CEP Clin Exp Pediatr Vol. 67, No. 9, 465–473, 2024
https://doi.org/10.3345/cep.2024.00577

Original article

Interleukin (IL)-1B and IL-1 receptor antagonist gene polymorphisms in children with primary immune thrombocytopenia

Seham Mohamed Ragab, MD[1], Wafaa Moustafa Abo Elfotoh, MD[1], Mahmoud Ahmed El-Hawy, MD[1], Eman Abdelfatah Badr, MD[2], Saara Khairat Ali Mostafa, MSc[1], Mai El-Sayad Abd El-Hamid, MD[1]

[1]Pediatrics Department, Faculty of medicine, Menoufia University, Shebin Elkom, Menoufia, Egypt; [2]Biochemistry Department, Menoufia University, Shebin Elkom, Menoufia, Egypt

Background: The pathophysiology and susceptibility of children to primary immune thrombocytopenia (ITP) are linked to polymorphisms of the interleukin (IL)-1B and IL-1 receptor (IL-1R) antagonist genes.

Purpose: To investigate the association between the susceptibility and severity of primary ITP in children and the IL-1B and IL-1R antagonist gene polymorphisms.

Methods: This comparative case-control study was conducted at the Menoufia University Hospital Hematology and Oncology Unit, Pediatric Department, between August 2022 and September 2023. The children were divided into patients (28 boys, 22 girls) who received hospital and outpatient clinic care and controls (50 healthy age- and sex-matched children).

Results: The mutant homozygous GG genotype and mutant G allele of rs16944 of the *IL1B* gene were considerably greater in patients than in controls (P<0.001). Furthermore, the mutant homozygous II/II genotype and heterozygous I/II genotype of the IL-1R antagonist gene were considerably greater in the case versus control group. The mutant II allele was significantly more prevalent in patients versus controls (P<0.001).

Conclusion: IL-1B and IL-1R antagonists may have a major impact on the development of immune thrombocytopenia. Furthermore, we found a relationship between IL-1B and IL-1R antagonist gene polymorphisms and the etiology of and children's susceptibility to primary immune thrombocytopenia.

Key words: Child, Interleukin, Gene polymorphisms, Primary immune thrombocytopenia

Key message

- Polymorphisms in interleukin (IL)-1B and IL-1 receptor (IL-1R) antagonists may significantly affect the pathogenesis of immune thrombocytopenia (ITP).
- IL-1B and IL-1R antagonist gene polymorphisms are correlated with severity and susceptibility to primary ITP in children.

Introduction

Immune thrombocytopenia (ITP) is an acquired thrombocytopenia that raises the risk of bleeding. Kohli et al.[1] define it as an isolated peripheral low platelet count of fewer than 100×10^9/L with no clear secondary etiology. It may have no symptoms or manifest with varied degrees of bleeding, ranging from diffuse skin purpura to severe mucosal or internal bleeding.[2] Its pathophysiology is mostly linked to platelet destruction and impaired bone marrow platelet production.[3,4]

ITP is an immune-mediated disease induced by autoantibodies against glycoproteins (GPs) IIb/IIIa and GPIb/IX found in platelet membranes.[5] B lymphocytes of the ill person generate antiplatelet antibodies, whereas T cells play a crucial part in etiopathogenesis.[6] The generation of autoantibodies by B cells, numerous dysfunctions in cellular immunity, and cytokine dysregulation are thought to play essential roles in the pathogenesis of ITP.[7]

According to Cai et al.,[8] cytokines have a significant role in controlling the balance between T helper 1 (Th1) and T helper 2 (Th2) cells. Th1 and Th2 imbalances in ITP lead to autoreactive B-cell differentiation; glucocorticoid therapy can assist in reestablishing Th1 and Th2 levels.[9]

Corresponding author: Mai El-Sayad Abd El-Hamid, MD, Pediatrics Department, Faculty of medicine, Menoufia University, Shebin Elkom, Menoufia, 6132415, Egypt
✉ Email: mai.elsaiad@med.menofia.edu.eg, https://orcid.org/0000-0002-9520-5708
Received: 1 April 2024, Revised: 11 June 2024, Accepted: 12 June 2024

A proinflammatory response, including the production of autoreactive antibodies and increased levels of IL-2 and interferon-γ, is triggered by the activation of CD4+ T cells.[10]

On chromosome 2q14 are IL-1B and IL-1 receptor (IL-1R) antagonists. Macrophages produce the proinflammatory cytokine IL-1 beta during systemic inflammatory responses.[11] By increasing cytokines including IL-6 and IL-12, it regulates immune and inflammatory responses.[12]

Three related genes make up the IL-1 family: IL-1A, IL-1B, and IL-1Ra. Each of these genes functions differently in autoimmune diseases and ITPs.[12] An enhanced cytokine response brought on by cytokine network disruption is another documented aspect of ITP pathophysiology. ITP etiopathogenesis has been linked to genetic variations in cytokine genes. Additionally, aberrant T cell and cytokine function are linked to most autoimmune illnesses, including ITP, and play a crucial role in the development of the disease. Cytokines are linked to ITP's severity and chronicity in addition to its etiogenesis.[6]

The etiology of ITP is influenced by polymorphisms in genes that encode cytokines, including IL-1, IL-4, human leukocyte antigen, tumor necrosis factors, and IL-10.[13] Members of the IL-1 cytokine family, IL-1A, IL-1Ra, and IL-1B, are crucial to the pathogenesis of ITP.[14] Thus, this study investigated the association between the susceptibility and severity of primary ITP in children and the IL-1B and IL-1R antagonist gene polymorphisms.

