Role of Platelet Endothelial Aggregation Receptor 1 Polymorphisms in Idiopathic Thrombocytopenic Purpura: Is There an Association?

Abstract

Background: Genetic risk factors are implicated in the etiology and pathogenesis of immune thrombocytopenic purpura (ITP). Platelet endothelial aggregation receptor 1 (PEAR1) plays an important role in regulating megakaryopoiesis and thrombopoiesis. rs12041331 and rs12566888 single-nucleotide polymorphisms of PEAR1 are associated with megakaryocyte differentiation and platelet function. **Materials and Methods:** To conduct this study, 68 peripheral blood samples of patients with ITP (56 acute and 12 chronic) were collected. The amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) was used to detection of rs12041331 and rs12566888 PEAR1 polymorphisms. **Results:** Statistically significant differences were not seen between rs12041331 and rs12566888 genotypes in acute and chronic groups ($P = 0.778$, $P = 0.844$). The frequency of rs12041331 AG/AA genotypes and the rs12566888 GT genotype was more in acute ITP patients; on the other hand, the rs12566888 TT genotype was more in the chronic group. The highest platelet counts and platelet distribution width (PDW) were related to the rs12041331 AG allele. GT and TT of rs12566888 had more PDW and platelet count, respectively. Mean platelet volume values between alleles of both the polymorphisms were constant and did not differ much. In general, no statistically significant differences were observed between genotypes of polymorphisms and platelet parameters. **Conclusions:** There was no association between rs12041331 and rs12566888 with platelet parameters in ITP patients and the severity of this disease. Further investigation with a larger size is recommended.

Keywords: *Genotype, immune thrombocytopenic purpura, megakaryocyte, platelet endothelial aggregation receptor 1, platelet, polymorphisms*

Sahar Najafi[1,2], Mohammad Ali Jalali Far[1,2], Gholam Abbas Kaydani[2], Kaveh Jaseb[1], Najmaldin Saki[1,2]

[1]*Thalassemia and Hemoglobinopathy Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences,* [2]*Department of Clinical Laboratory Sciences, School of Allied Medical Sciences, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

Introduction

Immune thrombocytopenic purpura (ITP) is an acquired autoimmune bleeding disorder associated with platelet counts $<10 \times 10^9$/L without an underlying cause or any apparent illness.[1] The annual incidence of ITP in children and adults is 1.9–6.4 and 3.3 cases/1000 people, respectively.[2,3] ITP is divided into two categories based on the pathological mechanism, the causative agent, and the duration of the disease: acute and chronic.[4] ITP in children is acute and self-limiting and generally does not require treatment, but adult ITP is chronic and has an increasing course and needs treatment in most cases.[5,6] The pathophysiology of ITP is not yet fully understood. However, autoreactive antibodies against platelets and megakaryocytes, especially glycoproteins IIb/IIIa and Ib/IX, are known as the main causes of platelet destruction in the spleen

and disruption of bone marrow (BM) megakaryocyte maturation which leads to thrombocytopenia.[7,8] Examination of peripheral blood (PB) and BM of ITP patients shows macrothrombocytopenia and an increase in immature megakaryocytes, respectively.[9]

Genetic disorders are involved in the occurrence and pathogenesis of ITP. Single-nucleotide polymorphisms cause a series of changes in the human genome that eventually lead to several autoimmune disorders, including ITP.[10] Platelet endothelial aggregation receptor 1 (PEAR1) is a recently discovered gene involved in platelet function, homeostasis, and platelet counts.[11,12] PEAR1 is a type 1 receptor of the epidermal growth factor (EGF) family located on the surface of active and inactive platelets, alpha-platelet granules, endothelial cells, and megakaryocytes and plays a role in platelet aggregation.[13-15] PEAR1 contains 23 exons, 22 introns, 15 extracellular

