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Research Paper

Systematic Review and Meta-Analysis on the Association between Polymorphisms in Genes of IL-12 Signaling Pathway and Hepatocellular Carcinoma Risk

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Abstract

We performed an updated meta-analysis and systematic review to explore the associations between polymorphisms in genes of IL-12 signaling pathway and hepatocellular carcinoma (HCC) risk. Diverse databases were retrieved to identify entire available studies, and odds ratios (ORs) correspondence with 95% confidence intervals (CIs) were performed to assess their associations. Finally, 6 polymorphisms in five genes of the IL-12 signaling pathway were extracted from 39 case-control studies, 26 publications. We identified that *STAT4*-rs7574865 polymorphism was significantly associated with an increased risk of HCC in allelic contrast, dominant, homozygote and recessive models. However, we failed to uncover any significant association between other polymorphisms in genes of IL-12 signaling pathway and HCC risk, including *IL18*-rs1946518 and -rs187238, *IFN-γ*-rs2430561, *IL12A*-rs568408, *IL12B*-rs3212227 and *STAT4*-rs7574865. When the subgroup analysis was conducted based on Hardy-Weinberg Equilibrium (HWE) status, we identified that *IFN-γ*-rs2430561 polymorphism was significantly associated with an increased risk of HCC in homozygote and recessive models of these studies whose control groups were conformed to HWE. To sum up, our study suggests that *STAT4*-rs7574865 is a risk factor for HCC. Further well-designed large sample size studies are warranted to shed new light on these findings.

Key words: IL-12 signaling pathway; polymorphism; Hepatocellular Carcinoma; risk

Introduction

Primary liver cancer is the sixth most frequent cancer around the world and the second ordinary cause of cancer-related death. Of them, approximately 70 to 85% of primary liver cancer cases are hepatocellular carcinoma (HCC) [1, 2]. Due to the high infection of hepatitis B virus (HBV), the high prevalence rate of HCC was observed in East Asia, Southeast Asia and sub-Saharan Africa [3-5]. In 2015, about 500,000 individuals were newly diagnosed, and lead to 420,000 death in China [6-9].

Cytokines are a family of proteins, which are familiarly concerned with both innate and adaptive immune responses to fight against infections. With the background of chronic hepatic inflammation, cytokines comprehensively participate in tumorigenesis process, including IL-6, IL-12, IL-18 and etc. [10-12]As a key immunoregulatory cytokine, IL-12 is consisted of two subunits, IL-12-p35 and IL-12-p40, which are translated from IL-12A gene and IL-12B gene, and were link with each other through covalent bond [13, 14]. IL-12 is an early pro-inflammatory cytokine, mainly secreted by antigen-presenting cells to amplify inflammatory signals. When IL-12 binds to IL-12R complex, the JAK

kinase (Tyk-2 and Jak-2) will be activated, thus contributes to the phosphorylation of IL-12R. Study also demonstrated that tyrosine phosphorylation of STAT4 protein, another pivotal molecular of IL-12 signaling pathway, which could regulate gene transcription through DNA homodimerization or translocation in nucleus [15]. IL-12 also induces the expression of IFN-γ in T and NK cells through activating JAK/STAT4 pathway and plays a fundamental role in the differentiation of naive T cells to Th1 cells [16, 17]. As a synergistic manner, IL-18 could together with IL-12 stimulates IFN-γ production by Th1 and NK cells, in addition, IL-12 could also up-regulate IL-18R expression promoting the secreting of IFN-γ [18].

As for IL-18, which is a cytokine initially known as an inducer of IFN-γ, plays important roles during both Th1 and Th2 responses [19]. These studies demonstrated that genes of IL-12 signaling pathway could functionally work together, contributing to the anti-infection process, and dysregulation of one or more genes in this pathway potentially can influence the whole pathway and thus result in tumorigenesis process. In addition, more evidence has been pointed out that IL-12 signaling pathway plays a pivotal role during anti-HBV-infection, and might contribute to the HCC pathogenesis [20, 21].

Till now, plenty of studies have examined the associations between polymorphisms in genes of IL-12 signaling pathway and HCC risk, however, these results were controversial and inconsistent. Such as, for *IL12B*-rs3212227 polymorphism, in Yang *et al.*'s [22] work, they suggested that this genetic polymorphism may have an independent effect HCC risk in a Chinese population, on the contrary, another study showed that it has no statistically difference between HCC cases and cancer-free chronic HCV patient groups[23]. As for *STAT4*, Chanthra *et al.* [24] found out that *STAT4*-rs7574865 polymorphism was related to an increased risk of HCC progression, a results consistent with Clark *et al.*'s work [25]. However, in another study conducted by Chen *et al.*[26], they failed to validate the function of rs7574865 polymorphism in STAT4 on the risk of HCC. Due to the heterogeneity within cancer subtypes, the diverse ethnicities of patient cohorts and the small sample sizes, the studies concerned about polymorphisms in genes of IL-12 signaling pathway and HCC risk were not consistent. To overcome these limitations, we exhaustively collected all available genetic polymorphisms in genes of IL-12 signaling pathway and their relevant eligible studies about HCC risk, and performed an updated meta-analysis to comprehensively demonstrate the associations between genetic variations of genes in IL-12 signaling pathway and HCC risk.

Materials and Methods

Literature filtrating and distinguishing of relevant studies

In order to identify all available studies regarding the relationships between genetic polymorphisms in genes of IL-12 signaling pathway and HCC risk, comprehensively literature search was conducted on diverse online databases, including PubMed, Embase, Science Direct and Google Scholar published up to May 30, 2018 by applying below MeSH terms: ('genes' OR 'abbreviations of genes') AND ('cancer' OR 'adenocarcinoma' OR 'tumor' OR 'carcinoma' OR 'neoplasms') AND ('variant' OR 'mutation' OR 'polymorphism' OR 'SNP' OR 'genotype'). Language of eligible studies was restricted to English and Chinese. All of the retrieved articles were reviewed by reading the title and abstract. In addition, full texts of these possibly relevant studies were further read for suitability in current work. Furthermore, in order to identify more eligible studies, the references of each enrolled study were also searched manually.

