Meta-Analysis

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Associations between hepatocellular carcinoma risk and rs3212227 and rs568408 polymorphisms: a systematic review and meta-analysis

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Abstract

Background: Interleukin-12 (IL-12) is considered to be a risk factor for cancer; however, its role in hepatocellular carcinoma (HCC) remains unknown. This study aimed to explore the impacts of the IL-12 rs3212227 and rs568408 gene polymorphisms on HCC.

Methods: We searched PubMed, Embase, Web of Science, and Chinese Knowledge Infrastructure databases for studies on the associations between HCC and IL-12 rs568408 and rs3212227 polymorphisms published prior to 1 May 2020. The effects of the polymorphisms on HCC susceptibility were presented as odds ratios (ORs) and associated 95% confidence intervals.

Results: Seven studies were ultimately included, including 2375 cases and 3445 controls. The rs3212227 polymorphism was significantly associated with the risk of HCC in both the dominant model (CC+AC vs. AA, OR=1.22) and the allele model (C vs. A, OR=1.12). Combined analysis of rs568408 yielded a significant relative risk for HCC in the dominant (AA+AG vs. GG, OR=1.13), recessive (AA vs. AG+GG, OR=1.72), allele (A vs. G, OR=1.29), heterozygote (AG vs. GG, OR=1.27), and homozygote models (AA vs. GG, OR 1.17).

Conclusion: The IL-12 rs3212227 and rs568408 gene polymorphisms are associated with an increased risk of HCC.

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Introduction

Hepatocellular carcinoma (HCC) is a fatal disease that poses a major health threat worldwide. Hepatitis B virus (HBV) is a major risk factor for HCC,[1] with an estimated 75%–85% of HCC cases attributable to persistent infection with HBV.[2] Growing evidence has also highlighted the role of genetic polymorphisms in HCC, suggesting an important role for genetic factors in carcinogenesis.[3,4] However, the potential value of genetic polymorphisms in HCC remains to be elucidated.

Cytokines are important immunoregulatory substances that produce inflammatory responses, thus influencing the occurrence of chronic inflammation, the tumor microenvironment,[5] and the pathogenesis of HCC.[6] Interleukin-12 (IL-12) comprises two subunits (p35 and p40) connected by a disulfide bond to produce an isoprene dimer cytokine with multiple biological effects.[7] Gene polymorphisms have been identified in the promoter region, intron, and 3'-untranslated region of the portion of the IL-12 gene that codes for the p40 subunit.[8] IL-12 plays a major role not only in inducing an appropriate immune responses against viral infections (including HBV), but also in the antitumor immune response.[9] IL-12 has been shown to inhibit the proliferation and metastasis of various malignant tumors, *in vitro* and *in vivo*,[10] and to inhibit the differentiation of natural killer and Th2 T helper cells, contributing to a strong anti-tumor effect.[11] IL-12 is encoded by two genes, *IL12A* and *IL12B*, and numerous studies have demonstrated that the *IL12A* and *IL12B* genes include multiple functional polymorphic sites that might affect the occurrence and progression of non-Hodgkin lymphoma,[12] tuberculosis,[13] autoimmune disease,[14] gastric carcinoma,[15] lung carcinoma,[16] and other malignancies.[17] Tan et al. showed that the *IL12A* rs568408 variant may be a marker single-nucleotide polymorphism (SNP) associated with an increased risk of poor HBV clearance and HBV-related HCC development.[9] Meanwhile, Ben-Selma et al.[18] suggested that *IL-12A* rs568408 and gene–gene interactions between *IL-12A* rs568408 and *IL-12B* rs3212227 contributed to the outcome of chronic HBV infection, indicating their potential usefulness as predictive and diagnostic biomarkers of HCC. Additional evidence has shown that the IL-12 signaling pathway plays a pivotal role during HBV infection and may contribute to the pathogenesis of HCC.[19,20] An accurate analysis of the distribution of IL-12 gene polymorphisms among human populations from various geographical regions may reveal a correlation between these polymorphisms and the occurrence of HCC.

Researchers have previously investigated the role of IL-12 in the development and progression of HCC;[21] however, the specific association between IL-12 gene polymorphisms and susceptibility to HCC remains controversial. We conducted a meta-analysis of related case-control studies with large sample sizes to obtain additional

evidence to allow the analysis of the association between IL-12 gene polymorphisms and susceptibility to HCC, to provide a scientific basis for the early diagnosis and treatment of HCC.

Methods and materials

Search strategy

Previous reports on the associations between the IL-12 rs568408 and rs3212227 polymorphisms and HCC were collected by searching the PubMed, Embase, Web of Science, Chinese Knowledge Infrastructure (CNKI), and Wanfang databases using the following key words: ("interleukin-12" OR "Interleukin-12" OR "IL-12" OR "IL12" OR "rs568408" OR "rs3212227"), and ("polymorphisms" OR "polymorphism" OR "SNP" OR "variation" OR "variant" OR "mutation" OR "genetic" OR "genotype"), and ("hepatocellular carcinoma" OR "HCC" OR "liver cancer"). According to the scope statements on Medline and Embase, other subject headings were included based on previous indexes and keywords that contained synonyms for the words included in the range description, to ensure that topic headings were used correctly, according to their definitions.

