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Rs3746444 T>C locus in miR-499 increases the susceptibility to hepatocellular carcinoma: A meta-analysis 14812 subjects

Jiang JK *et al.* MiR-499 rs3746444 T>C SNP and HCC

Abstract

BACKGROUND

Recently, many investigations have suggested that the rs3746444 T>C locus in the microRNA (miR)-499 gene may contribute to the occurrence of cancer. However, reports on the association between rs3746444 and hepatocellular carcinoma (HCC) are conflicting.

AIM

To further understand and explore the potential correlation between the single-nucleotide polymorphism of rs3746444 and the incidence of HCC.

METHODS

In this meta-analysis, we obtained electronic literature by searching the PubMed, Embase and Chinese BioMedical Disc databases (through May 20, 2022). All eligible case-control, prospective cohort or nested case-control studies with sufficient data for calculating the odds ratios with their 95% confidence intervals were included.

RESULTS

Ultimately, a total of 17 independent studies were included. We identified that rs3746444 was associated with the development of HCC (C *vs* T: $P = 0.019$ and CC/CT *vs* TT: $P = 0.016$). In Asian individuals, rs3746444 was associated with susceptibility to HCC (C *vs* T: $P = 0.013$ and CC/CT *vs* TT: $P = 0.016$). In addition, this study identified that the miR-499 rs3746444 locus was associated with susceptibility to HCC in the normal/healthy control subgroup (C *vs* T: $P = 0.034$ and CC/CT *vs* TT: $P = 0.024$).

CONCLUSION

In summary, this meta-analysis highlights that rs3746444 in the miR-499 gene is involved in the occurrence of HCC, especially in Asian individuals.

Key Words: Polymorphism; MicroRNA-499; Hepatocellular carcinoma; Meta-analysis; Susceptibility

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Core Tip: Many investigations have suggested that the rs3746444 T>C locus in the microRNA (miR)-499 gene may contribute to the occurrence of cancer. However, reports on the association between rs3746444 and hepatocellular carcinoma (HCC) are conflicting. This meta-analysis highlights that rs3746444 in the miR-499 gene is involved in the occurrence of HCC, especially in Asian individuals.

INTRODUCTION

In 2020, liver cancer (LC) was the seventh most frequent malignancy, with 905677 new cases worldwide^[1]. Accordingly, LC is ranked as the second leading cause of malignancy-related deaths, as it has resulted in death for 830180 individuals^[1]. The incidence rates of LC and LC-related deaths remain higher in China than other parts of the world^[2]. Hepatocellular carcinoma (HCC) is the predominant subtype of LC, accounting for approximately 75%-85% of primary LC cases^[3]. Overall, the survival rate of HCC remains poor. To date, the etiology of HCC is not well established. Although it has been reported that chronic virus infection^[4], type 2 diabetes^[5,6], obesity^[7,8], smoking^[9,10], heavy alcohol intake^[11-13] and aflatoxin-contaminated food stuffs^[14] may contribute to the occurrence of HCC, other risk factors may also lead to the development of HCC, such as hereditary factors^[15-18].

MicroRNAs (miRs) are noncoding RNAs of approximately 22 nucleotides in size. They may be implicated in the regulation of target genes and are involved in a number of cellular processes (*e.g.*, growth, proliferation, differentiation, apoptosis, migration and invasion)^[19-24]. Recently, several investigations have reported that the expression

profiling of serum miRs could be used as a marker for hepatitis C virus-related cases of HCC^[25]. Variants within miRs may alter target recognition, transcription, or posttranscriptional processing and then lead to malignant diseases^[26]. Additionally, most of the established miRs may influence many target genes; single-nucleotide polymorphisms (SNPs) in miRs could affect the level of multifarious proteins. MiR-499 is located within chromosome 20q. MiR-499 is involved in infection and inflammatory diseases^[27]. Rs3746444 T>C in miR-499 was identified to be correlated with the development of ankylosing spondylitis^[28], arthritis susceptibility^[29], and bronchial asthma^[27].

