Tumor necrosis factor rs361525 (-238G>A) polymorphism contributes to hepatocellular carcinoma susceptibility

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ABSTRACT

الأهداف: تحديد العلاقة الموجودة بين التعدد الشكلي الجيني لمعامل تنخر الأورام (TNF)-238G>A) وزيادة خطر الإصابة بسرطان الخلايا الكبدية.

الطريقة: أُجريت هذه الدراسة المقطعية بمستشفى زونجشان في جامعة فيودان، شنغهاي، الصين وذلك خلال الفترة من ديسمبر 2009م إلى مايو 2010م. لقد عملنا بحثاً شاملاً لجميع الدراسات التي تناولت دراسة العلاقة بين معامل تنخر الأورام TNF rs361525 -238G>A وزيادة خطر الإصابة بسرطان الخلايا الكبدية. وتم تحديد نموذج المؤثرات الثابتة (fixed effect model)، ونموذج المؤثرات العشوائية (random effect model) وذلك اعتماداً على اختبار التجانس (homogeneity) بين هذه الدراسات التي تضمنها التحليل الجمعي (meta analysis)، في حين تم تحديد التباين بين هذه الدراسات بواسطة اختبار كيو (Q test)، وI^2، وتم تعيين تحيز النشر (publication bias) بواسطة اختبار إيغير للانحدار الخطي.

النتائج: شمل هذا التحليل 708 حالة مصابة بسرطان الخلايا الكبدية و1349 حالة غير مُصابة، وقد أشار إلى وجود علاقة كبيرة بين معامل تنخر الأورام (TNF rs361525 (-238G>A) وزيادة خطر الإصابة بهذا السرطان وذلك مع اختلاف النماذج الجينية التالية: مقارنة تباين الزيجوت: (OR=1.70, 95% CI: 1.20-2.35, P heterogeneity=0.292)، ومقارنة النموذج السائد: (OR=1.69, 59% CI: 1.20–2.35, P heterogeneity=0.270)، ومقارنة النموذج السائد كلياً: (OR=1.62, 95% CI: 1.16–2.28, P heterogeneity=0.391)، ومقارنة الأليل: (OR=1.62, 95% CI: 1.18–2.23, P heterogeneity=0.253)، وبعد القيام بتحليل هذه النماذج الأربعة لم يتم تعيين كلاً من التباين أو تحيز النشر.

خاتمة: يُثبت هذا التحليل الجمعي وجود العلاقة بين التعدد الشكلي الجيني لمعامل تنخر الأورام TNF rs361525 -238G>A وزيادة خطر الإصابة بسرطان الخلايا الكبدية في أحد المجتمعات الصينية.

Objective: To assess the effect of the common polymorphisms of the tumor necrosis factor (TNF)-238G>A with hepatocellular carcinoma (HCC) risks.

Methods: The study design was cross-sectional, and carried out in Zhongshan Hospital Fudan University, Shanghai, China from December 2009 to May 2010. A comprehensive search was conducted to identify all studies on the association of TNF rs361525 (-238G>A) polymorphism with HCC risk. The fixed or random effect pooled measure was selected based on homogeneity testing among studies. Heterogeneity among studies was evaluated using Q test and I^2. Publication bias was estimated using a modified Egger's linear regression test.

Results: This current analysis including 708 HCC and 1,349 controls on TNF rs361525 (-238G>A) showed a significantly increased risk of HCC in different genetic models (heterozygote comparison: odds ratio [OR]=1.70, 95% confidence interval [CI]: 1.21-2.39, P heterogeneity=0.292; dominant model comparison: OR=1.68, 95% CI: 1.20-2.35, P heterogeneity=0.270; complete overdominant model comparison: OR=1.62, 95% CI: 1.16-2.28. P heterogeneity=0.391; and allele comparison: OR=1.62, 95% CI: 1.18-2.23, P heterogeneity=0.253). Neither heterogeneity nor publication bias was detected when analyses were performed on all 4 models.

Conclusion: This meta-analysis supports TNF rs361525 (-238G>A) polymorphism being associated with HCC in an Asian population.

Saudi Med J 2010; Vol. 31 (10): 1101-1105

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Received 7th July 2010. Accepted 6th September 2010.

