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The association between hepatitis B mutants and hepatocellular carcinoma

A meta-analysis

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Abstract

Background: More and more studies focus on the relationship between hepatitis B virus (HBV) basal core promoter/precore (BCP/PC) mutations, but it remains controvercial, we conducted a meta-analysis to investigate the features of hepatitis B virus basal core promoter/precore mutations on the progression of hepatocellular carcinoma (HCC).

Methods: A comprehensive search was conducted for articles published between January 1, 2005 and December 31, 2015 using the following databases: PubMed, Embase, Cochrane Library, Wanfang, and China National Knowledge Infrastructure. Medical subject heading terms were prioritized in setting the search strategy. Search terms included ("hepatitis B virus"), ("mutation or mutations or mutant"), and ("hepatocellular carcinoma" or "liver cancer" or hepatoma). A meta-analysis of pooled results from case–control studies examined the association between mutations G1896A, A1762T, G1764A, and A1762T/G1764A and the risk of HCC.

Results: We included 29 articles for analysis and found that G1896A (summary odds ratios [OR] = 2.04, 95% confidence interval [CI] = 1.41–2.95), A1762T (summary OR = 3.96, 95% CI = 1.98–7.92), G1764A (summary OR = 3.48, 95% CI = 1.99–6.09), and A1762T/G1764A (summary OR = 3.96, 95% CI = 2.77–5.65) are each associated with a statistically significant increase in the risk of HCC.

Conclusion: In summary, we found that G1896A, A1762T, G1764A, and A1762T/G1764A are associated with an increased risk of HCC.

Abbreviations: CHB = chronic hepatitis B, CI = confidence interval, HBeAg = hepatitis B E antigen, HBV = hepatitis B virus, HCC = hepatocellular carcinoma, NOS = Newcastle–Ottawa Scale, OR = odds ratios.

Keywords: hepatitis B virus, hepatocellular carcinoma, meta-analysis, mutant

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third cause of cancer mortality. Despite dramatic improvement in the treatment of chronic hepatitis B (CHB), HCC still remains a major cause of morbidity and mortality, contributing to approximately 350,000 deaths worldwide each year.[1] It has been estimated that 50% to 80% of these deaths are attributable to hepatitis B virus (HBV).[2]

The mechanism of HBV-related carcinoma involves several factors, and viral mutation plays an important role in the process of carcinoma development. It has been demonstrated that mutations in the HBV genome, which are important to the development of HCC, invariability occur in the basal core promoter (BCP), enhancer II, pre-S sequences, and precore region.[3–5] Accumulated evidence indicates that the most common mutations are a G to A substitution at nucleotide 1896 (G1896A) in the precore region, an A to T mutation at nucleotide 1762 (A1762T), a G to A mutation at nucleotide 1764 (G1764A), and the A1762T/G1764A double mutation in the BCP region.[6–8] These mutations may prevent the production of Hepatitis B E antigen (HBeAg) by introducing a premature stop codon into the open reading frame or may increase the transcription of pregenomic ribonucleicacid (RNA) by the removing of the nuclear receptor-binding motif, contributing to an inefficient immune response that ultimately leads to hepatocarcinogenesis.[9,10]

However, several published studies showed no significant association between these mutations and HCC.[11–14] And 1 study found no significant difference in BCP mutations between HCC and non-HCC patients with HBV genotype C, even though the mutant ratio increased with disease progression.[15]

There have been some meta-analyses investigating the relationship between mutations G1896A, A1762T, and

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G1764A and HCC, but the results were conflicting, and each of these studies was published before 2015.[3,5,16,17] Some studies have shown genotype to be a confounding factor, but only 1 meta-analysis completed a subgroup analysis by genotype.[5] Therefore, it is important to update these meta-analyses to provide a more comprehensive understanding of the relationship between these mutations and HCC. In this meta-analysis, we analyzed the relationship between the risk of developing HCC and mutations G1896A, A1762T, and G1764A, in addition to the A1762T/G1764A double mutation. To avoid the confounding effect of genotype, we also completed a subgroup analysis by genotype.