Methods

The Hematology and Oncology Unit, Pediatric Department, Menoufia University Hospitals conducted comparative case-control research. Our research was conducted in partnership with the Biochemistry and Molecular Biology Department, Faculty of Medicine, Menoufia University, from August 2022 to September 2023. The children studied were divided into 2 groups: the patients' group, which consisted of 50 ITP cases (28 boys and 22 females) who got inpatient treatment and outpatient clinic follow-up. Their ages ranged from 1 year to 18 years, with a median age of 8 years. The control group consisted of 50 healthy children of similar ages and genders with no history of medical difficulties.

1. Ethical consideration

The study was authorized by the faculty ethical committee, and signed informed consent was supplied by each patient's legal guardian. Under Institutional Review Board clearance number 2/20222 PEDI59, the Menoufia University Faculty of Medicine's ethical scientific committee approved the study plan.

2. Inclusion criteria

Children diagnosed with primary ITP ranged in age from 1 year to 18 years old and were treated according to our pediatric hematology unit protocol.

Primary ITP was described as an isolated case of thrombocytopenia without bone marrow abnormalities and in the absence of other thrombocytopenia-causing factors. A platelet counts of less than 100,000/μL was deemed abnormal.[15]

3. Exclusion criteria

Children with other autoimmune or chronic diseases, thrombocytopenia caused by other conditions (e.g., leukemia, Fanconi syndrome), and patients who had undergone ITP-specific medication before hospitalization.

4. All enrolled children were subjected to the following points of evaluation

Careful history taking focusing on personal history including name, age, and sex, history of disease illness including time, onset, course, and duration, bleeding site and grade, association with fever, arthralgia, arthritis, weight loss, and bone ache, medication, history of previous bleeding, previous surgery, febrile illness and contact with coronavirus disease 2019 patients, history of drug intake and transfusion of blood products or hospital admission, family history of similar condition.

Thorough clinical examination with emphasis on general condition, including appearance, facies, mucosal membrane and limbs, bleeding symptoms, and severity of bleeding; grading was performed by Buchanan et al.[16] Skin examination for purpura and ecchymosis, or color changes such as pallor, jaundice, and cyanosis; lymph node inspection for site, size, shape, and consistency; abdominal examination for hepatosplenomegaly and masses; and systemic examination for any abnormalities. Hematological investigations were performed on all patients at the time of presentation, after 7 days, and 1 month, as well as once for the control group that enrolled in the trial, including a complete blood count using automated Sysmex XN-1000 hematology analyzer (Sysmex America, Inc. Sysmex America, Lincolnshire, IL, USA) and inspection of the peripheral blood smear, platelet indices (mean platelet volume [MPV], platelet distribution width [PDW], and plateletcrit [PCT]). Platelet distribution curves were used to generate platelet indices, which were then acquired by automated hematology analyzers and reported alongside the platelet count.

A measure of platelet size, function, and activation is MPV, or MPV. 7.2–11.7 fL is the typical mean cell volume.[17] PDW, which varies with platelet activation and captures the heterogeneity in platelet shape, is a direct indicator of platelet size variability. PDW typically ranges from 9.4% to

16.0%.[18] PCT is a percentage that represents the volume that platelets occupy in blood. It is computed using the following formula: PCT=platelet count×MPV/10,000. PCT typically ranges between 0.11 to 0.29%.[19]

5. Molecular genetic study

Real-time polymerase chain reaction was used to detect rs16944 of the IL1B single nucleotide polymorphism (SNP), which included genotyping for the SNP. Genomic DNA was extracted from all frozen whole blood samples using Quick-g DNA TM Miniprep Kit, USA (catalog No. D3024).

1) Principles

The allelic discrimination assay genotypes the 2 possible variants at a SNP site in a target template sequence using 2 primer/probe pairs in each reaction. There is no set target sequence quantity. Unknown samples are split into 2 groups using the allelic discrimination assay: homozygous (samples containing only one allele, or allele), and heterozygous (samples containing both alleles 1 and 2). IL1Ra 86bp detection by employing variable time number repeats.

Blue dots represent the wild genotype (AA), red spots represent the mutant genotype (GG), and green dots represent the heterozygous genotype (AG) (Fig. 1). Furthermore, Fig. 2 displayed the following from left to right: lane 1: DNA ladder (100 bp), lane 2: negative control, and lane 3–10: patients' DNA sample products. In terms of interpretation, lanes 6, 7, 8 showed one band at 410 bp indicating wild (I/I) genotype, lane 10 showed one band at 240 bp indicating mutant (II/II) genotype, and lanes 3, 4, 5, 9 showed 2 bands at 410 bp and 240 bp suggesting heterozygous (I/II) genotype (Fig. 2).

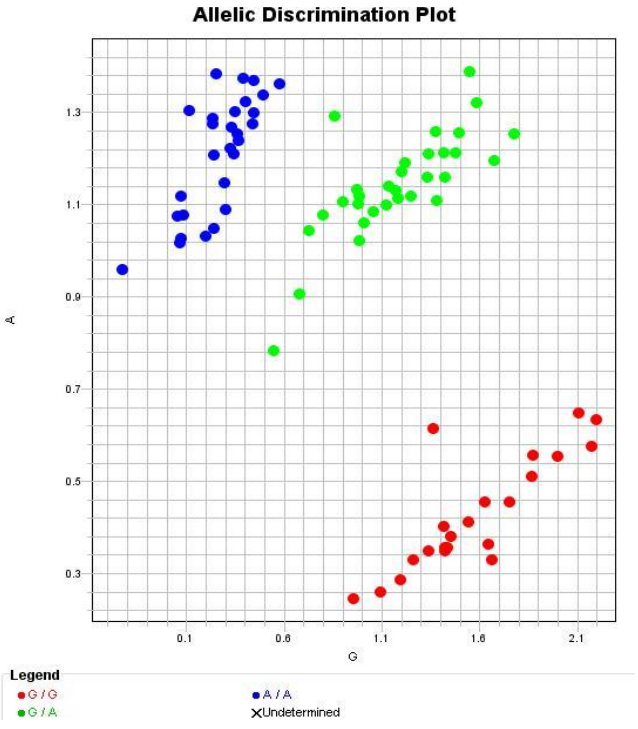

Fig. 1. Allelic discrimination plot of rs16944 of *IL1B* gene.

