REVIEW

Meta-analysis of associations of interleukin-28B polymorphisms rs8099917 and rs12979860 with development of hepatitis virus-related hepatocellular carcinoma

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Background: This meta-analysis aimed to assess available evidence on possible associations of interleukin-28B polymorphisms rs8099917 and rs12979860 with development of hepatitis virus-related hepatocellular carcinoma (HCC).

Methods: PubMed, EMBASE, Google Scholar, and the Chinese National Knowledge Infrastructure databases were systematically searched to identify relevant studies. Meta-analyses were performed to examine the association of interleukin-28B rs8099917 G/T and rs12979860 T/C polymorphisms with development of hepatitis virus-related HCC. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated.

Results: A total of ten studies involving 2,529 cases and 2,412 controls were included. The G-allele and GT genotype of rs8099917 were significantly associated with increased risk of hepatitis B virus (HBV)-related HCC (allelic model, OR 1.49, 95% CI 1.13–1.96, P=0.005; heterozygous model, OR 1.39, 95% CI 1.04–1.88, P=0.03). Conversely, the TT genotype was found to be significantly associated with lower risk of HBV-related HCC (dominant model, OR 0.68, 95% CI 0.51–0.91, P=0.01). Similar results were observed in the subgroup of Chinese patients and controls. In the pooled data set, the T-allele and TT genotype of rs12979860 showed a significant association with increased HCC risk (allelic model, OR 1.36, 95% CI 1.05–1.78, P=0.02; recessive model, OR 1.75, 95% CI 1.28–2.39, P=0.005; homozygous model, OR 1.99, 95% CI 1.41–2.80, P<0.001). Subgroup analysis based on ethnicity and etiology showed rs12979860 polymorphism to be significantly associated with HCC risk in Caucasians, especially hepatitis C virus-related HCC, according to all five genetic models. In contrast, only the TT genotype of rs12979860 was found to be significantly associated with increased risk of HBV-related HCC, especially in Asians.

Conclusion: The G-allele of rs8099917 may confer elevated risk of HBV-related HCC, while the wild-type TT genotype may protect against the disease. The T-allele of rs12979860 may increase the risk of HCC, in Caucasians, especially hepatitis C virus-related HCC. The TT genotype of rs12979860 may confer increased risk of HBV-related HCC, especially in Asians. These conclusions should be verified in large, well-designed studies.

Keywords: interleukin-28B, polymorphisms, hepatitis virus-related hepatocellular carcinoma, meta-analysis

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common causes of cancer-related mortality worldwide.[1] It is well known that infection with hepatitis B virus (HBV)

3249

or hepatitis C virus (HCV) is a major cause of chronic hepatitis, which can progress to HCC.[2] Since only a fraction of infected patients develop HCC during their lifetime, genetic factors may play a role in tumor development.

Interferon (IFN) treatment is usually the preferred approach for eliminating HBV and HCV in chronic hepatitis patients and thereby inhibiting HCC progression.[3,4] IFN-α, IFN-β, and IFN-λ are multifunctional cytokines responsible for transcription of subsets of genes, and they play a crucial role in antiviral immune responses involving activation of the JAKSTAT and MAPK pathways.[5,6] IFN-λ shows both antiviral and antitumor activities in experimental models: it has been found to inhibit HBV and HCV replication,[7] while activation of type III IFN group which consists of IFN-λ1, IFN-λ2, and IFN-λ3 induces apoptosis.[8]

Genome-wide association studies have shown that polymorphisms near the interleukin-28B (IL28B) gene on chromosome 19, which codes for IFN-λ3, are powerful predictors not only of sustained virologic response to combination therapy with Pegylated interferon (PEG-IFN) and ribavirin but also of spontaneous HCV clearance in patients with chronic hepatitis C infection.[9,10] Some studies have also suggested that IL28B polymorphisms are related to serological response to PEGylated IFN and to HCV recovery in patients with chronic hepatitis B infection, and that these relationships to HCV recovery are the opposite of those observed with HBV recovery.[11,12]

Since IL28B polymorphisms affect responses to IFN, and since IFN therapy affects the prognosis of patients chronically infected with HBV or HCV, such polymorphisms may also affect the risk of developing HCC. Thus, several case–control studies have focused on the association of two common polymorphisms near the IL28B gene, rs8099917 and rs12979860, with onset of hepatitis virus-related HCC.[13–22] These studies have not provided a clear answer because of limited sample size and heterogeneity in patient ethnicity and HCC etiology.

Therefore, we conducted the present meta-analysis of available data to gain a more reliable picture of associations of polymorphisms rs8099917 and rs12979860 with HCC risk. We also meta-analyzed the data according to whether the patients had HBV- or HCV-related HCC. To the best of our knowledge, this is the first meta-analysis to analyze association of the rs12979860 polymorphism with HCC risk in subgroups of patients with HBV- or HCV-related disease.