2. Materials and methods

2.1. Search strategy for original articles

The search was conducted for articles published between January 1, 2005 and December 31, 2015 using the following databases: PubMed, Embase, Cochrane Library, Wanfang, and China National Knowledge Infrastructure. Medical subject heading terms were prioritized in setting the search strategy. The search terms included ("hepatitis B virus"), ("mutation or mutations or mutant"), and ("hepatocellular carcinoma" or "liver cancer" or hepatoma). A detailed search strategy is included in the attached file. Besides, we examined the reference citations in the retrieved articles in an effort to identify additional eligible studies. A meta-analysis of the pooled results from case–control and cohort studies investigated the associations between mutation in A1762T, G1764A, and G1896A and the risk of HCC.

Two authors (FW and QZ) independently selected studies and discussed them with ML when inconsistencies were found. Articles that satisfied the following criteria were included: (1) case–control or cohort studies, (2) HCC and control subjects (CHB patients), (3) BCP A1762T, G1764A, and A1762T/G1764A double mutations (for HBV mutation), and precore G1896A, (4) HCC outcomes, and (5) available full texts. In addition, if the study duration and source of population recruitment overlapped greatly in 2 or more papers by the same authors, we only included the study with the largest number of HCC patients. The following exclusion criteria were applied: (1) studies including patients coinfected with hepatitis A, C, D, E virus or human immune deficiency virus, or patients with alcohol-related liver disease, (2) studies including patients coinfected with immune diseases, and (3) Newcastle–Ottawa Scale (NOS) score less than 5. See Fig. 1 for a detailed flow diagram describing the screening process according to the PRISMA 2009 Statement.

2.2. Data extraction

Data were independently extracted by 2 investigators (FW and QZ). The following information was abstracted from each included publication: first author, publication year, country or

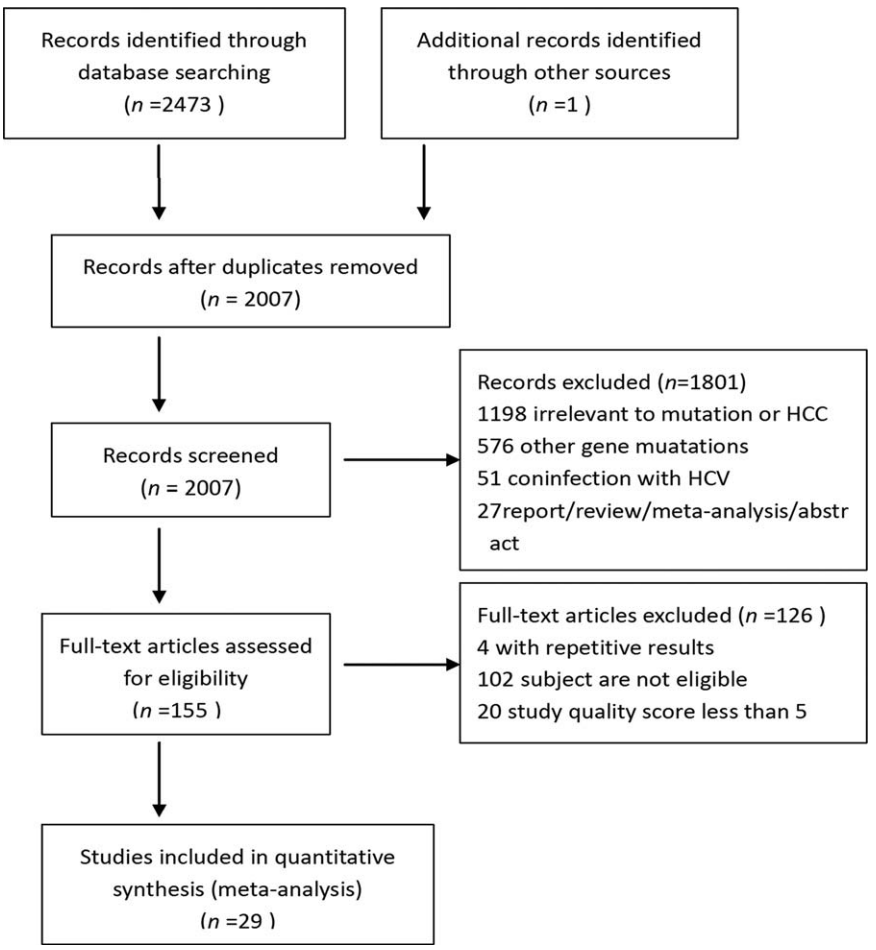

Figure 1. Flowchart for article screening in the meta-analysis.

