

# Toll-like receptor 7 single nucleotide polymorphism rs3853839 in pediatric patients with immune thrombocytopenia

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## ABSTRACT

**Background.** Immune thrombocytopenia (ITP) is a multifactorial disease involving environmental and genetic factors. This study aimed to evaluate the association of a single nucleotide polymorphism (SNP) rs3853839 in the Toll-like receptor 7 (*TLR7*) gene with susceptibility to ITP and its clinical features.

**Methods.** This retrospective, observational, case-control study was conducted on 172 pediatric patients with ITP and 170 healthy children. Genomic DNA was extracted from peripheral blood and genotyped via a snapshot technique.

**Results.** The serum *TLR7* mRNA in the case group ( $1.129 \pm 0.536$ ) was significantly higher than that in the control group ( $0.851 \pm 0.298$ ) ( $p < 0.001$ ). Female patients with the GG genotype and male patients with the G/(-) genotype demonstrated the highest level of *TLR7* mRNA ( $1.478 \pm 0.522$  and  $1.280 \pm 0.590$ , respectively) ( $p < 0.0001$ ), whereas female patients with the CC genotype and male patients with the C/(-) genotype showed the lowest level of *TLR7* mRNA ( $0.752 \pm 0.171$  and  $0.732 \pm 0.218$ , respectively) ( $p < 0.0001$ ). The severity and chronic progression of ITP was significantly increased in female patients with the GG genotype and male patients with the G/(-) genotype ( $p < 0.05$ ). However, *TLR7* rs3853839 polymorphism was not significantly associated with corticosteroid sensitivity and disease recurrence ( $p > 0.05$ ).

**Conclusions.** This study suggests that *TLR7* rs3853839 may be a key genetic factor in the susceptibility and severity of ITP disease, providing new insights into disease progression and severity prediction. These findings present significant insights into the pathogenesis of ITP and may serve as a foundation for developing personalized treatment strategies tailored for pediatric patients with ITP.

**Key words:** immune thrombocytopenia (ITP), *TLR7*, genetic polymorphism, genotype.

Primary immune thrombocytopenia (ITP) is a highly complex autoimmune disease.<sup>1</sup> Its etiology is still not fully understood. Genetic and environmental factors play essential roles in the pathogenesis of ITP.<sup>2</sup> The clinical features of ITP can include increased antibody-mediated platelet destruction or antibody-mediated inhibition of platelet production,

leading to insufficient platelet production.<sup>3</sup> The International Working Group (IWG) defines ITP as newly diagnosed (diagnosis to 3 months), persistent (3 to 12 months from diagnosis), or chronic (lasting >12 months).<sup>4</sup>

Toll-like receptors (TLRs) play essential roles in responses against microbial agents,

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inflammatory pathways, and the regulation of innate immune responses.<sup>5</sup> Disturbances in the innate immune response can precipitate the onset and progression of ITP.<sup>6</sup> TLRs induce the development and differentiation of T-cell subsets, including Th1, Th2, Th17, and Tregs. They also modulate the development and functions of Tregs through mediated signals and can impact the development of atopic disorders. Dysregulated TLR expression or genetic variations can contribute to imbalances in Th1 or Th2 immunity levels.<sup>7-9</sup> In patients with ITP, there is an observed imbalance in CD4+ T cell subsets, characterized by a skewed Th1/Th2 balance that favors Th1 and a skewed Th17/Treg balance that favors Th17. Consequently, there is an elevated production of Th1 and Th17 cytokines.<sup>10,11</sup>

Toll-like receptor 7 (*TLR7*) gene is found in X chromosome Xp22.2, which encodes the TLR7 protein. TLR7 serves as an intracellular pattern recognition receptor and is associated with multiple polymorphisms that are potentially associated with human disease.<sup>12</sup> In the investigation into the pathogenesis of ITP, whole-blood gene expression profiling from ITP patients was conducted, revealing the involvement of TLR7 in the pathogenesis of ITP.<sup>13</sup>

Although studies have shown that TLR7 may play an important role in the pathogenesis of ITP, the specific genetic variation and its functional effects still need further exploration. Previous studies on ITP gene polymorphism mainly focused on the *PTPN22* gene<sup>14</sup>, *HDAC3* gene<sup>15</sup>, and *FOXP3* gene<sup>16</sup>, while the study of *TLR7* gene polymorphism and ITP has not been reported.

