



Assessment of IL-17F rs763780 gene polymorphism in immune thrombocytopenia



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ABSTRACT

Interleukin-17F rs763780 (7488A/G) gene polymorphism obviously affecting the expression and activity of IL17F and may affect primary immune thrombocytopenia (PIT) susceptibility and its clinical features in Egyptian children and adults. 105 ITP patients divided into (63 pediatric and 42 adult patient) and 112 age and sex matched healthy controls were enrolled in this case control study. All patients were subjected to history taking; clinical examination, CBC, bone marrow aspiration and genotyping of IL17F rs763780 polymorphism by (PCR-RFLP) technique. Our results revealed significant decrease in the mutant heterozygous genotype AG and also in IL-17F mutant allele G frequency in ITP patient group and associated with increased risk for ITP compared with the control group ($P = 0.04$ and $P = 0.005$ respectively). Furthermore, the mutant allele G frequency was significantly decreased in childhood onset than adult onset ITP (OR = 0.31, $P = 0.02$) and also was significantly lower in chronic ITP when compared with newly diagnosed and persistent ITP ($P = 0.005$). Patients with the AA genotype showed severe thrombocytopenic state at diagnosis than those with the AG genotype ($P = 0.04$). We concluded from our results that interleukin-17F rs763780 (7488A/G) polymorphism is strongly correlated with susceptibility and severity of ITP.

1. Introduction

ITP is an autoimmune disease characterized by immune mediated platelet destruction in the periphery [1]. In children it is usually benign and self-limiting disease [2]. The incidence of ITP is $\cong 2-12/100,000$ per year for adults and children respectively, and its mortality rate is 1–3% per year in severely affected cases [3,4].

ITP pathogenesis is heterogeneous and very complicated [1]. The most common mechanism involves auto-reactive B cells producing autoantibodies targeting glycoproteins (GP such as GPIIb/IIIa, GPIb/IX and/or GPIa/IIa) expressed on platelet membrane [5,6]. Subsequently, autoantibody-coated platelets are cleared by splenic macrophages in Fc γ R-dependent mechanism [7].

T-cells also, play a crucial role via mediating peripheral platelet destruction and megakaryocyte destruction/inhibition in the bone marrow [8]. Previous studies focused on the role of auto-reactive CD4+Th cell and its response against platelet GPIIb-IIIa antigen by helping B cells to produce autoantibodies [9,10], and also, through increasing cytokine imbalance, especially in ITP patients with some evidence of higher levels of circulating pro-inflammatory cytokines [11–13].

CD4+Th cells subsets in ITP patients are perturbed, such as decreased number and function of Treg [14]. However, some groups reported up-regulation of Th17 cells in ITP patients [15–18]. It was noted that Treg cells play a fundamental role in preventing autoimmune diseases by maintenance of immune tolerance; in the contrary Th17

Abbreviations: BMA, bone marrow aspiration; CBC, complete blood count; CD4+Th, CD4+ T helper; CI, Confidence Interval; CR, complete response; FC γ R, Fc gamma receptor; FET, Fisher exact test; GCs, glucocorticoids; GP, glycoproteins; IL-17F, interleukin 17F; ITP, immune thrombocytopenic purpura; NR, no response; OR, odd ratio; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; PIT, primary immune thrombocytopenia; R, response; S, significant; SD, standard deviation; SLE, systemic lupus erythematosus; SNPs, single nucleotide polymorphism; Th17, T helper 17; TNF- α , tumor necrosis factor- α ; Treg, T regulatory

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cells may play the opposite role. They mediate inflammation and tissue injury through secretion of IL-17 and other pro-inflammatory cytokines [19].

Interleukin 17F (IL-17F) is a potent pro-inflammatory cytokine, and is one of the IL-17 cytokine family which comprises six members: IL-17A to 17F. It is mainly produced by Th17 subset cells in addition to other types of cells [20]. Previous studies focused on the critical pro-inflammatory effect of IL-17F and its correlation with inflammatory and autoimmune diseases such as systemic lupus erythematosus [21] rheumatoid arthritis [22] and asthma [23]. The encoded gene for interleukin 17F is mapped to the human chromosome arm of 6p12.2, which consists of 3 exons and 2 introns [24]. IL-17F at rs763780 (7488A/G) polymorphism is located within the coding region of IL-17F gene and causes a His-to-Arg substitution at amino acid 161. In vitro functional analysis revealed that IL-17 expression and activity may be suppressed in carriers of the mutant G allele [25]. Our study has been conducted to analyze IL-17F polymorphism at rs763780 (7488A/G) among Egyptian ITP patients and healthy individuals to evaluate its contribution to ITP susceptibility and its correlation with clinical features and disease pathogenesis.