Methods
Search strategy
PubMed, EMBASE, Google Scholar, and the Chinese National Knowledge Infrastructure (CNKI) databases were systematically searched to identify clinical and experimental case–control studies of possible associations of IL28B polymorphisms rs8099917 or rs12979860 with risk of hepatitis virus-related HCC published through January 15, 2016. No language restrictions were imposed. The following search terms were used: rs8099917; rs12979860; these two terms in combination with polymorphism, polymorphisms, SNP, variant, variants, variation, genotype, genetic or mutation; and all the earlier terms in combination with hepatocellular carcinoma and liver cancer. Reference lists in identified articles and reviews were also searched manually to identify additional eligible studies.

Inclusion criteria
To be included in our review and meta-analysis, studies should 1) have used a case–control design to assess the association of rs8099917 or rs12979860 polymorphisms with HCC risk in humans, 2) be available as full-text articles and report sufficient data for estimating an odds ratio (OR) with 95% confidence interval (CI), and 3) report genotype frequencies. If multiple publications were identified from the same research group and apparently involved the same population, only the most recent study was used in the meta-analysis.

Data extraction
Two authors (YZ and SLZ) independently extracted the following data from included studies: first author's family name, year of publication, country of origin, testing methods, P for Hardy–Weinberg equilibrium in controls, type of control population, numbers of cases and controls, and frequencies of genotypes in cases and controls. Discrepancies were resolved by consensus. Only those studies that met the predetermined inclusion criteria were included.

Statistical analysis
Following previous approaches,[23–25] we calculated the unadjusted OR with 95% CI from the genotype frequencies in cases and controls in order to assess the strength of association of rs8099917 and rs12979860 polymorphisms with HCC risk. The significance of pooled ORs was determined using the Z test, with $P < 0.05$ defined as the significance threshold. Meta-analysis was conducted using a fixed-effect model when P was > 0.10 for the Q test, indicating lack of heterogeneity among studies; otherwise, a random-effect model was used. All statistical tests were performed using Review Manager 5.2 (Cochrane Collaboration, London, UK).

Publication bias was assessed using Begg's funnel plot and Egger's weighted regression, generated using Stata 12.0 (Stata Corp, College Station, TX, USA). $P<0.05$ was considered statistically significant.

Results

Description of studies

Figure 1 illustrates the search and selection criteria for studies in the meta-analysis. A total of 368 potentially relevant publications up to January 15, 2016 were systematically identified in the PubMed, EMBASE, Google Scholar, and CNKI databases. Of these, we excluded 349 based on review of the titles and abstracts. The study by He et al was excluded because it was a meta-analysis,[26] and three studies were excluded because they did not have a case–control design.[27–29] The article by Shi et al was excluded because it did not report precise genotypes,[30] and the article by Lee et al was excluded because it did not report usable data.[31] Three articles investigating the association between IL28B polymorphism and the combination of HCC and liver cirrhosis were excluded.[32–34]

In the end, ten studies[13–22] were included in the meta-analysis (Table 1). Five studies[13–17] were conducted in Asian populations (1,041 cases and 907 controls) and evaluated the association between rs8099917 polymorphism and HCC risk. Nine studies,[14–22] involving 1,503 cases and 1,505 controls, evaluated the association between rs12979860 polymorphism

and HCC risk. The distribution of genotypes in controls was consistent with Hardy–Weinberg equilibrium ($P>0.05$) in all but two studies.

Meta-analysis of the association between rs8099917 polymorphism and HCC risk

Meta-analyses of the possible association between rs8099917 polymorphism and risk of HCC are summarized in Table 1; the overall results are summarized in Table 2. All 1,041 cases in the five studies examining rs8099917 polymorphism[13–17] had HBV-related HCC. Fixed-effect meta-analysis of the total population showed the rs8099917 polymorphism to be significantly associated with increased risk of HBV-related HCC according to the allelic model (OR 1.49, 95% CI 1.13–1.96, $P=0.005$; Figure 2) and heterozygous model (OR 1.39, 95% CI 1.04–1.88, $P=0.03$). These results suggest that the G-allele of rs8099917 may increase susceptibility to HBV-related HCC. In contrast, fixed-effect meta-analysis using the dominant model suggested a protective role of the wild-type TT genotype of rs8099917 in patients with HBV-related HCC (OR 0.68, 95% CI 0.51–0.91, $P=0.01$).

