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Association between COX-2 Gene Polymorphisms and Risk of Hepatocellular Carcinoma Development

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Running head: COX-2 polymorphisms and HCC risk

Strengths and limitations of this study

- Eight polymorphic variants of cyclooxygenase-2 gene were studied.
- Limited by lacking of gene-gene and gene-environment interaction data.

Abstract

Objective: To investigate the association between cyclooxygenase-2 (COX-2) polymorphism and risk to hepatocellular carcinoma (HCC) development.

Design: Systematic review and meta-analysis of COX-2 polymorphism and risk to HCC development among individuals with or without HCC.

Data sources: EMBASE, PubMed, Public Library of Science, SCOPUS, Web of Knowledge, and Chinese National Knowledge Infrastructure for all clinical and experimental case-control studies of COX-2 polymorphism and HCC risk. Studies published until March 2015 were included.

Review method: Ten studies were included for data extraction. The studies included in this review were majorly from Asian countries.

Results: A total of 2538 individuals with HCC and 3714 individuals without HCC were found to satisfy the inclusion criteria and included in the review. The association of specific genotypes in the eight polymorphic variants of COX-2 and risk to HCC development were analyzed. GG genotype at the A-1195G polymorphism might be associated with increased risk to HCC development; the OR across all studies was 0.87 (0.75 to 1.01) for G-allele vs. A-allele, 0.71 (0.71 to 0.95) for GG vs. AA, 0.72 (95%CI 0.57 to 0.91) for the GG vs. GA + AA, and 1.05 (0.77 to 1.44) for AA vs. GA + GG. Similar results were found when the meta-analysis was repeated separately for Chinese subgroup. However, evidence about the association between variants in G-765C, T+8473C, A-1290G, G-899C, and introns 1, 5, and 6 polymorphisms and risk to HCC development need more reliable data to demonstrate.

Conclusions: Only COX-2 A-1195G gene polymorphism might be associated with risk to HCC development. These conclusions should be verified in further studies.

Keywords: cyclooxygenase-2; hepatocellular carcinoma; meta-analysis; polymorphism; susceptibility

Introduction

Hepatocellular carcinoma (HCC) is a significant cause of cancer morbidity and mortality worldwide. The estimated incidence of new HCC cases each year is more than 0.5 million (1). China is one of the regions with highest incidence of HCC (>20 per 100,000 people), which accounts for more than 50% of the total cases (2,3). Epidemiologically, HCC is strongly associated with hepatitis B or C virus infection, alcohol consumption, and metabolic disease. However, not all individuals with these factors appear to have the same risk of developing HCC. HCC is a multifactorial disease. Nowadays, many studies revealed that gene polymorphisms may also contribute to the risk of hepatocarcinogenesis (4,5). Namely, patients with HCC exhibits a high degree of genetic heterogeneity.

Cyclooxygenase-2 (COX-2) is an inducible enzyme that converts arachidonic acid to prostaglandins, which are potent mediators of inflammation. COX-2 is normally absent in most tissue cells. It is induced in response to inflammatory cytokines, mitogens, angiogenic growth factors, and tumor promoters (6,7). Increased COX-2 expression has been associated with the early stages of hepatocarcinogenesis (8,9). However, the association of COX-2 genotypes polymorphism with risk to HCC has not been well revealed.

Recently, a number of studies (10-19) have examined whether an association exists between the COX-2 polymorphism and risk to HCC. These studies have arrived at different conclusions, with some suggesting a significant association and others no association. Since individual case-control studies may fail to detect complicated genetic relationship because of small sample size, this review aims to comprehensively assess the literature examining a possible link between the COX-2 polymorphism and risk to HCC.

Methods

Literature Search strategy

All clinical and experimental case-control studies of COX-2 polymorphism and HCC risk published through March 20, 2015 were identified through systematic searches in EMBASE, PubMed, Public Library of Science (www.plosmedicine.org), SCOPUS, Web of Knowledge, and Chinese National Knowledge Infrastructure. No language restrictions were imposed. The following search terms were used to identify studies: cyclooxygenase-2 *or* COX-2, gene *or* polymorphism *or* variation *or* genotype *or* genetic *or* mutation, "hepatocellular carcinoma" *or* "liver cancer" *or* HCC. We also searched the Catalog of Published Genome-Wide Association Studies (GWAS) (www.genome.gov/gwastudies) of the US National Human Genome Research Institute. Reference lists of these articles and relevant literature from review articles were also searched to identify additional relevant publications.

Inclusion criteria

Only full-length research study satisfied the following criteria will be included in this review: (a) it assessed the association between COX-2 polymorphism and risk to HCC development; (b) they used a case-control or cohort design in which cases were HCC patients and controls were healthy individuals, or with chronic hepatitis B or C, or with cirrhosis; (c) they focused on human beings; (d) they provided sufficient published data for estimating an odds ratio (OR) with a 95% confidence interval (95%CI). In the case of multiple studies apparently based on the same case or control population, we included only the study with the largest number of participants. Conference abstracts or other forms of summary publication were not included. If there was incomplete data on genotype frequency in this study, we would try to contact the authors to collect these data (20).

Data extraction

Two authors (J-HZ, J-TT) independently searched the literature and indentified eligible articles based on our inclusion criteria. These two author also independently

extracted the following data from included studies: first author's family name, year of publications, genotyping methods, source of controls (population-based and hospital-based), numbers and genotypes of cases and controls, and Hardy-Weinberg equilibrium (HWE) of controls. Extracted data were compared and discrepancies were resolved by discussion.

Statistical Methods and Bias Testing

As described in detail previously (20,21), the unadjusted OR with 95%CI was used to assess the strength of the association between the COX-2 polymorphism and HCC susceptibility based on the genotype frequencies in cases and controls. The meta-analysis examined the association of different genotypes at different locus of COX-2 with HCC risk by comparing the alleles, comparing homozygous genotypes, and applying recessive and dominant genetic models.

Pooled ORs were calculated using fixed- or random-effect models, and the significance of those ORs was assessed using the Z-test, and $P<0.05$ was considered statistically significant. We used a chi squared-based Q-test to assess heterogeneity among studies. In this test, $P>0.10$ was taken to suggest that effect sizes were larger than those expected by chance (22,23), indicating the absence of statistical heterogeneity. In this case, a pooled OR was calculated for each study using the fixed-effect model. Otherwise, the random-effect model was used to calculate pooled ORs. HWE in the control group was assessed using the asymptotic test, with $P < 0.05$ considered significant. As much as possible, the meta-analysis was performed according to the PRISMA guidelines (24).

As described in detail previously (20,21), to detect associations that might be masked in the overall sample, we performed subgroup analyses based on subsets of the included studies defined according to ethnicity. To assess the reliability of the outcomes in the meta-analysis, a sensitivity analysis was performed by excluding one study at a time.

Publication bias was assessed by visual inspection of Begg's funnel plots. Small-study bias was assessed by Harbord's modified test (25). All statistical tests for this meta-analysis were performed using Stata 11.0 (Stata-Corp, College Station, USA) and RevMan 5.3 (Cochrane Collaboration).

Results

Description of studies

Several research databases were searched to identify studies assessing the possible association between the polymorphism in the COX-2 gene and risk to HCC. A total of 562 studies were identified, none of which was a GWAS. This list was reduced to 22 after removing duplicates and screening based on the title and abstract review. These articles were read in full, and 8 studies were removed because they did not include control group, while another 3 studies were removed because overlapping patients were analyzed or was with incomplete data. In the end, 10 studies were included into analysis (Fig. 1) (10-19). The main characteristics of the included studies are shown in Tables 1-3. All the studies were reported that cases and controls were matched on age and gender.

The studies involved 2538 individuals with HCC and 3714 individuals without HCC. The A-1195G polymorphism in the COX-2 gene and risk of HCC development was reported by 8 studies (10-17) (Table 1), G-765C in 6 studies (16,17,19,20,23,24) (Table 2), and T+8473C in 3 studies (Table 3) (10-12).

Quantitative data synthesis

A-1195G

Although the polymorphism in the allelic contrast model only slightly affect HCC development risk (OR = 0.87, 95%CI = 0.75-1.01, *P* = 0.07), the GG genotype was significantly associated with increased risk in the homozygote comparison (OR = 0.71,

95%CI = 0.71-0.95, *P* = 0.02) and recessive genetic model (OR = 0.72, 95%CI = 0.57-0.91, *P* = 0.006; Fig. 2). However, the AA genotype was not associated with higher or lower HCC development risk in the dominant genetic model (OR = 1.05, 95%CI = 0.77-1.44, *P* = 0.74). The results after deleting each study were similar to those obtained across all studies. We loosely classified the study population as Chinese and non-Chinese based on the ethnicity of the participants. Meta-analysis of subgroup found that Chinese population have the same phenomena as the total population. However, the A-1195G polymorphism in the COX-2 gene was not associated with either increased or reduced risk of HCC development in non-Chinese population (Table 4).

G-765C

With respect to COX-2 G-765C polymorphism, significant association was not observed in all of the six studies (C- vs. G-allele: OR = 1.32, 95%CI 0.76 to 2.30; CC vs. GC+GG: OR = 0.88, 95%CI 0.16 to 4.75; CC vs. GG: OR = 0.93, 95%CI 0.16 to 5.35; GG vs. CC+GC: OR = 0.48, 95%CI 0.14 to 1.59). Since the two non-Chinese studies (10,13) were with small sample size and GG genotype was zero in three studies (11,14,17), subgroup analysis were not performed (Table 4).

T+8473C

With respect to COX-2 T+8473C polymorphism, significant association was also not observed in all the three studies (C- vs. T-allele: OR = 0.99, 95%CI 0.86 to 1.14; CC vs. CT+TT: OR = 1.31, 95%CI 0.83 to 2.07; CC vs. TT: OR = 1.25, 95%CI 0.78 to 1.98; TT vs. CT+CC: OR = 1.05, 95%CI 0.89 to 1.24) (Table 4).

Other locus

The study by Chang *et al*. (11) also reported other three locus polymorphism in the COX-2 gene: intron 1, intron 5, and intron 6. This study showed that, for each of the six genotypes, no differences in distribution between the HCC and control groups were found. The locus polymorphism of A-1290G was reported by one study with 270

cases and 540 healthy controls (17). This study did not found significant association between COX-2 A-1290G polymorphism and risk of HCC. The locus polymorphism of C-899G in the COX-2 gene was also reported only by one study with 300 patients with chronic hepatitis B, 300 patients with liver cirrhosis, 300 patients with HCC, and 300 healthy controls (19). This study found that COX-2 -899C genotype may increase the susceptibility of individuals to HCC.

Publication bias and small-study bias

Begg's funnel plots were prepared for the 8 studies to assess publication bias for studies about A-1195G polymorphism of COX-2 and HCC risk. The shape of the funnel plots appeared to be symmetrical for allele contrast, homozygous comparison, and recessive and dominant genetic models, suggesting the absence of publication bias. Small-study bias tests showed no significant bias ($P = 0.790$) (Fig. 3).

Discussion

Some studies reported an association between the COX-2 gene polymorphism and HCC development risk, while others found no such association. The most likely reason for the inconsistencies among these studies is the small sample size. To help resolve these conflicting results using a larger sample size, we conducted systematic review of published studies. In this review, we included 10 studies investigating the association of eight polymorphic variants of COX-2 and the susceptibility of HCC development. We found that GG genotype of A-1195G in the COX-2 gene was associated with increased risk of HCC development, especially in Chinese population. However, we did not found a compelling evidence of an association between other COX-2 gene polymorphisms and risk of HCC development.

As is known, polymorphisms in the COX-2 promoter may have an important effect on gene transcriptional activity by changing the binding capacity of certain nuclear proteins, thereby affecting COX-2 expression. Even though the exact molecular

mechanism still remains unclear, several polymorphisms of COX-2 have been published previously, and the results are still conflicting. Previous meta-analysis of 8 studies revealed that COX-2 C+202T polymorphism is associated with a lower prostate cancer risk in Caucasians (26). Another meta-analysis of 25 studies found that COX-2 A-1195G polymorphism is a low penetration risk factor of cancer (27). However, COX-2 C-765G and T+8473C polymorphisms are significantly associated with increased risk of digestive system cancers (28,29). The meta-analysis by Bu *et al.* (30) included 5 (10-12,15,17) of the 10 included studies of this review. They found an association between COX-2 A-1195G polymorphism and HCC risk, especially in Asians. In this update review with larger sample size, other 5 studies (13,14,16,18,19) were included. GG genotype at the A-1195G polymorphism was also associated with increased risk of HCC development across all studies. We also investigated other seven polymorphic variants (G-765C, T+8473C, intron 1, intron 5, intron 6, A-1290G, C-899G) of COX-2. Although COX-2 C-899G polymorphism may increase the risk of HCC, this result only based on one study. In order to demonstrate the association between COX-2 C-899G polymorphism and risk of HCC development, more reliable data with large sample size are needed.

HCC involves complex, multistep and heterogeneous malignant tumorigenesis. The etiology of HCC involves various host and environmental factors. Furthermore, host and environmental factors may interact synergistically in HCC pathogenesis and progression (4). Several studies in this review indicate that COX-2 polymorphisms can interact with environmental factors to module HCC risk. Among individuals with a drinking history, COX-2 -765 C allele carriers had a significantly higher risk for HCC compared with G allele (18,31). Though single gene polymorphism and risk of HCC was not found in the study by Fan *et al.* (12), demographic interactions were observed. Among individuals younger than 55 years, A-allele of COX-2 A-1195G polymorphism is a high penetration risk factor of HCC, while among female individuals, C-allele of COX-2 T+8473C is a low penetration risk factor of HCC. About the gene-gene interactions, no significant differences in the frequencies of any

combined genotypes was observed between HCC cases and healthy controls (11). The joint effects of COX-2 genotypes and smoking or alcohol drinking were also not found (11). Moreover, no significant differences in COX-2 C-899G genotype distribution interactions with age, sex, and smoking history was found (19). Therefore, whether the interactions of gene-gene and gene-environment of COX-2 polymorphism may contribute to the risk of HCC is unknown.

