RESEARCH ARTICLE

Tumor Necrosis Factor-α 238 G/A Polymorphism and Risk of Hepatocellular Carcinoma: Evidence from a Meta-analysis

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Abstract

Background: Tumor necrosis factor-α (TNF-α) plays a very important role in the development and progression of cancer. Many epidemiological studies have evaluated associations between the TNF-α 238 G/A polymorphism and hepatocellular carcinoma (HCC) risk, but the published data are inconclusive. Therefore, we performed the present meta-analysis. Methods: Electronic searches of several databases were conducted for all publications on the association between TNF-α 238 G/A polymorphism and HCC through July 2012. Asummary odds ratio (OR) with its 95% confidence interval (CI) were calculated to evaluate the strength of this association. Results: Eleven case-control studies with a total of 1,572 HCC cases and 1,875 controls were finally included in this meta-analysis. Overall, the TNF-α 238 G/A polymorphism was significantly associated with increased risk of hepatocellular carcinoma in three genetic comparison models (For A versus G: OR 1.32, 95%CI 1.04-1.69, *P* = 0.02, I^2 = 40%; for AG versus GG: OR 1.32, 95%CI 1.02-1.71, *P* = 0.03, I^2 = 40%; for AA/AG versus GG: OR 1.33, 95%CI 1.03-1.72, *P* = 0.03, I^2 = 41%) when all studies were pooled. Subgroup analysis by ethnicity further showed that there was a significant association between the TNF-α 238 G/A polymorphism and risk of HCC in Asians under three genetic comparison models (For A versus G: OR 1.30, 95%CI 1.00-1.68, *P* = 0.05, I^2 = 45% for AA/AG versus GG: OR 1.31, 95%CI 1.00-1.71, *P* = 0.05, I^2 = 46%). Conclusions: This meta-analysis provided convincing evidence that the TNF-α 238 G/A polymorphism is associated with increased susceptibility to HCC. However, more well-designed studies with large sample size are needed to validate this association in Caucasians.

Keywords: Tumor necrosis factor-α - hepatocellular carcinoma - single nucleotide polymorphism - meta-analysis

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Introduction

Liver cancer is the sixth most common cancer worldwide (748,300 new cases per year) and the third most common cause of cancer-related deaths causes (695,90 0 deaths per year) (Jemal et al., 2011). Hepatocellular carcinoma, which is the dominant histological type of liver cancer, accounts for 70-85% of primary malignancies in liver (El-Serag, 2011). Besides, hepatocellular carcinoma is particularly burdensome in the Asia-Pacific region, and control policy or preventive interventions are urgently needed in this region (Bridges et al., 2011; Wiangnon et al., 2012). 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 (Harkisoen et al., 2012). 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 (Wang et al., 2010a). Tumor necrosis factor-α (TNF-α) is the most important pro-inflammatory cytokine involved in the growth, differentiation, cellular function and survival of many cells, and it has been reported to play an important role in the pathogenesis of cancer (Walczak, 2011; Aggarwal et al., 2012). There are several common single nucleotide polymorphisms (SNP) in the TNF-α gene, including −238 (rs361525), −308 (rs1800629), −857 (rs1799724), and −1031 (rs1799964) positions, which could regulate TNF-α transcription and production (Qidwai et al., 2011). It has been well demonstrated that those SNPs can affect the functions of relevant proteins and further influence the host susceptibility to various cancers (Fan et al., 2011; Wang et al., 2011). Many epidemiological studies have evaluated the association between TNF-α 238 G/A polymorphism and hepatocellular carcinoma risk (Heneghan et al., 2003; Wang et al., 2003; Niro et al., 2005; Jeng et al., 2007; Kummee et al., 2007; Jeng et al., 2009; Wang et al., 2010b; Chen et al., 2011). However, published data were contradictory, and the association between TNF-α 238 G/A polymorphism and hepatocellular carcinoma risk was still inconclusive Therefore, we conducted a systematic review and meta-analysis to get a more precise estimate of the association between TNF-α 238 G/A polymorphism and hepatocellular carcinoma risk.