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Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third most common cause of cancer death.[1] Studies suggest that HCC is related to many factors, including chronic hepatitis B or C caused by viral infection, exposure to aflatoxin, and genetic polymorphism, among which variants of pro- and anti-inflammatory cytokines such as interleukin (IL) and tumor necrosis factors (TNF) are the most extensively investigated. Tumor necrosis factors play a critical role in the pathogenesis of HCC.[2] The TNF are thought to be determined largely by polymorphisms at different positions within the regulatory regions or signal sequences of the TNF gene,[3] which have been mapped to the class III region of the major histocompatibility complex (MHC) between the HLA-B and -DR loci. Recent studies have reported the presence of at least 44 polymorphisms of the TNF gene.[4] Polymorphisms located at positions –238(G>A) of the TNF promoter region (rs361525) appear to notably influence the expression of TNF, and are considered as functional single nucleotide polymorphisms (SNPs).[5] However, previous observations in various populations have produced conflicting results regarding the effect of TNF genetic variation on the risk of developing HCC.[6-11] The objective, therefore, of this meta-analysis was to explore the association of TNF rs361525 (-238G>A) polymorphisms with the risk of HCC.

Methods. The study design was cross-sectional and carried out in Zhongshan Hospital Fudan University, Shanghai from December 2009 to May 2010. Ethical approval was obtained from the Zhongshan Hospital Medical Ethics Committee.

Search strategy. A search to May 2010 was conducted for relevant available articles published in English or Chinese from 6 databases, namely, PubMed, ISI Web of Knowledge, HuGE Navigator Web, CNKI (China National Knowledge Infrastructure), VIP (Database of Chinese Scientific and Technical Periodicals) and CBM (China biomedical literature database) using key words such as: "TNF" or "tumor necrosis factor"; "liver cancer" or "hepatocellular carcinoma"; and "polymorphism" or "variation". Eligible studies included had to meet the following criteria: (a) an unrelated case-control study and (b) available genotype frequency.[12]

Data extraction. The following information was extracted from each study: first author, year of publication, ethnicity (nationality) of study population, and characteristics of the patients and the controls.

Statistics. Allele frequencies of the TNF rs361525 (-238G>A) polymorphism in the studies were determined using the allele counting method. The chi-square test was used to determine whether observed genotype frequencies conformed to the Hardy–Weinberg equilibrium (HWE). Crude odds ratios (OR) with 95% confidence intervals (CI) were used to assess the relationship between the TNF rs361525 (-238G>A) polymorphism and HCC risk. Because of the small sample sizes in individual studies, low frequency of variant homozygotes, and practical clinical value, meta-analyses were performed using the 4 models: heterozygote comparison (AG versus GG), dominant model (AG+AA versus GG), complete overdominant models (AG versus GG+AA) and additive model (A versus G). The heterogeneity was formally investigated by Q-testing and the I^2 statistic. For the Q statistic, if $p<0.10$, the heterogeneity was considered statistically significant. The I^2 metric is independent of the number of studies in the meta-analysis (I^2=0, no heterogeneity; I^2=25%, low heterogeneity; I^2=50%, moderate heterogeneity; I^2=75%, high heterogeneity).[13] The I^2 describes the proportion of total variation attributable to between-study differences or heterogeneity as opposed to random error or chance. In the presence of substantial heterogeneity (I^2>50%), the DerSimonian and Laird random effect model was adopted as the pooling method, in other cases, the inverse-variance fixed effect model was used. These 2 models provided similar results when between-study heterogeneity was absent. Furthermore, meta-regression with restricted maximum likelihood estimation was performed to explore the potentially important sources of between-study heterogeneity. Publication bias was evaluated with Begg's test for the funnel plot and Egger's linear regression asymmetry test. Sensitivity analyses were performed to assess the stability of the results. All statistical analyses were performed by Stata 10.0 software (Stata Corp, Fudan University, Shanghai, China).