*TLR7* expression is associated with *TLR7* rs3853839 C/G polymorphism, which has been shown to impact *TLR7* mRNA turnover in genetic studies.<sup>17</sup> The rs3853839 C/G SNP, which is located in the 3' untranslated region of the *TLR7* gene, has been associated with increases in *TLR7* mRNA and protein expression.<sup>18</sup> Numerous studies have indicated

a correlation between the rs3853839 C/G SNP and various autoimmune diseases, including systemic lupus erythematosus (SLE)<sup>12</sup>, knee osteoarthritis<sup>19</sup>, and autoimmune thyroid disease.<sup>20</sup> To our knowledge, to date no studies have been undertaken to clarify the relationship between *TLR7* rs3853839 C/G polymorphism and ITP. Therefore, it is essential to investigate the rs3853839 C/G single nucleotide polymorphism to enhance our understanding of the pathogenesis and clinical characteristics associated with ITP.

The main objective of this study was to evaluate the association of the *TLR7* rs3853839 C/G SNP with susceptibility to ITP and its clinical features.

## Materials and Methods

### Study design

This retrospective, observational, case-control study was conducted on 172 pediatric patients with ITP, and 170 healthy children were included as a control group. The study was conducted from January 2020 to January 2023. All the patients agreed to participate in the study and signed a written informed consent form before enrollment. The diagnosis of ITP is based on the updated international consensus report.<sup>21</sup> In this study, the clinical information of the children was collected, and all the children were followed for at least 1 year. Patients with a follow-up duration of less than one year or incomplete records were excluded from the study.

These definitions were developed in accordance with ITP Working Group guidelines to study the clinical characteristics and follow-up treatment outcomes of ITP.<sup>21</sup>

### Study definitions

1. Primary ITP: isolated thrombocytopenia (peripheral blood platelet count  $<100 \times 10^9$  /L) in the absence of other conditions associated with thrombocytopenia.

2. Newly diagnosed ITP: within 3 months from diagnosis.
3. Persistent ITP: between 3 and 12 months from diagnosis.
4. Chronic ITP: lasting for >12 months.
5. Severe ITP: presenting with bleeding sufficient to start treatment or occurrence of new bleeding symptoms necessitating additional treatment mediation with an alternative agent or an increased dose.

### **Response to treatment**

All children with ITP were administered high-dose dexamethasone through intravenous infusion. The treatment was given at 0.6 mg/kg daily, with a maximum dose allowed as 40 mg over four days. The response after steroid treatment was evaluated as follows:

*Corticosteroid sensitivity:* after standard glucocorticoid treatment, the platelet count significantly increased and stabilized at a safe level ( $\geq 30 \times 10^9/L$ ), effectively managing bleeding symptoms.

*Corticosteroid resistance:* platelet counts below  $30 \times 10^9/L$ , less than a twofold increase of baseline platelet count, or the presence of bleeding symptoms.

*Recurrence:* decrease in platelet counts below  $30 \times 10^9/L$  or less than twice the baseline or the presence of bleeding symptoms following treatment response.

### **Sample collection**

The sterile venipuncture was used to extract 3-5 mL of venous blood from each patient into an EDTA anticoagulant tube. A blood DNA extraction kit (Qiagen, Maryland, USA) was used to store the samples in the same vacuum tank at  $-20^\circ\text{C}$  until DNA extraction.

### **DNA amplification and isolation**

Whole-blood DNA was extracted via the QIAamp DNA Mini Kit (Qiagen, Hilden,

Germany). DNA concentration and purity were determined using a spectrophotometer NanoDrop 1000 (Thermo Fisher Scientific, Waltham, MA, USA), and all DNA samples were diluted to working concentrations of 50 ng/ $\mu\text{L}$ .

### **Amplification PCR and snapshot assay**

The SNaPshot assay is a mini-sequencing method widely used to detect polymorphisms in individual genes or the whole genome. The assay is sensitive and straightforward, and the results can be automatically evaluated using software.<sup>22</sup> Genotyping of the *TLR7* rs3853839 gene locus was performed via the Snapshot technique.