Table 1

Modified NOS for the assessment of the study quality.

Facet	Item	Details
Selection	(1) Is the case definition adequate?	(a) Yes, with independent validation ◆ (b) Yes, e.g., record linkage or based on self reports (c) No description
	(2) Representativeness of the cases	(a) Consecutive or obviously representative series of cases ◆ (b) Potential for selection biases or not stated
	(3) Selection of controls	(a) Community controls ◆ (b) Hospital controls (c) No description
	(4) Definition of controls	(a) No history of disease (endpoint) ◆ (b) No description of source
	(5) Number of case subject	(a) ≥50 ◆ (b) <50
Comparability	(6) Comparability of cases and controls on the basis of the design or analysis	(a) Study controls for ———— (select the most important factor) ◆ (b) Study controls for any additional factor (this criteria could be modified to indicate specific control for a second important factor.) ◆
Exposure	(7) Ascertainment of exposure	(a) Secure record (e.g., surgical records) ◆ (b) Written self-report or medical record only (c) No description
	(8) Same method of ascertainment for cases and controls	(a) Yes ◆ (b) No
	(9) Mutation detection method	(a) DNA sequence or line probe assay ◆ (b) RFLP, PAGE, self-made chips, microwell hybridization, and direct electrophoresis

area of the sample, source (hospital or community-based), mean age, sex, number of included patients, and controls, and the quality score of the study.

2.3. Assessment of study quality

Two investigators (FW and QZ) independently rated the quality of each retrieved study using a modified NOS for case–control studies. We modified the scale to fit our study by removing the exposure (structured interview where blind to case/control status) and nonresponse rate criteria and adding number of case subjects and mutation detection. The modified NOS is a 10-point scoring system based on 9 items, see Table 1. Higher scores indicate increasing study quality; studies with a score of 8 or higher were classified as high-quality studies, those with a score of 5 to 7 were classified as medium-quality studies, and those with an overall score of 4 or less were classified as low-quality studies (for the purposes of this analysis) and excluded. Discrepancies were resolved by discussion with a third investigator (ML).

2.4. Statistical analysis

The effect measures of interest were odds ratios (OR) and the corresponding 95% confidence intervals (CIs) of case–control studies. The heterogeneity of the included articles was evaluated using the I^2 statistics and P value. If the value of I^2 was less than 50% and the P value was more than 0.1, a fixed-effects model was employed, otherwise, a random-effects model was used.[18] The Galbraith plot was used to determine the main sources of heterogeneity.[19] Publication bias was evaluated using funnel plots and Egger test. A P value less than 0.1 was assumed to indicate statistically significant publication bias.[20] All statistical analyses were performed using R (version 3.3.0, R Foundation for Statistical Computing, Beijing, China), and all the tests were 2-sided.

2.5. Ethics statement

Ethical approval was not required for this meta-analysis since participants have not been affected directly.

3. Results

3.1. Study selection and characteristics of the studies included in the meta-analysis

We identified 2474 potentially relevant articles but excluded 2445 articles, leaving 29 case–control articles for analysis.[2,4,9,14,21–45] A summary of the 29 included studies is given in Table 2.

3.2. Mutations and HCC risk

For the mutation G1896A, 18 studies were included in this meta-analysis. The random-effects model was used, because the heterogeneity existed among the included studies ($I^2=80.2\%$, $P<.001$). Significant correlation was found between the mutation G1896A, a G to A substitution at nucleotide 1896, and the occurrence of HCC. G1896A increases the risk of HCC (summary OR = 2.04, 95% CI = 1.41–2.95), see Fig. 2A.

For the mutation A1762T, 10 studies were included in this meta-analysis. The random-effects model was used, because the heterogeneity existed among the included studies ($I^2=83.5\%$, $P<.001$). Significant correlation was found between A1762T, an A to T mutation at nucleotide 1762, and the occurrence of HCC. A1762T increases the risk of HCC (summary OR = 3.96, 95% CI = 1.98–7.92), see Fig. 2B.