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domains of the EGF family, and five intracellular domains rich in proline.[16] This receptor, through its extracellular part, causes platelet adhesion and platelet aggregation, consequently affecting megakaryopoiesis and neoangiogenesis.[17,18] When PEAR1 binds to its receptor through extracellular domains, the intracellular domains are phosphorylated by Src family kinases, triggering the PI3K/PTEN signaling pathway and stabilizing the fibrinogen receptor (α IIb β3), after which fibrinogen causes platelet aggregation.[11,17,19,20] PEAR1 also regulates the proliferation of megakaryocytic progenitors through this signaling.[17,21] Eptifibatide (antiplatelet drug and α IIb β3 antagonist) blocks PI3K/AKT/PTEN signaling and inhibits platelet aggregation.[22] PEAR1 polymorphisms can impair platelet activity through unknown mechanisms; for example, rs12041331 and rs12566888 are two significant intronic polymorphisms in PEAR1 associated with changes in platelet activity and megakaryocytic differentiation.[11,23] In a previous study, we theorized that PEAR1 polymorphisms might inhibit α IIb β3 activation, impair platelet aggregation, and the mechanism of coagulation drugs' action by inactivating the PI3K/AKT/PTEN pathway. As a result, platelet aggregation can be controlled using PEAR1 signaling inhibitors.[18] More than 15% of platelet function changes are affected by rs12041331, and rs125668888 is the only polymorphism in high linkage disequilibrium rs12041331in all PEAR1 gene region.[24]

Since the PEAR1 gene is a new subject for future studies, its effects on megakaryopoiesis,[11] platelet count,[12] and platelet aggregation[25] indicate that PEAR1 plays important roles in platelet-related activities. For the first time, the present study investigates the role of rs12566888 and rs12041331 polymorphisms in ITP by examining platelet parameters and their differences in acute and chronic ITP patients.

Materials and Methods

Selection of patients

In this study, 68 PB of patients with ITP (56 acute and 12 chronic) admitted to Baghaei 2 Hospital, Ahvaz, Iran, between 2017 and 2019 were collected [Figure 1]. Inclusion criteria for selecting patients were according to guidelines for the investigation and management of ITP, and thrombocytopenia was only due to having ITP, not other cases. The diagnosis of ITP was based on the following criteria: the presence of thrombocytopenia (platelet counts <100 × 109/L), patient's history, physical examination, the average concentration of hemoglobin, white blood cells counts, PB smear examination, a normal or increased number of megakaryocytes with normal myeloid, and erythroid progenitors in BM smears.[26] All patients received prednisolone and intravenous immunoglobulin. Patients with thrombocytopenia having similar conditions such as viral infections (including human immunodeficiency virus), hematologic disorders (myeloproliferative disorders,
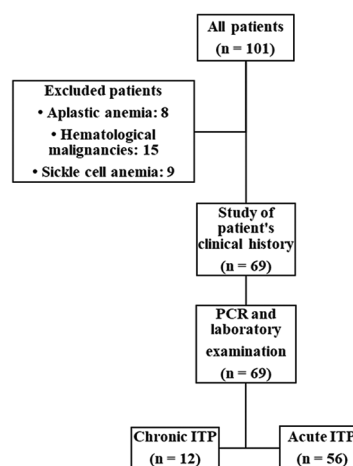
Figure 1: Flow diagram showing patient selection, excluded, and polymerase chain reaction and laboratory examination. Immune thrombocytopenic purpura indicates immune thrombocytopenia purpura

aplastic anemia), and systemic lupus erythematosus using BM aspiration assay were excluded. Additional data including age, symptoms, complete blood count (at time of diagnosis and after treatment), and BM smear were collected. The Medical Ethics Committee approved the study of Jundishapur Ahvaz University (IR.AJUMS. REC.1398.572). All participants signed informed consent.

Sample collection and DNA extraction

Two microliter PB from patients was collected into ethylenediaminetetraacetic acid (EDTA) (5%) anticoagulated tubes. DNA was extracted from blood samples using a kit (F. Hoffman-La Roche Ltd.) based on the manufacturer's protocol. Concentrations of all extracted DNAs were measured using a NanoDrop One Microvolume ultraviolet–visible spectrophotometer (Thermo Fisher, USA) with a concentration of 100–200 ng/μL and 1.8–2.0 ratio in 260/280 nm.

