VEGFA rs3025039 and biliary atresia susceptibility in Chinese population: a systematic review and meta-analysis

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ABSTRACT

Background Previous studies have suggested an association between vascular endothelial growth factor A (VEGFA) rs3025039 polymorphism and biliary atresia (BA). However, this conclusion is controversial and there is no published pooled evidence of this association.

Methods This study was conducted and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses. The protocol was registered with PROSPERO (International Prospective Register of Systematic Reviews). A thorough search was performed on databases including PubMed, Embase, and Chinese Biomedical Database up to August 2020. This study included 846 cases of BA and 2821 controls concerning VEGFA rs3025039 polymorphism. We selected relevant studies based on the following inclusion criteria: (1) the study design was case-control and cohort and (2) the patients carried standard clinical diagnoses of BA, etc. The exclusion criteria were as follows: (1) patients with other related diseases, (2) lack of requisite information and (3) duplicate data. The OR (odd ratio) and the corresponding 95% CI (confidence interval) were calculated to estimate the association.

Results This study on VEGFA rs3025039 polymorphism in the Chinese population included 846 cases and 2821 controls. The results showed that there was no significant association between rs3025039 and susceptibility to BA under four genetic models. The results of the subgroup analysis were similar to the overall results.

Conclusions This meta-analysis shows that rs3025039 was not associated with susceptibility to BA in the Chinese population. Further validation may entail additional research.

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INTRODUCTION

Biliary atresia (BA) is a type of progressive obliterative disorder in neonates that interferes with the function and anatomy of the intrahepatic and extrahepatic bile ducts.1 2 This destructive inflammatory obliterative cholangiopathy frequently leads to hepatic fibrosis and end-stage liver disease. If untreated, BA with progressive liver cirrhosis is uniformly fatal.3 The clear etiology of this disorder is not well understood. Genetic and immunological factors, infections, and other environmental factors might lead to BA, suggesting that it has a complex etiology.3 4 Hereditary factors participate in the pathogenesis of BA. Multiple single-nucleotide polymorphisms on the genes, including ADD3, XPNPEP1, VEGFA, and EFEMP1, are associated with risk of BA.4

The human VEGFA gene is one of the members of the VEGF (vascular endothelial growth factor) family located on chromosome 6p21.3. This gene comprises a type of heparin-binding protein that is in the form of a disulfide-linked homodimer. VEGFA participates in various developmental processes, including endothelial cell proliferation, cell migration,
and apoptosis.\textsuperscript{3-7} It may also participate in the pathogenesis of BA because it can function as a proinflammatory cytokine.\textsuperscript{8} Polymorphisms of this functional gene may affect expression regulation, leading to various incidences and severities of disease.\textsuperscript{9} Thus, clarifying the effects of alterations within the \textit{VEGFA} gene may provide markers for diagnosis and treatment to reverse progression of BA.

Previous studies have explored the correlation between rs3025039 within the \textit{VEGFA} gene and susceptibility to BA; however, the results remain controversial due to the lack of consistency among the studies. In China, rs3025039 has been found to be associated with susceptibility to BA.\textsuperscript{10,11} Interestingly, a recent publication reported no significant correlation between rs3025039 and BA in the southern Chinese population.\textsuperscript{12} This discrepancy needs further verification by increasing the sample size. In the mean time, a meta-analysis is a suitable method to summarize previous genetic association studies (GAS) and to draw relatively reliable conclusions.\textsuperscript{13,14} Therefore, the present metanalysis may provide evidence regarding the association of \textit{VEGFA} rs3025039 polymorphism with susceptibility to BA.

**METHODS**

This study protocol was registered with an international registration platform of systematic review, PROSPERO (International Prospective Register of Systematic Reviews). We conducted and reported the study according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement\textsuperscript{15} (see online supplemental table S1).

**Patient and public involvement**

Patients and the public were not involved in this study.

**Data sources and searches**

PubMed, Embase, and the Chinese Biomedical Database (CBD) were searched from inception until August 17, 2020 (see online supplemental material for full details on search strategy). We also considered references in the included studies and related reviews. We did not impose limitations on the language of papers, time period of follow-up, and published state. We reran the same searches before the final analyses and retrieved additional studies for inclusion.