Similar results, with even higher statistical significance, were observed when we meta-analyzed the subgroup of studies involving Chinese populations (958 cases and 743 controls): the OR and 95% CI were 1.61 and 1.19–2.18 for the allelic model ($P=0.002$), 1.54 and 1.07–2.22 for the heterozygous
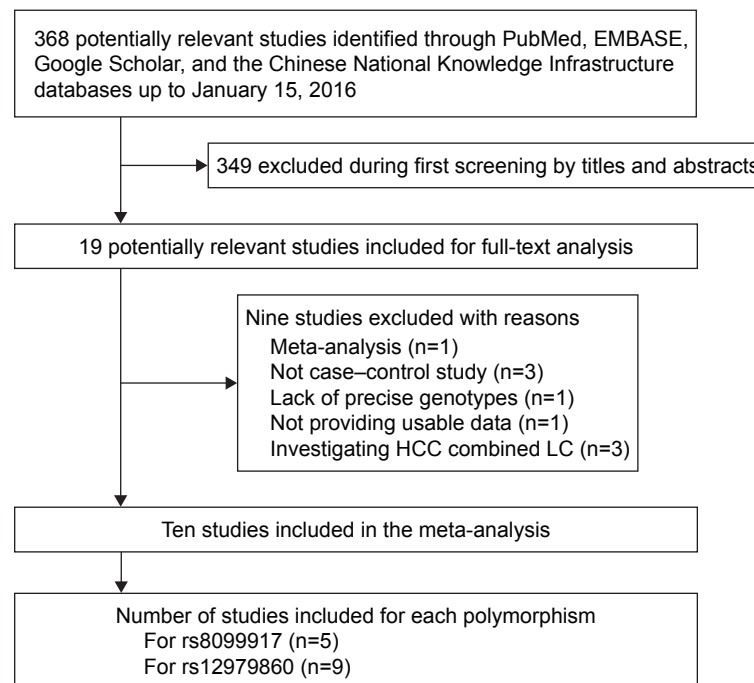
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│ 368 potentially relevant studies identified through PubMed,   │
│ EMBASE, Google Scholar, and the Chinese National Knowledge    │
│ Infrastructure databases up to January 15, 2016               │
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                │      │ abstracts                                              │
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│ 19 potentially relevant studies included for full-text analysis│
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                │      │   Meta-analysis (n=1)                                  │
                │      │   Not case–control study (n=3)                         │
                │      │   Lack of precise genotypes (n=1)                      │
                │      │   Not providing usable data (n=1)                      │
                │      │   Investigating HCC combined LC (n=3)                  │
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│ Number of studies included for each polymorphism              │
│   For rs8099917 (n=5)                                         │
│   For rs12979860 (n=9)                                        │
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Figure 1 Flowchart of study inclusion.
Abbreviations: HCC, hepatocellular carcinoma; LC, liver cirrhosis.

Table 1 Characteristics of studies in the meta-analysis

Author	Year	Country	Methods	P (HWE)	Type of control group	Cases/controls	Case genotype			Control genotype		
rs8099917 (1,041 cases and 907 controls)							**TT**	**GT**	**GG**	**TT**	**GT**	**GG**
Jiao et al[13]	2011	People's Republic of China	PCR	0.602	Healthy	99/144	87	12	0	132	12	0
Ren et al[14]	2012	People's Republic of China	TaqMan	0.001	Healthy	154/47	127	21	6	44	2	1
Chen et al[15]	2012	People's Republic of China	Real-time PCR	0.757	Healthy	406/244	356	48	2	218	25	1
Wang et al[16]	2012	People's Republic of China	PCR	0.458	Healthy	299/308	255	41	3	283	25	0
Kimkong et al[17]	2015	Thailand	PCR-RFLP	0.291	Patients with self-limiting HBV infection	83/164	72	10	1	139	25	0
rs12979860 (1,503 cases and 1,505 controls)							**CC**	**CT**	**TT**	**CC**	**CT**	**TT**
Fabris et al[18]	2011	Italy	PCR-RFLP	0.386	Patients with liver cirrhosis	85/171	23	50	12	79	78	14
Agundez et al[19]	2012	Spain	Real-time PCR	0.383	Patients with CHC virus infection	134/97	52	66	16	37	49	11
Eurich et al[20]	2012	Germany	TaqMan	0.219	Patients with CHC virus infection	61/106	13	31	17	35	57	14
Ren et al[14]	2012	People's Republic of China	TaqMan	0.761	Healthy	154/47	115	29	10	43	4	0
Chen et al[15]	2012	People's Republic of China	Real-time PCR	0.369	Healthy	406/244	348	53	5	213	29	2
Wang et al[16]	2012	People's Republic of China	PCR	<0.001	Healthy	298/290	97	198	3	80	210	0
Akkiz et al[21]	2014	Turkey	PCR-RFLP	0.732	Healthy	187/208	80	83	24	92	91	25
De Re et al[22]	2014	Italy	TaqMan	0.076	Healthy	95/178	28	49	18	75	89	14
Kimkong et al[17]	2015	Thailand	PCR-RFLP	0.312	Patients with self-limiting HBV infection	83/164	72	10	1	140	24	0

Abbreviations: HWE, Hardy–Weinberg equilibrium; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; HBV, hepatitis B virus; CHC, chronic hepatitis C.

model (P=0.02), and 0.61 and 0.43–0.87 for the dominant model (P=0.07), respectively. This suggests an association between rs8099917 polymorphism and HBV-related HCC risk in Chinese population. Meta-analysis results for the rs8099917 polymorphism in Chinese population are summarized in Table 2.

