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The relationship between toll-like receptors 9 gene rs5743836 polymorphism and lymphoma risk: a meta-analysis

Minchao Yan[1], Qin Jin[1], Yan Zhou[1], Shuping Mo[1], Lun Tang[1], Gang Zhang[1], Qinyan Fu[1] and Hui Zeng[1,2*]

Abstract

Objectives We performed this meta-analysis to investigate the potential relationship between the polymorphism of the rs5743836 gene of toll-like receptors 9 (*TLR9*) and the risk of lymphoma.

Methods Statistical analysis of all data was performed using Stata 15.0. Heterogeneity tests for all selected studies were performed using the Chi-square-based Q test ($P < 0.05$ suggesting heterogeneity) and the I-square test, and the pooled odds ratios (ORs) were calculated. Sensitivity analysis was also performed to evaluate the stability of the pooled results by funnel plot. Begg's regression test was also performed for possible publication bias in three genetic models.

Results We found that the *TLR9* gene rs5743836 was significantly associated with the risk of lymphoma in the dominant genetic model (OR = 1.54, 95%CI = 1.03–2.32, $P = 0.036$). However, we found that the *TLR9* gene rs5743836 was not significantly associated with lymphoma risk in the recessive genetic model (OR = 1.04, 95%CI = 0.65–1.65, $P = 0.873$) and the allele genetic model (OR = 1.28, 95%CI = 0.93–1.76, $P = 0.130$). We also performed a sensitivity analysis by removing each eligible study, we found that there was no significant change in the merging effect and pooled ORs, which indicates good stability of the results of this study. Publication bias was tested using Begg's funnel plot, and the results suggested that no publication bias was observed in dominant genetic models (TC + CC vs. TT, $P = 0.3486$), recessive genetic models (CC vs. TC + TT, $P = 0.829$), and allelic genetic model (C vs. T, $P = 0.463$).

Conclusions In conclusion, the results of this meta-analysis indicated that the *TLR9* gene rs5743836 was significantly associated with lymphoma risk in the dominant genetic model.

Keywords Toll-like receptors 9, rs5743836, Polymorphism, Lymphoma, Meta-analysis

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Introduction

Lymphoma is a malignant tumor originating in the lymphatic system, divided mainly into two types: Hodgkin lymphoma (HL) (approximately 10 -15%) and non-Hodgkin lymphoma (NHL) (approximately 85 -90%) [1]. In recent years, the incidence rate and mortality from lymphoma have increased in recent years [2, 3]. There were 553,000 new cases of non-Hodgkin lymphoma and 250,000 deaths in 2022 [2]. In China, the mortality rate of lymphoma is on the rise with age and is increasing at a rate of 4.5% annually [4]. Therefore, lymphoma has become an important public health threat worldwide. The study pointed out that the influencing factors of the incidence rate of HL include geographical, socioeconomic, ethnic, gender and age differences [5, 6]. Epidemiological studies have shown that the occurrence and development of HL are related to genetic and environmental factors [7].

Evidence suggested that nuclear factor kB (NF-kB) [8, 9] and Epstein bar virus (EBV) may be related to the pathogenesis of HL [10]. In short, toll like receptor 9 (TLR9) can recognize and activate viral CpG islands, signal through myeloid differentiation primary response protein 88 (MYD88), and activate NF kB [11, 12]. *TLR9* gene was located at chromosome 3p21.3 [13]. The rs5743836 (*TLR9-1237T > C*) is a special single nucleotide polymorphism (SNP) of the *TLR9* gene, and its relationship with lymphoma risk has gradually become a focus of research in recent years. In the forementioned studies, the results have shown that this SNP does not show an association with the risk of developing HL or significantly increases the risk of developing HL and NHL [14–18]. But to our knowledge, no meta- analysis was performed on this topic. So, we performed this meta- analysis to investigate the relationship between *TLR 9* gene SNPs and lymphoma risk.

