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BRIEF ARTICLE

Interleukin-10 gene polymorphisms and hepatocellular carcinoma susceptibility: A meta-analysis

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Abstract

AIM: To assess the association between *Interleukin-10* (*IL-10*) gene *IL-10-1082* (*G/A*), *IL-10-592*(*C/A*), *IL-10-819* (*T/C*) polymorphisms and hepatocellular carcinoma (HCC) susceptibility.

METHODS: Two investigators independently searched the Medline, Embase, China National Knowledge Infrastructure, and Chinese Biomedicine Database. Summary odds ratios (ORs) and 95% confidence intervals (95% CIs) for IL-10 polymorphisms and HCC were calculated in a fixed-effects model (the Mantel-Haenszel method) and a random-effects model (the DerSimonian and Laird method) when appropriate.

RESULTS: This meta-analysis included seven eligible studies, which included 1012 HCC cases and 2308 controls. Overall, IL-10-1082 G/A polymorphism was not associated with the risk of HCC (AA *vs* AG + GG, OR = 1.11, 95% CI = 0.90-1.37). When stratifying for ethnicity, the results were similar (Asian, OR = 1.12, 95% CI = 0.87-1.44; non-Asian, OR = 1.10, 95% CI = 0.75-1.60). In the overall analysis, the IL-10 polymorphism at position -592 (C/A) was identified as a genetic risk factor for HCC among Asians; patients carrying the IL-10-592*C allele had an increased risk of HCC (OR = 1.29, 95% CI = 1.12-1.49). No association was observed between the IL-10-819 T/C polymorphism and HCC susceptibility (TT *vs* TC + CC, OR = 1.02, 95% CI = 0.79-1.32).

CONCLUSION: This meta-analysis suggests that IL-10-592 A/C polymorphism may be associated with HCC among Asians. IL-10-1082 G/A and IL-10-819 T/C polymorphisms were not detected to be related to the risk for HCC.

Key words: Hepatocellular carcinoma; Interleukin-10; Gene polymorphism; Meta-analysis

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INTRODUCTION

Hepatocellular carcinoma (HCC), which is the fifth most common cancer and the third leading cause of cancer-

related death worldwide, is a global health problem[1,2]. The estimated annual number of cases exceeds 500 000, with a mean annual incidence of around 3%-4%[3]. Patients with HCC have a poor prognosis, with a five-year survival rate of 5% in developing countries in 2002[1] because of the lack of effective therapy in most patients[4]. Aetiologically, carcinogenesis of HCC is a complex, multistep and multifactor process, in which many factors are implicated. As we know, chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) is the most well-established environmental risk factor for HCC worldwide. However, only a fraction of HBsAg carriers eventually develop HCC and only 2.5% of HCV infected individuals develop HCC later in life[5]. The exact mechanism of hepatocarcinogenesis is still incompletely understood, and the risk factors for HCC still need to be further elucidated.

Interleukin-10 (IL-10), whose encoding gene is located on chromosome 1 (1q31-1q32), is an immunoregulatory cytokine produced by Th2 cells, monocytes/macrophages, and regulatory T cells. It plays an anti-inflammatory action by inhibiting the synthesis of cytokines such as IL-1α, IL-1β, IL-6, IL-8, IL-12 and tumor necrosis factor-alpha (TNF-α) in activated macrophage and interferon gamma (IFNγ) by T cells[6], and it also has some antifibrotic properties[7]. The risk for HCC increases with the severity of hepatic inflammation and chronic inflammation developing through the action of various inflammatory mediators is known as a cofactor of carcinogenesis[8,9]. It also has been suggested that chronically HCV-infected patients who received either short- or long-term therapy with recombinant IL-10 showed a decrease in hepatic inflammation and fibrosis[10,11]. The production of cytokines (including IL-10) is under genetic control and varies among individuals as a function of polymorphisms within the regulatory regions of the various genes that determine the transcriptional activation[12-15]. The promoter of the *IL-10* gene contains three biallelic polymorphisms at positions -1082 (base G to A, dbSNP No. rs1800896), -819 (base C to T, dbSNP No. rs1800871), and -592 (base C to A, dbSNP No. rs1800872) from the transcription start site, and these influence the capacity to produce IL-10[16]. *IL-10* gene polymorphisms have been reported to be associated with breast cancer[17], cervical cancer[18], multiple myeloma[19], cutaneous malignant melanoma[20], oral squamous cell carcinoma[21] and gastric carcinoma[22].