The Criteria of Inclusion and Exclusion

Publications inclusion criteria were demonstrated as: (1) patients were diagnosed by histopathology testing, and control group should be cancer-free, age-matched and sex-matched; (2) case-control studies which focus on the associations between polymorphisms in genes of IL-12 pathway and HCC risk; (3) enrolled articles should have sufficient genotype data, in order to calculate odds ratios (ORs) and 95% confidence intervals (CIs). On the contrast, publications should be excluded when they were: (1) Reviews or conference papers; (2) only case study; or (3) have no sufficient data.

Extracting of Data and Assessing of Article Quality

Data extraction and quality evaluation of each enrolled publications were conducted by Yao Xiao and Guodong Liu, independently. All the disagreements should be solved after discussion. Furthermore, the following information will be extracted from each publication, including name of the first author, publication year, ethnicity, allele and genotype distribution and Hardy-Weinberg equilibrium (HWE).

Meta-Analysis

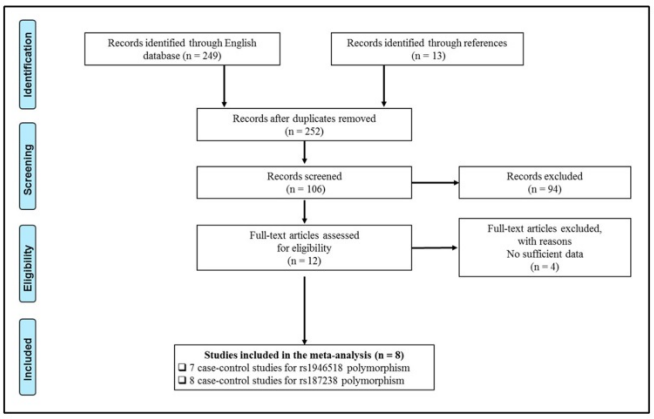
The associations between polymorphisms in gene of IL-12 signaling pathway and HCC risk was assessed by ORs and 95%CI. And the significance of

pooled ORs was determined by Z-test. Bonferroni correction was applied to adjust the *P*-value of Z-test, and *P*-adjust less than $1.67*10^{-3}$ [0.05/(five genetic models * six polymorphisms)] was considered as statistical significant[27]. Five genetic models were used to calculate their associations, including allele (M vs. W), homozygous (MM vs. WW), heterozygous (MW vs. WW), dominant (MW + MM vs. WW), and recessive models (MM vs. WW + WW) (W refers to wild allele and M refers to mutated allele). After that, stratified analyses were also conducted by different cancer type, ethnicity or source of control. Heterogeneity assumption was checked by I^2 test and Q statistic test. When $I^2 \leq 50\%$ and $P \geq 0.1$, the heterogeneity could be ignored, then, the fixed-effect model will be applied; Otherwise, the random-effect model will be selected [28]. Moreover, publication bias was appraised with the help of Egger's regression test and Begg's funnel plot, and the stability of results was confirmed by sensitivity analysis [29]. All statistical analyses were conducted by the Stata software (version 12.0; Stata Corporation, College Station, TX).

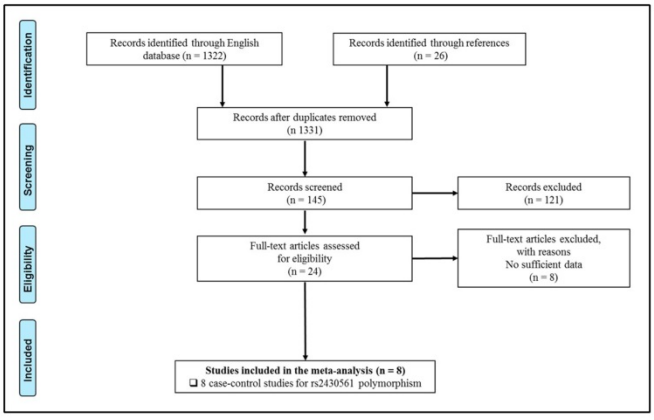
Results

Study identification and characteristics of enrolled publications

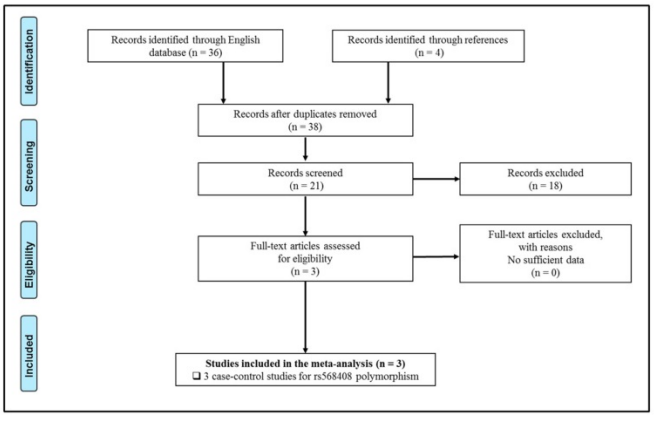
347 publications were identified after initial screening. After scoring out duplicates by reading the titles and abstracts, 310 publications were removed. Then, 37 full-text publications were assessed for eligibility. Among them, 11 publications were further excluded because relevant studies for one polymorphism were less than three. Finally, 26 publications comprising 39 case-control studies were enrolled for current meta-analysis, and the study selection process was presented in **Figure 1**.



