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Single nucleotide polymorphism of Notch1 gene rs3124599 allele is associated with the severity of CVA6-related HFMD in the Chinese Han population

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Abstract

Background There is evidence suggesting that Notch1 signaling pathway contributes to the development of hand, foot, and mouth disease (HFMD); however, the role of Notch1 gene polymorphisms in the severity of coxsackievirus A6 (CVA6)-related HFMD remains unclear. This study aimed to investigate the correlation between Notch1 gene polymorphisms and the severity of CVA6-related HFMD.

Methods A total of 196 patients (Chinese Han population) diagnosed with CVA6-related HFMD through nucleic acid testing were included in this study. Among them, 97 patients were classified as severe cases, while 99 cases were categorized as mild. The mRNA levels of Notch1 in the peripheral blood leukocytes of HFMD patients were detected by quantitative real-time polymerase chain reaction (qRT-PCR), and the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was utilized for genotyping of rs3124599, rs3124603, and rs3124591.

Results The frequencies of rs3124599 alleles were G (39.0%) and A (61.0%), while the frequencies of rs3124599 genotypes were GG (12.2%), GA (53.6%), and AA (34.2%), respectively. In the recessive model, the frequency of rs3124599 AA genotypes significantly increased in severe patients, compared to mild patients ($P<0.05$). Due to the low frequency of alleles for rs3124591 and rs3124603 in patients, as well as the absence of any difference in their distribution between the two groups ($P>0.05$), no additional statistical analysis was performed. After adjusting for age and sex, patients with rs3124599 AA genotype had a significantly higher risk of severe HFMD in comparison to G allele carriers (GA/GG), with an odds ratio (95% confidence interval) of 2.010 (1.094, 3.691). Meanwhile, the mRNA levels of Notch1 were found to be significantly higher in severe patients compared to mild patients ($P<0.05$), and a positive correlation was observed between Notch1 mRNA levels and the peripheral blood monocyte count ($r=0.42$, $P<0.001$). Additionally, there were significant differences observed in Notch1 mRNA levels and peripheral blood monocyte

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counts between patients with the AA genotype of rs3124599 and those with the GA genotype or G allele carriers ($P < 0.05$).

Conclusion In the Chinese Han population, there is a strong correlation between the Notch1 rs3124599 allele and the severity of CVA6-related HFMD. This correlation may be attributed to genetic polymorphism of rs3124599 regulating Notch1 transcription levels. These findings reveal the important role of Notch1 gene polymorphism in CVA6 infection, establishing a scientific foundation for the precise control of severe HFMD.

Keywords SNP, rs3124599, Hand, foot, and mouth disease, Notch1

Introduction

Hand, foot, and mouth disease (HFMD) is an infectious disease caused by *enteroviruses*. It exhibits high contagion rates among children under 5 years old, posing a significant threat to global public health, particularly in the Asia-Pacific region [1, 2]. The primary clinical manifestations of HFMD include fever, vesicular rashes on the hands, feet, and buttocks, as well as oral mucosal ulcers [3]. Although HFMD is typically mild and self-limiting, it can lead to severe complications associated with high morbidity and mortality [4]. It is noteworthy that some surviving cases may also suffer from long-term sequelae. In recent years, coxsackievirus A6 (CVA6) has emerged as a prominent etiological agent responsible for HFMD outbreaks in China and globally. Compared to typical viral strains, it exhibits a wider range of impact on the population and leads to more severe manifestations of illness [2, 5].

In addition to immune factors, host genetic factors also play a pivotal role in the pathogenesis and severity of HFMD. Furthemore, mounting evidence suggests that genetic polymorphisms in several genes, including interleukin (IL)-6, interleukin-10, and interleukin-17F, are associated with both the susceptibility and severity of HFMD [6–8]. Therefore, it is particularly important to understand how to utilize this relationship for early identification and prediction of HFMD severity.

The Notch signaling pathway, which is an evolutionarily conserved cellular signaling, serves as a key regulator of various cellular processes, including cell differentiation, proliferation, and apoptosis [9]. Notch1, a member of the Notch protein family, plays a crucial role in regulating the fate of hematopoietic stem cells, including silence, self-renewal, and differentiation into blood cells [10]. A previous study indicated that the Notch1 signaling pathway may be involved in regulating the function of $CD14^+$ monocytes and contributing to the adverse outcome of HFMD [11]. Another study suggests that Notch signaling regulates the development of immune cells, and may influence the progression of HFMD by affecting the quantity and status of peripheral lymphocytes [12]. Meanwhile, Notch1 exerts a significant influence on the regulation of innate immune responses [13]. Crosstalk between the Notch1 signaling pathway and the nuclear factor-kappa B (NF-κB) signaling pathway can facilitate NLRP3 activation, leading to subsequent release of IL-18 and IL-1β, thereby participating in the inflammatory response during tissue damage [14].