Materials and methods

Literature search

We systematically searched for relevant publications, the publication date was before 11 May 2024, using PubMed, Web of Science, Cochrane Library and China's national knowledge infrastructure. We use the following search terms: ("TLR9" OR "rs5743836" OR "1237T > C" OR "1237T/C" OR "T1237C") and ("variant" OR "polymorphism" OR "mutation") and ("lymphoma"). The search was limited to publications in English or Chinese.

Literature inclusion and exclusion criteria

The inclusion and exclusion criteria used for literature selection were as follows: 1. inclusion criteria: (1) case- control study focused on the relationship between the *TLR9* gene rs5743836 and lymphoma risk; (2) the genotype or allele frequencies of *TLR9*- rs5743836 were available in the article. 2. Exclusion criteria: (1) those were not case- control studies, such as reviews, letters, case reports, meta-analyses, or; (2) duplicated publications; (3) the genotype or allele frequencies of *TLR9*-rs5743836 were not available in the article.

Data extraction

Two investigators separately and independently read the literature and extracted information from all eligible publications by a standard data-collection form, the following information were extracted, including publication year, the name of first author, country or populations, sample size of case and control group, lymphoma types, Hardy-Weinberg equilibrium (HWE), the genotype or allele frequencies of *TLR9*- rs5743836. If inconsistent results were obtained, the third investigator will review the literature to ensure data accuracy and resolve conflicts.

Quality assessment

Two colleagues separately and independently evaluated the quality of all articles enrolled according to the quality assessment rules [19], based on the source of cases and controls, lymphoma types, *p* values of HWE test in controls, sample size, and Newcastle-Ottawa Scale (NOS) score, which scored from 0 to 9. If these two colleagues concluded inconsistent data during the article evaluation process, the third colleague will resolve the above inconsistent results.

Statistical analysis

In this study, Stata 15.0 software was used for all analyzes and the pooled OR (95%CI) was calculated to investigate the significance of the association between the *TLR9* gene rs5743836 and lymphoma risk in three genetic models, including the dominant model (CC + TC vs. TT), the recessive model (CC vs. TT + TC) and the allele model (C vs. T). Heterogeneity testing was performed for all selected studies using the Chi-square-based Q test ($P < 0.05$ suggesting heterogeneity) and the I-square test [20]. The pooled ORs were calculated with the fixed-effects model (Mantel–Haenszel) when heterogeneity did not exist, while with the DerSimonian and Laird random-effects model when heterogeneity existed [21, 22]. Sensitivity analysis was also performed to evaluate the stability of the pooled results by funnel plot and Begg's regression test was performed for possible publication bias in three genetic models [23, 24].

Results

Basic information on eligible studies

Altogether, two investigators searched 40 related studies and a total of 20 duplicate studies were excluded from this study. Then 20 related literatures were detailed read and 14 of the records were excluded from the database for the following reasons: not involved in rs5743836 or