Our data revealed that COX-2 A-1195G gene polymorphism may be a risk factor for hepatocarcinogenesis, but the complete picture is more complex. Seven (11,12,14,15,17-19) of the ten included individuals are Chinese. China has among the highest incidences of HCC in the world, as well as a high prevalence of hepatitis B virus infection and dietary exposure to aflatoxin B1, which are the two main risk factors for HCC (32-34). Some of the included controls are with hepatitis B or C virus infection, or cirrhosis. Duo to the sample size of these controls are small, subgroup analysis based on liver disease background was not performed. In addition, polymorphisms in numerous other genes, such as those encoding microsomal epoxide hydrolase (4) and epidermal growth factor (5) are also associated with the risk of HCC. It may be that any single nucleotide polymorphism such as COX-2 A-1195G is insufficient on its own to cause HCC, though it does increase the risk of the disease.

As stated before, some of the included controls had one or more of the following: alcoholic liver disease, HBV or HCV infection, and cirrhosis. Since the studies in included in this review often did not report detailed statistics on the proportion of HCC or control subjects with these background conditions, we could not perform subgroup analysis to separate the contribution of COX-2 polymorphism from that of possible confounders like HBV or HCV infection.

Some other limitations of this review should be considered too. Although we searched all the eligible records, the number of included studies was still relatively small. Subgroup stratification analysis of other COX-2 gene polymorphism was not

performed. Moreover, meta-analysis was not carried out for 5 polymorphic variants of COX-2. Second, the results may be affected by additional confounding factors, such as tumor status, age or gender, but most studies either did not report these baseline data or aggregated them in different ways, making it impossible to include them into pooled analysis. Moreover, the distribution of genotypes among controls did not show HWE in several studies. Finally, because of the lack of the individual original data, our meta-analysis was based on unadjusted data and a more precise analysis stratified by clinical manifestation and environmental factors has not been performed.

In conclusion, this review suggests that COX-2 A-1195G gene polymorphism, instead of other 7 polymorphic variants of COX-2, might be a risk factor of HCC development. However, since this review included few studies, large, well-designed studies are warranted to re-evaluate these associations.

This study is in accordance with the PRISMA guidelines (Checklist S1).

Author contributions: HFL, JTT, and HLT contributed equally to this work; JHZ conceived and designed the experiments; HFL, JTT, HLT and JHZ performed the research; BDX, LQL and JHZ performed the statistical analysis; HFL and JHZ wrote the manuscript; all authors have read and approved the final manuscript.

Declaration of interest: The authors report no conflicts of interest.

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References

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin 64: 9-29, 2014
2. Zhang CY, Huang TR, Yu JH, Zhang ZQ, Li JL, et al. Epidemiological analysis

of primary liver cancer in the early 21st century in Guangxi province of China. Chin J Cancer 29: 545-550, 2010

3. Zhong JH, Ke Y, Gong WF, Xiang BD, Ma L, et al. Hepatic resection associated with good survival for selected patients with intermediate and advanced-stage hepatocellular carcinoma. Ann Surg 260: 329-340, 2014

4. Zhong JH, Xiang BD, Ma L, You XM, Li LQ, et al. Meta-analysis of microsomal epoxide hydrolase gene polymorphism and risk of hepatocellular carcinoma. PLoS One 8: e57064, 2013

5. Zhong JH, You XM, Gong WF, Ma L, Zhang Y, et al. Epidermal growth factor gene polymorphism and risk of hepatocellular carcinoma: a meta-analysis. PLoS One 7: e32159, 2012

6. Williams CS, Mann M, DuBois RN. The role of cyclooxygenases in inflammation, cancer, and development. Oncogene 18: 7908-7916, 1999

7. Trifan OC, Hla T. Cyclooxygenase-2 modulates cellular growth and promotes tumorigenesis. J Cell Mol Med 7: 207-222, 2003

8. Bae SH, Jung ES, Park YM, Kim BS, Kim BK, et al. Expression of cyclooxygenase-2 (COX-2) in hepatocellular carcinoma and growth inhibition of hepatoma cell lines by a COX-2 inhibitor, NS-398. Clin Cancer Res 7: 1410-1418, 2001

9. Sung YK, Hwang SY, Kim JO, Bae HI, Kim JC, et al. The correlation between cyclooxygenase-2 expression and hepatocellular carcinogenesis. Mol Cells 17: 35-38, 2004

10. Akkiz H, Bayram S, Bekar A, Akgollu E, Ulger Y. Functional polymorphisms of cyclooxygenase-2 gene and risk for hepatocellular carcinoma. Mol Cell Biochem 347: 201-208, 2011

11. Chang WS, Yang MD, Tsai CW, Cheng LH, Jeng LB, et al. Association of cyclooxygenase 2 single-nucleotide polymorphisms and hepatocellular carcinoma in Taiwan. Chin J Physiol 55: 1-7, 2012

12. Fan XJ, Qiu XQ, Yu HP, Zeng XY, Yang Y, et al. [Association of COX-2 gene SNPs with the risk of hepatocellular carcinoma]. Chin J Cancer Rrev Treat 18:

405-409, 2011

13. Gharib AF, Karam RA, Abd El Rahman TM, Elsawy WH. COX-2 polymorphisms -765G-->C and -1195A-->G and hepatocellular carcinoma risk. Gene 543: 234-236, 2014
14. Li YH, Meng W. The Association of Survivin and COX-2 Gene with Hepatocellular Carcinoma. Fudan University: Master Dissertation. 2011.
15. Liu LF, Zhang JL, Lin JS. [The reIationship between Cyclooxygenase-2 gene -1195G/A genotype and risk of HBV-induced HCC: a case-control study in Han Chinese people]. Chin J Gastroenterol Hepatol 19: 333-335, 2010.
16. Mohamed FZ, Hussein YM, El-Deen IM, Sabea MS. Cyclooxygenase-2 single-nucleotide polymorphisms and hepatocellular carcinoma in Egypt. Mol Biol Rep 41: 1461-1468, 2014.
17. Xu DK, Zhang XM, Zhao P, Cai JC, Zhao D, et al. [Association between single nucleotide polymorphisms in promoter of COX-2 gene and hereditary susceptibility to hepatocellular carcinoma]. Chin J Hepatobiliary Surg 14: 587-589, 2008.
18. He J, Zhang Q, Ren Z, Li Y, Li X, et al. Cyclooxygenase-2 -765 G/C polymorphisms and susceptibility to hepatitis B-related liver cancer in Han Chinese population. Mol Biol Rep 39: 4163-4168, 2012.
19. He JH, Li YM, Zhang QB, Ren ZJ, Li X, et al. Cyclooxygenase-2 promoter polymorphism -899G/C is associated with hepatitis B-related liver cancer in a Chinese population of Gansu province. Chin Med J (Engl) 124: 4193-4197, 2011.
20. Zhong JH, Rodriguez AC, Yang NN, Li LQ. Methylenetetrahydrofolate reductase gene polymorphism and risk of type 2 diabetes mellitus. PLoS One 8: e74521,2013
21. Zhong JH, Zhang ZM, Li LQ. mEH Tyr113His polymorphism and the risk of ovarian cancer development. J Ovarian Res 6: 40, 2013
22. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 327: 557-560, 2003

23. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 21: 1539-1558, 2002

24. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS Med 6: e1000100, 2009

25. Harbord RM, Egger M, Sterne JA. A modified test for small-study effects in meta-analyses of controlled trials with binary endpoints. Stat Med 25: 3443-3457, 2006

26. Zhang H, Xu Y, Zhang Z, Liu R, Ma B. Association between COX-2 rs2745557 polymorphism and prostate cancer risk: a systematic review and meta-analysis. BMC Immunol 13: 14, 2012

27. Tang Z, Nie ZL, Pan Y, Zhang L, Gao L, et al. The Cox-2 -1195 G > A polymorphism and cancer risk: a meta-analysis of 25 case-control studies. Mutagenesis 26: 729-734, 2011.

28. Dong J, Dai J, Zhang M, Hu Z, Shen H. Potentially functional COX-2-1195G>A polymorphism increases the risk of digestive system cancers: a meta-analysis. J Gastroenterol Hepatol 25: 1042-1050, 2010.

29. Wang XF, Huang MZ, Zhang XW, Hua RX, Guo WJ. COX-2-765G>C polymorphism increases the risk of cancer: a meta-analysis. PLoS One 8: e73213, 2013.

30. Bu X, Zhao C. The association between cyclooxygenase-2 1195 G/A polymorphism and hepatocellular carcinoma: evidence from a meta-analysis. Tumour Biol 34: 1479-1484, 2013.

31. Song X, Cheng SH, Liu C, Liu YL, Sun JJ. Cyclooxygenase-2 Polymorphism and Susceptibility to Hepatocellular Carcinoma. Practic J Cancer 26: 255-258, 2011.

32. Wang FS, Fan JG, Zhang Z, Gao B, Wang HY. The global burden of liver disease: The major impact of China. Hepatology 60: 2099-2108, 2014.

33. Zhong JH, Zhong QL, Li LQ, Li H. Adjuvant and chemopreventive therapies

for resectable hepatocellular carcinoma: a literature review. Tumour Biol 35: 9459-9468, 2014.

34. Zhong JH, Rodríguez AC, Ke Y, Wang YY, Wang L, et al. Hepatic resection as a safe and effective treatment for hepatocellular carcinoma involving a single large tumor, multiple tumors, or macrovascular invasion. Medicine (Baltimore) 94:e396, 2015.

Figure legends

Figure 1. Flow chart of study selection.

Figure 2. Forest plots describing the association of A-1195G COX-2 polymorphism with HCC (GG vs. GA + AA).

Figure 3. Analysis to detect small-scale study bias across all included studies, based on the allele contrast genetic model.

Table 1. Main characteristics of studies about cyclooxygenase-2 A-1195G polymorphism and the risk of hepatocellular carcinoma.

Study	Country	Source of control	Genotyping method	P_{HWE}	Cases / Controls	No. of cases			No. of controls		
						GG	GA	AA	GG	GA	AA
Akkiz 2011[10]	Turkey	HB	PCR-RFLP	0.71	129/129	2	36	91	2	32	95
Chang 2012[11]	Taiwan	PB	PCR-RFLP	0.57	298/298	70	144	84	71	145	81
Fan 2011[12]	China	HB	TaqMan genotyping platform	0.52	780/780	204	390	186	205	381	194
Gharib 2014[13]	Egypt	PB	PCR-RFLP	0.86	120/130	17	60	43	31	66	33
Li 2011[14]	China	PB	PCR-RFLP	0.15	178/196	31	88	59	54	88	54
Liu 2010[15]	China	HB and PB	PCR-RFLP	0.56	210/420	31	110	69	101	216	103
Mohamed 2014[16]	Egypt	HB and PB	PCR-RFLP	< 0.001	75/125	12	49	14	40	22	63
Xu 2008[17]	China	PB	PCR-RFLP	0.14	270/540	52	125	93	119	287	134

Abbreviations: PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; PB, population-based; HB, hospital-based; PHWE, Hardy-Weinberg equilibrium of controls.

Table 2. Main characteristics of studies about cyclooxygenase-2 G-765C polymorphism and the risk of hepatocellular carcinoma.

Study	Country	Source of control	Genotyping method	P_{HWE}	Cases / Controls	No. of cases			No. of controls		
						GG	GA	AA	GG	GA	AA
Akkiz 2011[10]	Turkey	HB	PCR-RFLP	0.009	129/129	4	46	79	15	39	75
Chang 2012[11]	Taiwan	PB	PCR-RFLP	0.13	298/298	0	36	262	0	48	250
Gharib 2014[13]	Egypt	PB	PCR-RFLP	0.58	120/100	4	30	86	6	39	85
He 2012[18]	China	PB	PCR-RFLP	0.59	300/300	10	67	223	2	37	261
Li 2011[14]	China	HB	PCR-RFLP	0.60	178/196	0	26	152	0	14	182
Xu 2008[17]	China	PB	PCR-RFLP	0.58	270/540	0	37	233	0	25	515

Abbreviations: PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; PB, population-based; HB, hospital-based; P_{HWE}, Hardy-Weinberg equilibrium of controls.

Table 3. Main characteristics of studies about cyclooxygenase-2 T+8473C polymorphism and the risk of hepatocellular carcinoma.