**Eligibility criteria**

We established the inclusion and exclusion criteria based on discussion studies. We selected relevant studies based
on the following inclusion criteria: (1) the study design was case–control and cohort; (2) the patients carried a standard clinical diagnosis of BA; (3) the studies explored the target association; and (4) the authors presented enough data on genotype distribution. The criteria used to exclude studies were as follows: (1) patients with other related diseases; (2) lack of requisite information; and (3) duplicate data.

Study selection
At the first stage, duplicates from three electronic databases were screened and removed independently by three reviewers (SH, YY, and LM). At the second stage, the title and abstract of each of the remaining studies were reviewed independently by the same reviewers (SH, YY, and LM) to select eligible studies. At the final stage, the same reviewers (SH, YY, and LM) independently retrieved and assessed the potentially eligible full text of the remaining publications. Any discrepancy with regard to eligibility of articles was discussed among the three reviewers in consultation with a third reviewer (RD or SZ).

Data extraction
All data were recorded independently by three reviewers (SH, YY, and LM) in accordance with a record form, with regard to (1) study characteristics (author information, publication time, sample size, country, and ethnic origin); (2) genotype data (number with different genotypes, minor allele frequency, results of the Hardy-Weinberg equilibrium (HWE) of the control group, and genotyping methods). All related data were found in the original studies so we did not need to contact the study authors to request for missing data.

Quality score assessment
Three reviewers (SH, YY, and LM) independently appraised the quality of the GAS using a checklist revised from previous studies, which was done on the basis of genetic factors and epidemiological requirements. The checklist covered essential items aimed at the quality of the GAS, including representativeness and ascertainment of study subjects, genotyping, HWE, and association analysis. The total score ranged from 0 (worst) to 13 (best). Detailed information is shown in online supplemental table S2.

Statistical analysis
In a GAS, research candidates should be categorized into three groups (BB, Bb, and bb) and usually B is used as the susceptibility allele. Previous studies have suggested that the C allele increased BA susceptibility; therefore, we estimated the association between rs3025039 and susceptibility to BA using four different genetic models, namely a per-allele model (C vs T), a homozygous model (CC vs TT), a dominant model (CC+CT vs TT), and a recessive model (CC vs CT+TT). We measured the effects using OR (odd ratio) and 95% CI (confidence interval) using a fixed-effect or a random-effect model. We assessed heterogeneity using the Cochrane Q statistic and the inconsistency index (I²). A p value <0.1 indicated substantial heterogeneity.
We conducted subgroup analyses according to the several study subject areas. We performed a sensitivity analysis by excluding every publication individually to evaluate the reliability and stability of the overall OR. We appraised publication bias using Egger’s test and Begg’s test and visual inspection of funnel plots. The meta package (V.4.9.7) in R software (V.3.6.1) was used to complete all analyses. In addition to heterogeneity, p<0.05 (two-tailed) indicated significance.

RESULTS
Search findings
We conducted the search process and reported the findings according to the PRISMA statement (see figure 1). We identified 41 papers after an initial search. At the first stage, we removed 10 duplicate articles, leaving 31 articles of potential relevance. At the second stage, we excluded 5 papers that did not involve patients with BA, 5 studies on animal experiments, 7 traditional reviews, and 10 studies that were not about polymorphisms. At the final stage, we excluded one paper due to insufficient information for inclusion. Finally, we included three articles for data extraction and meta-analysis.

Study characteristics
Table 1 shows the main data of the three studies (846 cases and 2821 controls). All of the included studies were conducted in the Chinese population. The genotype distributions of the controls in the three studies were consistent with HWE, but one did not provide exact data. Of all studies, two involved southern Chinese individuals, and one study involved northwestern Chinese individuals. All studies used hospital-based controls. The total scores for the three studies ranged from 6 to 9 (see table 1 and online supplemental table S3).

Heterogeneity test
There was a significant between-study heterogeneity in four genetic models of rs3025039 polymorphism (I² range: 66.2%–87.9%, p=0.0003–0.0518; see table 2). Therefore, we used a random-effects model to combine the associations between rs3025039 polymorphism and risk of BA.

