

# Quantitative assessment of the effect of epidermal growth factor 61A/G polymorphism on the risk of hepatocellular carcinoma

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**Abstract.** The association between hepatocellular carcinoma (HCC) and the epidermal growth factor (EGF) 61A/G polymorphism has been analyzed in several studies, but results remain inconsistent. Therefore, the aim of the present study was to quantitatively summarize the association between the EGF 61A/G polymorphism and the risk of HCC. The PubMed and EMBASE databases were searched for studies published prior to May 1, 2014. The overall, subgroup and sensitivity analyses were conducted using Comprehensive Meta-Analysis software, version 2.2. In total, 12 published case-control studies, consisting of 2,095 patients with HCC and 3,766 control individuals, were included in the present study. Meta-analysis of the included studies revealed that EGF 61A/G polymorphism contributed to the risk of HCC under all four genetic models, consisting of the G vs. A (OR, 1.25; 95% CI, 1.11-1.40), GG vs. AA (OR, 1.53; 95% CI, 1.26-1.85), GG vs. AG + AA (OR, 1.34; 95% CI, 1.13-1.58) and GG + AG vs. AA (OR, 1.27; 95% CI, 1.08-1.49) comparisons. Subgroup analysis further suggested that EGF 61A/G polymorphism was associated with the risk of HCC in patients and control individuals with liver disease, based on ethnicity and source of control, respectively. No other significance in residual subgroup analysis was observed. The present meta-analysis suggests that the EGF 61A/G polymorphism is associated with an increased risk of HCC and may be a potential marker for liver disease, such as hepatitis B virus infection, hepatitis C virus infection and liver cirrhosis.

## Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and develops predominately in individuals

with liver cirrhosis (1). Cirrhosis is the strongest known risk factor for HCC, particularly cirrhosis resulting from infection with hepatitis C virus (HCV) or hepatitis B virus (HBV) (2,3). Additionally, heavy alcohol consumption, diabetes, obesity and tobacco use have been considered to contribute to the local burden of HCC (4,5). However, only a small number of people exposed to these risk factors develop HCC, suggesting that other environmental and genetic factors may play a role in HCC development. For this reason, the pathogenesis of HCC has not been fully elucidated.

Additionally, numerous clinicians rely on serological  $\alpha$ -fetoprotein testing and abdominal ultrasound imaging for HCC screening (6). However, these screening tools demonstrate low sensitivity and specificity (7-9) and the diagnoses of HCC are made late in the course of the disease. Therefore, early identification of molecular markers associated with an increased risk of HCC has been proposed as an alternative strategy for the diagnosis of HCC.

Epidermal growth factor (EGF) was first isolated in 1962 (10) and plays a critical role in liver tissue regeneration (11). In previous years, numerous studies have revealed that the EGF signaling pathway with the EGF 61A/G polymorphism (rs4444903), a commonly functional single-nucleotide polymorphism (SNP) in the 5'-untranslated region of the EGF gene, is associated with the risk of tumorigenesis in multiple human cancers (12-14). Studies have also reported that the EGF 61A/G polymorphism plays an important role in the occurrence of liver cancer. At present, there are three published meta-analyses that have investigated the association between the EGF 61A/G polymorphism and risk of cancer, including HCC (15-17). However, none of these studies searched a sufficient number of published studies and are not limited to HCC. Therefore, the studies are not conclusive in resolving the role of the EGF 61A/G polymorphism in HCC. Thus, the present meta-analysis was performed to address the association between the frequency of the EGF 61A/G polymorphism and the risk of HCC, and to complete an in-depth subgroup analysis of the study population characteristics.

## Materials and methods

**Inclusion criteria.** The present meta-analysis was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (18). Studies that

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met all of the following criteria were included: (1) Use of a cohort or case-control design; (2) sufficient data for examining an odds ratio (OR), with its 95% confidence interval (CI); (3) assessment of the EGF 61A/G polymorphism and HCC risk; and (4) the diagnosis of HCC was confirmed histologically, pathologically or cytologically. The titles and abstracts of all relevant studies were evaluated, and case reports, editorials and reviews were excluded.