The polymorphism of the Notch1 gene has been extensively studied in tumor diseases due to its significant role in the pathogenesis of these diseases. [15, 16]. The study found that single nucleotide polymorphism (SNPs) rs3124599 in Notch1 is associated with the risk of lung cancer in non-smoking females from northeast China [24]. In addition, SNPs of Notch1 (rs3124599 and rs3124591) have also demonstrated a significant association with the risk of nephrotic syndrome [17]. However, limited study has been conducted to examine the association between SNPs in Notch1 and the severity of HFMD. Considering that CVA6 is currently the main pathogen causing HFMD in China, this study aims to investigate the relationship between Notch1 rs3124599, rs3124603, and rs3124591 polymorphisms and the severity of CVA6-related HFMD. Our findings have the potential to contribute towards early identification and prevention of severe illness in patients with CVA6-related HFMD.

Materials and methods

Subjects

From July to September 2018, a total of 99 severe HFMD patients (two patients were excluded from the study due to unqualified samples) who were hospitalized at the Affiliated Children's Hospital of Zhengzhou University in Henan Province, China, were included in this study. Additionally, 99 mild HFMD patients were randomly selected as controls from the outpatient departments of hospitals during the same period. Ultimately, a total of 196 patients were included in the study. All samples exhibited positive results for CVA6 nucleic acid detection and were diagnosed based on "Hand, Foot, and Mouth Disease Diagnosis and Treatment Guidelines (2018 version)" [18]. Mild cases were defined as those in which patients had rashes on their hands, feet, mouth, and buttocks, with or without fever. Patients with severe disease exhibited central nervous system complications such as meningitis, encephalitis, encephalomyelitis, as well as respiratory or circulatory manifestations including dyspnea, cyanosis, bloody sputum, lung rales, and shock. All

patients underwent a general physical examination and routine laboratory tests. Approximately 3 mL of peripheral blood was collected before treatment on the day of admission, which was then placed in a tube containing 2% ethylenediaminetetraacetic acid and stored at -80 °C for further use.

This study was approved by the Ethics Committee of the Affiliated Children's Hospital of Zhengzhou University (No.2023-H-K19), and written informed consent was obtained from both the participants and their parent/legal guardian(s).

DNA extraction

The DNA was extracted from peripheral blood leukocytes using a modified phenol-chloroform method. To enhance the efficiency of the extraction process, we have implemented the following modifications compared to the conventional approach: (1) In order to significantly reduce the incubation time of the sample, 6 mol/L NaI and 15% SDS were used instead of proteinase K. (2) The DNA extraction process began with the use of a phenol-chloroform-isoamyl alcohol solution, followed by a second round with chloroform-isoamyl alcohol solution. This approach effectively minimizes sample contamination from phenol and reduces DNA loss caused by the solubility of phenol in water. (3) The separation of DNA and RNA was achieved by exploiting their differential solubility in the high-concentration NaCl solution, which eliminates the need for RNase, improving cost-effectiveness and processing speed. Minimal traces of RNA were detected, but they had no impact on the subsequent experiments.

Selection of SNPs

The SNPs of Notch1 were searched by frequency on the National Center for Biotechnology Information (NCBI), and the top 20 SNPs with the highest frequency were selected. After excluding SNPs that result in silent mutations, the remaining SNPs were assessed for linkage disequilibrium (LD) within the Han Chinese in Beijing (CHB) population using the National Institutes of Health (NIH) website. After conducting a comprehensive evaluation of the LD results and relevant literature, we have selected the SNPs that will be included in this study.

Genotyping

The Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique was used to genotype gene polymorphisms, and specific primers for three SNPs were designed based on the NCBI database (Table 1). The PCR-RFLP reaction was performed in the T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA) under the following conditions: 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 57 °C for 15 s, 72 °C for 1 min, and a final extension step of 72 °C for 8 min. The PCR products were digested with different restriction endonucleases under the conditions listed in Table 1. The digested products were then separated by electrophoresis on a 3% agarose gel containing 0.5 μg/ml ethidium bromide. The DYCP-31DN horizontal electrophoresis apparatus (Liuyi, Beijing, China) was utilized for electrophoresis analysis. The results of the electrophoresis were visualized under UV light to assess fragment separation and identify any potential polymorphisms.