Association between rs3025039 and risk of BA
Three studies, including 846 cases and 2821 controls, examined the association between rs3025039 and susceptibility to BA. There was no statistically significant association in any of the genetic models. The association between rs3025039 and susceptibility to BA was not significant in southern Chinese patients (C vs T: OR=1.64, 95% CI 0.50 to 5.37; CC vs TT: OR=1.40, 95% CI 0.27 to 7.14; CT+CC vs TT: OR=1.06, 95% CI 0.40 to 2.80; CC vs CT+TT: OR=1.72, 95% CI 0.48 to 6.14). For the northwestern Chinese subgroup, we could not perform a meta-analysis because there was only one study.

Subgroup analysis
We performed a subgroup analysis by area. Table 3 details the results. Southern Chinese individuals were the subjects in two studies, while one study involved northwestern Chinese individuals. The association between rs3025039 and susceptibility to BA was not significant in southern Chinese patients (C vs T: OR=1.64, 95% CI 0.50 to 5.37; CC vs TT: OR=1.40, 95% CI 0.27 to 7.14; CT+CC vs TT: OR=1.06, 95% CI 0.40 to 2.80; CC vs CT+TT: OR=1.72, 95% CI 0.48 to 6.14). For the northwestern Chinese subgroup, we could not perform a meta-analysis because there was only one study.

Table 2 Main results of the meta-analysis

| Studies (n) | Comparison model | Test of association | Test of heterogeneity | Test of publication bias |
|------------|------------------|---------------------|-----------------------|-------------------------|
|            |                  | OR      | 95% CI   | P value | P value | I² (%) | Begg’s test | Egger’s test |
| 1          | C vs T           | 1.50    | 0.90 to 2.49 | 0.121   | 16.52   | 0.001   | 87.9      | 0.602       | 0.422       |
| 2          | CC vs TT         | 2.02    | 0.58 to 6.99 | 0.269   | 7.49    | 0.024   | 73.3      |             |             |
| 3          | CT+CC vs TT      | 1.78    | 0.60 to 5.32 | 0.299   | 2.96    | 0.052   | 66.2      |             |             |
| 4          | CC vs CT+TT      | 1.53    | 0.89 to 2.63 | 0.124   | 5.92    | 0.001   | 86.3      |             |             |

CI, confidence interval; OR, odd ratio.

Figure 2 Forest plot of per-allele model of the association between VEGFA rs3025039 polymorphism and biliary atresia.

VEGFA, vascular endothelial growth factor A.
Sensitivity analysis
A sensitivity analysis was performed to evaluate the effects of individual studies on the pooled OR. The pooled OR with 95% CI changed after omitting Liu et al (OR=2.03, 95% CI 1.03 to 3.98), suggesting that this study was a source of heterogeneity (I² decreased from 87.9% to 66.9%) (figure 3).

Publication bias
Both tests supported the absence of publication bias (p value for Begg’s test=0.602, p value for Egger’s test=0.422; table 2). However, the shape of the funnel plot was asymmetric (figure 4). Because a funnel plot requires at least five studies and because we considered only three studies, there was a possibility that there was a possibility for the funnel plot to show asymmetry. Therefore, we considered only the results of the Begg’s and Egger’s tests.

DISCUSSION
We conducted a systematic review of the association between VEGFA rs3025039 polymorphism and susceptibility to BA. Lee et al were the first to identify VEGFA as a susceptibility locus for BA in Chinese patients. This result has biological plausibility; VEGFA is a mediator of the pathogenesis of BA. As a proinflammatory cytokine, VEGFA participates in various processes including cell proliferation, cell migration, and apoptosis, all of which occur in cell-mediated immune-inflammatory diseases, such as BA. There are several independent verifications of this association, but the conclusions remain controversial because the results of different studies in China were conflicting.

We found a difference between cases and controls in comparisons of all genotypes of rs3025039, suggesting that rs3025039 polymorphism may not correlate with susceptibility to BA. A subgroup analysis by area further suggested no significant association between rs3025039 and susceptibility to BA. Given that only one study included the subgroup of northwestern Chinese, our study did not demonstrate a solid correlation between rs3025039 and BA in northwestern Chinese patients.

