Epidemiology and Infection

cambridge.org/hyg

Association between TLR2 + 2477G/A polymorphism and bacterial meningitis: a meta-analysis

Xiaochun Jin[1], Shuzhou Yin[1], Youtao Zhang[2] and Xu Chen[2]

[1]Department of Anesthesiology, Suzhou Kowloon Hospital, Shanghai Jiaotong University School of Medicine, Suzhou, 215028, People's Republic of China and [2]Department of Clinical Laboratory, First Affiliated Hospital of Soochow University, Suzhou, 215006, People's Republic of China

Original Paper

Cite this article: Jin X, Yin S, Zhang Y, Chen X (2018). Association between TLR2 + 2477G/A polymorphism and bacterial meningitis: a meta-analysis. *Epidemiology and Infection* **146**, 642–647. https://doi.org/10.1017/S0950268818000298

Received: 12 December 2017
Revised: 17 January 2018
Accepted: 24 January 2018
First published online: 19 February 2018

Key words:
Bacterial meningitis; meta-analysis; polymorphism; toll-like receptor 2

Author for correspondence:
Xu Chen, E-mail: 934944882@qq.com

Abstract

Toll-like receptor 2 (TLR2) is a key member of TLRs, which is crucial in the initial inflammatory response against bacteria. TLR2, is also the initial barrier against bacterial infection and plays an important role in recognising a variety of bacterial lipoproteins. Several studies have been performed to investigate the TLR2 + 2477G/A polymorphism and bacterial meningitis susceptibility. Unfortunately, the results of previous studies were controversial. Therefore, we performed a meta-analysis to derive a more precise estimation of the association. The association between the TLR2 + 2477G/A polymorphism and bacterial meningitis susceptibility was assessed by odds ratios together with their 95% confidence intervals (CI). Six studies were enrolled in the present meta-analysis. Overall, no significant association between TLR2 + 2477G/A polymorphism and bacterial meningitis risk were found under allele contrast (A *vs.* G: OR = 1.15, 95% CI = 0.93–1.43, $P = 0.202$), recessive genetic model (AA *vs.* AG/GG: OR = 1.12, 95% CI = 0.90–1.41, $P = 0.313$). The significant association was found between TLR2 + 2477G/A polymorphism and pneumococcal meningitis risk under allele contrast (A *vs.* G: OR = 1.54, 95% CI = 1.01–2.36, $P = 0.046$), recessive genetic model (AA *vs.* AG/GG: OR = 1.63, 95% CI = 1.03–2.57, $P = 0.035$). We conclude that TLR2 + 2477G/A polymorphism is not associated with meningococcal meningitis risk but contributes an increased risk of pneumococcal meningitis.

Introduction

Bacterial meningitis (BM) is a severe infectious disease of the central nervous system occurring mainly in young children. It makes a bad effect on children's hearing and learning abilities [1, 2]. Although tremendous advance in antimicrobial therapy has been emerging, BM is still responsible for considerable mortality worldwide. However, the exact aetiology of BM remains unknown.

Studies have demonstrated that genetic variations in microbial recognition genes may be relevant with altered host responses to infection [3]. The pathogen recognizing receptors (PRRs) can generate increased or decreased inflammatory response to infection in epithelial and macrophages cells [4]. Toll-like receptors (TLRs) is an important family member of PRRs. They are composed of transmembrane proteins and expressed mainly on human immune cells including macrophages and dendritic, B, T and some other nonimmune cells [5, 6]. The above studies have confirmed that TLRs could adjust inflammatory reactions and activate immune response to eliminate infectious pathogens [7]. As a key member of TLRs, TLR2 plays an important role in recognising a variety of bacterial lipoproteins. A single nucleotide polymorphism of TLR2 + 2477G/A has been most widely studied and considered related to reduced cellular activation in the presence of the TLR2 ligand lipopeptide. And it is well-known that host genetic factors are closely related to the susceptibility to infectious disease caused by infectious pathogens.

In recent years, several literatures focused on the association between TLR2 + 2477G/A polymorphism and BM susceptibility. However, the results of previous studies were controversial. Hence, in the present study, we perform this meta-analysis on all published case-control studies to derive a more precise estimation of TLR2 + 2477G/A polymorphism with BM risk.