For the mutation G1764A, 10 studies were included in the meta-analysis. The random-effects model was used, because the heterogeneity existed among the included studies ($I^2=68.5\%$, $P<.001$). Significant correlation was found between G1764A, a G to A mutation at nucleotide 1764, and the occurrence of HCC.

Table 2

A summary of the 29 included studies.

No.	First author, y	Country or area	Source	Mean age, y	Male, %	No. of case/control	Quality score
1	Asim, 2010	India	Hospital and Medical College	53.6	89.3	150/136	7
2	Bahramali, 2008	Iran	One hospital	37.3	79	7/30	6
3	Bai, 2011	China	Two hospital	47.9	85.5	152/136	8
4	Chen, 2006	Taiwan	One hospital	45.2	88.0	50/38	8
5	Chen, 2008	Taiwan	One hospital	50.7	82.5	80/160	9
6	Ding, 2007	China	Community-based	–	–	42/158	8
7	Gao, 2015	China	One hospital	46.6	–	23/40	6
8	Guo, 2008	China	Community-based	39.9	94.8	58/71	9
9	Jang, 2012	Korea	Two hospital	52.9	78.7	75/75	8
10	Jin, 2012	China	Two hospital	62.4	90	60/60	7
11	Khan, 2013	Saudi Arabia	Different hospitals	68	83.6	55/127	6
12	Kusakabe, 2011	Japan	Six public health center areas	58.8	85	13/466	8
13	Lee, 2012	Korean	One hospital	44.3	83	135/135	8
14	Li, 2013	China	One hospital	–	–	102/105	9
15	Lin, 2005	Taiwan	One hospital	58	81.2	32/49	7
16	Liu, 2006	Taiwan	One hospital	54.0	83.5	200/160	7
17	Lyu, 2013	Korea	One center	55	80.8	318/234	7
18	Qu, 2014	China	Community-based	44	100	152/131	10
19	Shan, 2014	China	Five hospitals	49.2	82.8	88/104	8
20	Tanaka, 2006	Japan; HK	Two country	53.9	83.8	148/180	8
21	Tangkijvanich, 2010	Thailand	One hospital	55.7	86.7	60/60	9
22	Tong, 2007	Taiwan	One center	53.3	83.2	101/67	6
23	Utama, 2009	Indonesia	Five hospital	49.6	89.6	48/61	7
24	Wang, 2015	China	One hospital	50.5	62.6	41/75	6
25	Wei, 2013	China	One hospital	58.5	75.3	52/25	7
26	Xu, 2010	China	One hospital	41	90.0	60/120	9
27	Zheng, 2011	China	Twelve hospitals	50.7	83.3	156/185	8
28	Zhou, 2012	China	One hospital	–	–	85/157	6
29	Zhu, 2010	China	One hospital	43.6	–	20/35	7