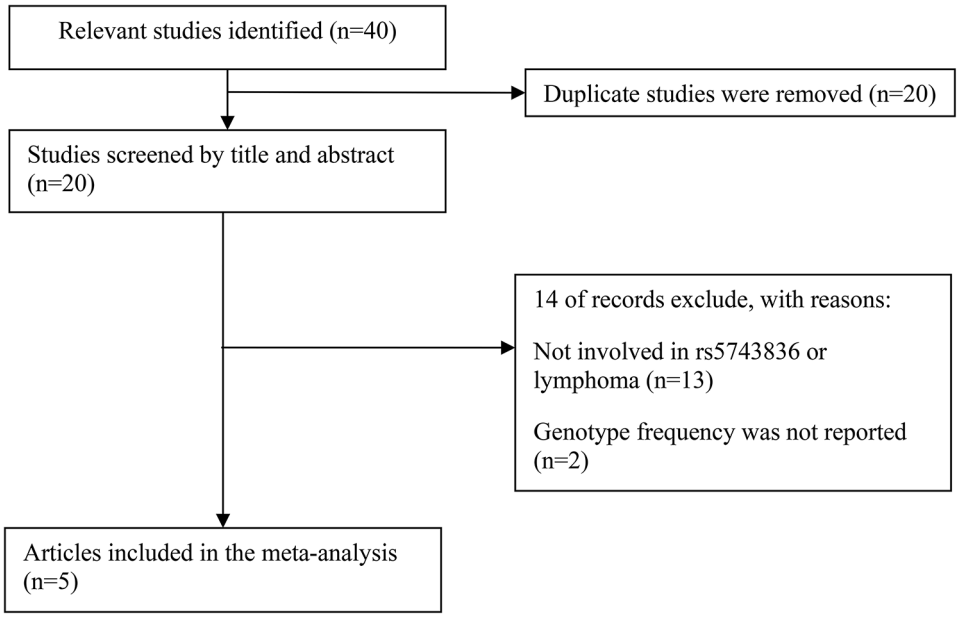

Fig. 1 Flow diagram of the details of the study

Table 1 Main characters of studies included in the meta-analysis (*TLR-9* gene rs5743836)

Author	Year	Ethnicity	Sample size		Cases					Controls					NOS score	Lymphoma types	HWE test for controls
			Case	Control	TT	TC	CC	T	C	TT	TC	CC	T	C			
Nieters	2006	German	678	667	507	156	15	1170	186	475	181	11	1131	203	8	lymphoma	0.182
Carvalho	2012	Portuguese	797	1160	551	244	2	1346	248	934	217	9	2085	235	8	B-cell NHL	0.349
Carvalho	2012	Italian	494	468	345	135	14	825	163	379	81	8	839	97	8	B-cell NHL	0.139
Carvalho	2012	US	801	972	579	209	13	1367	235	674	275	23	1623	321	8	B-cell NHL	0.415
Rahman	2014	Egypt	100	100	41	58	1	140	60	74	26	0	174	26	8	B-cell NHL	0.135
Nielsen	2015	North Denmark	355	307				213	47				412	56	6	B-cell NHL	> 0.05
Al-Khatib	2022	Arab Jordanian	130	234	87	39	4	593	65	183	46	5	523	85	8	HL	0.306

Note: NHL, non-Hodgkin lymphoma; cHL, classical Hodgkin lymphoma; HL, Hodgkin lymphoma

lymphoma ($n = 13$), and genotype frequency was not reported ($n = 2$). Finally, a total of 5 publications were included in the meta-analysis (Fig. 1). In these 5 studies enrolled, one study was separate into 3 studies according to the population of this study, and one study just provided allele frequencies in the allele model. Lastly, a total of 6 studies were included in the dominant model, 6 studies were included in recessive model, and 7 studies were included in the allele model. As shown in Table 1, the genotype distribution of all publications was consistent with HWE (all *P* values were greater than 0.05).

Results of the meta-analysis

Heterogeneity testing was performed among enrolled studies for three genetic models (dominant, recessive, and allele model). As shown in Fig. 2, we found that the *TLR9* gene rs5743836 was significantly associated with the risk of lymphoma in the dominant genetic model (OR = 1.54, 95%CI = 1.03–2.13, $P = 0.036$). However, we found that the *TLR9* gene rs5743836 was not significantly associated with lymphoma risk in the recessive genetic model (OR = 1.04, 95%CI = 0.65–1.65, $P = 0.873$) and the allele genetic model (OR = 1.28, 95%CI = 0.93–1.76, $P = 0.130$).

Sensitivity analysis

We also performed sensitivity analysis by removing each eligible study, we found that there was no significant change in the merging effect and pooled ORs, indicating good stability of the results of this study (Fig. 3).

Publication bias

Publication bias was tested using the Begg funnel graph, and the results suggested that no publication bias was observed in dominant genetic models (TC + CC versus TT, $P = 0.348$), recessive genetic models (CC *versus* $TC + TT$, $P = 0.829$), and allelic genetic model C (C *versus* T, $P = 0.463$) (Fig. 4).