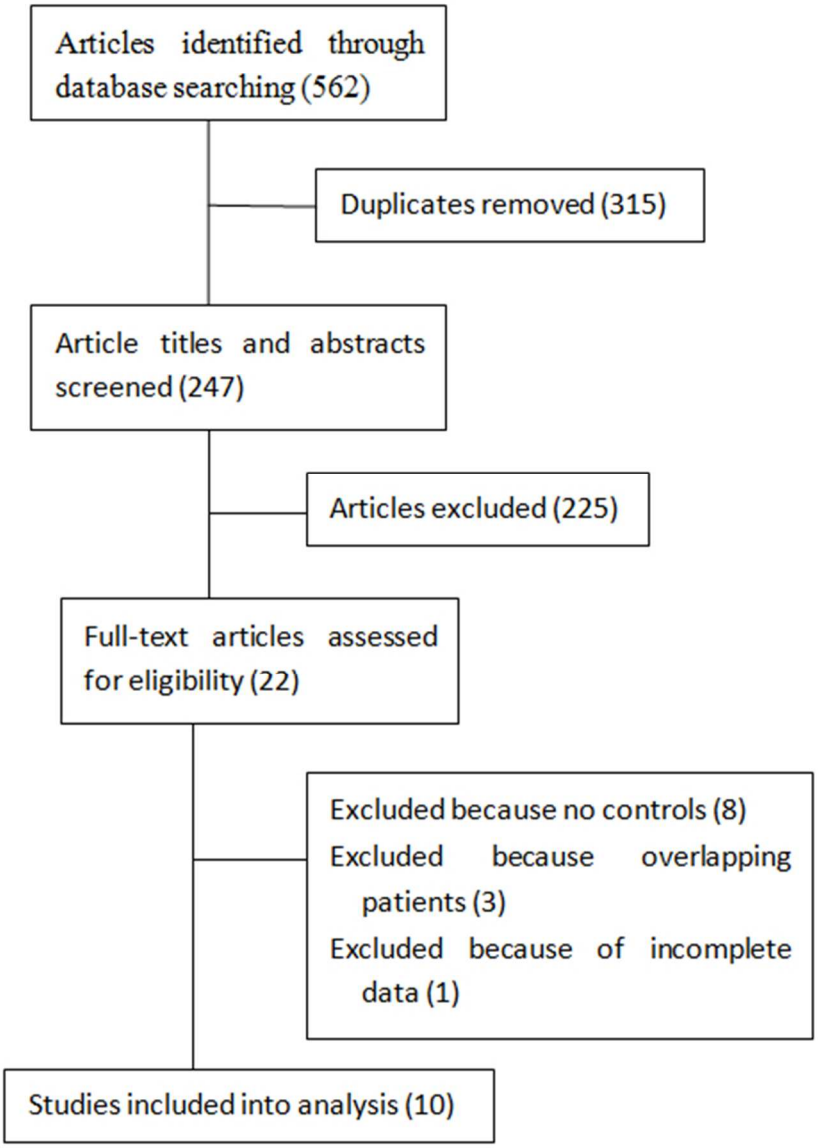
Study	Country	Source of control	Genotyping method	P_{HWE}	Cases / Controls	No. of cases			No. of controls		
						CC	TC	TT	CC	TC	TT
Akkiz 2011[10]	Turkey	HB	PCR-RFLP	0.16	129/129	8	56	65	9	62	58
Chang 2012[11]	Taiwan	PB	PCR-RFLP	< 0.001	298/298	0	103	195	0	97	201
Fan 2011[12]	China	HB	TaqMan genotyping platform	0.22	780/780	36	235	509	25	258	497

Abbreviations: PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; PB, population-based; HB, hospital-based; P_{HWE}, Hardy-Weinberg equilibrium of controls.

Table 4. Overall and stratified meta-analyses of the association between COX-2 polymorphisms and risk of hepatocellular carcinoma.

Comparison	Population	No. of Study	Test of association			Model	Test of heterogeneity	
			OR	95%CI	*P*		*P*	I^2
COX-2 A-1195G								
G-allele vs.	Overall	8	0.87	0.75-1.01	0.07	R	0.008	63
A-allele	Chinese	5	**0.84**	**0.72-0.98**	**0.03**	R	0.03	64
	Non-Chinese	3	1.00	0.63-1.59	0.99	R	0.02	74
GG vs. GA	Overall	8	**0.72**	**0.57-0.91**	**0.006**	R	0.05	51
+ AA	Chinese	5	0.79	0.62-1.00	0.05	R	0.07	54
	Non-Chinese	3	**0.49**	**0.31-0.78**	**0.003**	F	0.66	0
GG vs. AA	Overall	8	**0.71**	**0.71-0.95**	**0.02**	R	0.03	56
	Chinese	5	**0.71**	**0.51-0.98**	**0.04**	R	0.02	65
	Non-Chinese	3	0.77	0.32-1.84	0.56	R	0.13	52
AA vs.	Overall	8	1.05	0.77-1.44	0.74	R	< 0.001	79
GA+GG	Chinese	5	1.23	0.98-1.55	0.07	R	0.06	57
	Non-Chinese	3	0.69	0.24-2.03	0.51	R	< 0.001	90
COX-2 G-765C								
C-allele vs.	Overall							
G-allele		6	1.32	0.76-2.30	0.33	R	< 0.001	88
CC vs.	Overall							
GC+GG		3	0.88	0.16-4.75	0.88	R	0.007	80
CC vs. GG	Overall	3	0.93	0.16-5.35	0.94	R	0.005	81
GG vs.	Overall							
CC+GC		6	0.48	0.14-1.59	0.23	R	< 0.001	97
COX-2 T+8473C								
C-allele vs.	Overall							
T-allele		3	0.99	0.86-1.14	0.91	F	0.67	0
CC vs. CT +	Overall							
TT		3	1.31	0.83-2.07	0.25	F	0.37	0
CC vs. TT	Overall	3	1.25	0.78-1.98	0.35	F	0.33	0
TT vs. CT +	Overall							
CC		3	1.05	0.89-1.24	0.58	F	0.57	0

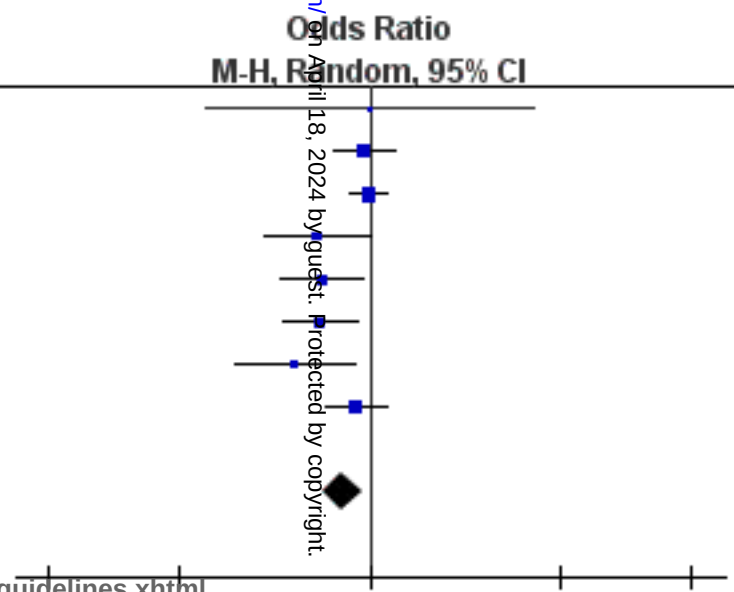
Abbreviations: OR, odds ratio; CI, confidence interval; R, random-effect model; F, fixed-effect model.

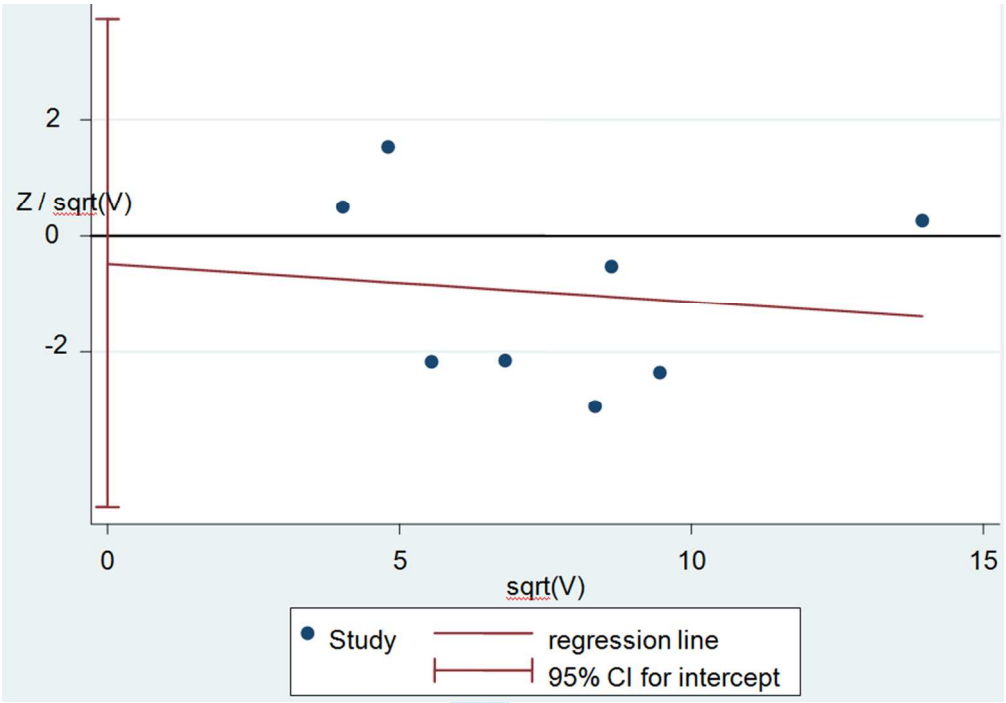


Study or Subgroup	Cases Events	Cases Total	Controls Events	Controls Total	Weight	Odds Ratio M-H, Random, 95% CI
Akkiz 2011	2	129	2	129	1.3%	1.00 [0.14, 7.21]
Chang 2012	70	298	74	298	16.4%	0.93 [0.64, 1.35]
Fan 2011	204	780	205	780	22.3%	0.99 [0.79, 1.24]
Gharib 2014	17	120	31	130	8.9%	0.53 [0.27, 1.01]
Li 2011	31	178	54	196	12.4%	0.55 [0.34, 0.91]
Liu 2010	31	210	101	420	14.1%	0.55 [0.35, 0.85]
Mohamed 2014	12	75	40	125	7.7%	0.40 [0.20, 0.83]
Xu 2008	52	270	119	540	16.8%	0.84 [0.59, 1.22]
Total (95% CI)		**2060**		**2618**	**100.0%**	**0.72 [0.57, 0.91]**
Total events	419		626			

Heterogeneity: Tau² = 0.05; Chi² = 14.19, df = 7 (P = 0.05); I² = 51%

Test for overall effect: Z = 2.72 (P = 0.006)





PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a meta-analysis.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	n/a
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	4
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	4
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	5
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	5-6

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	6
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	6
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	7
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	7
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	7
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	8
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	8
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	9
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	10
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	11
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	1

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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Association between COX-2 Gene Polymorphisms and Risk of Hepatocellular Carcinoma Development: a meta-analysis

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Primary Subject Heading:	Gastroenterology and hepatology
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Keywords:	cyclooxygenase-2, hepatocellular carcinoma, polymorphism, susceptibility

Association between COX-2 Gene Polymorphisms and Risk of Hepatocellular Carcinoma Development: a meta-analysis

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Running head: COX-2 polymorphisms and HCC risk

Strengths and limitations of this study

- Eight polymorphic variants of cyclooxygenase-2 gene were studied.
- Limited by lacking of gene-gene and gene-environment interaction data.

Abstract

Objective: To investigate the association between cyclooxygenase-2 (COX-2) polymorphism and risk to hepatocellular carcinoma (HCC) development.

Design: Systematic review and meta-analysis of COX-2 polymorphism and risk to HCC development among individuals with or without HCC.

Data sources: EMBASE, PubMed, Public Library of Science, SCOPUS, Web of Knowledge, and Chinese National Knowledge Infrastructure for all clinical and experimental case-control studies of COX-2 polymorphism and HCC risk. Studies published until March 2015 were included.

Review method: Ten studies were included for data extraction. The studies included in this review were mainly from Asian countries.

Results: A total of 2538 individuals with HCC and 3714 individuals without HCC were found to satisfy the inclusion criteria and included in the review. The associations of specific genotypes in the eight polymorphic variants of COX-2 and risk to HCC development were analyzed. GG genotype at the A-1195G polymorphism might be associated with increased risk to HCC development: the OR across all studies was 0.87 (0.75 to 1.02) for G-allele vs. A-allele, 0.72 (0.53 to 0.97) for GG vs. AA, 0.72 (95%CI 0.57 to 0.92) for the GG vs. GA + AA, and 1.05 (0.77 to 1.44) for AA vs. GA + GG. Similar results were found when the meta-analysis was repeated separately for Chinese subgroup. However, evidence about the associations between variants in G-765C, T+8473C, A-1290G, G-899C, and introns 1, 5, and 6 polymorphisms and risk to HCC development need more reliable data to demonstrate.

Conclusions: Only COX-2 A-1195G gene polymorphism might be associated with risk to HCC development. These conclusions should be verified in further studies.

Keywords: cyclooxygenase-2; hepatocellular carcinoma; meta-analysis; polymorphism; susceptibility

Introduction

Hepatocellular carcinoma (HCC) is a significant cause of cancer morbidity and mortality worldwide. The estimated incidence of new HCC cases each year is more than 0.5 million (1). China is one of the regions with highest incidence of HCC (>20 per 100,000 people), which accounts for more than 50% of the total cases (2,3). Epidemiologically, HCC is strongly associated with hepatitis B or C virus infection, alcohol consumption, and metabolic disease. However, not all individuals with these factors appear to have the same risk of developing HCC. HCC is a multifactorial disease. Nowadays, many studies revealed that gene polymorphisms may also contribute to the risk of hepatocarcinogenesis (4,5). Namely, patients with HCC exhibit a high degree of genetic heterogeneity.

Cyclooxygenase-2 [COX-2, also known as prostaglandin endoperoxide synthases or prostaglandin H synthases (PTGSs)] is an inducible enzyme that converts arachidonic acid to prostaglandins, which are potent mediators of inflammation. COX-2 is normally absent in most tissue cells. It is induced in response to inflammatory cytokines, mitogens, angiogenic growth factors, and tumor promoters (6,7). Increased COX-2 expression has been associated with the early stages of hepatocarcinogenesis (8,9). However, the association of COX-2 genotypes polymorphism with risk to HCC has not been well revealed.

Recently, a number of studies (10-19) have examined whether an association exists between the COX-2 polymorphism and risk to HCC. These studies have arrived at different conclusions, with some suggesting a significant association and others no association. Since individual case-control studies may fail to detect complicated genetic relationship because of small sample size, this review aims to comprehensively assess the literature examining a possible link between the COX-2 polymorphism and risk to HCC.