Materials and methods

Search strategy

PubMed, Embase and CNKI (China National Knowledge Infrastructure) databases were searched using the terms as follows: ('TLR2' or 'Toll-like receptor 2') in combination with ('polymorphism' or 'variant' or 'mutation') and in combination with 'Bacterial meningitis' updated on December 2017 for all publications on the association between TLR2 + 2477G/A polymorphism and BM

© Cambridge University Press 2018

CAMBRIDGE UNIVERSITY PRESS

risk. To identify other relevant studies, additional literatures were identified through scanning the references of original literatures which were included in the present Meta-analysis. Review articles were also examined and inspected to find other eligible literatures.

Inclusion and exclusion criteria

The issues below were inclusion criteria for the present literature selection: (a) a case-control study; (b) assessment of the relationship between TLR2 + 2477G/A polymorphism and BM risk; (c) offering distribution of genotypes or other data to compute odds ratios (ORs) and 95% CIs. Accordingly, studies were excluded if one of the following exclusion criteria existed: (a) studies that contained overlapping data; (b) not offering necessary data such as the distribution of alleles or genotypes; (c) studies in which family members had been investigated because of linkage disequilibrium.

Data extraction

All the data were independently reviewed and extracted by two investigators (Xu Chen and Xiaochun Jin). And the result was reviewed by a third investigator (Youtao Zhang). From each study, the following information was recorded: first author, publication year, country, ethnicity; the number of cases and controls, allele frequency and genotype distribution in cases and controls, genotyping methods and evidence of Hardy–Weinberg equilibrium (HWE) in control subjects. Different ethnic descents were categorised as Caucasians, Asian, African or Mixed population.

Statistical analysis

The OR and corresponding 95% confidence interval (95% CI) were calculated to assess the association strength between TLR2 + 2477G/A polymorphism and BM risk. As the genotype AA was rare in the Caucasian population, we evaluated the BM risk with TLR2 + 2477G/A polymorphism by two genetic models including allele contrast (A *vs.* G) and recessive genetic model (AA vs.AG/GG).

The χ^2-test based Q-statistic and I^2 statistics were applied to assess heterogeneity among eligible studies [8, 9]. If there was no obvious heterogeneity, the fixed-effects model would be applied to calculate summary OR [10]. If not, the random-effects model would be used [11]. To detect the possible source of heterogeneity, we examined the sources of heterogeneity such as publication year, the source of control, genotyping method, type of BM and sample size.

Sensitivity analysis was conducted to appraise the stability of the results and identify potentially influential studies. Any single study from the meta-analysis was deleted each time to calculate the influence of the individual dataset to the pooled OR. Funnel plots and Egger's linear regression test were applied to detect the potential publication bias [12]. An asymmetric plot infers a possible publication bias. The significance of the intercept was determined by the Student *t* test suggested by Egger ($P < 0.05$ was considered representative of statistically significant publication bias). All analyses were conducted using Stata software (version 12.0; StataCorp LP, College Station, TX, USA).

Results

Eligible studies

A flow diagram of the search process is shown in Figure 1. We searched all the eligible studies using three databases including PubMed, Embase and Chinese National Knowledge Infrastructure (CNKI). Based on the predefined search strategy and inclusion criteria, a total of six studies were finally included in our meta-analysis [13–18]. And all of the six studies came from PubMed and Embase. None of them came from CNKI. All of the six studies investigated the Caucasian population. Several genotyping methods were used in the eligible studies such as TaqMan probe, PCR-RFLP and direct sequencing. The genotype frequency in controls of all studies was consistent with HWE ($P > 0.05$). The main characteristics of all the case-control studies included in our meta-analysis were listed in Table 1.

