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ORIGINAL RESEARCH

The Potential Role of TNF-α (rs361525 and rs1800629) in Hepatocellular Carcinoma: Multivariate Analysis (Meta-Analysis)

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Abstract

Purpose Tumor necrosis factor-α has been suggested to play a crucial role in the development andprogression of hepatocellular carcinoma (HCC). Previous reports have indicated that rs361525 and rs1800629 might be risk factors for various cancers. Increasing studies have been conducted on the association of these two SNPs with HCC risk but the results remain inconclusive.
Methods In order to detect association between TNF- α and HCC, a meta-analysis was performed. Five studies with 541 cases and 795 controls were used for rs361525, while six studies including 925 cases and 1307 controls were collected for investigating rs1800629. The grouping of countries from data were obtained was done by Principal Coordinate Analysis plot (PCA). Moreover, association between geographical area and grouping of genotypes was determined by Canonical Correspondence Analysis (CCA).
Results Our meta-analysis showed that rs361525 and rs1800629 were not significantly associated with the risk of HCC. CCA analysis illustrated that there was not any correlation between genotype distribution and geographical distance for rs1800629 but there was significant correlation between genotype distribution and geographical features for rs361525. PCA analysis for both SNPs showed India and Korea were placed near each other and also China and Brazil were in same part of PCA plot.
Conclusion To sum up, this meta-analysis suggests that the rs361525 and rs1800629 are not associated with HCC development while geographical distance effect on rs361525 genetic inheritance but not effect on rs1800629. However, it is necessary to conduct further studies with larger sample. Moreover, gene-gene and gene-environment interactions should also be considered.

Keywords TNF-α · SNP · HCC · Meta-analysis

Introduction

Hepatocellular carcinoma (HCC) is a primary cancer of the liver and the fifth most common cancer worldwide [1], ranking third in global cancer-related death [2]. HCC is a multi-factorial diseases [3] and various risk factors such as hepatitis B virus (HBV), hepatitis C virus (HCV), alfatoxin B (AFB1) intake, and heavy alcohol intake have been associated with [4, 5]. In addition, exposure to environmental factors is thought to be one of the important risk factors. However, though people are exposed to the environmental risk factors and life styles, HCC develops only in a small proportion of the exposed people and it indicates that internal factors such as genetic variations like TNF-α polymorphisms might play critical roles in its carcinogenic mechanisms [6]. Tumor necrosis factor alpha (TNF-α) is a member of the TNF/TNFR cytokine superfamily [7] and is secreted by macrophages and T lymphocytes, is a potent pro-inflammatory cytokine and immune modulator, and exhibits a wide range of biological activities, including protection from infection, immune surveillance against tumors, and stimulation of inflammatory responses [8]. Intriguingly, it has been observed that TNF-α might play paradoxical roles in human cancers. Therapeutically, local accumulation of a high dose of TNF-α in tumor tissues seems to exhibit potent anti-neoplastic actions [9]. However, endogenous levels of TNF-α have been reported to contribute to the tissue remodeling for tumor growth and promote cancer metastasis [10]. As TNF-α production is partially governed by genetic factors [11], the role of polymorphisms of the TNF-α promoter in determining disease susceptibility or serving as a marker of disease severity has been a subject of intensive research in recent years. The TNF-α gene

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exhibits two important functional single nucleotide polymorphisms (SNPs) located at position − 238 and − 308 in its promoter region for rs361525 and rs1800629 respectively. rs361525G > A and rs1800629 G > A have been considered to influence the TNF-α transcriptional activity [12–17]. In recent years, several studies focused on the association between TNF-α rs361525G > A polymorphism and HCC risk [18–25]. However, the results of those studies remain inconclusive, potentially due to the possible small effect of the polymorphism on HCC risk or the relatively small sample size in each of published studies. In the present study, we performed a meta-analysis to obtain a more precise estimation of the association.

