Received: 29 December 2021 | Revised: 9 March 2022 | Accepted: 21 March 2022

DOI: 10.1002/jcla.24385

RESEARCH ARTICLE

WILEY

TLR4 polymorphisms as potential predictors of atopic dermatitis in Chinese Han children

Jianrong Shi[1] | Lin He[2] | Ran Tao[1] | Huiwen Zheng[3] | Wei Li[1] | Shuangshuang Huang[1] | Yunling Li[3] | Shiqiang Shang[1]

[1]Department of Clinical Laboratory, The Children's Hospital, Zhejiang University School of Medicine, National Clinical Research Center For Child Health, Hangzhou, China

[2]Zhejiang University School of Medicine, Hangzhou, China

[3]Department of Dermatology, The Children's Hospital, Zhejiang University School of Medicine, National Clinical Research Center For Child Health, Hangzhou, China

Correspondence
Shiqiang Shang, Department of Clinical Laboratory, The Children's Hospital, Zhejiang University School of Medicine, National Clinical Research Center For Child Health, Hangzhou 310052, Zhejiang, China.
Email: shangsq@zju.edu.cn

Funding information
Zhejiang Provincial Natural Science Foundation of China, Grant/Award Number: LY21H040002 and LGD20H010002

Abstract

Background: Toll-like receptor 4 (TLR4) is considered to be involved in the pathogenesis and progression of atopic dermatitis (AD). In the present study, we evaluated the relationship between *TLR4* gene polymorphisms and the susceptibility or severity of AD among Chinese Han children.

Methods: A total of 132 AD patients and 100 healthy controls were enrolled in this study. Four single-nucleotide polymorphisms (rs19277914, rs11536891, rs7869402, and rs11536889) of the *TLR4* gene were genotyped by multiplex PCR combined with next-generation sequencing.

Results: Our results showed that a significantly reduced risk for AD was associated with C allele [$p = 0.008$; odds ratio (OR) = 0.41, C vs. T], TC genotype ($p = 0.022$; OR = 0.41, TC vs. TT), and TC + CC genotype ($p = 0.010$; OR = 0.39, TC + CC vs. TT) of *TLR4* rs11536891. The frequency of the haplotype GCCG (rs1927914–rs11536891–rs7869402–rs11536889) in AD patients was lower than that in the controls ($p = 0.010$; OR = 0.38). Moreover, the results indicated that a higher risk of severe AD was related to the T allele ($p = 0.019$; OR = 2.97, T vs. C) and the TC genotype ($p = 0.021$; OR = 3.34, TC vs. CC) of *TLR4* rs7869402. A risk haplotype of *TLR4* (GTTG) was found in severe AD patients ($p = 0.010$; OR = 5.26).

Conclusions: Our data suggested that *TLR4* rs11536891 polymorphism was associated with the susceptibility to AD in Chinese Han children. And *TLR4* rs7869402 might confer the severity of pediatric AD patients.

KEYWORDS
atopic dermatitis, polymorphisms, susceptibility, Toll-like receptor 4

1 | INTRODUCTION

Atopic dermatitis (AD), also known as eczema, is the most common chronic inflammatory skin disease, adversely affecting 10%–20% of the pediatric population worldwide.[1] The disorder, characterized by intense itching and recurrent eczematous lesions, seriously reduces pediatric patients' quality of life and increases health care burden.[2,3] Although the exact pathophysiology of AD remains unclear, genetic predisposition, immune dysregulation, and epidermal barrier dysfunction have been thought to be the critical factors in the pathogenesis and development of AD.[1,4,5]

Jianrong Shi and Lin He contributed equally to this work.