Quantitative synthesis of data

The summary results for the association of TLR2 + 2477G/A polymorphism with BM risk are shown in Table 2. Overall, no significant association between TLR2 + 2477G/A polymorphism and BM risk were found under allele contrast (A *vs.* G: OR = 1.15, 95% CI = 0.93–1.43, $P = 0.202$) (Fig. 2), recessive genetic model (AA *vs.* AG/GG: OR = 1.12, 95% CI = 0.90–1.41, $P = 0.313$) (Fig. 3). The significant association was found between TLR2 + 2477G/A polymorphism and pneumococcal meningitis (PM) risk under allele contrast (A *vs.* G: OR = 1.54, 95% CI = 1.01–2.36, $P = 0.046$) (Fig. 2), recessive genetic model (AA *vs.* AG/GG: OR = 1.63, 95%CI = 1.03–2.57, $P = 0.035$) (Fig. 3).

Test of heterogeneity

There was no significant heterogeneity in TLR2 + 2477G/A polymorphism with BM risk under allele contrast ($P_{\text{heterogeneity}} = 0.063$, $I^2 = 44.4\%$) and recessive genetic model ($P_{\text{heterogeneity}} = 0.086$, $I^2 = 40.7\%$). To explore the sources of tiny heterogeneity, we evaluated allele contrast by considering possible sources including publication year, genotyping methods, type of BM, the source of control and sample size. We found that type of BM ($\chi^2 = 4.39$; df = 1; $P = 0.036$) could substantially influence the initial tiny heterogeneity.

Sensitivity analysis

Sensitivity analysis was conducted by sequential removal of individual eligible studies which were included in the present meta-analysis. In the present meta-analysis, any single study could not influence the overall results qualitatively, indicating robustness and reliability of our results.

Publication bias

Begg funnel plot was created to assess the publication bias of selected literatures. Although slightly asymmetrical funnel plots were detected in our results ($P = 0.107$) (Figs 4 and 5), Egger's test did not exhibit obvious publication bias in the allele comparison model ($P = 0.194$) and the recessive model quantitatively ($P = 0.167$).

Discussion

In the present study, we critically performed a meta-analysis of TLR2 + 2477G/A with BM risk. To the best of our knowledge, the present meta-analysis was the first to explore the association between TLR2 + 2477G/A polymorphism and BM risk. The results of our meta-analysis showed TLR2 + 2477G/A polymorphism was not associated with meningococcal meningitis risk but

https://doi.org/10.1017/S0950268818000298 Published online by Cambridge University Press

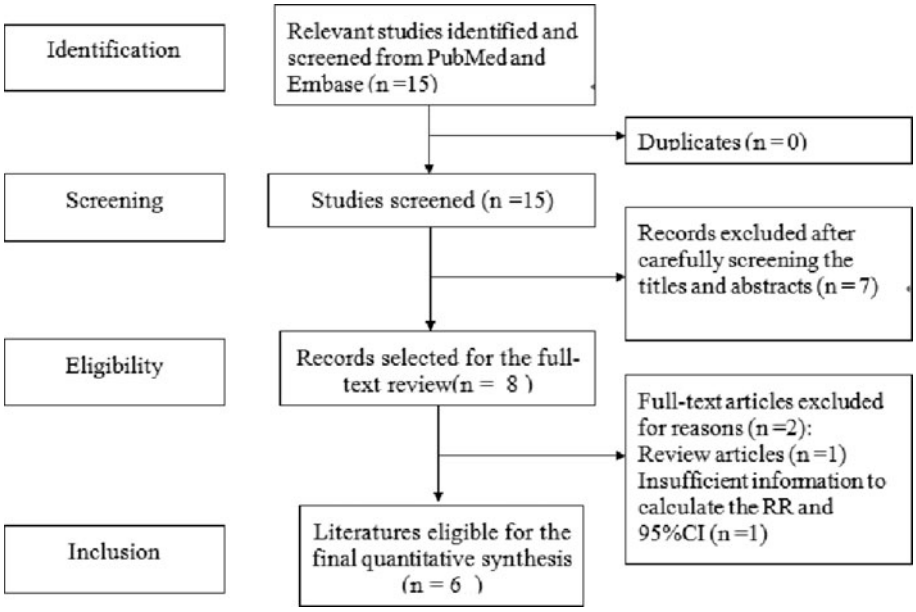


Fig. 1. Flow diagram for identification of eligible studies for this meta-analysis.