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Materials and Methods

Identification and eligibility of relevant studies

We searched Pubmed, EMBASE and Wangfang Medicine databases for all possible case-control studies. We used the keywords and subject terms: "hepatocellular carcinoma" or "liver cancer" or "liver tumor") and ("polymorphism" or "variant" or "genotype" or "polymorphism") and ("tumor necrosis factor" or "TNF-α" or "rs361525"), and the literature search was last updated on 15 July, 2012. There was no language limitation in the literature search. All eligible studies were retrieved, and their bibliographies were checked for other relevant publications. Review articles and bibliographies of other relevant studies identified were searched by hand to find additional eligible studies. The following criteria were used to select the eligible studies: (1) case-control studies involving the association between TNF-α 238 G/A polymorphism and hepatocellular carcinoma risk; (2) availability of genotype frequencies of TNF-α 238 G/A polymorphism for estimating an odds ratio (OR) with 95% confidence interval (CI) or OR with its 95%CI. If two or more studies reported the same patients populations, only the most recent or complete study was included into this meta-analysis. Reviews, non-case–control studies or studies involving cancer cells and animal models were all excluded from this meta-analysis.

Data extraction

The final eligible articles selected for meta-analysis were carefully evaluated independently by two reviewers. Discrepancies were adjudicated by the consensus among all reviewers. Data retrieved from the reports included first author's name, publication year, ethnicity of study population (categorized as Caucasians and Asians), study-design (sources of controls), genotyping method, types of cancer, number of cases and controls, and genotype frequencies of TNF-α 238 G/A polymorphism.

Statistical methods

Hardy-Weinberg equilibrium (HWE) in the controls was tested by a Chi-square test which compared the observed and expected genotype frequencies of the controls (Salanti et al., 2005). To get a more comprehensive assessment of the association between TNF-α 238 G/A polymorphism and hepatocellular carcinoma susceptibility, five different comparison model were used: the allele comparison model (A versus G), the homozygote comparison model (AA versus GG), the heterozygote comparison model (AG versus GG), the dominant genetic model (AA/AG versus GG), and the recessive genetic model (AA versus AG/GG). The I^2 statistic to quantify the proportion of the total variation due to heterogeneity were calculated, and a I^2 value of more than 50% was interpreted as significant heterogeneity among studies (Higgins et al., 2003). When the effects were assumed to be homogenous, the fixed-effects model was used (Mantel-Haenszel method) (Mantel et al., 1959). If obvious heterogeneity was present, the random-effects model was used (DerSimonian-Laird method) (DerSimonian et al., 1986). The statistical significance of the summary OR was determined using the Z-test and a *P* value of less than 0.05 was considered significant. Stratified analyses for different types of cancers were conducted to estimate cancer-specific ORs. Stratified analyses by ethnicity were also performed to estimate ethnic-specific ORs. Publication bias was evaluated with funnel plot. All analyses were conducted using Review Manager (version 5.1.0; The Cochrane Collaboration, Oxford, England), and *P* values were two sided.

Results

Characteristics of studies

137 abstracts were identified from Pubmed, EMBASE and Wangfang Medicine databases. According to eligibility criteria, only eleven case-control studies with a total of 1,572 hepatocellular carcinoma cases and 1,875 controls were finally included into this meta-analysis (Heneghan et al., 2003; Wang et al., 2003; Niro et al., 2005; Huang et al., 2007; Jeng et al., 2007; Kummee et al., 2007; Jeng et al., 2009; Jung et al., 2009; Wang et al., 2010b; Chen et al., 2011; Wang et al., 2012). These studies were published between 2003 and 2012 and all were hospital-based case-control studies. There were 10 studies from Asians and only one study from Caucasians (Niro et al., 2005). All publications were written in English except for two studies in Chinese (Huang et al., 2007; Wang et al., 2012). The most common testing method was the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), which was used in 8 of those 11 studies. The genotype distributions in the controls of all studies were in agreement with HWE.

Meta-analysis

There was no obvious between-study heterogeneity in all five comparison models (All $I^2 < 50\%$), thus the fixed-effects model was used to pool the data. Overall, TNF-α 238 G/A polymorphism was significantly associated with increased risk of hepatocellular carcinoma in three genetic comparison models (For A versus G: OR 1.32, 95%CI 1.04-1.69, $P = 0.02$, $I^2 = 40\%$; for AG versus GG: OR 1.32, 95%CI 1.02-1.71, $P = 0.03$, $I^2 = 40\%$; for AA/AG versus GG: OR 1.33, 95%CI 1.03-1.72, $P = 0.03$, $I^2 = 41\%$) when all studies were pooled into the meta-analysis (Figure 1, Figure 2, and Figure 3). However, the pooled ORs were not significant under the other two models (For AA versus GG: OR 1.59, 95%CI 0.45-5.68, $P = 0.47$, $I^2 =$