Results. ***Eligibility.*** Six case-control studies (published from 2003 to May 2010) on the association between TNF rs361525 (-238G>A) polymorphism and HCC risk, including 708 HCC and 1,349 controls, were selected from publication searching.[6-11] The main characteristics of these studies are presented in **Table 1**. Of the 6 studies, 5 were published in English and one

Disclosure. This study was financially supported by the National Basic Research Program of China (2007CB936000), the Major National Science and Technology Projects (2009ZX10004-301, 2008ZX10002-017), the Shanghai Science and Technology Commission (10410709400, 10411950100), the Shanghai Talent Development Foundation (2009-035), and the National Nature Science Foundation of China (No. 30772505 & No. 30872503).

Table 1 - Pathogens isolated from the ascitic fluid of cirrhotic patients with SBP from 1996-2002.

Author (year)	Ethinicity (Country)	Case n	Distribution of TNF-238G>A genotype						Frequency of TNF-238G>A alleles				HWE *P*-value
			Cases			Control			Cases		Controls		
			GG n (%)	AG n (%)	AA n (%)	GG n (%)	AG n (%)	AA n (%)	G n (%)	A n (%)	G n (%)	A n (%)	
Jung (2009)[10]	Korea	227/365	193 (85)	34 (15)	0	336 (92.1)	28 (7.7)	1 (0.2)	420 (92.5)	34 (7.3)	700 (95.9)	30 (4.1)	0.61
Huang (2007)[11]	China	100/150	88 (88)	12 (12)	0	143 (95.3)	7 (4.7)	0	188 (94)	12 (6)	293 (97.7)	7 (2.3)	0.77
Kummee (2007)[9]	Thailand	50/350	44 (88)	5 (10)	1 (2)	328 (93.7)	21 (6)	1 (0.3)	93 (93)	7 (7)	677 (96.7)	23 (3.3)	0.30
Jeng (2007)[8]	Taiwan	108/108	102 (94.4)	6 (5.5)	0	106 (98.1)	2 (1.9)	0	210 (97.2)	6 (3.8)	214 (99.1)	2 (0.9)	0.92
Wang (2003)[7]	Japan	125/204	111 (88.8)	13 (10.4)	1 (0.8)	178 (87.3)	24 (11.8)	2 (0.9)	235 (94)	15 (6)	380 (93.1)	28 (6.9)	0.25
Heneghan (2003)[6]	China	98/172	96 (98)	2 (2)	0	168 (97.7)	4 (2.3)	0	194 (99)	2 (1)	340 (98.8)	4 (1.1)	0.88
Total		**708/1349**	**634 (90)**	**72 (10.1)**	**2 (0.3)**	**1259 (93.3)**	**86 (6.4)**	**4 (0.3)**	**1340 (94.6)**	**76 (5.4)**	**2604 (96.5)**	**94 (3.5)**	

Table 2 - Meta-analysis results of the association of TNFA-238G>A polymorphisms and HCC of 6 studies.

Genetic model	OR	95% confidence interval	P value for herterogeneity	I^2	Egger's *P*-value
Heterozygote comparison AG/GG	1.70	1.21-2.39	0.292	18.70	0.98
Dominant model (AA+AG)/GG	1.68	1.20-2.35	0.270	21.80	0.909
Complete overdominant AG/(AA+GG)	1.62	1.16-2.28	0.391	3.80	0.973
Additive model A/G	1.62	1.18-2.23	0.253	24.10	0.789

study was published in Chinese;[11] the case patients in one study were all hepatitis B surface antigen (HBsAg) positive.[9] In 2 of the studies, case patients were partly HBsAg positive.[6,11] In another study, all patients suffered from chronic infection with hepatitis C virus (HCV).[7] In 2 of the studies, case patients were HBsAg positive as well as chronically infected with HCV.[8,10] The patients in all 6 studies were entirely or partly positive for HBsAg and/or HCV. The control groups of the 6 studies were all matched with the same character. Three studies also included a healthy control group that included people who were infected with neither hepatitis B nor C virus.[6,7,9]

Meta-analysis results. The variant genotypes and allele frequencies of TNF rs361525 (-238G>A) in the individual study are presented in **Table 1**. The AA genotype frequency was observed as being too low; among most studies no AA homozygous individuals were present in the panel. Overall, for TNF rs361525 (-238G>A) polymorphism, the frequency of GG homozygous individuals was 90%. The frequency of AG-heterozygous individuals was 10%, and the frequency of AA-homozygous individuals was 0.3% in the cases studied. In the controls, the frequencies of GG homozygous individuals was 93.3%, of AG heterozygous individuals was 6.4%, and of AA homozygous individuals was 0.3%. The G allele was the most common for TNF rs361525 (-238G>A) polymorphism. The results of the pooled analysis are summarized in **Table 2**. The results indicate the statistically significant association between the increased HCC risk and variant

