Systematic Review and Meta-Analysis

Medicine

OPEN

Association between the risk of hepatitis virus-related hepatocellular carcinoma and EGF polymorphism

A PRISMA-compliant updated meta-analysis

Qinjing Wang, MD[a], Lingling Xu, MD[b], Qianbo Wu, MD[b], Min Zhang, MD[b], Jing Zhang, MD[c,*]

Abstract

Background: The study aims to provide a comprehensive account of the association between the epidermal growth factor (EGF) + 61A/G polymorphism (rs4444903) and susceptibility to virus-related hepatocellular carcinoma (HCC).

Methods: Electronic searching of the Chinese National Knowledge Infrastructure, Wanfang, Chinese Scientific Journal Database (VIP), PubMed, Web of Science, and Embase was conducted to select eligible studies. Odds ratios (OR) and 95% confidence intervals (95%CI) were calculated to assess the strength of the association.

Results: In this study, a total of 18 articles were included with 2692 cases and 5835 controls for assessing the association between rs4444903 and HCC risk. The pooled results showed that the EGF + 61A/G polymorphism was significantly associated with the risk of virus-related HCC in all genetic models. Stratified analyses were conducted based on ethnicity, study quality, source of controls, type of controls, number of cases and genotyping method. The results showed that EGF + 61A/G polymorphisms significantly affect HCC susceptibility in different stratified populations. High heterogeneity was observed across included studies, and meta-regression analysis demonstrated that race, type of controls, and study quality contribute to the observed heterogeneity.

Conclusion: This pooled analysis found that EGF + 61A/G polymorphism was significantly associated with the risk of HCC.

Abbreviations: CI = confidence interval, EGF = epidermal growth factor, HBV = hepatitis B virus, HCC = hepatocellular carcinoma, OR = odds ratio.

Keywords: epidermal growth factor, ethnicity, hepatocellular carcinoma, meta-analysis, polymorphism

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common and lethal cancer worldwide.[1] The estimated annual number of patients with HCC has increased by more than 500,000 cases. Although significant advances in the diagnosis and treatment, the prognosis of HCC patients remains poor and the 5-year survival rate in developing countries is only 5%.[2,3] Multiple factors have been demonstrated to be associated with the development of HCC, such as chronic infection with hepatitis B (HBV) or hepatitis C virus, excessive alcohol consumption, high cigarette smoking and many etiological factors.[4] Additionally, HCC has been proven to be induced by inflammation, and virus-associated HCC is the most common type of liver cancer. In China, more than 80% of HCC patients were associated with chronic HBV infection.[5,6]

Today, most diagnoses of virus-related HCC are made after the disease has progressed substantially, and there is no effective therapy for most virus-related HCC patients currently.[7–9] Therefore, effective screening of high-risk populations for chemoprevention is of great significance to the treatment of virus-related HCC.[7,10] Serum alpha-fetoprotein measurement and liver imaging are currently the main methods for screening high-risk groups. However, due to the low sensitivity and specificity, the effectiveness is questionable and limited.[11–13] In order to improve prevention and treatment strategies, the identification of molecular markers associated with the risk of virus-related HCC is necessary.

In recent years, several important signaling pathways have been systematically studied in virus-related HCC. These pathways regulate physiological processes such as the growth and differentiation of tumor cells, the regeneration of blood vessels,

Both the authors have given their consent for publication.

The authors have no funding and conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

[a] *Department of Geriatric Oncology, Jiangnan Hospital Affiliated to Zhejiang University of Traditional Chinese Medicine (Xiaoshan Hospital of Traditional Chinese Medicine), Hangzhou, Zhejiang, China,* [b] *Department of Pediatrics, Tongde Hospital of Zhejiang Province, Hangzhou, Zhejiang, China,* [c] *Department of Oncology, Jiangnan Hospital Affiliated to Zhejiang University of Traditional Chinese Medicine (Xiaoshan Hospital of Traditional Chinese Medicine), Hangzhou, Zhejiang, China.*

**Correspondence: Jing Zhang, Department of Oncology, Jiangnan Hospital Affiliated to Zhejiang University of Traditional Chinese Medicine (Xiaoshan Hospital of Traditional Chinese Medicine), Hangzhou, Zhejiang 310016, China (e-mail: dakaishiye2008@126.com).*

How to cite this article: Wang Q, Xu L, Wu Q, Zhang M, Zhang J. Association between the risk of hepatitis virus-related hepatocellular carcinoma and EGF polymorphism: A PRISMA-compliant updated meta-analysis. Medicine 2022;101:42(e31280).

Received: 18 April 2022 / Received in final form: 18 September 2022 / Accepted: 20 September 2022

http://dx.doi.org/10.1097/MD.0000000000031280

and the migration of tumor cells.[14] Epidermal growth factor (EGF) plays a significant role in cell proliferation, differentiation and tumorigenesis of epithelial tissues.[15] The EGF + 61A > G polymorphism (rs4444903) is a functional SNP in the 5' untranslated region of the EGF gene.[16,17] It results in higher EGF levels in individuals with EGF genotype G/G in comparison to the A/A genotype.[18] Studies have shown that EGF signal pathway plays an important role in the occurrence of HCC. It was involved in the stimulation of the proliferation of epidermal and epithelial cells, which has a strong relationship with embryo growth, tissue repair, regeneration, and tumorigenesis.[19,20] The transient profile of EGF RNA accumulation suggested that the elevated EGF levels may catalyze a cascade of events preceding the first wave of liver DNA replication in hepatocytes isolated by collagenase perfusion.[21] The EGF could activate the EGF receptor as a ligand with biological effect through signal transduction.[22] Baghdadi I et al have proved that the cirrhotic patients with a GG genotype in rs4444903 had a significantly high risk to develop HCC.[23]

In this study, we performed a meta-analysis of all eligible studies to clarify the relationship between EGF polymorphism and the risk of virus-related HCC.

2. Methods

The Preferred Reporting Items for Systematic Reviews and Meta-analyses criteria were used for this meta-analysis.[24]

2.1. Literature-searching strategy

We performed a computerized literature search by 2 independent researchers in the following 6 electronic databases: Chinese National Knowledge Infrastructure, Wanfang, VIP, Pubmed, Web of Science, and Embase from their start date to September 2022. We used the following keywords and medical subject heading terms: ("Epidermal growth factor" or "EGF") and ("polymorphism" or "variant" or "SNP" OR "mutation") and ("hepatocellular carcinoma" or "liver cell carcinoma" or "liver cancer").

2.2. Inclusion and exclusion criteria

Studies included in the meta-analysis had to meet the following inclusion criteria: evaluating the association between EGF polymorphism and virus-related HCC risk, using unrelated individuals, providing sufficient data for estimating an odds ratio (OR) with its 95% confidence interval (CI), using case-control, cohort or cross-sectional design, published in English or Chinese. The corresponding authors were contacted to obtain missing information, and some studies were excluded if critical missing information was not obtained. Reviews, case reports, family-based studies, case-only studies, and studies without sufficient data were excluded. When a study reported results on different sub-populations based on ethnicity or geographical region, we treated each sub-population as a separate comparison. If more than 1 article was published using the same subjects, only the study with the largest sample size was selected.