2. Material and methods

A case-control study conducted during the period from November 2016 to December 2017. The study included 217 participants; they were divided into 2 groups:

- (a) Patients Group: Included 105 Egyptian ITP patients (63 pediatric and 42 adult patients) attending the pediatric and internal medicine hematology outpatient clinic, Benha University Hospitals (65 women and 40 men; mean age 15, range 3–44 years). ITP diagnosis was established via history taking together with physical examination and laboratory investigations that met the ITP diagnostic criteria [26]. Primary immune thrombocytopenia was defined as thrombocytopenia (platelet count $< 100 \times 10^9/L$), normal or increased marrow megakaryocytes, and no secondary immune or non-immune abnormalities known to cause the thrombocytopenic state.
- (b) Control Group: Included 112 age and sex-matched healthy controls (64 women and 48 men; mean age 16, range 3–49 years).

Informed consent was obtained from the patients' legal guardians or the patients themselves, and this study was approved by local ethical committee of Benha University. The study conformed to the guidelines of the Helsinki Declaration. Exclusion criteria comprised secondary causes of PIT, such as systemic lupus erythematosus (SLE), infants with age < 6 months, manifestations of active infection and splenomegaly.

We recorded the age of ITP onset for each patient according to patient history. PIT diagnosis was established via history taking together with physical examination and laboratory investigations. Inclusion criteria were obtained for these patients and incorporated a Complete Blood Count (CBC) results that revealed an isolated thrombocytopenia ($100 \times 10^9/L$) without anemia or leucopenia and Bone Marrow Aspirate (BMA) that showed a megakaryocytic morphology compatible with the diagnosis of PIT. Furthermore; patient's history showed no concomitant autoimmune disorders, no medications known to cause thrombocytopenia, no associated viral disorders or suspicion of malignancy.

The severity of bleeding tendency and thrombocytopenia was assessed according to the previously described criteria [27]. Clinical and laboratory characteristics of ITP patients are shown in Tables 1 and 2.

Steroid treatment was given to 86 patients (81.9%) as a first line therapy while 19 patients achieved spontaneous remission of thrombocytopenia without any treatment, the routine usage of glucocorticoids (GCs) were methylprednisolone (0.8 mg/kg of body weight) or prednisone (1 mg/kg of body weight) for 2 to 3 months or

dexamethasone 40 mg/d for 4 consecutive days (for adult patients).

The response criteria were analyzed according to the criteria of the ITP International Working Group [26]. A complete response (CR) is defined as any platelet count of at least $100 \times 10^9/L$, and a response (R) was defined as any platelet count between 30 and $100 \times 10^9/L$ or at least doubling of the baseline count. No response (NR) was defined as any platelet count lower than $30 \times 10^9/L$ or less than doubling of the baseline count. The glucocorticoids NR patients received with immunosuppressive drugs such as Azathioprine as well as recombinant human thrombopoietin. None of our patients was splenectomized. All patients were followed up in the outpatient department for at least 12 months from diagnosis. Then, a further classification was done on our patients based on the duration of the disease. They were classified into newly diagnosed ITP if remission within 3 months, chronic ITP if course of the disease was > 12 months, and others were classified into persistent ITP as shown in Table 2.

2.1. Genomic DNA extraction and genotyping

Two milliliters of anti-coagulated peripheral venous blood were withdrawn under complete aseptic conditions from all ITP cases as well as controls for genomic DNA extraction, DNA extraction was done from peripheral blood leucocytes using the QUICK-gDNA MiniPrep Kit 50 preps (ZYMO RESEARCH) (Epigenetics COMPANY, USA) (Lot No: ZRC 184087) (Catalog NO: D3024&D3025) manufacturers' instructions were followed and extracted DNA samples were stored at $-20^\circ C$.

IL-17F + A/G genotyping was carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method using PCR thermal cycler (Piko-Real 24 Thermo Fisher Scientific, Finland). Amplification reaction was performed with 200 ng of genomic DNA in a 50- μ l PCR mixture using 10 pmol of each primer (Biosearch technologies, USA), the following pairs of primers were used: forward: 5'-GTG TAG GAA CTT GGG CTG CAT CAA T-3' and reverse: 3'-AGC TGG GAA TGC AAA CAA AC-3'. The PCR cycles conditions were as follows: $94^\circ C$ for 3 min; 35 cycles at $94^\circ C$ for 30 s, $60^\circ C$ for 30 s and $72^\circ C$ for 30 s; and a final elongation step at $72^\circ C$ for 7 min. The PCR products were visualized after electrophoresis in 2% agarose gel and ethidium bromide staining under UV light. The amplified PCR products were digested with the NlaIII restriction endonuclease (New England BioLabs Inc.) and analyzed in 2% agarose gel. Three patterns were observed following digestion and electrophoresis: NlaIII digestion of PCR product yielded 52, 130 and 288 bp for allele A, whereas for allele G 52 and 418 bp fragments were observed. Homozygous GG genotype: gives two bands at 418, 52 bp, homozygous AA genotype: gives three bands at 288, 130, 52 bp and heterozygous GA genotype: gives four bands at 418, 288, 130, 52 bp (Fig. 1).