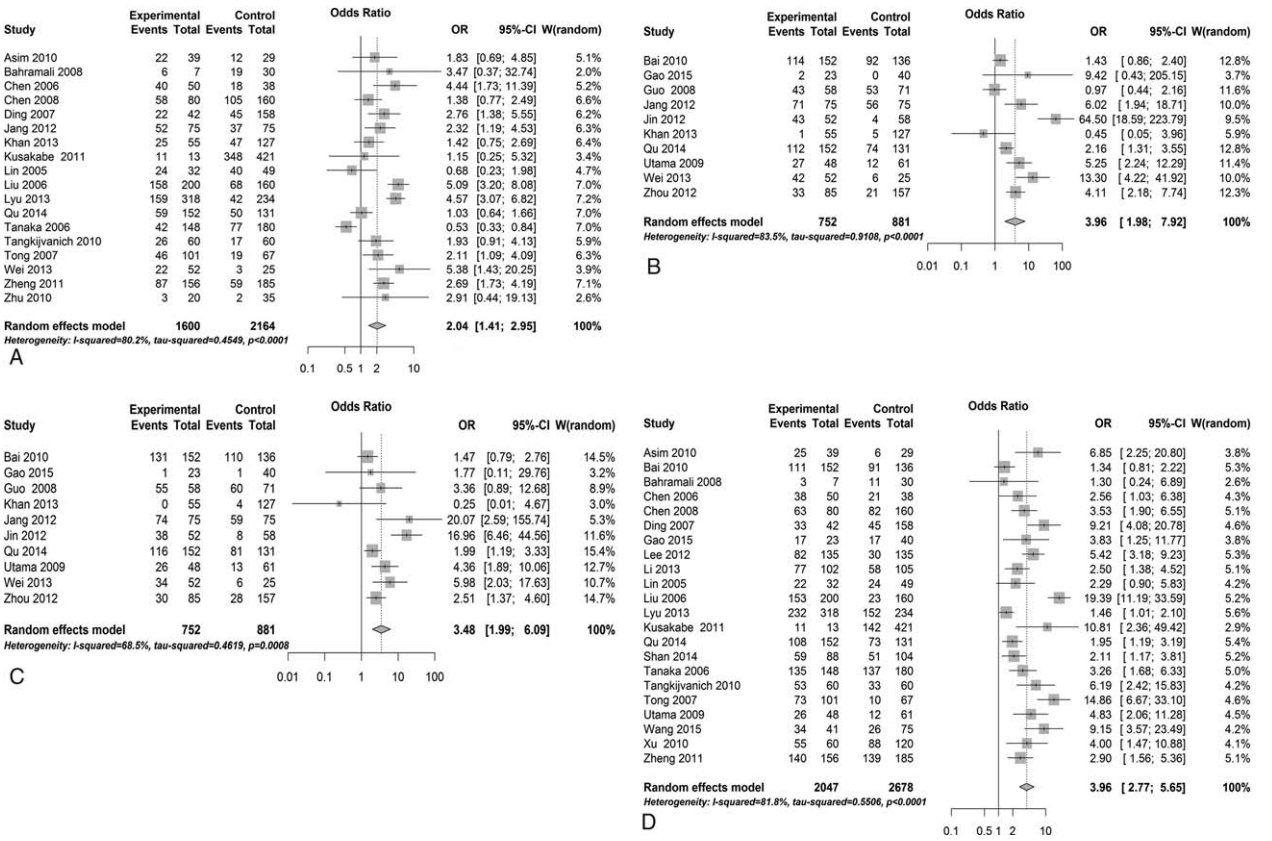

Figure 2. (A) Odds ratios (OR) of hepatocellular carcinoma (HCC) for G1896A. (B) OR of HCC for A1762T. (C) OR of HCC for G1764A. (D) OR of HCC for A1762T/G1764A double mutation.

G1764A increases the risk of HCC (summary OR = 3.48, 95% CI = 1.99–6.09), see Fig. 2C.

For the double mutation A1762T/G1764A, 22 studies were included in the meta-analysis. The random-effects model was used, because the heterogeneity existed among the included studies ($I^2 = 81.8\%$, $P < .001$). Significant correlation was found between A1762T/G1764A and the occurrence of HCC, the double mutation increases the risk of HCC (summary OR = 3.96, 95% CI = 2.77–5.65), see Fig. 2D.

To identify the source of heterogeneity, we used the Galbraith plot. We observed 4, 4, 3, and 6 outliers in the above 4 mutations, which were the main sources of heterogeneity, see Fig. 3. We then