2.3. Data extraction

All data were extracted independently by 2 investigators. Disagreement was resolved by discussion. The following data were extracted: authors, name of the journal, year of publication, ethnicity, country of study population, inclusion and exclusion criteria, characteristics of cases and controls, numbers of HCC cases and controls, matching criteria, source of controls, HCC confirmation, study design, genotyping methods, genotype frequencies of cases and controls, and interactions between environmental factors or genes.

2.4. Quality score assessment

The quality of the studies was independently assessed by the same 2 investigators. Any disagreement was resolved by discussion between the 2 investigators. The total scores ranged from 0 (worst) to 24 (best). Studies scoring <16 were classified as "low quality" and those scoring ≥16 as "high quality."

2.5. Statistical analysis

The unadjusted OR with 95%CI was used to assess the strength of the association between the EGF polymorphism and the risk of virus-related HCC. The pooled ORs were performed under the allelic contrast (G vs A), codominant model (homozygote comparison: GG vs AA, heterozygote comparison: GA vs AA), dominant model (GG + GA vs AA), and recessive model (GG vs GA + AA), respectively. Heterogeneity between studies was measured using a Q statistic test and an I^2 statistic. $P < .10$ was considered representative of significant statistical heterogeneity due to the low power of the statistic. I^2 ranges between 0% and 100% and represents the proportion of between-study variability that can be attributed to heterogeneity rather than chance. I^2 values of 25%, 50%, and 75% were defined as low, moderate and high estimates. If the significant Q-statistic indicated heterogeneity across studies, the random-effects model was used, otherwise, the fixed-effects model was adopted. The Z test was used to assess the significance of the pooled OR and a $P < .05$ was considered significant.

Subgroup analyses were stratified by racial descent, study quality, source of controls, type of controls, and the number of cases, respectively. Furthermore, metaregression analysis was performed to investigate 5 potential sources of heterogeneity including ethnicity (Asian populations vs non-Asian populations), study quality (high-quality studies vs low-quality studies), source of controls (hospital-based vs Population-based), type of controls (healthy controls vs controls with chronic liver diseases) and the number of cases (<100 vs ≥100).[25] Statistical significance was defined as a $P < .10$ because of the relatively weak statistical power.

To evaluate the stability of the results, sensitivity analyzes were performed by sequential omission of individual studies under various comparisons in the overall and Asian populations, respectively. Publication bias was investigated by funnel plot. Funnel plot asymmetry was assessed by the method of linear regression test. The Hardy-Weinberg equilibrium was tested using the χ^2 test. All P values were 2-sided. Data analyzes were performed using the software Stata version 11.0 software.

3. Results

3.1. Eligible studies

As shown in Figure 1, a total of 1124 articles were initially obtained by searching the databases. After duplicate checks by Endnote 20, 905 articles remained. We subsequently excluded 882 articles based on browsing the titles and abstracts. According to the inclusion criteria, 5 of the remaining 23 records were further excluded based on a full-text review. In total, 21 studies (18 articles) with 2692 virus-related HCC cases and 5835 controls were finally included in this meta-analysis.[23,26-42]

3.2. Characteristics of study

The characteristics of the 21 included studies are shown in Table 1. Of all eligible studies, 11 were conducted in Asian populations, 2 in European populations, 5 in African populations, and 3 in mixed populations. In all studies, the cases were histologically confirmed (17 studies) or diagnosed by elevated

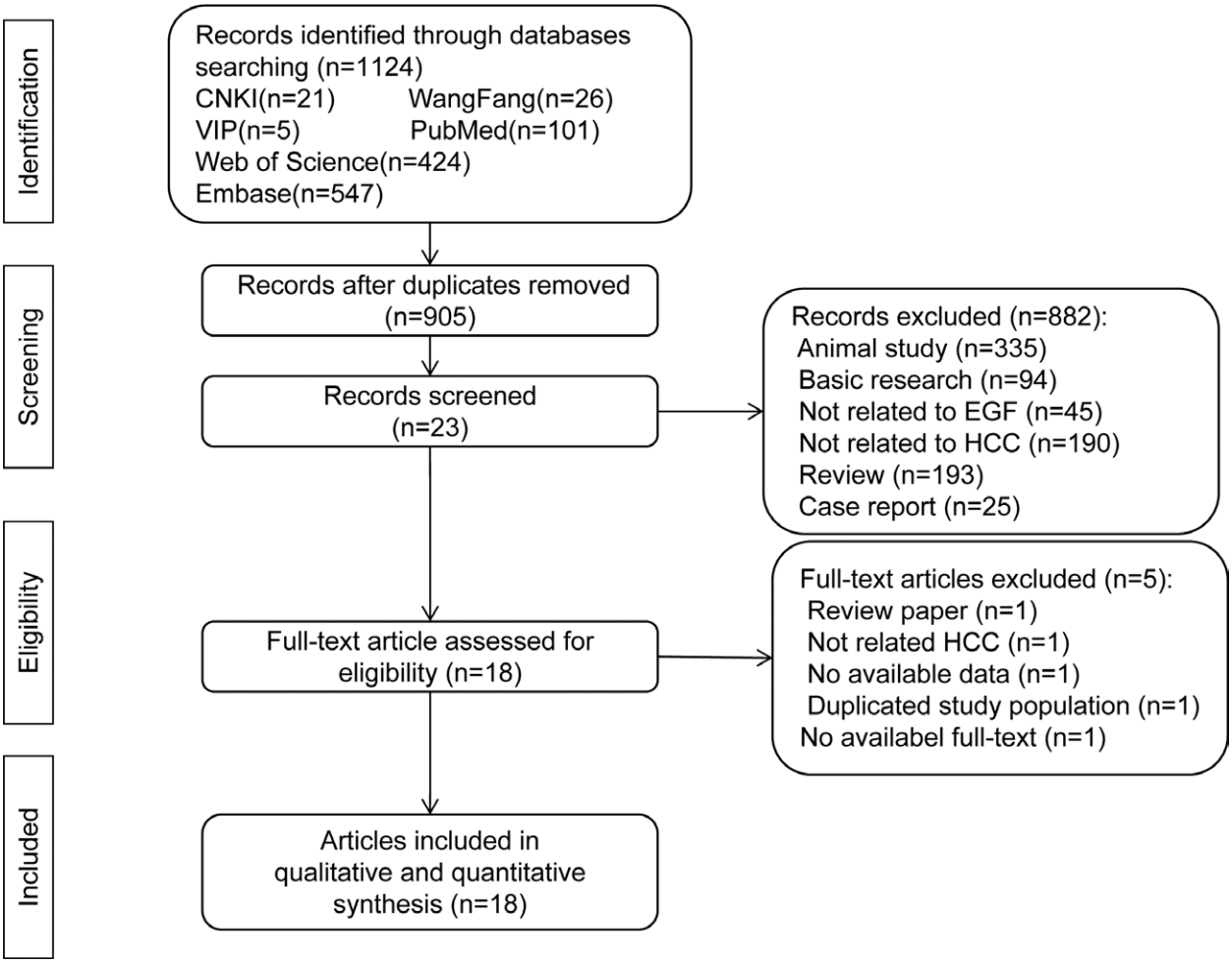

Figure 1. Flow diagram of the study selection process.