Over the last decade, a number of studies have assessed the association between the *IL-10* gene polymorphism and HCC risk in different populations; however, the results are inconsistent and inconclusive[23-29]. Different methodologies have been used, but, in particular, most of the studies used a small sample size and it is therefore not surprising that there has been a lack of replication in the various studies. By using all the available published data to increase the statistical power, it was hypothesized that a meta-analysis might allow plausible candidate genes to be excluded and causative genes to be identified with reliability. We have therefore taken a meta-analysis in which all the published case-control studies are processed to confirm whether the polymorphisms of *IL-10* gene promoter increased the risk of HCC.

MATERIALS AND METHODS

Literature search strategy

We searched the PubMed, Embase, China National Knowledge Infrastructure and Chinese Biomedicine databases for all articles on the association between IL-10 polymorphisms and HCC risk (last search update 30th November 2010). The following key words were used: "Interleukin-10" or "IL-10", "liver cancer" or "hepatocellular carcinoma", and "polymorphism" or "variant". The search was without restriction on language, conducted on human subjects. The reference lists of reviews and retrieved articles were hand searched at the same time. We did not consider abstracts or unpublished reports. If more than one article was published by the same author using the same case series, we selected the study where the most individuals were investigated.

Inclusion and exclusion criteria

We reviewed abstracts of all citations and retrieved studies. The following criteria were used to include published studies: (1) evaluating the association between *IL-10* gene polymorphism and HCC; (2) case-control design; and (3) sufficient genotypes data were presented to calculate the odds ratio (OR) and confidence interval (CI). Participants could be of any age. Studies were excluded if one of the following existed: (1) the design was based on family or sibling pairs; (2) the genotype frequency was not reported; or (3) there was insufficient information for extraction of data. The HCC definition used in the individual studies was accepted and we documented this in our analysis.

Data extraction

All data were extracted independently by two reviewers (Wei Y and Liu F) according to the inclusion criteria listed above. Disagreements were resolved by discussion between the two reviewers. The following characteristics were collected from each study: first author, year of publication, country of the first or corresponding author, ethnicity, number of cases and controls, genotyping methods, matching variables, and evidence of Hardy-Weinberg equilibrium (HWE) (Table 1). Different ethnicities descents were categorized as Asian and non-Asian.

Statistical analysis

The statistical analysis was conducted using STATA 11.0 (Stata Corp LP, College Station, TX, United States); $P < 0.05$ was considered statistically significant. HWE in the controls was tested by the chi-square test for goodness of fit, and a $P < 0.05$ was considered as significant disequilibrium. For IL-10-1082 polymorphism and IL-10-819 polymorphism, analyses were performed for AA *vs* AG + GG

genotype and TT *vs* TC + CC genotype, respectively. Because lack of separated AG and GG genotype frequency for two studies, A allele *vs* G allele could not be performed for IL-10-1082 polymorphism. Similarly, T allele *vs* C allele could not be performed for IL-10-819 polymorphism. For IL-10-592 polymorphism, we examined the contrast of the allelic effect of C (minor allele) *vs* A (common allele), and also examined the contrast of CC *vs* AC + AA genotypes. These contrasts correspond to the recessive and dominant effects of the C allele, respectively.

The OR and 95% CI were estimated for each study in a random-effects model or in a fixed-effects model. Heterogeneity among studies was examined with the χ^2-based Q testing and I^2 statistics[30]. $P < 0.1$ was considered significant for the χ^2-based Q testing and I^2 was interpreted as the proportion of total variation contributed by between-study variation. If there was a significant heterogeneity ($P < 0.1$), we selected a random-effects model (the DerSimonian and Laird method) to pool the data. If not, we selected a fixed-effects model (the Mantel-Haenszel method) to pool the data. Publication bias was examined with funnel plots and with the Begg's and Egger's tests[31-33]. If there is evidence of publication bias, the funnel plot is noticeably asymmetric. For the Begg's and Egger's tests the significance level was set at 0.05.

RESULTS

Studies included in the meta-analysis

There were 72 papers relevant to the search words. *Via* the steps of screening the title and reading the abstract, eight studies were identified[23-29,34]. Of these, one study was excluded because of no allele or genotype frequency; thus, seven eligible studies[23-29] which included 1012 HCC cases and 2308 controls were found to match our inclusion criteria. Four of seven publications indicated HWE in their subjects[23-25,27]; we calculated HWE for the remained three publications and found that only Migita's study relating to IL-10-1082 polymorphism was inconsistent with Hardy-Weinberg disequilibrium ($P = 0.004$). The flow chart of selection of studies and reasons for exclusion is presented in Figure 1. Studies had been carried out in China, Japan, Korea, Tunisia and the United States. Characteristics of studies included in the meta-analysis are presented in Tables 1 and 2.