Methods

Literature Search strategy

All clinical and experimental case-control studies of COX-2 polymorphism and HCC risk published through March 31, 2015 were identified through systematic searches in EMBASE, PubMed, Public Library of Science (www.plos.org), SCOPUS, Web of Knowledge, and Chinese National Knowledge Infrastructure. Due to a lot of papers were published by the Public Library of Science in the recent decade, we also searched this database. No language restriction was imposed. The following search terms were used to identify studies: cyclooxygenase-2 *or* COX-2, gene *or* polymorphism *or* variation *or* genotype *or* genetic *or* mutation, "hepatocellular carcinoma" *or* "liver cancer" *or* HCC. Detailed database search strategies of EMBASE are shown in table 1. We also searched the Catalog of Published Genome-Wide Association Studies (GWAS) (www.genome.gov/gwastudies) of the US National Human Genome Research Institute. Reference lists of these articles and relevant literature from review articles were also searched to identify additional relevant publications.

Inclusion criteria

Only full-length research study satisfied the following criteria would be included in this review: (a) it assessed the association between COX-2 polymorphism and risk to HCC development; (b) they used a case-control or cohort design in which cases were HCC patients and controls were healthy individuals, or with chronic hepatitis B or C, or with cirrhosis; (c) they focused on human beings; (d) they provided sufficient published data for estimating an odds ratio (OR) with a 95% confidence interval (95%CI). In the case of multiple studies apparently based on the same case or control population, we included only the study with the largest number of participants. Conference abstracts or other forms of summary publication were not included. If there was incomplete data on genotype frequency in this study, we would try to contact the authors to collect these data (20).

Data extraction

Two authors (S-CL, J-TT) independently searched the literature and indentified eligible articles based on our inclusion criteria. These two authors also independently extracted the following data from included studies: first author's family name, year of publications, genotyping methods, source of controls (population-based and hospital-based), numbers and genotypes of cases and controls, and Hardy-Weinberg equilibrium (HWE) of controls. Extracted data were compared and discrepancies were resolved by discussion with a third author (J-HZ).

Statistical Methods and Bias Testing

As describing previously (20,21), the unadjusted OR with 95%CI was used to assess the strength of the association between the COX-2 polymorphism and HCC susceptibility based on the genotype frequencies in cases and controls. The meta-analysis examined the association of different genotypes at different loci of COX-2 with HCC risk by comparing the alleles, comparing homozygous genotypes, and applying recessive and dominant genetic models.

Mantel-Haenszel estimate was used to give a pooled OR using the fixed- or random-effect models, and the significance of this OR was assessed using the Z-test, and $P<0.05$ was considered statistically significant. I^2 was used to estimate total variation across studies due to heterogeneity in percentage (22,23). Less than 25% was considered as low level of heterogeneity, 25% to 50% as moderate level of heterogeneity, and higher than 50% as high level of heterogeneity. $I^2 > 50\%$ could suggest heterogeneity and suggest using a random effect estimate (22,23). Otherwise, the fixed-effect model was used to calculate pooled ORs. HWE in the control group was assessed using the chi-square goodness-of-fittest, with $P < 0.05$ considered significant. As much as possible, the meta-analysis was performed according to the PRISMA guidelines (24).

As describing previously (20,21), to detect associations that might be masked in the

overall sample, we performed subgroup analyses based on ethnicity. Meta-regression was performed to exam the effect of ethnicity to compare results from the meta-analyses. To assess the reliability of the outcomes in the meta-analysis, a sensitivity analysis was performed by excluding one study at a time.

Publication bias was assessed by visual inspection of Begg's funnel plots. An asymmetric plot suggested possible publication bias, in which case Egger's test was used (25). All statistical tests for this meta-analysis were performed using Stata 11.0 (Stata-Corp, College Station, USA) and RevMan 5.3 (Cochrane Collaboration).

Results

Description of studies

Several research databases were searched to identify studies assessing the possible association between the polymorphism in the COX-2 gene and risk to HCC. A total of 562 studies were identified, none of which was a GWAS. This list was reduced to 22 after removing duplicates and screening based on the title and abstract review. These articles were read in full, and 8 studies were removed because they did not include control group, while another 4 studies were removed because overlapping patients were analyzed or was with incomplete data. No study which was published in a language other than in Chinese or in English was excluded. In the end, 10 studies were included into analysis (fig. 1) (10-19). Four of them were published in Chinese (12,14,15,17). Other five studies were published in English (10,11,13,16,18,19). The main characteristics of the included studies are shown in tables 2-4. All the studies were reported that cases and controls were matched on age and gender.

The studies involved 2538 individuals with HCC and 3714 individuals without HCC. The A-1195G polymorphism in the COX-2 gene and risk of HCC development was reported by 8 studies (10-17) (table 2), G-765C in 6 studies (10,11,13,14,17,18) (table 3), and T+8473C in 3 studies (table 4) (10-12).

Quantitative data synthesis

A-1195G

Although the polymorphism in the allelic contrast model only slightly affect HCC development risk (OR = 0.87, 95%CI = 0.75-1.02, *P* = 0.09), the GG genotype was significantly associated with increased risk across the genetic models tested: the OR across all studies was 0.72 (95%CI 0.53 to 0.97) for the GG vs. AA and 0.72 (95%CI 0.57 to 0.92) for GG vs. GA + AA (Fig. 2). However, the AA genotype was not associated with higher or lower HCC development risk: the OR across all studies was 1.05 (95%CI 0.77 to 1.44) for AA vs. GA + GG (table 5). The results after deleting each study were similar to those obtained across all studies. We loosely classified the study population as Chinese and non-Chinese based on the ethnicity of the participants. Meta-analyses of subgroups found that Chinese population has the same phenomena as the total population. However, the A-1195G polymorphism in the COX-2 gene was not associated with either increased or reduced risk of HCC development in non-Chinese population (table 5). Meta-regression also supported our results (table 6).

G-765C

With respect to COX-2 G-765C polymorphism, significant association was not observed in all of the six studies (C- vs. G-allele: OR = 1.32, 95%CI 0.76 to 2.30; CC vs. GC+GG: OR = 0.88, 95%CI 0.16 to 4.75; CC vs. GG: OR = 0.93, 95%CI 0.16 to 5.35; GG vs. CC+GC: OR = 0.48, 95%CI 0.14 to 1.59). Since the two non-Chinese studies (10,13) were with small sample size and GG genotype was zero in three studies (11,14,17), subgroup analyses were not performed (table 5).

T+8473C

With respect to COX-2 T+8473C polymorphism, significant association was also not observed in all the three studies (C- vs. T-allele: OR = 0.99, 95%CI 0.86 to 1.14; CC vs. CT+TT: OR = 1.31, 95%CI 0.83 to 2.07; CC vs. TT: OR = 1.25, 95%CI 0.78 to

1.98; TT vs. CT+CC: OR = 1.05, 95%CI 0.89 to 1.24) (table 5).

Other loci

The study by Chang *et al*. (11) also reported three loci polymorphism in the COX-2 gene: intron 1, intron 5, and intron 6. This study showed that, for each of the six genotypes, no differences in distribution between the HCC and control groups were found. The locus polymorphism of A-1290G was reported by one study with 270 cases and 540 healthy controls (17). This study did not find significant association between COX-2 A-1290G polymorphism and risk of HCC. The locus polymorphism of C-899G in the COX-2 gene was also reported only by one study with 300 patients with chronic hepatitis B, 300 patients with liver cirrhosis, 300 patients with HCC, and 300 healthy controls (19). This study found that COX-2 -899C genotype may increase the susceptibility of individuals to HCC.

Publication bias and small-study bias

Begg's funnel plots were prepared for the 8 studies to assess publication bias for studies about A-1195G polymorphism of COX-2 and HCC risk. The shape of the funnel plots appeared to be symmetrical for allele contrast, homozygous comparison, and recessive and dominant genetic models, suggesting the absence of publication bias. Moreover, Egger's test also suggested no publication bias (table 6).

Discussion

Some studies reported an association between the COX-2 gene polymorphism and HCC development risk, while others found no such association. The most likely reason for the inconsistencies among these studies is the small sample size. To help resolve these conflicting results using a larger sample size, we conducted systematic review of published studies. In this review, we included 10 studies investigating the association of eight polymorphic variants of COX-2 and the susceptibility of HCC development. We found that GG genotype of A-1195G in the COX-2 gene was associated with increased risk of HCC development, especially in Chinese population.

However, we did not find a compelling evidence of an association between other COX-2 gene polymorphisms and risk of HCC development.

As is known, the polymorphisms in the COX-2 promoter may have an important effect on gene transcriptional activity by changing the binding capacity of certain nuclear proteins, thereby affecting COX-2 expression. Even though the exact molecular mechanism still remains unclear, several polymorphisms of COX-2 have been published previously, and the results are still conflicting. Previous meta-analysis of 8 studies revealed that COX-2 C+202T polymorphism is associated with a lower prostate cancer risk in Caucasians (26). Another meta-analysis of 25 studies found that COX-2 A-1195G polymorphism is a low penetrance risk factor of cancer (27). However, COX-2 C-765G and T+8473C polymorphisms are significantly associated with increased risk of digestive system cancers (28,29). The meta-analysis by Bu *et al*. (30) included 5 (10-12,15,17) of the 10 included studies of this review. They found an association between COX-2 A-1195G polymorphism and HCC risk, especially in Asians. In this update review with larger sample size, other 5 studies (13,14,16,18,19) were included. GG genotype at the A-1195G polymorphism was also associated with increased risk of HCC development across all studies. We also investigated other seven polymorphic variants (G-765C, T+8473C, intron 1, intron 5, intron 6, A-1290G, C-899G) of COX-2. Although COX-2 C-899G polymorphism may increase the risk of HCC, this result only based on one study. In order to demonstrate the association between COX-2 C-899G polymorphism and risk of HCC development, more reliable data with large sample size are needed.

HCC involves complex, multistep and heterogeneous malignant tumorigenesis. The etiology of HCC involves various host and environmental factors. Furthermore, host and environmental factors may interact synergistically in HCC pathogenesis and progression (4). Several studies in this review indicate that COX-2 polymorphisms can interact with environmental factors to module HCC risk. Among individuals with a drinking history, COX-2 -765 C allele carriers had a significantly higher risk to

HCC development compared with G allele (18,31). Though single gene polymorphism and risk to HCC was not found in the study by Fan *et al*. (12), demographic interactions were observed. Among individuals younger than 55 years, A-allele of COX-2 A-1195G polymorphism is a high penetrance risk factor to HCC development, while among female individuals, C-allele of COX-2 T+8473C is a low penetrance risk factor to HCC development. About the gene-gene interactions, no significant difference in the frequencies of any combined genotypes was observed between HCC cases and healthy controls (11). The joint effects of COX-2 genotypes and smoking or alcohol drinking were also not found (11). Moreover, no significant difference in COX-2 C-899G genotype distribution interactions with age, sex, or smoking history was found (19). Therefore, whether the interactions of gene-gene and gene-environment of COX-2 polymorphism may contribute to the risk of HCC is unknown.

Our data revealed that COX-2 A-1195G gene polymorphism may be a risk factor for hepatocarcinogenesis, but the complete picture is more complex. Seven (11,12,14,15,17-19) of the ten included individuals are Chinese. China has among the highest incidences of HCC in the world, as well as a high prevalence of hepatitis B virus infection and dietary exposure to aflatoxin B1, which are the two main risk factors for HCC (32-34). Some of the included controls are with hepatitis B or C virus infection, or cirrhosis. Duo to the sample size of these controls are small, subgroup analysis based on liver disease background was not performed. In addition, polymorphisms in numerous other genes, such as those encoding microsomal epoxide hydrolase (4) and epidermal growth factor (5) are also associated with the risk of HCC. It may be that any single nucleotide polymorphism such as COX-2 A-1195G is insufficient on its own to cause HCC, though it does increase the risk of the disease.

As stated before, some of the included controls had one or more of the following: alcoholic liver disease, HBV or HCV infection, and cirrhosis. Since the studies included in this review often did not report detailed statistics on the proportion of

HCC or control subjects with these background conditions, we could not perform subgroup analysis to separate the contribution of COX-2 polymorphism from that of possible confounders like HBV or HCV infection. In addition, it's hard to assess the quality of the include studies, which may also lead to bias.

Some other limitations of this review should be considered too. Although we searched all the eligible records, the number of included studies was still relatively small. Subgroup stratification analysis of other COX-2 gene polymorphism was not performed. Moreover, meta-analysis was not carried out for 5 polymorphic variants of COX-2. Second, the results may be affected by additional confounding factors, such as tumor status, age or gender, but most studies either did not report these baseline data or aggregated them in different ways, making it impossible to include them into pooled analysis. Moreover, the distribution of genotypes among controls did not show HWE in several studies. Finally, because of the lack of the individual original data, our meta-analysis was based on unadjusted data and a more precise analysis stratified by clinical manifestation and environmental factors has not been performed.

In conclusion, this review suggests that COX-2 A-1195G gene polymorphism, instead of other 7 polymorphic variants of COX-2, might be a risk factor of HCC development. However, since this review included few studies, large, well-designed studies are warranted to re-evaluate these associations.

This study is in accordance with the PRISMA guidelines (Checklist S1).

Author contributions: SCL conceived and designed the experiments; SCL, JTT, HLT and JHZ performed the research; BDX, LQL and XGL performed the statistical analysis; SCL and JHZ wrote the manuscript; all authors have read and approved the final manuscript.

Declaration of interest: The authors report no conflicts of interest.

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Data sharing statement: No additional data available.