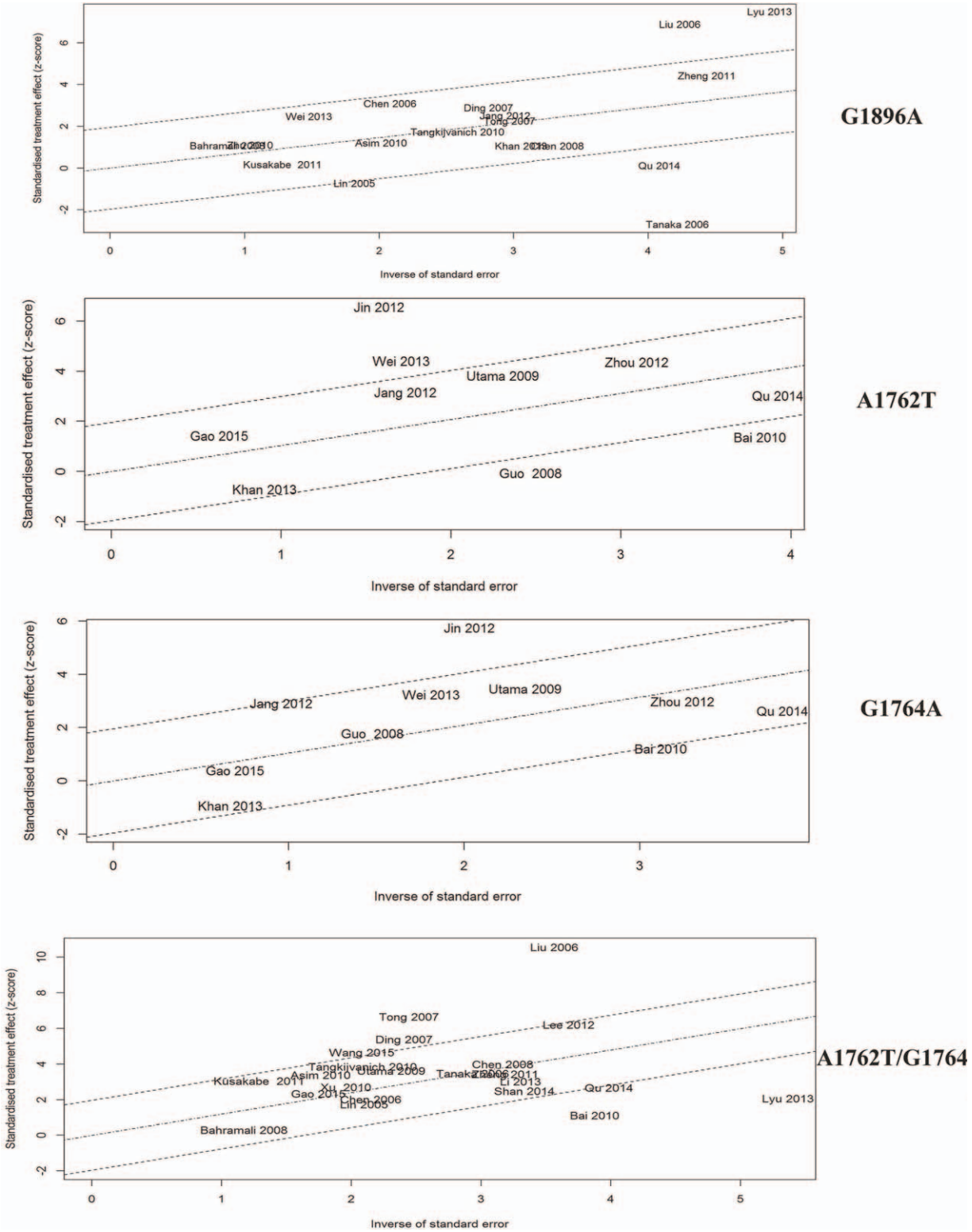

Figure 3. Galbraith plots for heterogeneity test of G1896A, A1762T, G1764A, and A1762T/G1764A double mutation.

Table 3

Subgroup analysis by genotype.

Mutation	Genotype	No. of study	n case/n control	OR (95% CI)
G1896A	A	0		
	B	1	218/251	1.38 (0.07, 26.21)
	C	6	737/819	1.76 (0.74, 4.23)
	D	2	52/137	1.74 (0.90, 3.38)
A1762T	A	0		
	B	1	85/157	4.11 (2.18, 7.74)
	C	2	127/100	8.52 (3.76, 19.30)
	D	1	45/107	0.46 (0.05, 4.08)
G1764A	A	0		
	B	1	85/157	2.51 (1.37, 4.6)
	C	2	127/100	9.07 (3.53, 23.27)
	D	1	45/107	0.25 (0.01, 4.79)
A1762T/G1764A	A	1	17/26	8.55 (0.83, 189.83)
	B	4	178/441	13.94 (8.28, 23.48)
	C	8	828/969	4.19 (2.23, 7.89)
	D	2	78/105	3.93 (1.75, 8.80)