Table 1. Main characteristics of all case-control studies included in meta-analysis

Literature	Ethnicity	Country	Genotyping methods	Source of control	Sample size	Matching criteria	HWE (*P* value)
Ewelina (2017)	Caucasian	Poland	direct sequencing	PB	39/49	Age, sex, ethnicity	0.825
Imran (2016)	Caucasian	India	direct sequencing	HB	130/130	Ethnicity	0.052
Orriols (2013)	Caucasian	Spain	PCR-RFLP	HB	20/66	Age, ethnicity	0.527
Stephen (2013)	Caucasian	USA	direct sequencing	HB	271/325	sex, ethnicity	0.471
Well (a) (2013)	Caucasian	Netherlands	TaqMan	PB	46/1141	Age, sex, ethnicity	0.267
Well (b) (2012)	Caucasian	Netherlands	TaqMan	PB	393/1141	Age, sex, ethnicity	0.267

PB. population-based; HWE, Hardy-Weinberg equilibrium; RFLP, Restricted Fragment Length Polymorphism.

Table 2. Meta-analysis of the TLR2 + 2477G/A polymorphism with BM risk

			Test of association				Test of heterogeneity		
Comparison	Type of BM	N	OR	95% CI	*P*	Mode	χ^2	*P*	I^2
A *vs*. G	Overall	10	1.15	0.93–1.43	0.202	Random	16.20	0.063	44.4
	PM	4	1.54	1.01–2.36	0.046	Fixed	3.95	0.266	24.1
	MM	4	1.18	0.90–1.53	0.224	Fixed	2.90	0.408	0
	Other	2	0.52	0.26–1.06	0.071	Random	2.43	0.119	58.8
AA *vs*. AG/GG	Overall	10	1.12	0.90–1.41	0.313	Random	15.19	0.086	40.7
	PM	4	1.63	1.03–2.57	0.035	Fixed	4.47	0.215	32.9
	MM	4	1.13	0.86–1.50	0.379	Fixed	1.29	0.732	0
	Other	2	0.51	0.25–1.05	0.068	Random	2.48	0.115	59.7

PM, pneumococcal meningitis; MM, meningococcal meningitis.

contributed an increased risk of pneumococcal meningitis in the Caucasian population.

How might the TLR2 + 2477G/A polymorphism affect susceptibility to BM? A gene function study demonstrated that TLR2 + 2477G/A polymorphism could weaken tyrosine phosphorylation, dimerization with TLR6, MyD88 recruitment and nuclear factor κB activation [19, 20]. All of the above actions bring about abnormal intracellular signaling and impaired cytokine secretion in response to lipopeptides, peptidoglycan and other ligands, which may result in the development of BM. Additionally, animals models have indicated that defective TLR2 signaling should be responsible for the increased risk to BM. All of these findings

https://doi.org/10.1017/S0950268818000298 Published online by Cambridge University Press