The total volume of each PCR mixture was 25  $\mu\text{L}$ : 5  $\mu\text{L}$  of DNA, 12.5  $\mu\text{L}$  of prepared Taq Red PCR master mix (Bioline), 1  $\mu\text{L}$  of each primer, and 5.5  $\mu\text{L}$  of nuclease-free water. The forward primer sequence of *TLR7* rs3853839 was as follows: AACCAATTGCTTCCGTGTCA. The reverse primer sequence of *TLR7* rs3853839 was as follows: GTTGCTGTATCAAGTGTGCAGA. The PCR cycle conditions were as follows: 1 cycle of  $95^\circ\text{C}$  predenaturation for 30 seconds followed by 40 cycles of  $95^\circ\text{C}$  denaturation for 10 seconds and  $60^\circ\text{C}$  annealing and extension for 30 seconds. The PCR products of each SNP were examined via 2.0% agarose gel electrophoresis and digested with the corresponding restriction enzymes (Thermo Scientific, USA) according to the manufacturer's protocol. The digested products were electrophoresed on a 3% agarose gel containing ethidium bromide and then visualized via UV transmission.

### **Statistical analysis**

The Helmholtz Centre website (Germany) was used to calculate the Hardy-Weinberg equilibrium (HWE) of *TLR7* rs3853839 genotypes. SPSS 23 software (IBM Corp, NY, USA) was used for statistical analysis of the current data. Normally distributed measurement data are presented as the means  $\pm$  SDs. The chi-square test was used to compare

categorical variables and assess the distribution of *TLR7* SNPs in the association between *TLR7* rs3853839 and ITP susceptibility, corticosteroid sensitivity, disease severity, disease recurrence, and disease progression. The risk associated with individual genotypes or alleles was calculated as the odds ratio (OR) with their 95% confidence intervals (95% CI). Differences between various groups were compared via a one-way analysis of variance and  $p < 0.05$  indicated a statistically significant difference.

## Results

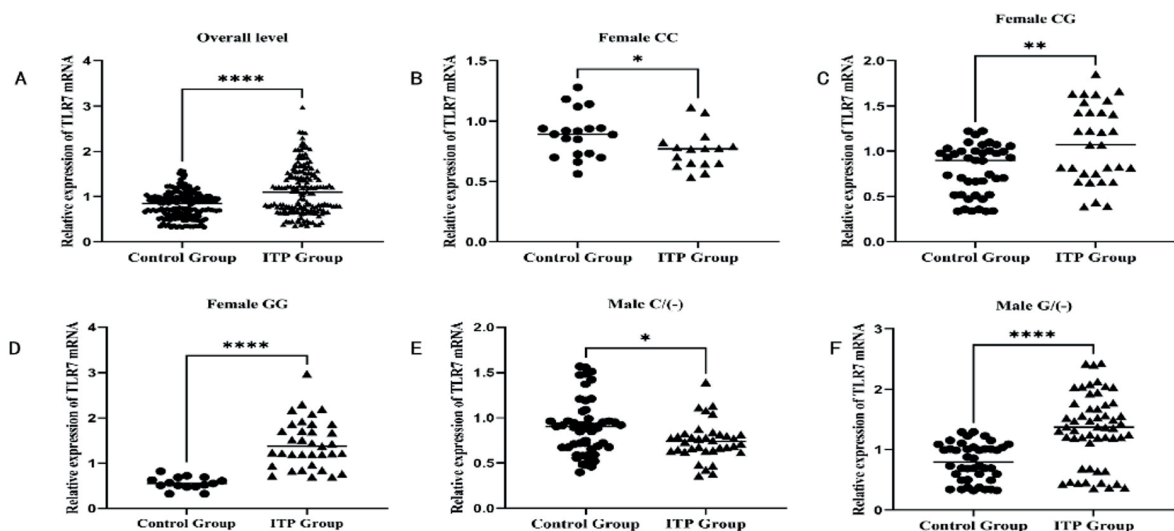
### Essential characteristics of the research subjects