Toll-like receptors (TLRs), a family of transmembrane proteins, recognize pathogen-associated molecular patterns and then enable the specific innate and adaptive immune responses.[6] It is well established that TLRs are expressed in a variety of immune/inflammatory cells and associated with different infectious and immune-mediated diseases.[7] Among ten functional TLRs, TLR4 has been shown to be linked to atopic diseases including AD through mediating pro-inflammatory signals and immune intervention.[7] Panzer et al.[8] reported that the expression of TLR4 increased gradually from the basement layer to the upper stratum spinosum, including the granular layer in AD patients, while it was mainly located in the basement layer in normal skin. Moreover, the level of TLR4 expression in peripheral blood monocytes of patients with AD was higher than that of healthy subjects regardless of the severity of the disease.[9] The results of an animal experiment explored that TLR4 could attenuate the hapten-induced skin inflammation through the inhibition of langerin-positive dendritic cells migration, suggesting that TLR4 might serve as a modulator of inflammatory and immune response during AD development.[6]

The human *TLR4* receptor gene is located on the chromosome (9q32-33). *TLR4* single-nucleotide polymorphisms (SNPs) have been indicated to be involved in the pathogenesis of various inflammatory and immune-related disorders such as rheumatoid arthritis, asthma, and ulcerative colitis.[10–14] Nevertheless, the results of the genetic researches on the effect of *TLR4* SNPs in systemic vasculitis were inconsistent.[15–19] To our knowledge, previous studies on the relationship between *TLR4* SNPs and AD have been only performed in Italian children, Russians, Ukrainian children, and German population.[20–22] Up to now, there have been no studies on the linkage between *TLR4* gene polymorphisms and AD in Chinese population. Therefore, the present study is carried out in this direction to explore the association of *TLR4* gene SNPs with the susceptibility and severity of AD in Chinese Han children, so as to estimate the population-specific predictive effect of *TLR4* genetic variation on AD.

2 | MATERIALS AND METHODS

2.1 | Subjects

In this study, the children with AD visiting the dermatology clinic of the Children's Hospital of Zhejiang University School of Medicine were recruited from February 2021 to August 2021. The inclusion criteria were defined as follows: patients newly diagnosed with AD based on the AD diagnostic criteria of Hanifin and Rajka[2]; less than 18 years old; all subjects and their parents agreed to participate in the study. Children with other skin diseases or any other systemic inflammatory and autoimmune disorders or a history of dermatological medication were excluded. The severity of AD was evaluated according to the Severity Scoring of Atopic Dermatitis (SCORAD) index.[2] In order to analyze the correlation between *TLR4* gene SNPs and the AD severity, the cases were divided into two subgroups, and named as mild-to-moderate subgroup (index: 0–50) and severe subgroup (index >50).[23]

During the same period, 100 unrelated healthy controls with no history of atopic or any other autoimmune diseases were selected from the children who underwent routine physical examination in the same hospital. All cases and controls enrolled in this study were of Chinese Han ethnicity. Their demographic and clinical information including gender and age were collected and described in Table 1. The study was adopted by the Institutional Ethics Committee of the Children's Hospital of Zhejiang University School of Medicine in accordance with the principles of the Declaration of Helsinki (2021-IRB-009).

2.2 | Genomic DNA extraction and genotype assessment

Peripheral blood samples from each subject were collected in ethylenediaminetetraacetic acid (EDTA) vials (1 ml). Two hundred microlitres of peripheral blood was taken from each sample for DNA extraction and genotype assessment. Genomic DNA was extracted using the Biospin Genomic DNA Purification Kit (BIOER Technology; #BSC06S1). Genomic DNA concentration and purity were determined by Nanodrop1000c spectrophotometer (Thermo Scientific). The integrity of DNA was then detected by agarose gel electrophoresis (1%). The qualified DNA samples were stored at −20°C until the genotype analysis and then at −80°C for long-term storage.

Four SNPs in *TLR4* gene were selected including rs1927914, rs11536891, rs7869402, and rs11536889. Genotyping was evaluated by multiplex PCR combined with next-generation sequencing on Illumina X-10, a high-throughput genotyping platform of Shanghai BioWing Applied Biotechnology Company (http://www.biowing.com.cn/).[24] The primers for TLR4 rs19

TABLE 1 The main demographics and clinical characteristics of AD patients and controls

Characteristics	Number	Gender			Age	
		Male [*n* (%)]	Female [*n* (%)]	*p* value	Mean ± SD	*p* value
Controls	100	66 (66.00)	34 (34.00)	0.131	4.9 ± 1.9	0.232
AD patients	132	73 (55.30)	59 (44.70)		2.6 ± 2.1	
Mild-to-moderate	99	54 (54.55)	45 (45.45)	0.919	2.6 ± 2.2	0.723
Severe	33	19 (57.58)	14 (42.42)		2.5 ± 1.9	

Abbreviations: AD, atopic dermatitis; SD, standard deviation.

