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ORIGINAL ARTICLE

Programmed Cell Death 1 (PDCD1) gene polymorphisms in Children with Chronic Immune Thrombocytopenia

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ABSTRACT

Background: Patients with chronic immune thrombocytopenia (cITP) have troubles with their immune system. Nevertheless, the exact role of the immune checkpoint pathway in the development of cITP remains unknown. We aimedto investigate the impact of programmed death-1 (PD-1, PDCD1) SNPs (rs1386349 and rs2227982) on the susceptibility and clinical features of cITP. **Methods:** Thirty cITP pediatric patients and thirty sex and age-matched healthy children were included as a control group. Routine laboratory investigations were done at the Clinical Pathology Department, Faculty of Medicine, Zagazig University, and detecting PDCD1 polymorphisms using a polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) system. **Results:** Regarding SNP (rs2227982), 80.0% of the cases group had genotype CC, 20.0% had genotype CT, and no one had genotype TT. 90.0% of the control group had genotype CC, 10.0% had genotype CT, and no one had genotype TT, 90.0% of the cases group had Allele C, and 10.0% had Allele T, 95.0% of the control group had Allele C, 5.0% had Allele T, according to SNP (rs1386349), 83.3% of the cases group had genotype CC, 13.3% had genotype CT, and 3.3% had genotype TT, 86.7% of the control group had genotype CC, 13.3% had genotype CT, and no one had genotype TT, 90.0% of the cases group had Allele C, and 10.0% had Allele T, 93.3% of the cases in the control group had Allele C, and 6.7% had Allele T. **Conclusions** The Pd1 gene polymorphisms (rs1386349 and rs2227982) do not affect susceptibility or clinical features among cITP patients.

Keywords Immune thrombocytopenia, Programmed Death-1, Single-nucleotide polymorphisms.

INTRODUCTION

The reticuloendothelial system removes platelets "opsonized with antiplatelet antibody" prematurely in immune thrombocytopenia (ITP), an autoimmune disease. Research has revealed that a combination of destruction and underproduction might cause ITP. [1].

When no other medical conditions are present, and the platelet count is less than $100x10^9$/L, the International Working Group (IWG) considers this condition immune thrombocytopenia [2].

Three types of immune thrombocytopenia have been identified: recently diagnosed (within three months of the diagnosis), persistent (between three and twelve months

from the diagnosis), and chronic (lasting more than twelve months from the start of diagnosis) [3].

On the surface of active T lymphocytes lies the protein known as programmed cell death 1 (PD-1), an inhibitory molecule on T cells and a member of the CD28 family [4]. An essential role for PD-1 in immunotolerance mechanisms was demonstrated in PD-1-deficient mice that develop autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, and chronic ITP. These mice also show that PD-1 inhibits the immune system [5]. By interacting with its ligands "PD-L1 and PD-L2," PD1 has been found to inhibit lymphocyte activation and cytokine production [6].

Single nucleotide polymorphisms (SNPs) in the PD1 gene may influence the function of PD-1 by altering the structure or the expression of PD-1 and affecting the susceptibility to diseases. One single nucleotide polymorphism (SNP) in the putative enhancer-like region that causes PD-1 transcriptional activity to be reduced in human T cells is the PDCD1 +7209 C/T (rs41386349). Reduced expression and decreased function of the cytoplasmic domain of PD-1 resulted from the PDCD1 +63379 C/T (rs2227982) in Exon 5 [7].

So, this study aimed to clarify the influence of PDCD1 SNPs (rs41386349 and rs2227982) on the risk of developing chronic immune thrombocytopenia in children and to investigate the association between PDCD1 SNPs (rs41386349 and rs2227982) and susceptibility to develop chronic ITP.

METHODS

Patients

The study was carried out on 30 children diagnosed with chronic immune thrombocytopenia who were admitted and followed up at the pediatric hematology unit, Zagazig University Hospital, as well as 30 age and sex-matched children who served as a control group from December 2023 to June 2024. The study protocol was approved by the Ethical Committee of the Faculty of Medicine, Zagazig University (IRB#6347)

Inclusion criteria: Both males and females aged more than one and less than 18 years old were included, as were children with chronic immune thrombocytopenia (platelet count less than 100 x 109/L) for more than 12 months.