Only studies published before 1 May 2020 were included in the meta-analysis. We also screened the reference lists to identify related titles.

Selection criteria

Studies included in the meta-analysis had to fulfill the following inclusion criteria: 1) case-control study; 2) investigation of the association between IL-12 rs3212227 or rs568408 polymorphism with HCC; and 3) complete data, including numbers of patients in the case and control groups, distribution of genotype frequency, and related statistical indicators. If multiple publications referred to the same study sample, the study with the largest sample was selected for inclusion in the meta-analysis.

Data extraction

Cunqing Kong and Miao Chen independently screened the literature and extracted the following data: first author, year of publication, country, ethnicity, baseline characteristics of the control group, sample size of the case and control groups, genetic frequency, Hardy–Weinberg equilibrium (HWE) values for the control group, HCC risk; rs3212227 polymorphism; rs568408 polymorphism; rs3212227 gene mutation (A to C); and rs568408 gene mutation (C to A).

This was not a primary research study, and ethical approval and informed consent were therefore not required.

Statistical analysis

All statistical analyses were performed using Stata software, version 12.0 (StataCorp LP, College Station, TX, USA) and Review Manager 5.3 (Cochrane Collaboration). All binary variables were presented as summary risk ratios (RR) with 95% confidence intervals (CI), calculated using a fixed-effects model except in the event of high heterogeneity, in which case a random-effects model was used. The associations between IL-12 polymorphisms and HCC risk were evaluated using recessive, dominant, homozygote, heterozygote, and allelic models. Unadjusted odds ratios (ORs) and 95%CIs were used to describe the susceptibility of patients with rs3212227 or rs568408 polymorphisms to HCC. HWE values in the control group were determined by Fisher's exact test and the effect of combining OR values on HCC was determined by the Z-test. Heterogeneity was evaluated using fixed-effect ($I^2<50\%$) and random-effect models ($I^2\geq50\%$). Subgroup analysis was performed to determine the effect of ethnicity

on the associations of rs3212227 and rs568408 polymorphism with HCC. We calculated the summary RR and 95%CI using a fixed-effect model, or a random-effect model in cases of high heterogeneity. The influence of study quality on the results was assessed by sensitivity analysis. The extent of heterogeneity was determined as the total percentage of variation between studies, measured with the I^2 statistic. I^2 values were categorized as low if I^2 (0%–25%), moderate (25%–50%), and high (50%–90%). The Q-statistic was used to assess the presence of heterogeneity. A PQ statistic $\geq$0.05 was considered to indicate no significant heterogeneity among the included studies.

Results

Description of included studies

The search process and inclusion criteria for this study are shown in Figure 1. A total of 522 studies written in English or Chinese and published in the searched databases prior to 1 May 2020 were systematically screened (Figure 1). Seven studies[9,21–26] were finally included in this meta-analysis (Table 1). These seven studies demonstrated the association between the rs3212227 polymorphism and HCC risk (2375 cases, 3445 controls) and three studies[9,21,22] also evaluated the relationship between the rs568408 polymorphism and HCC risk (1342 cases, 2026 controls). The genetic distribution of the rs568408 polymorphism in all but two controls[9,22] was consistent with the HWE (P>0.05).

Meta-analysis of association between rs3212227 polymorphism and HCC risk

The associations between rs3212227 polymorphism and HCC risk for five models and various subgroups are shown in Table 2. All seven studies included in the meta-analysis (2,375 cases) reported an association between rs3212227 polymorphism and HCC. A fixed-effect analyses showed that rs3212227 was associated