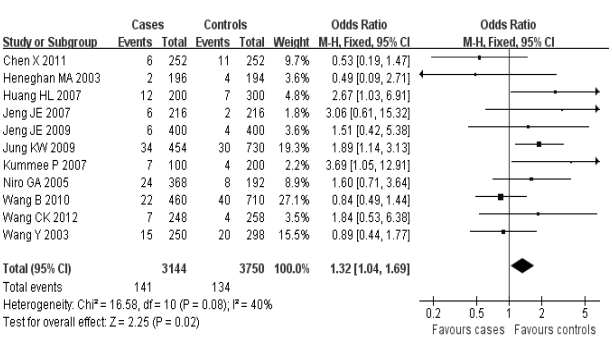

Figure 1. Forest Plot in the Meta-analysis of TNF-α 238 G/A Polymorphism and Hepatocellular Carcinoma Risk under the Allele Comparison Model (A versus G)

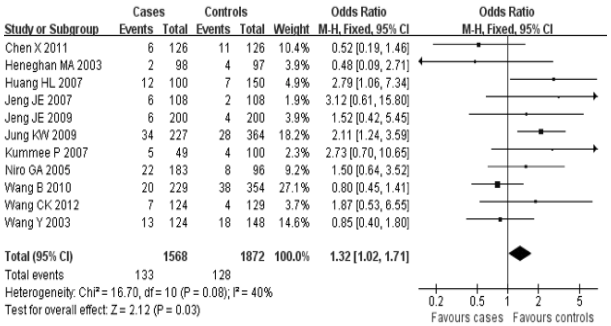

Figure 2. Forest Plot in the Meta-analysis of TNF-α 238 G/A Polymorphism and Hepatocellular Carcinoma Risk under the Heterozygote Comparison Model (AG versus GG)

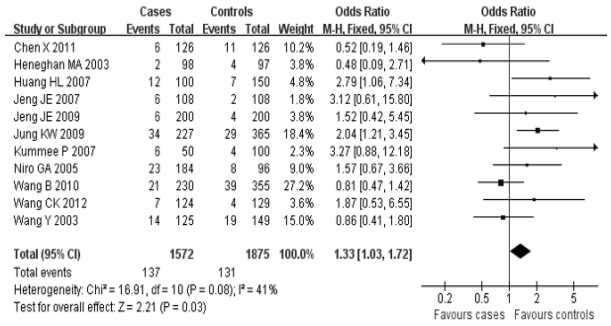

Figure 3. Forest Plot in the Meta-analysis of TNF-α 238 G/A Polymorphism and Hepatocellular Carcinoma Risk under the Dominant Genetic Model (AA/AG versus GG)

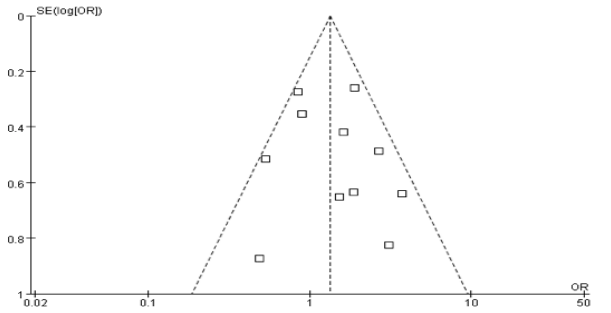

Figure 4. Funnel Plots in the Meta-analysis of the Association Between TNF-α 238 G/A Polymorphism and Hepatocellular Carcinoma Risk

0%; for AA versus GG/AG: OR 1.55, 95%CI 0.44-5.52, $P = 0.50$, $I^2 = 0\%$).

Subgroup analysis by ethnicity shown that there was an significant association between TNF-α 238 G/A polymorphism and risk of hepatocellular carcinoma in Asians under three genetic comparison models (For A versus G: OR 1.30, 95%CI 1.00-1.68, $P = 0.05$, $I^2 = 45\%$ for AA/AG versus GG: OR 1.31, 95%CI 1.00-1.71, $P = 0.05$, $I^2 = 46\%$). However, there was no association between TNF-α 238 G/A polymorphism and risk of hepatocellular carcinoma in Caucasians in all genetic comparison models (All P values were more than 0.05).

Publication bias

Funnel plot was conducted to assess the publication bias of the literature in this meta-analysis. As shown in Figure 4, the shape of the funnel plot seemed symmetrical in the allele comparison model (A versus G), which indicated low risk of publication bias in this meta-analysis (Figure 4).