6. Statistical analysis

With IBM SPSS Statistics ver. 22.0 (IBM Co., Armonk, NY, USA), the collected data was processed, tabulated, and statistically examined. The Shapiro-Wilk test was used to normalize quantitative data, which was then expressed as mean±standard deviation and compared using the analysis of variance test. Fisher exact test for variables with minuscule, expected quantities and the chi-square test for numerical and percentage qualitative data were used for comparison. A statistical significance test called the Mann-Whitney test is used to compare 2 groups that have independent nonparametric data and quantitative factors. The Kruskal-Wallis test is utilized to compare more than 2 groups when employing independent nonparametric data and quantitative variables. In comparison to the odds of the event occurring in the absence of that exposure, the odds ratio shows the odds that an outcome will occur given a specific exposure. *P* values less than 0.05 were considered significant, while values more than that threshold were considered nonsignificant.

Results

Fig. 3 depicts a flowchart representing the study population. Of the 118 children, 18 were excluded from the study (7 patients declined consent and 11 patients did not meet the inclusion criteria), leaving 100 children who were divided into 2 groups: 50 children with ITP who received treatment at Menoufia University Hospital and outpatient clinic follow-up, and 50 healthy children of similar age and gender who served as controls (Fig. 3).

In this investigation, there was no significant difference in

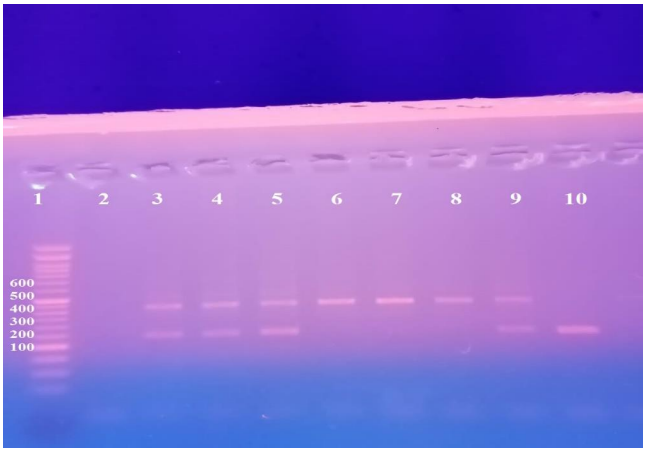

Fig. 2. Agarose gel electrophoresis of interleukin-1 receptor antagonist gene (VTNR).

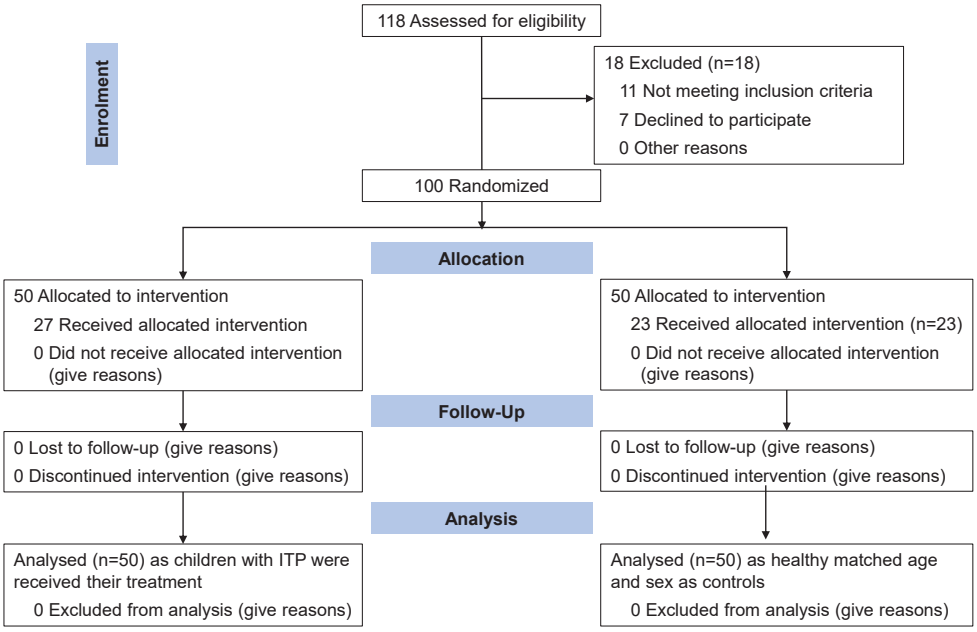

Fig. 3. Flowchart of the studied pediatric.

Table 1. Demographic data of the studied cases and controls

Variable	Cases (n=50)	Control (n=50)	Test of Sig.	*P* value
Sex, n (%)			0.161[a]	0.688
Male	28 (56.0)	26 (52.0)		
Female	22 (44.0)	24 (48.0)		
Age at examination (yr)			1,168.0[b]	0.571
Range	2.0–17.0	2.0–16.0		
Mean±SD	9.11±3.95	9.58±4.21		
Median (IQR)	8.0 (6.0–12.0)	10.0 (5.0–13.0)		
Residence, n (%)			2.098[a]	0.148
Rural	42 (84.0)	36 (72.0)		
Urban	8 (16.0)	1 4 (28.0)		

SD, standard deviation; IQR, interquartile range.
[a]Chi-square test (χ^2). [b]Mann-Whitney *U* test.