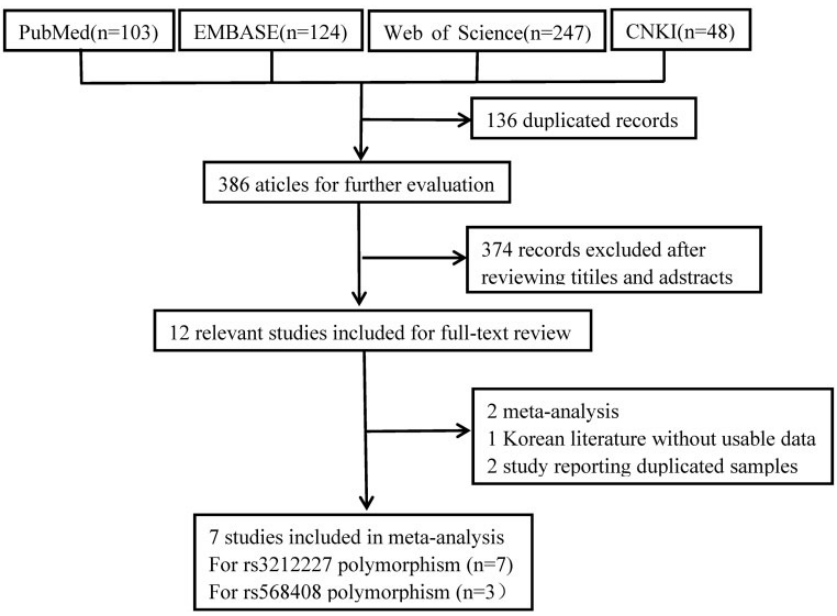

Figure 1. Flow diagram of literature research and selection process.

Table 1. Characteristics of studies included in this meta-analysis.

First author	Year	Country	Ethnicity	Genotyping method	*P* for HWE	Type of control group	Cases	Controls	NOS score
IL12A (rs568408)									
Liu[22]	2011	China	Asian	PCR-RFLP	Disequilibrium	Cancer-free	869	891	7
Tan[9]	2015	China	Asian	IMLDR	Disequilibrium	Healthy, CHB	395	979	6
Elsayed[21]	2016	Egypt	African	PCR-RFLP	Equilibrium	Healthy, CHC	78	156	5
IL12B (rs3212227)									
Nieters[23]	2005	China	Asian	PCR-RFLP	Equilibrium	Cancer-free	249	250	7
Ognjanovic[24]	2009	America	North American	Taqman assays	No checking	Healthy	117	223	5
Liu[22]	2011	China	Asian	PCR-RFLP	Equilibrium	Cancer-free	869	891	9
Yang[4]	2011	China	Asian	Taqman assays	Equilibrium	Cancer-free	608	612	8
Saxena[25]	2014	India	Asian	PCR-RFLP	Equilibrium	Healthy, CHB	59	336	6
Tan[9]	2015	China	Asian	IMLDR	Equilibrium	Healthy, CHB	395	977	7
Elsayed[21]	2016	Egypt	African	PCR-RFLP	Equilibrium	Healthy, CHC	78	156	6

PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; IMLDR, improved multiplex ligase detection reaction; HWE, Hardy–Weinberg equilibrium; CHB, chronic hepatitis B; CHC, chronic hepatitis C; NOS, Newcastle–Ottawa Scale.

Table 2. Meta-analysis of association of rs3212227 polymorphisms with risk of hepatocellular carcinoma.

Genotype comparison and genetic model	Group and subgroups	Sample size (n)		OR [95% CI]	*P* value	Analysis model	Test of heterogeneity	
		Case	Control				I^2 (%)	*P* value
Dominant model	Overall	2337	3362	1.22 [1.07–1.38]	0.003	Fixed	0	0.40
(CC+AC vs AA)	Asian	2142	2983	1.22 [1.07–1.38]	0.002	Fixed	0	0.50
	Other	195	379	1.04 [0.74–1.47]	0.82	Random	78	0.03
Recessive model	Overall	1971	2889	1.15 [0.99–1.33]	0.06	Fixed	0	0.75
(CC vs AG+AA)	Asian	1893	2733	1.15 [0.99–1.33]	0.07	Fixed	0	0.60
	Other	79	156	1.19 [0.59–2.40]	0.63			
Allele model	Overall	3942	5778	1.12 [1.03–1.21]	0.01	Fixed	25	0.26
(C vs A)	Asian	3786	5466	1.13 [1.04–1.23]	0.005	Fixed	12	0.33
	Other	156	312	0.84 [0.56–1.27]	0.41			
Heterozygote model	Overall	1971	2889	0.96 [0.86–1.08]	0.53	Fixed	47	0.11
(AC VS AA)	Asian	1893	2733	0.94 [0.83–1.06]	0.29	Fixed	0	0.65
	Other	78	156	1.77 [0.98–3.19]	0.06			
Homozygote model	Overall	1554	2348	1.13 [0.98–1.30]	0.88	Fixed	33	0.22
(CC vs AA)	Asian	1491	2218	1.18 [1.02–1.35]	0.02	Fixed	0	0.54
	Other	63	130	1.77 [0.98–3.19]	0.06			

OR, odds ratio; 95%CI, 95% confidence interval.