Material and Methods

Literature Search

We searched the articles using the search terms "tumor necrosis factor α," "TNFα," "polymorphism," "Hepatocellular Carcinoma," "HCC," "rs361525," and "rs1800629" in PubMed, Science Direct, and Google Scholar Databases. The results were limited to papers published in English and the last search was updated on October 5, 2016. All searched studies' bibliographies were checked for other relevant publications. Review articles were hand-searched to find additional eligible studies. Only published studies with full text articles were included. When overlapping data of the same patient population were included in more than one publication, only the most recent or complete study was used in this meta-analysis. Totally, five papers for rs361525 and six papers for rs1800629 with same protocols were found.

Inclusion and Exclusion Criteria

Studies meeting the following criteria were included in the meta-analysis: (1) case-control studies comparing HCC cases with healthy or non-cancer controls; (2) studies evaluating the association between TNF-α-238G/A and − 308G/A polymorphisms and HCC risk; and (3) sufficient genotype data of TNF-α-238G/A and − 308G/A polymorphisms were reported. The exclusion criteria were used as follows: (1) case-only studies; (2) case reports, letters, or reviews; (3) studies with incomplete data or no usable data; (4) studies containing overlapping data; and (5) control population including patients with malignant tumors.

Data Extraction

Information was extracted from all eligible publications according to the inclusion criteria listed above. The following characteristics were collected from each study: first author's surname, publication date, country of origin, ethnicity, total number of cases and controls, and numbers of cases and controls with the GG, GA, and AA genotypes, respectively.

Statistical Method

A meta-analysis to investigate the association between TNF-α rs361525 and rs1800629 polymorphisms and hepatocellular carcinoma susceptibility risk was performed. The Hardy-Weinberg equilibrium (HWE) was assessed by SNP STAT. The odds ratio (OR) of these SNPs and HCC risk was estimated for each study to assess the strength of the association. The significance of the ORs was determined

Table 1 Characteristics of studies in the meta-analysis

Author	Year	Country	Sample size (Ca/Co)	Case			Control			HWE
				AA	GA	GG	AA	GA	GG	
rs361525										
Shin [1]	2015	South Korea	157/201	1	26	130	1	20	180	0.01
Teixeria [27]	2013	Brazil	111/202	0	41	70	0	39	163	0.004
Sghaier [28]	2015	Tunisian	100/200	30	49	21	45	93	62	0.36
Li [29]	2016	China	88/82	0	18	70	0	16	66	0.01
Pangrahi [30]	2014	India	85/110	3	24	58	4	8	98	0.007
rs1800629										
Saleh Shahbazi [33]	2016	Iran	409/483	80	215	114	22	233	228	0.01
Saxena [34]	2014	India	60/139	0	4	56	0	11	128	0.01
Shin [1]	2015	South Korea	157/201	1	23	133	2	18	181	0.01
Teixeira [27]	2013	Brazil	111/202	0	30	81	3	26	173	0.01
Sghaier [28]	2015	Tunisia	100/200	28	47	25	48	72	80	0.01
Li [29]	2016	China	88/82	1	30	57	0	11	71	0.01