α-fetoprotein and different iconography changes (abdominal ultrasound and triphasic computed tomography). All controls were free of cancer. Four studies used healthy populations as controls, 5 studies used patients with chronic liver diseases (HBV infection, hepatitis C virus infection, and cirrhosis) as controls, and 12 studies used healthy subjects and patients with chronic liver diseases as controls. The sample size of the total participants ranged from 75 to 1774, with a mean of 406. Quality scores for individual studies ranged from 11.5 to 21, with 10 of the 21 studies classified as high quality. Twenty studies used peripheral blood, and 1 study used FFPE to extract genome DNA. Fourteen studies used the polymerase chain reaction-restriction fragment length polymorphism assay, 6 studies used the Taqman method, and 1 study used Matrix-Assisted Laser Desorption/Ionization Time of flight mass spectrometry to genotype the EGF + 61 A/G polymorphism. The genotype distribution in the controls of all studies was consistent with Hardy-Weinberg equilibrium.

3.3. Meta-analysis results

The pooled results of all studies showed that the EGF + 61A/G polymorphism was significantly associated with the susceptibility of virus-related HCC under all genetic models (G vs A: OR = 1.56, $P < .001$, 95%CI: 1.26–1.94, $I^2 = 86.8\%$; GG vs GA + AA: OR = 1.67, $P < .001$, 95%CI: 1.29–2.15, $I^2 = 79\%$; GG + GA vs AA: OR = 1.67, $P < .001$, 95%CI: 1.26–2.20, $I^2 = 70.2\%$; GG vs AA: OR = 2.18, $P < .001$, 95%CI: 1.50–3.16, $I^2 = 76.6\%$; GA vs AA: OR = 1.20, $P < .001$, 95%CI: 1.03–1.39, $I^2 = 23.7\%$) (Table 2).

In subgroup analyzes based upon ethnicity, significantly associations were observed between EGF + 61A/G polymorphism and the risk of virus-related HCC in Asian populations (G vs A: OR = 1.15, $P < .001$, 95%CI: 1.02–1.29, $I^2 = 40.1\%$), European populations (G vs A: OR = 1.59, $P < .001$, 95%CI: 1.05–2.41, $I^2 = 0.0\%$) and African populations (G vs A: OR = 4.46, $P < .001$, 95%CI 1.53–13.02, $I^2 = 93\%$) (Fig. 2). When stratifying by study quality, the results showed that EGF + 61A/G polymorphism was associated with the risk of virus-related HCC both in high-quality studies (G vs A: OR = 1.20, $P < .001$, 95%CI: 1.08–1.34, $I^2 = 24.2\%$) and in low-quality studies (G vs A: OR = 1.98, $P < .001$, 95%CI: 1.25–3.14, $I^2 = 91.7\%$). In subgroup analyzes by source of controls, the results showed that the EGF + 61A/G polymorphism was significantly associated with the risk of virus-related HCC in hospital-based studies (G vs A: OR = 1.72, $P < .001$, 95%CI: 1.29–2.29, $I^2 = 88.8\%$), but not in population-based studies (G vs A: OR = 1.12, $P = .202$, 95%CI: 0.99–1.27, $I^2 = 0.0\%$). Furthermore, according to the type of controls, a significant association was observed between EGF + 61A/G polymorphism and the risk of virus-related HCC when the controls had chronic liver diseases (G vs A: OR = 2.02, $P < .001$, 95%CI: 1.32–3.09, $I^2 = 82.7\%$), and when the controls were healthy individuals (G vs A: OR = 1.40, $P < .001$, 95%CI: 1.10–1.79, $I^2 = 86.4\%$) (Table 2).

Table 1

Main characteristics of eligible studies included in the meta-analysis.

First author	Yr	Country (ethnicity)	Source of controls	Type of controls	Sample origin	Genotyping methods	Sample size (case/control)	Genotype frequency (case/control)			G allele frequency	HWE	Quality score
								GG	GA	AA			
Tanabe-FRA	2008	France (European)	HB	Cirrhosis	Peripheral bolld	PCR-RFLP	44/77	15/12	17/37	12/28	39.60%	Y	13.5
Tanabe-USA	2008	USA (mixed)	HB	HBV/HCV/ Cirrhosis	Peripheral bolld	PCR-RFLP	59/148	23/32	27/65	9/51	43.60%	y	14.5
Qi	2009	China (Asian)	HB and PB	Healthy/HBV	Peripheral bolld	PCR-RFLP	215/380	102/182	98/160	15/38	68.90%	Y	21
Wang-GX	2009	China (Asian)	HB	Healthy/HBV	Peripheral bolld	PCR-RFLP	376/477	190/208	154/221	32/48	66.80%	Y	17.5
Wang-JS	2009	China (Asian)	HB	Healthy/HBV	Peripheral bolld	PCR-RFLP	186/198	107/93	65/88	14/17	69.20%	Y	18
Li	2010	China (Asian)	HB and PB	Healthy/ Cirrhosis	Peripheral bolld	PCR-RFLP	186/338	96/161	82/145	8/32	69.10%	Y	19.5
Abu Dayyeh	2011	USA (mixed)	HB	HCV	Peripheral bolld	PCR-RFLP	66/750	26/178	25/350	15/222	47.10%	Y	16.5
Chen	2011	China (Asian)	HB	Healthy/HBV/ Cirrhosis	Peripheral bolld	PCR-RFLP	120/240	62/106	51/110	7/24	67.10%	Y	19
Abbas	2012	Egypt (African)	HB	Healthy/HCV/ Cirrhosis	Peripheral bolld	PCR-RFLP	20/60	7/9	9/28	4/23	38.30%	Y	12
Cmet	2012	Italy (European)	HB and PB	Healthy/HBV	Peripheral bolld	PCR-RFLP	18/361	4/66	10/172	4/123	42.10%	Y	16
Shi	2012	China (Asian)	HB	Healthy	Peripheral bolld	PCR-RFLP	73/117	18/13	31/52	24/52	33.30%	Y	13.5
El-Bendary	2013	Egypt (African)	HB	HCV/Cirrhosis	Peripheral bolld	PCR-RFLP	133/105	57/9	43/36	33/60	25.70%	Y	12
Suenaga	2013	Japan (Asian)	HB	Healthy/HBV/ HCV	Peripheral bolld	PCR-RFLP	208/290	108/161	89/104	11/25	73.40%	Y	11.5
Wu	2013	China (Asian)	HB and PB	Healthy/HBV	Peripheral bolld	TaqMan	404/1370	206/647	153/576	45/147	68.20%	Y	17.5
Yuan-USA	2013	USA (mixed)	HB	Healthy	Peripheral bolld	TaqMan	117/225	28/63	61/102	28/60	50.70%	Y	19
Yuan-CHN	2013	China (Asian)	HB	Healthy/HBV/ HCV	Peripheral bolld	TaqMan	250/245	25/20	99/107	126/118	30.00%	Y	15
Wei	2016	China (Asian)	HB	HCV	Peripheral bolld	MALDI-TOF-MS	47/213	30/101	15/98	2/14	72.1%	Y	12.5
El Sergany	2017	Egypt (African)	HB	Healthy	Peripheral bolld	TaqMan	50/50	42/2	5/6	3/42	49.50%	Y	15.5
Gholizadeh	2017	Iranian (Asian)	HB	Healthy	FFPE/ Healthy	PCR-RFLP	40/106	4/34	25/48	11/24	51%	Y	13
Asar	2020	Egypt (African)	HB	Healthy/HCV	Peripheral bolld	TaqMan	30/60	11/11	10/34	9/15	48.89%	Y	13
Baghdadi	2020	Egypt (African)	HB	Healthy/ Cirrhosis	Peripheral bolld	TaqMan	50/25	20/4	23/13	7/8	56%	Y	21