Polymerase chain reaction

rs12041331 and rs12566888 polymorphisms were detected by amplification refractory mutation system–polymerase chain reaction (ARMS-PCR). The primer sequences are shown in Table 1. FlexCycler thermocycler carried out the PCR reaction as following: 10.5 μL distilled water, 12 μL PCR master mix, 2 μL DNA, and 0.25 μL of each forward and reverse primers and H_2O up to a final volume of 25 μL. The PCR reaction was carried out at 95°C for 5 min (initial denaturation), 95°C for 45 s, followed by 30 cycles of 61°C for 45 s, 72°C for 60 s, and 72°C for the final extension for 3 min at 72°C.

The amplified DNA was separated by electrophoresis using a 2% agarose gel in × 1 tris-borate-EDTA buffer (PAYA PAZHOOHESH, Iran) to check for the presence or absence of PCR products. rs12566888 and rs12041331 produced the single band with 528-bp and 518-bp lengths, respectively.

Table 1: List of primers adopted for amplification refractory mutation system-polymerase chain reaction of rs12041331 and rs12566888

Polymorphisms	Primers	Sequence	Size of PCR product (bp)
rs12041331	Forward	5'-CGTTTTGGGGGATCAGGTTC-3'	518
	Reverse-N	5'-AAGCAGAGAGTAAGGGCGAC-3'	
	Reverse-M	5'-AAGCAGAGAGTGAGGGTGAT-3'	
rs12566888	Forward	5'-ATGGCTTTAGTGAGTGGATTGT-3'	528
	Reverse-N	5'-CTCTCTCTCTCATCACTTCCTGTC-3'	
	Reverse-M	5'-CTCTCTCTCTCATCACTTCCTGTA-3'	

PCR: Polymerase chain reaction, N: Normal, M: Mutant

DNA sequencing

Sanger sequencing was used for confirmation of PCR amplicons, and all the samples were sequenced using the following protocol: 5 µL forward primer, 5 µL reverse primer, 0.7 µL Big dye, 4 µL buffer, 3 µL H2O, and 5 µL formamide. ABI-3130XL sequenced the final reaction, and DNA analysis was performed using ABI PRISM 3130 × 1 DNA Analyzer (Applied Biosystems, Foster City, CA, USA) [Figure 2]. The result of the sequence data was visualized by UGENE software.

Statistical analysis

Frequency descriptive methods, including frequency distribution table, charts, central indices, and appropriate dispersion to describe the variables studied, were discussed. Kruskal–Wallis test was used to determine the correlation between PEAR1 variants (rs12041331and rs12566888) with platelet parameters. A Chi-square test was used to determine the relationship between qualitative variables between acute and chronic ITP groups. The significance P value was considered <0.05, and the odds ratio with a 95% confidence interval (95% confidence interval) was calculated. All the statistical analyses were processed by SPSS software (statistical package, version 24) IBM Corporation, Armonk, NY, USA.

Results

Prevalence of rs12041331 and rs12566888 polymorphisms in acute and chronic groups

According to age and disease pathogenesis, patients were divided into acute and chronic groups (56 acute and 12 chronic). Clinical information and laboratory data of the patients are shown in Table 2. The results showed no statistically significant difference between the mean frequency of rs12041331 and rs12566888 genotypes in both the groups (P = 0.778, 0.844, respectively) [Table 3].
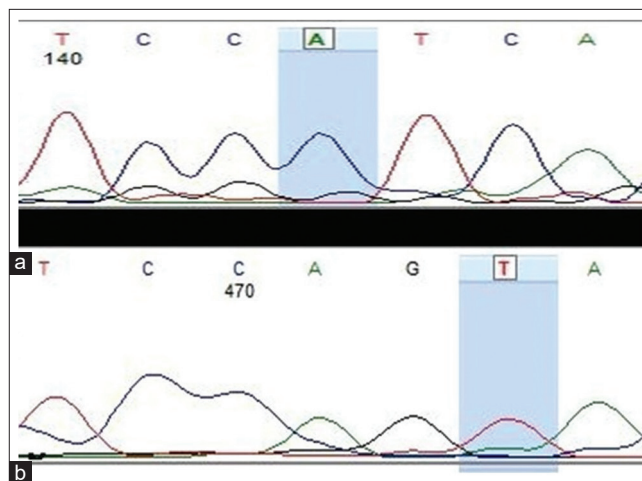
rs12041331

