Abstract: Hepatocellular carcinoma (HCC) is a heterogeneous disease with substantial genetic constitution. Previous work has evaluated the effect of prostaglandin-endoperoxide synthase 2 (PTGS2) variants (−765G/C, −1195A/G, and +8473T/C) on the development of HCC, but the conclusions are inconsistent. We conducted a meta-analysis in this work. Data from 7 case-control studies were combined to assess the association between PTGS2 variants and HCC. The risk of HCC (OR and 95% CI) was estimated using either the fixed- or the random-effects model according to the Q test. No significant association was identified for −765G/C and +8473T/C. However, we identified a significantly decreased risk in relation to the GG genotype of −1195A/G (OR = 0.70, 95% CI = 0.50–0.98 for GG versus AA). We also observed a similar decrease (OR = 0.47, 95% CI = 0.23–0.95 for GG versus AA) in Caucasian samples. Variant −1195A/G in the promoter PTGS2 may protect against the malignant progression of HCC. This significant association suggests that −1195A/G could be used as a biomarker of HCC.

(introduction)

Hepatitis virus infection, hepatitis B virus and hepatitis C virus in particular, is a known cause of hepatocellular carcinoma (HCC), an end-stage complication of fibrotic and chronic inflammatory liver disease. The interferon alpha treatment, however, is not effective for all patients. It works only among sustained responders by suppressing replication of hepatitis virus. There is wide difference in individual susceptibility toward HCC among hepatitis virus-infected populations, suggesting the possible role of genetic variation in the disease pathogenesis.

Prostaglandin-endoperoxide synthase 2 (PTGS2) is a pro-inflammatory enzyme induced by prostaglandins involved in cell proliferation, tumorigenesis, progression, and metastasis. The inducible enzyme enables the transformation of arachidonic acid into prostaglandins. The PTGS2 gene, also known as cyclooxygenase-2 (COX-2), is situated at human chromosome 1q25.2-q25.3. It is suggested that increased serum PTGS2 levels lead to upregulation of the prostaglandin EP1 receptor, which in turn decreases PTGS2 levels through certain pathways. The levels significantly increase in cancerous tissues and this increase may constitute a mechanism that facilitates carcinogenesis. In addition, cyclooxygenase inhibitors could down-regulate serum concentrations of potent angiogenic factors, thus suppressing angiogenesis and precluding tumor growth. Therefore, the PTGS2 gene may contribute to the progression of human cancer.

A case-control study of Chinese samples suggested that PTGS2 −765G/C and −1195A/G increase the genetic susceptibility to HCC, with −765GC and −1195AA associated with 2.89 and 1.57 times higher risk, respectively. Conversely, a decreased risk was detected for −765G/C and no association was found for −1195A/G and +8473T/C in Turkish samples. The controversial results are probably caused by sampling variance and population heterogeneity. In this study, meta-analysis was used to assess the association of the variants in the promoter region of the PTGS2 gene with HCC susceptibility.

METHODS

Literature Search Strategy, Inclusion and Exclusion Criteria, and Data Extraction

This study was approved by the Institutional Review Board of Shandong Provincial Hospital affiliated to Shandong University, Jinan, Shandong, China. Literature searches were performed through a 3-stage strategy to identify all potentially relevant papers. At stage 1, we systematically searched Embase, PubMed, Science Direct, and Wangfang databases, with the last search completed on November 16th, 2014. The key words including PTGS2, cyclooxygenase-2, HCC, liver cancer, and polymorphism were used. Two reviewers scanned the retrieved articles by title, abstract, and full texts whenever necessary to single out all case-control studies examining the association between one or more PTGS2 variants and HCC incidence. At stage 2, we checked the reference lists of each case-control study to gain new data. At stage 3, we contacted the corresponding author of an Italian study by e-mail, as the genetic data were incompletely reported in the original article.
The inclusion criteria designed for the human case–control studies included: used at least one of the PTGS2 variants to estimate the risk of HCC, clearly reported the genotype frequency, and the samples used in the study must be unique without any subsequent updates; if there were, the study with the largest sample size was considered in the final analysis. We excluded the studies for various reasons: only HCC patients were investigated, systematic reviews, animal studies, editorials, summary abstracts without complete data, and case reports.

Data on the following items were recorded for the case–control studies: surname of first author, journal and year of publication, study design, country of origin, ethnicity, proportion of men and women, selection of controls, methods used to determine the genotype of PTGS2 variants, genotyped cases and controls, and count of genotypes. Data were collected by 2 independent reviewers and subsequently checked by a third reviewer. In case of discrepancies, an expert in this filed was invited to make a final decision.