HB = hospital-based, HBV = control subjects were hepatitis B virus carriers, HCV = control subjects were hepatitis C virus carriers, HWE = Hardy-Weinberg equilibrium in control population, MALDI-TOF-MS = matrix-assisted laser desorption/ ionization time of flight mass spectrometry, N = no, PB = population-based, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism, Y = yes.

3.4. Heterogeneity analysis

The Q-statistic indicated statistically significant heterogeneity across all studies under all genetic models except for heterozygote comparison (Table 2). However, in the subgroup analyses by ethnicity, the between-study heterogeneity was not observed in Asian and European populations. Moreover, meta-regression indicated that both ethnicity, type of control, and study quality significantly contributed to the heterogeneity (Table 3).

3.5. Sensitivity analysis and publication bias

Begg's funnel plots were generated and Egger's test was performed on the final set of 21 studies to assess publication bias. The results showed that the risk of publication bias may exist in the overall population, but a low risk of publication bias in Asian populations (Fig. 3 and Table 4). Sensitivity analysis was performed by sequential omission of individual studies. Pooled ORs were consistently significant in general populations or Asian populations by omitting 1 study at a time under the allelic contrast, recessive model, and homozygote comparison, suggesting the robustness of our results (Fig. 4).

4. Discussion

HCC is a complex disease in which the environment and the host interact with multiple genes.[43] Currently recognized risk factors for HCC include liver virus infections, aflatoxins, alcoholic liver cirrhosis, etc.[4] However, only a small number of people exposed to the above risk factors eventually develop HCC, which indicates that host genetic factors may play an important role in the pathogenesis of HCC. Accumulating evidence has

Table 2

Main results of meta-analysis for EGF + 61A/G polymorphism and HCC risk.

Subgroup	No. comparisons	Sample size (case/control)	GG vs GA + AA		GG + GA vs AA		GG vs AA		GA vs AA		G vs A	
			OR (95% CI)	I^2 (%)	OR (95% CI)	I^2 (%)	OR (95% CI)	I^2 (%)	OR (95% CI)	I^2 (%)	OR (95% CI)	I^2 (%)
Overall	21	2680/5835	1.67 (1.29–2.15)*	79#	1.67 (1.26–2.20)*	70.2#	2.18 (1.50–3.16)*	76.6#	1.20 (1.03–1.39)*	23.7	1.56 (1.26–1.94)*	86.8#
Racial descent												
Asian	11	2105/3974	1.21 (1.01–1.45)*	52.5#	1.18 (0.99–1.42)	6.5	1.39 (1.07–1.82)*	34	1.11 (0.92–1.32)	4.5	1.15 (1.02–1.29)*	40.1
European	2	62/438	2.07 (0.98–4.38)	12.6	1.61 (0.84–3.12)	0	2.51 (1.10–5.72)*	0	1.30 (0.64–2.63)	0	1.59 (1.05–2.41)*	0
African	5	271/300	6.97 (2.34–20.71)*	79.8#	4.12 (1.23–13.81)*	86.5#	9.74 (2.43–39.12)*	82.9#	1.55 (0.99–2.41)	60.8#	4.46 (1.53–13.02)*	93#
Mixed	3	242/1123	1.55 (0.79–3.06)	77.3#	1.57 (0.96–2.58)	46.9	1.94 (0.87–4.33)	73.1#	1.37 (0.94–2.00)	11	1.45 (0.93–2.26)	77.1#
Study quality												
High quality	9	1720/4003	1.26 (1.07–1.48)*	38.4	1.26 (1.04–1.52)*	0	1.46 (1.13–1.89)*	29.2	1.16 (0.95–1.42)	0	1.20 (1.08–1.34)*	24.2
Low quality	12	960/1832	2.34 (1.30–4.20)*	85.6#	2.03 (1.22–3.38)*	80.7#	3.00 (1.48–6.08)*	82.3#	1.24 (1.00–1.54)*	40	1.98 (1.25–3.14)*	91.7#
Source of controls												
Population-based	4	823/2449	1.12 (0.95–1.32)	0	1.37 (0.90–2.10)	41.9	1.35 (0.93–1.95)	21.6	1.18 (0.88–1.58)	50.9#	1.12 (0.99–1.27)	0
Hospital-based	17	1857/3386	1.94 (1.38–2.73)*	81.6#	1.75 (1.24–2.45)*	73.6#	2.44 (1.53–3.89)*	78.8#	1.20 (1.01–1.43)*	20.3	1.72 (1.29–2.29)*	88.8#
Type of controls												
Healthy controls	15	2124/4919	1.47 (1.10–1.95)*	78.5#	1.40 (1.03–1.90)*	69.1#	1.68 (1.14–2.47)*	72.9#	1.08 (0.92–1.27)	20.4	1.40 (1.10–1.79)*	86.4#
Patients with chronic liver diseases	6	556/916	2.39 (1.33–4.28)*	79.4#	2.77 (1.99–3.86)*	0	4.13 (2.29–7.45)*	52#	1.89 (1.32–2.70)*	0	2.02 (1.32–3.09)*	82.7#
Number of cases												
>100	17	2530/5640	1.42 (1.14–1.77)*	72.1#	1.44 (1.17–1.78)*	45.9	1.78 (1.29–2.45)*	67.7#	1.18 (1.01–1.37)*	0	1.33 (1.12–1.56)*	76.7#
≤100	4	150/195	6.99 (1.51–32.40)*	84.3#	4.44 (0.70–28.16)	89.9#	9.73 (1.31–71.99)*	86.7#	1.53 (0.83–2.82)	70.6#	4.58 (0.95–22.09)	94.7#
Genotyping methods												
PCR-RFLP	14	1732/3647	1.56 (1.17–2.07)*	75.6#	1.68 (1.35–2.09)*	22.6	2.14 (1.46–3.13)*	65.5#	1.39 (1.14–1.70)*	0	1.44 (1.17–1.77)*	78.1#
TaqMan	6	901/1975	2.41 (1.14–5.11)*	87.7#	1.87 (0.91–3.84)	88.3#	2.74 (1.08–6.95)*	88#	0.98 (0.78–1.23)	61.9#	2.15 (1.15–4.01)*	94.4#
MALDI-TOF-MS	1	47/213	1.96 (1.02–3.76)*	/	1.58 (0.35–7.21)	/	2.08 (0.45–9.67)	/	1.07 (0.22–5.19)	/	1.66 (0.96–2.86)	/

95% CI, 95% confidence interval, EGF = epidermal growth factor, HCC = hepatocellular carcinoma, MALDI-TOF-MS = matrix-assisted laser desorption/ ionization time of flight mass spectrometry, OR = odds ratio, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism assay.