Exclusion criteria: Patients with secondary immune thrombocytopenia, patients with other diseases affecting the platelet count, and parents who refused to participate or sign the written consent.

Methods

Complete history taking and a thorough clinical examination were performed on all subjects. Routine laboratory investigations included a complete blood count (CBC) using the cell counters (Sysmex XT 1800, China). Prothrombin time(PT), Partial thromboplastin time(PTT), International Normalized Ratio INR (CS2500, Sysmex Corporation, Japan).Erythrocyte sedimentation rate (vision ESR analyzer, YHLO BIOTECH CO, China). C-reactive protein (Cobas 6000, Hitachi, Japan). Liver function test, Kidney function test (Cobase8000C 702, Technologies corporation, Tokyo Japan). Human Immunodeficiency Virus antibody test (Cobas 6000 EU3, Hitachi, Japan). Hepatitis C virus antibody test (Cobas 8000C 702, Technologies corporation, Tokyo Japan). Peripheral blood film. Bone marrow aspiration with Leishman-stained film examination. Detection of PDCD1 polymorphisms utilizing a polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) system.

For the detection of PD1 gene single-nucleotide polymorphisms, after obtaining written consent from patients and control parents, two ml of EDTA anticoagulated whole blood samples were collected.

DNA Extraction:

QIAamp Mini spin column was placed in a clean 1.5 ml microcentrifuge tube, followed by adding 200 µl of elution buffer (AE) then incubated at room temperature for 1 min, then centrifuged at 8000 rpm for one minute. (QIAGEN) according to the manufacturer protocol.

Extracted DNA visualization:

Submarine gel electrophoresis (SCIE-PLAS, Japson, India) was performed to visualize extracted genomic DNA using 2% agarose gel and an Ultraviolet transilluminator (Biometra, TFX-35M, FRANCE).

DNA amplification using polymerase chain reaction (PCR)

A 3 µl genomic DNA was pipetted into the master mix solution. Placing the samples into the pre-heated and calibrated thermal cycler (Biometra, Jena, Germany) applying the following protocol: Initial denaturation at 95°C for 5 minutes, denaturation at 95°C for 30 seconds, anneal primers at 60°C for 45 seconds, extend primers at 72°C for 1minute, and elongation at 72°C for 10 minutes. Amplified DNA visualization on Ultraviolet transilluminator (Biometra, TFX-35M, FRANCE) was carried out, and amplicon size for SNP (rs1386349) and SNP (rs 2227982) was 250 bp.

Restriction fragment length polymorphism (RFLP)

Restriction to SNP (rs 1386349) by enzyme BSH fast digest (Thermo Scientific, USA) that cuts at sequence 5. C G...C G ..3 and 3... G C...G C... 5 and restriction to SNP (rs2227982) by enzyme BPU10I fast digest (Thermo Scientific, USA) that cuts at sequence 5... C C ...T N A G C... 3 and 3... G G A N T ...C G... 5 were carried out. Reaction mixture: included *Nuclease water17* µl, Buffer 2 µl, Enzyme 1 µl, DNA 10 µl, and all were added to each other with gentle mixing and spin down. Incubation was done at 37°C in a heat block for 5min for SNP (rs 1386349). Incubation was done at 37°C in a heat block for 15 minutes for SNP (rs2227982).

RFLP results in visualization:

RFLP visualization: Submarine gel electrophoresis (SCIE-PLAS, Japson, India) was performed to visualize restricted genomic DNA using 2% agarose gel and Ultraviolet transluminator (Biometra, TFX-35M, FRANCE. For SNP (rs 1386349) and SNP (rs2227982), The normal genotype appeared as two bands at 50 and 200 bp. The mutated genotype appeared as one band at 250 bp. The heterozygous genotype appeared in three bands: 50 bp, 200 bp, and 250 bp.

Statistical analysis:

We used IBM SPSS software package version 20.0 to analyze the data fed into the computer. Numbers and percentages were used to describe the qualitative data. The X2-chi-square test was utilized to compare qualitative data. The quantitative data was compared using Student's t-test. Fisher's Exact or Monte Carlo correction is used in Correction for chi-square when more than 20% of the cells have an expected count of less than 5. Mann Whitney test (U) For abnormally distributed quantitative variables, to compare between two studied groups. Odd ratio (OR): Used to calculate the odds and 95% Confidence Interval of an event occurring in one risk group to the odds of it occurring in the non-risk group.