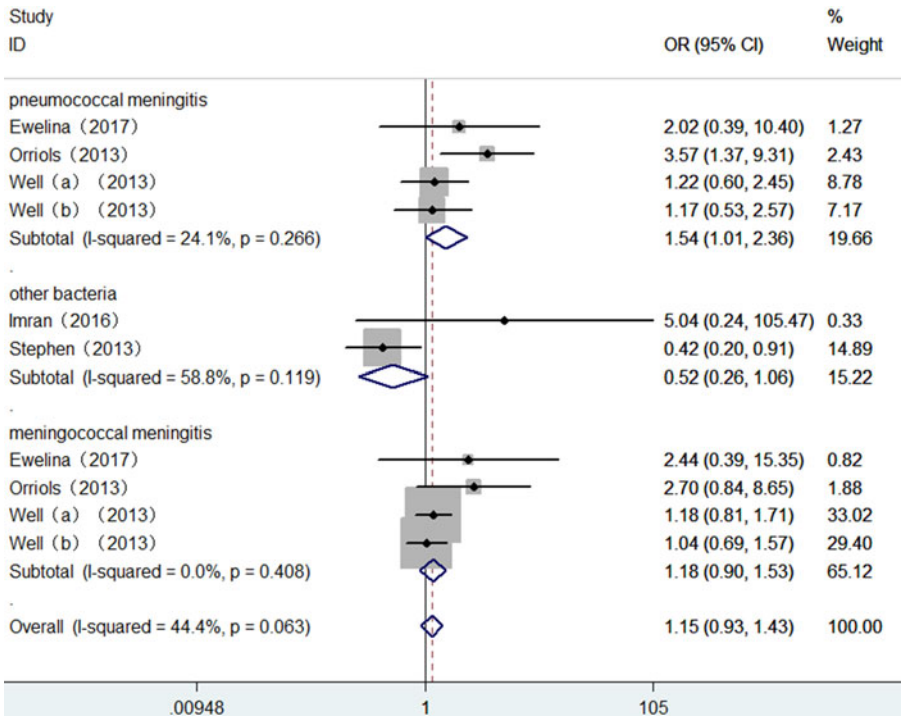


Fig. 2. Forest plot of TLR2 + 2477G/A polymorphism on BM risk (allele contrast A *vs.* G).

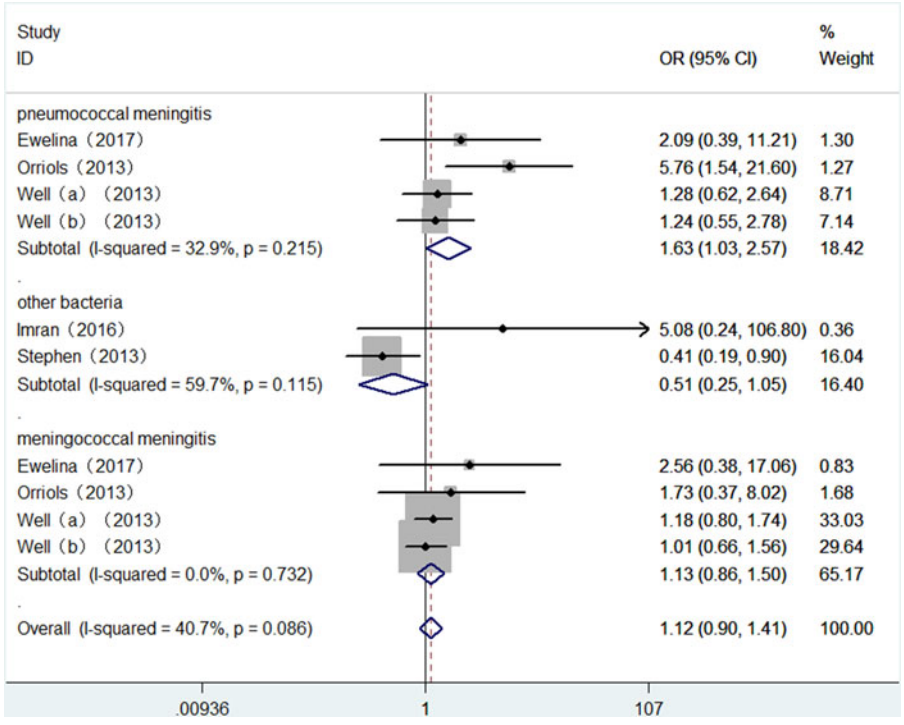


Fig. 3. Forest plot of TLR2 + 2477G/A polymorphism on BM risk (recessive genetic model: AA *vs.* AG/GG).

accord to the TLR2 + 2477G/A polymorphism with PM. Nevertheless, how could one explain the negative association between TLR2 + 2477G/A polymorphism and MM? The streptococcus pneumoniae belongs to the gram-positive bacterium, whose cell wall contains peptidoglycan and teichoic acid. And the Neisseria meningitides is a part of gram-negative bacterium, whose cell wall consists of lipoprotein, lipopolysaccharide and peptidoglycan. The different structure of cell wall may explain the difference between PM and MM. But no matter what happens, we should be cautious about the present results as the sample size may be insufficient.