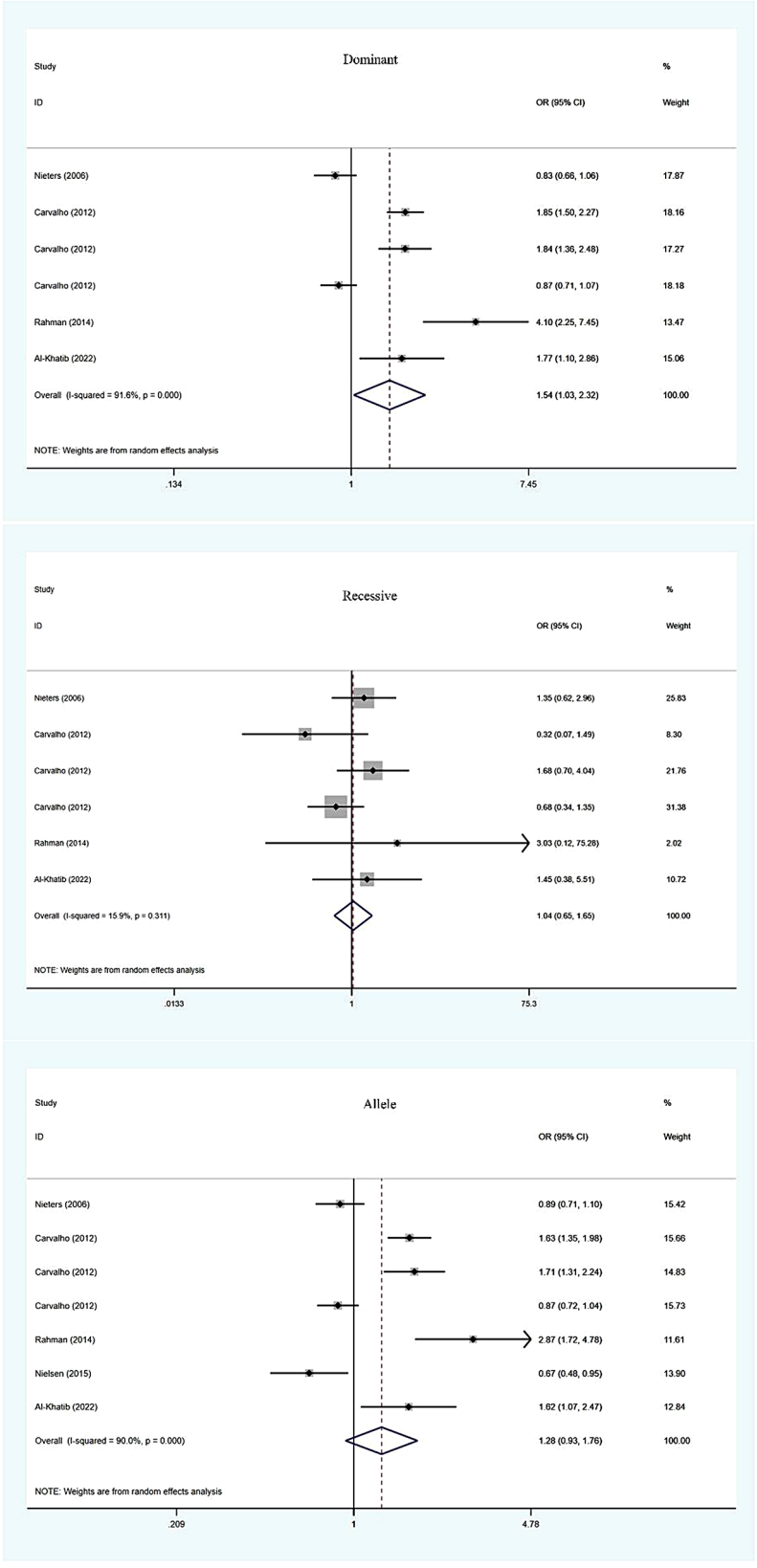

Fig. 2 Forest plots for the meta- analysis of the association between *TLR9* gene rs5743836 polymorphism and lymphoma risk under three genetic models (dominant, recessive and allele models)

