

# Association of TLR3 gene 1377C/T (rs3775290) and TLR7 gene C/G (rs3853839) polymorphism with hand, foot, and mouth disease caused by human enterovirus 71 infection susceptibility and severity in the Chinese Han population

## A meta-analysis of case-control studies

Haokun Tian, BA<sup>a</sup>, Weikai Xu, BA<sup>a</sup>, Lequan Wen, BA<sup>a,\*</sup> , Lirui Tang, BA<sup>a</sup>, Xinyuan Zhang, BA<sup>a</sup>, Tiangang Song, BA<sup>b</sup>, Changsen Yang, BA<sup>b</sup>

### Abstract

**Background:** Several case-control studies have been conducted on the relationship between rs3775290 C/T and rs3853839 C/G single nucleotide polymorphisms of the Toll-like receptor (TLR) gene and hand, foot, and mouth disease (HFMD) susceptibility and severity. This meta-analysis aimed to offer a systemic review of HFMD susceptibility and severity among the Chinese Han population associated with the C/T (rs3775290) polymorphism of the TLR3 gene or C/G (rs3853839) polymorphism of the TLR7 gene.

**Methods:** A computer search was conducted using PubMed, Web of Science, Embase, CNKI, CBM, VIP, and WanFang databases. The time ranges were from database establishment to 30/7/2021. Articles selected according to the inclusion and exclusion criteria underwent data extraction and methodological quality evaluation. RevMan 5.4 and Stata 16.0 were adopted for meta-analysis, and the incorporated odds ratio (OR) values and 95% confidence intervals (CIs) were calculated. Sensitivity and publication bias assessments were performed.

**Results:** 8 articles with 9 studies were selected. Among them, there were 858 cases and 577 controls in TLR3 rs3775290 studies as well as 2151 cases and 1554 controls in TLR7 rs3853839 studies. Regarding rs3775290 of TLR3, susceptibilities of the severe type of T-possessing individuals were larger than those of C-possessing individuals [OR = 1.34, 95%CI (1.10, 1.64),  $P = .004$ ]. The susceptibility of individuals with the severe TT genotype was 1.61 times that of individuals with the CC genotype [95%CI (1.07, 2.43),  $P=0.02$ ], while susceptibility to HFMD was not influenced by the genotype. In terms of the rs3853839 of the TLR7 gene, C allele carriers have a higher risk of developing HFMD than G allele carriers. The susceptibility to HFMD in CC+CG individuals was 1.24 times than that in GG individuals [95%CI (1.07, 1.43),  $P = .004$ ]. However, no relationship was found between this polymorphism and severity of the severe type. No significant publication bias was observed in this study.

**Conclusions:** rs3775290 (C/T) of TLR3 is associated with susceptibility to the severe type, whereas rs3853839 (C/G) of TLR7 is associated with susceptibility to HFMD. However, owing to the limited quantity and quality of the research, the aforementioned conclusions are yet to be justified by more high-quality research.

**Abbreviations:** DAMPs = damage related molecular patterns, dsRNA = double stranded RNA, EV71 = enterovirus 71, HRS = tyrosine kinase substrates, PAMPs = pathogens related molecular patterns, SNP = single nucleotide polymorphism, ssRNA = single-stranded RNA, TLR = toll-like Receptor, UTR = untranslated region.

**Keywords:** enterovirus 71, hand, foot and mouth disease, single nucleotide polymorphism, TLR3, TLR7

HT, WX, and LW are equal contributors.

The authors state no conflict of interest.

On reasonable request, datasets are available from the corresponding author.

<sup>a</sup> Joint program of Nanchang University and Queen Mary University of London, Nanchang University, 461 Bayi Avenue, Nanchang, Jiangxi, China, <sup>b</sup> Joint program of Nanchang University and Queen Mary University of London, Nanchang University, 1299 Xuefu Avenue, Nanchang, Jiangxi, China.

\*Correspondence: Lequan Wen, Joint program of Nanchang University and Queen Mary University of London, 461 Bayi Avenue, Nanchang, Jiangxi, China (e-mail: l.wen@se18.qmul.ac.uk).

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## 1. Introduction

Hand, foot, and mouth disease (HFMD) has the potential to occur year-round in all regions, especially in summer and autumn. Many epidemiological studies have been conducted on HFMD. Based on previous research, coxsackievirus group A type 4 to 7, 9, 10, and 16; group B type 1 to 3 and 5; part of echovirus; and enterovirus 71 (EV71) are the main pathogenic serotypes of enterovirus that cause HFMD. Among these, coxsackievirus group A type 16 and EV71 are the most common, and EV71 is one of the main pathogens responsible for the global outbreak of HFMD.<sup>[1-3]</sup> The epidemic areas of HFMD are mainly concentrated in tropical, subtropical, and temperate regions. Relevant research shows that the incidence density of diseases caused by EV71 infection is about 15.17/1000 persons per year, with a severe disease rate of about 6%.<sup>[4]</sup> Populations in all regions of the world are susceptible to EV71 infection. However, the type of EV71 infection in East Asian countries such as China is mainly C4; B3, B4, and B5 in Southeast Asian countries such as Malaysia and Singapore; and the B1, C1, and C2 genotype in European and North American countries.<sup>[5]</sup> HFMD has the highest incidence in children under 5 years of age and is characterized by fever and rash or herpes on the hands, feet, and mouth; susceptibility decreases with age.<sup>[1,2]</sup> Most patients have mild symptoms and a good prognosis, but a few children have neurological or circulatory complications and even have the risk of death.<sup>[2]</sup> Based on the severity of the clinical condition, HFMD can be divided into ordinary and severe cases. In ordinary cases, the symptoms are mild, and the prognosis is relatively good. Severe cases are mainly seen in children and are often complicated by neurological and respiratory diseases, such as encephalitis, meningitis, and pulmonary edema. At the same time, it also places a great burden on the circulatory system and causes cardiopulmonary dysfunction, such as myocarditis.<sup>[6]</sup> Furthermore, some studies have demonstrated that the central nervous system diseases and cardiopulmonary failure caused by HFMD can significantly reduce intelligence and delay development.<sup>[7]</sup>