References

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin 64: 9-29, 2014
2. Zhang CY, Huang TR, Yu JH, Zhang ZQ, Li JL, et al. Epidemiological analysis of primary liver cancer in the early 21st century in Guangxi province of China. Chin J Cancer 29: 545-550, 2010
3. Zhong JH, Ke Y, Gong WF, Xiang BD, Ma L, et al. Hepatic resection associated with good survival for selected patients with intermediate and advanced-stage hepatocellular carcinoma. Ann Surg 260: 329-340, 2014
4. Zhong JH, Xiang BD, Ma L, You XM, Li LQ, et al. Meta-analysis of microsomal epoxide hydrolase gene polymorphism and risk of hepatocellular carcinoma. PLoS One 8: e57064, 2013
5. Zhong JH, You XM, Gong WF, Ma L, Zhang Y, et al. Epidermal growth factor gene polymorphism and risk of hepatocellular carcinoma: a meta-analysis. PLoS One 7: e32159, 2012
6. Williams CS, Mann M, DuBois RN. The role of cyclooxygenases in inflammation, cancer, and development. Oncogene 18: 7908-7916, 1999
7. Trifan OC, Hla T. Cyclooxygenase-2 modulates cellular growth and promotes tumorigenesis. J Cell Mol Med 7: 207-222, 2003
8. Bae SH, Jung ES, Park YM, Kim BS, Kim BK, et al. Expression of cyclooxygenase-2 (COX-2) in hepatocellular carcinoma and growth inhibition of hepatoma cell lines by a COX-2 inhibitor, NS-398. Clin Cancer Res 7: 1410-1418, 2001
9. Sung YK, Hwang SY, Kim JO, Bae HI, Kim JC, et al. The correlation between cyclooxygenase-2 expression and hepatocellular carcinogenesis. Mol Cells 17:

35-38, 2004

10. Akkiz H, Bayram S, Bekar A, Akgollu E, Ulger Y. Functional polymorphisms of cyclooxygenase-2 gene and risk for hepatocellular carcinoma. Mol Cell Biochem 347: 201-208, 2011
11. Chang WS, Yang MD, Tsai CW, Cheng LH, Jeng LB, et al. Association of cyclooxygenase 2 single-nucleotide polymorphisms and hepatocellular carcinoma in Taiwan. Chin J Physiol 55: 1-7, 2012
12. Fan XJ, Qiu XQ, Yu HP, Zeng XY, Yang Y, et al. [Association of COX-2 gene SNPs with the risk of hepatocellular carcinoma]. Chin J Cancer Rrev Treat 18: 405-409, 2011
13. Gharib AF, Karam RA, Abd El Rahman TM, Elsawy WH. COX-2 polymorphisms -765G-->C and -1195A-->G and hepatocellular carcinoma risk. Gene 543: 234-236, 2014
14. Li YH, Meng W. The Association of Survivin and COX-2 Gene with Hepatocellular Carcinoma. Fudan University: Master Dissertation. 2011.
15. Liu LF, Zhang JL, Lin JS. [The relationship between Cyclooxygenase-2 gene -1195G/A genotype and risk of HBV-induced HCC: a case-control study in Han Chinese people]. Chin J Gastroenterol Hepatol 19: 333-335, 2010.
16. Mohamed FZ, Hussein YM, El-Deen IM, Sabea MS. Cyclooxygenase-2 single-nucleotide polymorphisms and hepatocellular carcinoma in Egypt. Mol Biol Rep 41: 1461-1468, 2014.
17. Xu DK, Zhang XM, Zhao P, Cai JC, Zhao D, et al. [Association between single nucleotide polymorphisms in promoter of COX-2 gene and hereditary susceptibility to hepatocellular carcinoma]. Chin J Hepatobiliary Surg 14: 587-589, 2008.
18. He J, Zhang Q, Ren Z, Li Y, Li X, et al. Cyclooxygenase-2 -765 G/C polymorphisms and susceptibility to hepatitis B-related liver cancer in Han Chinese population. Mol Biol Rep 39: 4163-4168, 2012.
19. He JH, Li YM, Zhang QB, Ren ZJ, Li X, et al. Cyclooxygenase-2 promoter polymorphism -899G/C is associated with hepatitis B-related liver cancer in a

Chinese population of Gansu province. Chin Med J (Engl) 124: 4193-4197, 2011.

20. Zhong JH, Rodriguez AC, Yang NN, Li LQ. Methylenetetrahydrofolate reductase gene polymorphism and risk of type 2 diabetes mellitus. PLoS One 8: e74521,2013

21. Zhong JH, Zhang ZM, Li LQ. mEH Tyr113His polymorphism and the risk of ovarian cancer development. J Ovarian Res 6: 40, 2013

22. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 327: 557-560, 2003

23. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 21: 1539-1558, 2002

24. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS Med 6: e1000100, 2009

25. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 315: 629–634, 1997.

26. Zhang H, Xu Y, Zhang Z, Liu R, Ma B. Association between COX-2 rs2745557 polymorphism and prostate cancer risk: a systematic review and meta-analysis. BMC Immunol 13: 14, 2012

27. Tang Z, Nie ZL, Pan Y, Zhang L, Gao L, et al. The Cox-2 -1195 G > A polymorphism and cancer risk: a meta-analysis of 25 case-control studies. Mutagenesis 26: 729-734, 2011.

28. Dong J, Dai J, Zhang M, Hu Z, Shen H. Potentially functional COX-2-1195G>A polymorphism increases the risk of digestive system cancers: a meta-analysis. J Gastroenterol Hepatol 25: 1042-1050, 2010.

29. Wang XF, Huang MZ, Zhang XW, Hua RX, Guo WJ. COX-2-765G>C polymorphism increases the risk of cancer: a meta-analysis. PLoS One 8: e73213, 2013.

30. Bu X, Zhao C. The association between cyclooxygenase-2 1195 G/A

polymorphism and hepatocellular carcinoma: evidence from a meta-analysis. Tumour Biol 34: 1479-1484, 2013.

31. Song X, Cheng SH, Liu C, Liu YL, Sun JJ. Cyclooxygenase-2 Polymorphism and Susceptibility to Hepatocellular Carcinoma. Practic J Cancer 26: 255-258, 2011.

32. Wang FS, Fan JG, Zhang Z, Gao B, Wang HY. The global burden of liver disease: The major impact of China. Hepatology 60: 2099-2108, 2014.

33. Zhong JH, Zhong QL, Li LQ, Li H. Adjuvant and chemopreventive therapies for resectable hepatocellular carcinoma: a literature review. Tumour Biol 35: 9459-9468, 2014.

34. Zhong JH, Rodríguez AC, Ke Y, Wang YY, Wang L, et al. Hepatic resection as a safe and effective treatment for hepatocellular carcinoma involving a single large tumor, multiple tumors, or macrovascular invasion. Medicine (Baltimore) 94:e396, 2015.

Figure legends

Figure 1. Flow chart of study selection.

Figure 2. Forest plots describing the association of A-1195G COX-2 polymorphism with HCC (GG vs. GA + AA).

Table 1 EMBASE search strategies

Database	Time span of search	Search strategy
EMBASE (Ovid SP)	1990 to March 2015	1. exp CYCLOOXYGENASE-2 2. (cyclooxygenase-2* or COX-2*).mp. [mp=title, abstract, subject headings, heading word, original title, drug trade name, drug manufacturer] 3. 1 or 2 4. (gene* or polymorphism* or variation* or genotype* or genetic* or mutation*).mp. [mp=title, abstract, subject headings, heading word, original title, drug trade name, drug manufacturer] 5. exp liver cell carcinoma/ 6. exp liver tumor/ 7. (((liver or hepatic or hepatocellular or hepato-cellular) and (carcinom* or cancer* or neoplasm* or malign* or tumo*)) or HCC).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer] 8. 5 or 6 or 7 9. 3 and 4 and 8

Table 2 Main characteristics of studies about cyclooxygenase-2 A-1195G polymorphism and the risk of hepatocellular carcinoma

Study	Country	Source of control	Genotyping method	P_{HWE}	Cases / Controls	No. of cases			No. of controls		
						GG	GA	AA	GG	GA	AA
Akkiz 2011[10]	Turkey	HB	PCR-RFLP	0.71	129/129	2	36	91	2	32	95
Chang 2012[11]	Taiwan	PB	PCR-RFLP	0.57	298/298	70	144	84	72	145	81
Fan 2011[12]	China	HB	TaqMan genotyping platform	0.52	780/780	204	390	186	205	381	194
Gharib 2014[13]	Egypt	PB	PCR-RFLP	0.86	120/130	17	60	43	31	66	33
Li 2011[14]	China	PB	PCR-RFLP	0.15	178/196	31	88	59	54	88	54
Liu 2010[15]	China	HB and PB	PCR-RFLP	0.56	210/420	31	110	69	101	216	103
Mohamed 2014[16]	Egypt	HB and PB	PCR-RFLP	< 0.001	75/125	12	49	14	40	22	63
Xu 2008[17]	China	PB	PCR-RFLP	0.14	270/540	52	125	93	119	287	134

Abbreviations: PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; PB, population-based; HB, hospital-based; PHWE, Hardy-Weinberg equilibrium of controls.

Table 3 Main characteristics of studies about cyclooxygenase-2 G-765C polymorphism and the risk of hepatocellular carcinoma

Study	Country	Source of control	Genotyping method	P_{HWE}	Cases / Controls	No. of cases			No. of controls		
						GG	GA	AA	GG	GA	AA
Akkiz 2011[10]	Turkey	HB	PCR-RFLP	0.009	129/129	4	46	79	15	39	75
Chang 2012[11]	Taiwan	PB	PCR-RFLP	0.13	298/298	0	36	262	0	48	250
Gharib 2014[13]	Egypt	PB	PCR-RFLP	0.58	120/100	4	30	86	6	39	85
He 2012[18]	China	PB	PCR-RFLP	0.59	300/300	10	67	223	2	37	261
Li 2011[14]	China	HB	PCR-RFLP	0.60	178/196	0	26	152	0	14	182
Xu 2008[17]	China	PB	PCR-RFLP	0.58	270/540	0	37	233	0	25	515

Abbreviations: PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; PB, population-based; HB, hospital-based; P_{HWE}, Hardy-Weinberg equilibrium of controls.

Table 4 Main characteristics of studies about cyclooxygenase-2 T+8473C polymorphism and the risk of hepatocellular carcinoma

Study	Country	Source of control	Genotyping method	P_{HWE}	Cases / Controls	No. of cases			No. of controls		
						CC	TC	TT	CC	TC	TT
Akkiz 2011[10]	Turkey	HB	PCR-RFLP	0.16	129/129	8	56	65	9	62	58
Chang 2012[11]	Taiwan	PB	PCR-RFLP	< 0.001	298/298	0	103	195	0	97	201
Fan 2011[12]	China	HB	TaqMan genotyping platform	0.22	780/780	36	235	509	25	258	497

Abbreviations: PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; PB, population-based; HB, hospital-based; P_{HWE}, Hardy-Weinberg equilibrium of controls.

Table 5 Overall and stratified meta-analyses of the association between COX-2 polymorphisms and risk of hepatocellular carcinoma

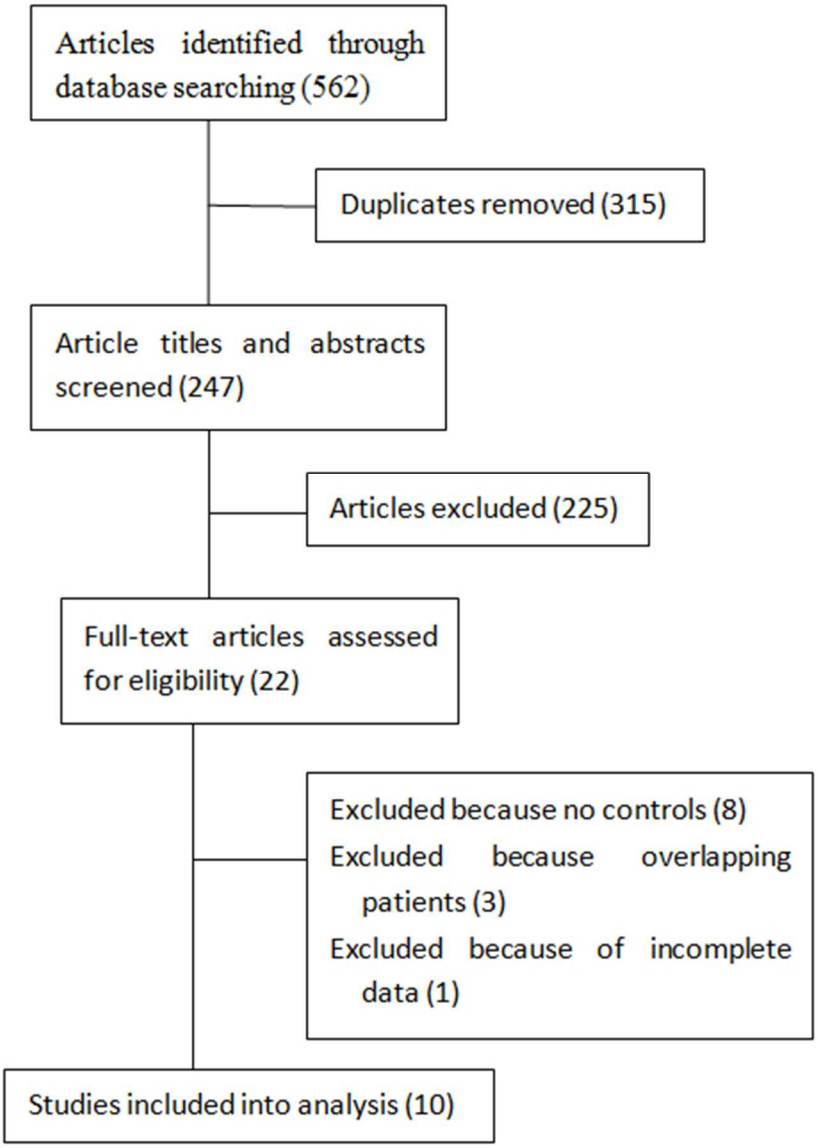
Comparison	Population	No. of Study	Test of association*			Model	Test of heterogeneity	
			OR	95%CI	*P*		*P*	I^2
COX-2 A-1195G (rs689466)								
G-allele vs.	Overall	8	0.87	0.75-1.02	0.09	R	0.007	64
A-allele	Chinese	5	**0.84**	**0.72-0.99**	**0.04**	R	0.02	65
	Non-Chinese	3	1.00	0.63-1.59	0.99	R	0.02	74
GG vs. GA	Overall	8	**0.72**	**0.57-0.92**	**0.008**	R	0.04	52
+ AA	Chinese	5	0.79	0.62-1.01	0.06	R	0.06	55
	Non-Chinese	3	**0.49**	**0.30-0.78**	**0.003**	F	0.66	0
GG vs. AA	Overall	8	**0.72**	**0.53-0.97**	**0.03**	R	0.02	57
	Chinese	5	**0.71**	**0.51-0.99**	**0.05**	R	0.02	66
	Non-Chinese	3	0.77	0.32-1.84	0.56	R	0.13	52
AA vs.	Overall	8	1.05	0.77-1.44	0.74	R	< 0.001	79
GA+GG	Chinese	5	1.23	0.98-1.55	0.07	R	0.06	57
	Non-Chinese	3	0.69	0.24-2.03	0.51	R	< 0.001	90
COX-2 G-765C (rs20417)								
C-allele vs.	Overall							
G-allele		6	1.32	0.76-2.30	0.33	R	< 0.001	88
CC vs.	Overall							
GC+GG		3	0.88	0.16-4.75	0.88	R	0.007	80
CC vs. GG	Overall	3	0.93	0.16-5.35	0.94	R	0.005	81
GG vs.	Overall							
CC+GC		6	0.48	0.14-1.59	0.23	R	< 0.001	97
COX-2 T+8473C (rs5275)								
C-allele vs.	Overall							
T-allele		3	0.99	0.86-1.14	0.91	F	0.67	0
CC vs. CT +	Overall							
TT		3	1.31	0.83-2.07	0.25	F	0.37	0
CC vs. TT	Overall	3	1.25	0.78-1.98	0.35	F	0.33	0
TT vs. CT +	Overall							
CC		3	1.05	0.89-1.24	0.58	F	0.57	0

