

## Original Article

# Associations between Cox-2 rs20417 and rs5275 polymorphisms and the risk of hepatocellular carcinoma: a meta analysis

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**Abstract:** Genetic polymorphisms of cyclooxygenase-2 (Cox-2) gene have been implicated in the susceptibility to hepatocellular carcinoma (HCC), but the findings from published studies are conflicting and inconclusive. To obtain a more precise estimate of the association of Cox-2 polymorphisms with HCC risk, we performed a meta-analysis of eight eligible case-control studies identified through an extensive online database search of PubMed, Embase, Cochrane Library, Web of Science, China National Knowledge Infrastructure, Wanfang and Chinese Biomedicine Database; after exclusion, 2324 cases and 2604 controls were included. The pooled odds ratios with corresponding 95% confidence intervals were calculated to assess associations, using fixed- or random-effect models. In addition, subgroup analysis by ethnicity and sensitivity analysis were performed. Our results showed that the Cox-2 rs20417 (-765 G/C) polymorphism was not associated with HCC risk in the studied genetic contrast modes (C vs. G, GC vs. GG, and CC + GC vs. GG). No significant association was found with ethnic groups examined ( $P > 0.05$ ). Similarly, no significant association of the Cox-2 rs5275 (+ 8473 T/C) polymorphism and HCC risk was found under any of the studied contrasts (C vs. T, TC vs. TT, CC vs. TT, CC + TC vs. TT, CC vs. TC + TT). The present meta-analysis, combining all currently available data, suggests no significant associations of either Cox-2 polymorphism with HCC risk. Further studies with a larger sample size are needed to determine the association in different ethnicities.

**Keywords:** Cox-2, polymorphisms, hepatocellular carcinoma, meta-analysis

## Introduction

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer, accounting for approximately 6% of all human cancers and half a million deaths per year, representing a major worldwide health problem [1, 2]. Although research carried out on the well-established risk factors, including hepatitis B virus (HBV), hepatitis C virus (HCV), aflatoxin exposure, smoking, and alcohol abuse, has contributed to improvements in the diagnosis and treatment of HCC, the inability to detect HCC at an early stage and the lack of effective monitoring for HCC progression may contribute to nearly identical incidence and mortality rates [3-6]. Accordingly, efforts to identify specific methods for the diagnosis and prevention of HCC are necessary. A growing body of evidence

has indicated the importance of individual susceptibility to the development of cancer and has demonstrated that genetic polymorphisms can predict cancer risk and may enable early diagnosis of cancers [7, 8].

Cyclooxygenases, also known as prostaglandin endoperoxide H synthases or prostaglandin G/H synthases, are key enzymes that mediate the conversion of free arachidonic acid into prostaglandin H<sub>2</sub> and other eicosanoids [9-11]. Cyclooxygenase-2 (COX-2) is the principal member of the cyclooxygenase family known to influence cancer development and progression through the regulation of several biologic processes, such as apoptosis inhibition, inflammation, immune response suppression, tumor cell invasion, metastasis, and angiogenesis [12]. A large volume of research data has demonstrat-

ed that COX-2 is normally absent in hepatic tissue, although overexpression is often observed in HCC, indicating a potential role in hepatocarcinogenesis [13-16]. The expression of COX-2 is regulated by a complex signal transduction pathway, in which many nuclear proteins interact with the Cox-2 promoter region and play a decisive role in gene transcription [17]. Therefore, single nucleotide polymorphisms (SNPs) in the Cox-2 promoter may exert profound effects on transcriptional activity by altering the binding capacity of certain nuclear proteins, thereby affecting expression of the COX-2 enzyme, and resulting in variability in individual susceptibility to cancer [18]. Numerous epidemiologic investigations assessing the association of the rs20417 (-765 G > C) and rs5275 (+ 8473 T/C) polymorphisms in the promoter region of Cox-2 with HCC risk have been carried out in recent years. However, the evidence of a genetic association remains weak, due to the paucity of data and conflicting results of published studies. Hence, the current meta-analysis was performed to clarify the association of these Cox-2 polymorphisms with HCC risk, by combined analysis of relevant published studies.