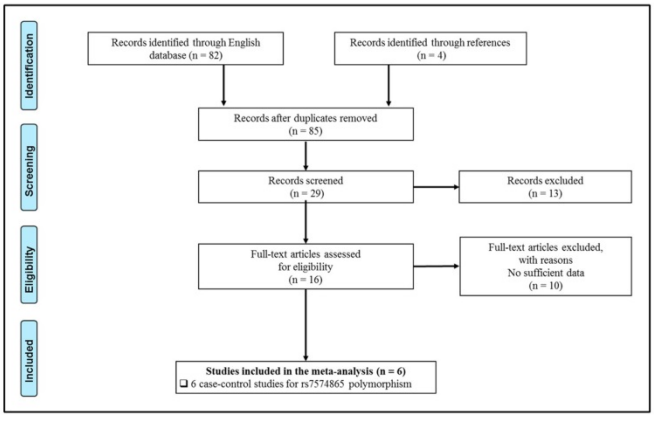
IL18



IFN-γ



IL12B



STAT4

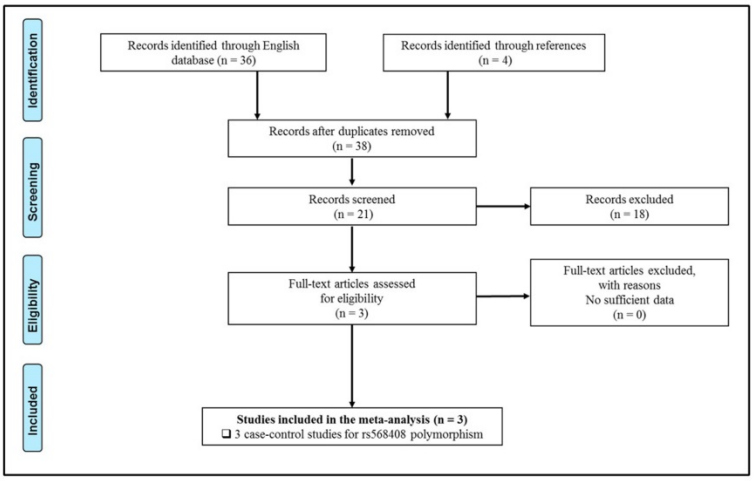


Figure 1. Study selection process for each gene enrolled.

The general demographical characteristics of all eligible publications were summarized in **Table 1**, including *IL18*-rs1946518/ rs187238 [30-37], *IFN-γ*-rs2430561 [35, 38-44], *IL12A*-rs568408 [45-47], *IL12B*-rs3212227 [42, 43, 45-49] and *STAT4*-rs7574865 [25, 26, 50-53]. In addition, the quality of each enrolled study was assessed by Newcastle-Ottawa Scale (NOS), and the outcomes were presented in (**Table S1**).

Meta-analysis

The detail results of current meta-analysis were shown in **Table 2**. The overall results suggested that *STAT4-rs7574865* polymorphism conferred a statistically increased risk of HCC in allelic (M vs. W: OR = 1.270, 95%CI = 1.166-1.384, $P_A = 1.760\times10^{-8}$), homozygous (MM vs. WW: OR = 1.651, 95%CI: 1.352-2.016, $P_A = 6.561\times10^{-7}$), recessive (MM vs. MW+WW: OR = 1.330, 95%CI = 1.168-1.516, $P_A = 4.680\times10^{-7}$) and dominant models (MW + MM vs. WW: OR = 1.470, 95%CI = 1.213-1.781, $P_A = 7.092\times10^{-5}$). Moreover, subsection analysis performed on source of control demonstrated that the P-B groups were more susceptible to develop HCC in allelic (M vs. W: OR = 1.320, 95%CI = 1.193-1.461, $P_A = 7.210\times10^{-8}$), homozygous (MM vs. WW: OR = 1.687, 95%CI: 1.326-2.146, $P_A = 1.593\times10^{-5}$), recessive (MM vs. MW+WW: OR = 1.417, 95%CI = 1.242-1.616, $P_A = 1.961\times10^{-7}$) and dominant models (MW + MM vs. WW: OR = 1.447, 95%CI = 1.148-1.824, $P_A = 1.354\times10^{-3}$, **Figure 2**), respectively. However, negative results were identified when subgroup analyses were conducted based on HWE, ethnicity and source of control.

Although overall results failed to uncover any positive association between *IL18*-rs1946518/ -rs187238, *IFN-γ*-rs2430561, *IL12A*-rs568408 and *IL12B*-rs3212227 polymorphisms and HCC risk, similar to *STAT4*-rs7574865 polymorphism, we identified that *IL18*-rs187238 polymorphism was related to an increased risk of HCC in H-B groups in allelic (M vs. W: OR = 1.604, 95%CI = 1.223-2.103, $P_A = 7.535\times10^{-4}$), heterozygous (MW vs. WW: OR = 1.665, 95%CI: 1.227-2.258, $P_A = 1.058\times10^{-3}$) and dominant models (MW + MM vs. WW: OR = 1.678, 95%CI = 1.246-2.260, $P_A = 6.950\times10^{-4}$). For *IFN-γ*-rs2430561 polymorphism, although overall analysis failed to uncover any positive result, when the subgroup analysis was conducted based on HWE status, we found that for these studies whose control groups conformed to HWE, were significantly associated with an increased risk of HCC in homozygous (MM vs. WW: OR = 2.375, 95%CI = 1.393-4.050, $P_A = 1.450\times10^{-3}$) and recessive models (MM vs. MM+MW: OR = 2.331, 95%CI = 1.471-3.691, $P_A = 2.991\times10^{-4}$), respectively.

Sensitivity Analysis and Publication Bias

Sensitivity analyses were performed to assess the impact of separate case-control study on the data pools (including *IL18*-rs1946518/rs187238, *IFN-γ*-rs2430561, *IL12A*-rs568408, *IL12B*-rs3212227 and *STAT4*-rs7574865 polymorphisms), and the results showed that the pooled ORs and 95%CIs were not been significantly influenced after removing each case-control study in sequence (**Table S2** and **Figure S1**). Moreover, to evaluate the publication bias, Begg's funnel plot and Egger's regression test were performed for each genetic polymorphism. By observing the shape of Begg's funnel plot, no evidence of publication bias was identified for any polymorphism, which was further verified by Egger's regression test (**Table S3** and **Figure S2**).

Table 1. Characteristics of eligible enrolled studies.