RESULTS:

The male number in cases was 16 (53.3%), and in control was 14 (46.7%); the female number in cases was 14(46.7%), and in control was 16 (53.3%). According to age, the range in cases was (3 to 17) and in control was (7 to 18) the mean in cases was (10.63 ± 3.76) and in control was (12± 3.11).According to weight, the range in cases was (14 to 80) and in control was (17 to 85) the mean in cases was (42.60 ± 19.61) and in control was (47.7± 16.91). According to

height, the range in cases was (100 to 170) and in control was (117 to 165) the mean in cases was (141.2 ± 17.05) and in control was (142.83±13.81). There was a statistically non-significant difference between the two studied groups regarding demographic data in Table (1).

Table (2) shows according to signs of bleeding in cases purpura was 23 (76.7 %) ,ecchymosis was 29 (96.7%) ,epistaxis was 12(40%) ,bleeding per gum was 3(10%) , menstruation was 4 (13.3%) , according to fever (relapsing) was 2 (6.7%), splenomegaly was 4 (13.3%).

Table (3) shows that the median of platelets among cases was (22×10 cubed over ml close paren, and in control, it was (280××10^3/ml). There is a significant decrease in platelet counts among patient groups compared with control (P < 0.001). The median of WBCs among cases was (15.6×10^3/ml) and among control was (8.3××10^3/ml) There is a significant increase in WBCs among the patient group compared with the control (P < 0.001). According to the Hb range among cases: It was (5-13g/dl)) and in control was (10-14 g/dl)); the mean value in cases was (10.23 ± 1.72) and in control was (11.30 ± 1.06), P was (0.005) so statistically significant differences were found between the two studied groups in platelet and Hb at diagnosis. According to PT: The range in cases was (11-13sec) and in control was (11-13 sec), the mean value in cases was (11.97 ± .73) and in control was (12 ± .67), and P was (0.854). According to PTT: The range in cases was (26-34sec) and in control was (26-34sec), the mean value in cases was (29.23 ± 2.21) and in control was (30.20 ± 2.04), P was (.084). So, statistically non-significant differences were found between the two studied groups in terms of PT and PTT at diagnosis.

Table 4 shows a comparison between the two studied groups according to SNP (rs 1386349). There, 25 (83.3%) of the cases group had genotype CC, 4 (13.3%) had genotype CT, and 1 (3.3%) had genotype TT. There, 26 (86.7%) of the control group had genotype CC, 4 (13.3%) had genotype CT, and no one had genotype TT. There were 54 (90.0%) of the cases group had Allele C, and 6 (10.0%) had Allele T. There were 56 (93.3%) in the control group having Allele C, 4 (6.7%) having Allele T, with no statistically significant differences between the two studied groups and SNP-1(rs 1386349).

According to SNP (rs2227982), 24 (80.0%) of the cases group had genotype CC, 6 (20.0%) had genotype CT, and no one had genotype TT. There were 27 (90.0%) of the control group had genotype CC, 3 (10.0%) had genotype CT and no one had genotype TT. There were 54 (90.0%) of the cases group had Allele C and 6 (10.0%) had Allele T. There were 57 (95.0%) of the control group having Allele C, 3 (5.0%) having Allele T, with statistically non-significant differences between the two studied groups and SNP (rs2227982) (Table 5).

Table 6 shows the relation between SNP–1 (rs1386349) and SNP–2 (rs2227982) in the case group (n=30): there were statistically non-significant differences between CC genotypes and (CT+TT) genotypes related to rs1386349 and rs2227982 regarding splenomegaly, platelets count, and the response to therapy.

Table(1): Comparison between the cases and control groups according to demographic data

Demographic data	Cases (n = 30)		Control (n = 30)		Test of Sig.	P
	No.	%	No.	%		
Sex						
Male	16	53.3	14	46.7	χ^2= 0.267	0.606
Female	14	46.7	16	53.3		
Age (years)						
Range(Min. – Max).	3.0 –17.0		7.0 – 18.0		t= 1.536	0.130
Mean ± SD.	10.63 ± 3.76		12.0 ± 3.11			
Weight (kg)						
Min. – Max.	14.0 – 80.0		17.0 – 85.0		t= 1.079	0.285
Mean ± SD.	42.60 ± 19.61		47.70 ± 16.91			
Height (cm)						
Min. – Max.	100.0 – 170.0		117.0 – 165.0		t= 0.408	0.685
Mean ± SD.	141.20 ± 17.05		142.83 ± 13.81			

p: If p-value is less than or equal to 0.05 (the significance level). SD: Standard deviation
χ^2: Chi-square test t: Student t-testsig: Significance