### Materials and methods

#### *Literature and search strategy*

A comprehensive electronic search of PubMed, Embase, the Cochrane Library, the Web of Science, the China National Knowledge Infrastructure, Wanfang, and the Chinese Biomedicine Database was conducted to identify studies linking Cox-2 polymorphisms and HCC risk. Studies available online involving human HCC, and written in English or Chinese, were retrieved up to July 2014. The following query was used to carry out the electronic database search: ["cyclooxygenase-2" or "Cox-2"] and ["liver carcinoma" or "liver cancer" or "hepatocellular carcinoma" or "HCC"] and ["polymorphism" or "polymorphisms" or "SNP" or "variant" or "genotype"]. Other potentially eligible studies were identified through individual and manual searches of reference lists of major textbooks, reviews, and included articles. In the case of overlapping studies, only the study with the largest sample size was included.

#### *Inclusion criteria*

Studies were required to meet each of the following eligibility criteria for inclusion in the cur-

rent meta-analysis: (1) studies were case-control or cohort studies evaluating the association of Cox-2 rs20417 or rs5275 polymorphisms with HCC risk; (2) malignant tumor diagnoses were pathologically or histologically confirmed; and (3) sufficient data were provided for the calculation of odds ratio (OR) with 95% confidence interval (CI).

#### *Exclusion criteria*

Studies were excluded from the meta-analysis for the following reasons: (1) control populations were not included; (2) outcomes of comparative analyses were not reported or difficult to determine; (3) investigations were based on incomplete raw data or overlapping studies; and (4) abstracts, case reports, comments, letters, reviews, or editorials.

#### *Data extraction*

Using a standardized form, the following data were extracted from each included publication by two independent investigators: first author, year of publication, region, ethnicity (categorized as Caucasians, Asians, and others), sample size, source of controls, genotyping method, allele or genotype frequencies, and evidence of Hardy-Weinberg equilibrium (HWE). Any discrepancies were resolved by discussion or consultation with a third investigator until a consensus was reached.

#### *Statistical analysis*

Individual or pooled OR with 95% CI was calculated to assess the strength of the associations between Cox-2 rs20417 and rs5275 polymorphisms and HCC risk using Review Manager version 5.2 software (provided by The Cochrane Collaboration, Oxford, England) (<http://www.cochrane.org/software/revman.htm>). Inter-study heterogeneity was estimated using a chi-square-based Q-statistic test, with  $P_h$ -values of < 0.1 indicating significant heterogeneity [19, 20]. The significance of the pooled statistical data was determined by the Z-test, with  $P$ -values < 0.05 considered significant, using the fixed-effect or random-effect model in the absence ( $P_h > 0.1$ ) or presence ( $P_h < 0.1$ ) of heterogeneity, respectively [21, 22]. Subgroup analysis according to the ethnicity was also performed. The potential for publication bias was measured using Begg's funnel plot, in

**Table 1.** Summary of characteristics of all included studies in the meta-analysis

| First author | Year | Ethnicity | Genotype-case  | Genotype-control | Source of control | Genotype method | Cox-2 polymorphism | HWE test |
|--------------|------|-----------|----------------|------------------|-------------------|-----------------|--------------------|----------|
|              |      |           | VR Ho/Ht/WT Ho | VR Ho/Ht/WT Ho   |                   |                 |                    |          |
| Chang        | 2012 | Asian     | 0/36/262       | 0/48/250         | hospital          | PCR-RFLP        | rs20417            | Y        |
|              | 2012 | Asian     | 0/103/195      | 0/97/201         | hospital          | PCR-RFLP        | rs5275             | N        |
| Gharib       | 2014 | African   | 4/30/86        | 6/39/85          | hospital          | PCR-RFLP        | rs20417            | Y        |
| Akkiz        | 2011 | Caucasian | 4/46/79        | 15/39/75         | population        | PCR-RFLP        | rs20417            | N        |
|              | 2011 | Asian     | 8/56/65        | 9/62/58          | population        | PCR-RFLP        | rs5275             | Y        |
| Fan          | 2011 | Asian     | 36/235/509     | 25/258/497       | hospital          | Taqman          | rs5275             | Y        |
| Xu           | 2008 | Asian     | 0/37/233       | 0/25/515         | population        | PCR-RFLP        | rs20417            | Y        |
| He           | 2012 | Asian     | 10/67/223      | 2/37/261         | population        | PCR-RFLP        | rs20417            | Y        |