As the main cause of HFMD is viral infection, EV71 must break through the human immune system to cause HFMD. Many immune reactions are involved in this process. Toll-like receptors (TLRs) are important protein molecules involved in innate immunity and also serve as a bridge between innate and cellular immunity. TLRs are single transmembrane noncatalytic proteins that can recognize conserved molecules derived from microorganisms.<sup>[8]</sup> They act as a natural immune system and monitor and recognize various disease-related molecular patterns.<sup>[8,9]</sup> When microorganisms break through the physical barriers of the body, TLRs can recognize them and activate the body to produce immune responses. TLR3 and Notch signaling are 2 important mediators of EV71-induced HFMD, the actions of which are involved in the modulation of CD14+ monocyte function and inflammatory responses. The TLR3-Notch-Src signaling pathway may regulate inflammatory responses.<sup>[10]</sup> In addition, pro-inflammatory cytokine production may be promoted by modulating the TLR7-mediated signaling pathway.<sup>[11]</sup> Thus, changes in TLR may affect the normal function of the immune system, leading to differences in the characteristics of different HFMD patients.

Gene polymorphisms include single-nucleotide polymorphisms (SNPs), polymorphic repetitive elements, small-scale insertions and deletions, and microsatellite variations. They may affect the susceptibility, severity, prognosis, and response to drugs in certain diseases. Currently, many case-control studies have reported that TLR gene polymorphisms are associated with the characteristics of many immune-related diseases, such as asthma,<sup>[12]</sup> acute rheumatic fever,<sup>[13]</sup> rheumatoid arthritis,<sup>[14]</sup> Hashimoto thyroiditis,<sup>[15]</sup> and many other infectious diseases that influence the immune system,<sup>[16,17]</sup> as well as other diseases such as Parkinson syndrome.<sup>[18]</sup>

At present, there have been several case-control studies on the relationship between rs3775290 C/T and rs3853839 C/G single nucleotide polymorphisms of TLR, susceptibility to HFMD, and susceptibility to the severe disease type. However, owing to the uneven quality of similar studies, small sample sizes, or regional and ethnic differences, their conclusions are inconsistent, limiting their credibility. The purpose of this study was to conduct a meta-analysis of case-control studies related to rs3775290 and rs3853839 polymorphisms of TLR3 and TLR7 genes and the susceptibility to HFMD and the severe type caused by EV71 infection, in order to provide more reliable evidence for basic research and clinical treatment.

## 2. Methods

### 2.1. Inclusion criteria

(1) Study design: Published case-control studies. (2) Contents: The correlation between TLR3 rs3775290 and TLR7 rs3853839 polymorphisms and susceptibility to HFMD and severe type was evaluated. (3) Participants: For the meta-analysis assessing susceptibility to HFMD, the case group included patients with HFMD diagnosed by clinical and pathological diagnosis, and the control group consisted of healthy people, regardless of sex, race, age, and disease history; for the meta-analysis evaluating the susceptibility to severe type, the mild group consisted of patients with mild HFMD diagnosed by clinical and pathological diagnosis, and the severe group included patients with severe symptoms or complications including myocarditis, encephalitis, and other severe diseases. (4) For the original research, the statistical method was properly applied, the data quality was reliable, the result expression was clear, and data of various genotype frequencies, odds ratio (OR) values, and 95% confidence interval (CI) were calculated.

### 2.2. Exclusion criteria

(1) The analyzed data were incomplete or missing and could not be obtained by contacting the original author. (2) Nonhuman studies. (3) Repeat of published and retrieved literature. (4) For repeated publications by the same author, the one with the highest quality and largest sample size was selected.

### 2.3. Retrieval strategy

PubMed, Web of Science, EMBASE, CNKI, CBM, VIP, and Wanfang databases were searched using a computer. The retrieval time limit was from database establishment to July 30, 2021. References included in the literature were traced to supplement the relevant literature. The retrieval process adopts a combination of free and subject words. The search terms included HFMD, human enterovirus 71, human enterovirus a, EV71, EV-71, toll-like receptor 3, toll-like receptor 7, TLR3, TLR7, TLR3, TLR7 polymorphism, TLR3 variants, TLR7 variants, TLR3 SNP, TLR7 SNP, rs3775290, and rs3853839. Using PubMed as an example, Table 1 lists the specific retrieval strategies.