**Search strategy.** All cohort studies and case-control studies of the EGF 61A/G polymorphism and risk of HCC published prior to May 1, 2014 were identified through systematic searches in the PubMed (National Institutes of Health, Bethesda, MA, USA) and EMBASE (Elsevier, Amsterdam, Netherlands) databases, using the following search strategy: ('epidermal growth factor' or 'EGF') AND 'polymorphism' AND ('hepatocellular carcinoma' or 'liver cancer' or 'HCC'). In addition, the reference lists of relevant publications were manually searched by two independent investigators.

**Data extraction.** For each study, the first author, year of publication, ethnicity of the population, type of control, number of patients and control individuals, genotyping method and Hardy Weinberg equilibrium (HWE) was extracted for the control group. The results were compared and discrepancies were resolved by consensus between two independent investigators.

**Statistical analysis.** The odds ratios (ORs) and relative 95% confidence intervals (CIs) were used to assess the strength of associations between the EGF 61A/G polymorphism and the risk of HCC by comparing five genetic models, which consisted of the G vs. A, AG vs. AA, GG vs. AA, GG vs. AG + AA, and AG + GG vs. AA models. Subgroup analysis was also performed based on the ethnicity and type of controls. Heterogeneity among the studies used was tested using the  $I^2$  test (19).  $I^2 < 40\%$  indicated an acceptable heterogeneity among the included studies in the present meta-analysis and the fix-effect model was used, otherwise the random-effect model was used.

The sensitivity analysis was conducted by omitting any single included study each turn. Publication bias was assessed by visual inspection of the funnel plots of the primary outcome and the Egger's test (20). The funnel plot was considered to be asymmetrical if the intercept of the Egger's regression line significantly deviated from zero, with a P-value of  $< 0.05$ . HWE in the control group was assessed using Fisher's exact test, with  $P < 0.05$  considered to indicate a statistically significant difference. All statistical tests for the present meta-analysis were conducted using Comprehensive Meta-Analysis software, Version 2.2 (Biostat, Inc., Englewood, NJ, USA).

## Results

**Study selection and patient characteristics.** The combined search yielded 132 studies, 123 of which were excluded as they clearly did not satisfy the inclusion criteria or were overlapping references (two or more publications from the same institute or duplicate publication using different languages). The publications by Zhong *et al* (15), Tanabe *et al* (21) and Yuan *et al* (22) all involved two independent case-control studies and were

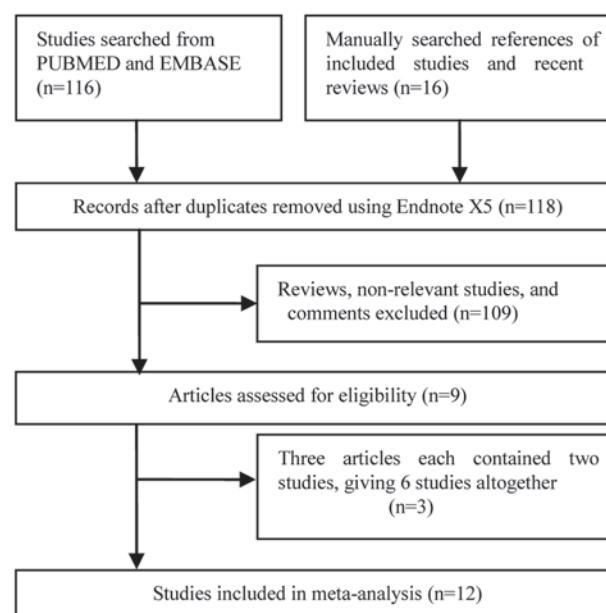


Figure 1. Flowchart of the study selection process.

overall considered to be six single studies. Finally, a total of 12 studies (15,21-28) that examined the association between the EGF 61A/G polymorphism and the risk of HCC were included in the current meta-analysis (Fig. 1).