TABLE 2 Allele frequencies of four TLR4 gene SNPs in AD cases and the controls

SNPs	Allele A/B	MAF AD	MAF Controls	HWE-*p* AD	HWE-*p* Controls	OR (95% CI)	*p* value
rs1927914	G/A	0.38	0.44	0.58	0.69	1.29 (0.89–1.87)	0.183
rs11536891	C/T	0.05	0.12	1.00	1.00	0.41 (0.20–0.81)	0.008*
rs7869402	T/C	0.07	0.06	1.00	1.00	0.95 (0.45–1.99)	0.889
rs11536889	C/G	0.25	0.24	0.35	0.26	1.06 (0.69–1.64)	0.780

Note: A: minor alleles; B: major alleles;

Abbreviations: AD, atopic dermatitis; CI, confidence interval; HWE-*p*, *p*-value of Hardy-Weinberg equilibrium; MAF, minor allele frequency; OR, odds ratio; SNP, single nucleotide polymorphism; TLR4, Toll-like receptor 4.

*Statistically significant ($p < 0.05$).

TABLE 3 Genotype analysis of TLR4 rs11536891 in codominant, dominant, and recessive models in AD cases and the controls

Genotype model	AD *n* = 132 [*n* (%)]	Control *n* = 100 [*n* (%)]	OR (95% CI)	*p* value
Codominant				
TT	118 (89.39)	76 (76.77)	1.00	
TC	14 (10.61)	22 (22.22)	0.41 (0.20–0.85)	0.022*
CC	0 (0)	1 (1.01)	-	
Dominant				
TT	118 (89.39)	76 (76.77)	1.00	
TC + CC	14 (10.61)	23 (23.23)	0.39 (0.19–0.81)	0.010*
Recessive				
TT + TC	132 (100.00)	98 (98.99)	1.00	
CC	0 (0)	1 (1.01)	-	0.190

Abbreviations: AD, atopic dermatitis; CI, confidence interval; OR, odds ratio; TLR4, Toll-like receptor 4.

*Statistically significant ($p < 0.05$).

27914 were 5′-GTTGATGGAGTCTACAAGAGTTTG-3′ (forward) and 5′-TTGTAAAGCTTTTAGGACAGTGTC-3′ (reverse). The primers for TLR4 rs11536891 were 5′-TCAAAACTGGAAA TATGACCACAG-3′ (forward) and 5′-ACACACACTTTCAAATA CACACAG-3′ (reverse). The primers for TLR4 rs7869402 were 5′-TGGGATCCCTCCCCTGTAGC-3′ (forward) and 5′-AGGAGCAT TGCCCAACAGG-3′ (reverse). The primers for TLR4 rs11536889 were 5′-CTTTAGGGAGACACAGATGGCTG-3′ (forward) and 5′-GAACATTCTTTTCTGGGAACCTTC-3′ (reverse).

2.3 | Statistical analysis

The software of Statistical Package for Social Science (SPSS) version 22.0 for Windows (IBM) was used to perform the statistical analysis. Continuous variables were expressed as mean ± standard deviation, and *t* test or variance analysis was used for comparison between groups. The categorical variables were expressed as percentage or ratio. Fisher's exact test or Pearson's chi-square test was used for comparison between groups. Genotypic frequencies of all subjects were checked for Hardy-Weinberg equilibrium (HWE) before analysis. HWE, linkage-disequilibrium, and haplotypes were performed by using Haploview version 4.2 program ($D' > 0.5$ and $r^2 > 0.33$ means strong linkage disequilibrium). All *p* values were bilateral, and $p < 0.05$ was the threshold for statistical significance. The correlation between *TLR4* polymorphisms and susceptibility or severity of AD, was assessed under different genetic models (codominant, dominant, recessive). The unconditional logistic regression analysis was used to calculate the odds ratio (OR) and 95% confidence intervals (CIs) of the different groups or subgroups.