### 2.4. Article screening and data extraction

Articles were independently screened by 2 researchers, with their data extracted, which was later cross-checked. Any differences were settled through discussion or negotiation with a third party. During literature screening, the title was checked as a priority. After excluding the evidently irrelevant literature, further reading of the abstract and full text was conducted to determine whether inclusion was warranted. If necessary, the original study author was contacted by email or telephone to obtain information that was not published but very important to this

**Table 1.**  
The retrieval strategy using the PubMed database.

#1	"Hand, foot and mouth disease"[Mesh]
#2	HFMD
#3	"Enterovirus A, human"[Mesh]
#4	EV71
#5	EV-A71
#6	#1 OR #2 OR #3 OR #4 OR #5
#7	TLR3
#8	TLR7
#9	#7 OR #8
#10	#6 AND #9

study. Data extraction included the following basic information of the included study: the first author of the study, the year of publication, the research location, race, detection methods, the numbers of included cases in the case and control groups, and the number of cases corresponding to each genotype.

### 2.5. Literature quality evaluation

The quality of case-control studies was evaluated by referring to the Oxford Critical Appraisal Skill Program (Oxford CASP, 2004) of the Oxford evidence-based medical center in the United Kingdom.<sup>[19]</sup> The following points were assessed: (1) Whether the diagnostic criteria are clearly explained. (2) Group matching. (3) The control group was comparable to the case group. (4) Whether the gene detection method was reasonable. (5) The sample size is sufficient. (6) Sufficient data. Following identical standards, 2 researchers were required to perform evaluations and cross-checking.

### 2.6. Statistical methods

RevMan 5.4 and Stata 16.0 were used for the meta-analysis. The chi-squared test was used to analyze the heterogeneity among the research results (the test level was  $\alpha = 0.05$ ). If there was no heterogeneity among the research results, the fixed-effect model was used for the meta-analysis. If there was heterogeneity, the random effects model was used for the meta-analysis. The OR value and 95%CI of the allele and genotype frequency of each study were calculated, and the Hardy-Weinberg balance of the control group was calculated.  $P < 0.05$  signifies statistical significance. The method of exclusion was used for the sensitivity analysis, and the combined effect was estimated and compared with the combined effect before exclusion. Begg and Egger tests were used to evaluate publication bias in the literature.

### 2.7. Ethics and dissemination

As this meta-analysis is based on published data, it does not require ethical approval. We expect to publish this article on peer-reviewed journal.

## 3. Results

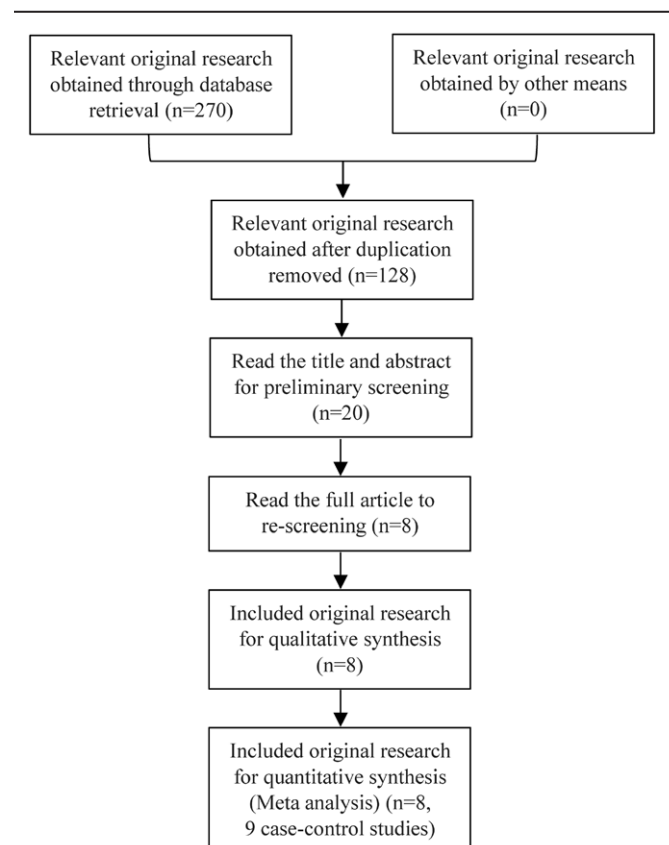
### 3.1. Literature search and quality evaluation results

In total, 270 relevant studies were initially performed. After layer-by-layer screening, 8 studies<sup>[11,20-26]</sup> were finally included, including 9 case-control studies with a total of 3009 cases and 2131 controls. Coincidentally, all the included studies were about EV71 HFMD in the Chinese Han population. Among them, there were 4 case-control studies involving TLR3 rs3775290, with a total of 858 cases and 577 controls, 3 of which assessed susceptibility and 4 assessed severity. There were

5 case-control studies involving TLR7 rs3853839, with a total of 2151 cases and 1554 controls, 4 of which assessed susceptibility and 5 assessed severity. The literature screening process and results are shown in Figure 1, and the basic characteristics of the included studies are shown in Tables 2 and 3. The results of the literature quality evaluation showed that the diagnostic criteria included in the literature were clear, the case-control groups were comparable, the gene detection methods were reasonable, and the results were clear. In the included literature, all subjects were Han Chinese. Nine studies had allele data, of which 7 control groups were in Hardy-Weinberg equilibrium, and 2 control groups did not agree with the Hardy-Weinberg equilibrium.