This study included 172 pediatric patients with ITP and 170 children in the control group. Cases and controls were matched in age and gender. The mean age of disease onset among cases was  $6.065 \pm 3.283$  years, while in the control group, it was  $5.939 \pm 3.344$  years ( $p = 0.688$ ) (Table I). The male-to-female ratio in the case group was 1.17:1, while in the control group, it was 1.24:1 ( $p = 0.725$ ) (Table I). The serum *TLR7*

mRNA in the case group ( $1.129 \pm 0.536$ ) was significantly higher than that in the control group ( $0.851 \pm 0.298$ ) ( $p < 0.001$ ) (Table I and Fig. 1). Female patients with the GG genotype and male patients with the G/(-) genotype demonstrated the highest level of *TLR7* mRNA ( $1.478 \pm 0.522$  and  $1.280 \pm 0.590$ , respectively) ( $p < 0.0001$ ). Whereas female patients with the CC genotype and male patients with the C/(-) genotype showed the lowest level of *TLR7* mRNA ( $0.752 \pm 0.171$  and  $0.732 \pm 0.218$ , respectively) ( $p < 0.0001$ ) (Table I and Fig. 1). The remaining clinical and laboratory data of the case group encompassed platelet count, initial bleeding event, corticosteroid sensitivity, disease severity at the time of sampling, disease recurrence, and disease progression, which are shown in Table I.

### *TLR7* rs3853839 PCR amplification products and SNaPshot sequencing results

SNaPshot sequencing results indicate that blue peaks correspond to the G base, and black peaks correspond to the C base. Both blue and black peaks signify the CG base. As the *TLR7* gene is situated on the X chromosome, female children



**Fig. 1.** Comparison of relative expression of *TLR7* mRNA between control and ITP groups with different genotypes.

A: Children in the control and ITP groups. B: Female children in the control and ITP groups with *TLR7* rs3853839 CC genotype. C: Female children in the control and ITP groups with *TLR7* rs3853839 CG genotype. D: Female children in the control and ITP groups with *TLR7* rs3853839 GG genotype. E: Male children in the control and ITP groups with *TLR7* rs3853839 C/(-) genotype. F: Male children in the control and ITP groups with *TLR7* rs3853839 G/(-) genotype. ITP, immune thrombocytopenia; *TLR7*, toll-like receptor 7.

**Table I.** Clinical and demographic characteristics of participants.

Characteristics	ITP group (n=172)	Control group (n=170)	p value
Age of disease onset (yrs, M±SD)	6.065±3.283	5.939±3.344	0.688
Sex (male: female)	1.17:1	1.24:1	0.725
Relative expression of <i>TLR7</i> mRNA (M±SD)	1.129±0.536	0.851±0.298	<0.0001
<i>TLR7</i> mRNA expression by genotype (M±SD)			
Female			
GG	1.478±0.522	0.583±0.143	<0.0001
CG	1.068±0.421	0.917±0.304	0.0604
CC	0.752±0.171	0.903±0.151	0.0146
Male			
G/(-)	1.280±0.590	0.831±0.295	<0.0001
C/(-)	0.732±0.218	0.889±0.325	0.0495
Platelet count (×10 <sup>9</sup> /L)	37.34±23.14	151.6±39.38	N/A
≤30×10 <sup>9</sup> /L (n, %)	90 (52.33)	N/A	
>30×10 <sup>9</sup> /L (n, %)	82 (47.67)	N/A	
Initial bleeding event (n, %)			N/A
Purpura	108 (62.79)	N/A	
Ecchymosis	38 (22.09)	N/A	
Wet purpura	26 (15.12)	N/A	
Corticosteroid sensitivity (n, %)			N/A
Complete response	75 (43.60)	N/A	
Response	32 (18.60)	N/A	
No response	65 (37.80)	N/A	
Disease severity at sampling (n, %)			N/A
Severe	70 (40.70)	N/A	
Non-severe	102 (59.30)	N/A	
Disease recurrence (n, %)			N/A
Recurrent	50 (29.07)	N/A	
Non-recurrent	122 (70.93)	N/A	
Disease progression (n, %)			N/A
Chronic ITP	55 (31.98)	N/A	
Non-chronic ITP	117 (68.02)	N/A	

ITP, immune thrombocytopenia; M±SD, mean ± standard deviation; N/A, not applicable; *TLR7*, Toll-like receptor 7.

exhibited the GG, CC, and CG genotypes (Fig. 2, A, B, and C), while male children showed the G/(-) and C/(-) genotypes (Fig. 2, D and E).