2.2. Statistical methods

The statistical analysis was conducted using STATA version 11 (STATA corporation, College Station, Texas). The collected data were summarized in terms of mean \pm Standard Deviation (SD) and range for quantitative data and frequency and percentage for qualitative data. Comparisons between the different study groups were carried out using the Chi-square test (χ^2) and Fisher Exact Test (FET) to compare proportions as appropriate and the corresponding Odd Ratio (OR) and 95% Confidence Interval (CI) were estimated. The Student *t*-test (*t*) and the Mann-Whitney test were used to detect differences between two groups regarding parametric and non-parametric data respectively. The Kruskal-Wallis test was used to detect differences between more than two groups regarding non-parametric data. For genetic association analyses, both polymorphisms were tested for deviations from Hardy-Weinberg equilibrium using the HWE program. A *P*-value < 0.05 was considered statistically significant (S).

Table 1
Laboratory characteristics of the study subjects:

Variable	Cases (no. = 105)		Controls (no. = 112)		Mann-Whitney	P
	Mean ± SD	Range	Mean ± SD	Range		
Hb (g/dL)	11.43 ± 1.67	7.5–15.8	12.18 ± 1.28	8.9–16	3.42	< 0.001 (HS)
WBCs ($\times 10^9/L$)	8.78 ± 12.47	3–132	7.03 ± 2.04	3.8–15.2	1.23	0.22
PLTs ($\times 10^9/L$)	30.52 ± 21.53	4–79	238.71 ± 62.79	132–436	12.72	< 0.001 (HS)

HS: highly significant.

Table 2
Clinical parameters of Idiopathic Thrombocytopenic Purpura (ITP) patients.

Variable	Cases (no. = 105)		
	No.	%	
Age of onset (years)	Childhood (< 18 y)	63	60
	Adult (> 18 y)	42	40
Severity of ITP according to platelets ($\times 10^9/L$)	Mild (platelets count 50–100 $\times 10^9/L$)	30	28.57
	Moderate (platelets count 30–50 $\times 10^9/L$)	20	19.05
	Severe (platelets count 10–30 $\times 10^9/L$)	29	27.62
	v. severe (platelets count < 10 $\times 10^9/L$)	26	24.76
Type of bleeding	Asymptomatic	28	26.67
	Petechiae & ecchymosis	30	28.57
	Epistaxis	47	44.76
Treatment with GCs	No	19	18.1
	Yes	86	81.9
Response to GCs therapy (no. = 86)	NR	4	4.65
	R	34	39.53
	CR	48	55.81
Immunosuppressive drugs + thrombopoietin treated patients	4	4.65	
Splenectomised patients Course	Newly diagnosed	0	0
	Persistent	31	29.52
	Chronic	47	44.76

GCs: glucocorticoids; ITP: primary immune thrombocytopenia.

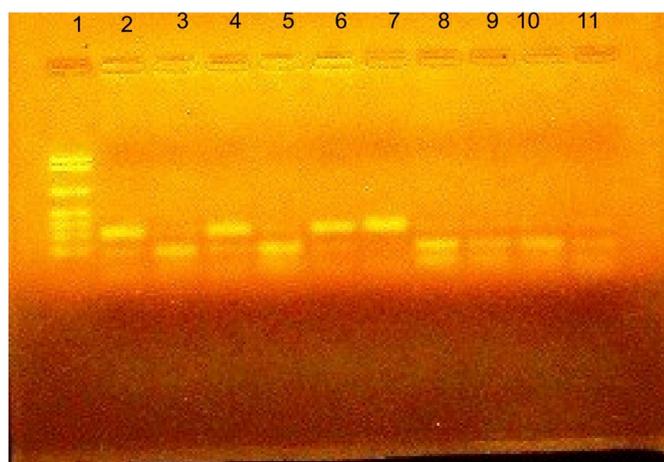


Fig. 1. Genotyping of the *IL-17F* rs763780 polymorphism.
 • Lane 1: shows DNA Ladder 100 bp (SiZer™-100 INtRON Biotechnology, cat. No. 24073).
 • Lanes 2, 4, 6, show heterozygous GA genotype: bands of (418, 288, 130, 52 bp).
 • Lanes 3, 5, 8, 9, 10, 11 show homozygous AA genotype: bands of (288, 130, 52 bp).
 • Lane 7 show homozygous GG genotype: 1 band of (418, 52 bp).