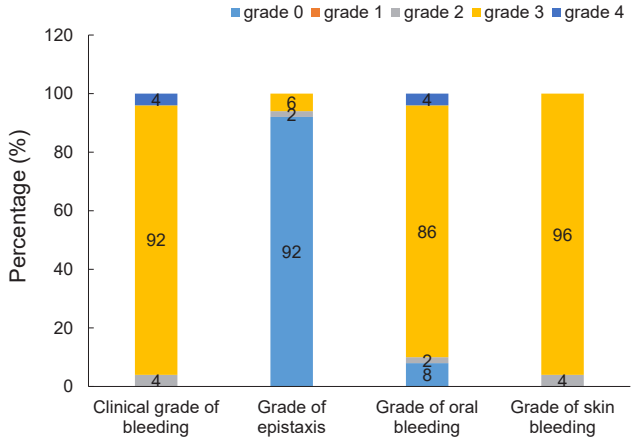

Fig. 4. Distribution of immune thrombocytopenic purpura (ITP) cases according to grades of bleeding (n=50)

age, sex, or residence between the case and control groups (Table 1). Furthermore, skin bleeding (100% of instances) was the most common, followed by oral bleeding (92%),

Table 2. rs16944 of *IL1B* gene polymorphism among cases versus controls

Gene polymorphism	Cases (n=50)	Control (n=50)	χ^2	*P* value
rs16944 of IL1B			21.186	**<0.001**
AA®	10 (20.0)	32 (64.0)		
AG	22 (44.0)	13 (26.0)		
GG	18 (36.0)	5 (10.0)		
P_0[a]	0.493	0.060		
Allele			25.418	**<0.001**
A	42 (42.0)	77 (77.0)		
G	58 (58.0)	23 (23.0)		

Values are presented as number (%) unless otherwise indicated.
IL, interleukin; ®, reference group.
P value for chi-square (χ^2) for goodness of fit.
[a]HW, Hardy-Weinberg equilibrium.
Boldface indicates a statistically significant difference with *P*<0.05.

with epistaxis accounting for just 8%. The clinical grade of overall bleeding was moderate degree (grade 3) in most of the cases (92%). Also, mild grades range from 0–2 was (4%), while moderate to severe grades range from 3–4 (96%) (Fig. 4).

Also, the mutant homozygous GG genotype and mutant G allele of rs16944 of the *IL1B* gene were substantially greater in patients compared to controls (*P*<0.001) (Table 2). Furthermore, children carrying the mutant homozygous GG and heterozygous AG genotypes had a significantly increased risk of developing ITP (OR, 11.52 and 5.4, respectively). In addition, ITP sufferers are 4.6 times more likely than controls to carry the mutant gene (Table 3). Furthermore, the mutant homozygous II/II genotype and heterozygous I/II genotype of the IL-1R antagonist gene were considerably greater in cases compared to the control group. The mutant II allele was more significant in patients than

Table 3. Odds ratio among cases and controls according to rs16944 of *IL1B* genotype and alleles

Gene polymorphism	Cases (n=50)	Control (n=50)	*P* value	OR (95% CI)
rs16944 of IL1B				
AA®	10 (20.0)	32 (64.0)		1.000
AG	22 (44.0)	13 (26.0)	**0.001**	5.415 (2.018–14.531)
GG	18 (36.0)	5 (10.0)	**<0.001**	11.520 (3.405–38.980)
Allele distribution				
A	42 (42.0)	77 (77.0)		1.000
G	58 (58.0)	23 (23.0)	**<0.001**	4.623 (2.507–8.526)

Values are presented as number (%) unless otherwise indicated.
OR, odds ratio; CI, confidence interval; IL, interleukin; ®, reference group.
Boldface indicates a statistically significant difference with $P<0.05$.

Table 4. IL-1R antagonist gene polymorphism among cases versus controls

IL-1R antagonist genotype	Cases (n=50)	Control (n=50)	χ^2	*P* value
IL-1R			34.443	**<0.001**
I/I®	8 (12.0)	37 (74.0)		
I/II	31 (74.0)	11 (22.0)		
II/II	11 (14.0)	2 (4.0)		
P_0[a)]	0.084	0.332		
Allele			32.175	**<0.001**
I	47 (47.0)	85 (85.0)		
II	53 (53.0)	15 (15.0)		

Values are presented as number (%) unless otherwise indicated.
IL-1R, interleukin-1 receptor; ®, reference group; HW, Hardy-Weinberg equilibrium.
P_0, *P* value for chi-square test (χ^2) for goodness of fit.
[a)]HW, Hardy-Weinberg equilibrium.
Boldface indicates a statistically significant difference with $P<0.05$.

controls ($P<0.001$) (Table 4).

Heterozygous form I/II genotypes have a significantly increased risk of developing ITP (OR, 25.43 and 13), respectively. ITP cases are also about 6.3 times more likely to carry mutant alleles than healthy children (Table 5). Furthermore, ITP cases with overall moderate to severe (grade 3–4) bleeding were significantly associated with an increase in heterozygous AG (22/50) and mutant homozygous GG (18/50) genotypes compared to cases carrying wild-type homozygous AA genotypes (8/50). All cases with epistaxis (grade 1–4) carried the mutant homozygous (GG) genotype of rs16944 of the *IL1B* genotype. There was no significant difference among the other parameters (Table 6). Furthermore, ITP patients with epistaxis (grade 1–4) are substantially related to an increase in the mutant homozygous (II/II) genotype of the IL-1R antagonist gene. There was no significant difference among the other parameters (Table 7).