CI = confidence interval, OR = odds ratios.

omitted those studies and recalculated each correlation using a fixed-effects model. Even with statistical heterogeneity eliminated, significant correlations were still found, and the above mutations were again shown to increase the risk of HCC ($OR_{adjusted\ G1896A}=2.10$, 95% CI=1.71–2.59, $I^2=12.9\%$, $P_{heterogeneity}=.312$; $OR_{adjusted\ A1762T}=3.16$, 95% CI=2.28–4.37, $I^2=45.0\%$, $P_{heterogeneity}=.106$; $OR_{adjusted\ G1764A}=2.60$, 95% CI=1.90–3.57, $I^2=19.9\%$, $P_{heterogeneity}=.278$; $OR_{adjusted\ A1762T/G1764A}=3.22$, 95% CI=2.68–3.86, $I^2=23.6\%$, $P_{heterogeneity}=.186$).

In the subgroup analysis by genotype, a single study indicated that in patients with genotype A HBV infection, there was no statistically significant correlation between the risk of HCC and A1762T/G1764A. For patients with genotype B or C HBV infection, the risk of HCC was associated with A1762T, G1764A, and A1762T/G1764A. These mutations increase the risk of HCC. For genotype D, only the double mutation, A1762T/G1764A, increases the risk of HCC, see Table 3.

3.3. Publication bias

We found no existent bias via application of Egger test ($t_{G1896A}=0.14$, $P=.889$; $t_{A1762T}=1.34$, $P=.217$; $t_{G1764A}=0.86$, $P=.413$; $t_{A1762T/G1764A}=1.64$, $P=.101$). The funnel plot appeared symmetrical as well, see Fig. 4.

4. Discussion

This meta-analysis shows that the mutations G1896A, A1762T, G1764A, and A1762T/G1764A are each associated with a statistically significant increase in the risk of HCC. Therefore, these mutations may have utility as potential biomarkers for predicting the occurrence of HCC and provide patients at high-

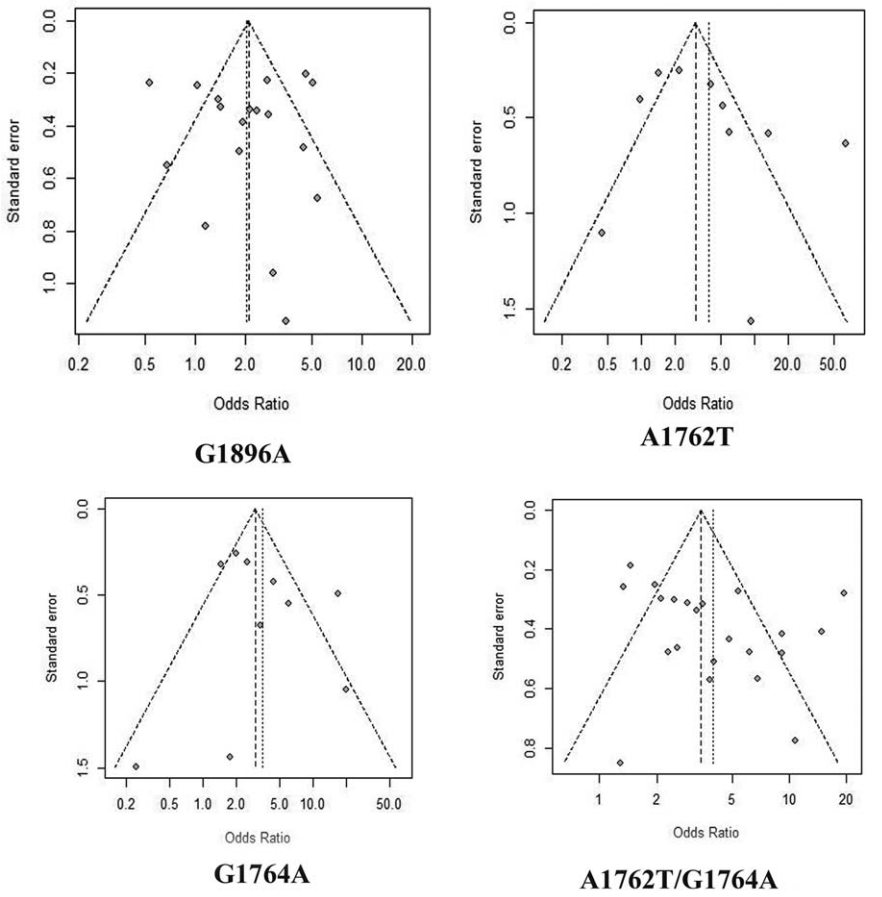

Figure 4. Funnel plots.

risk for developing the disease with the benefits of early diagnosis and treatment.