Assessment of Notch1 expression with quantitative real-time polymerase chain reaction

The blood specimens were centrifuged at 4000 rpm for 10 min at a temperature of 4 °C to separate the blood cells. Total RNA was extracted from the peripheral blood leukocytes using the TRIzol method. Subsequently, the concentration of total RNA was measured using a NanoDrop ND-2000 (Thermo Fisher Scientific, Waltham, MA, USA). Reverse transcription (RT) was then performed with Hifair II 1st-strand cDNA synthesis supermix (Yeasen, Shanghai, China).

The expression level of Notch1 was determined by quantitative real-time polymerase chain reaction (qRT-PCR) using the instrument (Serial No. q225-0207, Kubo, Beijing, China). The qRT-PCR reaction system (10 mL) consisted of diethyl phosphorocyanidate (DEPC) water (2.6 μL), upstream and downstream primers (0.2 μL each), SYBR Green Master Mix (5 μL, Cat No.11202ES03 Yeasen, Shanghai, China), and sample cDNA (2 μL). The cycle process was conducted with 40 cycles of 95 °C for 5 min, 95 °C for 10 s, and 60 °C for 30 s. Finally, the transcription levels of relevant genes were normalized to that of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene and were calculated by the cycle threshold ($2^{-\Delta\Delta CT}$) method.

Table 1 Specific primers and enzymatic reaction conditions for SNPs

SNP ID	Forward primer	Reverse primer	Enzyme	Company	Response time	Fragment length
rs3124599	CTCATTGACATCGTGCTGGC	AAACGTCAGTCTCGTCCCTG	MluI	Beyotime, China	1 h	349 bp 100 bp
rs3124603	GTCGCACTCATCGATGTCCA	TCATGGACACCTTGGTCTGG	BstUI	Thermo Fisher, USA	30 min	182 bp 53 bp
rs3124591	ACGTAGGAAAACCCTGGCTC	CCGACCAGAGGAGCCTTTTT	MnlI	Yeasen, China	1 h	165 bp 40 bp

Statistical analysis

Data were analyzed using the SPSS 25.0 (IBM, Chicago, IL, USA). The chi-square, t-tests and fisher's exact test were used to evaluate the basic information of patients in the two groups. The t-test was used for quantitative data, while the chi-square test and fisher's exact test were used for count data. Hardy-Weinberg equilibrium is utilized to calculate whether the selected sample is from a random population. The genotype and allele frequencies between the patients with mild and severe HFMD were compared using the chi-square test and fisher's exact test. The association between SNPs and the risk of severe HFMD was evaluated by calculating odds ratios (OR) and 95% confidence intervals (95% CI) using logistic regression. Student's t-test was used to estimate the differences in the relative expression levels of Notch1 between the genotype groups. $P<0.05$ was considered statistically significant.

Results

Demographic characteristics of study participants

This study enrolled a total of 196 HFMD patients (120 males) between 7 and 156 months old (average 26.5±14.1 months). Among them, 99 cases were classified as mild cases, while the remaining 97 cases were in the severe group. No significant difference was observed between the mild and severe groups in terms of age and gender ($P>0.05$). Other clinical characteristics are shown in Table 2.

Screening of SNPs

In order to eliminate the influence of linkage disequilibrium and select the most meaningful SNPs, we chose the top 12 non-silent mutant SNPs with the highest frequency for inclusion in LD analysis, while also computing the FORGEdb scores of these SNPs. After conducting a comprehensive evaluation of the LD results and relevant literature, the present study ultimately identified three SNPs (Fig. 1): rs3124599 (G>A), rs3124603 (T>C), and rs3124591 (C>T). Among them, rs3124591 is located within the 3' untranslated region (3'UTR), while both rs3124599 and rs3124603 are located within the intronic region (Fig. 1).

Association between Notch1 SNPs and HFMD severity

The DNA extraction was performed using a modified phenol-chloroform method, resulting in extracted DNA samples with an average concentration of 148.3±43.8 ng/μL, and exhibiting a 260/280 ratio of 1.90±0.17. Compared to conventional methods, the modified method demonstrates simplicity, cost-effectiveness, rapidity, and enhanced suitability for large-scale clinical investigations.