VR, variant; WT, wild-type; Ht, heterozygote; VR Ho, variant homozygote; WT Ho, wide-type homozygote; Y, in agreement with HWE (Hardy-Weinberg equilibrium); N, in disagreement with HWE.

which the standard error of logOR of each study was plotted against its logOR, and an asymmetric plot suggested possible publication bias [23]. The funnel plot asymmetry was further assessed using Egger's linear regression test (a *P*-value of < 0.05 was considered significant) [24]. Begg's and Egger's tests were both performed using Stata 12.0 software (Stata Corporation, College Station, Texas, USA). In order to enhance the credibility of the results, sensitivity analysis was also performed by sequential omission of individual studies in the analysis of various contrasts.

## Results

### Study characteristics

Initially, 32 potentially relevant publications investigating the association between Cox-2 rs20417 and rs5275 polymorphisms and the risk of HCC were retrieved according to the search strategy. The titles, abstracts, and full texts of all retrieved publications were carefully reviewed and screened using defined criteria. Six studies met the defined inclusion criteria and were included in the current meta-analysis [11, 25-29]. As more than one case-control study was included in the publications by Chang *et al.* [25] and Akkiz *et al.* [26], each was considered as a separate study in the meta-analysis. Therefore, a total of eight case-control studies from six publications (four in English and two in Chinese) were used in this study, involving 2324 cases and 2604 controls (rs20417, 1117 cases and 1397 controls; rs5275, 1207 cases and 1207 controls). Of these, six case-control studies were conducted in Asian populations, and one each in African

and Caucasian populations. Polymorphisms in control subjects were in agreement with HWE in all studies, except for those of Chang *et al.* [25] and Akkiz *et al.* [26]. Detailed characteristics of the included studies are summarized in **Table 1**.

### Meta-analysis results

The primary results of the meta-analysis are presented in **Table 2** and **Figure 1**. In total, five studies, including 1117 cases and 1397 controls, examining the association between the Cox-2 rs20417 polymorphism and HCC risk, were included in our meta-analysis, we found no significant association using three different allele or genotype contrasts: C vs G, GC vs. GG, and CC + GC vs. GG (Allele contrast, C vs. G: OR = 1.24, 95% CI = 0.67-2.27,  $P_h < 0.001$ ; Ht vs. WT Ho, GC vs. GG: OR = 1.34, 95% CI = 0.75-2.38,  $P_h < 0.001$ ; Dominant model, CC + GC vs. GG: OR = 1.29, 95% CI = 0.70-2.38,  $P_h < 0.001$ ). This association was not examined using CC vs. GG or CC vs. GC + GG contrasts due to the low frequency of the CC genotype or C allele in cases and controls. When stratified by ethnicity, meta-analysis showed no significant results ( $P > 0.05$ ). A meta-analysis of 1207 cases and 1207 controls from three studies was carried out to test the association of the rs5275 polymorphism with HCC. No significant association was found using the following allele and genotype contrasts: C vs. T; TC vs. TT; TC vs. TT; dominant model, CC + TC vs. TT; recessive model, CC vs. TC + TT. (Allele contrast, C vs. T: OR=0.99, 95% CI = 0.86-1.14,  $P_h = 0.67$ ; Ht vs. WT Ho, TC vs. TT: OR = 0.93, 95% CI = 0.78-1.10,  $P_h = 0.51$ ; VR Ho vs. WT Ho, CC vs. TT: OR = 1.25, 95% CI = 0.78-1.98,  $P_h = 0.33$ ; Dominant model,