Of the total patients, 24 patients (20 acute, 4 chronic) were carriers for heterozygotes genotype (AG) and 2 acute patients had homozygous genotype (AA). The AG genotype was more in acute ITP patients (35.7%) compared with the chronic group (33.3%), while the GG

Table 2: Clinical presentations and laboratory data of patients

Characteristics	Patients with ITP (*n*=68), mean±SD	
	Chronic (*n*=12)	Acute (*n*=56)
Sex, *n* (%)		
Male	1 (8.3)	23 (41.1)
Female	11 (91.7)	33 (58.9)
Age (range)	28.92±11.704 (17-71)	8.34±12.493 (1-12)
Platelet (10³/µl)	40.250±9.3820	25.732±18.2024
WBC (×10⁹/L)	9.225±4.4108	9.341±3.4771
RBC (×10³/L)	4.2342±0.79085	4.2863±0.68128
Hb (g/dL)	11.767±2.5443	11.155±1.6902
HCT (%)	35.950±8.0870	33.588±5.0788
MCV (fL)	84.4350±5.58630	78.7179±6.46160
MCH (pg)	28.1950±3.19528	26.5814±2.97155
MCHC (g/dL)	32.6525±2.62838	33.0163±1.84761

ITP: Immune thrombocytopenia purpura, WBC: White blood cell, RBC: Red blood cell, Hb: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: MCH concentration, SD: Standard deviation

Figure 2: Evaluation of rs12041331 and rs12566888 polymorphisms by direct sequencing technique in patients with immune thrombocytopenia. (a) rs12041331. (b) rs12566888

allele in the chronic group (66.75%) was higher than acute (60.7%). Homozygous AA genotype only detected in acute patients (3.6%) [Figure 3].

rs12566888

Twenty-one patients (18 acute, 3 chronic) were carriers for

heterozygotes genotype (GT) and 4 (3 acute, 1 chronic) had a homozygous genotype (TT). The GT genotypes were more frequent in acute ITP patients (32.1%) than chronic patients (25%). In comparison, TT (8.3%) and GG (66.7%) genotypes' frequencies were more in the chronic group than acute group (5.4% and 62.5% 0, although statistically significant differences were not observed [Table 3 and Figure 4].

The association between rs12041331 and rs12566888 polymorphisms with platelet-related parameters in patients

The results of our evaluation showed no statistically significant relationship between rs12041331 and rs12566888 with platelet parameters (platelet count, mean platelet volume [MPV], and platelet distribution width [PDW]) [Table 4].

rs12041331

The AG genotype (31.5 ± 20.97) without a significant correlation showed a higher average platelet count compared with the AA genotype (13.5 ± 9.19) ($P = 0.293$). MPV values between alleles of rs12041331 were constant and did not differ much ($P = 0.962$). The PDW in individuals carrying the AG (17.3 ± 1.46) was higher than in carriers for AA (16.35 ± 0.49), without a statistically significant difference between genotypes and PDW ($P = 0.139$).

rs12566888

Platelet count was higher in the presence of TT genotype (29.25 ± 19.38) than in GT (29.14 ± 20.12) ($P = 0.958$). MPV values between GT and TT were constant and did not differ much ($P = 0.996$). The PDW in individuals carrying the GT (17.24 ± 1.25) was higher than in carriers for TT (16.57 ± 0.68), without a statistically significant difference between genotypes and PDW ($P = 0.188$).