### 3.2. Meta-analysis results

**3.2.1. Relationship between rs3775290 polymorphism of the TLR3 gene and susceptibility to HFMD.** According to the results of the meta-analysis, there was no significant difference in the risk of HFMD between the codominant model TT versus CC group [OR = 1.20, 95%CI (0.84, 1.69),  $P = .31$ ], dominant model TT+TC versus CC group [OR = 1.23, 95%CI (0.97, 1.57),  $P = .09$ ], or recessive model TT versus TC+CC group [OR = 1.11, 95%CI (0.81, 1.52),  $P = .53$ ]. Taking the TT+TC versus CC comparison as an example, the forest plot is shown in Figure 2A. In addition, the T allele relative to the C allele showed an OR = 1.15 [95%CI (0.96, 1.37),  $P = .12$ ], the TT versus TC comparison showed an OR = 1.02 [95%CI (0.72, 1.44),  $P = .92$ ], and the TC versus CC comparison showed an OR = 1.23 [95%CI (0.95, 1.60),  $P = .12$ ]. In all the above models, the  $P$  value of the statistical test was higher than 0.05, which shows that there was no difference in the relationship



**Figure 1.** Literature screening process and results.

**Table 2.**

**Basic characteristics of included studies of the relationship between rs3775290 and HFMD susceptibility and severity.**

Included study	Research place	Race	Test method	Number (severe/mild)	Severe group genotype			Mild group genotype			Number (case/control)	Case group genotype			Control group genotype			Hardy-Weinberg equilibrium
					TT	TC	CC	TT	TC	CC		TT	TC	CC	TT	TC	CC	
He HF 2017	China	East Asian	iMLDR	78/99	14	39	25	9	40	50	177/225	23	79	75	27	83	115	No
Yuan AY 2017	China	East Asian	PCR-RFLP	59/128	14	23	22	31	52	45	187/232	47	75	65	54	96	82	Yes
Bai YB 2019	China	East Asian	PCR-RFLP	76/90	14	38	24	9	35	46	166/120	23	73	70	15	47	58	Yes
Chen GP 2021	China	East Asian	PCR-RFLP	176/152	23	80	73	18	62	72	/	/	/	/	/	/	/	/

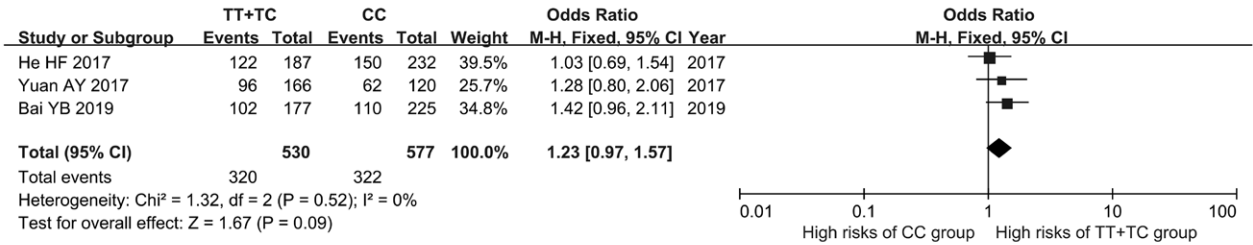
**Table 3.**

**Basic characteristics of included studies of the relationship between rs3853839 and HFMD susceptibility and severity.**

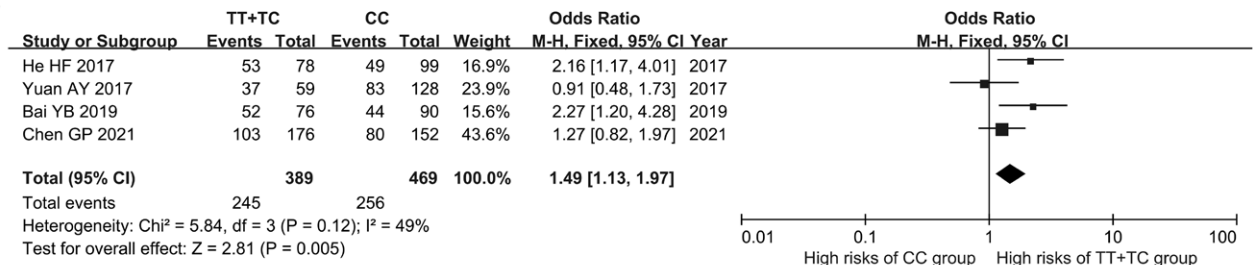
Included study	Research place	Race	Test method	Number (severe/mild)	Severe group genotype			Mild group genotype			Number (Case/Control)	Case group genotype			Control group genotype			Hardy-Weinberg equilibrium
					CC	CG	GG	CC	CG	GG		CC	CG	GG	CC	CG	GG	
Li YP 2017	China	East Asian	Bi-PASA	108/72	26	11	71	14	13	45	180/201	40	24	116	26	23	152	No
Zhang YL 2017	China	East Asian	Unmentioned	341/439	79	64	198	58	65	316	780/748	137	129	514	109	114	525	Yes
Zhang YH 2019	China	East Asian	Unmentioned	162/193	40	29	93	74	25	94	355/300	51	47	257	50	42	208	Yes
Han Y 2020	China	East Asian	Unmentioned	181/327	24	71	86	15	95	217	508/305	39	166	303	7	90	208	Yes
Chen GP 2021	China	East Asian	Unmentioned	176/152	33	21	122	28	26	98	/	/	/	/	/	/	/	/