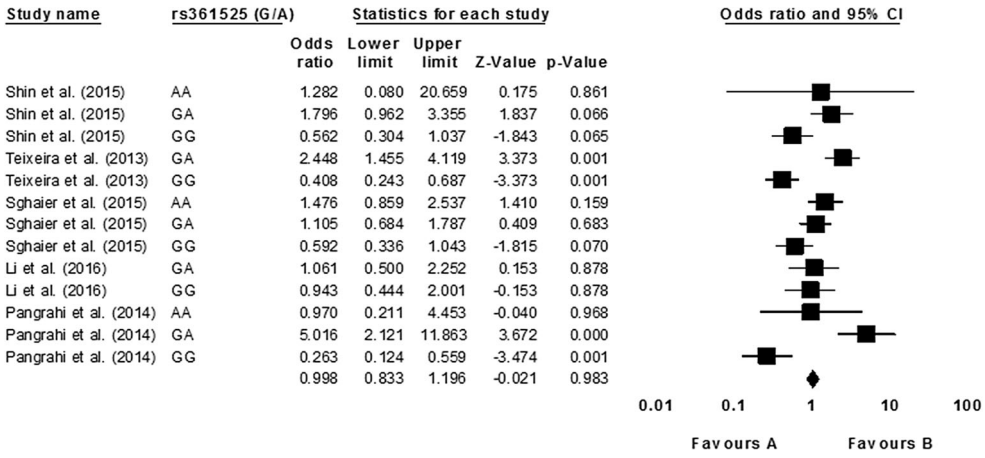

Fig. 1 Meta-analysis for the relationship between rs361525 and HCC risk. The solid squares represent odds ratios (ORs) from individual studies; the diamonds are shown as overall effect

using the *Z* test, and a *P* value of less than 0.05 was considered significant. Statistical analysis was undertaken using CMA software (Comprehensive Meta-analysis version 2). In addition, clustering analysis, PCA, and CCA were done by PAST software V2.7 [26] to examine which countries are more close to each other according to genotype distribution and correlation between geographical distance and genotype distribution, respectively.

Results

A comprehensive literature search of the electronic databases, including PubMed, Google Scholar, and Science Direct were performed and initially identified 15 relevant articles. After reviewing the full texts of those reports, five case-control studies with a total of 1336 subjects (541 HCC cases and 795 controls) were finally included into the meta-analysis for rs361525 [1, 27–32] and six studies with 925 cases and 1307 controls met the inclusion criteria and were used in the meta-analysis for rs1800629 [1, 27–29, 33, 34]. Table 1 showed characteristics of studies in the meta-analysis. According to this table, just one study (Tunisia) for rs361525 was in HWE ($P > 0.05$). In all studies except the Saleh Shahbazi study, GG genotype was increased significantly and also AA and GA genotypes were decreased. It showed why those studies were not in HWE.

The main results of meta-analysis of the association between the rs361525 and HCC risk is shown in Fig. 1. Our meta-analysis of the five studies showed that rs361525 was not significantly associated with the risk of HCC. AA genotype not demonstrated significant association with HCC and also was not observed in two studies (Li and Teixeira). AG and GG genotypes illustrated significant association just in two studies (Panigrahi and Teixeira, $P = 0.001$).

The association between the rs1800629 and HCC risk is shown in Fig. 2. Meta-analysis showed that rs1800629 was not significantly associated with the risk of HCC. AA genotype demonstrated significant association with HCC just in Saleh Shahbazi study (Iran, $P = 0.000$) and this genotype did not show any association with HCC in other studies and also this genotype was not observed in Indian population. AG