#Random effect estimate.

*Significant results, P value < .05.

proved the important role of genetic factors in the occurrence and development of tumors.[44,45] EGF activates the EGF pathway by combining with transmembrane EGF receptors to promote cell proliferation and differentiation, thereby enhancing the carcinogenic rate of various carcinogens. In recent years, research on the relationship between the EGF + 61A/G polymorphism and malignant tumor susceptibility has gradually increased.[46] including HCC. However, inconsistent findings concerning the association between EGF + 61A/G polymorphism and susceptibility to HCC were observed across the studies.

Herein, 21 cohorts (18 articles) with 2692 virus-related HCC and 5835 controls were included in this meta-analysis to analysis the association between EGF + 61A/G polymorphism and the susceptibility of virus-related HCC. The results suggested that EGF + 61A/G polymorphism was significantly associated with the risk of virus-related HCC. Given the considerable heterogeneity across studies, meta-regression analysis was performed and found the contribution of race, type of controls, and research quality to the heterogeneity. Moreover, the stratified analysis further sheds light on the effect of different variables on the relationship between EGF + 61A/G polymorphism and HCC susceptibility. The sensitivity analysis further strengthened the validity of these positive correlations in the overall population and the Asian population, indicating the credibility of our results.

It is possible that the effects of genetic factors related to cancer are different across various ethnic populations. A large

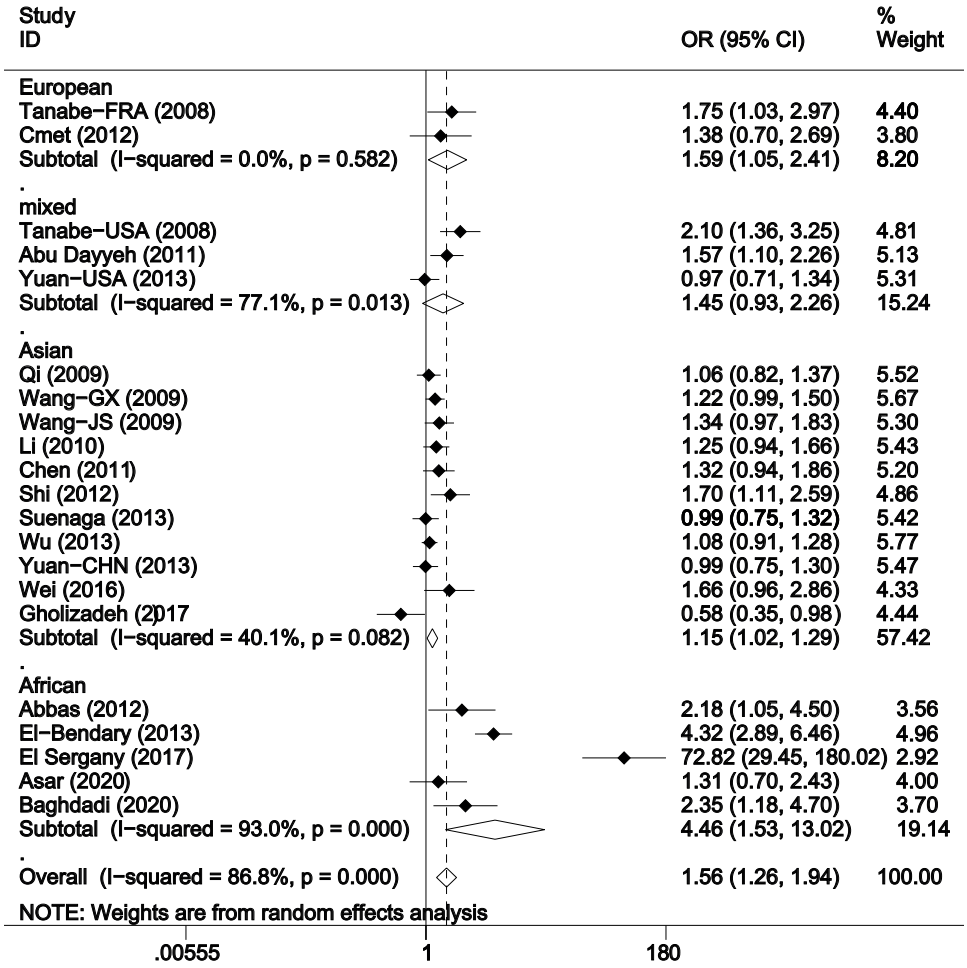

Figure 2. Forest plot for the association between EGF + 61A/G polymorphism and the risk of HCC risk stratified according to different ethnicities (G vs A). For each study, the OR estimate and its 95%CI are plotted with a diamond (◆) and a horizontal line. The size of a box (gray square) is proportional to the weight that the study has in calculating the summary effect estimate. The center of the diamond indicates the OR and the ends of the diamond correspond to the 95%CI. EGF = epidermal growth factor, HCC = hepatocellular carcinoma, OR = odds ratio.

Table 3

Main results of meta-regression for EGF + 61A/G polymorphism and HCC risk.

Factor	GG vs GA + AA		GG + GA vs AA		GG vs AA		GA vs AA		G vs A	
	t	*P*	*t*	*P*	*t*	*P*	*t*	*P*	*t*	*P*
Racial descent	−2.43	**.025**	−1.76	.095	−2.29	**.034**	−1.29	.212	−2.17	**.043**
Source of controls	1.07	.298	0.36	.722	0.73	.473	−0.08	.939	0.84	.41
Type of controls	0.91	.373	2.12	**.048**	1.7	.106	2.77	**.012**	0.71	.486
Genotyping methods	0.68	.507	−0.03	.975	0.25	.803	−2.05	.054	0.73	.477
Sample size	−1.22	.236	−0.89	.386	−1.15	.265	−0.50	.626	−1.11	.28
Quality score	2.6	**.018**	1.9	.073	2.34	**.031**	0.75	.461	2.54	**.02**

The bold value is <0.05 and considered to be significant.