Gene	Polymorphism	First author	Year	Ethnicity	Source of Control	Cancer Type	Case			Control		
							WW	WM	MM	WW	WM	MM
IL18	rs1946518	Teixeira *et al.[35]*	2013	mixed	P-B	HCC	38	56	18	85	105	12
	rs1946518	Lau *et al.*[27]	2016	Asian	H-B	HCC	88	167	87	148	276	135
	rs1946518	Bao *et al.*[25]	2015	Asian	P-B	HCC	37	73	43	41	76	48
	rs1946518	Migita *et al.*[29]	2009	Asian	P-B	HCC	13	26	8	20	30	13
	rs1946518	Chen *et al.*[26]	2012	Asian	P-B	HCC	47	126	55	83	156	61
	rs1946518	Karra *et al.*[28]	2015	African	P-B	HCC	70	152	49	102	144	34
	rs1946518	Zhang *et al.*[32]	2016	Asian	P-B	HCC	32	55	22	23	66	38
	rs187238	Kim *et al.*[31]	2009	Asian	H-B	HCC	37	17	2	434	122	2
	rs187238	Teixeira *et al.[35]*	2013	mixed	P-B	HCC	57	48	7	100	84	18
	rs187238	Lau *et al.* [27]	2016	Asian	H-B	HCC	266	73	3	476	78	5
	rs187238	Bao *et al.* [25]	2015	Asian	P-B	HCC	122	28	3	106	54	5
	rs187238	Migita *et al.* [29]	2009	Asian	P-B	HCC	43	3	1	52	10	1
	rs187238	Chen *et al.* [26]	2012	Asian	P-B	HCC	159	59	10	173	115	12
	rs187238	Karra *et al.* [28]	2015	African	P-B	HCC	123	134	14	159	108	13
	rs187238	Zhang *et al.* [32]	2016	Asian	P-B	HCC	82	25	2	99	24	4
IFN-γ	rs2430561	Teixeira *et al.*[30]	2013	Caucasian	P-B	HCC	40	50	21	79	82	41
	rs2430561	Kim *et al.*[35]	2013	Asian	H-B	HCC	133	31	6	131	38	2
	rs2430561	Migita *et al.*[36]	2005	Asian	H-B	HCC	41	7	0	157	31	0
	rs2430561	Ben-Ari *et al.*[39]	2003	Caucasian	P-B	HCC	3	7	0	18	24	6
	rs2430561	Nieters *et al.*[37]	2005	Asian	H-B	HCC	155	94		164	86	
	rs2430561	Saxena *et al.*[38]	2014	Asian	P-B	HCC	15	28	16	52	77	17
	rs2430561	Bouzgarrou *et al.*[34]	2009	African	P-B	HCC	17	21	20	33	47	23
	rs2430561	Bahgat *et al.*[33]	2015	Egyptian	P-B	HCC	10	24	16	6	15	4
IL12A	rs568408	Elsayed *et al.*[40]	2016	Egyptian	P-B	HCC	42	26	10	84	7	1
	rs568408	Tan *et al.*[42]	2015	Asian	P-B	HCC	313	76	6	511	161	14
	rs568408	Liu *et al.*[41]	2011	Asian	P-B	HCC	504	277	21	631	220	10
IL12B	rs3212227	Saxena *et al.*[38]	2014	Asian	P-B	HCC	19	31	9	63	71	14
	rs3212227	Elsayed *et al.*[40]	2016	Egyptian	P-B	HCC	41	22	15	38	40	14
	rs3212227	Nieters *et al.*[37]	2005	Asian	H-B	HCC	56	193		72	178	
	rs3212227	Tan *et al.*[42]	2015	Asian	P-B	HCC	104	201	90	200	347	139
	rs3212227	Ognjanovic *et al.*[43]	2009	mixed	P-B	HCC	57	60		128	95	
	rs3212227	Liu *et al.*[41]	2011	Asian	P-B	HCC	249	422	160	272	414	158
	rs3212227	Yang *et al.*[44]	2011	Asian	H-B	HCC	156	309	143	195	302	115
STAT4	rs7574865	Chanthra *et al.*[45]	2015	Asian	P-B	HCC	19	86	87	28	100	62
	rs7574865	Chen *et al.*[21]	2013	Asian	H-B	HCC	35	217	249	75	327	370
	rs7574865	Chen *et al.*[46]	2015	Asian	P-B	HCC	40	211	257	343	1333	1298
	rs7574865	Clark *et al.*[20]	2013	Asian	H-B	HCC	20	102	117	28	92	86
	rs7574865	Kim *et al.*[47]	2015	Asian	P-B	HCC	20	103	160	306	1251	1293
	rs7574865	Liao *et al.*[48]	2014	Asian	P-B	HCC	25	93	104	27	113	97

P-B: population-based; H-B: hospital-based; HCC: Hepatocellular Carcinoma; W: wild allele; M: mutant allele.

Table 2. Results of meta-analysis.

Gene	Polymorphism	Comparison	Subgroup	N	P_H	P_A	Random	Fixed
IL18	rs187238	M vs. W	Overall	8	0.000	9.976×10^{-1}	1.000 (0.736-1.359)	1.033 (0.904-1.181)
	rs187238	M vs. W	Asian	6	0.000	8.103×10^{-1}	0.948 (0.615-1.462)	0.951 (0.801-1.130)
	rs187238	M vs. W	H-B	2	0.577	**7.535×10^{-4}**	1.604 (1.223-2.103)	1.597 (1.216-2.096)
	rs187238	M vs. W	P-B	6	0.002	3.018×10^{-1}	0.841 (0.605-1.168)	0.906 (0.777-1.056)
	rs187238	M vs. W	Y	7	0.000	6.355×10^{-1}	0.926 (0.674-1.272)	0.997 (0.868-1.145)
	rs187238	WM vs. WW	Overall	8	0.000	9.445×10^{-1}	0.986 (0.657-1.480)	1.054 (0.897-1.240)
	rs187238	WM vs. WW	Asian	6	0.000	6.487×10^{-1}	0.881 (0.509-1.522)	0.924 (0.757-1.129)
	rs187238	WM vs. WW	H-B	2	0.946	**1.058×10^{-3}**	1.665 (1.227-2.258)	1.665 (1.227-2.259)
	rs187238	WM vs. WW	P-B	6	0.000	4.054×10^{-1}	0.814 (0.501-1.322)	0.887 (0.733-1.074)
	rs187238	WM vs. WW	Y	7	0.000	7.038×10^{-1}	0.917 (0.588-1.432)	1.023 (0.865-1.209)
	rs187238	WM+MM vs. WW	Overall	8	0.000	9.645×10^{-1}	0.991 (0.671-1.463)	1.050 (0.897-1.228)
	rs187238	WM+MM vs. WW	Asian	6	0.000	7.008×10^{-1}	0.903 (0.535-1.522)	0.933 (0.769-1.132)
	rs187238	WM+MM vs. WW	H-B	2	0.790	**6.950×10^{-4}**	1.678 (1.246-2.260)	1.676 (1.243-2.258)
	rs187238	WM+MM vs. WW	P-B	6	0.000	3.724×10^{-1}	0.815 (0.519-1.278)	0.883 (0.735-1.062)
	rs187238	WM+MM vs. WW	Y	7	0.000	6.719×10^{-1}	0.913 (0.601-1.389)	1.011 (0.860-1.189)
	rs187238	MM vs. WW	Overall	8	0.298	9.495×10^{-1}	1.034 (0.641-1.667)	0.987 (0.654-1.488)
	rs187238	MM vs. WW	Asian	6	0.212	8.754×10^{-1}	1.086 (0.516-2.283)	0.955 (0.540-1.691)
	rs187238	MM vs. WW	H-B	2	0.051	3.368×10^{-1}	3.216 (0.296-34.892)	1.934 (0.618-6.050)
	rs187238	MM vs. WW	P-B	6	0.806	6.383×10^{-1}	0.906 (0.58-1.416)	0.900 (0.579-1.398)
	rs187238	MM vs. WW	Y	7	0.885	6.731×10^{-1}	0.920 (0.600-1.408)	0.913 (0.599-1.392)
	rs187238	MM vs. WM+WW	Overall	8	0.431	9.058×10^{-1}	1.012 (0.671-1.526)	0.976 (0.650-1.464)
	rs187238	MM vs. WM+WW	Asian	6	0.299	8.563×10^{-1}	1.145 (0.587-2.234)	1.054 (0.598-1.857)
	rs187238	MM vs. WM+WW	H-B	2	0.055	3.757×10^{-1}	2.878 (0.278-29.858)	1.777 (0.570-5.542)
	rs187238	MM vs. WM+WW	P-B	6	0.921	6.329×10^{-1}	0.906 (0.584-1.405)	0.900 (0.583-1.389)
	rs187238	MM vs. WM+WW	Y	7	0.963	6.419×10^{-1}	0.912 (0.600-1.388)	0.906 (0.598-1.373)