A database was created according to the information extracted from each study. The detailed characteristics of the included studies are summarized in Table I. Overall, 2,095 patients with HCC and 3,766 control individuals were retrieved. Seven of the studies enrolled Chinese individuals (15,22-26), three studies involved a mixed population, including Caucasian, Hispanic and Asian populations and individuals of African descent (21,22,27), one study enrolled only Caucasians (21) and one enrolled only Egyptian individuals (28). The genotype distributions in the controls for all studies were consistent with the HWE expectations.

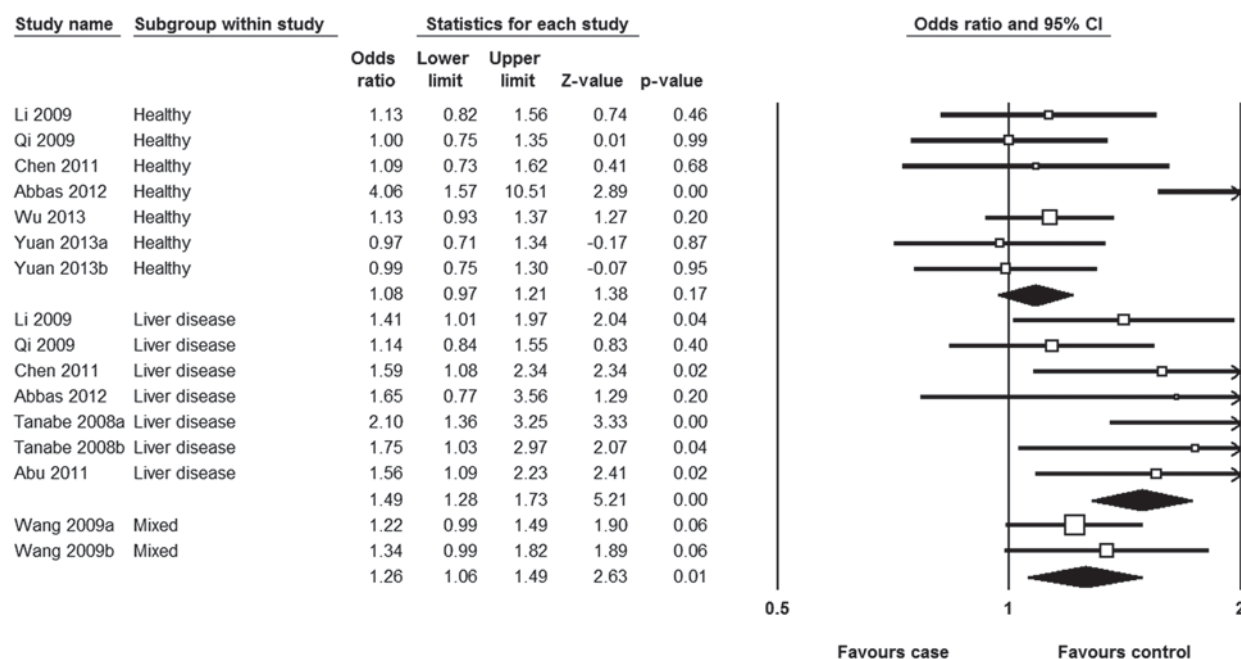
**Overall analysis.** The evaluation of association between the EGF 61A/G polymorphism and the risk of HCC is reported in Table II. Calculation of overall ORs in the total population demonstrated that the EGF 61A/G polymorphism was associated with increased risk of HCC in the total population in the G vs. A (OR, 1.25; 95% CI, 1.11-1.40), GG vs. AA (OR, 1.53; 95% CI, 1.26-1.85), GG vs. AG + AA (OR, 1.34; 95% CI, 1.13-1.58) and GG + AG vs. AA (OR, 1.27; 95% CI, 1.08-1.49) models.

**Subgroup analysis.** The results were similar between the ethnicities, with the overall results in the Chinese population being similar to those of the other ethnicities. No significant association was observed between the EGF 61A/G polymorphism and HCC risk in the mixed population. When stratifying by source of controls, the EGF 61A/G polymorphism was associated with an increased risk of HCC in the control individuals with a liver disease. However, the meta-analysis revealed that there was no association between the EGF 61A/G polymorphism and the risk of HCC in healthy and mixed controls (Table II; Fig. 2).