#### ***TLR7 rs3853839 C/G genotype, allele, and gene frequency distributions***

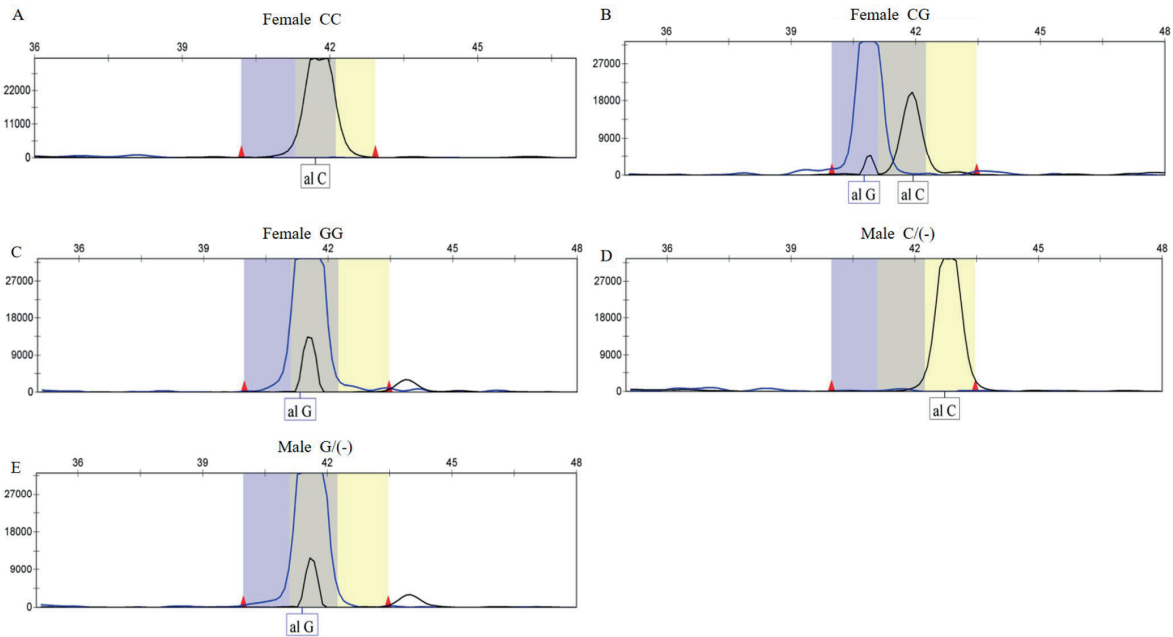
The *TLR7* rs3853839 GG genotype frequency in female patients was significantly higher compared to the control group ( $\chi^2=6.505$ ,

$p=0.039$ ) (Table II). The *TLR7* rs3853839 G/(-) genotype frequency in male patients was significantly higher compared to the control group ( $\chi^2=3.968$ ,  $p=0.046$ ; OR=1.857, 95% CI: 1.034-3.336) (Table II). The *TLR7* rs3853839 G allele gene frequency in ITP patients was significantly higher compared to the control group ( $\chi^2=6.146$ ,  $p=0.013$ ; OR=1.567, 95% CI: 1.098-2.238) (Table II). The *TLR7* rs3853839 GG



carrying gene frequency in ITP patients was significantly higher compared to the control

group ( $\chi^2=5.826$ ,  $p=0.016$ ; OR=2.337, 95% CI: 1.164-4.693) (Table II).



**Fig. 2.** Sequencing peak map of the *TLR7* rs3853839 gene locus.

A: Female children with *TLR7* rs3853839 CC genotype. B: Female children with *TLR7* rs3853839 CG genotype. C: Female children with *TLR7* rs3853839 GG genotype. D: Male children with *TLR7* rs3853839 C/(-) genotype. E: Male children with *TLR7* rs3853839 G/(-) genotype. Denoting blue peaks signify the G base, and black peaks signify the C base. Both blue and black peaks signify the CG base.

**Table II.** Frequency distribution of the *TLR7* rs3853839 G/C genotype, allele, and carrying gene in the ITP group and control group (n, %).

<i>TLR7</i> -C/G SNP Genotype	ITP group (n=172)	Control group (n=170)	$\chi^2$	p value	OR	95% CI
Female	82	76	6.505	0.039	-	-
GG	33 (40.24)	17 (22.37)				
CG	33 (40.24)	44 (57.89)				
CC	16 (19.52)	15 (19.74)				
Male	90	94	3.968	0.046	1.824	1.007-3.303
G/(-)	59 (65.56)	48 (51.06)				
C/(-)	31 (34.44)	46 (48.94)				
Allele	254	246				
G	158 (62.20)	126 (51.22)	6.146	0.013	1.567	1.098-2.238
C	96 (37.80)	120 (48.78)				
Carrying gene	82	76	5.826	0.016	2.337	1.164-4.693
GG	33 (40.24)	17 (22.37)				
CG+CC	49 (59.76)	59 (77.63)				

CI, confidence interval; ITP, immune thrombocytopenia; OR, odds ratio; SNP, single nucleotide polymorphism; *TLR7*: Toll-like receptor 7.