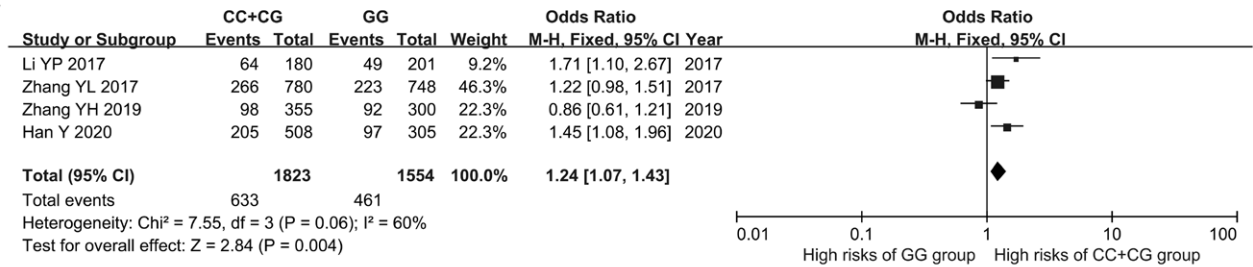
**A**



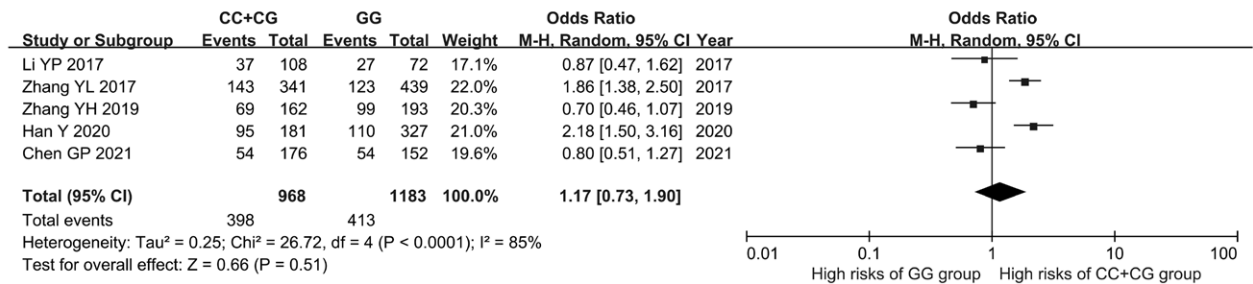
**B**



**C**



**D**



**Figure 2.** Relationship between rs3775290 and rs3853839 polymorphism of the TLR3 gene and HFMD susceptibility and severity. (A) rs3775290 TT+TC vs CC and susceptibility. (B) rs3775290 TT+TC vs CC and severity. (C) rs3853839 CC+CG vs GG and susceptibility. (D) rs3853839 CC+CG vs GG and severity.

between the C/T polymorphism of rs3775290 of the TLR3 gene and the risk of HFMD (Table 4).

**3.2.2. Relationship between rs3775290 polymorphism of the TLR3 gene and severity of HFMD.**

Considering the severity of HFMD, the results of the meta-analysis showed that patients with genotype TT were 1.61 times more likely to develop severe HFMD than patients with genotype CC [OR = 1.61, 95%CI (1.07, 2.43), P = .02], and the result of a comparison of the dominant model TT+TC versus CC [OR = 1.49, 95%CI (1.13, 1.97), P = .005] was also statistically significant. However, for the recessive model TT versus TC+CC, there was no significant difference in the risk of developing severe HFMD [OR = 1.36, 95%CI (0.93, 2.00), P = .11]. Taking the TT+TC versus CC comparison as an example, a forest plot is shown in Figure 2B. The meta-analysis showed that patients with the T allele were more likely to develop severe HFMD than those with the

C allele [OR = 1.34, 95%CI (1.10, 1.64), P = .004]. Patients with genotype TT were 1.61 times more likely to develop severe HFMD than those with genotype CC [95%CI (1.07, 2.43), P = .02]. Patients with genotype TC were 1.44 times more likely to develop severe HFMD than those with genotype CC [95%CI (1.07, 1.94), P = .02] (Table 4).

**3.2.3. Relationship between rs3853839 polymorphism of the TLR7 gene and susceptibility to HFMD.**

According to the results of the meta-analysis, there was no significant difference in the risk of HFMD between the codominant model CC versus GG group (OR = 1.54, 95%CI [0.93, 2.55], P = .09) and the CC versus CG+GG group [OR = 1.48, 95%CI (0.93, 2.37), P = .10]. However, a significant difference was found in the risk of HFMD in the dominant model CC+CG versus GG. Populations with genotypes CC and CG were more likely to develop HFMD than those with genotype GG [OR = 1.24, 95%CI (1.07, 1.43), P = .004], as shown in Figure 2C. In addition, the study of the

**Table 4.****Relationship between rs3775290 polymorphism of the TLR3 gene and HFMD susceptibility and severity.**

Genotype	Susceptibility			Severity		
	OR	95%CI	P	OR	95%CI	P
T vs C	1.15	(0.96, 1.37)	0.12	1.34	(1.10, 1.64)	0.004
TT vs CC	1.20	(0.84, 1.69)	0.31	1.61	(1.07, 2.43)	0.02
TT vs TC	1.02	(0.72, 1.44)	0.92	1.17	(0.78, 1.78)	0.44
TC vs CC	1.23	(0.95, 1.60)	0.12	1.44	(1.07, 1.94)	0.02
(TT+TC) vs CC	1.23	(0.97, 1.57)	0.09	1.49	(1.13, 1.97)	0.005
TT vs (TC+CC)	1.11	(0.81, 1.52)	0.53	1.36	(0.93, 2.00)	0.11

C allele relative to the G allele showed an OR = 1.28 [95%CI (0.98, 1.69),  $P = .07$ ], the CC versus GG comparison showed an OR = 1.25 [95%CI (0.96, 1.62),  $P = .10$ ], and the CG versus GG comparison showed an OR = 1.16 [95%CI (0.97, 1.39),  $P = .10$ ]. Among the studies above, only statistical tests of the CC+CG versus GG group showed  $P < 0.05$ , while studies in other groups could not explain the association between the rs3853839 polymorphism and HFMD susceptibility. There was a slight correlation between the TLR7 rs3853839 G/C polymorphism and the risk of HFMD in the meta-analysis of the original studies (Table 5).