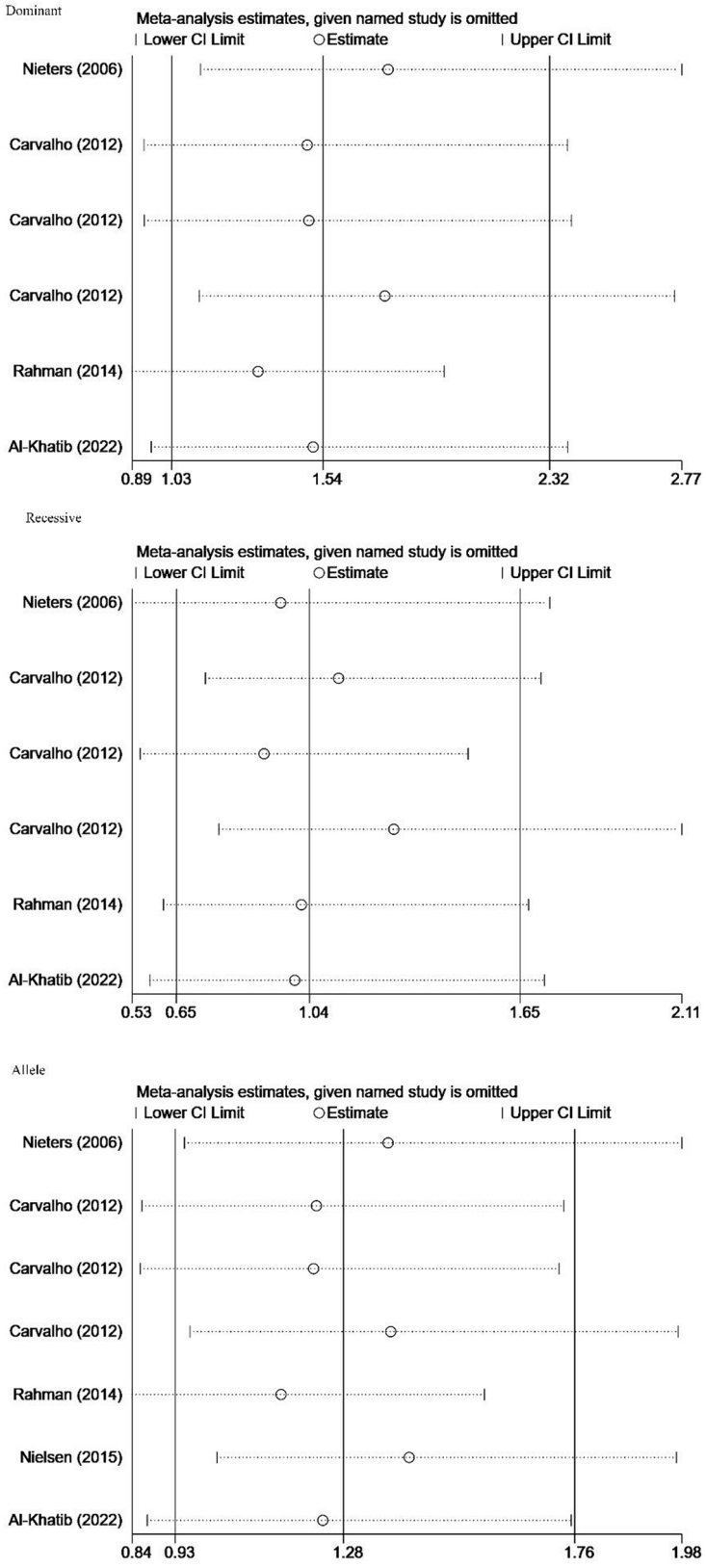

Fig. 3 Sensitivity analysis of the pooled ORs and 95% CI, omitting each dataset in the meta-analysis under three genetic models (dominant, recessive and allele models)