3 | RESULTS

3.1 | General characteristics

This case-control study enrolled 132 AD patients and 100 healthy controls. As presented in Table 1, there were 73 males and 59 females in the case group, with a mean age of 2.6 ± 2.1 years. The control children with a mean age of 4.9 ± 1.9 years, consisted of 66 males and 34 females. No significant difference was found between the cases and controls in gender and mean age. Among these cases, 99 (75.00%) were confirmed as mild-to-moderate and 33 (25.00%) were diagnosed as severe in severity. There was no significant difference in gender and average age between the mild-to-moderate subgroup and the severe subgroup.

The successful genotyping rate of the four SNPs was 99.57%–100%. The distribution of all SNP genotypes in the case group, the control group, and each subgroup conformed to the HWE test ($p > 0.05$).

3.2 | Gene polymorphisms in AD patients and healthy children

The allele frequencies of four *TLR4* SNPs in AD cases and controls were shown in Table 2. The minor allele frequency (MAF) of *TLR4* rs11536891 was significantly different between AD patients and the healthy children ($p = 0.008$). Compared with T allele, the C allele of rs11536891 in *TLR4* gene was associated with a reduced risk of AD (OR = 0.41, 95% CI: 0.20–0.81).

The genotype frequencies of four *TLR4* SNPs in AD cohort and healthy controls were further analyzed in three genetic models including codominant, dominant, and recessive models. As shown in Table 3, a significant relevance was found between *TLR4* rs11536891 and AD susceptibility in both codominant and dominant model. The risk of AD in children with TC genotype was lower than that in children with TT genotype (OR = 0.41, 95% CI: 0.20–0.85, $p = 0.022$). Additionally, the frequency of rs11536891 TC + CC genotype was significantly decreased as compared to that in TT genotype (OR = 0.39, 95% CI: 0.19–0.81, $p = 0.010$).

Nevertheless, the allele and genotype frequencies of the other three SNPs (rs1927914, rs7869402, and rs11536889) displayed no significant differences between AD patients and the controls.

Moreover, we estimated the linkage disequilibrium and haplotype construction. The four polymorphic sites of *TLR4* gene were in a state of linkage disequilibrium with one another in all participants (All $D' > 0.5$ and $r^2 > 0.33$). As shown in Table 4, among the five haplotypes, the frequency of GCCG composed of rs1927914, rs11536891, rs7869402, and rs11536889 in *TLR4* gene (*TLR4*/GCCG) was significantly lower in AD patients than in controls (OR = 0.38, 95% CI: 0.18–0.79, $p = 0.010$, GCCG vs. ATCG).

3.3 | Gene polymorphisms in mild-to-moderate cases and severe cases

As shown in Table 5, the MAF of *TLR4* rs7869402 was significantly different between mild-to-moderate cases and severe cases ($p = 0.019$). The T allele of rs7869402 was associated with a higher risk of severe AD, compared with C allele (OR = 2.97, 95% CI: 1.15–7.66).

As shown in Table 6, *TLR4* rs7869402 was significantly related to the severity of AD in genotype analysis ($p = 0.021$). The risk of severe AD in patients with TC genotype was significantly higher than that in patients with CC genotype (OR = 3.34, 95% CI: 1.22–9.14).

For allele and genotype frequency of the other three *TLR4* SNPs (rs1927914, rs11536891, and rs11536889), no significant differences were observed between severe subjects and mild-to-moderate subjects.

All four polymorphic sites of *TLR4* gene were in linkage disequilibrium with one another in AD patients(All $D' > 0.5$ and $r^2 > 0.33$). As shown in Table 7, among the five haplotypes, the frequency of GTTG formed by rs1927914, rs11536891, rs7869402, and rs11536889 in *TLR4* gene (*TLR4*/GTTG) was significantly higher in patients with severe AD (OR = 5.26, 95% CI: 1.51–16.67, $p = 0.010$, GTTG vs. ATCG).