Meta-analysis of the association between rs12979860 polymorphism and HCC risk

Meta-analyses of the possible association between rs12979860 polymorphism and HCC risk are summarized in Table 1, and overall results are summarized in Table 2. In the total population of 1,503 cases and 1,505 controls, rs12979860 polymorphism showed a significant relationship with HCC in the allelic model (OR 1.36, 95% CI 1.05–1.78, P=0.02; Figure 3), recessive model (OR 1.75, 95% CI 1.28–2.39, P=0.005), and homozygous model (OR 1.99, 95% CI 1.41–2.80, P<0.001).

In the Caucasian subgroup of 562 cases and 760 controls, rs12979860 polymorphism was associated with HCC risk in all five genetic models: allelic, OR 1.47, 95% CI 1.05–2.06, P=0.02 (Figure 3); recessive, OR 1.62, 95% CI 1.16–2.25, P=0.004; dominant, OR 0.69, 95% CI 0.49–0.97, P=0.03; homozygous, OR 1.84, 95% CI 1.29–2.64, P<0.001; and heterozygous, OR 1.30, 95% CI 1.02–1.66, P=0.03. These results suggest that the T-allele of rs12979860 may be a genetic risk factor for HCC in Caucasians. In contrast, in the

Asian subgroup of 941 cases and 745 controls, rs12979860 polymorphism was significantly related to HCC risk only in the recessive model (OR 3.51, 95% CI 1.09–11.23, P=0.03) and homozygous model (OR 3.54, 95% CI 1.11–11.34, P=0.03). These results suggest that the TT genotype of rs12979860 may be a genetic risk factor for HCC in Asians. Meta-analyses of association of rs12979860 polymorphism with HCC risk in different ethnic subgroups are summarized in Table 2.

A subset of studies that clearly reported whether their cases had HBV- or HCV-related HCC were meta-analyzed in order to examine whether the association between rs12979860 polymorphism and HCC risk depends on etiology. These included two studies on Caucasians involving 272 cases with HBV- or HCV-related disease,[18,21] a study by Fabris et al involving 35 HCV-related and 50 undefined cases,[18] and a study by Akkiz et al involving 24 HCV-related, 110 HBV-related, and 49 undefined cases.[21] Meta-analysis of data for each type of HCC showed that the rs12979860 polymorphism was associated with risk of HCV-related HCC according to four genetic models (Tables 2 and 3): allelic, OR 1.44, 95% CI 1.03–2.10, P=0.03; recessive, OR 1.82, 95% CI 1.04–3.20, P=0.04; homozygous, OR 2.26, 95% CI 1.04–4.92, P=0.04; and heterozygous, OR 1.37, 95% CI 1.03–1.84, P=0.03. These results suggest that the T-allele of rs12979860 may increase risk of HCV-related HCC. In contrast, rs12979860

Table 2 Overall meta-analysis of the association of rs8099917GT and rs12979860C/T polymorphisms with risk of hepatocellular carcinoma