3. Results

105 Egyptian patients diagnosed with primary immune thrombocytopenia were enrolled in this study. In addition to 112 age and sex matched healthy volunteers as a control group.

The distribution of *IL-17F* rs763780 genotype and allele frequencies in ITP patients and control group are shown in [Table 3](#). The mutant genotype AG was significantly lower in total ITP patients when compared with control group, while the homozygous (GG) mutant genotype was detected only in the control group, the frequency of the G allele was significantly lower among ITP patients than the control group, and also, its frequency was significantly lower in childhood onset ITP than in adulthood onset ITP. There was no statistically significant differences were detected between the male and female ITP patients as shown in [Table 4](#).

Regarding the association between the *IL-17F* 7488 AA/AG genotypes and the clinical features of ITP, patients with the AA genotype showed very severe and severe thrombocytopenic state at diagnosis than those with the AG genotype with a significant *P* value (*P* = 0.04) ([Table S1](#)). In addition, patients with AA genotype were presented with bleeding symptoms at diagnosis than those with the AG genotype with a significant *P* value (*P* = 0.007) ([Table S2](#)).

As regard treatment response, no significant associations were found between AA/AG genotype distribution or allele frequency and the response to glucocorticoids, (*P* = 0.29) ([Table 5](#)).

When patients were divided according to disease course into newly diagnosed, persistent and chronic, we found that chronic ITP patients had a significantly higher frequency of the *IL-17F* rs763780 AA genotype (*P* = 0.004), and also significantly lower mutant G allele frequency (*P* = 0.005) compared to newly diagnosed + persistent group ([Table 6](#)).

4. Discussion

IL17F is one of *IL17* cytokine family produced mainly by T helper 17 subset cells, the hallmark of the Th17 subset is the production of interleukin *IL-17A* and *IL-17F*, which share strong homology, and responsible of its pathogenic activity [20]. *IL-17* can induce the production of pro-inflammatory cytokines and recruit neutrophils and monocytes, together resulting in an immune-mediated inflammatory reaction through the expression of *IL-17* receptors [28].

Emerging evidence supports that the polymorphisms located within genes coding for *IL-17F* appeared to play a role in susceptibility to various autoimmune and inflammatory diseases such as inflammatory bowel disease [29], asthma [25,30] and rheumatoid arthritis [31,32].

Immune thrombocytopenia is an autoimmune and inflammatory disease characterized by immune-mediated platelet destruction. Although ITP has been associated with inflammation, only a few studies have identified the association between inflammation-related SNPs and the pathogenesis of ITP [33]. The (rs763780) at 7488 A/G *IL-17F* SNP localized in exon 3, this polymorphism causes a His-to-Arg substitution at amino acid 161 (H161R) and is also change amino acid in the protein sequence it can alter the conformation or the expression level of the

Table 3
Genotype and allele frequencies of IL-17F polymorphism in ITP patients and control group.

Genotype	Cases (No. = 105)		Childhood onset ITP (No. = 63)		Adult onset ITP (No. = 42)		Controls (No. = 112)		P ^{aa}	OR (95% CI)	P ^{ba}	OR (95% CI)
	No.	%	No.	%	No.	%	No.	%				
AA	96	91.43	58	92.06	38	90.48	89	79.46	0.02 (S)	1.00	0.10	1.00
AG	9	8.57	5	7.94	4	9.52	20	17.86	0.04 (S)	0.42 (0.181 to 0.964)	0.06	0.38 (0.11 to 1.14)
GG	0	0.0	0	0.0	0	0.0	3	2.68	0.11	0	0.28	0
AG + GG	9	8.57	5	7.94	4	9.52	23	20.53	0.01 (S)	0.36 (0.14 to 0.87)	0.03	0.33 (0.09 to 0.97)
A allele	201/210	95.71	121/126	96.03	80/84	95.24	198/224	88.39	0.005	0.34 (0.14 to 0.78)	0.02	0.31 (0.09 to 0.86)
G allele	9/210	4.28	5/126	3.97	4/84	4.76	26/224	11.61	(S)		(S)	

CI, confidence interval.

OR, odd ratio.

P^a: cases vs. controls.

P^b: childhood onset ITP vs. adulthood onset ITP.

S; significant differences (P < 0.05).

^a Chi-square test and Fisher exact test as appropriate.

protein [25].

Our study interrogated the associations between (rs763780) at 7488 A/G IL-17F gene polymorphisms and primary immune thrombocytopenia susceptibility and its role in the disease pathogenesis.