4 | DISCUSSION

Genetic variation,immune disorder, and epidermal barrier dysfunction have been considered to be involved in the pathogenesis of AD.[1,2,4,5] TLR4 is the main receptor of lipopolysaccharide, which participates in the perception of pathogens and triggers a pathogen-specific immune response.[6] Moreover, TLR4 is expressed on the surface of a multiple kinds of skin cells and implicated in the occurrence of several inflammatory skin diseases.[7] Both animal and clinical researches to date have established that TLR4, as an amplifier of inflammatory response[25] and a modular of immune response,[6] plays an important role in the initiation and progression of AD.[8,9] Additionally, previous studies have demonstrated that some

TABLE 4 Haplotype frequencies of TLR4 gene for risk of AD

Haplotypes	AD $n = 132$ [n (%)]	Control $n = 100$ [n (%)]	OR (95% CI)	p value
(rs1927914, rs11536891, rs7869402, rs11536889)				
ATCG	100 (38.13)	65 (33.26)	1.00	-
GTCG	66 (25.21)	51 (25.84)	0.89 (0.54 –1.45)	0.641
ATCC	62 (23.54)	45 (22.74)	0.96 (0.58–1.59)	0.879
GCCG	14 (5.30)	24 (12.16)	0.38 (0.18–0.79)	0.010*
GTTG	18 (6.74)	10 (5.24)	1.11 (0.46–2.70)	0.810

Note: The haplotype frequencies under 0.05 was not included in the haplotype analysis.

Abbreviations: AD, atopic dermatitis; CI, confidence interval; OR, odds ratio; TLR4. Toll-like receptor 4.

*Statistically significant ($p < 0.05$).

TABLE 5 Allele frequencies of four TLR4 SNPs in patients with mild-to-moderate and severe AD

SNPs	Allele A/B	MAF		HWE-*p*		OR (95% CI)	*p* value
		Mild-to-moderate	Severe	Mild-to-moderate	Severe		
rs1927914	G/A	0.35	0.45	0.19	0.48	1.52 (0.87–2.68)	0.143
rs11536891	C/T	0.05	0.08	1.00	1.00	0.58 (0.19–1.80)	0.341
rs7869402	T/C	0.05	0.14	1.00	1.00	2.97 (1.15–7.66)	0.019*
rs11536889	C/G	0.23	0.29	0.78	0.39	0.75 (0.40–1.40)	0.364

Note: A: minor alleles; B: major alleles.

Abbreviations: AD, atopic dermatitis; CI, confidence interval; HWE-*p*, *p*-value of Hardy-Weinberg equilibrium; MAF, minor allele frequency; OR, odds ratio; SNP, single nucleotide polymorphism; TLR4, Toll-like receptor 4.

*Statistically significant ($p < 0.05$).

TABLE 6 Genotype analysis of TLR4 rs7869402 in patients with mild-to-moderate and severe AD

Genotype	Mild-to-moderate, *n* (%)	Severe, *n* (%)	OR (95% CI)	*p* value
C/C	89 (89.90)	24 (72.73)	1.00	
T/C	10 (10.10)	9 (27.27)	3.34 (1.22–9.14)	0.021*

Abbreviations: AD, atopic dermatitis; CI, 95% confidence interval; OR, odds ratio; TLR4, Toll-like receptor 4.

*Statistically significant ($p < 0.05$).

TABLE 7 Haplotype frequencies of TLR4 gene in patients with mild-to-moderate and severe AD

Haplotypes (rs1927914, rs11536891, rs7869402, rs11536889)	Mild-to-moderate *n* = 99 [*n* (%)]	Severe *n* = 33 [*n* (%)]	OR (95% CI)	*p* value
ATCG	83 (42.25)	17 (25.75)	1.00	-
GTCG	49 (24.92)	17 (26.18)	1.89 (0.83–4.17)	0.130
ATCC	44 (22.40)	18 (26.86)	2.04 (0.93–4.55)	0.074
GCCG	9 (4.55)	5 (7.58)	2.86 (0.78–10.00)	0.121
GTTG	10 (5.05)	8 (11.70)	5.26 (1.51–16.67)	0.010*

Note: The haplotype frequencies under 0.05 was not included in the haplotype analysis.