Evaluation of IL-10 gene polymorphisms and association with HCC

There were six case-control studies[23,24,26-29] which had been performed to study the IL-10-1082 A/G polymorphism and HCC risk. Of these, four studies were performed in Asians, one study in Americans and one in Africans. Because adequate sample populations were unavailable for the American and African groups, we performed ethnicity-specific meta-analysis in the Asian and non-Asian populations. The combined results based on all studies showed that there was no association between IL-10-1082 A/G polymorphism and HCC risk (AA *vs* AG + GG, OR = 1.11, 95% CI = 0.90-1.37). When stratifying for ethnicity, the results were similar (Asian, OR = 1.12, 95% CI = 0.87-1.44; non-Asian, OR = 1.10, 95% CI = 0.75-1.60) (Figure 2).

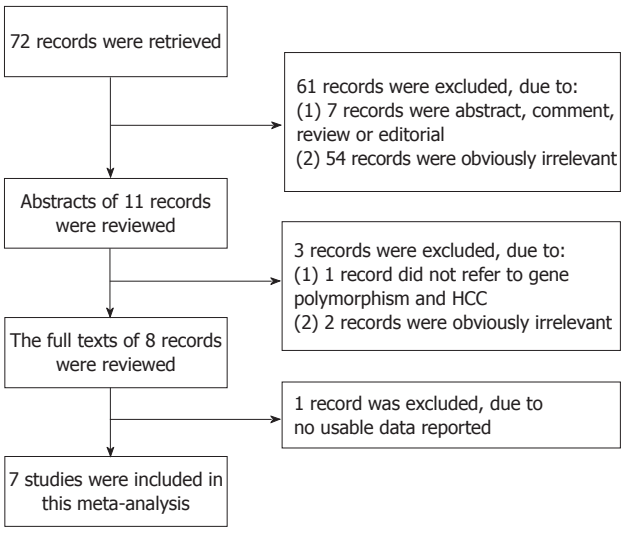

Figure 1 Flow chart of selection of studies and specific reasons for exclusion from the meta-analysis.

There were four case-control studies[25,26,28,29] which had been performed to study the IL-10-592 C/A polymorphism and HCC risk. Of these, all studies were performed in Asians. The combined results based on all studies showed that C allele carriers had an increased risk of HCC *vs* A allele carriers (OR = 1.29, 95% CI = 1.12-1.49) (Figure 3). Meanwhile, CC genotype carriers had an increased risk of HCC *vs* AC and AA genotype carriers (OR = 1.68, 95% CI = 1.25-2.26).

Only three studies were performed to study the IL-10-819 T/C polymorphism and HCC risk. The combined results based on all studies showed that there was no association between IL-10-819 T/C polymorphism and HCC risk (TT *vs* TC + CC, OR = 1.02, 95% CI = 0.79-1.32).

Sensitivity analysis

The influence of a single study on the overall meta-analysis estimate was investigated by omitting one study at a time, and the omission of any study made no significant difference, indicating that our results were statistically reliable.

Evaluation of heterogeneity and publication bias

Statistically significant heterogeneity was not observed between trials for all analysis with the χ^2-based Q testing and I^2 statistics. Review of funnel plots could not rule out the potential publication bias for all analyses. Publication bias was not evident when the Begg's rank correlation method and the Egger's weighted regression method were used except for analyzing IL-10-1082 polymorphism and HCC risk (Table 3).