Quality Assessment

We evaluated Hardy-Weinberg equilibrium (HWE) in control populations using the X² test to assess the quality of the studies included in this meta-analysis. A P value lower than 0.05 indicated significant HWE deviation. The studies were categorized into the high-quality group when P > 0.05; otherwise, they were considered low-quality studies.

Statistical Analysis

The fixed-effect model proposed by Mantel and Haenszel and the random-effect model described by DerSimonian and Laird were properly used to estimate the risk of HCC (OR and 95% CI; odds ratio and 95% confidence interval). The former model was performed when the Chi-square based Q statistic test and I² statistic indicated no notable heterogeneity between studies (PQ-test > 0.05 and I² < 50%); otherwise, the latter model was used. Subgroup analyses were performed according to ethnicity for PTGS2 765G/C. Sensitivity analyses were used to check if the single studies had significant influence on the combined risk estimates. Publication bias was determined using the funnel plots and the Egger linear regression test. All 2-sided P values less than 0.05 were considered significant. Statistical analyses were done using the Stata software package (version 12.0; Stata Corporation, College Station, TX) and the meta package for R (version 3.0.3; the R Foundation for Statistical Computing, Tsukuba, Japan).

RESULTS

Characteristics of Studies

A total of 39 records were initially identified, as shown in Figure 1. We scanned all titles and abstracts, excluding 29 articles as a result of expression-based study, using an animal model to investigate the etiology of HCC or irrelevant human cancer research. We further examined eligibility of the remaining 10 full texts. Three studies were excluded, because 2 were subsequently updated and 1 did not provide sufficient genetic data and no reply was received after contacting the authors. The pooling dataset therefore comprised 7 studies. Of these, 5 studies analyzed –765G/C, 6 investigated –1195A/G, and 3 studied +8473T/C. For –765G/C, 2 studies were conducted in Caucasians and 3 in Asians, with Akkiz et al deviating from HWE. With respect to –1195A/G, there were 4 Asian

![FIGURE 1. Flow chart for primary selection in this meta-analysis.](Image)
| Reference                  | Region | Ethnicity | Gender (Men/Women) | Selection of Controls | Polymorphism Site | Genotyping Methods | Cases/Controls | Quality |
|----------------------------|--------|-----------|--------------------|-----------------------|-------------------|-------------------|----------------|---------|
| Xu et al, 2008<sup>15</sup> | China  | Asian     | 234 (86.67)/36 (13.33) 468 (86.67)/72 (13.33) | Age and sex-matched, population-based | −765G/C, −1195A/G | PCR-RFLP | 270/540 | High |
| Akkiz et al, 2011<sup>16</sup> | Turkey | Caucasian | 105 (81.4)/24 (18.6) 105 (81.4)/24 (18.6) | Age, sex, alcohol consumption, smoking-matched, hospital-based | −765G/C, −1195A/G, +8473T/C | PCR-RFLP | 129/129 | Low<sup>a</sup> |
| He et al, 2011<sup>26</sup> | China  | Asian     | 204 (68.0)/96 (32.0) 192 (64.0)/108 (36.0) | Age, sex, region-matched, hospital-based | −765G/C, | PCR-TaqMan | 300/300 | High |
| Chang et al, 2012<sup>27</sup> | China  | Asian     | 213 (71.5)/85 (28.5) 213 (71.5)/85 (28.5) | Age, sex, alcohol consumption, smoking-matched, hospital-based | −765G/C, −1195A/G, +8473T/C | PCR-RFLP | 298/298 | Low<sup>i</sup> |
| Gharib et al, 2014<sup>28</sup> | Egypt  | Caucasian | 84 (70.0)/36 (30.0) 80 (61.6)/50 (38.4) | Age and sex-matched, population-based | −765G/C, −1195A/G | PCR-RFLP | 120/130 | High |
| Liu et al, 2010<sup>29</sup> | China  | Asian     | 177 (84.3)/33 (15.7) 175 (83.3)/35 (16.7) | Population-based | −1195A/G, | PCR-RFLP | 210/210 | High |
| Fan et al, 2011<sup>30</sup> | China  | Asian     | 639 (81.9)/141 (18.1) 618 (79.2)/162 (20.8) | Age, sex, region-matched, hospital-based | −1195A/G, +8473T/C | PCR-TaqMan | 780/780 | High |

PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism.