Discussion

Various factors such as genetic and molecular interactions can contribute to the incidence of ITP.[27] The presence of polymorphisms in the genes encoding factors related to platelet aggregation, megakaryocyte differentiation, and platelet count can contribute to bleeding in ITP patients.[12,28] PEAR1 is a known gene involved in the proliferation of megakaryocytes and thrombopoiesis by expressing a transmembrane protein on megakaryocytes' surface.[29,30] PI3K/AKT/PTEN is the signaling pathway involved in PEAR1 operation. In 2013, Kauskot *et al.* reported that removing PEAR1 increases the level of AKT and enhances the proliferation of megakaryocyte precursor cells. They also stated that PEAR1 is upregulated during megakaryopoiesis to inhibit megakaryocyte proliferation but does not affect megakaryocyte maturation.[17] Izzi *et al.*, by analyzing the differentiation process of megakaryocytes and examining the megakaryocyte markers, showed that the expression of PEAR1, unlike CD41a, reaches its maximum expression during differentiation. They also concluded that methylation of rs12041331 is associated with

Table 3: Frequency of platelet endothelial aggregation receptor 1 polymorphisms genotypes in patients with acute and chronic immune thrombocytopenic purpura

PEAR1 polymorphisms	Chronic ITP (*n*=12), *n* (%)	Acute ITP (*n*=56), *n* (%)	*P*
rs12041331 genotype frequency			
AG	4 (33.3)	20 (35.7)	0.778
AA	0	2 (3.6)	
GG	8 (66.7)	34 (60.7)	
rs12566888 genotype frequency			
GT	3 (25)	18 (32.1)	0.844
TT	1 (8.3)	3 (5.4)	
GG	8 (66.7)	35 (62.5)	

PEAR: Platelet endothelial aggregation receptor, ITP: Immune thrombocytopenic purpura

Table 4: Genotypes of rs12041331 and rs12566888 associations with platelet counts, mean platelet volume, and platelet distribution width

| PEAR1 polymorphisms	Platelet, mean±SD	*P*	MPV, mean±SD	*P*	PDW, mean±SD	*P*
rs12041331 genotype frequency						
AG	31.5±20.97	0.293	13.27±1.63	0.962	17.3±1.46	0.139
AA	13.5±9.19		13.3±3.818		16.35±0.49	
GG	27.16±15.87		13.33±2.048		16.18±3.05	
rs12566888 genotype frequency						
GT	29.14±20.12	0.958	13.37±1.66	0.996	17.24±1.25	0.188
TT	29.25±19.38		13.32±2.22		16.57±0.68	
GG	27.79±16.91		13.28±2.05		16.29±3.07	

MPV: Mean Platelet volume, PDW: Platelet distribution width, SD: Standard deviation, PEAR: Platelet endothelial aggregation receptor

Figure 3: Evaluation of rs12041331 polymorphism by amplification refractory mutation system–polymerase chain reaction products on the agarose gel. In this Figure, both wells belong to 1 specimen. In both wells, forward primers are the same, but their reverse primers are different. In addition, genotype can be diagnosed according to the band and the well: if the band appears in both wells, the genotype is AG; if the band appears in the forward well of the genotype, the genotype is wild genotype GG. If the band appears in the mutated forward well, the genotype is AA

Figure 4: Evaluation of rs12566888 polymorphism by amplification refractory mutation system–polymerase chain reaction products on the agarose gel. In this Figure, both wells belong to 1 specimen. In both wells, forward primers are the same, but their reverse primers are different. In addition, genotype can be diagnosed according to the band and the well: if the band appears in both wells, the genotype is GT; if the band appears in the forward well of the genotype, the genotype is wild genotype GG. If the band appears in the mutated forward well, the genotype is TT

increased PEAR1 expression.[11] Based on these findings, we can conclude that increasing PEAR1 expression is not beneficial for ITP patients because it prevents megakaryocyte proliferation and subsequently decreases platelet production, but low regulation of PEAR1 can increase platelet production. We suggest that future studies investigate platelet and megakaryocyte activity in ITP samples by knockout PEAR1 or stimulating its expression.