DISCUSSION

It has been recognized that the most important risk fac-

Table 1 Characteristics of studies included in the meta-analysis

Authors	Year	Design	Country	Ethnicity	No. of case	No. of control	Genotyping methods	Matching criteria
Bouzgarrou *et al*[23]	2009	HCC	Tunisia	African	58	145	AS-PCR	Age, sex, geographical area
Ognjanovic *et al*[24]	2009	PCC	United States	Caucasian	120	230	Taqman	Age, sex, race
Tseng *et al*[25]	2006	HCC	Taiwan, China	Asian	208	528	PCR-RFLP	Race
Migita *et al*[26]	2005	HCC	Japan	Asian	48	188	PCR-SSP	-
Nieters *et al*[27]	2005	HCC	China	Asian	250	250	AS-PCR	Age, sex, race, district of residence
Heneghan *et al*[28]	2003	HCC	China	Asian	98	175	-	Age, sex
Shin *et al*[29]	2003	HCC	Korea	Asian	230	792	MAPA	-

HCC: Hospital-based case-control; PCC: Population-based case-control; PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism; PCR-SSP: Polymerase chain reaction and sequence-specific primer typing; AS-PCR: Allele-specific polymerase chain reaction; MAPA: Multiplex automated primer extension analysis.

Table 2 *Interleukin-10* gene polymorphism and hepatocellular carcinoma susceptibility

Gene	Polymorphism	HCC group genotype[1]			Control group genotype[2]			HWE	Ref.
		AA	AG	GG	AA	AG	GG		
IL-10	-1082 G/A	39	79	-	67	147	-	Yes	[24]
IL-10	-1082 G/A	24	24	10	56	68	21	Yes	[23]
IL-10	-1082 G/A	42	5	1	176	10	2	No	[26]
IL-10	-1082 G/A	130	119	-	115	135	-	Yes	[27]
IL-10	-1082 G/A	86	12	0	160	15	0	Yes	[28]
IL-10	-1082 G/A	201	28	1	675	112	5	Yes	[29]
		AA	AC	CC	AA	AC	CC		
IL-10	-592 C/A	93	84	31	259	223	46	Yes	[25]
IL-10	-592 C/A	17	23	8	85	78	25	Yes	[26]
IL-10	-592 C/A	89	101	26	384	299	65	Yes	[29]
IL-10	-592 C/A	49	38	11	95	60	19	Yes	[28]
		TT	TC	CC	TT	TC	CC		
IL-10	-819 T/C	17	23	8	85	78	25	Yes	[26]
IL-10	-819 T/C	130	119	-	115	135	-	Yes	[27]
IL-10	-819 T/C	49	38	11	95	60	19	Yes	[28]

[1]Absolute number of patients; [2]Absolute number of controls; OR: Odds ratio; HCC: Hepatocellular carcinoma; HWE: Hardy-Weinberg equilibrium.

Table 3 Pooled odds ratio for *Interleukin-10* gene polymorphism and hepatocellular carcinoma susceptibility in meta-analyses

Comparison	No. of study	OR (95% CI)	*P*-Publication bias			Heterogeneity test[2]	
			P[1] value	Egger[3]	Begg[4]	*P* value	I^2
IL-10-1082							
AA *vs* AG + GG	6	1.11 (0.90, 1.37)	0.32	0.004	0.02	0.44	0
IL-10-592							
CC *vs* AA + AC	4	1.68 (1.25, 2.26)	0.001	0.43	1	0.11	50.80%
C allele *vs* A allele	4	1.29 (1.12, 1.49)	0.001	0.51	0.73	0.86	0
IL-10-819							
TT *vs* TC + CC	3	1.02 (0.79, 1.32)	0.88	0.13	0.3	0.14	48.90%

[1]Fixed effects models were used, weighted by the inverse variance. All statistical tests are two sided; [2]$P < 0.1$ is considered statistically significant for Q statistics; I^2 is interpreted as the proportion of total variation contributed by between-study variation; [3]Egger's test to evaluate publication bias, $P < 0.05$ is considered statistically significant; [4]Begg's test to evaluate publication bias, $P < 0.05$ is considered statistically significant. OR: Odds ratio.

tor for the development of HCC is cirrhosis[35]. Chronic infections with HBV and HCV are the most frequent causes of cirrhosis worldwide. A large number of cohort and case-control studies have shown that alcohol consumption causes liver cirrhosis and is an independent risk factor for HCC[36,37]. Epidemiological studies reported elevated HCC risks associated with exposure to aflatoxins after adjustment for HBV exposure[38]. Cigarette smoking has been causally associated with the risk of HCC[37]. Although so many environmental factors are found to correlate with the tumorigenesis of HCC, there still are a portion of patients without known risk factors who eventually developed HCC[39]. Previous study had shown an interaction of environmental factors and genetic predisposition in the development of HCC[40]. Therefore, genetic predisposition may contribute to the process of tumorigenesis.