Additionally, a number of investigations have suggested that the rs3746444 SNP in miR-499 may contribute to the occurrence of cancer. Liu *et al*^[30] reported that miR-499-5p could promote the metastasis of colorectal cancer and might be used as a vital target for colorectal cancer therapy. Additionally, a previous study identified that in HepG2 cells, miR-499 could inhibit the level of the E26 transformation specific sequence 1, which is an important proto-oncogene in the development of HCC^[31]. The miR-499 variant rs3746444 has been suggested to play an important role in the occurrence of various malignancies, such as adenocarcinoma of the esophagogastric junction^[32], prostate cancer^[33], cervical squamous cell carcinoma^[34], oral squamous cell cancer^[35], and lung cancer^[36]. Recently, a number of studies have focused on the relationship between rs3746444 in miR-499 and HCC^[36-40]; however, the obtained findings are conflicting. Several meta-analyses also reported controversial results. Some pooled analyses have suggested that the rs3746444 C allele could not confer a risk to HCC^[41-44]. However, other publications have reported that the rs3746444 C allele may contribute to the occurrence of HCC^[40,45-47]. These controversial findings may be due to the limited sample sizes included in these analyses. Recently, some case-control studies have been conducted to further explore this potential association^[48-50]. An updated meta-analysis is needed to shed new light on the relationship between rs3746444 in miR-499 and HCC regarding all available publications. Therefore, this meta-analysis involved a large sample size to verify whether the miR-499 rs3746444 SNP could influence the

occurrence of HCC. And these possible relationships might be beneficial to the prevention of liver carcinogenesis.

MATERIALS AND METHODS

Study research

In this meta-analysis, we obtained electronic literature by searching the PubMed, Embase and Chinese BioMedical Disc (CBM) databases (through May 20, 2022). We used the following keywords: (SNP OR variant OR polymorphism) AND (neoplasm OR carcinoma OR tumor OR cancer) AND (hepatocellular OR liver) AND (microRNA499 OR miR499 OR microRNA-499 OR miR-499 OR rs3746444). The references included in the retrieved publications and relevant reviews, as well as published meta-analyses, were hand-searched to obtain more related data. Due to no restriction on language, a large amount of data was collected. We also cited high-quality articles in *Reference Citation Analysis* (<https://www.referencecitationanalysis.com>).

Inclusion and exclusion criteria

The inclusion criteria for the eligible literature were as follows: (1) Assessing the relationship of rs3746444 in miR-499 with HCC susceptibility; (2) Full-text study; (3) Designed as a case-control study, a prospective cohort or a nested case-control study; and (4) Sufficient data could be used to calculate the odds ratios (ORs) with their 95% confidence intervals (CIs). When a publication contained more than one investigation, it was treated as an independent case-control study. Accordingly, letters, reviews, comments, non-case-control studies, studies that violated Hardy-Weinberg equilibrium (HWE), literature without sufficient data and duplicated data were excluded.

Data extraction

Two authors (Jiang JK and Lin J) reviewed the eligible literature and extracted the data independently. The following information was collected: The first author, year of publication, mean age (years), sex (male, %), drinking status (%), smoking status (%),

country/ethnicity, hepatitis B surface antigen (HBsAg) (positive, %), number of subjects, HWE, genotyping method and genotype data. In a case of a conflicting assessment, another author (Tang WF) took part in a discussion until a consensus opinion was obtained.

Statistical methods

The results of this meta-analysis were assessed in four genetic models: A dominant model (CC/TC *vs* TT), recessive model (CC *vs* TT/TC), homozygote comparison (CC *vs* TT) and allelic model (C *vs* T). The correlation between rs3746444 in miR-499 and HCC susceptibility was determined by using ORs and the corresponding 95% CIs. The heterogeneity among the eligible studies was assessed by using the I^2 test and Q test. For heterogeneity, the level of significance was $P < 0.1$ and/or $I^2 \geq 50\%$. When it was significant, we used a random-effects model (DerSimonian and Laird) to assess the association between rs3746444 in miR-499 and HCC susceptibility^[51,52]. Otherwise, we used a fixed-effects model (Mantel-Haenszel) to determine the potential association^[53]. In this study, a Galbraith radial plot was used to confirm the source of the heterogeneity. Sensitivity analysis was performed to explore whether an individual investigation might significantly influence the assessment. We used Egger's test and Begg's funnel plots to measure the possible bias among the publications. For publication bias, the level of significance was $P < 0.1$. STATA 12.0 software (Stata Corp., College Station, Texas) was used to conduct statistical analysis. All P -values were measured with two-sided tests. By using Power-SampleSize software, the power value ($\alpha = 0.05$) was also used to assess the stability of our study^[54]. We used the Newcastle-Ottawa Quality Assessment Scale to assess the quality of eligible studies and defined scores ≥ 7 stars as high-quality studies^[55].

RESULTS

Study characteristics

An electronic search of the CBM, PubMed and Embase databases obtained 117 publications. After the titles or abstracts were reviewed by two authors (Jiang JK and Lin J), 47 duplicates were removed. Fifty articles were excluded based on the inclusion criteria (Figure 1). Thus, 20 articles were reviewed in full text. Three publications were included after reading the references of eligible articles. However, 11 case-control studies were excluded for violating HWE. Finally, 13 publications with 17 independent case-control studies focusing on the relationship between the rs3746444 polymorphism and HCC risk were included^[40,48,50,56-65].