Subsequently, we used the PCR-RFLP technique for genotyping. The electrophoresis results of different SNPs are presented in Fig. 2, while the comprehensive electrophoretic profiles of all samples can be found in Supplementary Fig. 1. Table 3 provides a summary of the genotype distributions and allele frequencies for different Notch1 SNPs. No significant difference was observed in genotype distribution or allele frequency between patients with severe HFMD and mild HFMD. Additionally, the genotype distributions of each group were found to adhere to the Hardy–Weinberg equilibrium ($P>0.05$), showing that the selected sample represents a random population.

Notably, a significantly higher frequency of the rs3124599 AA genotype was observed in patients with severe HFMD compared to those with mild HFMD, following a recessive model ($P<0.05$). After adjusting for age and gender, a binary logistic analysis was performed,

Table 2 Comparison of some basic characteristics and personal history of disease in HFMD patient between severe and mild group

Characteristics	Mild Group (*N*=99)	Severe Group (*N*=97)	t / χ^2	*P*
Age (months)	25.6±11.4	28.3±19.1	1.2	0.22[a]
Gender (male/female)	62/37	57/40	0.3	0.58[b]
Duration of fever (days)	1.9±0.9	2.9±1.3	5.6	<0.01[a]
The highest body temperature (℃)	39.0±0.5	39.2±0.6	2.3	0.02[a]
WBC (10^9/L)	10.2±3.1	11.7±4.1	2.83	<0.01[a]
Lymphocyte (10^9/L)	3.3±2.0	3.9±2.1	2.0	0.04[a]
Monocyte (10^9/L)	0.2±0.4	0.5±0.3	5.0	<0.01[a]
Limb shaking	0 (0%)	31 (32.0%)	/	<0.001[c]
Vomiting	0 (0%)	25 (25.8%)	/	<0.001[c]
Convulsions	0 (0%)	68 (70.1%)	/	<0.001[c]
Myoclonus	0 (0%)	23 (23.7%)	/	<0.001[c]
Pulmonary rales	0 (0%)	5 (5.2%)	/	<0.001[c]
Meningitis encephalitis	0 (0%)	16 (16.5%)	/	<0.001[c]
Encephalomyelitis	0 (0%)	5 (5.2%)	/	<0.01[c]

[a] Groups were compared using the t-test; [b] Groups were compared using the chi-square test; [c] Groups were compared using the fisher's exact test; WBC: White blood cell