Table (2): Distribution of cases according to physical examination at the start of study

Physical examination	Case (n = 30)	
	No.	%
Bleeding		
Purpura	**23**	**76.7**
Echymosis	**29**	**96.7**
Epistaxis	**12**	**40.0**
Bleeding per gum	**3**	**10.0**
Menstruation	**4**	**13.3**
Intracranial hemorrhage	**0**	**0**
Fever(Relapsing)	**2**	**6.7**
Splenomegaly	**4**	**13.3**

Table (3) Comparison between the two studied groups according to blood count and screening of coagulation at diagnosis

	Cases (n = 30)	Control (n = 30)	Test of Sig.	P
Platelet × 10^3/ml Median (IQR)	22.50 (10.0 – 47.0)	280.0(190.0 – 320.0)	U= 0.00*	< 0.001*
WBCs × 10^3/ml Median(IQR)	15.6(11.0 – 20.90)	8.3(7.5 – 11.2)	U= 3.55*	< 0.001*
Hb(g/dl)				
Range (Min. – Max).	5.0 – 13.0	10.50 – 14.0	t= 2.900*	0.005*
Mean ± SD.	10.23 ± 1.72	11.30 ± 1.06		
PT (Sec) Min. – Max. Mean ± SD.	11.0 – 13.0 11.97 ± 0.73	11.0 – 13.0 12.0 ± 0.67	t= 0.184	0.854
PTT (Sec) Min. – Max. Mean ± SD.	26.0 – 34.0 29.23 ± 2.21	26.0 – 34.0 30.20 ± 2.04	t = 1.761	0.084

p: p-value for comparing between the two studied groups *: Statistically significant at p ≤ 0.05
IQR: **Inter quartile range** SD: **Standard deviationt: Student t-test** **U: Mann Whitney test**

Table (4): Comparison between the two studied groups according to SNP (rs 1386349)

(rs 1386349)	Cases (n = 30)		Control (n = 30)		χ^2 (p)	p_0	OR(95% C.I)
	No.	%	No.	%			
Genotype							
CC	25	83.3	26	86.7	1.018 (^{MC}p= 0.237)		1.000
CT	4	13.3	4	13.3		0.959	1.040 (0.234 –4.618)
TT	1	3.3	0	0.0		–	–
Allele							
C	54	90.0	56	93.3	0.436 (0.509)		1.000
T	6	10.0	4	6.7		0.512	1.557 (0.416 –5.819)

χ^2: **Chi-square test** MC: **Monte Carlo**OR: Odds ratio CI: Confidence interval
p_0: p-value for **OR**p: p-value for comparing between the two studied groups

Table (5): Comparison between the two studied groups according to SNP (rs 2227982).

SNP (rs2227982)	Cases (n = 30)		Control (n = 30)		χ^2 (^{FE}p)	p_0	OR(95% C.I)
	No.	%	No.	%			
SNP(rs2227982							
CC	24	80.0	27	90.0	1.176 (0.472)		1.000
CT	6	20.0	3	10.0		0.286	2.250 (0.507 –9.993)
TT	0	0.0	0	0.0		–	–
Allele							
C	54	90.0	57	95.0	1.081 (0.491)		1.000
T	6	10.0	3	5.0		0.308	2.111 (0.503 –8.866)

χ^2: **Chi-square test** MC: **Monte Carlo**OR: Odds ratio CI: Confidence interval
p_0: p-value for **OR**p: p-value for comparing between the two studied groups

Table (6): Relation between SNP (rs1386349) & SNP (2227982) and platelets count and splenomegaly in cases group (n=30).