**The relationship between TLR7 rs3853839 C/G gene polymorphism and corticosteroid sensitivity in pediatric patients with ITP**

All cases were classified into corticosteroid-sensitive and corticosteroid-resistant cohorts based on their reactivity to corticosteroid therapy (n=107 and 65, respectively). The distribution of genotypes between the two groups was compared; however, no significant correlation was observed between the TLR7 rs3853839 genotypes and corticosteroid sensitivity in pediatric patients with ITP ( $p>0.05$ , Table III). This indicates that the TLR7 rs3853839 polymorphism is not associated with ITP in relation to the response to corticosteroid therapy.

**The relationship between TLR7 rs3853839 C/G gene polymorphism and the severity of pediatric patients with ITP**

All cases were classified into the severe ITP group and non-severe ITP group (n=70 and 102, respectively). Among female ITP patients

with different genotypes of TLR7 rs3853839, the proportion of GG genotype in severe ITP patients was 19 (55.88%), which was significantly higher than that in non-severe ITP patients 14 (29.17%), and this difference was statistically significant ( $\chi^2=6.371$ ,  $p=0.041$ , Table III). Among male ITP patients with different genotypes of TLR7 rs3853839, the proportion of G/(-) genotype in severe ITP patients was 28 (77.78%), which was significantly higher than that in non-severe ITP patients 31 (57.41%), and this difference was again statistically significant ( $\chi^2=3.969$ ,  $p=0.046$ , Table III). This indicates a potential correlation between the TLR7 rs3853839 polymorphism and the severity of ITP disease.

**The relationship between TLR7 rs3853839 C/G gene polymorphism and disease recurrence**

All cases were classified into the recurrent ITP group and the non-recurrent ITP group (n=50 and 122, respectively). The distribution of genotypes between the two groups was compared; however, no significant correlation was observed between the TLR7 rs3853839

**Table III.** Relationship between TLR7 rs3853839 genotype and clinical indicators in pediatric patients with ITP, n (%).

Clinical indicators	Genotype (Female)					Genotype (Male)			
	GG (n, %)	CG (n, %)	CC (n, %)	$\chi^2$	p value	G/(-) (n, %)	C/(-) (n, %)	$\chi^2$	p value
Corticosteroid sensitivity	33 (40.24)	33 (40.24)	16 (19.51)	3.038	0.219	59 (65.56)	31 (34.44)	0.891	0.345
Corticosteroid-sensitive ITP group (n=107)	19 (41.31)	21 (45.65)	6 (13.04)			38 (62.30)	23 (37.70)		
Corticosteroid-resistant ITP group (n=65)	14 (38.89)	12 (33.33)	10 (27.78)			21 (72.41)	8 (27.59)		
Disease severity	33 (40.24)	33 (40.24)	16 (19.51)	6.371	0.041	59 (65.56)	31 (34.44)	3.969	0.046
Severe ITP group (n=70)	19 (55.88)	9 (26.47)	6 (17.65)			28 (77.78)	8 (22.22)		
Non-severe ITP group (n=102)	14 (29.17)	24 (50.00)	10 (20.83)			31 (57.41)	23 (42.59)		
Disease recurrence	33 (40.24)	33 (40.24)	16 (19.51)	1.183	0.553	59 (65.56)	31 (34.44)	0.300	0.584
Recurrent ITP group (n=50)	10 (37.04)	13 (48.15)	4 (14.81)			14 (60.87)	9 (39.13)		
Non-recurrent ITP group (n=122)	23 (41.82)	20 (36.36)	12 (21.82)			45 (67.16)	22 (32.84)		
Disease progression	33 (40.24)	33 (40.24)	16 (19.51)	8.968	0.011	59 (65.56)	31 (34.44)	4.333	0.037
Non-chronic ITP group (n=117)	17 (31.48)	28 (51.85)	9 (16.67)			37 (58.73)	26 (41.27)		
Chronic ITP group (n=55)	16 (57.14)	5 (17.86)	7 (25.00)			22 (81.48)	5 (18.52)		

genotypes and recurrence in pediatric patients with ITP ( $p>0.05$ , Table III). This indicates that the *TLR7* rs3853839 polymorphism is not associated with ITP in relation to the recurrence of ITP disease.