## Cox-2 polymorphisms and the risk of hepatocellular carcinoma

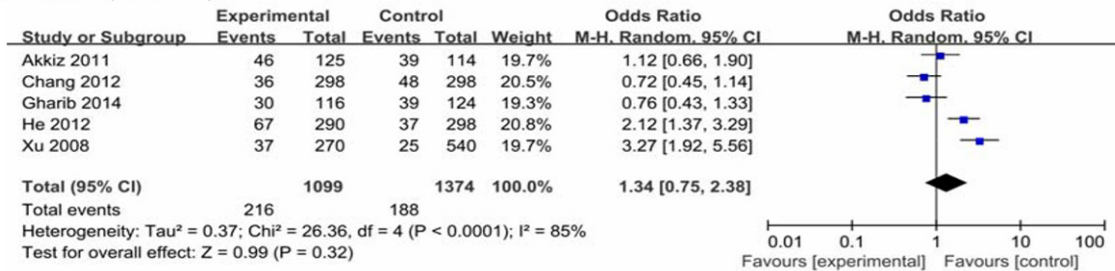
**Table 2.** Associations between rs20417 and rs5275 polymorphisms in Cox-2 and HCC risk

| Cox-2 polymorphisms | Study group | Sample size (case/control) | Allele contrast   |         | Ht vs. WT Ho      |        | VR Ho vs. WT Ho   |       | Dominant model    |         | Recessive model   |       |
|---------------------|-------------|----------------------------|-------------------|---------|-------------------|--------|-------------------|-------|-------------------|---------|-------------------|-------|
|                     |             |                            | OR [95% CI]       | $P_h$   | OR [95%CI]        | $P_h$  | OR [95% CI]       | $P_h$ | OR [95% CI]       | $P_h$   | OR [95% CI]       | $P_h$ |
| rs20417             | overall     | 1117/1397                  | 1.24 [0.67, 2.27] | < 0.001 | 1.34 [0.75, 2.38] | <0.001 | -                 | -     | 1.29 [0.70, 2.38] | < 0.001 | -                 | -     |
|                     | Asian       | 868/1138                   | 1.73 [0.75, 4.03] | < 0.001 | 1.70 [0.71, 4.05] | <0.001 | -                 | -     | 1.75 [0.72, 4.24] | < 0.001 | -                 | -     |
|                     | Other       | 249/259                    | 0.74 [0.55, 1.01] | 0.85    | 0.93 [0.63, 1.37] | 0.33   | -                 | -     | 0.82 [0.57, 1.17] | 0.66    | -                 | -     |
| rs5275              | overall     | 1207/1207                  | 0.99 [0.86, 1.14] | 0.67    | 0.93 [0.78, 1.10] | 0.51   | 1.25 [0.78, 1.98] | 0.33  | 0.95 [0.81, 1.13] | 0.57    | 1.31 [0.83, 2.07] | 0.37  |

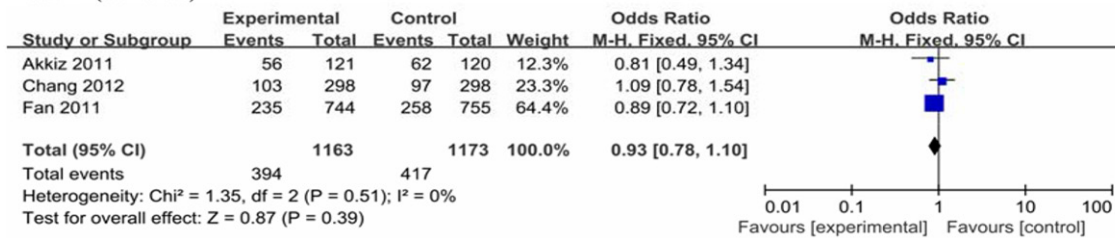
VR, variant; WT, wild-type; Ht, heterozygote; VR Ho, variant homozygote; WT Ho, wide-type homozygote.  $P_h$ ,  $P$  value of Q-test for heterogeneity test, and Random effects model was used when  $P$  value for heterogeneity test <0.1; otherwise, fixed effects model was used in the analysis.

## Cox-2 polymorphisms and the risk of hepatocellular carcinoma

### A rs20417 (GC vs. GG)



### B rs5275 (TC vs. TT)



**Figure 1.** Meta-analysis of the associations between Cox-2 rs20417 and rs5275 polymorphisms and HCC cancer risk (A, rs20417: GC vs. GG; B, rs5275: TC vs. TT).