Abbreviations: OR, odds ratio; CI, confidence interval; R, random-effect model; F, fixed-effect model.

*Mantel-Haenszel estimate was used to give a pooled odds ratio using the fixed- or random-effect models.

Table 6 Ethnicity meta-regression and publication bias of COX-2 A-1195G polymorphisms and risk of hepatocellular carcinoma

Variables	Coef.	Std. Err.	z	P	95% confidence interval
Meta-regression					
G-allele vs. A-allele	-0.107	0.271	-0.40	0.693	-0.639-0.424
GG vs. GA + AA	0.520	0.435	1.20	0.232	-0.3321-1.373
GG vs. AA	0.217	0.574	0.38	0.706	-0.909-1.342
AA vs. GA+GG	0.282	0.561	0.50	0.616	-0.819-1.382
Publication bias by Egger's test					
G-allele vs. A-allele	-0.059	0.210	-0.28	0.788	-0.573-0.455
GG vs. GA + AA	0.148	0.196	0.75	0.481	-0.3332-0.628
GG vs. AA	-0.017	0.323	-0.05	0.959	-0.807-0.772
AA vs. GA+GG	0.416	0.485	0.86	0.423	-0.770-1.603



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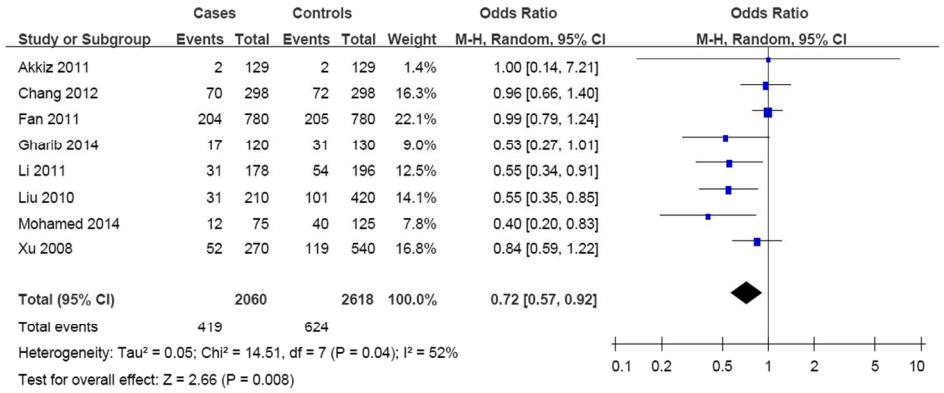

102x46mm (300 x 300 DPI)

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a meta-analysis.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	n/a
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	4
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	4
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	5
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	5-6

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	6
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	6
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	7
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	7
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	7
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	8
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	8
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	9
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	10
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	11
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	1

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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BMJ Open

Association between COX-2 Gene Polymorphisms and Risk of Hepatocellular Carcinoma Development: a meta-analysis

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Secondary Subject Heading:	Gastroenterology and hepatology, Genetics and genomics
Keywords:	cyclooxygenase-2, hepatocellular carcinoma, polymorphism, susceptibility

Association between COX-2 Gene Polymorphisms and Risk of Hepatocellular Carcinoma Development: a meta-analysis

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Running head: COX-2 polymorphisms and HCC risk

Strengths and limitations of this study

- Eight polymorphic variants of cyclooxygenase-2 gene were studied.
- Limited by lacking of gene-gene and gene-environment interaction data.

Abstract

Objective: To investigate the association between cyclooxygenase-2 (COX-2) polymorphism and risk to hepatocellular carcinoma (HCC) development.

Design: Systematic review and meta-analysis of COX-2 polymorphism and risk to HCC development among individuals with or without HCC.

Data sources: EMBASE, PubMed, Public Library of Science, SCOPUS, Web of Knowledge, and Chinese National Knowledge Infrastructure for all clinical and experimental case-control studies of COX-2 polymorphism and HCC risk. Studies published until March 2015 were included.

Review method: Ten studies were included for data extraction. The studies included in this review were mainly from Asian countries.

Results: A total of 2538 individuals with HCC and 3714 individuals without HCC were found to satisfy the inclusion criteria and included in the review. The associations of specific genotypes in the eight polymorphic variants of COX-2 and risk to HCC development were analyzed. GG genotype at the A-1195G polymorphism might be associated with decreased risk to HCC development: the OR across all studies was 0.87 (0.75 to 1.02) for G-allele vs. A-allele, 0.72 (0.53 to 0.97) for GG vs. AA, 0.72 (95%CI 0.57 to 0.92) for the GG vs. GA + AA, and 1.05 (0.77 to 1.44) for AA vs. GA + GG. Similar results were found when the meta-analysis was repeated separately for Chinese subgroup. However, evidence about the associations between variants in G-765C, T+8473C, A-1290G, G-899C, and introns 1, 5, and 6 polymorphisms and risk to HCC development need more reliable data to demonstrate.

Conclusions: Only COX-2 A-1195G gene polymorphism might be associated with decreased risk to HCC development. These conclusions should be verified in further studies.

Keywords: cyclooxygenase-2; hepatocellular carcinoma; meta-analysis; polymorphism; susceptibility

Introduction

Hepatocellular carcinoma (HCC) is a significant cause of cancer morbidity and mortality worldwide. The estimated incidence of new HCC cases each year is more than 0.5 million (1). China is one of the regions with highest incidence of HCC (>20 per 100,000 people), which accounts for more than 50% of the total cases (2,3). Epidemiologically, HCC is strongly associated with hepatitis B or C virus infection, alcohol consumption, and metabolic disease. However, not all individuals with these factors appear to have the same risk of developing HCC. HCC is a multifactorial disease. Nowadays, many studies revealed that gene polymorphisms may also contribute to the risk of hepatocarcinogenesis (4,5). Namely, patients with HCC exhibit a high degree of genetic heterogeneity.

Cyclooxygenase-2 [COX-2, also known as prostaglandin endoperoxide synthases or prostaglandin H synthases (PTGSs)] is an inducible enzyme that converts arachidonic acid to prostaglandins, which are potent mediators of inflammation. COX-2 is normally absent in most tissue cells. It is induced in response to inflammatory cytokines, mitogens, angiogenic growth factors, and tumor promoters (6,7). Increased COX-2 expression has been associated with the early stages of hepatocarcinogenesis (8,9). However, the association of COX-2 genotypes polymorphism with risk to HCC has not been well revealed.

Recently, a number of studies (10-19) have examined whether an association exists between the COX-2 polymorphism and risk to HCC. These studies have arrived at different conclusions, with some suggesting a significant association and others no association. Since individual case-control studies may fail to detect complicated genetic relationship because of small sample size, this review aims to comprehensively assess the literature examining a possible link between the COX-2 polymorphism and risk to HCC.

Methods

Literature Search strategy

All clinical and experimental case-control studies of COX-2 polymorphism and HCC risk published through March 31, 2015 were identified through systematic searches in EMBASE, PubMed, Public Library of Science (www.plos.org), SCOPUS, Web of Knowledge, and Chinese National Knowledge Infrastructure. Due to a lot of papers were published by the Public Library of Science in the recent decade, we also searched this database. No language restriction was imposed. The following search terms were used to identify studies: cyclooxygenase-2 *or* COX-2, gene *or* polymorphism *or* variation *or* genotype *or* genetic *or* mutation, "hepatocellular carcinoma" *or* "liver cancer" *or* HCC. Detailed database search strategies of EMBASE are shown in table 1. We also searched the Catalog of Published Genome-Wide Association Studies (GWAS) (www.genome.gov/gwastudies) of the US National Human Genome Research Institute. Reference lists of these articles and relevant literature from review articles were also searched to identify additional relevant publications.

Inclusion criteria

Only full-length research study satisfied the following criteria would be included in this review: (a) it assessed the association between COX-2 polymorphism and risk to HCC development; (b) they used a case-control or cohort design in which cases were HCC patients and controls were healthy individuals, or with chronic hepatitis B or C, or with cirrhosis; (c) they focused on human beings; (d) they provided sufficient published data for estimating an odds ratio (OR) with a 95% confidence interval (95%CI). In the case of multiple studies apparently based on the same case or control population, we included only the study with the largest number of participants. Conference abstracts or other forms of summary publication were not included. If there was incomplete data on genotype frequency in this study, we would try to contact the authors to collect these data (20).

Data extraction

Two authors (S-CL, J-TT) independently searched the literature and indentified eligible articles based on our inclusion criteria. These two authors also independently extracted the following data from included studies: first author's family name, year of publications, genotyping methods, source of controls (population-based and hospital-based), numbers and genotypes of cases and controls, and Hardy-Weinberg equilibrium (HWE) of controls. Extracted data were compared and discrepancies were resolved by discussion with a third author (J-HZ).

Statistical Methods and Bias Testing

As describing previously (20,21), the unadjusted OR with 95%CI was used to assess the strength of the association between the COX-2 polymorphism and HCC susceptibility based on the genotype frequencies in cases and controls. The meta-analysis examined the association of different genotypes at different loci of COX-2 with HCC risk by comparing the alleles, comparing homozygous genotypes, and applying recessive and dominant genetic models.

Mantel-Haenszel estimate was used to give a pooled OR using the fixed -effect models, while DerSimonian-Laird estimate for random effect models. The significance of OR was assessed using the Z-test, and $P<0.05$ was considered statistically significant. I^2 was used to estimate total variation across studies due to heterogeneity in percentage (22,23). Less than 25% was considered as low level of heterogeneity, 25% to 50% as moderate level of heterogeneity, and higher than 50% as high level of heterogeneity. $I^2 > 50\%$ could suggest heterogeneity and suggest using a random effect estimate (22,23). Otherwise, the fixed-effect model was used to calculate pooled ORs. HWE in the control group was assessed using the chi-square goodness-of-fittest, with $P < 0.05$ considered significant. As much as possible, the meta-analysis was performed according to the PRISMA guidelines (24).

As describing previously (20,21), to detect associations that might be masked in the

overall sample, we performed subgroup analyses based on ethnicity. Meta-regression was performed to exam the effect of ethnicity to compare results from the meta-analyses. To assess the reliability of the outcomes in the meta-analysis, a sensitivity analysis was performed by excluding one study at a time.

Publication bias was assessed by visual inspection of Begg's funnel plots. An asymmetric plot suggested possible publication bias, in which case Egger's test was used (25). All statistical tests for this meta-analysis were performed using Stata 11.0 (Stata-Corp, College Station, USA) and RevMan 5.3 (Cochrane Collaboration).

Results

Description of studies

Several research databases were searched to identify studies assessing the possible association between the polymorphism in the COX-2 gene and risk to HCC. A total of 562 studies were identified, none of which was a GWAS. This list was reduced to 22 after removing duplicates and screening based on the title and abstract review. These articles were read in full, and 8 studies were removed because they did not include control group, while another 4 studies were removed because overlapping patients were analyzed or was with incomplete data. No study which was published in a language other than in Chinese or in English was excluded. In the end, 10 studies were included into analysis (fig. 1) (10-19). Four of them were published in Chinese (12,14,15,17). Other five studies were published in English (10,11,13,16,18,19). The main characteristics of the included studies are shown in tables 2-4. All the studies were reported that cases and controls were matched on age and gender.

The studies involved 2538 individuals with HCC and 3714 individuals without HCC. The A-1195G polymorphism in the COX-2 gene and risk of HCC development was reported by 8 studies (10-17) (table 2), G-765C in 6 studies (10,11,13,14,17,18) (table 3), and T+8473C in 3 studies (table 4) (10-12).