The results of this study showed that the rare homozygous mutant (GG) genotype was only detected in control group, the heterozygous mutant genotype (AG), also the G allele frequency were more frequently observed in healthy control group more than ITP patients group with a significant P value, suggested that the mutant allele G of the IL-17F His161Arg polymorphism is inversely associated with development of ITP and have a protective effect, our results are in consistent with a study performed by Li et al. [34] in Chinese Han population and revealed also that the frequency of mutant allele G was significantly lower in ITP patients than in normal controls (total ITP 3.6% vs controls 7.7%, P = 0.026), although the difference of genotype frequency between patients and normal controls did not reach statistic difference, another previous research performed by Saitoh et al. [35] highlighted the role of IL17F (rs763780) at 7488 SNP on susceptibility of chronic ITP and found that the mutant genotype was associated with decrease risk of development of chronic ITP.

Our results revealed no differences were detected in genotypes or allele frequency between male and female ITP patients; this is consistent with Li et al. [34] who found also no differences in genotype frequency between male and female ITP patients.

In our results a significantly lower number of G allele was found in childhood onset ITP than in adulthood onset, while in the study

Table 4
Genotype and Allele frequencies of IL-17F polymorphism in male and female patients with ITP and normal control.

Genotype	Cases				Controls				P	OR (95% CI)
	Male (No. = 40)		Female (No. = 65)		Male (No. = 48)		Female (No. = 64)			
	No.	%	No.	%	No.	%	No.	%		
AA	37	92.5	59	90.77	38	79.17	51	79.69	1.00	0.8 (0.12 to 4.02)
AG	3	7.5	6	9.23	9	18.75	11	17.19		
GG	0	0.0	0	0.0	1	2.08	2	3.13		
AG + GG	3	7.5	6	9.23	10	20.83	13	20.32	1.00	0.8 (0.12 to 4.02)
A allele	77/80	96.25	124/130	95.38	85/96	88.54	113/128	88.28	1.00	0.8 (0.13 to 3.9)
G allele	3/80	3.75	6/130	4.62	11/96	11.46	15/128	11.72		

*Fisher exact test.

CI, confidence interval.

OR, odd ratio.

P: male ITP vs. female ITP.

Table 5
Genotype and Allele frequencies of IL-17F polymorphism association with treatment response in ITP patients:

Genotype	NR (no. = 4)		Response + complete response (no. = 82)		P ^a	OR (95% CI)
	No.	%	No.	%		
AA	3	75.0	76	92.68	0.29	4.22 (0.07 to 61.68)
AG	1	25.0	6	7.32		
A allele	7/8	87.5	158/164	96.34	0.29	3.76 (0.07 to 38.33)
G allele	1/8	12.5	6/164	3.6		

CI, confidence interval.

OR, odd ratio.

^a Fisher exact test.

performed by Li et al. [34] didn't show a significant difference.

As regard the disease course, our results revealed that all patients developed chronic ITP had AA wild genotype and the frequency of the G mutant allele was significantly lower in chronic ITP when compared with newly diagnosed and persistent ITP groups, this is in agreement with Liu et al. [35] who demonstrated in his study that the frequency of G mutant allele was decreased in patients with chronic ITP, and also, with a study done by Saitoh et al. [36] showed that the frequency of the IL-17F 7488 mutant Arg161 genotype was significantly lower among the chronic ITP patient group in comparison to the control group (0% vs. 4.8%, P < 0.05). This inferred that IL-17F 7488 mutant genotype

Table 6
Genotypes and Allele frequencies of IL-17F polymorphism association with course of ITP.

Genotype	Newly diagnosed + persistent (no. = 58)		Chronic (no. = 47)		P
	No.	%	No.	%	
AA	49	84.48	47	100.0	0.004
AG	9	15.52	0	0.0	(S)
A allele	107/116	92.24	94/94	100.0	0.005
G allele	9/116	7.76	0	0.0	(S)

P; newly diagnosed + persistent vs. chronic.

S: significant differences ($P < 0.05$).

has less ability to potentiate an immunologic reaction in the development of chronic ITP.

Regarding severity and clinical presentation, we found that patients with the IL-17F rs763780 wild AA genotype had a very severe and severe thrombocytopenic state and a bleeding manifestations at diagnosis than those with the IL-17F rs763780 AG genotype and this is in consistent with the results of a study performed by Saitoh et al. [36] in chronic ITP who found that the ITP patients had IL-17F rs763780 homozygous wild genotype was associated with severe thrombocytopenic state and bleeding tendency at diagnosis than mutant heterozygous genotype.

However, our results revealed no significant association between IL-17F polymorphism and GCs responses and this is also in agreement with Li et al. [34] who found the same results.