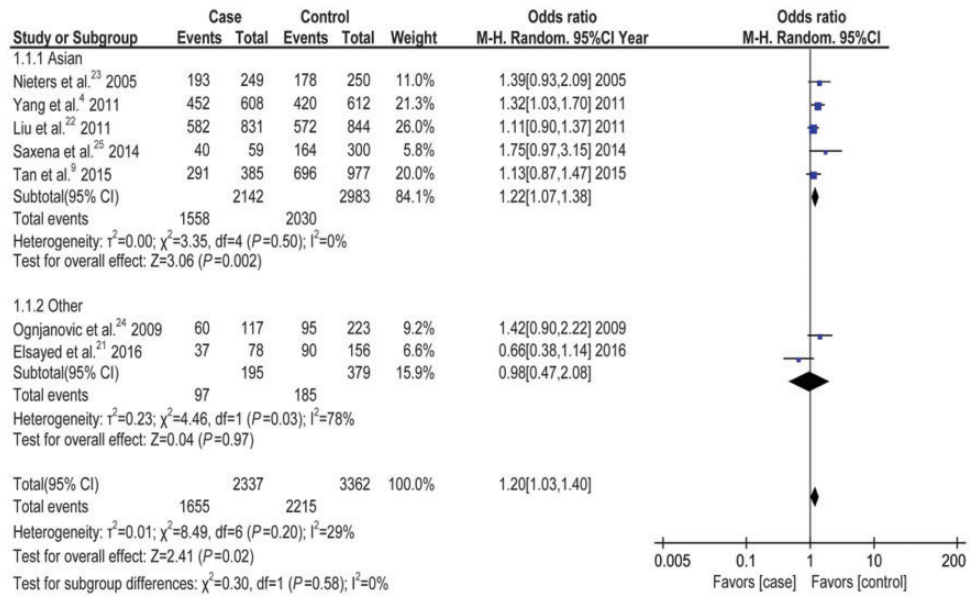

Figure 2. Association between rs3212227 polymorphism and hepatocellular carcinoma risk in the dominant model.
M-H, Mantel–Haenszel; CI, confidence interval.

with increased risk for HCC in the dominant model (CC+AC vs. AA, OR 1.22, 95%CI 1.07–1.38, $P = 0.003$) (Figure 2) and the allele model (C vs. A, OR 1.12, 95%CI 1.03–1.21, $P = 0.01$) (Table 2). These results suggested that the C-allele polymorphism of rs3212227 may increase susceptibility to HCC. However, fixed-effect analyses using the other models showed no significant association between rs3212227 polymorphism and HCC risk (recessive model: CC vs. AG+AA, OR 1.15, 95%CI 0.99–1.33; heterozygote model: AC vs. AA, OR 0.96, 95%CI 0.86–1.08; homozygote model: CC vs. AA, OR 1.13, 95%CI 0.99–1.30).

Subgroup analysis according to ethnicity revealed significant differences between Asian and other ethnicities (2,180 cases, 3,066 controls; dominant model: CC+AC vs. AA, OR 1.22, 95%CI 1.07–1.38, $P = 0.002$; allele model: C vs. A, OR 1.13, 95%CI 1.04–1.23, $P = 0.005$; homozygote model: CC vs. AA, OR 1.18, 95%CI 1.02–1.35, $P = 0.02$). These results indicated a significant association between the IL-12 rs3212227 polymorphism and HCC risk in the Asian population (Table 2).

Meta-analysis of association between rs568408 polymorphism and HCC risk

The associations between rs3212227 polymorphism and HCC risk for five models and various subgroups are shown in Table 3. The rs568408 polymorphism was significantly associated with increased risk of HCC in the overall study population (1,342 cases, 2,026 controls). The significant association between the rs568408 polymorphism and HCC risk was supported by ORs of 1.13 in the dominant model (AA+AG vs. GG: 95%CI 1.01–1.28, $P<0.001$) (Figure 3) and 1.72 in the recessive model (AA vs. AG+GG: 95%CI 1.07–2.78, $P = 0.003$).

Table 3. Meta-analysis of association of rs568408 polymorphisms with risk of hepatocellular carcinoma.

Genotype comparison and genetic model	Group and subgroups	Sample size (n)		OR [95% CI]	*P* value	Analysis model	Test of heterogeneity	
		Case	Control				I^2 (%)	*P* value
Dominant model	Overall	1275	1995	1.13 [1.01–1.28]	0.0008	Random	95	<0.001
(AA+AG vs GG)	Chinese	1197	1839	3.13 [2.00–4.90]	0.04	Random	95	<0.001
	Other	78	156	1.21 [1.08–1.36]	0.001	Random		
Recessive model	Overall	1197	1839	1.72 [1.07–2.78]	0.003	Random	68	0.04
(AA vs AG+GG)	Chinese	1275	1995	1.40 [0.81–2.42]	0.23	Random	74	0.05
	Other	78	156	3.68 [1.28–10.53]	0.02	Random		
Allele model	Overall	2550	3990	1.29 [1.12–1.48]	0.0003	Random	95	<0.001
(A vs G)	Chinese	2394	3678	1.18 [1.02–1.36]	0.03	Random	95	<0.001
	Other	156	312	4.08 [2.44–6.82]	<0.00001	Random		
Heterozygote model	Overall	1238	1958	1.27 [1.08–1.49]	0.0004	Random	94	<0.001
(AG vs GG)	Chinese	1170	1808	1.18 [1.00–1.39]	0.06	Random	95	<0.001
	Other	68	150	4.84 [2.40–9.78]	<0.00001	Random		
Homozygote model	Overall	890	1512	1.88 [1.17–3.03]	0.009	Random	79	0.009
(AA vs GG)	Chinese	844	1373	1.46 [0.85–2.51]	0.17	Random	81	0.02
	Other		52 139	5.28 [1.81–15.39]	0.002	Random		

OR, odds ratio; 95%CI, 95% confidence interval.