Table I. Characteristics of included studies in the meta-analysis.

First author, year (ref.)	Ethnicity	Hepatocellular carcinoma group				Type of control	Control group				Genotyping method	HWE
		Total	GG	AG	AA		Total	GG	AG	AA		
Tanabe, 2008a (21)	Mixed	59	23	27	9	Cirrhosis	148	32	65	51	PCR-RFLP	0.19
Tanabe, 2008b (21)	Caucasian	44	15	17	12	Cirrhosis	77	12	37	28	PCR-RFLP	0.97
Li, 2009 (25)	Chinese	186	96	82	8	Healthy	186	96	73	17	PCR-RFLP	0.56
						Cirrhosis	152	65	72	15		0.44
Qi, 2009 (24)	Chinese	215	102	98	15	Healthy	208	104	84	20	PCR-RFLP	0.64
						HBV infection	172	78	76	18		0.97
Zhong, 2012a (15)	Chinese	397	200	163	34	Mixed	480	209	222	49	PCR-RFLP	0.37
Zhong, 2012b (15)	Chinese	217	125	76	16	Mixed	200	94	89	17	PCR-RFLP	0.53
Chen, 2011 (26)	Chinese	120	62	51	7	Healthy	120	61	49	10	PCR-RFLP	0.97
						HBV infection	120	45	61	14		0.33
Abu, 2011 (27)	Mixed	66	26	25	15	HCV infection	750	180	350	220	Real-time PCR	0.08
Abbas, 2012 (28)	Egyptian	20	7	9	4	Healthy	20	2	6	12	PCR	0.37
						HCV infection or cirrhosis	40	7	22	11		0.48
Yuan, 2013a (22)	Mixed	117	28	61	28	Healthy	225	63	102	60	TaqMan	0.16
Yuan, 2013b (22)	Chinese	250	25	99	126	Healthy	245	20	107	118	TaqMan	0.53
Wu, 2013 (23)	Chinese	404	206	153	45	Healthy	623	291	256	76	TaqMan	0.09

ref., reference; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; HWE, hardy-weinberg equilibrium.



### Meta analysis

Figure 2. Forest plot describing the association of EGF 61A/G polymorphism with hepatocellular carcinoma based on the G vs. A genetic model.

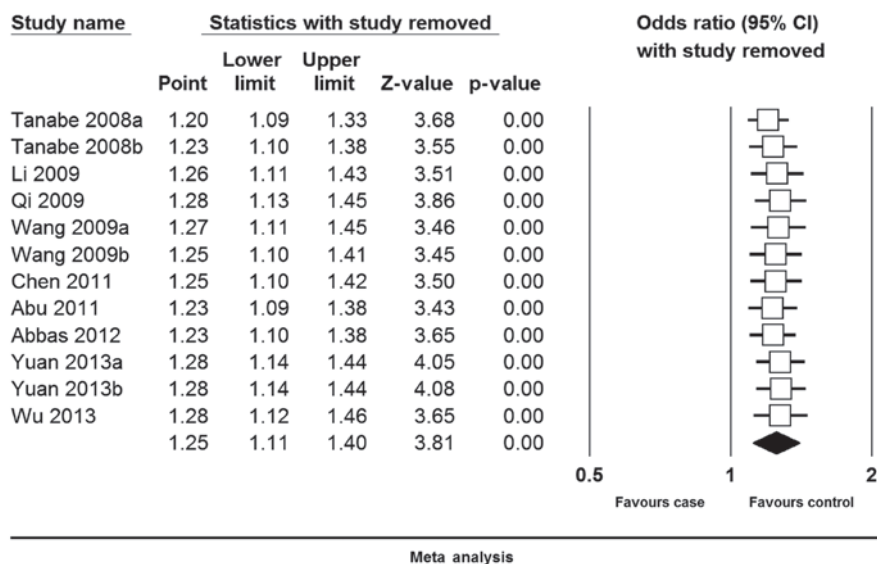


Figure 3. Forest plot of sensitivity analysis, performed by omitting each study in each turn, based on the G vs. A genetic model.