The heterogeneity is an important factor when performing meta-analysis. Finding the sources of heterogeneity and solving the heterogeneity are of great significance to final results. Overall, the heterogeneity is not significant. The publication year, genotyping methods, type of BM, the source of control and sample size were considered as the possible source of heterogeneity. We found the type of BM contributed substantial

https://doi.org/10.1017/S0950268818000298 Published online by Cambridge University Press

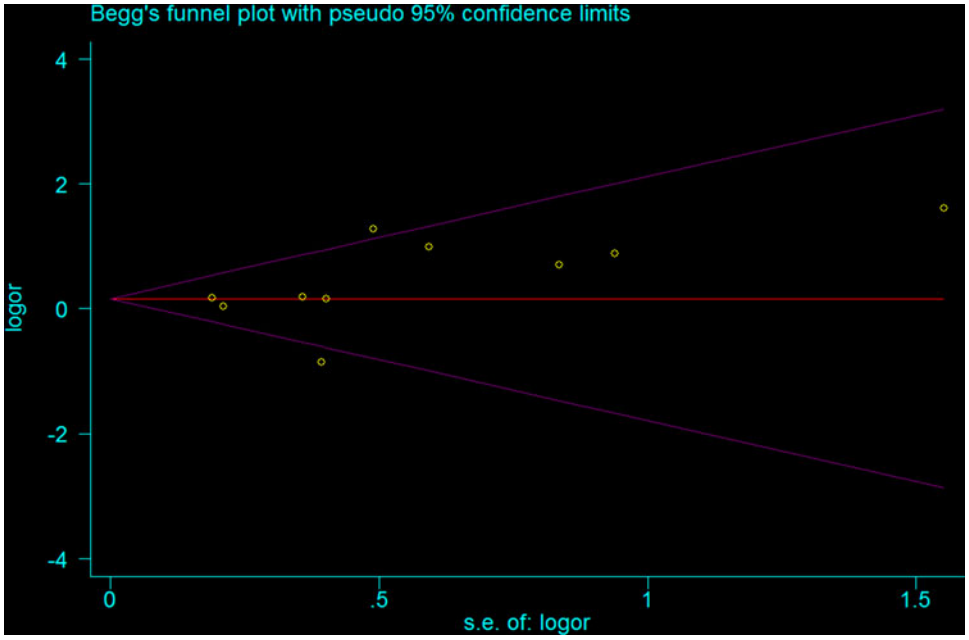


Fig. 4. Funnel plot of TLR2 + 2477G/A polymorphism on BM risk (allele contrast A *vs.* G).

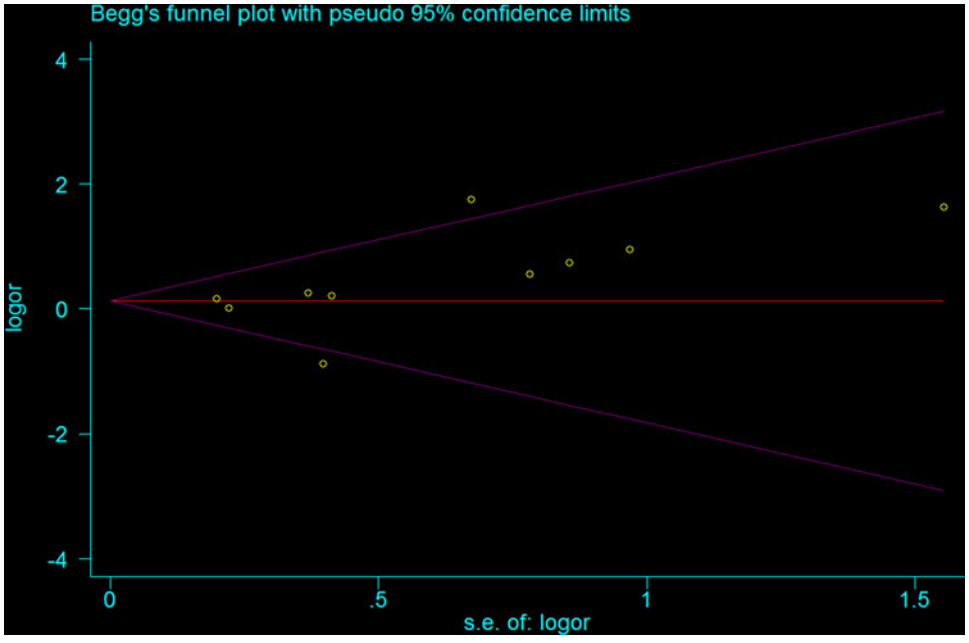


Fig. 5. Funnel plot of TLR2 + 2477G/A polymorphism on BM risk (recessive genetic model: AA *vs.* AG/GG).