Furthermore, using univariate logistic analysis, we discovered that platelet count after 7 days and platelet count at presentation were significant protective factors affecting ITP development (OR, 0.25, 0.95, and 0.79, respectively), whereas mutant genotypes of IL1B and IL-1R antagonist

Table 5. Odds ratios of cases versus control according to IL-1R antagonist genotype and alleles

IL-1R antagonist genotype	Cases (n=50)	Control (n=50)	*P* value	OR (95% CI)
IL-1R				
I/I®	8 (12.0)	37 (74.0)		1.000
I/II	31 (74.0)	11 (22.0)	**0.001**	13.034 (4.662–36.443)
II/II	11 (14.0)	2 (4.0)	**<0.001**	25.437 (4.697–137.771)
Allele distribution				
I	47 (47.0)	85 (85.0)		1.000
II	53 (53.0)	15 (15.0)	**<0.001**	6.390 (3.254–12.549)

Values are presented as number (%) unless otherwise indicated.
IL-1R, interleukin-1 receptor; OR, odds ratio; CI, confidence interval; ®, reference group.
P value for univariate regression analysis for comparison with reference genotype.
Boldface indicates a statistically significant difference with $P<0.05$.

appeared to be risk factors for ITP development (OR, 7.11 and 14.94). Using multivariate logistic analysis, only platelet count after 7 days was determined to be the most significant predictor of ITP ($P=0.001$, OR, 0.962) (Table 8).

Discussion

Bound to platelets and megakaryocytes, IgG autoantibodies cause ITP by targeting surface antigens such as GP αIIbβ3 (GPIIbIIIA) and GPIb-IX-V.[20,21] There is growing evidence that the pathophysiology of this disease involves faulty regulatory T cells (Tregs), imbalances in helper T cells, abnormalities in megakaryocyte maturation, inappropriate T-cell anergy, and cytotoxic T cells.[22,23]

Cytokine gene polymorphisms in Egyptian ITP patients have not been extensively studied. Thus, we looked at cytokine gene polymorphisms and their relationship to ITP susceptibility and disease severity in the current study. We found that, with 56% of respondents being male and 44% being female, there was no discernible sex preference. However, Elalfy et al.[24] discovered no gender preference in acute ITP, although chronic ITP was more common in females. Also, Ahmed and Younies[25] discovered that males and females were equally affected. Tantawy et al.[26] discovered that out of the 40 patients, 17 were males and 23 were females. Moreover, Libert et al.[27] concluded that chronic ITP was more frequent in females as they are exposed to autoimmune disorders more than males but in children ITP is equal in both sexes.

The current study found that skin bleeding (100% of instances) was the most common, followed by oral bleeding (92%), with epistaxis accounting for just 8%. In most patients (92%), the total clinical grade of bleeding was moderate. These findings are consistent with those described by Zahran and Alam et al.,[28] who found cutaneous symptoms in all 40 patients with acute ITP, including petechial bleed-

Table 6. Correlation between rs16944 of *IL1B* genotypes with different parameters among children with immune thrombocytopenic purpura

Variable	rs16944 of IL1B			Test of Sig.	*P* value
	AA (n=10)	AG (n=22)	GG (n=18)		
Sex				χ^2=0.413	0.813
Male	6 (60.0)	13 (59.1)	9 (50.0)		
Female	4 (40.0)	9 (40.9)	9 (50.0)		
Age at onset (yr)				χ^2=2.622	0.270
≤6	6 (60.0)	14 (63.6)	7 (38.9)		
>6	4 (40.0)	8 (36.4)	11 (61.1)		
Mean±SD	6.0±2.11	6.64±3.0	6.83±2.90	H=0.401	0.818
Median (range)	6.0 (2.0–9.0)	6.0 (2.0–15.0)	8.0 (2.0–10.0)		
Clinical grade of overall bleeding				χ^2=5.080	**0.035**[b]
Mild (0–2)	2 (20.0)	0 (0)	0 (0)		
Moderate to severe (3–4)	8 (80.0)	22 (100)	18 (100)		
Significance between groups		P_1=**0.031**[a], P_2=**0.049**[a], P_3=NA			
Epistaxis				χ^2=5.812	**0.036**[b]
Negative (0)	10 (100)	22 (100)	14 (77.8)		
Positive (1–4)	0 (0)	0 (0)	4 (22.2)		
Significance between groups		P_1=NA, P_2=0.265[a], P_3=**0.033**[a]			
Oral bleeding				χ^2=0.594	1.000[b]
Negative (0)	1 (10.0)	2 (9.1)	1 (5.6)		
Positive (1–4)	9 (90.0)	20 (90.9)	17 (94.4)		
Platelet (×10^3 cells/μL) at presentation				H=2.747	0.253
Mean±SD	22.80±12.78	16.77±9.53	17.06±4.99		
Median (range)	18.0 (3.0–47.0)	14.50 (0.0–48.0)	17.5 (11.0–27.0)		
PCT (%) at presentation				H=1.417	0.492
Mean±SD	0.15±0.04	0.17±0.06	0.16±0.06		
Median (range)	0.17 (0.07–0.19)	0.18 (0.07–0.23)	0.18 (0.06–0.23)		
MPV (fL) at presentation				H=1.382	0.501
Mean±SD	12.50±1.90	11.95±2.40	13.24±3.33		
Median (range)	12.0 (9.0–16.0)	12.0 (9.0–16.0)	13.0 (9.0–22.30)		
PDW (%) at presentation				H=0.270	0.874
Mean±SD.	17.17±4.39	17.45±4.43	16.78±4.33		
Median (range)	18.50 (10.0–22.0)	20.0 (10.0–22.0)	18.50 (8.0–22.0)		
Recovery from first line at presentation (steroids and IVIG)	(n=8)	(n=16)	(n=16)	χ^2=1.133	0.580[b]
Partial response	5 (62.5)	13 (81.3)	12 (75.0)		
Complete response	3 (37.5)	3 (18.8)	4 (25.0)		

Values are presented as number (%) unless otherwise indicated.
SD, standard deviation; Sig., significance; NA, not applicable; H, Kruskal-Wallace test; PCT, plateletcrit; MPV, mean platelet volume; PDW, platelet distribution width; IVIG, intravenous immunoglobulin.
P_1, comparing AA vs. AG; P_2, comparing AA vs. GG; P_3, comparing AG vs. GG.
[a]Fisher exact test. [b]Monte Carlo.
Boldface indicates a statistically significant difference with P<0.05.

ing and mild bruises. Only 9 patients suffered from mild epistaxis. Alam[29] discovered bruising in 85.3%, petechial rash in 79%, and epistaxis in 24%. Neunert et al.[30] also found the intercontinental cooperative ITP Study Group examined the therapy of children with persistent and chronic ITP and found no incidences of cerebral hemorrhage. Following diagnosis and at 28 days, 6 months, 12 months, and 24 months, 1,345 participants were gathered. Skin bleeding was the most common site of bleeding, followed by epistaxis. These findings contradict Sabhan et al.,[31] who found that epistaxis was present in 87% of newly diagnosed ITP patients.