Polymorphism	Allelic model				Recessive model				Dominant model				Homozygous model				Heterozygous model			
	OR	95% CI	Z (P-value)	I^2 (%)	OR	95% CI	Z (P-value)	I^2 (%)	OR	95% CI	Z (P-value)	I^2 (%)	OR	95% CI	Z (P-value)	I^2 (%)	OR	95% CI	Z (P-value)	I^2 (%)
rs8099917	**G versus T**				**GG versus GT + TT**				**TT versus GT + GG**				**GG versus TT**				**GT versus TT**			
Overall	1.49	1.13–1.96	2.82 (0.005)	23	2.76	0.78–9.80	1.57 (0.12)	0	0.68	0.51–0.91	2.56 (0.01)	24	2.91	0.82–10.30	1.65 (0.10)	0	1.41	1.04–1.89	2.24 (0.02)	23
Ethnicity																				
Chinese	1.61	1.19–2.18	3.08 (0.002)	13	3.61	0.78–16.67	1.65 (0.10)	0	0.61	0.43–0.87	2.70 (0.007)	31	3.84	0.83–17.71	1.72 (0.09)	0	1.54	1.07–2.22	2.32 (0.02)	35
rs12979860	**T versus C**				**TT versus CT + CC**				**CC versus CT + TT**				**TT versus CC**				**CT versus CC**			
Overall	1.36	1.05–1.78	2.29 (0.02)	74	1.75	1.28–2.39	3.48 (0.005)	6	0.77	0.58–1.03	1.76 (0.08)	59	1.99	1.41–2.80	3.95 (<0.001)	26	1.12	0.94–1.34	1.28 (0.20)	43
Ethnicity																				
Caucasian	1.47	1.05–2.06	2.27 (0.02)	75	1.62	1.16–2.25	2.87 (0.004)	34	0.69	0.49–0.97	2.14 (0.03)	49	1.84	1.29–2.64	3.34 (<0.001)	55	1.30	1.02–1.66	2.13 (0.03)	28
Asian	1.20	0.78–1.86	0.84 (0.40)	64	3.51	1.09–11.23	2.11 (0.03)	0	0.90	0.56–1.44	0.44 (0.66)	60	3.54	1.11–11.34	2.13 (0.03)	43	0.95	0.73–1.23	0.41 (0.69)	43
Chinese	1.32	0.75–2.34	0.97 (0.33)	0	3.28	0.95–11.36	1.88 (0.06)	0	0.81	0.44–1.52	0.65 (0.52)	73	3.33	0.96–11.53	1.90 (0.06)	0	1.09	0.65–1.84	0.33 (0.74)	61
Etiology																				
HCV-related HCC	1.44	1.03–2.10	2.15 (0.03)	66	1.82	1.04–3.20	2.09 (0.04)	52	0.64	0.42–0.99	1.99 (0.05)	55	2.26	1.04–4.92	2.05 (0.04)	68	1.37	1.03–1.84	2.15 (0.03)	30
HBV-related HCC	1.16	0.86–1.57	0.99 (0.32)	56	1.80	1.03–3.15	2.08 (0.04)	0	0.90	0.63–1.28	0.59 (0.55)	52	1.97	1.09–3.56	2.26 (0.02)	0	0.99	0.79–1.25	0.07 (0.94)	33

Abbreviations: OR, odds ratio; CI, confidence interval; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; HBV, hepatitis B virus.

polymorphism was associated with risk of HBV-related HCC in the recessive model (OR 1.80, 95% CI 1.03–3.15, P=0.04) and homozygous model (OR 1.97, 95% CI 1.09–3.56, P=0.02). These results suggest that the TT genotype of rs12979860 may increase risk of HBV-related disease.

Publication bias

Begg's funnel plot and Egger's test were performed to detect potential publication bias in the meta-analysis. No obvious asymmetry was observed in Begg's funnel plots for rs8099917 (Figure 4A) or rs12979860 (Figure 4B) according to the allelic model. Similarly, Egger's tests showed no significant risk of publication bias in the allelic model for rs8099917 (G-allele versus T-allele: t=0.51, P=0.647) or rs12979860 (T-allele versus C-allele: t=2.07, P=0.077).

Discussion

Few meta-analyses have focused on the association between rs8099917 polymorphism and risk of developing HCC, and individual studies have failed to reach a consensus.[13–17] Most of those studies suggest that rs8099917 polymorphism does not affect risk of HBV-related HCC,[13–15,17] but the present meta-analysis suggests that the G-allele of rs8099917 may be associated with increased risk, and that the wild-type TT genotype may protect against the disease. Similar results were observed specifically in Chinese population. It is worth noting that all five previous studies,[13–17] involving 1,041 cases and 907 controls, were conducted on Asian populations in which all cases had HBV-related HCC.

Two previous meta-analyses were published reporting that rs12979860 polymorphism is a genetic risk factor for hepatitis virus-related HCC.[26,35] These results should be interpreted with caution, since the meta-analyses included 118 cases from a case–control study in which the patients appeared to have had HCC and liver cirrhosis and to have been in end-stage liver disease.[32] This may make the meta-analysis results less accurate because genetic factors affect risk of HCC and liver cirrhosis differently. In addition, these meta-analyses did not include four recent case–control studies.[15,17,21,22] The present meta-analysis not only included these four studies but also excluded the study by El-Awady et al.[32]