Quantitative data synthesis

A-1195G

Although the polymorphism in the allelic contrast model only slightly affect HCC development risk (OR = 0.87, 95%CI = 0.75-1.02, *P* = 0.09), the GG genotype was significantly associated with decreased risk across the genetic models tested: the OR across all studies was 0.72 (95%CI 0.53 to 0.97) for the GG vs. AA and 0.72 (95%CI 0.57 to 0.92) for GG vs. GA + AA (Fig. 2). However, the AA genotype was not associated with higher or lower HCC development risk: the OR across all studies was 1.05 (95%CI 0.77 to 1.44) for AA vs. GA + GG (table 5). The results after deleting each study were similar to those obtained across all studies. We loosely classified the study population as Chinese and non-Chinese based on the ethnicity of the participants. Meta-analyses of subgroups found that Chinese population has the same phenomena as the total population. However, the A-1195G polymorphism in the COX-2 gene was not associated with either increased or reduced risk of HCC development in non-Chinese population (table 5). Meta-regression also supported our results (table 6).

G-765C

With respect to COX-2 G-765C polymorphism, significant association was not observed in all of the six studies (C- vs. G-allele: OR = 1.32, 95%CI 0.76 to 2.30; CC vs. GC+GG: OR = 0.88, 95%CI 0.16 to 4.75; CC vs. GG: OR = 0.93, 95%CI 0.16 to 5.35; GG vs. CC+GC: OR = 0.48, 95%CI 0.14 to 1.59). Since the two non-Chinese studies (10,13) were with small sample size and GG genotype was zero in three studies (11,14,17), subgroup analyses were not performed (table 5).

T+8473C

With respect to COX-2 T+8473C polymorphism, significant association was also not observed in all the three studies (C- vs. T-allele: OR = 0.99, 95%CI 0.86 to 1.14; CC vs. CT+TT: OR = 1.31, 95%CI 0.83 to 2.07; CC vs. TT: OR = 1.25, 95%CI 0.78 to

1.98; TT vs. CT+CC: OR = 1.05, 95%CI 0.89 to 1.24) (table 5).

Other loci

The study by Chang *et al*. (11) also reported three loci polymorphism in the COX-2 gene: intron 1, intron 5, and intron 6. This study showed that, for each of the six genotypes, no differences in distribution between the HCC and control groups were found. The locus polymorphism of A-1290G was reported by one study with 270 cases and 540 healthy controls (17). This study did not find significant association between COX-2 A-1290G polymorphism and risk of HCC. The locus polymorphism of C-899G in the COX-2 gene was also reported only by one study with 300 patients with chronic hepatitis B, 300 patients with liver cirrhosis, 300 patients with HCC, and 300 healthy controls (19). This study found that COX-2 -899C genotype may increase the susceptibility of individuals to HCC.

Publication bias and small-study bias

Begg's funnel plots were prepared for the 8 studies to assess publication bias for studies about A-1195G polymorphism of COX-2 and HCC risk. The shape of the funnel plots appeared to be symmetrical for allele contrast, homozygous comparison, and recessive and dominant genetic models, suggesting the absence of publication bias. Moreover, Egger's test also suggested no publication bias (table 6).

Discussion

Some studies reported an association between the COX-2 gene polymorphism and HCC development risk, while others found no such association. The most likely reason for the inconsistencies among these studies is the small sample size. To help resolve these conflicting results using a larger sample size, we conducted a systematic review of published studies. In this review, we included 10 studies investigating the association of eight polymorphic variants of COX-2 and the susceptibility of HCC development. We found that GG genotype of A-1195G in the COX-2 gene was associated with decreased risk of HCC development, especially in Chinese population.

However, we did not find a compelling evidence of an association between other COX-2 gene polymorphisms and risk of HCC development.

As is known, the polymorphisms in the COX-2 promoter may have an important effect on gene transcriptional activity by changing the binding capacity of certain nuclear proteins, thereby affecting COX-2 expression. Even though the exact molecular mechanism still remains unclear, several polymorphisms of COX-2 have been published previously, and the results are still conflicting. Previous meta-analysis of 8 studies revealed that COX-2 C+202T polymorphism is associated with a lower prostate cancer risk in Caucasians (26). Another meta-analysis of 25 studies found that COX-2 A-1195G polymorphism is a low penetrance risk factor of cancer (27). However, COX-2 C-765G and T+8473C polymorphisms are significantly associated with increased risk of digestive system cancers (28,29). The meta-analysis by Bu *et al*. (30) included 5 (10-12,15,17) of the 10 included studies of this review. They found an association between COX-2 A-1195G polymorphism and HCC risk, especially in Asians. In this update review with larger sample size, other 5 studies (13,14,16,18,19) were included. We found GG genotype at the A-1195G polymorphism was associated with decreased risk of HCC development across all studies. We also investigated other seven polymorphic variants (G-765C, T+8473C, intron 1, intron 5, intron 6, A-1290G, C-899G) of COX-2. Although COX-2 C-899G polymorphism may increase the risk of HCC, this result only based on one study. In order to demonstrate the association between COX-2 C-899G polymorphism and risk of HCC development, more reliable data with large sample size are needed.

HCC involves complex, multistep and heterogeneous malignant tumorigenesis. The etiology of HCC involves various host and environmental factors. Furthermore, host and environmental factors may interact synergistically in HCC pathogenesis and progression (4). Several studies in this review indicate that COX-2 polymorphisms can interact with environmental factors to module HCC risk. Among individuals with a drinking history, COX-2 -765 C allele carriers had a significantly higher risk to

HCC development compared with G allele (18,31). Though single gene polymorphism and risk to HCC was not found in the study by Fan *et al*. (12), demographic interactions were observed. Among individuals younger than 55 years, A-allele of COX-2 A-1195G polymorphism is a high penetrance risk factor to HCC development, while among female individuals, C-allele of COX-2 T+8473C is a low penetrance risk factor to HCC development. About the gene-gene interactions, no significant difference in the frequencies of any combined genotypes was observed between HCC cases and healthy controls (11). The joint effects of COX-2 genotypes and smoking or alcohol drinking were also not found (11). Moreover, no significant difference in COX-2 C-899G genotype distribution interactions with age, sex, or smoking history was found (19). Therefore, whether the interactions of gene-gene and gene-environment of COX-2 polymorphism may contribute to the risk of HCC is unknown.

Our data revealed that COX-2 A-1195G gene polymorphism may be a protective factor for hepatocarcinogenesis, but the complete picture is more complex. Seven (11,12,14,15,17-19) of the ten included individuals are Chinese. China has among the highest incidences of HCC in the world, as well as a high prevalence of hepatitis B virus infection and dietary exposure to aflatoxin B1, which are the two main risk factors for HCC (32-34). Some of the included controls are with hepatitis B or C virus infection, or cirrhosis. Duo to the sample size of these controls are small, subgroup analysis based on liver disease background was not performed. In addition, polymorphisms in numerous other genes, such as those encoding microsomal epoxide hydrolase (4) and epidermal growth factor (5) are associated with the risk of HCC. It may be that any single nucleotide polymorphism such as COX-2 A-1195G or epidermal growth factor 61*A/G is insufficient on its own to cause HCC.

As stated before, some of the included controls had one or more of the following: alcoholic liver disease, HBV or HCV infection, and cirrhosis. Since the studies included in this review often did not report detailed statistics on the proportion of

HCC or control subjects with these background conditions, we could not perform subgroup analysis to separate the contribution of COX-2 polymorphism from that of possible confounders like HBV or HCV infection. In addition, it's hard to assess the quality of the include studies, which may also lead to bias.

Low occurrences of genotypes within the COX-2 G-765C and COX-2 T+8473C polymorphisms may lead to null results in Table 5. Therefore, more reliable data with larger sample sizes are needed to give an idea of relationships involving COX-2 G-765C and COX-2 T+8473C polymorphisms whose analyses have suffered due to being underpowered and whose null results have to be treated with caution.

Some other limitations of this review should be considered too. Although we searched all the eligible records, the number of included studies was still relatively small. Subgroup stratification analysis of other COX-2 gene polymorphism was not performed. Moreover, meta-analysis was not carried out for 5 polymorphic variants of COX-2. Second, the results may be affected by additional confounding factors, such as tumor status, age or gender, but most studies either did not report these baseline data or aggregated them in different ways, making it impossible to include them into pooled analysis. Moreover, the distribution of genotypes among controls did not show HWE in several studies. Finally, because of the lack of the individual original data, our meta-analysis was based on unadjusted data and a more precise analysis stratified by clinical manifestation and environmental factors has not been performed.

In conclusion, this review suggests that COX-2 A-1195G gene polymorphism, instead of other 7 polymorphic variants of COX-2, might be a protective factor of HCC development. However, since this review included few studies, large, well-designed studies are warranted to re-evaluate these associations.

This study is in accordance with the PRISMA guidelines (Checklist S1).

Author contributions: SCL conceived and designed the experiments; SCL, JTT, HLT and JHZ performed the research; BDX, LQL and XGL performed the statistical analysis; SCL and JHZ wrote the manuscript; all authors have read and approved the final manuscript.

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References

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin 64: 9-29, 2014
2. Zhang CY, Huang TR, Yu JH, Zhang ZQ, Li JL, et al. Epidemiological analysis of primary liver cancer in the early 21st century in Guangxi province of China. Chin J Cancer 29: 545-550, 2010
3. Zhong JH, Ke Y, Gong WF, Xiang BD, Ma L, et al. Hepatic resection associated with good survival for selected patients with intermediate and advanced-stage hepatocellular carcinoma. Ann Surg 260: 329-340, 2014
4. Zhong JH, Xiang BD, Ma L, You XM, Li LQ, et al. Meta-analysis of microsomal epoxide hydrolase gene polymorphism and risk of hepatocellular carcinoma. PLoS One 8: e57064, 2013
5. Zhong JH, You XM, Gong WF, Ma L, Zhang Y, et al. Epidermal growth factor gene polymorphism and risk of hepatocellular carcinoma: a meta-analysis. PLoS One 7: e32159, 2012
6. Williams CS, Mann M, DuBois RN. The role of cyclooxygenases in inflammation, cancer, and development. Oncogene 18: 7908-7916, 1999
7. Trifan OC, Hla T. Cyclooxygenase-2 modulates cellular growth and promotes tumorigenesis. J Cell Mol Med 7: 207-222, 2003

8. Bae SH, Jung ES, Park YM, Kim BS, Kim BK, et al. Expression of cyclooxygenase-2 (COX-2) in hepatocellular carcinoma and growth inhibition of hepatoma cell lines by a COX-2 inhibitor, NS-398. Clin Cancer Res 7: 1410-1418, 2001

9. Sung YK, Hwang SY, Kim JO, Bae HI, Kim JC, et al. The correlation between cyclooxygenase-2 expression and hepatocellular carcinogenesis. Mol Cells 17: 35-38, 2004

10. Akkiz H, Bayram S, Bekar A, Akgollu E, Ulger Y. Functional polymorphisms of cyclooxygenase-2 gene and risk for hepatocellular carcinoma. Mol Cell Biochem 347: 201-208, 2011

11. Chang WS, Yang MD, Tsai CW, Cheng LH, Jeng LB, et al. Association of cyclooxygenase 2 single-nucleotide polymorphisms and hepatocellular carcinoma in Taiwan. Chin J Physiol 55: 1-7, 2012

12. Fan XJ, Qiu XQ, Yu HP, Zeng XY, Yang Y, et al. [Association of COX-2 gene SNPs with the risk of hepatocellular carcinoma]. Chin J Cancer Rrev Treat 18: 405-409, 2011

13. Gharib AF, Karam RA, Abd El Rahman TM, Elsawy WH. COX-2 polymorphisms -765G-->C and -1195A-->G and hepatocellular carcinoma risk. Gene 543: 234-236, 2014

14. Li YH, Meng W. The Association of Survivin and COX-2 Gene with Hepatocellular Carcinoma. Fudan University: Master Dissertation. 2011.

15. Liu LF, Zhang JL, Lin JS. [The reIationship between Cyclooxygenase-2 gene -1195G/A genotype and risk of HBV-induced HCC: a case-control study in Han Chinese people]. Chin J Gastroenterol Hepatol 19: 333-335, 2010.

16. Mohamed FZ, Hussein YM, El-Deen IM, Sabea MS. Cyclooxygenase-2 single-nucleotide polymorphisms and hepatocellular carcinoma in Egypt. Mol Biol Rep 41: 1461-1468, 2014.

17. Xu DK, Zhang XM, Zhao P, Cai JC, Zhao D, et al. [Association between single nucleotide polymorphisms in promoter of COX-2 gene and hereditary susceptibility to hepatocellular carcinoma]. Chin J Hepatobiliary Surg 14:

587-589, 2008.

18. He J, Zhang Q, Ren Z, Li Y, Li X, et al. Cyclooxygenase-2 -765 G/C polymorphisms and susceptibility to hepatitis B-related liver cancer in Han Chinese population. Mol Biol Rep 39: 4163-4168, 2012.

19. He JH, Li YM, Zhang QB, Ren ZJ, Li X, et al. Cyclooxygenase-2 promoter polymorphism -899G/C is associated with hepatitis B-related liver cancer in a Chinese population of Gansu province. Chin Med J (Engl) 124: 4193-4197, 2011.

20. Zhong JH, Rodriguez AC, Yang NN, Li LQ. Methylenetetrahydrofolate reductase gene polymorphism and risk of type 2 diabetes mellitus. PLoS One 8: e74521,2013

21. Zhong JH, Zhang ZM, Li LQ. mEH Tyr113His polymorphism and the risk of ovarian cancer development. J Ovarian Res 6: 40, 2013

22. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 327: 557-560, 2003

23. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 21: 1539-1558, 2002

24. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS Med 6: e1000100, 2009

25. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 315: 629–634, 1997.

26. Zhang H, Xu Y, Zhang Z, Liu R, Ma B. Association between COX-2 rs2745557 polymorphism and prostate cancer risk: a systematic review and meta-analysis. BMC Immunol 13: 14, 2012

27. Tang Z, Nie ZL, Pan Y, Zhang L, Gao L, et al. The Cox-2 -1195 G > A polymorphism and cancer risk: a meta-analysis of 25 case-control studies. Mutagenesis 26: 729-734, 2011.