Regarding the rs16944 IL-1B polymorphism, the results of this study suggest a link between the mutant homozygous (GG) and heterozygous (AG) genotypes of rs16944 IL-1B and susceptibility to ITP. Furthermore, offspring carrying the changed G allele had a 4.6-fold increased sensitivity to ITP. Yadav et al.[12] found a in primary ITP, there is a substantial correlation between the homozygous mutant genotype of IL-1B31 and severe ITP as compared to healthy controls (OR, 2.76; 95% CI, 1.076–7.10). Moreover, a significant probability of severe ITP was linked to the IL-B-31 mutant allele (OR, 1.64; 95% CI, 1.046–2.58), while neither severe nor non-severe ITP was linked to the IL-1B-511 genotype.

Table 7. Correlation between IL-1R antagonist genotype and different parameters

Variable	I/I (n=8)	I/II (n=31)	II/II (n=11)	Test of Sig.	*P* value
Sex				χ^2=1.422	0.559[b]
Male	3 (37.5)	18 (58.1)	7 (63.6)		
Female	5 (62.5)	13 (41.9)	4 (36.4)		
Age at onset (yr)				χ^2=2.433	0.308[b]
≤6	3 (37.5)	16 (51.6)	8 (72.7)		
>6	5 (62.5)	15 (48.4)	3 (27.3)		
Mean±SD	7.13±2.03	6.84±2.85	5.45±2.91	H=1.558	0.459
Median (range)	7.50 (4.0–10.0)	6.0 (2.0–15.0)	6.0 (2.0–10.0)		
Clinical grade of overall bleeding				χ^2=2.039	0.340[b]
Mild (0–2)	1 (12.5)	1 (3.2)	0 (0)		
Moderate to severe (3–4)	7 (87.5)	30 (96.8)	11 (100)		
Epistaxis				χ^2=10.639	**0.002**[b]
Negative (0)	8 (100)	31 (100)	7 (63.6)		
Positive (1–4)	0 (0)	0 (0)	4 (36.4)		
Significance between groups		P_1=NA, P_2=0.265[a], P_3=**0.003**[a]			
Oral bleeding				χ^2=0.620	1.000[b]
Negative (0)	0 (0)	3 (9.7)	1 (9.1)		
Positive (1–4)	8 (100)	28 (90.3)	10 (90.9)		
Platelet (×10^3 cells/μL) at presentation				H=1.141	0.565
Mean±SD	20.37±11.31	18.23±9.94	16.0±3.58		
Median (range)	18.50 (3.0–41.0)	18.0 (0.0–48.0)	17.0 (11.0–21.0)		
PCT (%) at presentation				H=1.378	0.502
Mean±SD	0.15±0.05	0.17±0.05	0.16±0.07		
Median (range)	0.17 (0.07–0.19)	0.18 (0.07–0.23)	0.18 (0.06–0.23)		
MPV (fL) at presentation				H=2.026	0.363
Mean±SD	11.75±1.83	12.26±2.31	13.85±3.86		
Median (range)	12.0 (9.0–14.0)	12.0 (9.0–17.0)	14.0 (9.0–22.30)		
PDW (%) at presentation				H=1.926	0.382
Mean±SD.	16.88±3.80	16.86±4.25	18.18±5.0		
Median (range)	16.50 (12.0–22.0)	20.0 (10.0–22.0)	20.0 (8.0–22.0)		
Recovery from first line at presentation (steroids and IVIG)	(n=7)	(n=22)	(n=11)	χ^2=0.498	0.892[b]
Partial response	5 (71.4)	16 (72.7)	9 (81.8)		
Complete response	2 (28.6)	6 (27.3)	2 (18.2)		

Values are presented as number (%) unless otherwise indicated.
IL-1R, interleukin-1 receptor; SD, standard deviation; Sig., significance; NA, not applicable; H, Kruskal-Wallace test; PCT, plateletcrit; MPV, mean platelet volume; PDW, platelet distribution width; IVIG, intravenous immunoglobulin.
P_1, comparison of I/I and I/II; P_2, comparison of I/I and II/II.
[a]Fisher exact test. [b]Monte Carlo.
Boldface indicates a statistically significant difference with P<0.05.

Table 8. Univariate and multivariate logistic regression analysis of parameters affecting primary immune thrombocytopenia in children (n=50 vs. 50)

Variable	Univariate		Multivariate[a]	
	P value	OR (95% CI)	*P* value	OR (LL–UL 95% CI)
Platelet count after 7 days	**<0.001**	0.952 (0.931–0.974)	0.001	0.962 (0.941–0.983)
Platelet count at presentation	**<0.001**	0.790 (0.705–0.886)	0.095	0.768 (0.563–1.047)
rs16944 of IL1B (heterozygous AG+ mutant GG)	**<0.001**	7.111 (2.885–17.526)	0.600	2.771 (0.062–124.602)
IL-1R antagonist (heterozygous I/II+ mutant II/II)	**<0.001**	14.942 (5.578–40.028)	0.477	4.435 (0.073–270.124)

OR, odds ratio; LL, lower limit; UL, upper limit; CI, confidence interval; IL-1R, interleukin-1 receptor.
[a]All variables with values of P<0.05 were included in the multivariate analysis.
Boldface indicates a statistically significant difference with P<0.05.