Obviously, these results should be confirmed in a more extended study, including larger cohorts and different ethnic populations in order to validate the role of this SNP as molecular contributor to the addressed pathology. A relatively small size of patients cohort analyzed in the present study constitutes the most important limitation of our work. It would also be of interest to relate the results of the polymorphism studies with the expression and serum concentration of IL-17F and other pro-inflammatory cytokines, such as TNF- α .

5. Conclusion

In conclusion, the results of our study strongly suggest that the (rs763780) SNP within the IL-17F genes might contribute to the susceptibility of ITP development and might affect the severity and the disease course, yet it has to be stated that further prospective studies with larger sample size and diverse populations are needed to validate the association between IL-17F (rs763780) SNP and the development of ITP disease.

Declaration of interest statement

The authors report no declarations of interest.

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Statement of contribution

- 1- Amira MN Abdelrahman: contributed to the design and implementation of the research, performed the analytical method, interpreted the data and discussed the results, design tables and figures, took the lead in writing the manuscript, and contributed to the final manuscript.
- 2- Fetnat Mahmoud Tolba: contributed to the design and implementation of the research, provided critical feedback and helped

shape the research, supervised the findings of this work, discussed the results and contributed to the final manuscript.

- 3- Safia Mohamed Diab: was involved in planning and supervised the findings of this work, discussed the results and contributed to the final manuscript.
- 4- Ola Galal Behairy: aided in choosing the patients appropriate for the study and data collection, aided in interpreting the results and contributed to the final manuscript.
- 5- Eman Rateb Abd Almonaem: aided in choosing the patients appropriate for the study and data collection, discussed the results and contributed to the final manuscript.
- 6- Mysara M Mogahed: aided in choosing the patients appropriate for the study and data collection, discussed the results and contributed to the final manuscript.
- 7- Shereen Abdel-sadek Mohamed: aided in data collection, contributed to sample preparation discussed the results and contributed to the final manuscript.

All authors give final approval of the version to be submitted and any revised version.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bcmd.2018.12.001>.

References

- [1] R. Stasi, M.L. Evangelista, E. Stipa, F. Buccisano, A. Venditti, S. Amadori, Idiopathic thrombocytopenic purpura: current concepts in pathophysiology and management, *Thromb. Haemost.* 99 (2008) 4–13.
- [2] D. Provan, R. Stasi, A.C. Newland, V.S. Blanchette, P. Bolton-Maggs, J.P. Bussell, B.H. Chong, D.B. Cines, T.B. Gernsheimer, B. Godeau, J. Grainger, I. Greer, B.J. Hunt, P.A. Imbach, G. Lyons, R. McMillan, F. Rodeghiero, M.A. Sanz, M. Tarantino, S. Watson, J. Young, D.J. Kuter, International consensus report on the investigation and management of primary immune thrombocytopenia, *Blood* 115 (2010) 168–186.
- [3] Y.C. Cohen, B. Djulbegovic, O. Shamai-Lubovitz, B. Mozes, The bleeding risk and natural history of idiopathic thrombocytopenic purpura in patients with persistent low platelet counts, *Arch. Intern. Med.* 160 (2000) 1630–1638.
- [4] M. Michel, Immune thrombocytopenic purpura. Epidemiology and implications for patients, *Eur. J. Haematol. Suppl.* 71 (2009) 3–7.
- [5] R. He, D.M. Reid, C.E. Jones, N.R. Shulman, Spectrum of Ig classes, specificities, and titers of serum antiglycoproteins in chronic idiopathic thrombocytopenic purpura, *Blood* 83 (1994) 1024–1032.
- [6] E.F. van Leeuwen, J.T. van der Ven, C.P. Engelfriet, A.E. von dem Borne, Specificity of autoantibodies in autoimmune thrombocytopenia, *Blood* 59 (1982) 23–26.
- [7] M. Kuwana, Y. Okazaki, Y. Ikeda, Splenic macrophages maintain the anti-platelet autoimmune response via uptake of opsonized platelets in patients with immune thrombocytopenic purpura, *J. Thromb. Haemost.* 7 (2009) 322–329.
- [8] L. Chow, R. Aslam, E.R. Speck, M. Kim, N. Cridland, M.L. Webster, P. Chen, K. Sahib, H. Ni, A.H. Lazarus, M.B. Garvey, J. Freedman, J.W. Semple, A murine model of severe immune thrombocytopenia is induced by antibody-and CD8+ T cell-mediated responses that are differentially sensitive to therapy, *Blood* 6 (2010) 1247–1253.
- [9] M. Kuwana, J. Kaburaki, Y. Ikeda, Autoreactive T cells to platelet GPIIb-IIIa in immune thrombocytopenic purpura. Role in production of anti-platelet autoantibody, *J. Clin. Invest.* 102 (1998) 1393–1402.
- [10] M. Kuwana, Y. Ikeda, The role of autoreactive T-cells in the pathogenesis of idiopathic thrombocytopenic purpura, *Int. J. Hematol.* 81 (2005) 106–112.
- [11] I. Malinowska, A. Obitko-Pludowska, E.S. Buescher, M. Wasik, R. Rokicka-Milewska, Release of cytokines and soluble cytokine receptors after intravenous anti-D treatment in children with chronic thrombocytopenic purpura, *The Hematology Journal* 2 (2001) 242–249.
- [12] H. Ogawara, H. Handa, K. Morita, M. Hayakawa, J. Kojima, H. Amagai, Y. Tsumita, Y. Kaneko, N. Tsukamoto, Y. Nojima, H. Murakami, High Th1/Th2 ratio in patients with chronic idiopathic thrombocytopenic purpura, *Eur. J. Haematol.* 71 (2003) 283–288.
- [13] S. Culic, B. Labar, A. Marusic, I. Salamunic, Correlations among age, cytokines, lymphocyte subtypes, and platelet counts in autoimmune thrombocytopenic purpura, *Pediatr. Blood Cancer* 47 (2006) 671–674.
- [14] B. Liu, H. Zhao, M.C. Poon, Z. Han, D. Gu, M. Xu, H. Jia, R. Yang, Z.H. Han, Abnormality of CD4 (+) CD25 (+) regulatory T cells in idiopathic thrombocytopenic purpura, *Eur. J. Haematol.* 78 (2003) 139–143.
- [15] J. Zhang, D. Ma, X. Zhu, X. Qu, C. Ji, M. Hou, Elevated profile of Th17, Th1 and Tc1 cells in patients with immune thrombocytopenic purpura, *Haematologica* 94 (2009) 1326–1329.