CC + TC vs. TT: OR = 0.95, 95% CI = 0.81-1.13,  $P_h = 0.57$ ; Recessive model, CC vs. TC+TT: OR = 1.31, 95% CI = 0.83-2.07,  $P_h = 0.37$ ).

#### Sensitivity analysis and publication bias

Sensitivity analysis was performed by sequential omission of individual studies to investigate the influence of each study on the overall OR. The significance of the pooled OR in the analysis of rs20417 and rs5275 was not excessively affected in any of the contrasts analyzed, indicating the robustness of the meta-analysis results. No evidence of publication bias was seen from the symmetrical graphics using Begg's funnel plot for either SNP (Figure 2); this was supported by the results of Egger's tests (rs20417, GC vs. GG:  $P = 0.817$ ; rs5275, TC vs. TT:  $P = 0.962$ ).

#### Discussion

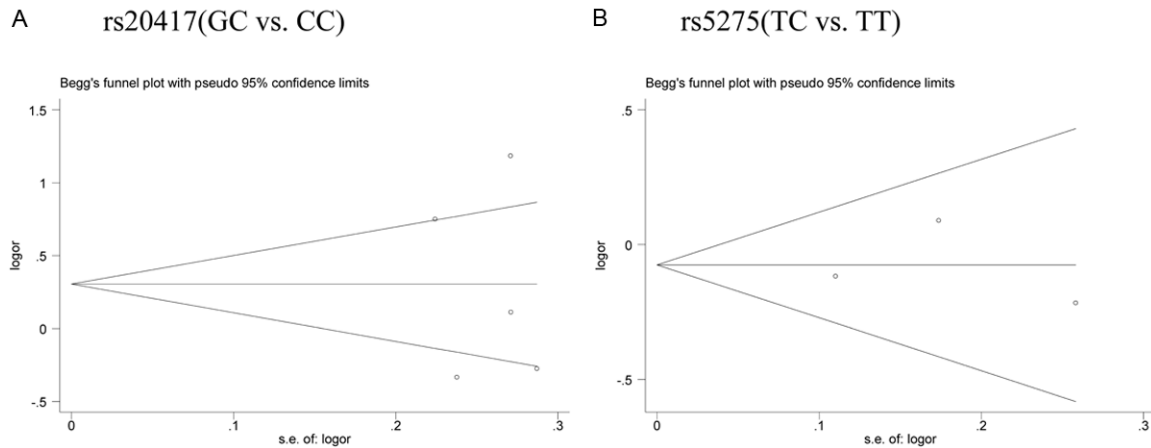
The genetic origin of HCC had been a focus of research in recent years, and several studies have found that genetic alterations may be useful indicators of early-stage disease, or may predict the risk of HCC [9, 30, 31]. Cox-2 was implicated in the formation of prostaglandins, which may contribute to the initiation and progression of cancer [32], and the overexpression of COX-2 was frequently observed in various types of cancer tissue, including HCC [33-35]. Given the important role of COX-2 in

many cellular functions, including inhibition of apoptosis, tumor growth, angiogenesis, invasion, and metastasis, it is biologically plausible that Cox-2 polymorphisms may be associated with an increased risk of HCC [36-38]. A polymorphism in the promoter region of Cox-2 could functionally upregulate the transcriptional activity of COX-2, indicating a possible mechanism by which Cox-2 may contribute to genetic susceptibility to HCC [39]. A previous meta-analysis, combining 5 individual studies, has suggested that a common polymorphism, Cox-2 -1195G > A, was associated with an increased risk of HCC [40]. Recently, the association between two other common variants of Cox-2, the rs20417 and rs5275 polymorphisms, and the risk of HCC has been investigated in numerous association studies. However, the results have been inconsistent, possibly due to limited sample size and ethnic variation. In order to address the inconsistencies and limitations of previously published studies, and to draw a more robust conclusion, the current meta-analysis was performed.

In the present study, our first meta-analysis included five case-control studies involving 1117 cases and 1397 controls to investigate the association between the Cox-2 rs20417 polymorphism and HCC risk. No significant association was found in our analysis, in contrast to some previous individual studies. Xu et



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**Figure 2.** Begg's funnel plots for publication bias test on the associations of between Cox-2 rs20417 and rs5275 polymorphisms and HCC cancer risk (A, rs20417: GC vs. GG; B, rs5275: TC vs. TT).