#### ***The relationship between TLR7 rs3853839 C/G gene polymorphism and disease progression***

All cases were classified into the chronic ITP group and the non-chronic ITP group ( $n=55$  and  $117$ , respectively). Among female ITP patients with different genotypes of *TLR7* rs3853839, the proportion of GG genotype in chronic ITP patients was  $16(57.14\%)$ , which was significantly higher than that in non-chronic ITP patients  $17(31.48\%)$ , and the difference was statistically significant ( $\chi^2=8.968$ ,  $p=0.011$ , Table III). Among male ITP patients with different genotypes of *TLR7* rs3853839, the proportion of G/(-) genotype in chronic ITP patients was  $22(81.48\%)$ , which was significantly higher than that in non-chronic ITP patients  $37(58.73\%)$ , and the difference was statistically significant ( $\chi^2=4.333$ ,  $p=0.037$ , Table III). The results showed that ITP patients with *TLR7* rs3853839 GG genotype and G/(-) genotype had a significantly increased risk of developing chronic ITP.

#### **Discussion**

Recent information has shown that activation of *TLR7* leads to increased levels of Th1 and Th17 cells, and their cytokines play a pivotal role in the pathogenesis of ITP.<sup>23,24</sup> However, to the best of our knowledge, there is a lack of studies exploring the specific mechanisms involved. In this study, we aimed to clarify the characteristics of this mechanism associated with the *TLR7* rs3853839 single nucleotide polymorphism. Our results indicate that the *TLR7* rs3853839 GG genotype in female patients and the G/(-) genotype in male patients are linked to an increased risk of ITP, a more serious disease condition, and chronic disease progression. This finding is reported for the first time in the literature, as far as we are aware.

The pathogenesis of primary ITP involves multistep procedures, with genetic factors playing a pivotal role despite it being an autoimmune disease.<sup>25</sup> The principal function of the *TLR7* gene is to regulate the innate immune response.<sup>4,26</sup> Excessive activation or impaired function of *TLR7* may lead to innate immune dysfunction and the breakdown of immune tolerance, subsequently resulting in the onset of autoimmune diseases.<sup>27</sup> The immune system homeostasis in ITP patients typically relies on the dynamic equilibrium between Th1 and Th2 cells. However, any disruption to this balance can potentially induce changes in ITP.<sup>11</sup> Yang's research indicates that the activation of *TLR7* can lead to the polarization of Th1 immune cells, potentially influencing the development of ITP.<sup>28</sup> *TLR7* has also been found to recognize endogenous single-stranded RNA. When it is activated, a series of reactions occur through the classical myeloid cell differentiation factor 88 pathway, which promotes an increase in inflammatory factors such as IL-6, TNF- $\alpha$ , and IFN- $\gamma$ , thus triggering the differentiation of Th17 cells and dendritic cells and ultimately leads to the immune inflammatory response.<sup>29,30</sup> Previous research has indicated a notable increase in the quantities of Th17 cells and their associated cytokines, IL-6 and TNF- $\alpha$ , in patients with ITP.<sup>23,24</sup> IL17 can induce the production of inflammatory cytokines such as IFN- $\gamma$ , IL-1, and IL-6, further increasing the production of antiplatelet antibodies in mice<sup>3,31</sup> and patients with ITP.<sup>32,33</sup> Therefore, the *TLR7* gene may cause ITP through these pathways, and its specific mechanism needs further study.

Rs3853839, located in the 3' UTR of *TLR7*, has been proven to be functional and related to an increase in *TLR7* mRNA and *TLR7* protein expression and the stimulation of IFN gene upregulation to cause the occurrence of disease.<sup>18,34</sup> The 3' UTR is an essential regulatory region for expressing many genes that regulate mRNA translation, degradation, and subcellular localization by influencing RNA-binding proteins or noncoding RNAs.<sup>35</sup> Raafat et al.<sup>36</sup> reported that miRNAs could



regulate TLR signaling through direct effects on expression or by modulation of downstream regulators, adaptor molecules, and cytokines. The *TLR7* rs3853839 SNP can potentially impact gene expression by abolishing, weakening, or creating miRNA binding sites.<sup>37</sup> The non-risk C allele of *TLR7* rs3853839 matches a predicted binding site of microRNA-3148 (miR-3148), resulting in fast transcript breakdown and reducing *TLR7* mRNA levels.<sup>38</sup>