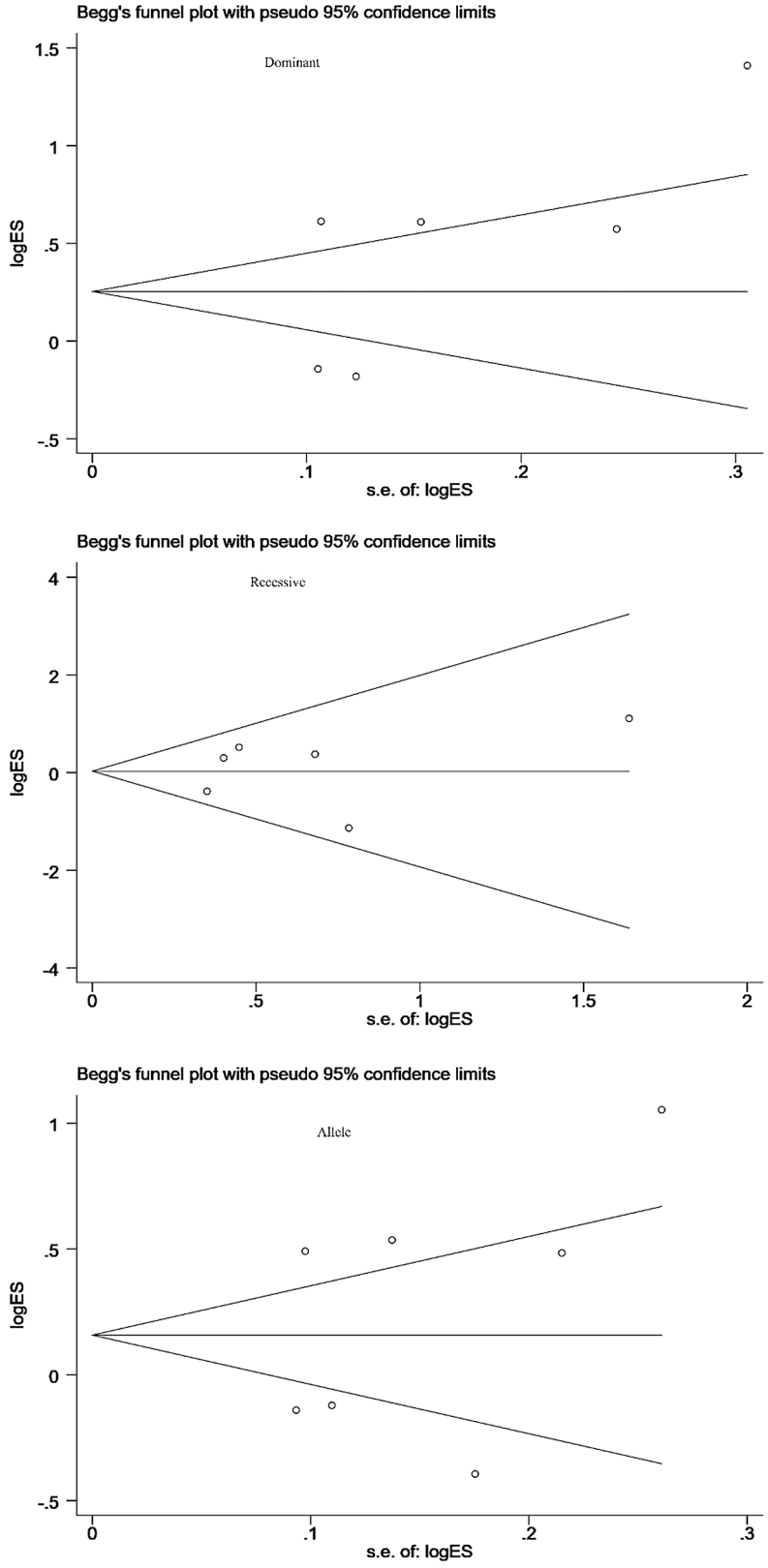

Fig. 4 Begg's funnel plot for the meta-analysis of association between TLR9 gene rs5743836 polymorphism and lymphoma risk under three genetic models (dominant, recessive and allele models)