*al.* found that individuals with a GC genotype had a markedly elevated risk of HCC compared to GG carriers [28]. He *et al.* found that carriers of the C allele in the rs20417 polymorphism had an increased risk of HBV-related liver cancer [29]. Conversely, a study by Akkız *et al.* [26] reported that the proportion of CC variant was significantly lower in patients with HCC than control subjects, suggesting that the Cox-2 rs20417 polymorphism may be associated with a reduced risk of HCC. Moreover, two previous studies by Gharib *et al.* [11] and Chang *et al.* [25] found no association between the Cox-2 rs20417 polymorphism and HCC risk, in line with the results of our meta-analysis. Small sample sizes likely account for discrepancies between studies. An assessment of the association between the Cox-2 rs20417 polymorphism and HCC risk found that more precise estimates were obtained using large sample numbers than individual analyses using smaller sample sizes. In light of differences in genetic backgrounds, which may contribute to variations in the association of SNPs with HCC, we also conducted analyses stratified by ethnicity. However, we failed to show any significant association between the Cox-2 rs20417 polymorphism and HCC risk in any population analyzed, indicating that ethnicity may not be involved in this association. However, only one study in a Caucasian [26] and one in an African population [11] were included in this meta-analysis. Therefore, future investigations into ethnic differences may validate the association of this polymorphism with HCC, especially in Caucasians and Africans.

In our second meta-analysis of three case-control studies, including 1207 cases and 1207 controls, we found no significant association between HCC and the rs5275 polymorphism, which was in accordance with the findings of the previous studies. We did not perform subgroup analysis due to the limited number of studies included in this meta-analysis. Therefore, no conclusions could be drawn regarding ethnic associations. Nevertheless, the current study is the first meta-analysis carried out to investigate the association between the Cox-2 rs5275 polymorphism and the risk of HCC in a large number of cases, using all eligible published studies. Collectively, the development of HCC is attributed to the interaction between environmental factors, such as hepatitis virus and life style [4, 40], and genetic susceptibility. The polymorphisms of Cox-2 studied here may not directly affect susceptibility to HCC, but rather they may be involved in gene-gene or gene-environment interactions in hepatocarcinogenesis, which may account for the negative associations found.

Despite considerable efforts made to explore the associations between Cox-2 rs20417 and rs5275 polymorphisms and HCC risks, several limitations must be acknowledged. First, heterogeneity can interfere with the interpretation of results of a meta-analysis. Although the likelihood was minimized by using a rigorous search strategy, explicit inclusion criteria, and strict data extraction and analysis, significant inter-study heterogeneity was found in nearly every comparison studied, especially for rs20417.

Second, there is no doubt that variations in individual susceptibility to HCC can be attributed to complex gene-gene and gene-environmental exposure interactions [4]. It has been well documented that the development of HCC is significantly associated with hepatitis virus infection and alcohol abuse [40]. However, we were unable to investigate the interactions of Cox-2 rs20417 and rs5275 polymorphisms with other genes and environmental factors involved in the development of HCC, due to a lack of necessary data. Third, there is a lack of available studies regarding these associations in different ethnicities. Only one study was carried out in a Caucasian and one in an African population for the rs20417 polymorphism, and the three studies of the rs5275 polymorphism were pooled in our meta-analysis, which limited our ability to draw more useful conclusions. Additionally, the results obtained in the present study are based on unadjusted estimations. Some major confounding variables, including age, smoking and alcohol status, hepatitis virus status, family history, and environmental factors should be taken into consideration for a more accurate analysis.

In summary, our meta-analysis combining all currently available data suggests no significant associations of Cox-2 rs20417 and rs5275 polymorphisms with HCC. However, further studies in different ethnic groups using large sample sizes and well-matched controls are greatly needed to clarify any associations. Future studies considering gene-gene and gene-environment interactions are encouraged to provide a more comprehensive understanding of the potential role of Cox-2 polymorphisms in the pathogenesis of HCC.

## Disclosure of conflict of interest

None.

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