In this study, not only did individuals with the modified IL-1B polymorphism allele exhibit heightened vulnerability to ITP, but they also exhibited a more severe variant of the illness, where ITP cases presented with overall moderate to severe bleeding are significantly associated with an increase of mutant allele-containing genotypes: heterozygous AG

(22/50) and homozygous GG (18/50) genotypes compared with cases carrying wild homozygous AA (8/50), also, all cases presented with epistaxis carried the mutant homozygous (GG) genotype of rs16944 of IL1B genotype. This indicates that the altered allele may affect the gene expression and/or alter the protein function resulting in more severe disease.

In terms of IL-1R antagonist, we discovered a substantial increase in mutant allele-containing genotype, heterozygous (I/II), and mutant homozygous (II/II) genotype variations of the IL-1R antagonist polymorphism in cases compared to the control group. Furthermore, the mutant II allele of the IL-1R antagonist polymorphism was considerably higher in patients compared to controls. Furthermore, ITP sufferers are around 6.3 times more likely to possess the mutant gene than healthy children. A previous Egyptian study by Al-Tawil et al.[11] found that interleukin-1 receptor antagonist gene polymorphisms in children and adolescents with primary ITP were associated with disease susceptibility, response to therapy, and outcome, with patients having significantly higher frequencies of the mutant allele and genotype than controls.

Moreover, our results agree with a previous study[32,33] that linked an IL-1R antagonist polymorphism to ITP. Additionally, a case-control study on both IL1B (IL-1B-511, IL-1B-31) and IL1RA polymorphisms was carried out by El Amawy and Shahin,[34] which showed a significant correlation between ITP and the genotype distributions or alleles of IL-1B-31, IL-1Ra, and IL-1B-511, as well as the fact that mutant genotypes of IL-1B-31, IL-1B-511, and IL-1R carry a significant risk for ITP by (18.62, 3.5, and 5.76 folds, respectively). On the other hand, Elsaadany et al.[35] looked at how genetic variants in IL-1B affected Egyptian children's development of primary ITP.

The study's drawbacks were a single center design, a very limited sample size for polymorphism analysis, and the exploration of just 2 polymorphisms due to the high cost of the research.

In conclusion, the current investigation suggests that polymorphisms in the IL-1B and IL-1R antagonists may play a major role in the pathophysiology of ITP. Additionally, there is a correlation between the severity and susceptibility of primary ITP in children and polymorphisms in the genes encoding the IL-1B and IL-1R antagonist.

Footnotes

Conflicts of interest: No potential conflict of interest relevant to this article was reported.

Funding: This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

ORCID:
Seham Mohamed Ragab https://orcid.org/0000-0001-7121-3984
Mahmoud Ahmed El-Hawy https://orcid.org/0000-0002-3420-922X
Eman Abdelfatah Badr https://orcid.org/0000-0001-9724-1596
Mai El-Sayad Abd El-Hamid https://orcid.org/0000-0002-9520-5708

References

1. Kohli R, Chaturvedi S. Epidemiology and clinical manifestations of immune thrombocytopenia. Hamostaseologie 2019;39:238-49.
2. Lambert C, Maitland H, Ghanima W. Risk-based and individualised management of bleeding and thrombotic events in adults with primary immune thrombocytopenia (ITP). Eur J Haematol 2024;112:504-15.
3. Lambert MP, Gernsheimer TB. Clinical updates in adult immune thrombocytopenia. Blood 2017;129:2829-35.
4. Neunert CE, Cooper N. Evidence-based management of immune thrombocytopenia: ASH guideline update. Hematology Am Soc Hematol Educ Program 2018;2018:568-75.
5. Schmidt DE, Heitink-Polle KMJ, Porcelijn L, van der Schoot CE, Vidarsson G, Bruin MCA, et al. Anti-platelet antibodies in childhood immune thrombocytopenia: prevalence and prognostic implications. J Thromb Haemost 2020;18:1210-20.
6. Rasheed BN, Eissa AA. Impact of IL-1Ra gene polymorphism on the etiology and fate of disease in children with immune thrombocytopenic purpura. J Immunol Res 2021;2021:505673.
7. Consolini R, Legitimo A, Caparello MC. The centenary of immune thrombocytopenia - Part 1: revising nomenclature and pathogenesis. Front Pediatr 2016;4:102.
8. Cai Y, Ji H, Zhou X, Zhao K, Zhang X, Pan L, et al. Interleukin-21 modulates balance between regulatory T cells and T-helper 17 cells in chronic hepatitis B virus infection. BMC Infect Dis 2023;23:719.
9. Dong H, Ren Z, Shao W, Duan G, Du A, Du B. Effect of ADSCs on Th17/Treg and T-bet/GATA-3 in model mice with primary immune thrombocytopenia. Cell Mol Biol (Noisy-le-grand) 2024;70:150-4.
10. Kostic M, Zivkovic N, Cvetanovic A, Marjanovi G. CD4+ T cell phenotypes in the pathogenesis of immune thrombocytopenia. Cell Immunol 2020;351:104096.
11. Al-Tawil MM, Kamal TM, Borham OM, Abd El-Ghany SM. Interleukin-1 receptor antagonist gene polymorphisms in Egyptian children and adolescents with primary immune thrombocytopenia: association with disease susceptibility, response to therapy, and outcome. J Pediatr Hematol Oncol 2023;45:e650-4.
12. Yadav DK, Tripathi AK, Gupta D, Shukla S, Singh AK, Kumar A, et al. Interleukin-1B (IL-1B-31 and IL-1B-511) and interleukin-1 receptor antagonist (IL-1Ra) gene polymorphisms in primary immune thrombocytopenia. Blood Res 2017;52:264-9.