The absence of significant findings may stem from the fact that we included only three original studies in this study. In addition, as the sample size increased, the association between rs3025039 and susceptibility to BA decreased in the three studies. We observed statistical significance in only two studies with small numbers of participants. Therefore, the statistical power was relatively low, suggesting that the association might be spurious. For the first reason, more original studies regarding the association between rs3025039 and susceptibility to BA are necessary to generate accurate results. However, for the second reason, the effect may be relatively small or not at all, and explicit exploration of this association may result in an unnecessary study. Therefore, it may be more cost-effective to devote more resources to explore other potential biomarkers.

Several limitations need to be acknowledged when considering the results of our study. First, the quantity and sample size of the included studies were insufficient to obtain high power for making a confirmatory conclusion, even though we undertook a comprehensive literature search. Second, all study subjects came from China only; therefore, we could not avoid potential selection bias. Third, residual confounders were possible because BA is a multifactorial and complicated disease, involving gene–environment and gene–gene interactions. We could not detect these effects due to limited information. Fourth, there was a lack of sufficient data; therefore, we did not conduct a subgroup analysis of familial and other BA types. Finally, there was significant heterogeneity that might distort the results. Several aspects may cause heterogeneity, including differences in experimental methods across studies. Therefore, readers should interpret our results with caution.

| Area    | Studies (n) | Comparison model       | OR  | 95% CI      |
|---------|-------------|------------------------|-----|-------------|
| Southern| 2           | C vs T                 | 1.64| 0.50 to 5.37|
|         | 2           | CC vs TT               | 1.40| 0.27 to 7.14|
|         | 2           | CT+CC vs TT            | 1.06| 0.40 to 2.80|
|         | 2           | CC vs CT+TT            | 1.72| 0.48 to 6.14|

Cl, confidence interval; OR, odd ratio.

Table 3 Results of the subgroup analysis

[Figure 3 Forest plot of per-allele model for sensitivity analysis.]

[Table 3] Results of the subgroup analysis

| Study                        | Odds Ratio | OR    | 95% CI    |
|------------------------------|------------|-------|-----------|
| Omitting Lee, H.C., 2010     | 1.21       | [0.75; 1.97] |
| Omitting Liu, B., 2017       | 1.64       | [0.50; 5.37] |
| Omitting Liu, F., 2018       | 2.03       | [1.03; 3.98] |
| Random effects model         | 1.50       | [0.90; 2.49] |
In conclusion, VEGFA rs3025039 polymorphism might not be associated with an elevated risk of BA in Chinese population. Obtaining a definitive conclusion regarding this association may entail additional research. Future studies are recommended to identify other possible genetic markers.

Contributors SH contributed to conceptualization, data curation, formal analysis, project administration, resources, investigation, methodology, software, visualization, writing - original draft. YY contributed to data curation, formal analysis, resources, funding acquisition, supervision, validation, writing - review and editing. GC contributed to resources, writing - review and editing. LM contributed to data curation, formal analysis, resources, investigation, methodology, software, visualization, writing - review and editing. GC contributed to resources, writing - review and editing. YH contributed to writing - review and editing. LM contributed to data curation, formal analysis, analysis, resources, funding acquisition, investigation, methodology, software, visualization, writing - review and editing. GC contributed to resources, writing - review and editing. ZS contributed to supervision, resources. GC contributed to resources, writing - review and editing. YY contributed to data curation, formal analysis, resources, funding acquisition, supervision, validation, writing - review and editing. ZS contributed to supervision, writing - review and editing. SH contributed to conceptualization, data curation, formal analysis, resources, funding acquisition, investigation, methodology, software, writing - review and editing. LM contributed to data curation, formal analysis, resources, investigation, methodology, visualization, writing - review and editing.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval This study is a systematic review and meta-analysis and the data from this study were extracted from the original studies. Moreover, there are no patients or animals involved in this study. Thus, this study does not require ethics approval.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available in a public, open access repository. As a meta-analysis, all of the data in this study can be found in table 1 and were extracted from the original studies.

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