heterogeneity to the final results. Then, we carefully evaluated literature quality of every included study and found that the heterogeneity may come from Stephen *et al.* who focus on population including Black non-Hispanic, White non-Hispanic and Hispanic [15]. It is well-known that ethnicity exerts a tremendous influence on allele frequency and genotype distribution. Nevertheless, the sample size of Stephen *et al.* is small. Future large, well designed epidemiological studies are required to investigate the polymorphism in other ethnicities such as Asian, African populations. Sensitive analysis demonstrated that any single study could not affect the final result of the present meta-analysis, indicating that our results were stable and reliable. The publication bias is another important factor that may influence the reliability of meta-analysis. We failed to find any evidence to demonstrate the existence of publication bias, suggesting that publication bias have little effect on the results of our study and the results of our meta-analysis are relatively stable.

Studies demonstrated that inevitable selection bias is existent in hospital-based controls, which were just represent a sample of ill-defined reference population and may not be representative of the study population or general population. Furthermore, it was easier to be influenced when the genotypes under investigation were associated with a disease condition. Hence, it is crucial

https://doi.org/10.1017/S0950268818000298 Published online by Cambridge University Press

and necessary to select population-based controls, which contributes to eliminating selection biases in such genetic association studies. In the present meta-analysis, the source of control brings little heterogeneity to the final results.

As we all know, HWE plays an important role in genetic association studies and gene polymorphism investigations. The genetic association studies which disobey the law of HWE may derive from genetic reasons such as nonrandom mating and the alleles reflect recent mutations. All of the above reasons may lead to the unreliable results of genetic association studies. In the present meta-analysis, all eligible studies conformed to the law of HWE, which contributes to the reliable results of the present meta-analysis.

Although comprehensive meta-analysis was conducted to demonstrate the association between TLR2 + 2477G/A polymorphism with BM risk, there are still some limitations that should be pointed out. Firstly, the primary studies included in our meta-analysis mainly investigated Caucasian population. Since TLR2 + 2477G/A polymorphism substantially varies across different ethnicities, more primary studies which focused on other ethnicities such as Asian population, African population, the mixed population should be carried out. Secondly, we should be cautious to the results because only six eligible studies were included in our meta-analysis. The sample size was relatively small and the only Caucasian population was investigated. Thirdly, because of lack of sufficient primary data, hence, subgroup analysis according to age, gender, radiation exposure and other confounding factors could not be performed in the present meta-analysis.

In spite of the limitations above, our meta-analysis had also several advantages. Firstly, a meta-analysis of the association of TLR2 + 2477G/A polymorphism on BM risk is statistically more powerful than any other single study. Secondly, the quality of our eligible studies was relatively high and the sensitivity analysis and publication bias analysis suggested the stability and credibility of the meta-analysis, which leads to a more convincing result. More important, the process of literature selection, data extraction and data analysis in the meta-analysis was well designed and conducted.

In conclusion, this is the first meta-analysis which investigates the association between TLR2 + 2477G/A polymorphism and BM risk. We conclude that TLR2 + 2477G/A polymorphism is not associated with meningococcal meningitis risk but contributes an increased risk of pneumococcal meningitis.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0950268818000298

Declaration of Interest. The authors declare that there are no competing interests associated with the manuscript.

Funding. The authors declare that there are no sources of funding to be acknowledged.

Author contributions. XC, XJ conceived and designed the experiments; XC, XJ, SY, YZ performed the experiments; SY, YZ analysed the data; XC, XJ, SY, YZ contributed reagents/materials/analysis tools; and XC, XJ, SY, YZ wrote the paper.