Discussion

Inflammation has been considered to be an important factor involving in the carcinogenesis, and, as the most common inflammatory cytokine, TNF-α has been implicated in both the development and progression of various cancers studies (Sethi et al., 2012; Suganuma et al., 2012). Because A allele of TNF-α at –238 in the promoter region was found to down-regulate gene expression, TNF-α 238 G/A polymorphism is believed to be involved in the in the carcinogenesis (Falvo et al., 2010; Qidwai et al., 2011). In the past decade, many studies have focused on the relationship between this variant and cancers. There are also many epidemiological studies evaluating the association between TNF-α 238 G/A polymorphism and hepatocellular carcinoma risk. However, results from these studies were ambiguous, and the association between TNF-α 238 G/A polymorphism and hepatocellular carcinoma risk was still inconclusive. Therefore, we performed a meta-analysis to evaluate the association between TNF-α 238 G/A polymorphism and hepatocellular carcinoma risk.

In the present meta-analysis, we performed a meta-analysis of 11 case-control studies with a total of 1,572 hepatocellular carcinoma cases and 1,875 controls. Overall, TNF-α 238 G/A polymorphism was significantly associated with increased risk of hepatocellular carcinoma in three genetic comparison models (For A versus G: OR 1.32, 95%CI 1.04-1.69, $P = 0.02$, $I^2 = 40\%$; for AG versus GG: OR 1.32, 95%CI 1.02-1.71, $P = 0.03$, $I^2 = 40\%$; for AA/AG versus GG: OR 1.33, 95%CI 1.03-1.72, $P = 0.03$, $I^2 = 41\%$) when all studies were pooled into the meta-analysis. Subgroup analysis by ethnicity shown that there was an significant association between TNF-α 238 G/A polymorphism and risk of hepatocellular carcinoma in Asians under three genetic comparison models (For A versus G: OR 1.30, 95%CI 1.00-1.68, $P = 0.05$, $I^2 = 45\%$ for AA/AG versus GG: OR 1.31, 95%CI 1.00-1.71, $P = 0.05$, $I^2 = 46\%$). Thus, this meta-analysis suggests that TNF-α 238 G/A polymorphism is associated with increased susceptibility to hepatocellular carcinoma.

TNF-α is produced by many types of cells, such as macrophages, neutrophils, fibroblasts, keratinocytes, NK cells, T and B cells, and tumour cells (Mocellin et al., 2008; Vujanovic, 2011; Sun, 2012). TNF-α is a potent pleiotropic proinflammatory cytokine that affects cell growth, cell differentiation, cellular function and survival (Mocellin et al., 2008; Sun, 2012). As an important inflammatory factor, the polymorphisms in TNF-α gene have been largely studied, and TNF-α polymorphisms have been suggested to be associated with risks of many diseases, including rheumatoid arthritis, ankylosing spondylitis, and hepatitis B (Marcos et al., 2009; Postal et al., 2011). There are also many studies suggesting TNF-α polymorphisms are associated with risks of cancers, however, most them focus on the TNF-α -308 polymorphism (Fan et al., 2011; Wang et al., 2011). The relation between TNF-α -238 polymorphism and risk

of cancer has not been well assessed. A comprehensive meta-analysis by Zhou et al. was published in 2011 to evaluate this association, and Zhou et al. reported that no significant association was found between the TNF-α-238 polymorphism and the risk for cancer (Zhou et al., 2011). However, Zhou et al. (2011) didn't include all eligible studies, and included only 4 studies on hepatocellular carcinoma, which could increase risk of bias in that meta-analysis. Our meta-analysis included 11 case-control studies with a total of 1,572 hepatocellular carcinoma cases and 1,875 controls, and the large sample size could provide enough statistical power to get a precise estimation on the association between TNF-α-238 polymorphism and hepatocellular carcinoma risk. Thus, our meta-analysis provided new and strong evidence for the key role of TNF-a-238 polymorphism in the development of hepatocellular carcinoma.

One main limitation in this meta-analysis should be considered when interpreting the findings. Currently, there are limited studies published to investigate the association between TNF-α-238 polymorphism and hepatocellular carcinoma risk in Caucasians. There was only one study by Niro et al. performed in Italy (Niro et al., 2005). The limited study with relatively small sample size could cause poor validation and increase the risk of random error. Thus, more well-designed studies with large sample sizes are needed to further identify this association among Caucasians. Besides, the associations between TNF-α-238 polymorphism and risks of other kinds of cancer are also worthy investigating and need further studies.

In summary, this meta-analysis suggests that TNF-α 238 G/A polymorphism is associated with increased susceptibility to hepatocellular carcinoma. However, more well-designed studies with large sample size are needed to validate this association in Caucasians.

Acknowledgements

The author(s) declare that they have no competing interests.

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