*genotypic data for variant −765G/C were not in Hardy-Weinberg equilibrium (HWE).

*genotypic data for variant +8473T/C were not in HWE.
TABLE 2. Main Results of the Meta-Analysis

| Variables     | No. of Studies | OR (95% CI) | P (Q-test) | OR (95% CI) | P (Q-test) | OR (95% CI) | P (Q-test) | OR (95% CI) | P (Q-test) |
|---------------|----------------|-------------|------------|-------------|------------|-------------|------------|-------------|------------|
| −765G/C       | 7              | 0.71 (0.17, 2.95) | 0.06       | 0.71 (0.17, 2.95) | 0.06       | 0.71 (0.17, 2.95) | 0.06       | 0.71 (0.17, 2.95) | 0.06       |
| +8473T/C      | 9              | 1.25 (0.74, 2.11) | 0.16       | 1.25 (0.74, 2.11) | 0.16       | 1.25 (0.74, 2.11) | 0.16       | 1.25 (0.74, 2.11) | 0.16       |
| +1195A/G      | 6              | 0.70 (0.50, 0.98) | 0.03       | 0.70 (0.50, 0.98) | 0.03       | 0.70 (0.50, 0.98) | 0.03       | 0.70 (0.50, 0.98) | 0.03       |
| +8473T/C      | 9              | 1.25 (0.78, 0.98) | 0.32       | 1.25 (0.78, 0.98) | 0.32       | 1.25 (0.78, 0.98) | 0.32       | 1.25 (0.78, 0.98) | 0.32       |
| +8473C        | 2              | 0.75 (0.65, 0.86) | 0.02       | 0.75 (0.65, 0.86) | 0.02       | 0.75 (0.65, 0.86) | 0.02       | 0.75 (0.65, 0.86) | 0.02       |
| +8473C        | 2              | 0.47 (0.25, 0.90) | 0.40       | 0.47 (0.25, 0.90) | 0.40       | 0.47 (0.25, 0.90) | 0.40       | 0.47 (0.25, 0.90) | 0.40       |
| −1195A/G      | 6              | 1.38 (0.65, 2.94) | 0.14       | 1.38 (0.65, 2.94) | 0.14       | 1.38 (0.65, 2.94) | 0.14       | 1.38 (0.65, 2.94) | 0.14       |
| −1195A/G      | 6              | 0.56 (0.30, 1.04) | 0.54       | 0.56 (0.30, 1.04) | 0.54       | 0.56 (0.30, 1.04) | 0.54       | 0.56 (0.30, 1.04) | 0.54       |

* OR and 95% CI were calculated based on data from three studies, as CC genotype downregulates the expression of PTGS2.

BIAS DIAGNOSTICS

We assessed the publication bias for −765G/C and −1195A/G. Symmetrical distribution was observed in all funnel plots (P > 0.05). However, the Egger test demonstrated evidence of high possibility of publication bias in the GC versus GG model of −765G/C (P = 0.001, Figure 4). Figure 5 shows the funnel plot constructed for −1195A/G using the GG versus AA model (P = 0.811).

DISCUSSION

This study, for the first time to our knowledge, has examined the association between PTGS2 gene variants (−765G/C, −1195A/G, and +8473T/C) and HCC susceptibility by the use of meta-analysis. A total of 7 case–control studies were incorporated. Of the PTGS2 variants studied, only one, corresponding to −1195A/G, was found to be associated with HCC. We noted that the presence of a GG genotype may reduce the risk to develop HCC, especially in Caucasians. Although the statistical power is relatively stronger compared to the previously published studies, some findings should be interpreted with caution due to the large heterogeneity and significant publication bias.

Several groups have investigated the hypothesis of a relationship between the variants in the PTGS2 gene and HCC. The first case–control study was published by Xu et al in 2008. In this analysis, 270 HCC patients and 540 controls subjects of Chinese race were analyzed to evaluate the effects conferred by −765G/C and −1195A/G, which were reported to be associated with significantly increased risk of HCC (OR = 1.57 and 2.89, respectively). In the independent studies following Xu et al, some replicated the significant association, while others did not. For example, Liu and Lin investigated the variant −1195A/G only and found 1.38-fold increased risk among 420 Chinese samples (210 cases and 210 controls) with the −1195A allele. In contrast, Fan et al showed no evidence supporting that −1195A/G is a risk factor for HCC, even though they identified 50% decreased risk associated with +8473T/C among 780 cases and 780 controls of Chinese ancestry. Such controversial results were also indicated in Caucasian samples. The wide disparity in the reported results may result from certain aspects, including methodological errors (eg, selection bias, genotyping errors, and population stratification), different ethnic groups, and limited sample size. Other plausible factors, such as poor study design, may also contribute to the inconsistency.