Abbreviations: AD, atopic dermatitis; CI, 95% confidence interval; OR, odds ratio; TLR4, Toll-like receptor 4.

*Statistically significant ($p < 0.05$).

TLR4 genetic variants are associated with the susceptibility to several atopic and autoimmune diseases, such as asthma, Henoch-Schönlein purpura, and rheumatoid arthritis.[10,26,27] As far as we know, however, only a few studies were carried out to investigate the predictive role of *TLR4* genetic risk in AD. For instance, a study performed in Italian children detected the prevalence of *TLR4*-D299G (14.9%) in the patients with AD.[20] Another genotyping study in Russia showed a strong association between AD and *TLR4* (Asp299Gly).[21] Besides, the meta-analysis study further presented a correlation between the polymorphism of *TLR4* rs4986790 and a high risk of AD in the Caucasian population.[22]

In Chinese Han children, our results revealed an association between *TLR4* polymorphisms and the susceptibility to AD. In contrast with T allele and TT genotype, children with C allele and TC genotype of *TLR4* rs11536891 have a lower risk of AD. There was a significantly decreased risk of having AD disease in children with haplotype of *TLR4*/GCCG, which were composed of SNP rs1927914, rs11536891, rs7869402, and rs11536889. Considering the differences in *TLR4* gene polymorphism in different ethnic populations, the other four SNPs instead of *TLR4* Asp299Gly (rs4986790) were selected according to the procedural screening program under the specific conditions, as well as the reports from the *TLR4* SNP-related studies.[12,27–29] So far, very few studies have focused on the relevance of these four *TLR4* SNPs with AD, even though they have been confirmed to play roles in the pathogenesis of the atopic and inflammatory diseases.[10,12,26,27] Thus, our results could provide baseline information for analyzing the etiologic effect and the risk predictive ability of *TLR4* gene polymorphisms on AD.

In addition to the etiology of AD, studies on prediction of the severity of AD in patients remains challenging. Several inflammation-associated gene polymorphisms are linked with the severity of AD, which could be used to predict potential severity.[30,31] Our results showed the association of C allele and CC genotype of *TLR4*

rs7869402 with severe AD. Meanwhile, the present study provided evidence that *TLR4*/GTTG might be the risk factor for severe AD patients. Molecular mechanism correlation studies indicated that TLR4-associated signaling pathways including janus kinase-signal transducer and activator of transcription (JAK-STAT), tumor necrosis factor (TNF), and mammalian target of rapamycin (mTOR) pathways, play certain roles in the development of atopic dermatitis.[32–34] That might be the potential reason why *TLR4* polymorphisms could be associated with the severity of AD, and the relevant mechanisms need to be explored in further investigation.

However, there are some shortcomings in the present study. The main limitation is that the sample size is still not enough to draw a more powerful conclusion, although the participants in this study have been proven to be representative in evaluating the compliance with HWE balance. In future, the multicenter studies with a larger sample size are needed to further investigate the relationship between genetic variation in *TLR4* and AD. Moreover, for ethical considerations, there is no in-depth examination on the expression of TLR4 in skin tissues of subjects, especially that in the skin of healthy controls. In addition, there were limited data on AD patients' characteristics such as serum immunoglobulin E, complement 3, complement 4, and cytokines (interferon-γ, interleukin-4, interleukin-10). This information would be helpful to analyze the severity and molecular mechanism of AD.

In conclusion, the present study revealed that the TC genotype of *TLR4* rs11536891 and the *TLR4*/GCCG haplotype confer the decreased susceptibility to AD in Chinese Han children. *TLR4* rs7869402 TC genotype and the *TLR4*/GTTG haplotype might be used to predict a high risk of severe AD in Chinese pediatric population.