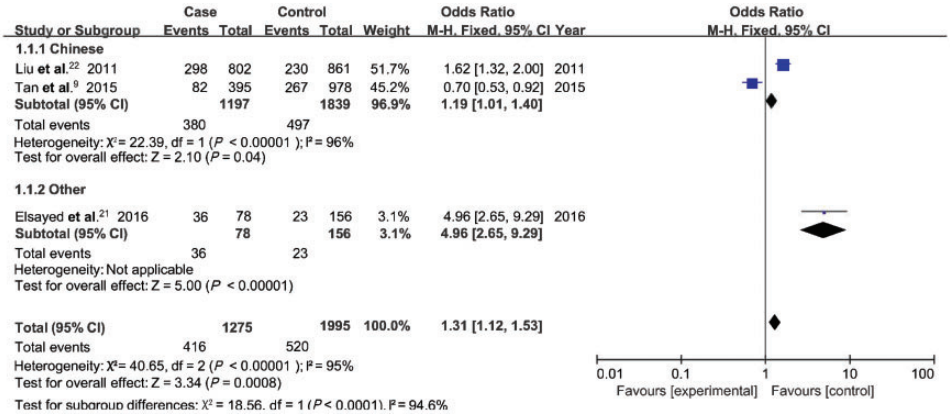

Figure 3. Association between rs568408 polymorphism and hepatocellular carcinoma risk in the dominant model.
M-H, Mantel–Haenszel.

Similar trends were observed for the allele (A vs. G: OR = 1.29, 95%CI 1.12–1.48, $P<0.001$), heterozygote (AG vs. GG: OR = 1.27, 95%CI 1.08–1.49, $P<0.001$), and homozygote models (AA vs. GG: OR = 1.17, 95%CI 1.88–3.03, $P = 0.009$) (Table 3).

Subgroup analysis of a study conducted in China that included 1264 cases and 1870 control showed that the rs568408 polymorphism was associated with HCC risk in the dominant (AA+AG vs. GG: OR 3.13, 95% CI 2.00–4.90, $P = 0.04$), allele (A vs. G: OR 1.18, 95%CI 1.02–1.36, $P = 0.03$), and heterozygote models (AG vs. GG: OR 1.18, 95%CI 1.00–1.39, $P = 0.06$) (Table 3). These results indicated that the A allele of rs568408 might be a genetic factor associated with increased risk of HCC in China.

Sensitivity analysis

Sensitivity analysis was conducted by excluding one study at a time to assess the influence on the overall OR. Sensitivity analysis of the studies investigating the rs3212227 polymorphism showed that the results were credible (Figure 4).

Discussion

Numerous studies have evaluated the associations between various polymorphisms of the IL-12 gene and HCC risk, but the results have been inconsistent. We therefore conducted a qualitative meta-analysis to explore the potential association between inherited variations in the IL-12 gene and HCC susceptibility in a large patient cohort. Our findings indicated that the rs3212227 and rs568408 polymorphisms were significantly correlated with HCC risk.

Elsayed et al.[21] and Liu et al.[22] previously showed that the IL-12 rs568408 A allele polymorphism was significantly correlated with HCC susceptibility. However, Tan et al.[9] reported conflicting findings, and found no significant association between this polymorphism and an increased risk for HCC. Xiao et al.[27] identified the *STAT4* rs7574865 polymorphism as a risk factor for HCC, but failed to find any significant association between HCC risk and other polymorphisms in genes related to the IL-12 signaling pathway, including *IL12A* rs568408 and *IL12B* rs3212227. In contrast, the current meta-analysis indicated that