**3.2.4. Relationship between rs3853839 polymorphism of the TLR7 gene and severity of HFMD.** According to the results of the meta-analysis, there was no significant difference in the severity of HFMD in the CC vs GG [OR = 1.38, 95%CI (0.70, 2.73),  $P = .35$ ], CC+CG versus GG [OR = 1.17, 95%CI (0.73, 1.90),  $P = .51$ ], and CC versus CG+GG comparisons [OR = 1.33, 95%CI (0.71, 2.48),  $P = .37$ ] (Fig. 2D). The study of C allele relative to G allele showed an OR = 1.18 [95%CI (0.73, 1.91),  $P = .49$ ], CC versus GG comparison showed an OR = 1.30 [95%CI (0.75, 2.24),  $P = .35$ ], and CG versus GG comparison showed an OR = 1.14 [95%CI (0.74, 1.76),  $P = .55$ ]. In all the above models, the  $P$  value of the statistical test was higher than 0.05, which shows that there was no difference in the relationship between the C/G polymorphism of rs3853839 of the TLR7 gene and the severity of HFMD (Table 5).

**3.2.5. Sensitivity analysis.** A one-by-one exclusion method was used for the sensitivity analysis. Taking CC+CG versus GG of rs3853839 susceptibility group as an example, after excluding one study, the minimum combined OR value was 1.34 [95%CI (1.14, 1.58)] (excluding Zhang YH 2019), and the maximum value was 1.17 [95%CI (0.99, 1.39)] (excluding Han Y 2020). The results showed heterogeneity among the included research results to some extent.

**3.2.6. Publication bias analysis.** The publication bias analysis of rs3853839 gene polymorphism of TLR7 and HFMD severity is shown in Table 6. Taking the CC+CG versus GG genotype as an example, a funnel plot of the CC+CG versus GG genotype

**Table 5.****Relationship between rs3853839 polymorphism of the TLR7 gene and HFMD susceptibility and severity.**

Genotype	Susceptibility			Severity		
	OR	95%CI	P	OR	95%CI	P
C vs G	1.28	(0.98, 1.69)	0.07	1.18	(0.73, 1.91)	0.49
CC vs GG	1.54	(0.93, 2.55)	0.09	1.38	(0.70, 2.73)	0.35
CC vs CG	1.25	(0.96, 1.62)	0.10	1.30	(0.75, 2.24)	0.35
CG vs GG	1.16	(0.97, 1.39)	0.10	1.14	(0.74, 1.76)	0.55
(CC+CG) vs GG	1.24	(1.07, 1.43)	0.004	1.17	(0.73, 1.90)	0.51
CC vs (CG+GG)	1.48	(0.93, 2.37)	0.10	1.33	(0.71, 2.48)	0.37

was drawn after Begg test. It was found that the 5 included studies completely appeared on the plot and were scattered and distributed centered on the combined OR value, which was basically symmetrical and in an inverted funnel shape. Because of the small number of studies with single SNP sites included in the meta-analysis, Egger test showed that  $t = 2.81$ ,  $P = .067 > 0.05$ , and the 95%CI (-0.25, 4.01) included 0, suggesting no publication bias (Fig. 3). Considering the TT+TC versus CC comparison of the rs3775290 gene polymorphism of TLR3 and the severity of HFMD, there was no significant publication bias, as indicated by the funnel plot of Begg test, and  $t = 1.92$ ,  $P = .195 > 0.05$ , and the 95%CI (-0.80, 2.10) included 0 in Egger test.

#### 4. Discussion

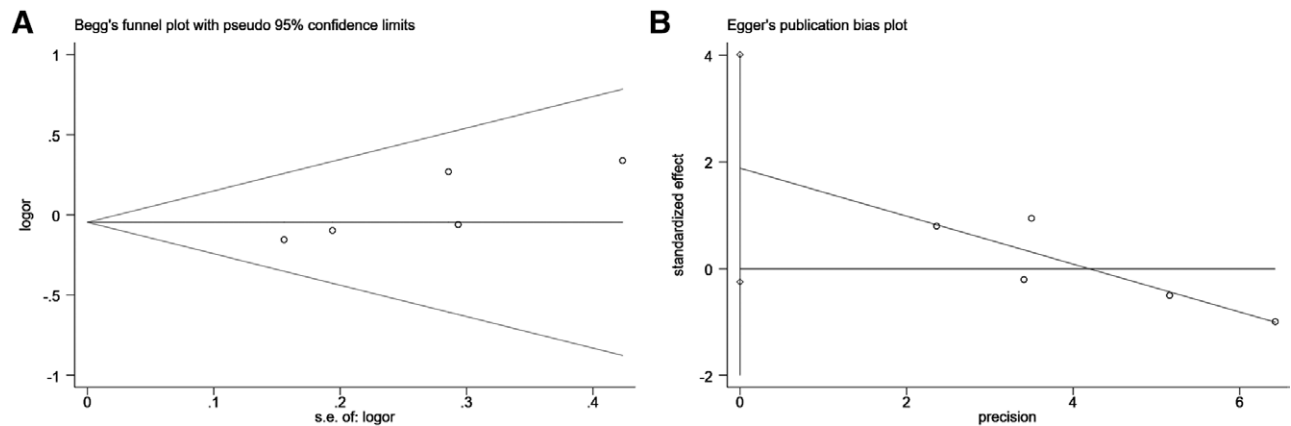
With the development of evidence-based medicine, meta-analyses have been increasingly applied in the medical field. They can be used as a powerful statistical tool by integrating the results of multiple samples on the same topic, which can increase the sample size, improve the statistical efficiency of the original results, and solve the problem of inconsistencies between individual research results.