ACKNOWLEDGMENTS

This study was supported by Zhejiang Provincial Natural Science Foundation of China (LY21H040002 and LGD20H010002).

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

Jianrong Shi and Lin He designed the research, completed the experimental part of the study, collected the laboratory parameters and patients' information, performed the statistical analysis, drew the figures, and drafted the manuscript. Ran Tao, Huiwen Zheng, Wei Li, Shuangshuang Huang, and Yunling Li completed the experimental part of the study and collected the laboratory parameters. Shiqiang Shang supervised the entire study and provided academic guidance throughout the study process.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Shiqiang Shang https://orcid.org/0000-0001-5389-6750

REFERENCES

1. Weidinger S, Novak N. Atopic dermatitis. *Lancet*. 2016;387(10023):1109-1122.
2. Langan SM, Irvine AD, Weidinger S. Atopic dermatitis. *Lancet*. 2020;396(10247):345-360.
3. Ramirez FD, Chen S, Langan SM, et al. Assessment of sleep disturbances and exhaustion in mothers of children with atopic dermatitis. *Jama Dermatol*. 2019;155(5):556-563.
4. Wang HN, Ji K, Zhang LN, et al. Inhibition of c-Fos expression attenuates IgE-mediated mast cell activation and allergic inflammation by counteracting an inhibitory AP1/Egr1/IL-4 axis. *J Transl Med*. 2021;19(1):261.
5. Kim J, Kim BE, Leung DYM. Pathophysiology of atopic dermatitis: clinical implications. *Allergy Asthma Proc*. 2019;40(2):84-92.
6. Lin L, Xie M, Chen X, et al. Toll-like receptor 4 attenuates a murine model of atopic dermatitis through inhibition of langerin-positive DCs migration. *Exp Dermatol*. 2018;27(9):1015-1022.
7. Kumar V. Going, Toll-like receptors in skin inflammation and inflammatory diseases. *EXCLI J*. 2021;20:52-79.
8. Panzer R, Blobel C, Folster-Holst R, et al. TLR2 and TLR4 expression in atopic dermatitis, contact dermatitis and psoriasis. *Exp Dermatol*. 2014;23(5):364-366.
9. Tsybikov NN, Petrisheva I, Fefelova EV, et al. Expression of TLR2 and TLR4 on peripheral blood monocytes during exacerbation of atopic dermatitis. *Allergy Asthma Proc*. 2015;36(6):e140-e145.
10. Yang H, Wei C, Li Q, et al. Association of TLR4 gene non-missense single nucleotide polymorphisms with rheumatoid arthritis in Chinese Han population. *Rheumatol Int*. 2013;33(5):1283-1288.
11. Tizaoui K, Kaabachi W, Hamzaoui K, et al. Association of single nucleotide polymorphisms in Toll-like receptor genes with asthma risk: a systematic review and meta-analysis. *Allergy Asthma Immunol Res*. 2015;7(2):130-140.
12. Senhaji N, Diakite B, Serbati N, et al. Toll-like receptor 4 Asp299Gly and Thr399Ile polymorphisms: new data and a meta-analysis. *BMC Gastroenterol*. 2014;14(1):206.
13. Noreen M, Shah MA, Mall SM, et al. TLR4 polymorphisms and disease susceptibility. *Inflamm Res*. 2012;61(3):177-188.
14. Noreen M, Arshad M. Association of TLR1, TLR2, TLR4, TLR6, and TIRAP polymorphisms with disease susceptibility. *Immunol Res*. 2015;62(2):234-252.
15. Dunstan E, Lester S, Rischmueller M, et al. TLR4 polymorphism is not associated with biopsy proven giant cell arteritis. *Clin Exp Rheumatol*. 2014;32:S26-S29.
16. Palomino-Morales R, Torres O, Vazquez-Rodriguez TR, et al. Association between toll-like receptor 4 gene polymorphism and biopsy-proven giant cell arteritis. *J Rheumatol*. 2009;36:1501-1506.
17. Ben Dhifallah I, Lachheb J, Houman H, et al. Toll-like receptor gene polymorphisms in a Tunisian population with Behçet's disease. *Clin Exp Rheumatol*. 2009;27:S58-S62.
18. Boiardi L, Atzeni F, Casali B, et al. Toll-like receptor 4 (TLR4) gene polymorphisms in Italian patients with Behçet's disease. *Clin Exp Rheumatol*. 2009;27:S43-S47.
19. Horie Y, Meguro A, Ota M, et al. Association of TLR4 polymorphisms with Behcet's disease in a Korean population. *Rheumatology*. 2009;48:638-642.
20. Salpietro C, Rigoli L, Miraglia DGM, et al. TLR2 and TLR4 gene polymorphisms and atopic dermatitis in Italian children: a multicenter study. *Int J Immunopathol Pharmacol*. 2011;24(4 Suppl):33-40.
21. Tyurin YA, Shamsutdinov AF, Kalinin NN, et al. Association of Toll-like cell receptors TLR2 (p.Arg753GLN) and TLR4 (p.Asp299GLY)