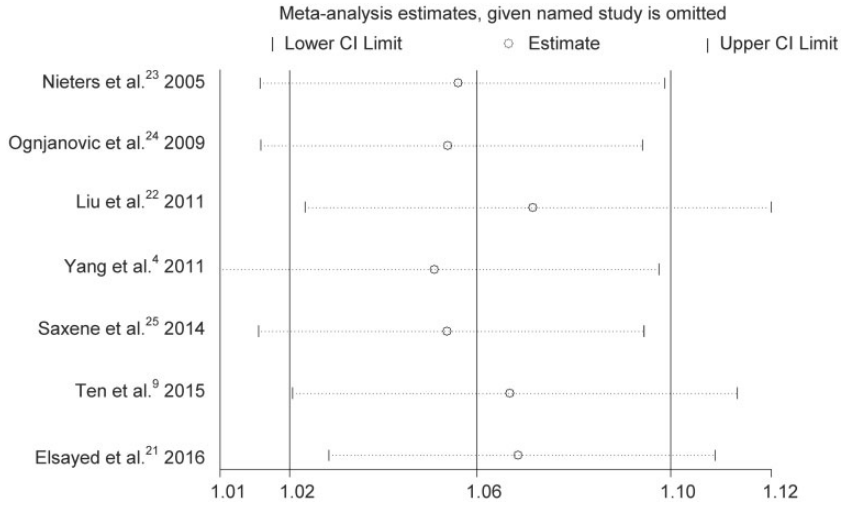

Figure 4. Sensitivity analysis for IL12 polymorphisms at rs3212227.
CI, confidence interval.

rs568408 polymorphisms in the IL-12 gene were significantly correlated with HCC risk, suggesting that the AA wild-type IL-12 genotype may protect against HCC tumorigenesis, especially among individuals of Chinese ethnicity. All 1,342 cases and 2,026 controls included in this meta-analysis were infected with HBV or hepatitis C virus (HCV), further suggesting that the IL-12 rs568408 polymorphism might increase the risk of HBV or HCV infection. Regarding the IL-12 rs3212227 polymorphism, several studies[21] found no significant association between this polymorphism and HCC susceptibility. However, Saxena et al.[25] concluded that mutation of the rs3212227 C allele increased the risk of HCC. Similarly, a previous meta-analysis[28] reported a significant association between the rs3212227 polymorphism and HCC tumorigenesis.

Notably, the current meta-analysis included seven studies with 2375 HCC patients and 3445 controls. Most of the included studies reported a significant association between the rs3212227 polymorphism and an increased risk of HCC, especially in Asian patients. Overall, the findings indicated that the IL-12 rs3212227 polymorphism contributes to the development of HCC.

This study demonstrated a clear increase in HCC risk associated with the IL-12 rs568408 polymorphism under all the genetic models investigated, further suggesting that IL-12 may contribute to the etiology of HCC. IL-12 has been shown to be involved in Th1 cell development and interferon-gamma (IFN-γ) production, as well as cell-mediated cytotoxicity,[29] and has demonstrated antitumor effects in animal models of melanoma,[30] renal carcinoma,[31] and colon carcinoma.[32] A previous study[33] demonstrated that IL-12 enhanced tumor control via the inhibition of tumor angiogenesis and the expansion of intertumoral T regulatory cell populations. As a tumor suppressor, IL-12 increases production of IFN-γ, which increases p53 expression, contributing to tumor cell apoptosis.[34] Wang et al.[35] showed that IL-12 downregulated the expression of STAT3 and

promoted macrophage polarization to the M1-like phenotype, resulting in HCC tumor growth.

Inherited genetic variations in *IL12A* and *IL12B* may dramatically alter the cytokine expression and protein structure. IL-12 has been shown to positively regulate the expression of the interferon regulatory factors IRF1 and IRF4, which have demonstrated tumor suppressor activity in cancer.[36,37] Moreover, the results of several animal studies indicated that IL-12 regulates the release of cytokines via nonspecific immune mechanisms, inhibiting carcinogenesis *in vivo*.[38,39] IL-12 is associated with the clearance of HBV and is highly expressed in hepatic cells in individuals infected with HBV.[40,41] Many IL-12 SNPs were recently found to be associated with HCC susceptibility, including rs3212227[23,42] and rs568408.[22] SNPs in the IL-12 gene regulate gene expression levels, leading to dysregulation of the immune response to infection and tumorigenesis.[9] These SNPs may also disrupt signaling pathways, dysregulate exonic mRNA, and alter protein expression, contributing to the occurrence of HCC.[43]

This study had several limitations. First, the control populations in the included studies did not necessarily comprise healthy individuals. Second, the *P*-values for the HWE test for the rs568408 polymorphism carried out by Liu et al.[22] and Tan et al.[9] were <0.05, similar to the results of Elsayed et al.,[21] which may have led to increased heterogeneity among the studies. Third, we did not adjust for factors that may have influenced the comparison, such as cancer status, sex, age, and of alcohol use. Fourth, the assays used to detect genetic polymorphisms may have varied in terms of sensitivity and specificity, which may have affected the reliability of the results. Fifth, the major studies investigating rs3212227 polymorphism focused entirely on Asian populations, suggesting that larger sample sizes and more sophisticated designs are needed to validate our findings.