EGF = epidermal growth factor, HCC = hepatocellular carcinoma.

number of studies have shown that the relationship between EGF + 61A/G polymorphism and HCC susceptibility differs between ethnicity. Tanabe KK et al[41] included 2 independent research populations, that one of the research populations was Caucasian, and the other research population was composed of whites, blacks, Asians, and Hispanics. Abu DB et al[38] compared white people to black people. Jiang G et al[46] studied Asian population, European population, and African population. The same result suggested that the EGF + 61A/G polymorphism was significantly associated with the risk of HCC under all genetic models, and the relationship between EGF + 61A/G polymorphism and HCC susceptibility differs between races. In this study, ethnicity was also identified as a potential source of heterogeneity by meta-regression and subgroup analyses. The results showed that the frequency of the EGF + 61G allele was highest in Asian populations, intermediate in European populations, and lowest in African populations. The higher prevalence of the EGF + 61G allele might lead to a higher HCC prevalence among Asian populations. In subgroup analyses based on ethnicity, a significant association was observed between EGF + 61A/G polymorphism and the risk of virus-related HCC in Asian and European populations.

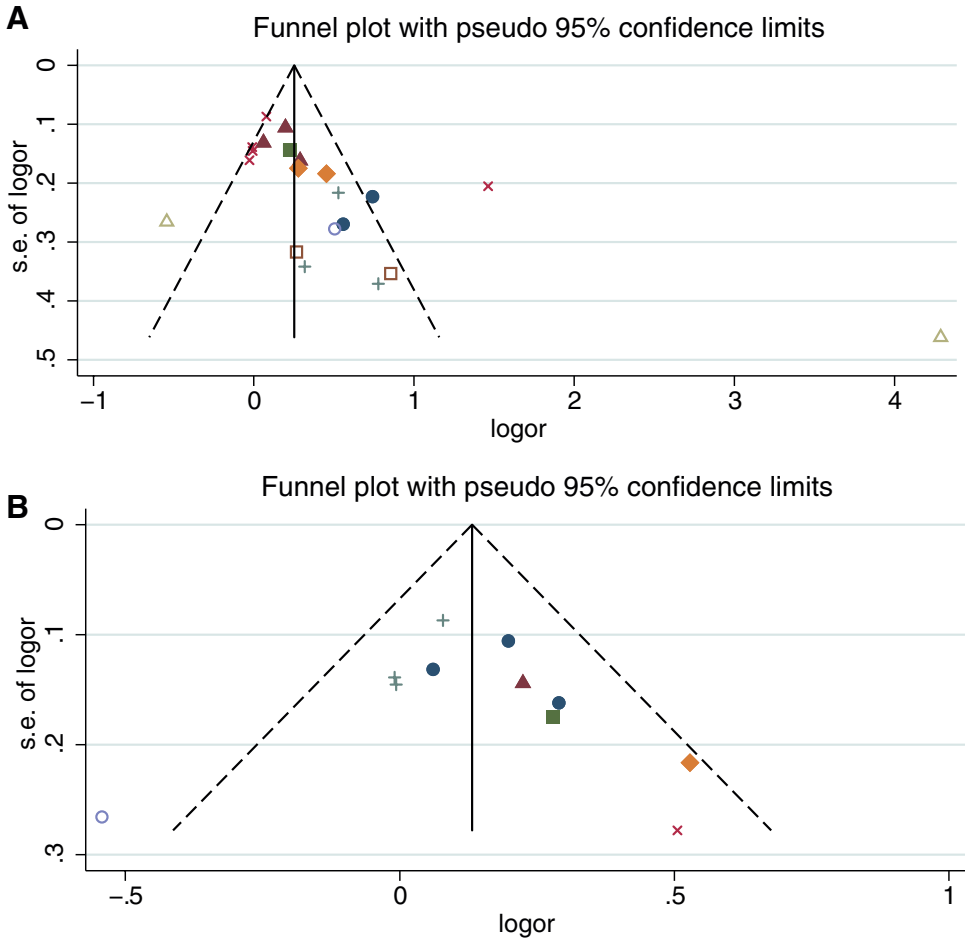

Figure 3. Begg funnel graph of Egger's test for publication bias of the EGF + 61A/G polymorphism and the risk of HCC risk (G vs A). (A) Overall populations; (B) Asian populations. The horizontal line in the funnel plot indicates summary estimate, whereas the sloping lines indicate the expected 95% confidence intervals for a given standard error. EGF = epidermal growth factor, HCC = hepatocellular carcinoma.

Table 4

The results of the Begg and Egger's test.

Population	Genetic model	Begg (zlp)	Egger (tlp)
All	G vs A	3.11|0.002	2.87|0.01
Asian	G vs A	1.09|0.276	0.43|0.68
All	GG vs GA + AA	2.39|0.017	2.78|0.012
Asian	GG vs GA + AA	0.93|0.35	0.04|0.968
All	GG + GA vs AA	2.08|0.037	2.90|0.009
Asian	GG + GA vs AA	1.09|0.276	2.48|0.035
All	GG vs AA	2.87|0.004	2.72|0.014
Asian	GG vs AA	1.56|0.119	0.73|0.482
All	GA vs AA	1.72|0.085	3.18|0.005
Asian	GA vs AA	1.40|0.161	2.59|0.029

There are several limitations in the present study. This study revealed that the EGF + 61A/G polymorphism was significantly associated with the risk of HCC in a hospital-based study, but not in a population-based study. Therefore, the results should be treated with caution, as the controls from hospital-based studies may not be representative of the general population. Larger population-based studies are needed to further confirm the association between EGF + 61A/G polymorphism and HCC susceptibility. Herein, 11 of the 21 eligible studies were classified as low-quality studies, which might not rule out the true influence of factors that could bias estimates and lead to erroneous conclusions. In addition, aside from genetic factors, there are other factors related to the development of HCC, such as exposure to aflatoxin B1, smoking, and habitual alcoholism, which should be considered. Finally, the number of studies included in the meta-analysis for European populations and African populations was relatively small, which may lead to low statistical power and generate fluctuation in estimation.

This study combined currently published research on the relationship between EGF + 61A/G polymorphism and HCC susceptibility and generated credible pooled results. However, due to the limitations mentioned above, more studies with a more rigorous design, larger sample size, and wider perspectives are required to obtain more reliable gene effects and more precision. The inherent relationship between EGF gene polymorphism and HCC susceptibility provides better preventive measures and treatment options for HCC.

5. Conclusion

In summary, this meta-analysis demonstrated that EGF + 61A/G polymorphism was significantly associated with the risk of HCC. Further studies with more rigorous designs, larger sample sizes, and wider perspectives are needed to validate our findings.

Acknowledgments

We are grateful to all researchers in the enrolled studies.