These included studies were published between 2012 and 2020, and in the eligible case-control studies, the participant number ranged from 100 to 1507. Table 1 shows the included terms in the eligible studies. In summary, 7 case-control studies involving Caucasian individuals were found^[50,56-58], and the others focused on Asian individuals^[40,48,59-65]. The distributions of the rs3746444 genotypes and alleles in the miR-499 SNP and the results of the quality assessment are summarized in Tables 1 and 2.

Main findings

The main results are summarized in Table 3. When we combined the included case-control studies, we identified that rs3746444 in miR-499 was associated with the development of HCC (C vs T: $P = 0.019$ and CC/CT vs TT: $P = 0.016$, Figure 2). In a subgroup analysis by different races, rs3746444 in miR-499 was found to be associated with susceptibility to HCC in the Asian population (C vs T: $P = 0.013$ and CC/CT vs TT: $P = 0.016$). When we considered the source of disease, the miR-499 rs3746444 locus was identified to be associated with susceptibility to HCC in normal/healthy control individuals (C vs T: $P = 0.034$ and CC/CT vs TT: $P = 0.024$) and hepatitis/virus-related control individuals (C vs T: $P = 0.007$, CC vs TT: $P = 0.014$ and CC/CT vs TT: $P = 0.018$).

Sensitivity analysis

To confirm the stability of our findings, we conducted a sensitivity analysis in this meta-analysis. We deleted an individual study in turn and calculated the ORs and CIs of the remaining studies to determine the influence of each datum. The findings suggested that these evaluations could not be altered by any eligible study (Figure 3).

6 *Publication bias*

By using Begg's and Egger's tests, publication bias among the eligible studies was determined. There was no significant bias among the eligible studies (Figure 4, $P > 0.1$, data not shown).

Heterogeneity

In this meta-analysis, significant heterogeneity was identified. We conducted stratified analyses to explore the source of heterogeneity. Newcastle-Ottawa Scale (Nos) was used to evaluate the literature quality. We found an association between hospital-based (HB) studies, high-quality studies (Nos ≥ 7.0), Asian individuals, and normal/healthy control subgroups and significant heterogeneity. The Galbraith radial plot test suggested that 4 outliers^[40,56,63,65] might contribute to the significant heterogeneity (Figure 5).

The power of the present study ($\alpha = 0.05$)

By using Power-Sample Size software, the power value ($\alpha = 0.05$) was also used to assess the stability of our study. As summarized in Table 3, in the overall comparison, the power value was more than 0.8 in the allele and dominant genetic models. In the subgroup analyses, the power value was more than 0.8 in Asian individuals and the normal/healthy control subgroups in the allele genetic model and in Asian individuals and the normal/healthy control subgroups in the dominant genetic model.

DISCUSSION

Recently, rs3746444 in miR-499 and its importance to the occurrence of HCC have been extensively investigated. However, several meta-analyses reported controversial

results, which might be due to the limited sample sizes included in these analyses. Recently, some case-control studies have been conducted to further explore this potential association in different populations. Thus, an updated meta-analysis should be conducted to shed new light on the relationship between rs3746444 in miR-499 and HCC. As summarized in Table 3, we identified that rs3746444 in miR-499 was associated with the development of HCC in the allele and the dominant genetic models (the value of power ≥ 0.8).

The merit of this updated meta-analysis was that the present pooled analysis included a larger sample size to verify whether the miR-499 rs3746444 SNP could influence the occurrence of HCC. In this study, we identified that the miR-499 rs3746444 SNP could confer a risk to HCC. Some meta-analyses have focused on the potential correlation between rs3746444 in miR-499 and the risk of HCC. A previous pooled analysis suggested that the rs3746444T allele in the miR-499 gene could not play a vital role in the tumorigenesis of HCC^[42]. However, other meta-analyses reported that rs3746444 in miR-499 might confer susceptibility to HCC^[40,45-47]. Additionally, some more recent case-control studies have been conducted to explore the potential association between rs3746444 in the miR-499 SNP and the risk of HCC^[48-50]. The potential association was more controversial. Thus, we included 28 independent case-control studies with 5948 cases and 8864 controls and conducted an updated meta-analysis to focus on the relationship between rs3746444 in the miR-499 SNP and the risk of HCC. In this study, we identified that the miR-499 rs3746444 SNP could confer a risk to HCC.