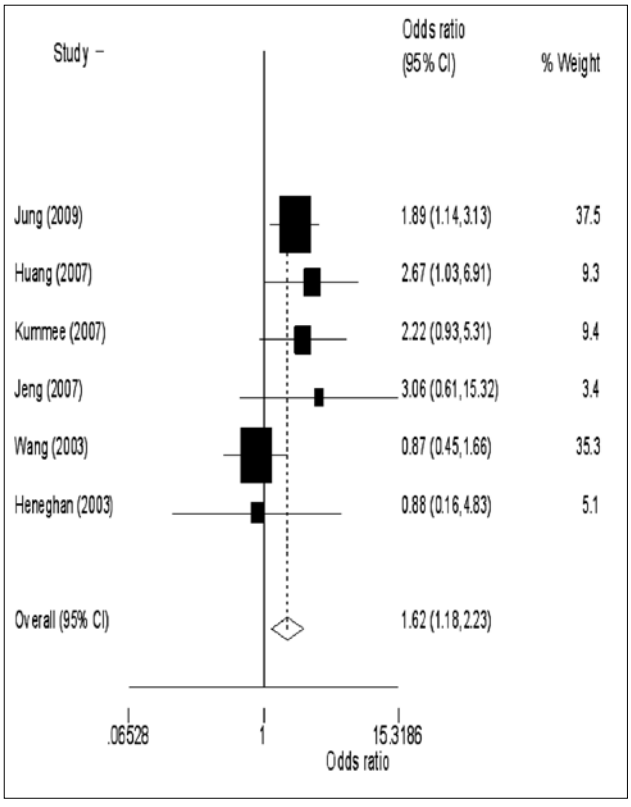

Figure 1 - Forest plot of hepatocellular carcinoma risk associated with the TNF-238G>A polymorphism (A/G). The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the study-specific weight (inverse of the variance). The diamond represents the pooled OR and 95% CI.

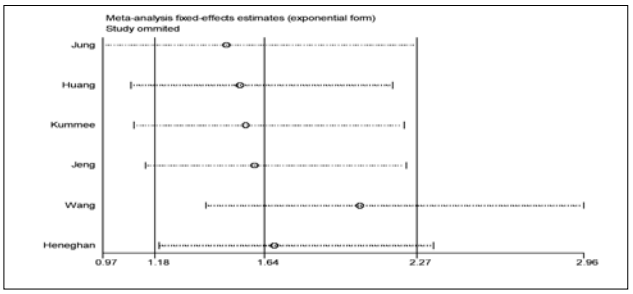

Figure 2 - Sensitivity analysis: examining the influence of individual studies of 9 studies (A/G). This figure shows the influence of each study on the meta-analysis, in which the meta-analysis estimates are computed by omitting one study at a time. By default, fixed-effects analyses are displayed.

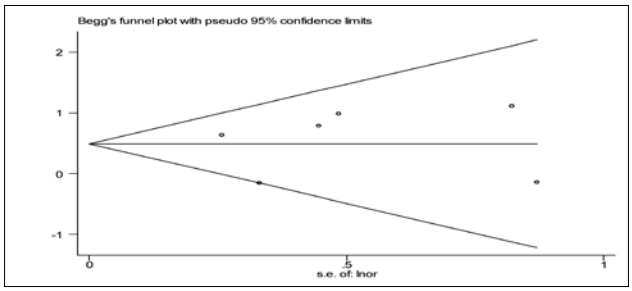

Figure 3 - Begg's funnel plot for publication bias test (A/G). Each point represents a separate study for the indicated association. lnOR logarithmically transformed of odds ratio. Horizontal line, mean effect size.