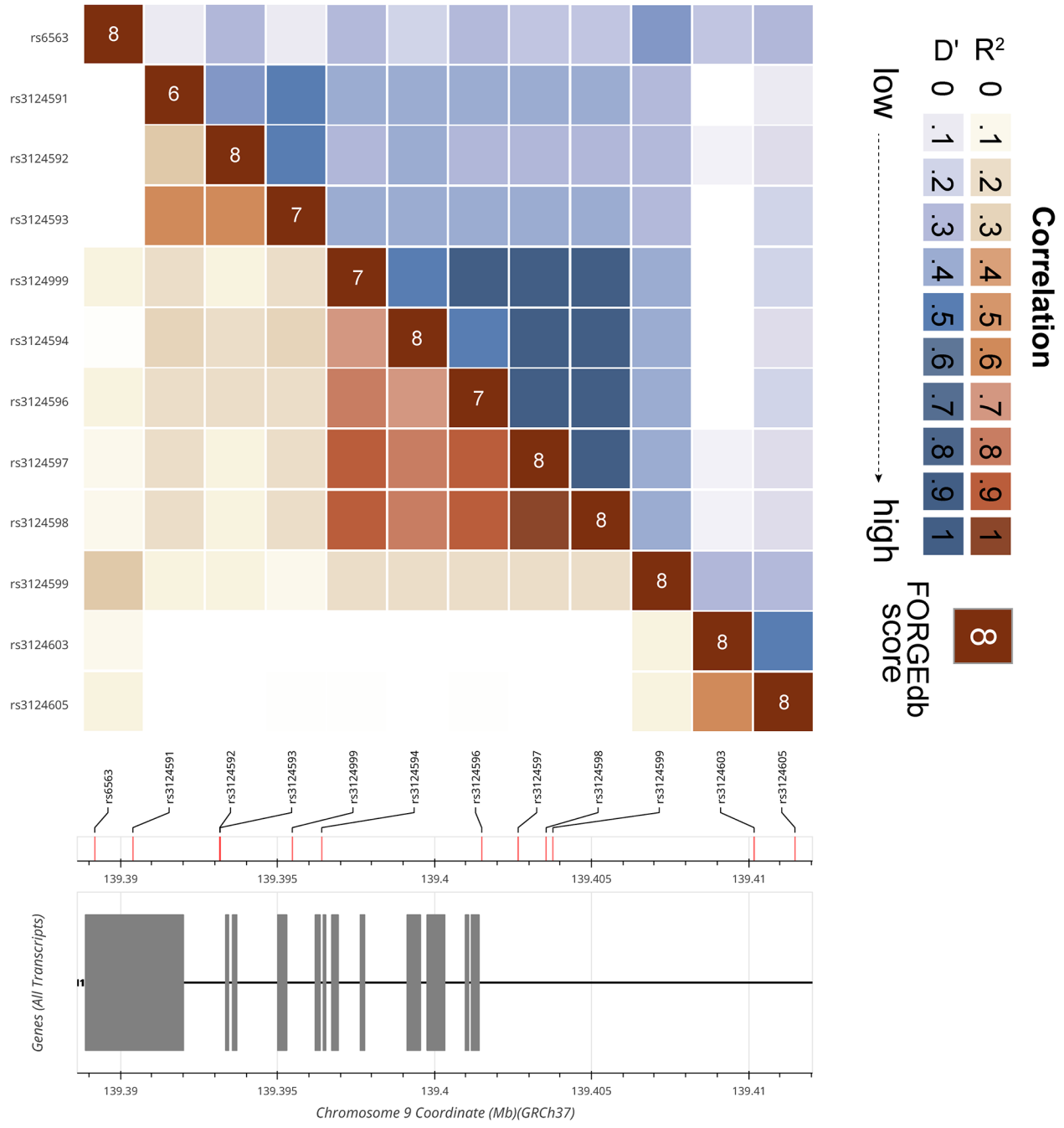

Fig. 1 Results of linkage disequilibrium analysis and the genomic location of each SNP

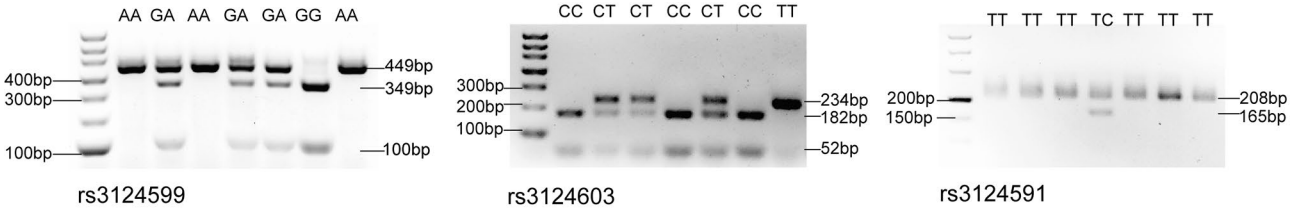

Fig. 2 Electrophoresis results of different SNPs

Table 3 Genotypes and allele frequencies of rs3124599, rs3124603, and rs3124591 polymorphism in the HFMD patients

SNP	Genes	Total (%)	Mild group (%)	Severe group (%)	χ^2	*P*
rs3124599	Genotype					
	GG	24 (12.2)	12 (12.1)	12 (12.4)	4.645	0.098[a]
	GA	105 (53.6)	60 (66.7)	45 (46.4)		
	AA	67 (34.2)	27 (27.2)	40 (41.2)		
	Allelic genes					
	G	153 (39.0)	84 (42.4)	69 (35.6)	1.936	0.164[a]
	A	239 (61.0)	114 (57.6)	125 (64.4)		
rs3124603	Genotype					
	TT	8 (4.1)	7 (8.0)	1 (1.0)	/	0.087[b]
	CT	38 (19.4)	17 (17.2)	21 (21.6)		
	CC	150 (76.5)	75 (75.8)	75 (77.3)		
	Allelic genes					
	C	341 (87.0)	171 (88.1)	170 (85.9)	0.452	0.501[a]
	T	51 (13.0)	23 (11.9)	28 (14.1)		
rs3124591	Genotype					
	CT	8 (4.1)	6 (6.1)	2 (2.1)	/	0.279[b]
	TT	188 (95.9)	93 (97.9)	95 (93.9)		
	Allelic genes					
	C	8 (2.0)	6 (3.0)	2 (1.0)	/	0.284[b]
	T	384 (98)	192 (97.0)	192 (99.0)		