Discussion

In this study, we found that the *TLR9* gene rs5743836 was significantly associated with the risk of lymphoma in dominant genetic model, but was not significantly associated with lymphoma risk in recessive and allele genetic models. Previously, several studies [14–18, 25, 26] have focused on the relationship between SNPs from the *TLR9* gene and lymphoma susceptibility, however, these previously mentioned studies conclude inconsistent results. In 2015, Nielsen et al. [26] indicated that the *TLR9* gene rs5743836 was associated with a lower diffuse large B cell lymphoma (DLBCL) risk. However, the others study [14, 16, 25] concluded contradictory results and suggested that *TLR9* gene rs5743836 was not associated with lymphoma risk. Nieters et al. [25] first indicated that the *TLR9* gene rs5743836 (-1237T > C) was not associated with the risk of lymphoma and the risk of several types of lymphoma. Martin et al. [16] indicated that *TLR9* rs5743836 was not associated with susceptibility to lymphoma. Carvalho et al. [14] demonstrated that rs5743836 has no significant impact on the risk of NHL in the American population. In addition, other studies [14, 15, 17, 18] also concluded inconsistent results. Carvalho et al. [14] also demonstrated that rs5743836 has a significant impact on the risk of NHL in the Italian and Portuguese populations. Mollaki et al. [15] suggested that the *TLR9* gene rs5743836 (-1237T > C) was associated with a greater risk of Hodgkin lymphoma. A study by Rahman et al. [18] conducted a case-control study among the Egyptian population, they found that rs5743836 polymorphism was a significant risk factor for B-NHL. Recently, Al Khatib et al. [17] conducted a study on the relationship between *TLR9* gene SNP and HL risk and prognosis, they compared the frequency of the rs5743836 (*TLR9*-1237T > C) gene between the case and the control group, and the results showed that the risk of HL statistically associated with minor alleles in both dominant and dominant models.

The detailed mechanism between *TLR9* gene rs5743836 and lymphoma risk was not well known. CpG oligonucleotides are believed to be TLR9 agonists and stimulate TLR9 expression. Signaling stimulated by exogenous or endogenous ligands through TLR9 plays a fundamental role in the host's immune response and subsequent lymphoma risk [27]. The signaling of the TLR pathway is essentially dependent on NF-kB [28]. Individuals carrying the *TLR9* gene rs5743836 promoter polymorphism variant "C" allele (*TLR9*-123 C) exhibit increased expression of TLR9 mRNA, transcriptional activity, and dysregulated immune responses because TLR9 increases the binding affinity for NF-kB by creating potential NF-κB-binding sites.

Several limitations also existed in this meta-analysis. First, we just selected articles with abstract written in English or Chinese, and those articles without English or Chinese abstract, written in other languages were not included in the study, which can lead to biased results. Second, just one study was performed in Asians (Arab Jordanian) and African (Egypt) respectively, and the others were al performed in Caucasians, so we were unable to group the selected articles according to race. In the future, more case- control studies were performed in Asians or African populations, subgroup analysis should be performed according to ethnicity to verified the results obtained in current study.

In conclusion, the results of this meta-analysis indicated that the *TLR9* gene rs5743836 was significantly associated with the risk of lymphoma in dominant genetic model, however, no significant association was obtained between the *TLR9* gene rs5743836 and lymphoma risk under recessive and allele genetic models.

Abbreviations

CNKI	China National Knowledge Infrastructure
NOS	Newcastle-Ottawa Scale
OR	Odds ratio
CI	Confidence interval. TLR9:toll-like receptors 9
TLR9	Toll-like receptors 9
HWE	Hardy-Weinberg equilibrium
SNPs	Single nucleotide polymorphisms

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Author contributions

Manuscript writing, articles search, editing and review were conducted by Minchao Yan and Hui Zeng, Qin Jin, Yan Zhou and Shuping Mo performed evaluation for the quality of the selected studies. Lun Tang, Gang Zhang and Qinyan Fu performed data analysis.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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