Gene	Polymorphism	Comparison	Subgroup	N	P_H	P_A	Random	Fixed
	rs1946518	W vs. M	Overall	7	0.012	2.739×10^{-1}	1.107 (0.923-1.329)	1.125 (1.014-1.249)
	rs1946518	W vs. M	Asian	5	0.083	9.618×10^{-1}	0.995 (0.822-1.206)	1.024 (0.905-1.158)
	rs1946518	W vs. M	P-B	6	0.009	3.427×10^{-1}	1.117 (0.889-1.404)	1.163 (1.027-1.317)
	rs1946518	W vs. M	Y	6	0.019	5.521×10^{-1}	1.060 (0.875-1.285)	1.094 (0.980-1.220)
	rs1946518	WM vs. WW	Overall	7	0.248	7.791×10^{-2}	1.162 (0.945-1.430)	1.169 (0.983-1.391)
	rs1946518	WM vs. WW	Asian	5	0.269	5.398×10^{-1}	1.063 (0.823-1.373)	1.069 (0.864-1.322)
	rs1946518	WM vs. WW	P-B	6	0.229	4.382×10^{-2}	1.206 (0.939-1.549)	1.235 (1.006-1.517)
	rs1946518	WM vs. WW	Y	6	0.164	1.043×10^{-1}	1.152 (0.900-1.473)	1.166 (0.969-1.404)
	rs1946518	WM+MM vs. WW	Overall	7	0.057	2.034×10^{-1}	1.176 (0.916-1.510)	1.202 (1.019-1.417)
	rs1946518	WM+MM vs. WW	Asian	5	0.112	5.578×10^{-1}	1.035 (0.768-1.396)	1.062 (0.869-1.297)
	rs1946518	WM+MM vs. WW	P-B	6	0.051	2.341×10^{-1}	1.203 (0.887-1.633)	1.275 (1.048-1.550)
	rs1946518	WM+MM vs. WW	Y	6	0.038	3.901×10^{-1}	1.135 (0.850-1.515)	1.176 (0.987-1.401)
	rs1946518	MM vs. WW	Overall	7	0.003	2.675×10^{-1}	1.268 (0.833-1.931)	1.269 (1.024-1.573)
	rs1946518	MM vs. WW	Asian	5	0.075	9.398×10^{-1}	0.985 (0.663-1.463)	1.043 (0.815-1.335)
	rs1946518	MM vs. WW	P-B	6	0.002	3.262×10^{-1}	1.307 (0.766-2.231)	1.369 (1.054-1.777)
	rs1946518	MM vs. WW	Y	6	0.016	5.732×10^{-1}	1.123 (0.751-1.679)	1.182 (0.946-1.477)
	rs1946518	MM vs. WM+WW	Overall	7	0.029	3.453×10^{-1}	1.155 (0.856-1.559)	1.141 (0.953-1.366)
	rs1946518	MM vs. WM+WW	Asian	5	0.350	9.856×10^{-1}	0.996 (0.799-1.240)	1.002 (0.818-1.227)
	rs1946518	MM vs. WM+WW	P-B	6	0.017	4.088×10^{-1}	1.179 (0.797-1.744)	1.178 (0.945-1.469)
	rs1946518	MM vs. WM+WW	Y	6	0.183	4.279×10^{-1}	1.062 (0.833-1.353)	1.078 (0.895-1.298)
IFN-γ	rs2430561	W vs. M	Overall	5	0.392	3.251×10^{-2}	1.239 (1.016-1.511)	1.237 (1.018-1.503)
	rs2430561	W vs. M	Asian	2	0.126	6.921×10^{-2}	1.328 (0.822-2.145)	1.336 (0.977-1.825)
	rs2430561	W vs. M	Caucasian	2	0.344	4.878×10^{-1}	1.111 (0.824-1.499)	1.111 (0.825-1.498)
	rs2430561	W vs. M	P-B	4	0.338	2.146×10^{-2}	1.300 (1.030-1.641)	1.288 (1.038-1.598)
	rs2430561	W vs. M	Y	4	0.489	1.284×10^{-2}	1.359 (1.068-1.730)	1.358 (1.067-1.728)
	rs2430561	WM vs. WW	Overall	5	0.796	9.487×10^{-1}	1.009 (0.752-1.355)	1.010 (0.753-1.354)
	rs2430561	WM vs. WW	Asian	2	0.324	7.893×10^{-1}	0.942 (0.614-1.445)	0.944 (0.617-1.443)
	rs2430561	WM vs. WW	Caucasian	2	0.734	5.353×10^{-1}	1.162 (0.722-1.870)	1.162 (0.723-1.869)
	rs2430561	WM vs. WW	P-B	4	0.884	5.403×10^{-1}	1.116 (0.784-1.589)	1.117 (0.785-1.589)
	rs2430561	WM vs. WW	Y	4	0.799	6.838×10^{-1}	0.928 (0.648-1.327)	0.929 (0.650-1.326)
	rs2430561	WM+MM vs. WW	Overall	8	0.943	2.454×10^{-1}	1.131 (0.916-1.397)	1.133 (0.918-1.398)
	rs2430561	WM+MM vs. WW	Asian	4	0.544	4.033×10^{-1}	1.114 (0.859-1.445)	1.116 (0.862-1.445)
	rs2430561	WM+MM vs. WW	Caucasian	3	0.959	4.531×10^{-1}	1.176 (0.770-1.797)	1.176 (0.770-1.797)
	rs2430561	WM+MM vs. WW	H-B	3	0.688	7.673×10^{-1}	1.044 (0.788-1.383)	1.043 (0.788-1.381)
	rs2430561	WM+MM vs. WW	P-B	5	0.938	1.585×10^{-1}	1.255 (0.911-1.727)	1.258 (0.914-1.730)
	rs2430561	WM+MM vs. WW	Y	5	0.755	3.910×10^{-1}	1.148 (0.828-1.594)	1.153 (0.833-1.596)
	rs2430561	WM+MM vs. WW	Overall	5	0.768	3.459×10^{-1}	1.138 (0.864-1.498)	1.141 (0.867-1.500)