genotypes in different genetic models. In heterozygote comparison, the variant genotype AG was associated with a statistically significant increased risk of HCC (**Table 2**). In addition, the significant main effects were also observed in both dominant and complete overdominant models (**Table 2**). Notably, the A allele was associated with an increased HCC risk compared with the G allele - additive model (**Figure 1**). There was no evidence of heterogeneity among studies using all 4 models or comparisons: P heterogeneity reached 0.253-0.391 and I^2 reached 3.8-24.1%, all below 25% (**Table 2**). Sensitivity analysis showed that the association between TNF rs361525 (-238G>A) and the risk of HCC of the 6 studies was robust: when anyone study was omitted, the 95% CI would not include 1.0 under all models. (**Figure 2**). Funnel plots and Egger's test were performed to assess publication bias (**Figure 3**). The data suggest that there was no evidence of publication bias: Egger's *p*-value reached 0.789-0.980 under various models (**Table 2**).

Discussion. Tumor necrosis factor (TNF) is a pleiotropic inflammatory cytokine with procarcinogenic properties. It is located on chromosome 6 at 6p21.3 and encodes within the MHC. Recent reports suggest that the MHC may contain tumor susceptibility genes and genetic elements that may contribute to some tumors.[14,15] The TNF rs361525 (-238G>A) gene polymorphism has been reported as being associated with several cancers including HCC;[8,16,17] studies of an association of TNF rs361525 (-238G>A) gene polymorphism with HCC have reported conflicting results.[6-11] This discrepancy among results is not surprising. Persistent difficulties in obtaining robust and replicable results in genetic effects are small, requiring studies with many thousands of subjects or meta-analyses to be detected.[18]

In the present study, a meta-analysis of 6 published studies was conducted to evaluate the association between TNF rs361525 (-238G>A) polymorphisms and HCC risk because no such meta-analysis has been published to date. In overall combination studies, it is found that the variant AG genotype was associated with a significant increased HCC risk in all 4 compared genetic models.

The TNF exhibits pro tumorigenic activity,[19] and it has been shown that dysregulation and overproduction of TNF could be involved in cancer development and progression.[20] Blood levels of TNF are seen to be significantly higher in patients with solid tumors including HCC.[19,21] In recent years, several TNF promoter polymorphisms have been identified and their association with susceptibility to solid tumors as well as with high TNF expression has been documented.[22-24] Notably, polymorphisms located at positions –238 of the TNF promoter region appear to influence the transcription and expression of TNF and is considered as a functional single nucleotide polymorphism (SNP).[5] The rare A allele has been shown to be associated with high production, suggesting that the TNF rs361525 (-238G>A) A allele may be a risk allele for HCC.[25] This meta-analysis found that the variant genotypes were associated with a significantly increased HCC risk in all 4 genetic models, namely, heterozygote comparison, dominant model, complete overdominant model, and additive model, suggesting that the TNF rs361525 (-238G>A) AG genotype or A allele is a risk allele for HCC, which is consistent with experimental findings. Another meta-analysis has reported that TNF rs361525 (-238G>A) polymorphism is associated with psoriatic arthritis,[26] which was consistent with the results recorded for this meta-analysis. Interestingly enough, neither significant heterogeneity nor publication bias was detected in all 4 compared genetic models performed on TNF rs361525 (-238G>A) polymorphism status, indicating that the pooled results may be robust and unbiased.

Some limitations of this meta-analysis should be addressed. First, the numbers of published studies were not sufficiently large enough for a comprehensive analysis. Second, this analysis only includes 6 studies. Third, all of the 6 studies in this meta-analysis were conducted on Asian populations. Despite these limitations, our meta-analysis also had some advantages. First, a substantial numbers of cases and controls were pooled from different studies, which significantly increased the statistical power of the analysis. The quality of case–control studies included in our current meta-analysis was satisfactory and met the established inclusion criterion.[6-11] Third, we did not detect any heterogeneity and publication bias indicating that the whole pooled result may be robust and unbiased.

In conclusion, this meta-analysis suggests that the TNF rs361525 (-238G>A) polymorphism is associated with HCC in Asian populations. Larger studies including different ethnic groups with a careful matching between HCC patients and controls should be considered in the future to confirm results obtained from this meta-analysis, and to further evaluate the effect of HCC status on the TNF rs361525 polymorphisms-associated HCC risk.

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