In our study, we demonstrated that the PTGS2 promoter variants being investigated, with the possible exception of −1195A/G, were not associated with the risk of developing HCC. Our findings were in agreement with most published papers, including a recent meta-analysis by Bu et al. This meta-analysis evaluated the effects of −1195A/G only and identified a statistically significant increase in the risk of HCC (OR ranged from 1.26 to 1.45). We identified a new Caucasian study and found significant effects in these populations. In addition, we assessed the association for −765G/C and +8473T/C, showing no evidence of a major effect.

The results of the present analysis seem to contradict the previous experimental studies. Akkiz et al reported that −765CC genotype downregulates the expression of PTGS2; it is the decreased promoter activity that may represent an
important mechanism to explain the significant reduction in the risk of HCC. It is suggested that aberrant expression of the PTGS2 gene induced by the genetic variations in its promoter region would result in chronic immune activation and inflammation, uncontrolled cell growth, suppressed apoptosis, pre-cancerous lesions, and subsequent tumor formation.\(^{32,33}\) Therefore, the PTGS2 genetic variants are likely to modulate HCC predisposition through up- or downregulating the expression of the gene they are mapped on.

There are several strengths in the meta-analysis. The first strength refers to the comprehensive evaluation of the association between PTGS2 variants and HCC susceptibility. We included all available data for the commonly studied variants to provide compelling evidence for the associations that remain elusive. The second strength is that we identified no genetic contributions for \(-765G/C\) and \(+8473T/C\), which was not reported in the early meta-analysis. Nevertheless, there was some evidence of notable between-study heterogeneity, and publication bias possibly caused by the failure to include pertinent unpublished reports. Some of our findings may be affected. This is the first limitation that should be taken into consideration when interpreting the present results. Second, the sample size is too low for some variant sites and subgroups, leading to less definite conclusions for some findings. Third, as none of the included studies stated adjusted ORs, the genetic association of interest was evaluated using unadjusted ORs. Finally, both environmental and inherited genetic risk factors are involved in the etiology of HCC. Nonetheless, the effect of
gene–environment interactions was not assessed and this may finally influence the findings we demonstrated in the present study. These limitations emphasize the need for continued clinical and basic research to provide new evidence for the molecular mechanisms underlying HCC incidence.

In summary, our meta-analysis suggests that the development of HCC may be associated with PTGS2 variant –1195A/G, but not –765G/C or +8473T/C. A larger study which takes gene–environment interactions and role of confounding factors into account is expected to validate our findings and to determine the role of these variants in HCC pathogenesis.