polymorphisms with indicators of general and local immunity in patients with atopic dermatitis. *J Immunol Res*. 2017;2017:8493545.
22. Zhang Y, Wang HC, Feng C, et al. Analysis of the Association of polymorphisms rs5743708 in TLR2 and rs4986790 in TLR4 with atopic dermatitis risk. *Immunol Invest*. 2019;48(2):169-180.
23. Oh DY, Schumann RR, Hamann L, et al. Association of the toll-like receptor 2 A-16934T promoter polymorphism with severe atopic dermatitis. *Allergy*. 2009;64(11):1608-1615.
24. Jie Z, Chen A, You X, et al. A panel of SNP markers for meat traceability of Halal beef in the Chinese market. *Food Control*. 2017;87:94-99.
25. Molteni M, Gemma S, Rossetti C. The role of toll-like receptor 4 in infectious and noninfectious inflammation. *Mediat Infamm*. 2016;6978936.
26. Christou E, Giardino G, Stefanaki E, et al. Asthma: an undermined state of immunodeficiency. *Int Rev Immunol*. 2019;38(2):70-78.
27. Xu H, Jiang G, Shen H, et al. Association of TLR4 gene polymorphisms with childhood Henoch-Schonlein purpura in a Chinese population. *Rheumatol Int*. 2017;37(11):1909-1915.
28. Oliveira J, Busson M, Etain B, et al. Polymorphism of Toll-like receptor 4 gene in bipolar disorder. *J Affect Disord*. 2014;152–154:395-402.
29. Li Z, Gao H, Liu Y, et al. Genetic variants in the regulation region of TLR4 reduce the gastric cancer susceptibility. *Gene*. 2021;767:145181.
30. Glen J, Trzeciak M, Sobjanek M, et al. Interleukin-13 promoter gene polymorphism -1112 C/T is associated with atopic dermatitis in Polish patients. *Acta Dermatovenerol Croat*. 2012;20(4):231-238.
31. Wilkowska A, Glen J, Zablotna M, et al. The association of GM-CSF -677A/C promoter gene polymorphism with the occurrence and severity of atopic dermatitis in a Polish population. *Int J Dermatol*. 2014;53(3):e172-e174.
32. Bao L, Zhang H, Chan LS. The involvement of the JAK-STAT signaling pathway in chronic inflammatory skin disease atopic dermatitis. *JAKSTAT*. 2013;2(3):e24137.
33. Park HJ, Shim HS, Park S, et al. Antidepressant effect and neural mechanism of Acer tegmentosum in repeated stress-induced ovariectomized female rats. *Anim Cells Syst (Seoul)*. 2020;24(4):205-213.
34. Mercurio L, Albanesi C, Madonna S. Recent updates on the involvement of PI3K/AKT/mTOR molecular cascade in the pathogenesis of hyperproliferative skin disorders. *Front Med (Lausanne)*. 2021;8:665647.

How to cite this article: Shi J, He L, Tao R, et al. *TLR4* polymorphisms as potential predictors of atopic dermatitis in Chinese Han children. *J Clin Lab Anal*. 2022;36:e24385. doi:10.1002/jcla.24385