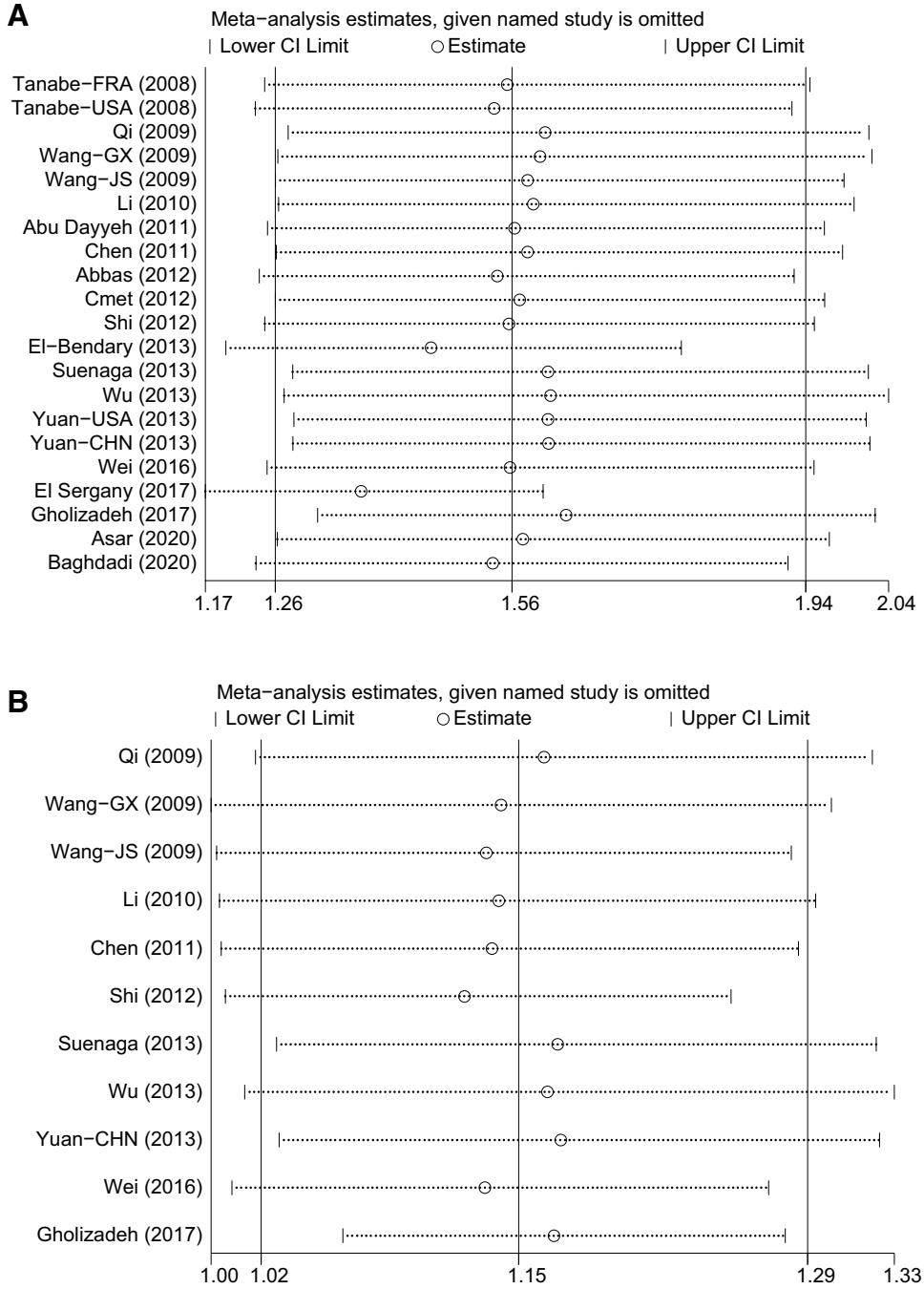

Figure 4. Visualization of the sensitivity analysis based on overall populations (A) and Asian populations (B).

Author contributions

Min Zhang and Qinjing Wang had full access to all the data in the study and takes responsibility for the integrity of the data and the precision of the data analysis; the concept and design were carried out by Lingling Xu, Min Zhang, and Qinjing Wang; acquisition of data was carried out by Lingling Xu and Qianbo Wu; analysis and interpretation of the data was performed by Lingling Xu and Qianbo Wu; drafting of the manuscript was carried out by Lingling Xu, Qinjing Wang, and Jing Zhang; critical review of the manuscript for important intellectual content was carried out by Min Zhang and Jing Zhang; statistical analysis was carried out by Lingling Xu, Qinjing Wang, and Qianbo Wu; administrative, technical or material support: none; supervision, None;

Conceptualization: Qinjing Wang, Lingling Xu, Min Zhang.
Data curation: Qianbo Wu, Min Zhang.
Formal analysis: Lingling Xu, Qianbo Wu, Min Zhang.
Funding acquisition: Lingling Xu.
Writing – original draft: Lingling Xu.
Writing – review & editing: Qinjing Wang, Min Zhang, Jing Zhang.

References

[1] Parkin DM, Bray F, Ferlay J, et al. Estimating the world cancer burden: Globocan 2000. Int J Cancer. 2001;94:153–6.
[2] Parkin DM, Bray F, Ferlay J, et al. Global cancer statistics, 2002. CA Cancer J Clin. 2005;55:74–108.
[3] Michielsen PP, Francque SM, van Dongen JL. Viral hepatitis and hepatocellular carcinoma. World J Surg Oncol. 2005;3:27.