The effect of rs12041331 and rs12566888 polymorphisms on PEAR1 expression and platelet function is unknown. Our study is the first research to examine the relationship between two PEAR1 polymorphisms and ITP disease. This study evaluated the relationship between rs12041331 and rs12566888 in the PEAR1 gene with platelet parameters (platelet counts, PDW, and MPV) and the investigation's prevalence of two polymorphism genotypes in acute and chronic ITP patients. No significant relationship was found in this study, but the prevalence of both the polymorphism genotypes in the acute group was higher than in the chronic group. In general, the frequency of rs12566888 in two groups of patients was higher than rs12041331. The highest frequency in both the polymorphisms was related to the GG allele, and the lowest frequency was related to the AA rs12041331 and TT rs12566888. Eicher *et al.* found that the GG rs12041331 was associated with increased PEAR1 expression, and the AA allele in this polymorphism was associated with decreased PEAR1 expression in human's platelets.[12] These findings suggest that rs12041331 genotypes may be associated with ITP. This hypothesis can be raised that the GG presence, unlike the AA, is associated with increased PEAR1 expression and decreased megakaryocyte differentiation. Findings have shown that polymorphisms in the PEAR1 gene can alter platelet activity and related parameters. Faraday *et al.* revealed the association between rs12041331 with platelet aggregation and PEAR1 protein expression on platelets.[24] In this regard, Würtz *et al.*
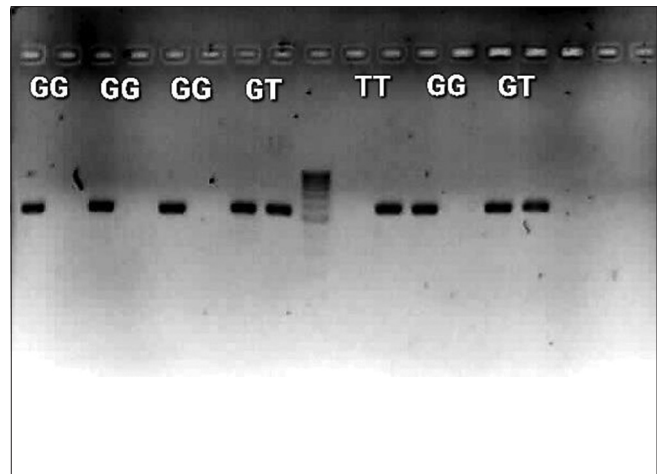
with evaluated patients with coronary artery disease showed that the AA allele of rs12041331 reduced platelet aggregation and increased platelet activation compared to the GG allele.[31] Johnson *et al.*, with a genome-wide study, showed that AA rs12041331 and TT rs12566888 to be associated with decreased platelet aggregation.[25] AA rs12041331 seems to be related to platelet activity. According to these studies, this genotype's presence may reduce the function of the coagulation system in ITP patients and increase bleeding in them by reducing platelet aggregation. Yao *et al.*, with the evaluation of adenosine diphosphate (ADP)-induced platelet aggregation, concluded that PEAR1 genetic variations were strongly associated with ADP-induced platelet aggregation and rs12566888 was significantly associated with platelet reactivity.[32]

Eicher *et al.*, with a large-scale meta-analysis, found that PEAR1 was not associated with platelet counts and MPV.[12] In the present study, by examining the rs12041331 and rs12566888 genotypes, it was found that without any significant relation, the platelet count in the AG rs12041331 and TT rs12566888 was higher than the other alleles. Like the Eicher study result, MPV values between alleles of both polymorphisms were constant and did not differ much. Our study data showed that PEAR1 was not associated with MPV. Izzi *et al.*, by measuring PEAR1 methylation, concluded that PEAR1 methylation with PDW is positively associated significantly. They did not find any association between PEAR1 (methylation) and platelet counts.[29] In our study, the allele AG in polymorphism rs12041331 and the GT in rs12566888 were associated with the highest amount of PDW.

Conclusions

Given that PEAR1 is expressed on two cells involved in the pathogenesis of ITP (platelets and megakaryocytes), it seems that genetic disorders in PEAR1 may be involved in the etiology of ITP. We did not find a significant relationship between rs12041331 and rs12566888 genotypes in the group of acute and chronic ITP, platelet count, MPV, and PDW for the first time. However, the higher prevalence of rs12041331 and rs12566888 in acute patients than chronic can indicate the role of PEAR1 in acute ITP. Therefore, it is possible that studies in other races or more specimens can lead to effective results.