28. Dong J, Dai J, Zhang M, Hu Z, Shen H. Potentially functional

COX-2-1195G>A polymorphism increases the risk of digestive system cancers: a meta-analysis. J Gastroenterol Hepatol 25: 1042-1050, 2010.

29. Wang XF, Huang MZ, Zhang XW, Hua RX, Guo WJ. COX-2-765G>C polymorphism increases the risk of cancer: a meta-analysis. PLoS One 8: e73213, 2013.

30. Bu X, Zhao C. The association between cyclooxygenase-2 1195 G/A polymorphism and hepatocellular carcinoma: evidence from a meta-analysis. Tumour Biol 34: 1479-1484, 2013.

31. Song X, Cheng SH, Liu C, Liu YL, Sun JJ. Cyclooxygenase-2 Polymorphism and Susceptibility to Hepatocellular Carcinoma. Practic J Cancer 26: 255-258, 2011.

32. Wang FS, Fan JG, Zhang Z, Gao B, Wang HY. The global burden of liver disease: The major impact of China. Hepatology 60: 2099-2108, 2014.

33. Zhong JH, Zhong QL, Li LQ, Li H. Adjuvant and chemopreventive therapies for resectable hepatocellular carcinoma: a literature review. Tumour Biol 35: 9459-9468, 2014.

34. Zhong JH, Rodríguez AC, Ke Y, Wang YY, Wang L, et al. Hepatic resection as a safe and effective treatment for hepatocellular carcinoma involving a single large tumor, multiple tumors, or macrovascular invasion. Medicine (Baltimore) 94:e396, 2015.

Figure legends

Figure 1. Flow chart of study selection.

Figure 2. Forest plots describing the association of A-1195G COX-2 polymorphism with HCC (GG vs. GA + AA).

Table 1 EMBASE search strategies

Database	Time span of search	Search strategy
EMBASE (Ovid SP)	1990 to March 2015	1. exp CYCLOOXYGENASE-2 2. (cyclooxygenase-2* or COX-2*).mp. [mp=title, abstract, subject headings, heading word, original title, drug trade name, drug manufacturer] 3. 1 or 2 4. (gene* or polymorphism* or variation* or genotype* or genetic* or mutation*).mp. [mp=title, abstract, subject headings, heading word, original title, drug trade name, drug manufacturer] 5. exp liver cell carcinoma/ 6. exp liver tumor/ 7. (((liver or hepatic or hepatocellular or hepato-cellular) and (carcinom* or cancer* or neoplasm* or malign* or tumo*)) or HCC).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer] 8. 5 or 6 or 7 9. 3 and 4 and 8

Table 2 Main characteristics of studies about cyclooxygenase-2 A-1195G polymorphism and the risk of hepatocellular carcinoma

Study	Country	Source of control	Genotyping method	P_{HWE}	Cases / Controls	No. of cases			No. of controls		
						GG	GA	AA	GG	GA	AA
Akkiz 2011[10]	Turkey	HB	PCR-RFLP	0.71	129/129	2	36	91	2	32	95
Chang 2012[11]	Taiwan	PB	PCR-RFLP	0.57	298/298	70	144	84	72	145	81
Fan 2011[12]	China	HB	TaqMan genotyping platform	0.52	780/780	204	390	186	205	381	194
Gharib 2014[13]	Egypt	PB	PCR-RFLP	0.86	120/130	17	60	43	31	66	33
Li 2011[14]	China	PB	PCR-RFLP	0.15	178/196	31	88	59	54	88	54
Liu 2010[15]	China	HB and PB	PCR-RFLP	0.56	210/420	31	110	69	101	216	103
Mohamed 2014[16]	Egypt	HB and PB	PCR-RFLP	< 0.001	75/125	12	49	14	40	22	63
Xu 2008[17]	China	PB	PCR-RFLP	0.14	270/540	52	125	93	119	287	134

Abbreviations: PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; PB, population-based; HB, hospital-based; PHWE, Hardy-Weinberg equilibrium of controls.

Table 3 Main characteristics of studies about cyclooxygenase-2 G-765C polymorphism and the risk of hepatocellular carcinoma

Study	Country	Source of control	Genotyping method	P_{HWE}	Cases / Controls	No. of cases			No. of controls		
						GG	GA	AA	GG	GA	AA
Akkiz 2011[10]	Turkey	HB	PCR-RFLP	0.009	129/129	4	46	79	15	39	75
Chang 2012[11]	Taiwan	PB	PCR-RFLP	0.13	298/298	0	36	262	0	48	250
Gharib 2014[13]	Egypt	PB	PCR-RFLP	0.58	120/100	4	30	86	6	39	85
He 2012[18]	China	PB	PCR-RFLP	0.59	300/300	10	67	223	2	37	261
Li 2011[14]	China	HB	PCR-RFLP	0.60	178/196	0	26	152	0	14	182
Xu 2008[17]	China	PB	PCR-RFLP	0.58	270/540	0	37	233	0	25	515

Abbreviations: PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; PB, population-based; HB, hospital-based; P_{HWE}, Hardy-Weinberg equilibrium of controls.

Table 4 Main characteristics of studies about cyclooxygenase-2 T+8473C polymorphism and the risk of hepatocellular carcinoma

Study	Country	Source of control	Genotyping method	P_{HWE}	Cases / Controls	No. of cases			No. of controls		
						CC	TC	TT	CC	TC	TT
Akkiz 2011[10]	Turkey	HB	PCR-RFLP	0.16	129/129	8	56	65	9	62	58
Chang 2012[11]	Taiwan	PB	PCR-RFLP	< 0.001	298/298	0	103	195	0	97	201
Fan 2011[12]	China	HB	TaqMan genotyping platform	0.22	780/780	36	235	509	25	258	497

Abbreviations: PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; PB, population-based; HB, hospital-based; P_{HWE}, Hardy-Weinberg equilibrium of controls.

Table 5 Overall and stratified meta-analyses of the association between COX-2 polymorphisms and risk of hepatocellular carcinoma

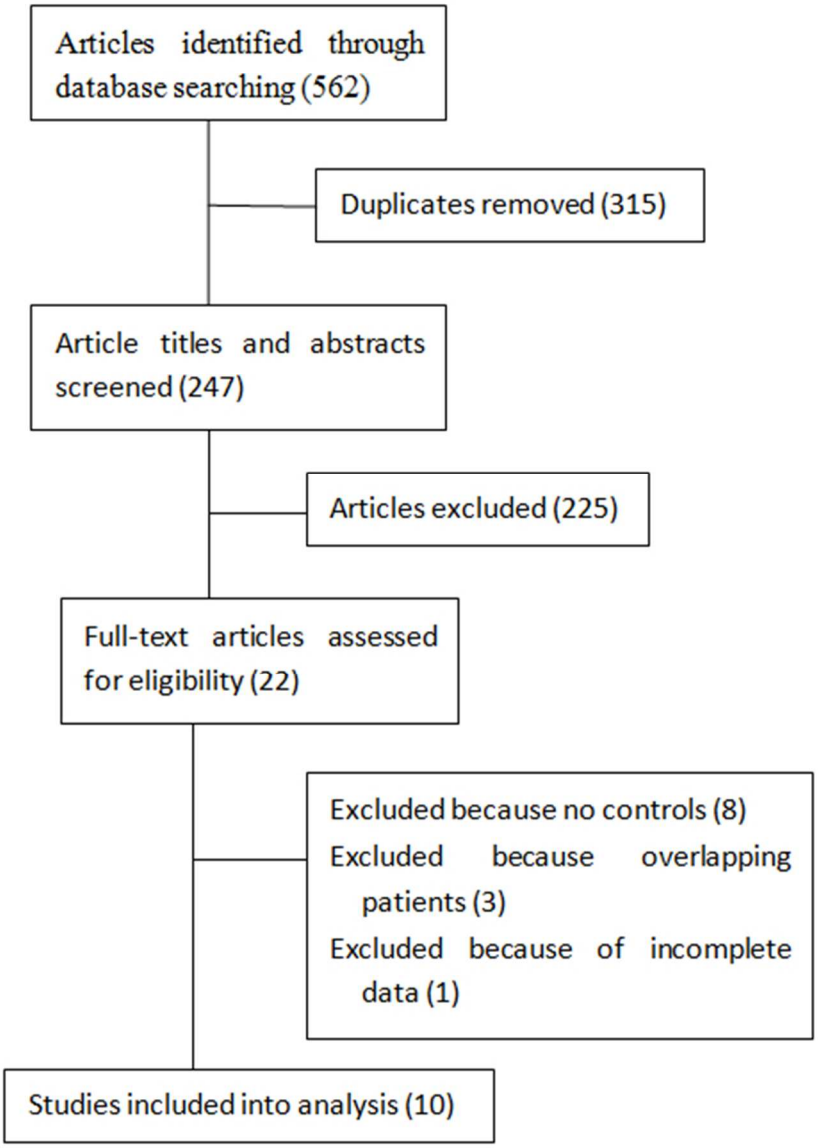
Comparison	Population	No. of Study	Test of association*			Model	Test of heterogeneity	
			OR	95%CI	*P*		*P*	I^2
COX-2 A-1195G (rs689466)								
G-allele vs.	Overall	8	0.87	0.75-1.02	0.09	R	0.007	64
A-allele	Chinese	5	**0.84**	**0.72-0.99**	**0.04**	R	0.02	65
	Non-Chinese	3	1.00	0.63-1.59	0.99	R	0.02	74
GG vs. GA	Overall	8	**0.72**	**0.57-0.92**	**0.008**	R	0.04	52
+ AA	Chinese	5	0.79	0.62-1.01	0.06	R	0.06	55
	Non-Chinese	3	**0.49**	**0.30-0.78**	**0.003**	F	0.66	0
GG vs. AA	Overall	8	**0.72**	**0.53-0.97**	**0.03**	R	0.02	57
	Chinese	5	**0.71**	**0.51-0.99**	**0.05**	R	0.02	66
	Non-Chinese	3	0.77	0.32-1.84	0.56	R	0.13	52
AA vs.	Overall	8	1.05	0.77-1.44	0.74	R	< 0.001	79
GA+GG	Chinese	5	1.23	0.98-1.55	0.07	R	0.06	57
	Non-Chinese	3	0.69	0.24-2.03	0.51	R	< 0.001	90
COX-2 G-765C (rs20417)								
C-allele vs.	Overall							
G-allele		6	1.32	0.76-2.30	0.33	R	< 0.001	88
CC vs.	Overall							
GC+GG		3	0.88	0.16-4.75	0.88	R	0.007	80
CC vs. GG	Overall	3	0.93	0.16-5.35	0.94	R	0.005	81
GG vs.	Overall							
CC+GC		6	0.48	0.14-1.59	0.23	R	< 0.001	97
COX-2 T+8473C (rs5275)								
C-allele vs.	Overall							
T-allele		3	0.99	0.86-1.14	0.91	F	0.67	0
CC vs. CT +	Overall							
TT		3	1.31	0.83-2.07	0.25	F	0.37	0
CC vs. TT	Overall	3	1.25	0.78-1.98	0.35	F	0.33	0
TT vs. CT +	Overall							
CC		3	1.05	0.89-1.24	0.58	F	0.57	0

Abbreviations: OR, odds ratio; CI, confidence interval; R, random-effect model; F, fixed-effect model.

*Mantel-Haenszel estimate was used to give a pooled odds ratio using the fixed -effect models, while DerSimonian-Laird estimate for random effect models.

Table 6 Ethnicity meta-regression and publication bias of COX-2 A-1195G polymorphisms and risk of hepatocellular carcinoma

Variables	Coef.	Std. Err.	z	P	95% confidence interval
Meta-regression					
G-allele vs. A-allele	-0.107	0.271	-0.40	0.693	-0.639-0.424
GG vs. GA + AA	0.520	0.435	1.20	0.232	-0.3321-1.373
GG vs. AA	0.217	0.574	0.38	0.706	-0.909-1.342
AA vs. GA+GG	0.282	0.561	0.50	0.616	-0.819-1.382
Publication bias by Egger's test					
G-allele vs. A-allele	-0.059	0.210	-0.28	0.788	-0.573-0.455
GG vs. GA + AA	0.148	0.196	0.75	0.481	-0.3332-0.628
GG vs. AA	-0.017	0.323	-0.05	0.959	-0.807-0.772
AA vs. GA+GG	0.416	0.485	0.86	0.423	-0.770-1.603



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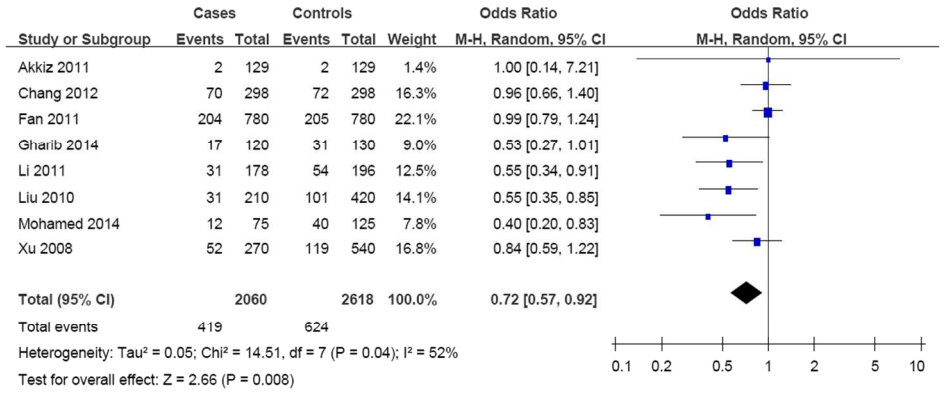

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a meta-analysis.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	n/a
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	4
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	4
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	5
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	5-6

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	6
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	6
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	7
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	7
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	7
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	8
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	8
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	9
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	10
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	11
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	1

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