The contribution of HBV to the pathogenesis of HCC is complex, especially in light of the identification of mutant variants of HBV that modulate carcinogenesis. Mutation G1896A may suppress the expression of HBeAg by inducing a stop codon. However, HBV DNA would still be synthesized, contributing to the progression of liver disease.[9] The overall results confirm this supposition, and the overall risk was higher than Liao et al[3] conclusion (OR = 1.458, $P < .05$) and differed from Yang et al[17] (OR = 0.77, no statistically significant association) and Liu et al.[5]

The A1762G/G1764A mutation increased transcription of pregenomic RNA by the removing of the nuclear receptor-binding motif, thus creating a binding site for hepatocyte nuclear factor.[10] The double mutation also affected the amino acid sequence of *HBV X* genes, upregulating Skp2 and down-regulating p21. The combination of these changes may contribute to the suppression of precore mRNA, and increase expression in pgRNA transcription, resulting in an increase in viral replication, which may eventually lead to HCC.[2,46] The results support the conclusion that each of these mutations (A1762T, G1764A, and A1762T/G1764A) plays a significant role in the progression of chronic HBV infection to HCC.[38,43,47,48] The risk of HCC associated with the double mutation A1762T/G1764A was similar to those described in previous meta-analyses.[5,17] The data show a similar risk of HCC associated with A1762T, G1764A, and A1762T/G1764A with OR of 3.96, 3.48, and 3.96, respectively. This may indicate that any 1 site of mutation of A1762T or G1764A constitutes a danger signal. Moreover, this result is different from Liu et al[5] conclusion that combined mutations increase the risk of HCC more than a single mutation.

In the subgroup analysis by genotype, we found something interesting. First, although the correlation between the mutation G1896A and HCC was statistically significant, the correlations between the risk of HCC and the mutation G1896A for genotypes B, C, and D were not. This mirrors the findings of Liao et al[3] study. One possible explanation is that there were not enough studies with subgroup analyses by genotype. Indeed, there were only 1, 6, and 2 studies with a subgroup analysis of genotypes B, C, and D, respectively. Genotype A may in fact be the most susceptible to HCC, but there were no studies with a subgroup analysis of genotype A alone. For patients with A1762T or G1764A, genotype C was most susceptible to HCC, while genotype B was most susceptible to HCC in patients with the double mutation A1762T/G1764A. In contrast, Liao et al[3] study concluded that genotype C was the most susceptible to HCC in patients with A1762T/G1764A. As there were so few studies with a subgroup analysis of genotype A for patients with A1762T, G1764A, or A1762T/G1764A, it was not possible to make a comparison of the risk with other genotypes. Nevertheless, the results of subgroup analysis remind us that it is important to assess the risk of HCC by genotype. We recommend that future studies stratify patients on the basis genotype and specifically recruit those with genotype A HBV infection.

Our study had 3 limitations. First, the majority of the included in our meta-analysis were conducted in Eastern Asia, so population bias cannot be avoided. Second, we only analyzed articles that published in English or Chinese. We did not include articles published in other languages due to the impracticability of getting accurate medical translation, it may ignore the potential high-quality studies published in other languages. Third, in the subgroup analysis by genotype, only limited studies were available; thus, the results seemed insufficient.

Despite these limitations, we find that mutations G1896A, A1762T, G1764A, and A1762T/G1764A are associated with a higher risk of HCC. Frequent examination of HBV patients for these mutations should be helpful in predicting the occurrence of HCC. Future research should focus on other regions and ethnic groups for the detection of HBV mutations for predicting the occurrence of HCC. And it is important to stratify patients on the genotype when studying the relationship between the mutations and the HCC.

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