Conflicts of interest

There are no conflicts of interest.

References

1. Terrell DR, Beebe LA, Vesely SK, Neas BR, Segal JB, George JN. The incidence of immune thrombocytopenic purpura in children and adults: A critical review of published reports. Am J Hematol 2010;85:174-80.

2. Rodeghiero F, Stasi R, Gernsheimer T, Michel M, Provan D, Arnold DM, *et al*. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: Report from an international working group. Blood 2009;113:2386-93.

3. Kühne T, Berchtold W, Michaels LA, Wu R, Donato H, Espina B, *et al*. Newly diagnosed immune thrombocytopenia in children and adults: A comparative prospective observational registry of the Intercontinental Cooperative Immune Thrombocytopenia Study Group. Haematologica 2011;96:1831-7.

4. Berchtold P, McMillan R, Tani P, Sommerville-Nielsen S, Blanchette VS. Autoantibodies against platelet membrane glycoproteins in children with acute and chronic immune thrombocytopenic purpura. Blood 1989;74:1600-2.

5. Imbach P, Kühne T, Müller D, Berchtold W, Zimmerman S, Elalfy M, *et al*. Childhood ITP: 12 months follow-up data from the prospective registry I of the Intercontinental Childhood ITP Study Group (ICIS). Pediatr Blood Cancer 2006;46:351-6.

6. Rosthøj S, Hedlund-Treutiger I, Rajantie J, Zeller B, Jonsson OG, Elinder G, *et al*. Duration and morbidity of newly diagnosed idiopathic thrombocytopenic purpura in children: A prospective Nordic study of an unselected cohort. J Pediatr 2003;143:302-7.

7. Provan D, Stasi R, Newland AC, Blanchette VS, Bolton-Maggs P, Bussel JB, *et al*. International consensus report on the investigation and management of primary immune thrombocytopenia. Blood 2010;115:168-86.

8. Mohammadi MB, Kaydani GA, Asnafi AA, Saki N. The correlation between overexpression of GATA-1 variants valine 205 methionine and R216Q with occurrence, prognosis, and therapeutic approaches for immune thrombocytopenic purpura. Clin Cancer Investig J 2019;8:241-6.

9. Vinayakamurthy S, Potluri R, Shivajirao P, Singh R, Pujahari R, Maniketh I. A study of megakaryocyte morphology in bone marrow aspiration smears of cases of thrombocytopenia. Med J DY Patil Univ 2017;10:51-7.

10. Aref S, El-Ghonemy MS, El-Aziz SA, Abouzeid T, Talaab M, El-Sabbagh A. Impact of serum immunoglobulins level and IL-18 promoter gene polymorphism among Egyptian patients with idiopathic thrombocytopenic purpura. Hematology 2017;22:99-104.

11. Izzi B, Pistoni M, Cludts K, Akkor P, Lambrechts D, Verfaillie C, *et al*. Allele-specific DNA methylation reinforces PEAR1 enhancer activity. Blood 2016;128:1003-12.

12. Eicher JD, Chami N, Kacprowski T, Nomura A, Chen MH, Yanek LR, *et al*. Platelet-Related Variants Identified by Exomechip Meta-analysis in 157,293 Individuals. Am J Hum Genet 2016;99:40-55.

13. Nanda N, Bao M, Lin H, Clauser K, Komuves L, Quertermous T, *et al*. Platelet endothelial aggregation receptor 1 (PEAR1), a novel epidermal growth factor repeat-containing transmembrane receptor, participates in platelet contact-induced activation. J Biol Chem 2005;280:24680-9.

14. Fisch AS, Yerges-Armstrong LM, Backman JD, Wang H, Donnelly P, Ryan KA, *et al*. Genetic variation in the platelet endothelial aggregation receptor 1 gene results in endothelial dysfunction. PLoS One 2015;10:e0138795.

15. Kardeby C, Damaskinaki FN, Sun Y, Watson SP. Is the endogenous ligand for PEAR1 a proteoglycan: clues from the sea. Platelets 2020:1-7.