	rs2430561	WM+MM vs. WW	Asian	2	0.181	5.545×10^{-1}	1.162 (0.665-2.032)	1.129 (0.755-1.687)
	rs2430561	WM+MM vs. WW	Caucasian	2	0.872	5.184×10^{-1}	1.157 (0.744-1.802)	1.157 (0.743-1.802)
	rs2430561	WM+MM vs. WW	P-B	4	0.853	1.790×10^{-1}	1.248 (0.899-1.731)	1.251 (0.902-1.734)
	rs2430561	WM+MM vs. WW	Y	4	0.610	4.380×10^{-1}	1.137 (0.812-1.591)	1.141 (0.817-1.593)
	rs2430561	MM vs. WW	Overall	5	0.271	1.164×10^{-2}	1.777 (1.088-2.903)	1.683 (1.123-2.523)
	rs2430561	MM vs. WW	Asian	2	0.916	3.909×10^{-3}	3.188 (1.460-6.964)	3.177 (1.449-6.968)
	rs2430561	MM vs. WW	Caucasian	2	0.298	6.153×10^{-1}	1.187 (0.615-2.293)	1.163 (0.645-2.098)
	rs2430561	MM vs. WW	P-B	4	0.198	2.618×10^{-2}	1.725 (0.989-3.011)	1.610 (1.058-2.449)
	rs2430561	MM vs. WW	Y	4	0.754	**1.450×10^{-3}**	2.375 (1.393-4.050)	2.372 (1.394-4.035)
	rs2430561	MM vs. WM+WW	Overall	5	0.144	7.219×10^{-3}	1.785 (1.075-2.966)	1.624 (1.140-2.313)
	rs2430561	MM vs. WM+WW	Asian	2	0.920	2.773×10^{-3}	2.871 (1.438-5.731)	2.880 (1.440-5.760)
	rs2430561	MM vs. WM+WW	Caucasian	2	0.151	6.576×10^{-1}	1.294 (0.512-3.269)	1.123 (0.671-1.880)
	rs2430561	MM vs. WM+WW	P-B	4	0.102	1.646×10^{-2}	1.713 (0.979-2.997)	1.562 (1.085-2.248)
	rs2430561	MM vs. WM+WW	Y	4	0.847	**2.991×10^{-4}**	2.331 (1.471-3.691)	2.333 (1.474-3.693)
STAT4	rs7574865	W vs. M	Overall	6	0.395	**1.760×10^{-8}**	1.270 (1.166-1.384)	1.272 (1.170-1.382)
	rs7574865	W vs. M	H-B	2	0.300	3.909×10^{-2}	1.172 (1.003-1.370)	1.169 (1.008-1.356)
	rs7574865	W vs. M	P-B	4	0.503	**7.210×10^{-8}**	1.320 (1.193-1.461)	1.321 (1.194-1.462)
	rs7574865	WM vs. WW	Overall	6	0.845	9.838×10^{-3}	1.302 (1.064-1.592)	1.303 (1.066-1.593)
	rs7574865	WM vs. WW	H-B	2	0.824	3.922×10^{-2}	1.462 (1.020-2.097)	1.461 (1.019-2.096)
	rs7574865	WM vs. WW	P-B	4	0.706	8.236×10^{-2}	1.234 (0.968-1.574)	1.239 (0.973-1.577)
	rs7574865	WM+MM vs. WW	Overall	6	0.841	**7.092×10^{-5}**	1.470 (1.213-1.781)	1.474 (1.217-1.785)
	rs7574865	WM+MM vs. WW	H-B	2	0.624	1.768×10^{-2}	1.520 (1.077-2.145)	1.518 (1.075-2.143)
	rs7574865	WM+MM vs. WW	P-B	4	0.623	**1.354×10^{-3}**	1.447 (1.148-1.824)	1.456 (1.157-1.833)
	rs7574865	MM vs. WW	Overall	6	0.755	**6.561×10^{-7}**	1.651 (1.352-2.016)	1.658 (1.359-2.024)
	rs7574865	MM vs. WW	H-B	2	0.479	1.355×10^{-2}	1.574 (1.101-2.251)	1.571 (1.098-2.248)
	rs7574865	MM vs. WW	P-B	4	0.564	**1.593×10^{-5}**	1.687 (1.326-2.146)	1.696 (1.334-2.156)
	rs7574865	MM vs. WM+WW	Overall	6	0.249	**4.680×10^{-7}**	1.330 (1.168-1.516)	1.322 (1.186-1.473)
	rs7574865	MM vs. WM+WW	H-B	2	0.324	1.889×10^{-1}	1.138 (0.938-1.380)	1.138 (0.938-1.380)
	rs7574865	MM vs. WM+WW	P-B	4	0.518	**1.961×10^{-7}**	1.417 (1.242-1.616)	1.417 (1.243-1.617)
IL12A	rs568408	W vs. M	Overall	3	0.000	1.096×10^{-1}	1.926 (0.863-4.299)	1.363 (1.180-1.574)
	rs568408	W vs. M	Asian	2	0.000	7.654×10^{-1}	1.105 (0.573-2.130)	1.235 (1.064-1.434)
	rs568408	WM vs. WW	Overall	3	0.000	1.450×10^{-1}	1.804 (0.816-3.988)	1.342 (1.135-1.587)
	rs568408	WM vs. WW	Asian	2	0.000	7.665×10^{-1}	1.112 (0.552-2.242)	1.245 (1.048-1.479)
	rs568408	WM+MM vs. WW	Overall	3	0.000	1.193×10^{-1}	1.982 (0.838-4.689)	1.386 (1.178-1.631)
	rs568408	WM+MM vs. WW	Asian	2	0.000	7.580×10^{-1}	1.123 (0.538-2.345)	1.261 (1.066-1.492)
	rs568408	WM+MM vs. WW	Overall	3	0.000	1.193×10^{-1}	1.982 (0.838-4.689)	1.386 (1.178-1.631)