Our meta-analysis of all included patients suggests that the T-allele of rs12979860 significantly increases risk of HCC. The T-allele also seems to increase risk of HCC in the subset of Caucasians, especially HCV-related HCC. Although we failed to detect a significant association between the T-allele and risk of HBV-related HCC in the allelic model, the TT genotype of rs12979860 was associated with elevated
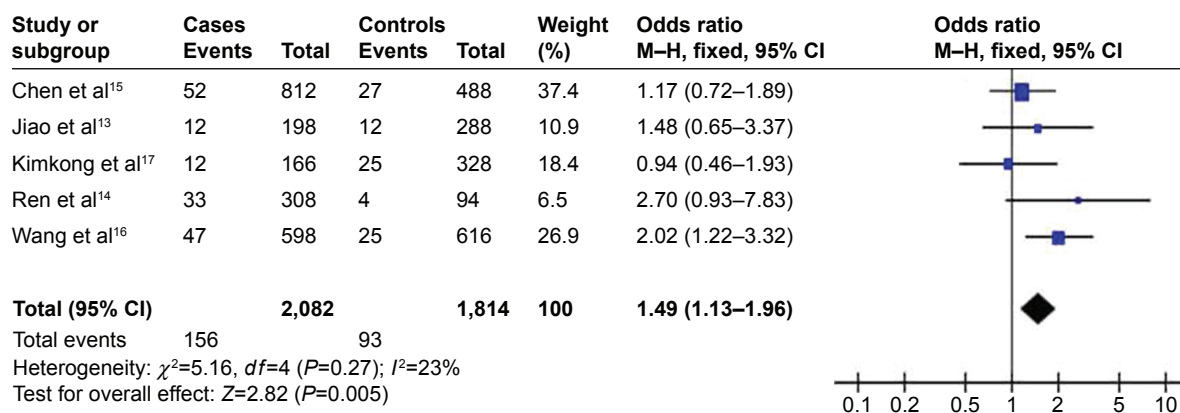
Study or subgroup	Cases Events	Total	Controls Events	Total	Weight (%)	Odds ratio M–H, fixed, 95% CI	Odds ratio M–H, fixed, 95% CI
Chen et al[15]	52	812	27	488	37.4	1.17 (0.72–1.89)	
Jiao et al[13]	12	198	12	288	10.9	1.48 (0.65–3.37)	
Kimkong et al[17]	12	166	25	328	18.4	0.94 (0.46–1.93)	
Ren et al[14]	33	308	4	94	6.5	2.70 (0.93–7.83)	
Wang et al[16]	47	598	25	616	26.9	2.02 (1.22–3.32)	
Total (95% CI)		**2,082**		**1,814**	**100**	**1.49 (1.13–1.96)**	
Total events	156		93				

Heterogeneity: $\chi^2=5.16$, $df=4$ ($P=0.27$); $I^2=23\%$
Test for overall effect: $Z=2.82$ ($P=0.005$)

0.1 0.2 0.5 1 2 5 10

Figure 2 Forest plot describing the association between rs8099917 polymorphism and hepatocellular carcinoma risk (G-allele versus T-allele).
Abbreviations: CI, confidence interval; M–H, Mantel–Haensze.

risk of HBV-related disease across all patients and in the subgroup of Asians. Therefore, our meta-analysis provides new evidence of the important role of IL28B rs12979860 polymorphism in hepatitis virus-related carcinogenesis.