[a] Groups were compared using the chi-square test; [b] Groups were compared using the fisher's exact test

Table 4 Genotype and allele frequency of rs3124599, rs3124603 polymorphism between groups in dominant and recessive models

Model	SNP	Genotype	Mild group (%)	Severe group (%)	χ^2	P_1	OR (95% CI)	P_2
Recessive	rs3124599	GG/GA	72 (72.7)	57 (58.8)	4.247	0.039[a]	2.010 (1.094, 3.691)	0.024
		AA	27 (27.3)	40 (41.2)				
	rs3124603	TT/CT	22 (22.7)	22 (22.2)	0.006	0.939[a]	1.121 (0.563, 2.229)	0.746
		CC	75 (77.3)	77 (77.8)				
Dominant	rs3124599	GG	12 (12.1)	12 (12.4)	0.003	0.957[a]	0.988 (0.419, 2.333)	0.242
		AA/GA	87 (87.9)	85 (87.6)				
	rs3124603	TT	1 (1.0)	7 (7.1)	/	0.065[b]	7.607 (0.908, 63.769)	0.061
		CC/CT	96 (99.0)	92 (92.9)				

P1: *P*-value for chi-square test and fisher' s exact test; P2: *P*-value for logistic regression; [a] Groups were compared using the chi-square test; [b] Groups were compared using the fisher's exact test; OR: odds ratios; CI: confidence intervals

revealing that patients with the rs3124599 AA genotype exhibited a significantly higher risk of severe HFMD compared to those with the GG/GA genotype, with OR (95% CI) of 2.010 (1.094, 3.691) (Table 4). Although there was no significant difference in the genotype and allele frequency distribution of rs3124591 and rs3124603 between the two groups of HFMD patients, a discernible trend can still be observed in the distribution of genotypes and allele frequencies. The limited sample size has resulted in insufficient research on rare alleles, thus emphasizing the necessity of expanding the sample size in subsequent investigations to facilitate more comprehensive analyses.

Comparison of different genotypes with Notch1 mRNA levels

To determine whether patients with severe HFMD showed increased expression of the Notch1 gene, qRT-PCR was performed on both mild and severe cases. The results showed a significantly higher relative expression level of Notch1 in the severe group compared to the mild group ($t=2.653$ $P=0.009$) (Fig. 3A).

The relative expression levels of Notch1 in HFMD patients with different genotypes were further compared. The relative expression level of Notch1 was significantly higher in patients with rs3124599 AA genotype compared to those with GA genotype ($t=2.051$ $P=0.042$) and G gene carriers (GA/GG) ($t=2.419$ $P=0.017$). Based on these results, we can observe a tendency for the relative expression level of Notch1 to increase with each additional A gene carried by the patients (Fig. 4A). Due to

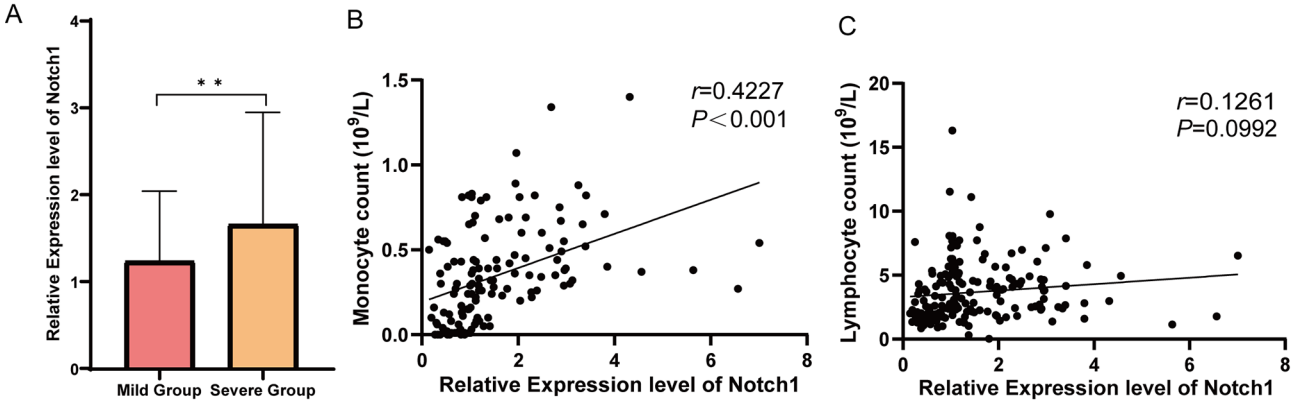

Fig. 3 Association of different genotypes with Notch1 mRNA expression (**A**) Relative expression levels of Notch1 and its distribution in mild and severe groups of HFMD patients. (**B, C**) Correlation of relative Notch1 expression levels with monocyte count, lymphocyte count in patients with HFMD. **: $P<0.01$