A genetic predisposition to HCC has been suggested by many studies[41,42]. Recent studies suggest that single nucleotide polymorphism may be related to the tu-

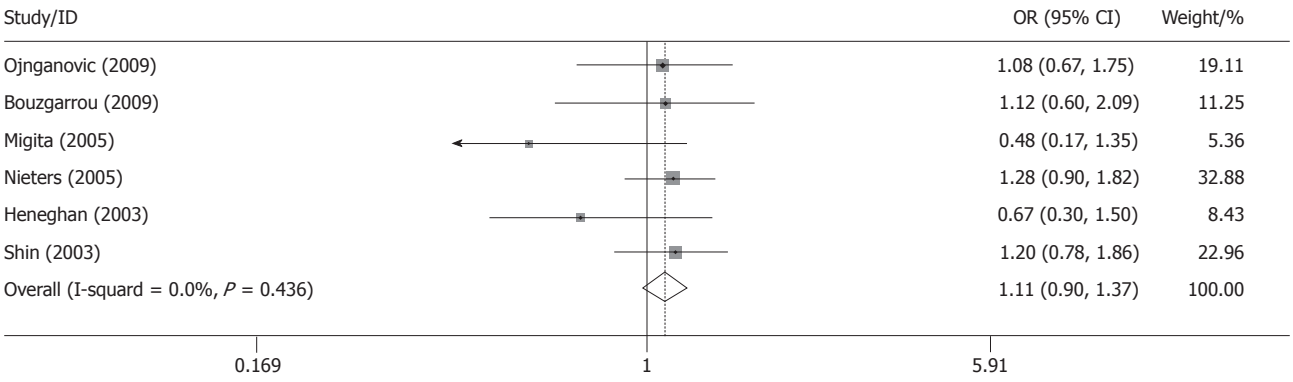

Figure 2 Odds ratios and 95% CI of individual studies and pooled data for the association of the Interleukin-10-1082 A/G polymorphism and hepatocellular carcinoma risk comparing AA genotype with AG + GG genotype.

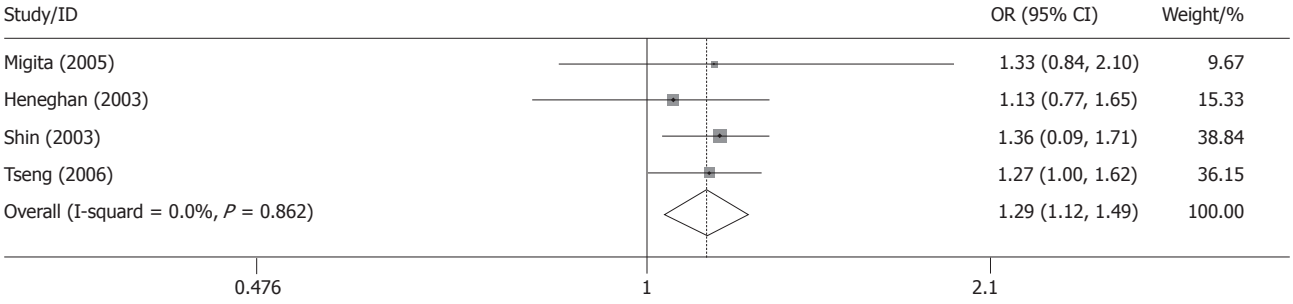

Figure 3 Odds ratios and 95% CI of individual studies and pooled data for the association of the Interleukin-10-592 C/A polymorphism and hepatocellular carcinoma risk comparing any C allele with A allele.

morigenesis of liver cancer[43,44]. The *IL-10* gene and its encoded protein is an immunoregulatory cytokine that plays an anti-inflammatory action, which has three SNPs at positions -1082, -592 and -819 promoter region. Until recently, a number of studies had been performed to analyse IL-10 polymorphisms and HCC risk. However, most of these studies were based on small sample sizes. Moreover, there were still some conflicting results. As a powerful statistical method, meta-analysis can provide a quantitative approach for pooling the results of different research on the same topic, and for estimating and explaining their diversity[45,46].