- [16] A.M. Rocha, C. Souza, G.A. Rocha, F.F. de Melo, N.C. Clementino, M.C. Marino, A. Bozzi, M.L. Silva, O.A. Filho, D.M. Queiroz, The levels of IL-17A and of the cytokines involved in Th17 cell commitment are increased in patients with chronic immune thrombocytopenia, *Haematologica* 96 (2011) 1560–1564.
- [17] X. Zhu, D. Ma, J. Zhang, J. Peng, X. Qu, C. Ji, M. Hou, Elevated interleukin-21 correlated to Th17 and Th1 cells in patients with immune thrombocytopenia, *J. Clin. Immunol.* 30 (2010) 253–259.
- [18] Y. Hu, D.X. Ma, N.N. Shan, Y.Y. Zhu, X.G. Liu, L. Zhang, S. Yu, C.Y. Ji, M. Hou, Increased number of Tc17 and correlation with Th17 cells in patients with immune thrombocytopenia, *PLoS One* 6 (2011) e26522.
- [19] J. Pene, S. Chevalier, L. Preisser, E. Venereau, M.H. Guilleux, S. Ghannam, J.P. Molès, Y. Danger, E. Ravon, S. Lesaux, H. Yssel, H. Gascan, Chronically inflamed human tissues are infiltrated by highly differentiated Th17 lymphocytes, *J. Immunol.* 180 (2008) 7423–7430.
- [20] A.G. Shabgah, E. Fattahi, F.Z. Shahneh, Interleukin-17 in human inflammatory diseases, *Postepy Dermatol. Alergol.* 31 (2014) 256–261.
- [21] E. Robak, L. Kulczycka-Siennicka, Z. Gerlicz, M. Kierstan, A. Korycka-Wolowicz, A. Sysa-Jedrzejowska, Correlations between concentrations of interleukin (IL)-17A, IL-17B and IL-17F, and endothelial cells and proangiogenic cytokines in systemic lupus erythematosus patients, *Eur. Cytokine Netw.* 24 (1) (2013) 60–68.
- [22] M. Jain, M. Attur, V. Furer, J. Todd, R. Ramirez, M. Lock, Q. Lu, S. Abramson, J. Greenberg, Increased plasma IL-17F levels in rheumatoid arthritis patients are responsive to methotrexate, anti-TNF, and T cell costimulatory modulation, *Inflammation* 38 (1) (2015) 180–186.
- [23] K. Ota, M. Kawaguchi, S. Matsukura, M. Kurokawa, F. Kokubu, J. Fujita, Y. Morishima, S.K. Huang, Y. Ishii, H. Satoh, N. Hizawa, Potential involvement of IL-17F in asthma, *J Immunol Res* 2014 (2014) 602846, <https://doi.org/10.1155/2014/602846> (8 pages).
- [24] T.A. Moseley, D.R. Haudenschild, L. Rose, A.H. Reddi, Interleukin-17 family and IL-17 receptors, *Cytokine Growth Factor Rev.* 14 (2) (2003) 155–174.
- [25] M. Kawaguchi, D. Takahashi, N. Hizawa, S. Suzuki, S. Matsukura, F. Kokubu, Y. Maeda, Y. Fukui, S. Konno, S. Huang, N. Nishimura, M. Adachi, IL-17F sequence variant (His161Arg) is associated with protection against asthma and antagonizes wild-type IL-17F activity, *J. Allergy Clin. Immunol.* 117 (4) (2006) 795–801.