Gene	Polymorphism	Comparison	Subgroup	N	P_H	P_A	Random	Fixed
	rs568408	WM+MM vs. WW	Asian	2	0.000	7.580×10^{-1}	1.123 (0.538-2.345)	1.261 (1.066-1.492)
	rs568408	MM vs. WW	Overall	3	0.007	2.029×10^{-1}	2.574 (0.601-11.033)	2.151 (1.285-3.603)
	rs568408	MM vs. WW	Asian	2	0.035	6.075×10^{-1}	1.404 (0.384-5.130)	1.576 (0.897-2.771)
	rs568408	MM vs. WM+WW	Overall	3	0.025	2.298×10^{-1}	2.158 (0.615-7.568)	1.957 (1.160-3.300)
	rs568408	MM vs. WM+WW	Asian	2	0.072	5.886×10^{-1}	1.356 (0.450-4.085)	1.487 (0.843-2.622)
IL12B	rs3212227	W vs. M	Overall	5	0.301	6.458×10^{-3}	1.129 (1.021-1.247)	1.127 (1.034-1.228)
	rs3212227	W vs. M	Asian	4	0.344	3.593×10^{-3}	1.141 (1.039-1.254)	1.139 (1.043-1.243)
	rs3212227	W vs. M	P-B	4	0.439	1.334×10^{-1}	1.081 (0.977-1.197)	1.081 (0.976-1.197)
	rs3212227	WM vs. WW	Overall	5	0.156	7.995×10^{-2}	1.115 (0.914-1.360)	1.133 (0.985-1.302)
	rs3212227	WM vs. WW	Asian	4	0.769	2.749×10^{-2}	1.174 (1.018-1.354)	1.174 (1.018-1.354)
	rs3212227	WM vs. WW	P-B	4	0.138	3.564×10^{-1}	1.048 (0.805-1.365)	1.080 (0.917-1.273)
	rs3212227	WM+MM vs. WW	Overall	7	0.282	2.390×10^{-3}	1.210 (1.049-1.394)	1.205 (1.068-1.360)
	rs3212227	WM+MM vs. WW	Asian	5	0.624	1.947×10^{-3}	1.224 (1.077-1.391)	1.224 (1.077-1.391)
	rs3212227	WM+MM vs. WW	H-B	2	0.906	3.978×10^{-3}	1.366 (1.105-1.688)	1.366 (1.105-1.688)
	rs3212227	WM+MM vs. WW	P-B	5	0.243	8.896×10^{-2}	1.139 (0.941-1.378)	1.136 (0.981-1.315)
	rs3212227	WM+MM vs. WW	Overall	5	0.181	1.900×10^{-2}	1.162 (0.970-1.392)	1.170 (1.026-1.335)
	rs3212227	WM+MM vs. WW	Asian	4	0.537	6.362×10^{-3}	1.206 (1.054-1.381)	1.207 (1.054-1.381)
	rs3212227	WM+MM vs. WW	P-B	4	0.221	2.023×10^{-1}	1.096 (0.884-1.358)	1.106 (0.947-1.291)
	rs3212227	MM vs. WW	Overall	5	0.429	6.126×10^{-3}	1.278 (1.073-1.522)	1.277 (1.072-1.520)
	rs3212227	MM vs. WW	Asian	4	0.323	5.045×10^{-3}	1.301 (1.067-1.586)	1.291 (1.080-1.543)
	rs3212227	MM vs. WW	P-B	4	0.605	1.250×10^{-1}	1.179 (0.957-1.451)	1.177 (0.956-1.448)
	rs3212227	MM vs. WM+WW	Overall	5	0.629	3.630×10^{-2}	1.176 (1.011-1.367)	1.175 (1.010-1.367)
	rs3212227	MM vs. WM+WW	Asian	4	0.476	4.563×10^{-2}	1.170 (1.004-1.365)	1.170 (1.003-1.364)
	rs3212227	MM vs. WM+WW	P-B	4	0.681	2.405×10^{-1}	1.116 (0.931-1.336)	1.114 (0.930-1.335)