The data from this meta-analysis clearly suggest that the IL-10-592 C allele is a genetic contributor to overall HCC susceptibility. We also observed that the C/C homozygote had a stronger association with HCC susceptibility than the C/A heterozygote and AA homozygote. Given the important roles of IL-10 in inflammation and tumor development, it was biologically plausible that IL-10-592 C/A polymorphism was associated with the risk of HCC by modulating IL-10 expression. Such evidence on the functionality of this polymorphism might lead to a better understanding of this association. The risk for HCC increases with the severity of hepatic inflammation and chronic inflammation developing through the action of various inflammatory mediators is known as a cofactor of carcinogenesis[8,9]. As mentioned above, the function of IL-10 is to inhibit immune response and inflammation. Meanwhile, previous studies suggested that IL-10/-592*C allele may be a marker for lower IL-10 production[29,47]. The genetic polymorphism may affect the severity of hepatic inflammation and cause higher HCC risk due to lower IL-10 production. In addition, this meta-analysis also suggests that IL-10-1082 A/G polymorphism was not associated with the risk of HCC. When stratifying for ethnicity, the results were similar. Similarly, no association was found between HCC susceptibility and IL-10-819 T/C polymorphism.

As previously described, ethnicity can strongly influence the distribution of cytokine gene polymorphisms[48]. This suggests that there are racial differences in genetic risk; the different genetic backgrounds and different environments the different ethnicities lived in may contribute to the ethnic discrepancy. In the meta-analysis, studies comprising IL-10 polymorphism (at position -592 and -819) and HCC risk were all performed in Asians. For IL-10-1082 polymorphism and HCC susceptibility, we stratified the result by race (Asian and non-Asian population), but the results were similar. This is probably because the number of studies from non-Asian populations were too small to detect the ethnic discrepancy, thus, caution should be adopted when explaining our results.

One of the major concerns in a sound meta-analysis is the degree of heterogeneity that exists between the component studies because non-homogeneous data are liable to result in misleading results. In the present study, the Q testing and I^2 statistics were carried out to test the

significance of heterogeneity. Fortunately, statistically significant heterogeneity was not observed between trials for all analysis with the χ^2-based Q testing and I^2 statistics. Moreover, we performed a sensitivity analysis by removing one study each time and rerunning the model to determine the effect on each overall estimate. The estimates changed little, which implied that our results were statistically reliable.

However, there are still some limitations in this meta-analysis. (1) We did not test for gene-to-environment interactions because of the issue of multiple testing and the lack of sufficient studies. It is possible for specific environmental and lifestyle factors to alter those associations between gene polymorphisms and HCC risk; (2) as in most meta-analyses, these results should be interpreted with caution because the populations from 5 countries and controls were not uniform; (3) the number of studies and the number of subjects in the studies included in the meta-analysis by specific subgroups were small, thus, caution should be adopted when explaining our results; and (4) meta-analysis is retrospective research that is subject to methodological limitations. In order to minimize the bias, we developed a detailed protocol before initiating the study, and performed a meticulous search for published studies by using explicit methods for study selection, data extraction and data analysis. Nevertheless, our results should be interpreted with caution.

This meta-analysis suggests that the IL-10-592 C/A polymorphism may be associated with HCC among Asians. The pooled ORs in this study - both with respect to the IL-10-592*C allele and the IL-10-592/CC homozygote - suggest a modest but definite genetic effect. It is critical that larger and well-designed multicentre studies based on different ethnic groups are needed to confirm our results.

COMMENTS

Background

Interleukin-10 (IL-10) is an immunoregulatory cytokine produced by Th2 cells, monocytes/macrophages, and regulatory T cells. The promoter of the *IL-10* gene contains three biallelic polymorphisms at positions -1082 (base G to A, dbSNP No. rs1800896), -819 (base C to T, dbSNP No. rs1800871), and -592 (base C to A, dbSNP No. rs1800872) from the transcription start site. These changes have been implicated as risk factors for hepatocellular carcinoma (HCC), but individual studies have been inconclusive or controversial. The aim of this meta-analysis was to clarify the effect of IL-10 polymorphisms on the risk of HCC.

Research frontiers

To date, a number of studies have assessed the association between the *IL-10* gene polymorphism and HCC risk in different populations; however, the results are inconsistent and inconclusive. No quantitative summary of the evidence has ever been performed.

Innovations and breakthroughs

IL-10-592 A/C polymorphism may be associated with HCC among Asians, while IL-10-1082 G/A and IL-10-819 T/C polymorphisms could not alter susceptibility to HCC.

Applications

It can be seen from this paper that IL-10-592 A/C polymorphism could alter susceptibility to HCC. It suggests that a common variant in the functional region of a definitively meaningful gene had an effect on human disease, such as cancer.

Peer review

The meta-analysis aimed at assessing the association between *IL-10* gene polymorphisms and HCC. This is an appealing issue, leading to interesting results.

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