13. Takahashi N, Saitoh T, Gotoh N, Nitta Y, Alkebsi L, Kasamatsu T, et al. The cytokine polymorphisms affecting Th1/Th2 increase the susceptibility to, and severity of, chronic ITP. BMC Immunol 2017;18:26.
14. Zalesak M, Danisovic L, Harsanyi S. Psoriasis and psoriatic arthritis-associated genes, cytokines, and human leukocyte antigens. Medicina (Kaunas) 2024;60:815.
15. Onisâi M, Vlădăreanu AM, Delcea C, Cior scu M, Bumbea H, Nicolescu A, et al. Perinatal outcome for pregnancies complicated with thrombocytopenia. J Matern Fetal Neonatal Med 2012;25:1622-6.
16. Buchanan GR, Adix L. Grading of hemorrhage in children with idiopathic thrombocytopenic purpura. J Pediatr 2002;141:683-8.
17. Rajantie J, Javela K, Joutsi-Korhonen L, Kekomäki R. Chronic thrombocytopenia of childhood: use of non-invasive methods in clinical evaluation. Eur J Haematol 2004;72:268-72.
18. Wiwanitkit V. Plateletcrit, mean platelet volume, platelet distribution width: its expected values and correlation with parallel red blood cell parameters. Clin Appl Thromb Hemost 2004;10:175-8.
19. Beyan C, Kaptan K, Ifran A. Platelet count, mean platelet volume, platelet distribution width, and plateletcrit do not correlate with optical platelet aggregation responses in healthy volunteers. J Thromb Thrombolysis 2006;22:161-4.
20. Lowe EJ, Buchanan GR. Idiopathic thrombocytopenic purpura diagnosed during the second decade of life. J Pediatr 2002;141:253-8.
21. Boylan B, Chen H, Rathore V, Paddock C, Salacz M, Friedman KD, et al. Anti-GPVI-associated ITP: an acquired platelet disorder caused by autoantibody-mediated clearance of the GPVI/FcRgamma-chain complex from the human platelet surface. Blood 2004;104:1350-5.
22. Zhang J, Ma D, Zhu X, Qu X, Ji C, Hou M. Elevated profile of Th17, Th1 and Tc1 cells in patients with immune thrombocytopenic purpura. Haematologica 2009;94:1326-9.
23. Cines DB, Gernsheimer T, Wasser J, Godeau B, Provan D, Lyons R, et al. Integrated analysis of long-term safety in patients with chronic immune thrombocytopaenia (ITP) treated with the thrombopoietin (TPO) receptor agonist romiplostim. Int J Hematol 2015;102:259-70.
24. Elalfy M, Elbarbary N, Khaddah N, Abdelwahab M, El Rashidy F, Hassab H, et al. Intracranial hemorrhage in acute and chronic childhood immune thrombocytopenic purpura over a ten-year period: an Egyptian multicenter study. Acta Haematol 2010;123:59-63.
25. Ahmed FE, Younies EH. Pediatrics neonatal care. History 2016;15:31-9.
26. Tantawy AA, Elsherif NHK, Kenny MA, Aboulfotouh KA, Hassan AE, Kabil ME. Silent bleeding in children and adolescents with immune thrombocytopenia: relation to laboratory parameters and health related quality of life. J Thromb Thrombolysis 2020;50:258-66.
27. Libert C, Dejager L, Pinheiro I. The X chromosome in immune functions: when a chromosome makes the difference. Nat Rev Immunol 2010;10:594-604.
28. Zahran AM, Elsayh KI. CD4+ CD25+High Foxp3+ regulatory T cells, B lymphocytes, and T lymphocytes in patients with acute ITP in Assiut Children Hospital. Clin Appl Thromb Hemost 2014;20:61-7.
29. Alam MM. Idiopathic thrombocytopenic purpura in children: a 10 years experience at tertiary care hospital. J Pak Med Assoc 2014;64:1358-62.
30. Neunert CE, Buchanan GR, Imbach P, Bolton-Maggs PH, Bennett CM, Neufeld E, et al. Bleeding manifestations and management of children with persistent and chronic immune thrombocytopenia: data from the Intercontinental Cooperative ITP Study Group (ICIS). Blood 2013;121:4457-62.
31. Sabhan AH, Al-Jadiry MF, Ghali HH, Abed WM, Al-Hadad SA. Chronic immune thrombocytopenic purpura in children overview of 60 patients. Pediatr Hematol Oncol J 2016;1:9-12.
32. Rocha AM, De Souza C, Rocha GA, De Melo FF, Saraiva IS, Clementino NC, et al. IL1RN VNTR and IL2-330 polymorphic genes are independently associated with chronic immune thrombocytopenia. Br J Haematol 2010;150:679-84.
33. Pesmatzoglou M, Lourou M, Goulielmos GN, Stiakaki E. DNA methyltransferase 3B gene promoter and interleukin-1 receptor antagonist polymorphisms in childhood immune thrombocytopenia. Clin Dev Immunol 2012;2012:352059.
34. El Amawy M, Shahin E. Assessment of IL-1B31 and IL RA gene polymorphism in immune thrombocytopenia. Benha Med J 2023;40:298-309.
35. Elsaadany ZA, Momen NN, Elmesawy OE, Abd Elhady M, Gad A. Studying the role of IL-1B genetic polymorphisms in the development of primary immune thrombocytopenia among Egyptian children. Gene Rep 2023;30:101736.

How to cite this article: Ragab SM, ElFotoh WMA, El-Hawy MA, Badr EA, Mostafa SKA, El-Hamid MEA. Interleukin (IL)-1B and IL-1 receptor antagonist gene polymorphisms in children with primary immune thrombocytopenia. Clin Exp Pediatr 2024;67:465-73.