REFERENCES

1. Schafer DF, Sorrell MF. Hepatocellular carcinoma. Lancet. 1999;353:1253–1257.
2. Parkin DM, Bray F, Ferlay J, et al. Global cancer statistics, 2002. CA Cancer J Clin. 2005;55:74–108.
3. Block TM, Mehta AS, Fimmel CJ, et al. Molecular viral oncology of hepatocellular carcinoma. Oncogene. 2003;22:5093–5107.
4. Schuppan D, Krebs A, Bauer M, et al. Hepatitis C and liver fibrosis. Cell Death Differ. 2003;10(Suppl 1):S59–S67.
5. Bruno S, Facciotto C. The natural course of HCV infection and the need for treatment. Ann Hepatol. 2008;7:114–119.
6. Wilt TJ, Shamliyan T, Shaukat A, et al. Management of chronic hepatitis B. Evid Rep Technol Assess (Full Rep). 2008;1:671.
7. Yu MW, Yeh SH, Chen PJ, et al. Hepatitis B virus genotype and DNA level and hepatocellular carcinoma: a prospective study in men. J Natl Cancer Inst. 2005;97:265–272.
8. Chen CJ, Yang HI, Su J, et al., Iloeje UH and Group R-HS. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA. 2006;295:65–73.
9. Missilia SB, Ostrowski M, Heathcote EJ. Disease progression in chronic hepatitis C: modifiable and nonmodifiable factors. Gastroenterology. 2008;134:1699–1714.
10. Falade-Nwulia O, Seagbe EC, Rinaldo CR, et al. Comparative risk of liver-related mortality from chronic hepatitis B versus chronic hepatitis C virus infection. Clin Infect Dis. 2012;55:507–513.
11. Hussain SP, Hofseth LJ, Harris CC. Radical causes of cancer. Nat Rev Cancer. 2003;3:276–285.
12. Sood R, Flint-Ashtamker G, Borenstein D, et al. Upregulation of prostaglandin receptor EP1 expression involves its association with cyclooxygenase-2. PLoS One. 2014;9:e91018.
13. Cao Y, Prescott SM. Many actions of cyclooxygenase-2 in cellular dynamics and in cancer. J Cell Physiol. 2002;190:279–286.
14. Sawaoka H, Tsuji S, Tsuji M, et al. Cyclooxygenase inhibitors suppress angiogenesis and reduce tumor growth in vivo. Lab Invest. 1999;79:1469–1477.
15. Xu DK ZX, Zhao P, Cai JC. Association between single nucleotide polymorphisms in promoter of COX-2 gene and hereditary susceptibility to hepatocellular carcinoma [article in Chinese]. Chin J Hepatobiliary Surg. 2008;14:840–843.
16. Akkiz H, Bayram S, Bekar A, et al. Functional polymorphisms of cyclooxygenase-2 gene and risk for hepatocellular carcinoma. Mol Cell Biochem. 2011;347:201–208.
17. Giacalone A, Montalto G, Giannitrapani L, et al. Association between single nucleotide polymorphisms in the cyclooxygenase-2, tumor necrosis factor-alpha, and vascular endothelial growth factor-A genes, and susceptibility to hepatocellular carcinoma. OMICS. 2011;15:193–196.
18. Salanti G, Amontunta G, Ntzani EE, et al. Hardy-Weinberg equilibrium in genetic association studies: an empirical evaluation of reporting, deviations, and power. Eur J Hum Genet. 2005;13:840–848.
19. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst. 1959;22:719–748.
20. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986;7:177–188.
21. Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. BMJ. 2003;327:557–560.
22. Munafò MR, Clark TG, Flint J. Assessing publication bias in genetic association studies: evidence from a recent meta-analysis. Psychiatry Res. 2004;129:39–44.
23. Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315:629–634.
24. Song X, Liu C, Liu LY, et al. Cyclooxygenase-2 polymorphisms and susceptibility to hepatocellular carcinoma. Pract J Cancer. 2011;26:255–258.
25. Mohamed FZ, Hussein YM, El-Deen IM, et al. Cyclooxygenase-2 single-nucleotide polymorphisms and hepatocellular carcinoma in Egypt. Mol Biol Rep. 2014;41:1461–1468.
26. He JH, Li YM, Zhang QB, et al. Cyclooxygenase-2 promoter polymorphism –996G/C is associated with hepatitis B-related liver cancer in a Chinese population of Gansu province. Chin Med J (Engl). 2011;124:4193–4197.
27. Chang WS, Yang MD, Tsai CW, et al. Association of cyclooxygenase 2 single-nucleotide polymorphisms and hepatocellular carcinoma in Taiwan. Chin J Physiol. 2012;55:1–7.
28. Gharib AF, Karam RA, Abd El Rahman TM, et al. COX-2 polymorphisms –765G→C and -1195A→G and hepatocellular carcinoma risk. Gene. 2014;543:234–236.
29. Liu LF, ZH, Liu 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. 2010;19:333–335.
30. Fan XJ, QX, Yu HP, Zeng XY. Association of COX-2 gene SNPs with the risk of hepatocellular carcinoma. Chin J Cancer Prev Treat. 2011;18:405–409.
31. Bu X, Zhao C. The association between cyclooxygenase-2 1195G/A polymorphism and hepatocellular carcinoma: evidence from a meta-analysis. Tumour Biol. 2013;34:1479–1484.
32. O’Byrne KJ, Dalgleish AG. Chronic immune activation and inflammation as the cause of malignancy. Br J Cancer. 2001;85:473–483.
33. Sui W, Zhang Y, Wang Z, et al. Antitumor effect of a selective COX-2 inhibitor, celecoxib, may be attributed to angiogenesis inhibition through modulating the PTEN/Pi3K/Akt/HIF-1 pathway in an H(2)(2) murine hepatocarcinoma model. Oncol Rep. 2014;31:2252–2260.