Toraih *et al*^[26] reported that in silico data analysis, the T to C substitution in the miR-499 rs3746444 SNP did not prominently affect the structure of the hairpin loop. Functional prediction revealed that different miR-499 rs3746444 alleles have different targets. The miR-499 rs3746444*C allele only has 58.2% of the gene targets of the rs3746444*T variant and generates 763 new gene targets. The miR-499 gene can target both alcohol dehydrogenase 1 beta polypeptide (ADH1B) and aldehyde dehydrogenase 1 family member A3 (ALDH1A3) genes. Pettinelli *et al*^[66] suggested that hepatic

ALDH1A3 was expressed at lower levels and was inversely correlated with the level of plasma retinol in nonalcoholic steatohepatitis cases, which may alter the risk for HCC. Recently, some studies have identified that the ADH1B gene may be involved in the development of HCC^[67-69]. A previous study indicated that rs3746444 in miR-499 was correlated with susceptibility to ulcerative colitis and that the expression of miR-499 was decreased (5-fold) in ulcerative colitis cases with the rs3746444 TC genotype compared with those with the rs3746444 TT variant^[70]. Taken together, these results indicate that the rs3746444 C allele in the miR-499 gene could decrease the expression of the miR-499 gene and alter the levels of the ADH1B and ALDH1A3 genes. Finally, this SNP could be implicated in the occurrence of HCC. However, the relationship between rs3746444 in miR-499 and HCC in different subgroups could not be well explained. In the future, more attention should be given to the potential mechanism by which hepatitis B virus infection acts in different ethnicities or statuses.

Since significant heterogeneity was found in this meta-analysis, subgroup analysis was performed to observe the major source of heterogeneity. The findings of the subgroup analysis indicated that the normal/healthy control, Asian and HB subgroups could greatly increase the heterogeneity. Additionally, the Galbraith radial plot identified 4 outliers^[40,56,63,65], which could contribute to the major source of heterogeneity.

There are some limitations in this meta-analysis. First, the electronic literature was only searched in the PubMed, Embase and CBM databases, and bias might have occurred. Second, all investigations have been conducted in Caucasian and Asian populations; thus, our findings were only appropriate for these populations. Third, due to insufficient data (*e.g.*, HBsAg, drinking, smoking, sex, age, body mass index and lifestyle) in this study, we did not consider these factors in the subgroup analysis. Fourth, due to the lack of environmental factors, we also did not take into account gene-environment interactions. Fifth, in this meta-analysis, significant heterogeneity was identified. Sixth, we did not pay close attention to the expression level of target genes, which could be controlled by rs3746444 T>C locus. Finally, in this study, we only

included the relationship between rs3746444 in miR-499 and HCC risk. The potential role of other vital miR loci can't be ignored.

CONCLUSION

In summary, this meta-analysis highlights that rs3746444 in miR-499 is involved in the occurrence of HCC, especially in Asian individuals. In the future, more investigations are needed to confirm our results.

ARTICLE HIGHLIGHTS

Research background

This meta-analysis highlights that rs3746444 in microRNA (miR)-499 is involved in the occurrence of hepatocellular carcinoma (HCC), especially in Asian individuals. These possible relationships might be beneficial to the prevention of liver carcinogenesis. In the future, more investigations are needed to confirm.

Research motivation

Recently, a number of studies have focused on the relationship between rs3746444 in miR-499 and HCC. However, the obtained findings are conflicting.

Research objectives

In summary, this meta-analysis highlights that rs3746444 in miR-499 is involved in the occurrence of HCC, especially in Asian individuals. In the future, more investigations are needed to confirm our results.

Research methods

This meta-analysis involved a large sample size to verify whether the miR-499 rs3746444 single-nucleotide polymorphism could influence the occurrence of HCC. These possible relationships might be beneficial to the prevention of liver carcinogenesis.

Research results

Reports on the association between rs3746444 and HCC are conflicting.

Research conclusions

The results of this meta-analysis were assessed³ in four genetic models: A dominant model (CC/TC *vs* TT), recessive model (CC *vs* TT/TC), homozygote comparison (CC *vs* TT) and allelic model (C *vs* T). The correlation between rs3746444 in miR-499 and HCC susceptibility was determined by using odd ratios⁷ and the corresponding 95% confidence intervals. We used a random-effects model (DerSimonian and Laird) to assess the association between rs3746444 in miR-499 and HCC susceptibility. Otherwise, we used a fixed-effects model (Mantel-Haenszel) to determine the potential association. We used² the Newcastle-Ottawa Quality Assessment Scale to assess the quality of eligible studies and defined scores ≥ 7 stars as high-quality studies.

Research perspectives

Reports on the association between rs3746444 and HCC are conflicting. This meta-analysis highlights that rs3746444 in miR-499 is involved in the occurrence of HCC, especially in Asian individuals. These possible relationships might be beneficial to the prevention of liver carcinogenesis.

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