The findings of this systematic review are limited by the designs of the included studies. First, the controls in four case–control studies were not healthy individuals, which increases the risk of heterogeneity because not all controls were chosen according to the same well-defined criteria. Second, the P-value associated with Hardy–Weinberg equilibrium was <0.05 in two included studies[14,16] (Table 1), suggesting that these study populations were not
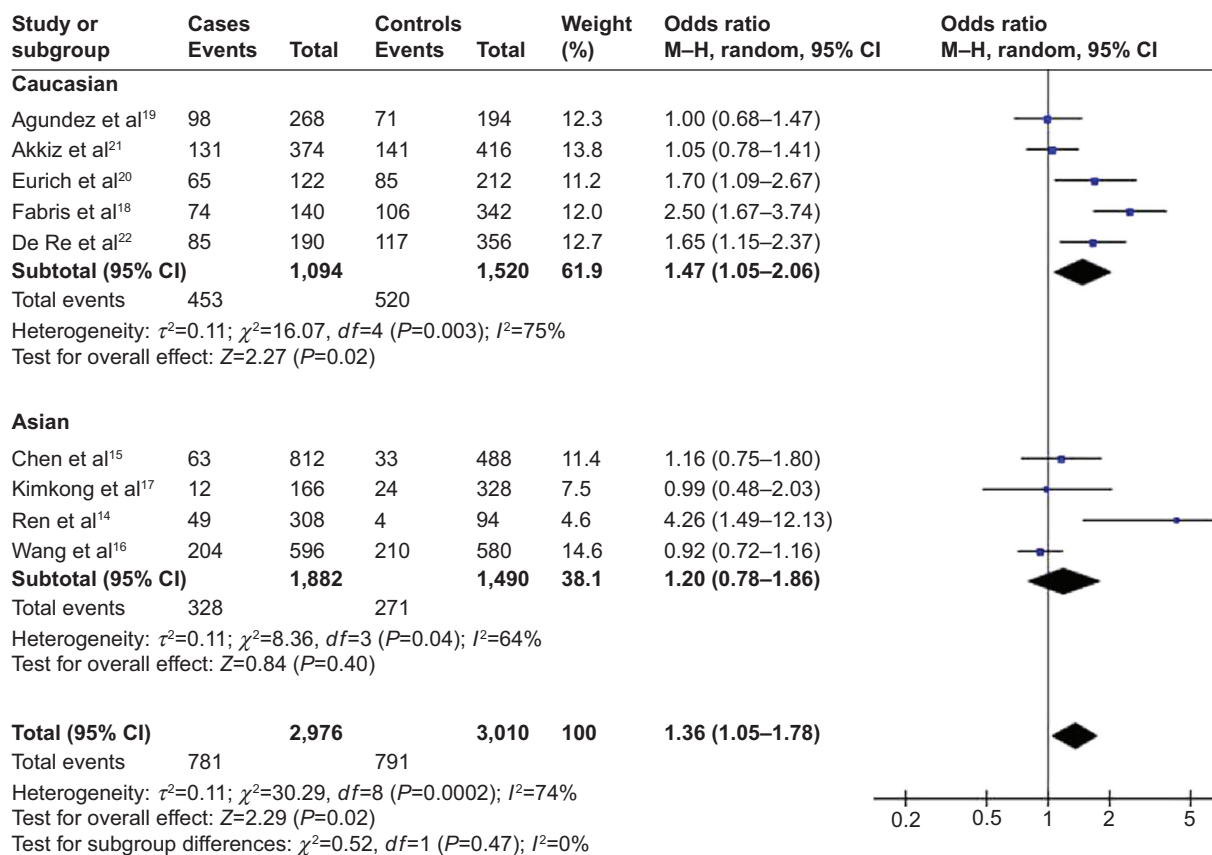
Study or subgroup	Cases Events	Total	Controls Events	Total	Weight (%)	Odds ratio M–H, random, 95% CI	Odds ratio M–H, random, 95% CI
Caucasian							
Agundez et al[19]	98	268	71	194	12.3	1.00 (0.68–1.47)	
Akkiz et al[21]	131	374	141	416	13.8	1.05 (0.78–1.41)	
Eurich et al[20]	65	122	85	212	11.2	1.70 (1.09–2.67)	
Fabris et al[18]	74	140	106	342	12.0	2.50 (1.67–3.74)	
De Re et al[22]	85	190	117	356	12.7	1.65 (1.15–2.37)	
Subtotal (95% CI)		**1,094**		**1,520**	**61.9**	**1.47 (1.05–2.06)**	
Total events	453		520				

Heterogeneity: $\tau^2=0.11$; $\chi^2=16.07$, $df=4$ ($P=0.003$); $I^2=75\%$
Test for overall effect: $Z=2.27$ ($P=0.02$)

Asian							
Chen et al[15]	63	812	33	488	11.4	1.16 (0.75–1.80)	
Kimkong et al[17]	12	166	24	328	7.5	0.99 (0.48–2.03)	
Ren et al[14]	49	308	4	94	4.6	4.26 (1.49–12.13)	
Wang et al[16]	204	596	210	580	14.6	0.92 (0.72–1.16)	
Subtotal (95% CI)		**1,882**		**1,490**	**38.1**	**1.20 (0.78–1.86)**	
Total events	328		271				

Heterogeneity: $\tau^2=0.11$; $\chi^2=8.36$, $df=3$ ($P=0.04$); $I^2=64\%$
Test for overall effect: $Z=0.84$ ($P=0.40$)

Total (95% CI)		**2,976**		**3,010**	**100**	**1.36 (1.05–1.78)**	
Total events	781		791				

Heterogeneity: $\tau^2=0.11$; $\chi^2=30.29$, $df=8$ ($P=0.0002$); $I^2=74\%$
Test for overall effect: $Z=2.29$ ($P=0.02$)
Test for subgroup differences: $\chi^2=0.52$, $df=1$ ($P=0.47$); $I^2=0\%$

0.2 0.5 1 2 5

Figure 3 Forest plot describing the association between rs12979860 polymorphism and hepatocellular carcinoma risk (T-allele versus C-allele).
Abbreviation: CI, confidence interval.

