results indicated that *TNF*-308 G/A gene polymorphism were significantly associated with the susceptibility to HCC among non-Asians but not for Asians. However, our results were inconsistent with the previous meta-analysis (40,41) showed that the *TNF*-308 G/A polymorphisms may be associated with HCC among Asians. Since then, several additional studies with large cohort populations have been reported. Simple differences in cohort populations and study design may also contribute to the disparate findings. We have improved upon that previous meta-analysis by including more recent related studies and by generally using a more comprehensive search strategy. Screening, study selection, and data extraction were performed independently and reproducibly by two reviewers. We also explored heterogeneity and potential publication bias in accordance with published guidelines.

Some limitations of this meta-analysis should be acknowledged. First, heterogeneity can interfere with the interpretation of the results of a meta-analysis. Although we minimized this likelihood by performing a careful search of published studies, using explicit criteria for a study's inclusion and performing strict data extraction and analysis, significant interstudy heterogeneity nevertheless existed in nearly every comparison. The presence of heterogeneity can result from differences in the selection of controls, age distribution, and prevalence of lifestyle factors. Although most controls were selected from healthy populations, some studies had selected controls among friends or family members of lung cancer patients or patients with other diseases. Further, only published studies were included in this meta-analysis. The presence of publication bias indicates that non-significant or negative findings might be unpublished. Finally, our results were based on unadjusted estimates; a more precise analysis should have been conducted if individual data were available, which would have allowed us to adjust using other covariates, including age, ethnicity, family history, environmental factors, and lifestyle.

Despite these limitations, this meta-analysis suggests that the *TNF*-308 G/A polymorphisms are not associated with HCC risk among Asians; however the gene polymorphisms are associated with non-Asians. Large-sample studies of different ethnic groups with carefully matched cases and controls should be considered in future association studies to confirm the results of our meta-analysis.

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None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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