This study systematically evaluated the susceptibility of individuals with polymorphisms in TLR3 and TLR7 genes to HFMD and susceptibility to the severe type. Polymorphism of rs3775290 is in exon 4 of the TLR3 gene, which is a synonymous mutation that does not lead to amino acid change,<sup>[27]</sup> while polymorphism of rs3853839 is in the 3' untranslated region (UTR) of the TLR7 gene and leads to changes in hsa-miR-298, which is a typical miRNA binding change of 3' UTR.<sup>[28]</sup> The results suggested that the susceptibility to the severe type increased considering the comparison of T allele relative to C allele in TLR3 rs3775290 polymorphism, and the difference was statistically significant. The risk of HFMD in the homozygous TT genotype was 1.61 times higher than that in the CC genotype, suggesting that the T allele of TLR3 rs3775290 could significantly increase the possibility of HFMD developing into a severe disease. However, in studies on susceptibility to HFMD, the rs3775290 gene polymorphism of TLR3 did not show statistical significance, indicating that there was no significant association between the 2. Meanwhile, at the TLR7 rs3853839 polymorphism site, the risk of HFMD was 1.24 times higher in the CC and CG genotypes than in the GG genotype, and the difference was statistically significant. However, in studies on the relationship between the severity of HFMD, the rs3853839 gene polymorphism of TLR7 showed no statistical significance, indicating that there was no significant association between the 2.

TLRs are biomolecules expressed on the surface of macrophages and dendritic cells that can recognize many types of pathogen-related molecular patterns (PAMPs) or damage-related molecular patterns (DAMPs) and respond to them. These pattern recognition receptors can bind to pathogen PAMPs and initiate intracellular signal transduction, leading to the expression and secretion of effector molecules, further leading to the activation of innate immune cells and secretion of a variety of proinflammatory cytokines, such as tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-12, and IL-6. These cytokines induce

**Table 6.****Publication bias analysis of the rs3853839 gene polymorphism of TLR7 and severity of HFMD.**

Genotype	Std. Err.	t	P	95%CI
CC vs GG	0.34	3.08	0.054	(-0.03, 2.16)
(CC+CG) vs GG	0.67	2.81	0.067	(-0.25, 4.01)
CC vs (CG+GG)	0.36	3.28	0.046	(0.04, 2.32)
C vs G	0.67	3.66	0.035	(0.32, 4.59)



**Figure 3.** Publication bias analysis results (Taking CC+CG vs GG genotype of rs3853839 and the severity of HFMD as an example).

inflammation and promote antigen presentation. Therefore, they are highly associated with viral infections.

EV71, a positive-sense single-stranded RNA (ssRNA) virus, is one of the main causes of HFMD. The pathogenesis of EV71 remains unclear, and many studies are underway. However, many studies have suggested that the host immune response plays an important role in the development of this disease. TLRs mediate pathogen recognition, which can induce immune and inflammatory responses in the host cells.<sup>[29]</sup> Intracellular TLRs are transported from the endoplasmic reticulum to the endosomal network, where they initiate complete signaling, resulting in an inflammatory response.<sup>[29,30]</sup> Studies have shown that the TLR3-Notch-Src signaling pathway may regulate inflammatory responses. The combination of TLR3 and Notch signaling modulates CD14+ monocyte function and inflammation during the development of HFMD.<sup>[10]</sup> When CD14+ monocytes are treated with TLR3 agonists such as poly(I:C), the activation of Notch1 and Notch2 is promoted. Furthermore, suppression of Notch signaling depresses poly(I:C)-induced inflammation without altering TLR3 expression. The TLR3-Notch-Src signaling pathway may regulate inflammatory responses that are witnessed in severe HFMD cases.<sup>[10]</sup> TLR7 has also been associated with EV71 infection. Viral ssRNA binding to TLR7, MyD88, and IRAK is linked to TLR7 and TRAF6, and TAK1 and TAB1/2 are recruited, thereby initiating multiple downstream pathways, including the p38 MAPK, NF- $\kappa$ B, and IRF3/7 pathways, to trigger IFNs and the production of proinflammatory cytokines. It is suggested that tyrosine kinase substrates (HRS) might be supportive of TLR7 signaling initiation, and p38, NF- $\kappa$ B, and IRF3 pathways induce IFN induction and proinflammatory factor production. Therefore, TLR7 crucially participates in immune and inflammatory responses to EV71-induced HFMD.<sup>[29]</sup> Therefore, TLR3 and TLR7 are strongly associated with EV71-induced HFMD. As the meta-analysis results of TLR3 and TLR7 polymorphisms showed different manifestations, it is speculated that different signaling pathways might be differently activated downstream after EV71 infection, which requires more basic follow-up research.