**Sensitivity analysis.** For the sensitivity analysis, each study involved in the meta-analysis was omitted each time to reflect the influence of the individual dataset to the pooled ORs. The corresponding pooled ORs were not qualitatively altered, indicating that the present results were statistically robust (Fig. 3).

**Publication bias.** Funnel plot and Egger's test were performed to assess the publication bias of literature. The shape of the funnel plot (Fig. 4) appeared to be asymmetrical for the EGF 61A/G polymorphism in the genotype comparison of G vs. A, indicating the presence of publication bias. Therefore, Egger's

test was performed to statistically assess the symmetry of the funnel plot. The result suggested that publication bias probably existed in the present study for the G vs. A ( $P=0.013$ ), GG vs. AA ( $P=0.004$ ), AG vs. AA ( $P=0.011$ ), GG + AG vs. AA ( $P<0.001$ ) and GG vs. AG + AA ( $P=0.051$ ) genotypes.

### Discussion

EGF has been hypothesized to promote hepatocyte transformation, and dysregulation of the EGF signaling pathway has been speculated to be important in early

Table II. Overall and subgroups meta-analysis of EGF 61A/G polymorphism and HCC risk.

Characteristic	n	G vs. A			GG vs. AA			AG vs. AA			GG vs. AG+AA			GG+AG vs. AA		
		OR	(95% CI)	I <sup>2</sup> , %	OR	(95% CI)	I <sup>2</sup> , %	OR	(95% CI)	I <sup>2</sup> , %	OR	(95% CI)	I <sup>2</sup> , %	OR	(95% CI)	I <sup>2</sup> , %
Overall	12	1.25	(1.11-1.40)	40.29	1.53	(1.26-1.85)	27.77	1.15	(0.96-1.36)	0.00	1.34	(1.13-.58)	40.62	1.27	(1.08-1.49)	14.57
Ethnicity																
Chinese	7	1.17	(1.06-1.28)	0.00	1.39	(1.11-1.74)	0.00	1.08	(0.88-1.33)	10.26	1.23	(1.08-1.40)	0.00	1.17	(0.97-1.42)	4.93
Mixed	3	1.44	(0.93-2.25)	76.86	1.93	(0.87-4.28)	72.79	1.36	(0.93-1.98)	12.47	1.54	(0.79-3.02)	76.82	1.57	(0.96-2.57)	46.96
Caucasian	1	1.75	(1.03-2.97)	NA	2.92	(1.06-8.06)	NA	1.07	(0.44-2.60)	NA	2.80	(1.17-6.73)	NA	1.52	(0.68-3.42)	NA
Egyptian	1	2.18	(1.05-4.50)	NA	4.47	(1.05-19.07)	NA	1.85	(0.50-6.79)	NA	3.05	(0.96-9.74)	NA	2.49	(0.74-8.36)	NA
Source of controls																
Liver disease	7	1.49	(1.28-1.73)	0.26	3.00	(2.11-4.26)	0.00	1.47	(1.07-2.04)	0.00	1.62	(1.31-1.99)	26.84	1.81	(1.33-2.45)	0.00
Healthy	7	1.08	(0.97-1.21)	31.77	1.55	(1.04-2.33)	46.61	1.14	(0.92-1.42)	35.00	1.06	(0.91-1.25)	0.00	1.17	(0.95-1.44)	38.31
Mixed	2	1.26	(1.06-1.49)	0.00	1.39	(0.93-2.07)	0.00	1.01	(0.67-1.52)	0.00	1.38	(1.11-1.72)	0.00	1.20	(0.82-1.76)	0.00

N, number of case-control study; OR, odds ratio; CI, confidence interval; NA, not available.