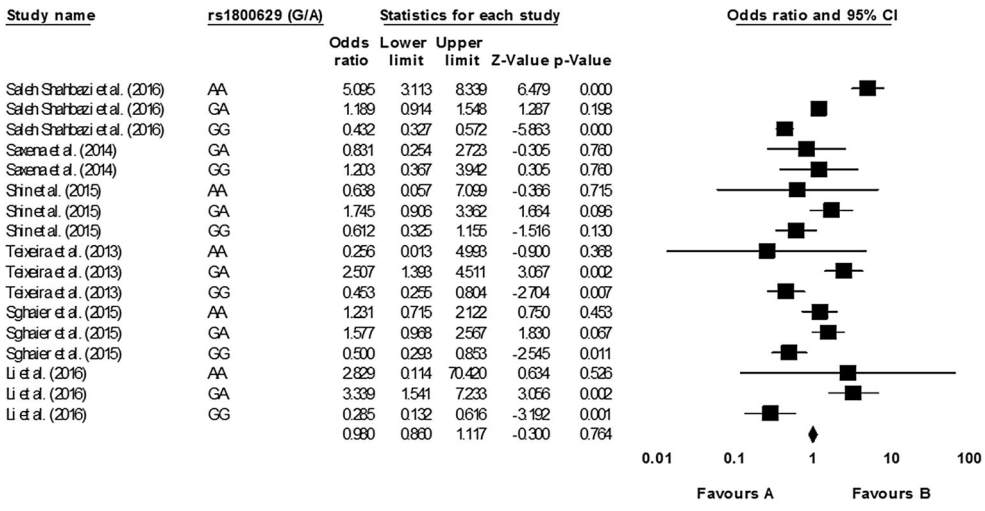

Fig. 2 Meta-analysis for the relationship between rs1800629 and HCC risk. The solid squares represent odds ratios (ORs) from individual studies; the diamonds are shown as overall effect

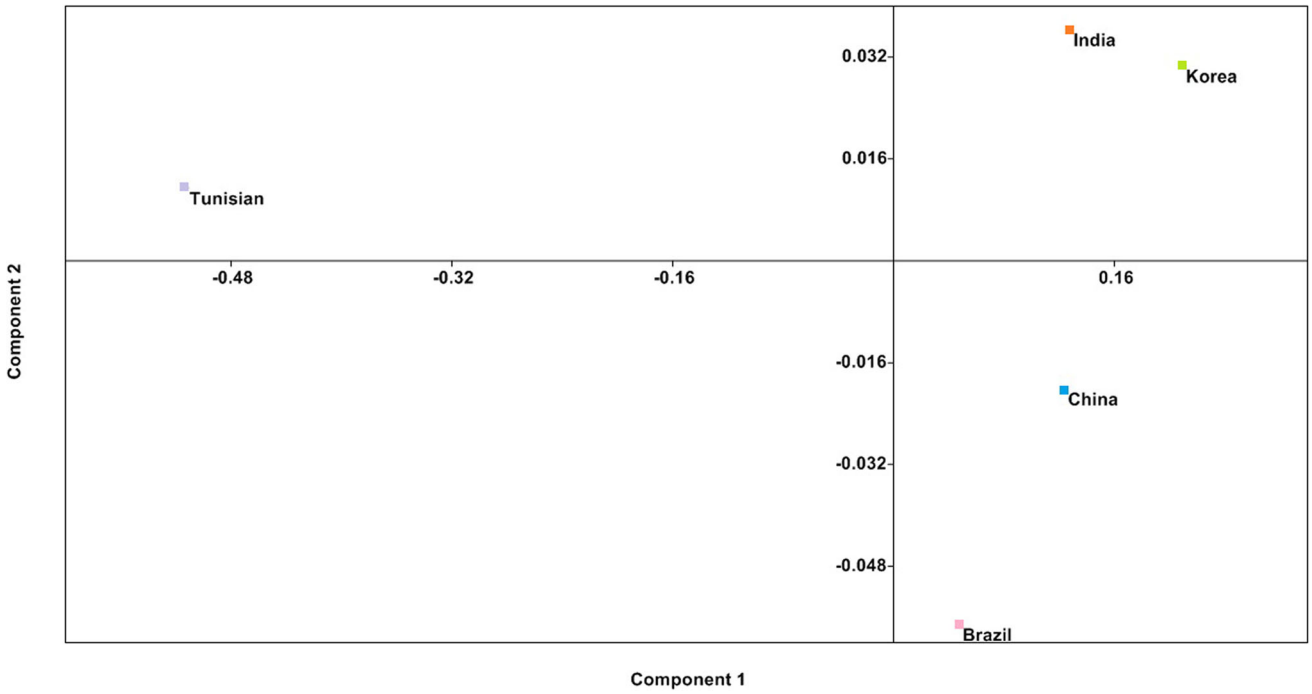

Fig. 3 PCA plot (orange: India, light green: Korea, blue: China, pink: Brazil and light purple: Tunisia)

genotype showed significant association just in two studies (Li and Teixeira, $P = 0.002$) and GG genotype was significant except in two studies (Shin $P = 0.130$ and Saxena $P = 0.750$).