Table 3 Genotype frequencies of the rs12979860 polymorphism according to etiology of hepatocellular carcinoma

Author	Year	Country	Method	P (HWE)	Type of control	Cases/ controls	Case genotype			Control genotype		
							CC	CT	TT	CC	CT	TT
HCV-related HCC (374 cases and 760 controls)							CC	CT	TT	CC	CT	TT
Fabris et al[18]	2011	Italy	PCR-RFLP	0.386	Patients with liver cirrhosis	35/171	6	21	8	79	78	14
Agundez et al[19]	2012	Spain	PCR-RFLP	0.383	Patients with CHC virus infection	134/97	52	66	16	37	49	11
Eurich et al[20]	2012	Germany	PCR-RFLP	0.219	Patients with CHC virus infection	61/106	13	31	17	35	57	14
Akkiz et al[21]	2014	Turkey	PCR-RFLP	0.732	Healthy	49/208	21	24	4	92	91	25
De Re et al[22]	2014	Italy	TaqMan	0.076	Healthy	95/178	28	49	18	75	89	14
HBV-related HCC (1,051 cases and 953 controls)							CC	CT	TT	CC	CT	TT
Ren et al[14]	2012	People's Republic of China	TaqMan	0.006	Healthy	154/47	115	29	10	43	4	0
Chen et al[15]	2012	People's Republic of China	Real-time PCR	0.369	Healthy	406/244	356	48	2	218	25	1
Wang et al[16]	2012	People's Republic of China	MALDI-TOF MS	<0.001	Healthy	298/290	97	198	3	80	210	0
Akkiz et al[21]	2014	Turkey	PCR-RFLP	0.732	Healthy	110/208	43	50	17	92	91	25
Kimkong et al[17]	2015	Thailand	PCR-RFLP	0.312	Patients with self-limiting HBV infection	83/164	72	10	1	140	24	0

Abbreviations: HWE, Hardy–Weinberg equilibrium; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; CHC, chronic hepatitis C; HBV, hepatitis B virus; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

representative of the broader target population. Third, the results may be affected by additional confounding factors, such as tumor status, sex, or age, but most studies either did not report these baseline data or aggregated the data in different ways, preventing their inclusion in the meta-analysis. Fourth, the studies used various assay methods to characterize the polymorphisms; these methods differ in sensitivity and specificity, which may affect the reliability of the results. Lastly, the fact that all five studies of the rs8099917 polymorphism in our meta-analysis[13–17] examined Asian populations highlights the need for large,

well-designed studies in Caucasian and African populations to verify our findings.

Conclusion

The G-allele of rs8099917 may be associated with elevated risk of HBV-related HCC, while the wild-type TT genotype of rs8099917 may protect against this disease. The T-allele of rs12979860 may increase risk of HCC in Caucasians, especially HCV-related HCC. The TT genotype of rs12979860 may increase risk of HBV-related HCC, especially in Asians. Further detailed and well-designed studies involving larger,
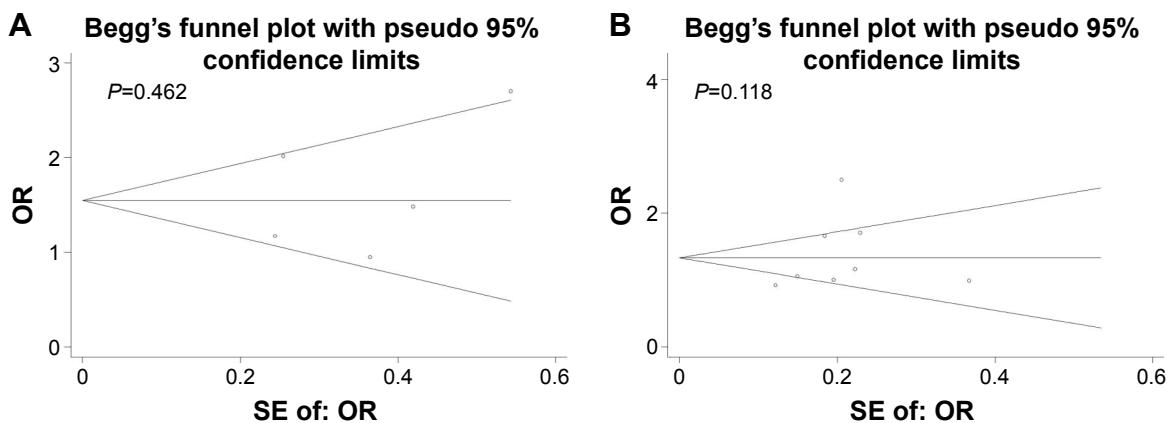
Figure 4 Begg's funnel plots according to allelic models.
Notes: Begg's funnel plots to detect publication bias in analysis of the association of (**A**) rs8099917 polymorphism and hepatocellular carcinoma risk according to the allelic model (G-allele versus T-allele), or the association of (**B**) rs12979860 polymorphism and hepatocellular carcinoma risk according to the allelic model (T-allele versus C-allele).
Abbreviations: OR, odds ratio; SE, standard error.

multiethnic samples are needed to clarify the influence of those two polymorphisms on HCC risk. Such studies should also explore gene–gene and gene–environment interactions that may mediate an association of rs809991 and rs12979860 with risk of developing hepatitis virus-related HCC.

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Disclosure

The authors report no conflicts of interest in this work.

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