This meta-analysis showed that IL-12 polymorphisms were strongly associated with HCC risk. The rs3212227 polymorphism appeared to be related to a higher risk of HCC, while the rs568408 polymorphism also showed a significant association with HCC, suggesting that rs568408 may also lead to increased susceptibility to HCC.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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References

1. Yeh FS, Yu MC, Mo CC, et al. Hepatitis B virus, aflatoxins, and hepatocellular carcinoma in southern Guangxi, China. *Cancer Res* 1989; 49: 2506–2509.
2. Yang HI, Lu SN, Liaw YF, et al. Hepatitis B e antigen and the risk of hepatocellular carcinoma. *N Engl J Med* 2002; 347: 168–174.
3. Pan X and Wang G. Correlations of IL-23R gene polymorphism with clinicopathological characteristics and prognosis of hepatocellular carcinoma patients after interventional therapy. *Genomics* 2019; 111: 930–935.

4. Yang Y, Luo C, Feng R, et al. The TNF-α, IL-1B and IL-10 polymorphisms and risk for hepatocellular carcinoma: a meta-analysis. *J Cancer Res Clin Oncol* 2011; 137: 947–952.
5. Kalvakolanu DV. Cytokine signaling in cancer: Novel players and pathways. *Cytokine* 2017; 89: 1–3.
6. Zekri ARN, El Deeb S, Bahnassy AA, et al. Role of relevant immune-modulators and cytokines in hepatocellular carcinoma and premalignant hepatic lesions. *World J Gastroenterol* 2018; 24: 1228–1238.
7. O'Shea JJ, Gadina M and Siegel RM. Cytokines and cytokine receptors. In: Rich RR, Fleisher TA, Shearer WT, et al (eds) *Clinical Immunology: Principles and Practice*. 5th ed. Elsevier, 2019, pp.127–155.
8. Yan J, Smyth MJ and Teng MW. Interleukin (IL)-12 and IL-23 and their conflicting roles in cancer. *Cold Spring Harb Perspect Biol* 2018: 10: a028530.
9. Tan A, Gao Y, Yao Z, et al. Genetic variants in IL12 influence both hepatitis B virus clearance and HBV-related hepatocellular carcinoma development in a Chinese male population. *Tumour Biol* 2016; 37: 6343–6348.
10. Jiang J, Zhang Y, Peng K, et al. Combined delivery of a TGF-β inhibitor and an adenoviral vector expressing interleukin-12 potentiates cancer immunotherapy. *Acta Biomater* 2017; 61: 114–123.
11. Veinalde R, Grossardt C, Hartmann L, et al. Oncolytic measles virus encoding interleukin-12 mediates potent antitumor effects through T cell activation. *Oncoimmunology* 2017; 6: e1285992.
12. Naif HM. Association of gene polymorphism and serum levels of IL-12p40 with the susceptibility to non-Hodgkin's lymphoma in Iraq. *Journal of Contemporary Medical Sciences* 2017; 3.
13. Taheri M, Naderi M, Hashemi M, et al. Association between IL12A rs568408, IL12B rs3212227 and IL-12 receptor rs383483 polymorphisms and risk of pulmonary tuberculosis. *Archives of Clinical Infectious Diseases* 2017; 12. DOI: 10.5812/archcid.39318.
14. Paradowska-Gorycka A, Sowinska A, Stypińska B, et al. IL-12B gene polymorphisms and IL-12 p70 serum levels among patients with rheumatoid arthritis. *Scand J Immunol* 2017; 85: 147–154.
15. Zheng Y, Wang M, Tian T, et al. Role of interleukin-12 gene polymorphisms in the onset risk of cancer: a meta-analysis. *Oncotarget* 2017; 8: 29795–29807.
16. Sepesi B, Ye Y, Mitchell KG, et al. Genetic variants in cytokine signaling pathways and clinical outcomes in early-stage lung cancer patients. *J Thorac Cardiovasc Surg* 2018; 155: 2635–2645.e15.
17. Shi X, Jia Y, Xie X, et al. Single-nucleotide polymorphisms of the IL-12 gene lead to a higher cancer risk: a meta-analysis based on 22,670 subjects. *Genes Genet Syst* 2018: 92: 173–187.
18. Ben-Selma BW, Laribi AB, Alibi S, et al. Interaction analysis of IL-12A and IL-12B gene variants with chronic hepatitis B infection in Tunisian patients. *Immunol Lett* 2020; 225: 50–56.
19. Wang HW, Gao HL, Wei XX, et al. Up-regulation of IL-12 expression in patients with chronic hepatitis B is mediated by the PI3K/Akt pathway. *Mol Cell Biochem* 2015; 407: 135–142.
20. Yin D, Wang Y, Sai W, et al. HBx-induced miR-21 suppresses cell apoptosis in hepatocellular carcinoma by targeting interleukin-12. *Oncol Rep* 2016; 36: 2305–2312.
21. Elsayed HM, Nabiel Y and Sheta T. IL12 gene polymorphism in association with hepatocellular carcinoma in HCV-infected Egyptian patients. *Immunol Invest* 2017; 46: 123–133.
22. Liu L, Xu Y, Liu Z, et al. IL12 polymorphisms, HBV infection and risk of hepatocellular carcinoma in a high-risk Chinese population. *Int J Cancer* 2011; 128: 1692–1696.
23. Nieters A, Yuan JM, Sun CL, et al. Effect of cytokine genotypes on the hepatitis B virus-hepatocellular carcinoma association. *Cancer* 2005; 103: 740–748.
24. Ognjanovic S, Yuan JM, Chaptman AK, et al. Genetic polymorphisms in the cytokine genes and risk of hepatocellular carcinoma