### ***TLR7 rs3853839 SNP in Non-Hematological Disorders***

It has been reported that the *TLR7* rs3853839 SNP is associated with human SLE.<sup>39</sup> Azab et al.<sup>12</sup> reported that the *TLR7* rs3853839 GG genotype and G allele were significantly associated with the pathogenesis of SLE in Egyptian patients. Yue et al.<sup>18</sup> reported that the *TLR7* rs3853839 C allele polymorphism is sex-specific and has a protective effect on the persistence of hepatitis C prevention in Chinese women. Xi et al.<sup>19</sup> revealed that the *TLR7* rs3853839 C/G polymorphism may play a role in susceptibility to knee OA. Shen's study conducted on patients with systemic lupus erythematosus revealed that individuals carrying the G mutant allele exhibited elevated *TLR7* mRNA transcripts. Heterozygous participants also had elevated levels of *TLR7* mRNA containing the G allele. These findings support the role of *TLR7* rs3853839 SNP in regulating *TLR7* mRNA expression.<sup>34</sup> El-Hefnawy et al.<sup>40</sup> indicated that the GG genotype of the *TLR7* rs3853839 SNP may be a genetic risk factor for severe COVID-19 and adverse clinical outcomes. Elevated *TLR7* mRNA expression in severe cases indicated its potential as a biomarker for COVID-19 prognosis. These findings also support the role of *TLR7* rs3853839 SNP in regulating *TLR7* mRNA expression in non-hematological disorders.

To our knowledge, this study represents the first demonstration of the association between *TLR7* rs3853839 C/G SNP and the susceptibility and clinical manifestations of ITP within the

Chinese Han population. We also observed that the GG and G/(-) genotypes are associated with an increased risk of ITP. The GG and G/(-) genotypes may result in heightened activation of *TLR7*, thus enhancing the self-reactivity of the immune system and increasing the risk of ITP. The GG genotype and G/(-) genotypes were shown to be substantially more common among severe patients in the current investigation. This phenomenon may be explained by excessive activation of *TLR7*, which may lead to a stronger inflammatory and autoimmune response, aggravating platelet destruction and production inhibition. This study also demonstrated that the GG and G/(-) genotypes may be predictive indicators for the chronic progression of ITP in pediatric patients. The continuous activation of *TLR7* may lead to chronic disorders of the immune system, promoting the chronicity of ITP. This finding provides new insights into the individualized treatment of ITP.

Our study had certain limitations. Firstly, the participants included in our study were exclusively from the Chinese Han population. A more comprehensive investigation involving multi-racial populations is imperative to authenticate the correlations between *TLR7* rs3853839 gene polymorphisms and ITP. Secondly, the biological functions of *TLR7* rs3853839 remain unknown, and further mechanistic research is essential to elucidate the impact of *TLR7* rs3853839 gene polymorphisms on the transcription, splicing, and translation efficiency of *TLR7*. The third and last limitation is that the time required to draw blood is not standard. Moreover, treatment modalities other than steroids could not be examined in this study. Despite this, we believe that our results accentuate the role of immunological derangements in its pathogenesis and will lead to further studies. The course of *TLR7* mRNA individually in the same patient throughout the disease course and the cut-off levels at presentation, which predict the potential severity and progressivity of ITP in an individual patient, even at presentation, can be examined in further studies. These will enable

individualized therapy in ITP, like avoidance of steroids but treatment with *TLR7* inhibitors.

In conclusion, this study suggests *TLR7* rs3853839 gene polymorphisms may be a potential genetic marker for ITP, affecting disease susceptibility, severity, and treatment response. These findings provide new insights into the pathogenesis of ITP and may provide a basis for developing personalized treatment strategies.

### Ethical approval

The study was approved by Medical Ethics Committee of Linyi People's Hospital (date: 10.26.2022, number: YX200343). Moreover, the participants' families were informed about the study, and their written consent was obtained.

### Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: JW, JL; data collection: JW, SW, GL, HS; analysis and interpretation of results: JW, JL; draft manuscript preparation: JW, SW, GL, HS, JL. All authors reviewed the results and approved the final version of the manuscript.

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### Conflict of interest

The authors declare that there is no conflict of interest.

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