In addition, there are numerous other polymorphisms in TLR3 and TLR7 genes that have been confirmed to be associated with many other diseases. For example, Lee et al found that the TLR3 rs5743313 polymorphism influences the risk of severe outcomes in H7N9/H1N1 influenza. The underlying mechanism might be that TLR3 gene polymorphism influences the level of inflammatory factors, thus affecting virus clearance and pro-inflammatory response signals at different disease stages.<sup>[31]</sup> Fan et al found that the TLR3 gene rs5743305 polymorphism may be associated with increased susceptibility to breast cancer due to the reduced ability of TLR3 to recognize double-stranded RNA (dsRNA) and induce apoptosis in

human breast cancer cells,<sup>[32]</sup> and Chen DN et al found that the TLR3 rs3775291 polymorphism was associated with the recurrence of breast cancer. In addition, considering the rs3775291 polymorphism, advanced dry age-related macular degeneration, hepatitis C virus (HCV) infection, chronic hepatitis caused by hepatitis B virus (HBV), and hepatitis B-related acute-on-chronic liver failure were confirmed to be affected mainly by the reduced signaling activity of TLR3 and the ability to bind to dsRNA or influence its glycosylation.<sup>[33–35]</sup> The susceptibility, severity, and prognosis of bacterial meningitis in children and non-small cell lung cancer have also been proven to be related to this factor.<sup>[36,37]</sup> Case-control studies and meta-analyses considering TLR7 also confirmed that its polymorphism is related to the characteristics of a large number of diseases, especially immune-related diseases such as HIV-1<sup>[38]</sup> and HBV<sup>[39]</sup> infection, tuberculosis,<sup>[17]</sup> asthma,<sup>[40]</sup> systemic lupus erythematosus,<sup>[41]</sup> and many types of cancer.<sup>[42,43]</sup> The main possible mechanisms were also associated with changes in dsRNA-binding capacity and inflammatory factor production related to signaling pathways, mainly NF- $\kappa$ B and MAPK.

Gene polymorphisms are widely present in humans, and even slightly different types of genes can affect the progression of some pathways. Therefore, some genetic polymorphisms present in the population may cause an increased susceptibility to disease, which explains the results of this meta-analysis.

A meta-analysis is a comprehensive analysis of published literature with similar research objectives, contents, and types, which is greatly influenced by the included literature, and publication bias is one of the common possible biases. In this study, no significant publication bias was found using Begg and Egger test. Therefore, the results have credibility. However, the following limitations still exist: First, all the literature included in this study was publicly published in Chinese or English. Potential language bias may have affected the results of the meta-analysis. In addition, cases and controls involved in this study mainly come from well-known hospitals in economically developed cities in China, which have a high level of diagnosis and treatment, and are responsible for the diagnosis and treatment of many severely ill patients transferred from subordinate hospitals. As a result, these hospitals generally have high admission rates for severely ill patients, leading to an admission rate bias in the original research. Therefore, the incidence rates of severe cases included in the original studies were higher than the general rate, and more multicenter case-control studies on HFMD are expected to be conducted in the future. However, this did not influence the genotyping and related analysis in the original research, which had only a limited impact on the meta-analysis. Furthermore, the number of articles on the



polymorphism of TLR3 rs3775290 and TLR7 rs3853839 and disease susceptibility is limited, leading to a small number of studies included in this meta-analysis and a small number of case and control groups, which reduced the representativeness of the conclusions. Moreover, subgroup analysis could not be performed on different ethnic and age groups, which reduced the comprehensiveness of the results. Considering the influence of ethnic factors, although studies on HFMD in other countries are lacking, some studies have shown that TLR3 rs3775290 is related to chronic HCV infection and can predict the outcome of HCV-specific cell-mediated immunity.<sup>[16]</sup> Among the Chinese Han people, the population with the TT genotype had a higher risk of chronic hepatitis caused by HCV infection, as indicated by the TT versus CC comparison with OR=1.63.<sup>[44]</sup> Among Egyptians, the conclusion is consistent with OR=2.64.<sup>[45]</sup> It seems that the genotypes of rs3775290 have the same characteristics in Chinese Han people and Egyptian patients with chronic hepatitis caused by HCV infection, which may also provide guidance for HFMD. However, more case-control studies are needed for verification.

In summary, as susceptibility genes of EV71-induced HFMD, TLR3 and TLR7 play an important role in the development of the disease. TLR3 rs3775290 was associated with susceptibility to severe HFMD, and TLR7 rs3853839 polymorphism was associated with susceptibility to HFMD. For rs3775290, the T allele may be a risk factor for the development of severe HFMD. For rs3853839, the population with CC and CG genotypes had a higher risk of HFMD than the GG group.

As an infectious disease, HFMD has attracted increasing attention, and some patients may experience serious sequelae. Studying the correlation between the incidence of HFMD and TLR3 rs3775290 and TLR7 rs3853839, in order to understand the pathogenesis and outcome mechanism of HFMD at the gene level, can provide new ideas for the research, prevention, and treatment of HFMD. In the future, it is expected that more multi-center, large-sample, and homogeneous case-control studies will be carried out to better evaluate the correlation between TLR3 and TLR7 gene polymorphisms and HFMD susceptibility and severity and provide more reliable evidence for basic research and clinical treatment.

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## Author contributions

HT is responsible for articles retrieval, combined analysis of included studies, and writing of article outline with the help of WX and LW. LT and XZ are responsible for writing and structure improvement of this article. TS and CY are responsible for data checking. LW determines the direction for the topic selection of this meta-analysis.

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