PCA analysis was done for rs361525 according to genotype frequencies (Fig. 3). Based on PCA analysis India and Korea were placed near each other because both of them were not significant in AA genotype ($P > 0.05$) and also China and Brazil were in same part of PCA plot because both of them did not have AA genotype and it was the reason of their similarity. Tunisia was located far away from other countries in different part of PCA plot and for all genotypes was not significant.

PCA and clustering analysis showed for rs1800629 in Fig. 4. Based on PCA and clustering analysis India and Korea were placed near each other because both of them were not significant in all genotypes ($P > 0.05$) and also China and Brazil were near each other because their P value for GA and GG genotypes were significant. Iran and Tunisia located in two different parts of PCA plot and these two countries were in one cluster with 0.16 distance.

CCA analysis and Mantel test were done to examine correlation between genotype distribution and geographical distance for these two SNPs (Fig. 5). For rs361525, Tunisia with the most longitude and the least latitude has different genetic structure among all mentioned countries and also Korea, India, and China were similar from geographical point of view thus it was assumed that geographical potential effect on

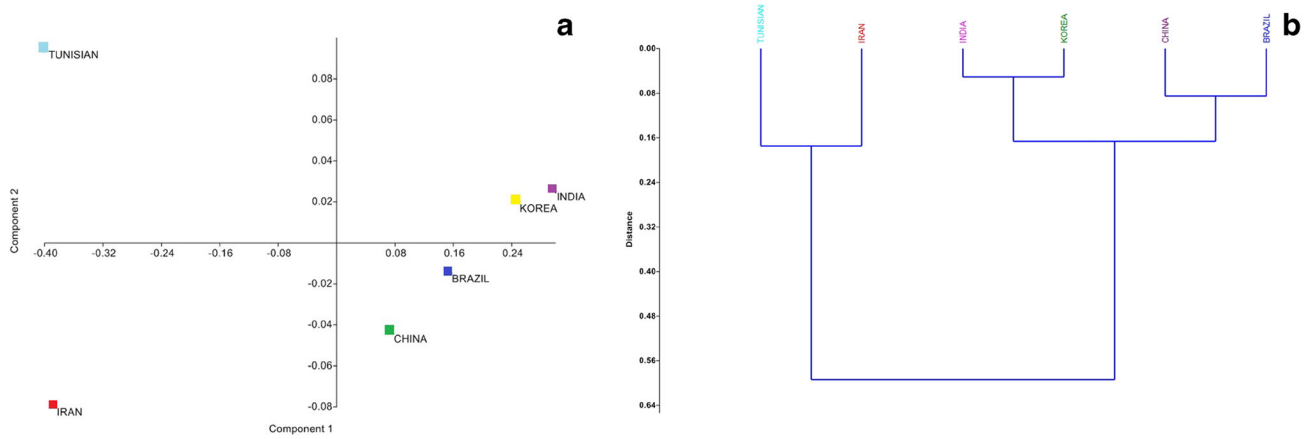

Fig. 4 PCA plot (**a**) and clustering analysis (**b**)