in low-risk non-Asians of USA. *Carcinogenesis* 2009; 30: 758–762.
25. Saxena R, Chawla YK, Verma I, et al. Effect of IL-12B, IL-2, TGF-β1, and IL-4 polymorphism and expression on hepatitis B progression. *J Interferon Cytokine Res* 2014; 34: 117–128.
26. Yan Y, Xiaoqiang Q, Hongping Y, et al. Polymorphism of TGF-β1 and IL-12B gene and risk of hepatocellular cancer in Guangxi—a case-control study. *Chin J Public Health* 2011: 1383–1385.
27. Xiao Y, Liu G and Gong L. Systematic review and meta-analysis on the association between polymorphisms in genes of IL-12 signaling pathway and hepatocellular carcinoma risk. *J Cancer* 2018; 9: 3583–3592.
28. Chen H, Cheng S, Wang J, et al. Interleukin-12B rs3212227 polymorphism and cancer risk: a meta-analysis. *Mol Biol Rep* 2012;39:10235–10242.
29. Zundler S, Neurath MF. Interleukin-12: Functional activities and implications for disease. *Cytokine Growth Factor Rev* 2015;26:559–568.
30. Lampreht Tratar U, Kos S and Kamensek U. Antitumor effect of antibiotic resistance gene-free plasmids encoding interleukin-12 in canine melanoma model. *Cancer Gene Ther* 2018; 25: 260–273.
31. Zhang Y, Luo CL, He BC, et al. Exosomes derived from IL-12-anchored renal cancer cells increase induction of specific antitumor response in vitro: a novel vaccine for renal cell carcinoma. *Int J Oncol* 2010; 36: 133–140.
32. Tasaki K, Yoshida Y, Maeda T, et al. Protective immunity is induced in murine colon carcinoma cells by the expression of interleukin-12 or interleukin-18, which activate type 1 helper T cells. *Cancer Gene Ther* 2000; 7: 247–254.
33. Cao X, Leonard K, Collins LI, et al. Interleukin 12 Stimulates IFN-γ–Mediated Inhibition of Tumor-Induced Regulatory T-Cell Proliferation and Enhances Tumor Clearance. *Cancer Res* 2009; 69: 8700–8709.
34. Takaoka A, Hayakawa S, Yanai H, et al. Integration of interferon-α/β signalling to p53 responses in tumour suppression and antiviral defence. *Nature* 2003; 424: 516–523.
35. Wang Q, Cheng F, Ma TT, et al. Interleukin-12 inhibits the hepatocellular carcinoma growth by inducing macrophage polarization to the M1-like phenotype through downregulation of Stat-3. *Mol Cell Biochem* 2016; 415: 157–168.
36. Lehtonen A, Lund R, Lahesmaa R, et al. IFN-α and IL-12 activate IFN regulatory factor 1 (IRF-1), IRF-4, and IRF-8 gene expression in human NK and T cells. *Cytokine* 2003; 24: 81–90.
37. Pathak S, Ma S, Trinh L, et al. IRF4 is a suppressor of c-Myc induced B cell leukemia. *PLoS One* 2011; 6: e22628.
38. Noguchi Y, Jungbluth A, Richards EC, et al. Effect of interleukin 12 on tumor induction by 3-methylcholanthrene. *Proc Natl Acad Sci U S A* 1996; 93: 11798–11801.
39. Nanni P, Nicoletti G, De Giovanni C, et al. Combined allogeneic tumor cell vaccination and systemic interleukin 12 prevents mammary carcinogenesis in HER-2/neu transgenic mice. *J Exp Med* 2001; 194: 1195–1206.
40. Becskei A and Grusby MJ. Contribution of IL-12R mediated feedback loop to Th1 cell differentiation. *FEBS Lett* 2007; 581: 5199–5206.
41. Yoshimoto T, Takeda K, Tanaka T, et al. IL-12 up-regulates IL-18 receptor expression on T cells, Th1 cells, and B cells: synergism with IL-18 for IFN-γ production. *J Immunol* 1998; 161: 3400–3407.
42. Han SS, Cho EY, Lee TS, et al. Interleukin-12 p40 gene (IL12B) polymorphisms and the risk of cervical cancer in Korean women. *Eur J Obstet Gynecol Reprod Biol* 2008; 140: 71–75.
43. Howell WM and Rose-Zerilli MJ. Cytokine gene polymorphisms, cancer susceptibility, and prognosis. *J Nutr* 2007; 137: 194S–199S.