- [26] F. Rodeghiero, R. Stasi, T. Gernsheimer, M. Michel, D. Provan, D. Arnold, J.B. Bussel, D.B. Cines, B.H. Chong, N. Cooper, B. Godeau, K. Lechner, M.G. Mazzucconi, R. McMillan, M.A. Sanz, P. Imbach, V. Blanchette, T. Kühne, M. Ruggeri, J.N. George, Standardization of terminology, definitions and outcome criteria in immunethrombocytopenic purpura of adults and children: report from an international working group, *Blood* 113 (11) (2009) 2386–2393.
- [27] J.J. Han, S.K. Baek, J.J. Lee, S.Y. Kim, K.S. Cho, H.J. Yoon, Long-term outcomes of a 5-year follow up of patients with immune thrombocytopenic purpura after splenectomy, *Kor. J. Hematol.* 45 (2010) 197–204.
- [28] S.P. Raychaudhuri, S.K. Raychaudhuri, M.C. Genovese, IL-17 receptor and its functional significance in psoriatic arthritis, *Mol. Cell. Biochem.* 359 (1–2) (2012) 419–429.
- [29] T. Arisawa, T. Tahara, T. Shibata, M. Nagasaka, M. Nakamura, Y. Kamiya, H. Fujita, M. Nakamura, D. Yoshioka, Y. Arima, M. Okubo, I. Hirata, H. Nakano, The influence of polymorphisms of interleukin-17A and interleukin-17F genes on the susceptibility to ulcerative colitis, *J. Clin. Immunol.* 28 (2008) 44–49.
- [30] J. Du, J.C. Han, Y.J. Zhang, G.B. Qi, H.B. Li, Y.J. Zhang, S. Cai, Single-nucleotide polymorphisms of IL-17 gene are associated with asthma susceptibility in an Asian population, *Medical science monitor, Med. Sci. Monit.* 22 (2016) 780.
- [31] K. Bogunia-Kubik, J.S. Wierkot, A. Malak, B. Wysoczanska, B. Nowak, S. Białowa, K. Gębura, L. Korman, P. Wiland, IL-17A, IL-17F and IL-23R gene polymorphisms in Polish patients with rheumatoid arthritis, *Arch. Immunol. Ther. Exp.* 63 (3) (2015) 215–221.
- [32] I.I.F.G. Da Silva, H.D. Angelo, E. Rushansky, M.H. Mariano, M.D.M.D. Maia, P.R.E. de Souza, Interleukin (IL)-23 receptor, IL-17A and IL-17F gene polymorphisms in Brazilian patients with rheumatoid arthritis, *Arch. Immunol. Ther. Exp.* (2017) 1–7.
- [33] J. Li, S. Ma, L. Shao, C. Ma, C. Gao, X.H. Zhang, M. Hou, J. Peng, Inflammation-related gene polymorphisms associated with primary immune thrombocytopenia, *Front. Immunol.* 8 (2017) 744, <https://doi.org/10.3389/fimmu.2017.00744>.
- [34] H. Li, Z. Zhou, W. Tai, W. Feng, D. Zhang, X. Gu, R. Yang, Decreased frequency of IL-17F rs763780 site allele G is associated with genetic susceptibility to immune thrombocytopenia in a Chinese population, *Clin. Appl. Thromb. Hemost.* 23 (5) (2017) 466–467.
- [35] S. Liu, Y.Z. Xiong, T. Li, Y. Li, S.Q. Gu, Y.M. Wang, K.H. Zhang, M. Hou, X.G. Liu, Interleukin-17A and-17F gene polymorphisms in Chinese population with chronic immune thrombocytopenia, *Ann. Clin. Lab. Sci.* 46 (3) (2016) 291–297.
- [36] T. Saitoh, N. Tsukamoto, H. Koiso, T. Mitsui, A. Yokohama, H. Handa, M. Karasawa, H. Ogawara, Y. Nojima, H. Murakami, Interleukin-17F gene polymorphism in patients with chronic immune thrombocytopenia, *Eur. J. Haematol.* 87 (3) (2011) 253–258.