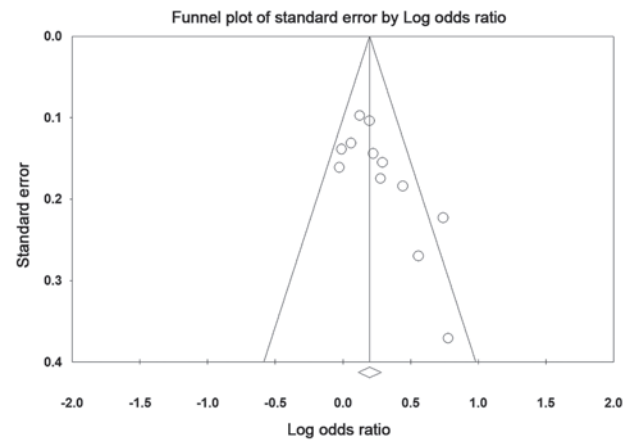


Figure 4. Funnel plot for the assessment of publication bias based on the G vs. A genetic model.

hepatocarcinogenesis (29,30). To the best of our knowledge, numerous previously published genetic studies have demonstrated a positive association between the EGF 61A/G polymorphism and risk of HCC, while other studies have found no notable evidence that this polymorphism increases the susceptibility to HCC. This encouraged the completion of the present meta-analysis. Meta-analysis is a method for combining relevant global studies to increase the statistical power and resolve the discrepancy issue of genetic association studies (31-34). In the present meta-analysis, a total of 12 case-control studies involving 2,095 patients and 3,766 control individuals were analyzed to provide a comprehensive assessment of the association between the EGF 61A/G polymorphism and HCC risk. The present results for the total population demonstrated that the EGF 61A/G polymorphism increased the risk of HCC. In addition, evaluation of heterogeneity was always conducted in statistical analysis. Thus, the subgroup meta-analyses were performed according to the ethnicity and source of the control individuals.

Subsequent to stratification by ethnicity, the present meta-analysis indicated that the A allele may reduce susceptibility to HCC in the Chinese population, but not in a mixed population. This finding in the mixed population is not in accordance with the results previously published by Zhong *et al* (15). In this previous meta-analysis, a significant association was indicated between the EGF 61A/G polymorphism and risk of HCC based on eight case-control studies. The considerably larger sample size of the present study may account for this difference. The frequency of the AA genotype varies extensively between different ethnicities, with a prevalence of 10% in those of Asian descent, ~30% in Caucasians, and 33% in those of African descent, suggesting a possible ethnicity-based difference. This may be the reason why no association with the EGF 61A/G polymorphism was detected among the mixed population. Although environmental factors may be the predominate factors in the development of HCC, the distribution of EGF genotypes in various ethnicities may also explain the increased prevalence of HCC in China (35).

In the stratified analysis by control source, the G allele was found to be significantly associated with an increased risk of HCC in the control individuals with liver diseases,



consisting of HBV and HCV infection and cirrhosis. However, there was no significant association between the EGF 61A/G polymorphism and the risk of HCC among the healthy control individuals. The present findings suggest that the EGF 61A/G polymorphism may be a potential marker in the context of liver disease, consisting of HBV infection, HCV infection and cirrhosis, rather than a susceptibility gene polymorphism. The consideration of the history of relevant diseases was also a strength of the present meta-analysis compared with the previous meta-analyses performed on this topic.

There are also limitations to the present study. First, one of the major concerns is bias, due to selective publication. Evident publication bias was detected in the G vs. A, GG vs. AA, AG vs. AA, and GG + AG vs. AA genotype comparisons. Secondly, the Caucasian and Egyptian populations were assessed in only one study each, and therefore the results must be interpreted with caution. Thirdly, the majority of studies were performed using the Chinese population and additional studies are required using alternative ethnic groups. Finally, although the heterogeneity in the present study was not large, it was present in the genetic models. The subgroup analysis indicated that the heterogeneity may result from the mixed subgroup. Although heterogeneity is extremely common in meta-analyses of genetic association, this requires consideration.

In summary, the present meta-analysis suggests that the EGF 61A/G polymorphism is associated with an increased risk of HCC. Based on the evidence obtained in the present meta-analysis, the EGF 61A/G polymorphism was found to be a potential marker for HCC in the context of liver disease, such as HBV and HCV infection and liver cirrhosis. Considering the limited objectives of the present meta-analysis, additional studies should be conducted with larger sample sizes and more healthy control designs or prospective cohort designs.

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