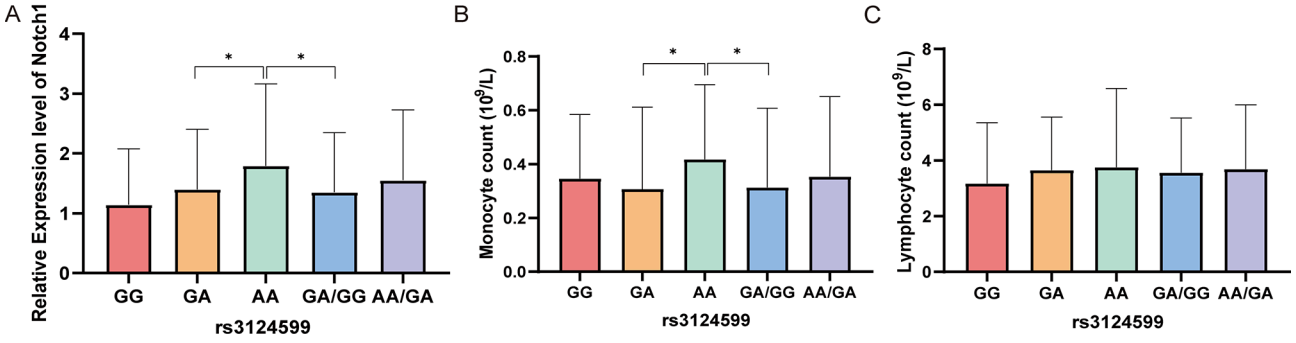

Fig. 4 Levels of relevant indicators in different genotype groups of rs3124599 (**A**) Relative expression levels of Notch1 in different genotype groups. (**B**) Monocyte count in different genotype groups. (**C**) Lymphocyte count in different genotype groups. *: $P<0.05$

the low frequency of alleles C and T for rs3124591 and rs3124603 in patients, as well as no difference observed in their distribution between the two groups ($P>0.05$), further statistical analysis was not conducted.

Considering the importance of Notch1 in monocyte and lymphocyte development, we examined the correlation between relative expression levels of Notch1 and monocyte counts as well as lymphocyte counts. The research data revealed a significant positive correlation between the relative expression levels of Notch1 and monocyte counts ($r=0.425$, $P<0.001$). However, no such association was observed between Notch1 expression levels and lymphocyte counts (Fig. 3B, C). Subsequent investigations revealed significant differences in monocyte counts among patients with different genotypes, but there were no differences in lymphocyte counts (Fig. 4B, C). Patients with the rs3124599 AA genotype exhibited a significantly higher monocyte count compared to individuals with the GA genotype ($t=2.087$, $P=0.039$) and G gene carriers ($t=2.081$, $P=0.039$).

Discussion

For a long time, HFMD was considered as a mild viral infection characterized by typical clinical manifestations that spontaneously resolved in a few days without complications. Over the past two decades, compelling evidence has emerged indicating that a proportion of HFMD patients inevitably progress to severe cases with high mortality rates, and some survivors also experience long-term sequelae. With the gradual emergence of highly pathogenic and contagious CVA6 as the primary pathogen of HFMD, growing concerns have arisen among individuals regarding this illness. Due to its significant impact on children, families, and society, a substantial research effort has been devoted in recent years to early detection of HFMD severity. SNPs have the potential to modify gene product sequences, regulate gene expression, and modulate gene function, thus influencing phenotypic traits [19]. An increasing number of studies have demonstrated that gene mutations and SNPs play a crucial role in the susceptibility and severity of infectious diseases [20–22].