References

1. **Somand D and Meurer W** (2009) Central nervous system infections. *Emergency Medicine Clinics of North America* **27**(1), 89–100, ix.
2. **de Jonge RC, *et al.*** (2010) Predicting sequelae and death after bacterial meningitis in childhood: a systematic review of prognostic studies. *BMC Infectious Diseases* **10**, 232.
3. **Brouwer MC, Read RC and van de Beek D** (2010) Host genetics and outcome in meningococcal disease: a systematic review and meta-analysis. *The Lancet Infectious Diseases* **10**(4), 262–274.
4. **Becker CE and O'Neill LA** (2007) Inflammasomes in inflammatory disorders: the role of TLRs and their interactions with NLRs. *Seminars in Immunopathology* **29**(3), 239–248.
5. **Jimenez-Dalmaroni MJ, Gerswhin ME and Adamopoulos IE** (2016) The critical role of toll-like receptors–from microbial recognition to autoimmunity: a comprehensive review. *Autoimmunity Reviews* **15**(1), 1–8.
6. **Underhill DM** (2004) Toll-like receptors and microbes take aim at each other. *Current Opinion in Immunology* **16**(4), 483–487.
7. **Jo EK** (2008) Mycobacterial interaction with innate receptors: TLRs, C-type lectins, and NLRs. *Current Opinion in Infectious Diseases* **21**(3), 279–286.
8. **Higgins JP, *et al.*** (2003) Measuring inconsistency in meta-analyses. *BMJ* **327**(7414), 557–560.
9. **Higgins JP and Thompson SG** (2002) Quantifying heterogeneity in a meta-analysis. *Statistics in Medicine* **21**(11), 1539–1558.
10. **Mantel N and Haenszel W** (1959) Statistical aspects of the analysis of data from retrospective studies of disease. *Journal of the National Cancer Institute* **22**(4), 719–748.
11. **DerSimonian R and Laird N** (1986) Meta-analysis in clinical trials. *Controlled Clinical Trials* **7**(3), 177–188.
12. **Begg CB and Mazumdar M** (1994) Operating characteristics of a rank correlation test for publication bias. *Biometrics* **50**(4), 1088–1101.
13. **Gowin E, *et al.*** (2017) Analysis of TLR2, TLR4, and TLR9 single nucleotide polymorphisms in children with bacterial meningitis and their healthy family members. *International Journal of Infectious Diseases: IJID: Official Publication of the International Society for Infectious Diseases* **60**, 23–28.
14. **Rizvi I, *et al.*** (2016) Vitamin D status, vitamin D receptor and toll like receptor-2 polymorphisms in tuberculous meningitis: a case-control study. *Infection* **44**(5), 633–640.
15. **Spector SA, *et al.*** (2013) Genetic variants in toll-like receptor 2 (TLR2), TLR4, TLR9, and FCgamma receptor II are associated with antibody response to quadrivalent meningococcal conjugate vaccine in HIV-infected youth. *Clinical and Vaccine Immunology: CVI* **20**(6), 900–906.
16. **Telleria-Orriols JJ, *et al.*** (2014) TLR2-TLR4/CD14 polymorphisms and predisposition to severe invasive infections by Neisseria meningitidis and Streptococcus pneumoniae. *Medicina Intensiva* **38**(6), 356–362.
17. **van Well GT, *et al.*** (2013) Single nucleotide polymorphisms in pathogen recognition receptor genes are associated with susceptibility to meningococcal meningitis in a pediatric cohort. *PloS ONE* **8**(5), e64252.
18. **van Well GT, *et al.*** (2012) Polymorphisms in toll-like receptors 2, 4, and 9 are highly associated with hearing loss in survivors of bacterial meningitis. *PloS ONE* **7**(5), e35837.
19. **Xiong Y, *et al.*** (2012) R753q polymorphism inhibits toll-like receptor (TLR) 2 tyrosine phosphorylation, dimerization with TLR6, and recruitment of myeloid differentiation primary response protein 88. *The Journal of Biological Chemistry* **287**(45), 38327–38337.
20. **Lorenz E, *et al.*** (2000) A novel polymorphism in the toll-like receptor 2 gene and its potential association with staphylococcal infection. *Infection and Immunity* **68**(11), 6398–6401.

https://doi.org/10.1017/S0950268818000298 Published online by Cambridge University Press