16. Keramati AR, Yanek LR, Iyer K, Taub MA, Ruczinski I, Becker DM, *et al*. Targeted deep sequencing of the PEAR1 locus for platelet aggregation in European and African American families. Platelets 2019;30:380-6.

17. Kauskot A, Vandenbriele C, Louwette S, Gijsbers R, Tousseyn T, Freson K, *et al*. PEAR1 attenuates megakaryopoiesis via control of the PI3K/PTEN pathway. Blood 2013;121:5208-17.

18. Ansari N, Najafi S, Shahrabi S, Saki N. PEAR1 polymorphisms as a prognostic factor in hemostasis and cardiovascular diseases. J Thromb Thrombolysis 2021;51:89-95.

19. Kauskot A, Di Michele M, Loyen S, Freson K, Verhamme P, Hoylaerts MF. A novel mechanism of sustained platelet αIIbβ3 activation via PEAR1. Blood, The Journal of the American Society of Hematology 2012;119:4056-65.

20. Getz TM, Manne BK, Buitrago L, Mao Y, Kunapuli SP. Dextran sulphate induces fibrinogen receptor activation through a novel Syk-independent PI-3 kinase-mediated tyrosine kinase pathway in platelets. Thromb Haemost 2013;109:1131-40.

21. Criel M, Izzi B, Vandenbriele C, Liesenborghs L, Kerckhoven SV, Lox M. Absence of Pear1 does not affect murine platelet function *in vivo*. Thromb Res 2016;146:76-83.

22. Nie XY, Li JL, Qin SB, Fu Y, Liang GK, Shi LW, *et al*. Genetic mutations in PEAR1 associated with cardiovascular outcomes in Chinese patients with acute coronary syndrome. Thromb Res 2018;163:77-82.

23. Johnson AD. Pairing megakaryopoiesis methylation with PEAR1. Blood 2016;128:890-2.

24. Faraday N, Yanek LR, Yang XP, Mathias R, Herrera-Galeano JE, Suktitipat B, *et al*. Identification of a specific intronic PEAR1

gene variant associated with greater platelet aggregability and protein expression. Blood 2011;118:3367-75.

25. Johnson AD, Yanek LR, Chen MH, Faraday N, Larson MG, Tofler G, *et al*. Genome-wide meta-analyses identifies seven loci associated with platelet aggregation in response to agonists. Nat Genet 2010;42:608-13.

26. Neunert C, Lim W, Crowther M, Cohen A, Solberg L Jr., Crowther MA, *et al*. The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia. Blood 2011;117:4190-207.

27. Rezaeeyan H, Jaseb K, Kaydani GA, Asnafi AA, Masbi MH, Saki N, *et al*. Involvement of interferon-γ+874A/T polymorphism in the pathogenesis of and therapeutic response to immune thrombocytopenia. Lab Med 2019;50:e42-9.

28. Herrera-Galeano JE, Becker DM, Wilson AF, Yanek LR, Bray P, Vaidya D, *et al*. A novel variant in the platelet endothelial aggregation receptor-1 gene is associated with increased platelet aggregability. Arterioscler Thromb Vasc Biol 2008;28:1484-90.

29. Izzi B, Noro F, Cludts K, Freson K, Hoylaerts MF. Cell-specific PEAR1 methylation studies reveal a locus that coordinates expression of multiple genes. International journal of molecular sciences 2018;19:1069.

30. Vandenbriele C, Kauskot A, Vandersmissen I, Criel M, Geenens R, Craps S, *et al*. Platelet endothelial aggregation receptor-1: A novel modifier of neoangiogenesis. Cardiovasc Res 2015;108:124-38.

31. Würtz M, Nissen PH, Grove EL, Kristensen SD, Hvas AM. Genetic determinants of on-aspirin platelet reactivity: Focus on the influence of PEAR1. PLoS One 2014;9:e111816.

32. Yao Y, Tang XF, Zhang JH, He C, Ma YL, Xu JJ, *et al*. Association of PEAR1 genetic variants with platelet reactivity in response to dual antiplatelet therapy with aspirin and clopidogrel in the Chinese patient population after percutaneous coronary intervention. Thromb Res 2016;141:28-34.