P-B: population-based; H-B: hospital-based; W: wild allele; M: mutant allele; HWE: Hardy-Weinberg equilibrium (Y: conform to HWE; N: not conform to HWE). Characters with bold mean statistically significant.

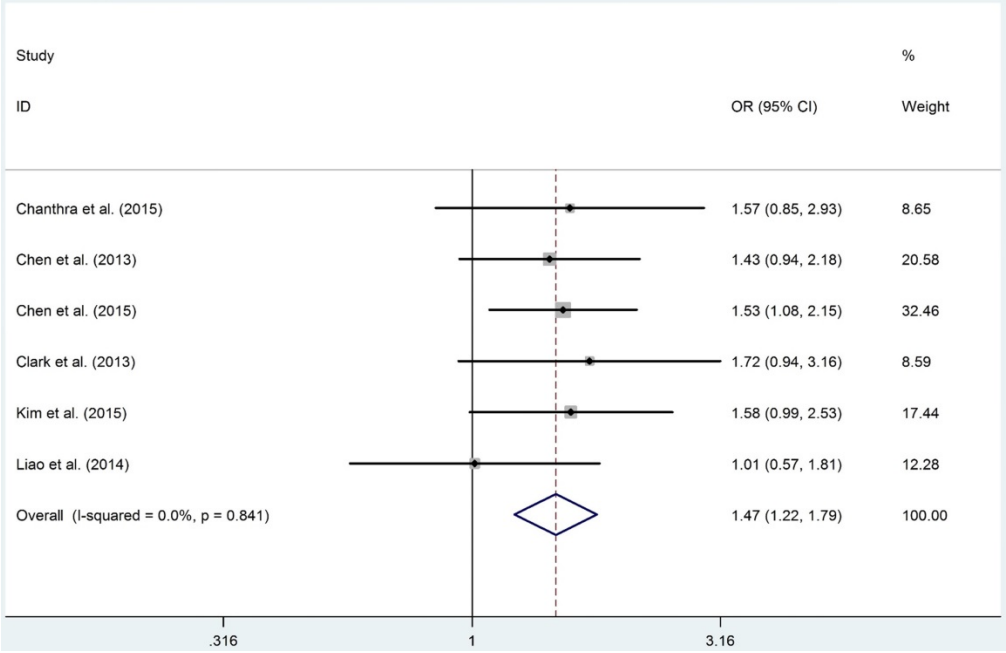


Figure 2. Forest plot of STAT4 gene rs7574865 polymorphism in dominant model (MW+MM vs. WW).

Discussion

IL-12 coordinates innate and adaptive immune responses in human beings and is regarded as an important immunomodulatory cytokine in immune system. STAT4 promotes the differentiation of naive $CD4^+$ T cells into Th-1 and cytotoxicity of NK cells, as well as the T cell proliferation [54], and its activation is mainly triggered by IL-12 signaling. In addition, IL-12 and IL-12R complex could functionally promote the phosphorylation of Jak kinase, promoting cell growth [55]. These genes are key genes of IL12 signaling pathway and could functionally work together to exert their function.

It was worth noting that HBV carriers have a greater than 100-fold increased relative risk of developing the HCC [56], and evidence has pointed out that IL-12 signaling pathway plays a pivotal role during anti-HBV-infection, and even on HCC tumorigenesis. In addition, evidence also suggested that genetic variations in genes of IL-12 signaling pathway were associated with HCC risk. Nevertheless, till now, no consistent conclusions had been acquired. Therefore, we collected all the

available studies and conducted current updated meta-analysis to comprehensively validate the associations between genetic polymorphisms in genes of IL-12 signaling pathway and HCC risk, trying to identify more genetic markers for the screening of HCC.

Here, we identified that *STAT4-rs7574865* polymorphism conferred a statistically increased risk of HCC. As for *IFN-γ*-rs2430561 polymorphism, although overall analysis failed to identify any positive result, we found that for these studies whose control groups conformed to HWE, were significantly associated with an increased risk of HCC. Besides, subgroup analyses based on source of control also identified that the P-B groups were more susceptible to develop HCC in allelic, homozygous, recessive and dominant models for *STAT4-rs7574865* polymorphism, while H-B groups were more susceptible to HCC risk for *IL18*-rs187238 polymorphism in allelic, heterozygous and dominant models, respectively, suggesting that the source of control was also one of the bias influencer.

STAT4 is the key member of STAT protein family, which could transduce signals of cytokine-receptor complexes, and could regulate the transcription of several genes. Through JAK/STAT signaling pathway, IL-12, IL-23 and IFN-1 could induce the response of STAT4, furthermore, the transcription and expression of a variety of genes would be regulated [57-60]. Currently, the influence of *STAT4*-rs7574865 polymorphism on HCC tumorigenesis have been performed on several previous studies, but the results were contraverial[25, 50, 51]. The current analysis revealed that the "M" allele of *STAT4*-rs7574865 polymorphism conferred to an increased risk of HCC. In addition, the pooled results also demonstrated that MM mutant genotype was 1.651 and 1.330-fold increased risk of HCC than WW and MW+WW genotypes, respectively.

IFN-γ plays a critical role in liver function, and it could impact the apoptosis and regeneration of hepatocyte [61]. The balance of STAT4 depended IFN-γ expression could affect both the antiviral and antitumor processes [62]. In previous study, some incompatible correlations were found between variants in *IFN-γ* gene and the risk of HCC. Saxena *et al.* [43] reported that the wild genotype (TT) distribution of *IFN-* γ-rs2430561 polymorphism had the highest frequency for HCC group (27.12 %), and was significantly higher than controls. On the contrast, no statistically significant difference in *IFN*-γ-rs2430561 genotype frequency was presented between chronic hepatitis patients and cirrhotic/HCC group in the study conducted by Bahgat *et al.*[38]. In current work, the overall pooled results suggested that there was no statistical connection between rs2430561 polymorphism and HCC risk, while the further subgroup analysis by HWE status found that for these studies whose controls conformed to HWE were significantly associated with an increased risk of HCC, suggesting that HWE status influenced the overall results, causing potential bias.

In this meta-analysis, publication retrieval was carefully done according to the pre-set strict inclusion standards. The advantages of current work should not be buried. Firstly, this is the first study concerned the relationships between all the available genetic polymorphisms in genes of IL-12 signaling pathway and HCC risk. Secondly, we used NOS form to evaluate the quality of each registered study, and low quality studies will be eliminated to further raise the credibility of pooled results. Thirdly, stratification analyses were performed based on ethnicity, source of controls and ethnicity, to decrease the impact of heterogeneity sources, thus we could obtain more accurate results. Fourthly, recognized formula was used to adjust the results, avoiding false positive results. Fifthly, sensitivity analysis was conducted to confirm the stability of current conclusions, and Egger's test and Begg's funnel plot were carried out to detect potential publication bias. On the contrast, several disadvantages should also be listed here. In the first place, there were no sufficient samples for several genetic polymorphisms, which might provide an untrustworthy result. What's more, we only enrolled publications written in English or Chinese, missing publications from other languages may cause potential bias. Last but not the least, we failed to obtain the detail histological subtypes of HCC patients, therefore, stratification analysis based on histological type cannot be conducted.

To conclude, the present meta-analysis suggests that the *STAT4*-rs7574865 polymorphism is a risk factor for HCC patients.

Supplementary Material

Supplementary figures and tables.
http://www.jcancer.org/v09p3583s1.pdf

Acknowledgments

Study design: L.G., Y.X.; Performed the study: Y.X. and G.L.; Analyzed the data: Y.X. and G.L.; Wrote and revised the paper: Y.X., G.L. and L.G.

Competing Interests

The authors have declared that no competing interest exists.

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