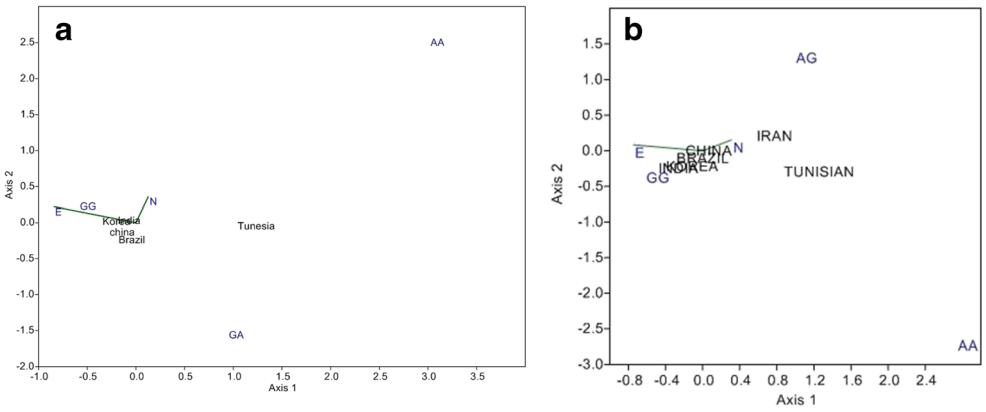

Fig. 5 CCA plot. **a** rs361525 and **b** rs1800629

genotype distribution and for its confirmation Mantel test was done. Based on mantel test result (correlation $R = 0.6407$, $P = 0.030$) there was significant correlation between genotype distribution and geographical distance.

Iran and Tunisia showed different genetic structure, it was thought there was correlation between geographical distance and genotype distribution so Mantel test was done for its confirmation and the result contradicted this hypothesis (correlation $R = 0.6407$, $P = 0.230$) for rs1800629. Thus, there is no correlation between geographical distance and genotype distribution for this SNP.

Discussion and Conclusion

Carcinogenesis of HCC is a complex process. The risk factors include chronic infection of hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, carcinogen exposure, excessive alcohol consumption, and a variety of genetic factors [35]. Previous studies have suggested that genetic factors also play vital roles in the development of hepatocellular carcinoma. Currently, many genetic polymorphisms have been investigated as possible risk factors of hepatocellular carcinoma, and some of them have been identified as candidate risk factors of hepatocellular carcinoma [32]. However, the understanding on the genetic background of hepatocellular carcinoma is still unclear and needs further researches. TNF-α, the most crucial inflammatory cytokine, plays a very important role in inflammation and in the pathogenesis of cancer [36]. However, the association of rs361525 and rs1800629 with susceptibility to HCC is still not conclusive to date. Two of the five studies for rs361525 included in this meta-analysis reported that the A allele is associated with a high risk of HCC. Nevertheless, the other three studies yet failed to demonstrate this relationship. Two studies for rs1800629 have been reported to be related to risk of HCC because of A allele which can change the transcription of TNF-α and TNF-α production. Because of these conflicting results, we performed this meta-analysis to precisely clarify the true association. For rs361525, our meta-analysis showed that there is no significant association between this SNP and HCC susceptibility and also HCC is not statistically affected or protected by AA genotype. This result was consisted with Shin et al. [1], Li et al. [29], Panigrahi et al. [30] but not consisted with Teixeria et al. [27] and Sghaier et al. [28]. According to what mentioned above, this SNP demonstrate various distributions among population with different geographical regions so genotype distribution will be changed by geographical distance and we cannot say that this SNP have association with HCC in whole world. Actually, GG genotype went to the East and its frequency was higher in those areas. Thus, this result could be because of inbreeding or founder effects. Another reason is that there are various genotypes of HCV in the world so, some of them are more frequent in some parts of world and are endemic on that regains. Its frequency in healthy subject was more than cases. For rs1800629, the result indicated that this SNP in not a risk factor for developing HCC. This result was consisted with Shin et al. [1] and Saxena et al. [34]. To sum up, this meta-analysis suggests that the rs361525 and rs1800629 are not associated with HCC development while geographical distance effect on rs361525 genetic inheritance but not effect on rs1800629 and population genetic background plays an important role in genotype distribution. However, it is necessary to conduct further studies with larger sample sizes using standardized unbiased genotyping methods, homogeneous HCC patients and well matched controls. Moreover, gene-gene and gene-environment interactions should also be considered.

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