The human Notch1 gene, which is located on chromosome 9q34.3, exhibits a considerable degree of

polymorphism, and certain of these polymorphisms have been reported to be associated with susceptibility to specific diseases. Liu et al. discovered that rs13999482 polymorphism in Notch1 is associated with the development of squamous cell carcinoma of the head and neck [23]. Cao's study showed a significant association between the rs3124591 TC genotype and high expression of Notch1 [16]. Quan's study demonstrated that rs312599 in Notch1 may serve as a novel biomarker for the susceptibility of small cell lung cancer in Chinese non-smoking females [24]. Additionally, previous investigations have revealed an association between rs3124603 and an increased susceptibility to noise-induced hearing loss within the Chinese population [25]. Despite the Notch1 gene plays a pivotal role in determining cellular fate, limited studies have reported on the association between Notch1 gene polymorphisms and the severity of HFMD.

In this study, we evaluated the association of polymorphisms in Notch1 rs3124599, rs3124603, and rs3124591 with the severity of HFMD. As expected, there was a strong correlation between the Notch1 rs3124599 AA genotype and the severity of HFMD. Subsequently, a higher mRNA level of Notch1 was observed in severe patients. Similarly, a previous study also found an increase in Notch1 mRNA levels among severe HFMD patients [11].

Although rs3124599 is located within the intron region and does not directly alter the protein sequence, it potentially exerts regulatory influence on Notch1 transcription, thereby modulating the expression of Notch1 [24]. The impact of SNPs in intronic regions of genes on the heterogeneous splicing process of mRNAs has been demonstrated [26]. By qRT-PCR, we observed a significantly higher mRNA expression level of Notch1 in peripheral blood leukocytes from patients with the rs3124599 AA genotype compared to those with the GA genotype and G gene carriers. It has been reported that carriers of the rs3124599 GG genotype have a 2.193 times higher risk of small cell lung cancer (SCLC) compared to carriers of the AA/AG genotype [24]. Additionally, Notch1, as a tumor suppressor gene, can inhibit the growth and metastasis of SCLC [27]. This suggests that individuals with the GG genotype may express a lower level of Notch1 compared to the AA/AG genotype. These results imply that gene polymorphisms in Notch1 may influence the severity of HFMD by affecting the transcriptional regulation of Notch1, subsequently impacting Notch1 protein expression.

Interestingly, a significant correlation was observed between the relative expression levels of Notch1 and monocyte counts, which persisted even after genotyping for rs3124599. It has been demonstrated that Notch1 signaling contributes to the activity of $CD14^{+}$ monocytes in EV71-infected HFMD and also associated with the poor prognosis of EV71-induced HFMD [10]. Meanwhile, it has also been reported that Notch1 exerts an influence on monocyte survival and differentiation into macrophages [28]. Our research findings suggest that Notch1 rs3124599 gene polymorphisms may influence the monocyte count in peripheral blood by modulating Notch1 transcription, thereby ultimately impacting the severity of HFMD. However, the confirmation of the specific mechanisms underlying this hypothesis requires further investigation.

Despite all the valuable results reported, this study had some limitations. The protein level of Notch1 was not tested in blood samples. Therefore, the analysis of Notch1 rs3124599 polymorphism in CVA6 infection is limited to the mRNA level rather than extending to the protein level. Although the importance of the polymorphisms rs3124591 and rs3124603 has been described in many articles, and even in this study, these alleles showed different distribution trends among different groups. However, due to their relatively low frequency in the Han population and limited sample size, we were unable to conduct a more in-depth investigation on them.

In conclusion, we found that genetic polymorphisms at rs3124599 of the Notch1 gene may influence the severity of CVA6-related HFMD by modulating the transcriptional activity of Notch1. The rs3124599 polymorphism in the Notch1 gene may serve as a genetic susceptibility marker for severe CVA6-related HFMD, thereby enabling early prediction of the severity of CVA6 infection.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-024-09640-2.

Supplementary Material 1

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Author contributions

All authors contributed to the study conception and design. Conceptualization, data curation and writing - original draft were performed by Zijie Li and Wangquan Ji. Resources and visualization were performed by Bowen Dai, Shouhang Chen, and Fang Wang. Investigation, methodology, supervision and writing - review & editing were performed by Guangcai Duan and Yuefei Jin. The first draft of the manuscript was written by Zijie Li and all authors commented on previous versions of the manuscript.

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publish, or preparation of the manuscript. No author received a salary from any of the funders.

Data availability
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study was approved by the Ethics Committee of the Affiliated Children's Hospital of Zhengzhou University (NO.2023-H-K19).

Competing interests
The authors declare no competing interests.

Consent for publication
Written informed consent was obtained from all participants or their parent/legal guardian(s) included in the study.

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