[4] Melero I, Crocenzi T, Welling T, et al. Phase I/II safety and antitumor activity of nivolumab in patients with advanced hepatocellular carcinoma (HCC): CA209-040. J Clin Oncol. 2015;33:A101.
[5] Papatheodoridis GV, Chan HL, Hansen BE, et al. Risk of hepatocellular carcinoma in chronic hepatitis B: assessment and modification with current antiviral therapy. J Hepatol. 2015;62:956–67.
[6] de Martel C, Maucort-Boulch D, Plummer M, et al. World-wide relative contribution of hepatitis B and C viruses in hepatocellular carcinoma. Hepatology. 2015;62:1190–200.
[7] Yang S, Lin Q, Lin W, et al. Effect of adjuvant interferon therapy on hepatitis B virus-related hepatocellular carcinoma: a systematic review. World J Surg Oncol. 2016;14:159.
[8] Li W, Deng R, Liu S, et al. Hepatitis B virus-related hepatocellular carcinoma in the era of antiviral therapy: the emerging role of non-viral risk factors. Liver Int. 2020;40:2316–25.
[9] Khemlina G, Ikeda S, Kurzrock R. The biology of hepatocellular carcinoma: implications for genomic and immune therapies. Mol Cancer. 2017;16:149.
[10] Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. Lancet. 2003;362:1907–17.
[11] Kim CK, Lim JH, Lee WJ. Detection of hepatocellular carcinomas and dysplastic nodules in cirrhotic liver: accuracy of ultrasonography in transplant patients. J Ultrasound Med. 2001;20:99–104.
[12] Trevisani F, D'Intino PE, Morselli-Labate AM, et al. Serum alpha-fetoprotein for diagnosis of hepatocellular carcinoma in patients with chronic liver disease: influence of HBsAg and anti-HCV status. J Hepatol. 2001;34:570–5.
[13] Elgenidy A, Afifi AM, Awad AK, et al. Utility of serum angiopoietin-2 as diagnostic marker of hepatocellular carcinoma: a systematic review and diagnostic test accuracy meta-analysis. Clin Res Hepatol Gastroenterol. 2022;46:101909.
[14] Zender L, Villanueva A, Tovar V, et al. Cancer gene discovery in hepatocellular carcinoma. J Hepatol. 2010;52:921–9.
[15] Zhong JH, You XM, Gong WF, et al. Epidermal growth factor gene polymorphism and risk of hepatocellular carcinoma: a meta-analysis. PLoS One. 2012;7:e32159.
[16] Xu W, Li Y, Wang X, et al. Association between EGF promoter polymorphisms and cancer risk: a meta-analysis. Med Oncol. 2010;27:1389–97.
[17] Zhang YM, Cao C, Liang K. Genetic polymorphism of epidermal growth factor 61A>G and cancer risk: a meta-analysis. Cancer Epidemiol. 2010;34:150–6.
[18] Almeida LO, Custódio AC, Santos MJ, et al. The A61G EGF polymorphism is associated with development of extraaxial nervous system tumors but not with overall survival. Cancer Genet Cytogenet. 2010;198:15–21.
[19] Limaye PB, Bowen WC, Orr AV, et al. Mechanisms of hepatocyte growth factor-mediated and epidermal growth factor-mediated signaling in transdifferentiation of rat hepatocytes to biliary epithelium. Hepatology. 2008;47:1702–13.
[20] Fisher DA, Lakshmanan J. Metabolism and effects of epidermal growth factor and related growth factors in mammals. Endocr Rev. 1990;11:418–42.
[21] Mullhaupt B, Feren A, Fodor E, et al. Liver expression of epidermal growth factor RNA. Rapid increases in immediate-early phase of liver regeneration. J Biol Chem. 1994;269:19667–70.
[22] Villanueva A, Newell P, Chiang DY, et al. Genomics and signaling pathways in hepatocellular carcinoma. Semin Liver Dis. 2007;27:55–76.
[23] Baghdadi I, Abu Ella K, El Shaaraway A, et al. Genetic polymorphism of epidermal growth factor gene as a predictor of hepatocellular carcinoma in Hepatitis C cirrhotic patients. Asian Pac J Cancer Prev. 2020;21:2047–53.
[24] Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ. 2009;339:b2535.
[25] Thompson SG, Sharp SJ. Explaining heterogeneity in meta-analysis: a comparison of methods. Stat Med. 1999;18:2693–708.
[26] Asar S, Elshayeb A, Elwazzan D, et al. Epidermal growth factor gene polymorphism in hepatitis C virus patients with hepatocellular carcinoma. J Hepatol. 2020;73:S326–7.
[27] Gholizadeh M, Khosravi A, Torabian P, et al. Association of the epidermal growth factor gene +61A>G polymorphism with hepatocellular carcinoma in an Iranian population. Gastroenterol Hepatol Bed Bench. 2017;10:284–8.
[28] El Sergany HF, Mohamed AM, Madkour NK, et al. Epidermal growth factor gene polymorphism in Egyptian patients with hepatocellular carcinoma related to hepatitis C. J Gastroenterol Hepatol Res 2017;6:2481–5.
[29] Wei H, Zhang ST, Qiao KY, et al. Genetic polymorphism of EGF rs4444903 influences susceptibility to HCV-related hepatocellular carcinoma in a Chinese Han population: a casecontrol study via MALDI-TOF MS assay. J Dig Dis. 2016;17:93–4.
[30] Suenaga M, Yamada S, Fujii T, et al. A functional polymorphism in the epidermal growth factor gene predicts hepatocellular carcinoma risk in Japanese hepatitis C patients. Onco Ther. 2013;6:1805–12.
[31] Wu J, Zhang W, Xu A, et al. Association of epidermal growth factor and epidermal growth factor receptor polymorphisms with the risk of hepatitis b virus-related hepatocellular carcinoma in the population of north China. Genet Test Mol Biomark. 2013;17:595–600.
[32] El-Bendary M, Neamatallah M, El-Maksoud MA, et al. 640 epidermal growth factor genetic polymorphism predicts risk of hepatocellular carcinoma in Egyptian patients with hcv (genotype 4)-related cirrhosis. J Hepatol. 2013;58:S261.
[33] Yuan JM, Fan Y, Ognjanovic S, et al. Genetic polymorphisms of epidermal growth factor in relation to risk of hepatocellular carcinoma: two case-control studies. BMC Gastroenterol. 2013;13:32.
[34] Abbas E, Shaker O, El Aziz GA, et al. Epidermal growth factor gene polymorphism 61A/G in patients with chronic liver disease for early detection of hepatocellular carcinoma: a pilot study. Eur J Gastroenterol Hepatol. 2012;24:458–63.
[35] Cmet S, Fabris C, Fattovich G, et al. Carriage of the EGF rs4444903 A>G functional polymorphism associates with disease progression in chronic HBV infection. Clin Exp Immunol. 2012;167:296–302.
[36] Shi HZ, Ren P, Lu QJ, et al. Association between EGF, TGF-β1 and TNF-α gene polymorphisms and hepatocellular carcinoma. Asian Pac J Cancer Prev. 2012;13:6217–20.
[37] Chen K, Wei Y, Yang H, et al. Epidermal growth factor +61 G/A polymorphism and the risk of hepatocellular carcinoma in a Chinese population. Genet Test Mol Biomark. 2011;15:251–5.
[38] Abu Dayyeh BK, Yang M, Fuchs BC, et al. A functional polymorphism in the epidermal growth factor gene is associated with risk for hepatocellular carcinoma. Gastroenterology. 2011;141:141–9.
[39] Li Y, Xie Q, Lu F, et al. Association between epidermal growth factor 61A/G polymorphism and hepatocellular carcinoma susceptibility in Chinese patients. Liver Int. 2010;30:112–8.
[40] Qi P, Wang H, Chen YM, et al. No association of EGF 5'UTR variant A61G and hepatocellular carcinoma in Chinese patients with chronic hepatitis B virus infection. Pathology. 2009;41:555–60.
[41] Tanabe KK, Lemoine A, Finkelstein DM, et al. Epidermal growth factor gene functional polymorphism and the risk of hepatocellular carcinoma in patients with cirrhosis. JAMA. 2008;299:53–60.
[42] Hongxue W. Genetic association between epidermal growth factor gene polymorphisms and hepatocellular carcinoma (in Chinese). Guangxi Province, China: Guangxi Medical University; 2009.
[43] Llovet JM, Bruix J. Molecular targeted therapies in hepatocellular carcinoma. Hepatology. 2008;48:1312–27.
[44] Ciardiello F, Tortora G. EGFR antagonists in cancer treatment. N Engl J Med. 2008;358:1160–74.
[45] Ji H, Li D, Chen L, et al. The impact of human EGFR kinase domain mutations on lung tumorigenesis and in vivo sensitivity to EGFR-targeted therapies. Cancer Cell. 2006;9:485–95.
[46] Jiang G, Yu K, Shao L, et al. Association between epidermal growth factor gene +61A/G polymorphism and the risk of hepatocellular carcinoma: a meta-analysis based on 16 studies. BMC Cancer. 2015;15:314.