	SNP–1(rs1386349)				Test of Sig.	OR	(95% C.I)		P
	CC (n = 25)		CT + TT (n = 5)						
	No.	%	No.	%					
Splenomegaly						1.00	Ref.		
No	21	84.0%	5	100.0%	0.923				0.462
Yes	4	16.0%	0	0.0%		0.808	0.670 - 0.976		
Platelets count					U= 0.725				0.481
Min. – Max.	2.0 – 128.0		2.0 – 43.0						
Mean ± SD.	34.44 ± 32.995		20.4 ± 15.34						
Response to treatment	24	96.0%	4	80.0%	χ^2(FE)=1.71	0.1667	0.009 to 3.239		0.310
	SNP–2 (2227982)				Test of Sig.	OR	(95% C.I)		p
	CC (n = 24)		CT + TT (n = 6)						
	No.	%	No.	%					
Splenomegaly									
No	20	83.3%	6	100.0%	χ^2(FE)= 1.154	0.769	0.623 to 0.949		0.388
Yes	4	16.7%	0	0.0%					
Platelets count					U=1.818				0.069
Min. – Max.	2.0 – 128.0		2.0 – 43.0						
Mean ± SD.	36.88 ± 32.25		13.0± 15.66						
Response to treatment	23	95.8%	5	83(.3%	χ^2(FE)=1.205	0.217	0.012 to 4.094		0.366

χ^2: **Chi-square test** FE: **Fisher Exact** **t: Student t-test** **SD:Standard deviation**
U: Mann Whitney test OR: Odds ratio CI: Confidence interval
p_0: p-value for **OR**p: p-value for comparing between the two studied groups

DISCUSSION:

In the current study, we aimed to investigate the impact of PDCD1 SNPs (rs 1386349, rs2227982) on the susceptibility and clinical features of cITP. The most common presenting symptom was Ecchymosis in 29 (96.7%) cases, followed by Purpura in 23(76.7%) cases, which coincides with the results in the study done by Bardis et al. [8], who found that from mucosal to mucocutaneous bleeding, patients with ITP exhibited a broad spectrum of clinical symptoms.

In the present study, statistically insignificant differences were found between the two studied groups regarding SNP (rs 1386349) and SNP (rs 2227982)) with c.ITP agrees with Chen et al. [9], who found no discernible variation in the allele frequency of any SNPs between controls and individuals with ITP. Also, Li's study found that the rs3087243 is unrelated to ITP vulnerability in the Chinese population [10].

It was also hypothesized that children and adults may experience ITP in different ways. One possible explanation is that adult ITP is typically more chronic and challenging to cure, in contrast to the benign and self-limiting nature of pediatric ITP [11].

Also, Craig et al. [12] revealed that most ITP episodes in children happen suddenly within a few weeks following a viral infection. In adults, chronic ITP is associated with chronic lymphocytic leukemia, lymphomas, SLE, and

infectious mononucleosis. Middle-aged women typically have a gradual development of chronic ITP. According to these, the events that set things in motion might be distinct. The underlying mechanism of thrombocytopenia, which is the breakdown of platelets coated with antibodies by mononuclear macrophages, seems comparable in adults and children.

In addition to acetazolamide and aspirin, several medications can induce autoimmune thrombocytopenia, which is similar to immune thrombocytopenia caused by phenytoin, digitoxin, methyldopa, meprobamate, sulfamethazine, rifampin, as well as quinidine [13].

In the present study, the most common presenting symptom was Ecchymosis in 29 (96.7%) cases, followed by Purpura in 23(76.7%) cases. Also, Harald et al. [14] differentiate between cITP in adults and children; they revealed that both are similar in clinical manifestation (extensive purpura, hemorrhage as petechiae and hematoma, epistaxis, hematuria, bloody stool, metro-menorrhagia, conjunctival purpura, retinal bleeding and ICH (<.5%). The same diagnostic tools: increased platelet size, reticulated platelets (platelet associated immunoglobulins, immature platelet fraction), a monoclonal antibody specific immobilization of platelet antigen (MAIPA): a qualitative method for platelet antibody detection) with spontaneous remission is approx. 80% within one year in children while is approx. 20-30% within one year in adults; cITP in children is standard in males with a bleeding tendency of 90%, while in adults, it is common in females with a bleeding tendency of 70%.

The present study showed a significant decrease in platelet counts among patient groups compared with control ($P < 0.001$). Bardis et al. [8] found that the number of PD-1 CD-4 + T-cells is much higher in the case group compared to the control group. Wang et al. [15] found that the fraction of PD-1 on CD4+T cells in the blood of ITP patients was significantly more significant than in healthy controls, and this result confirms the crucial role of the PD1 molecule in evidence of an autoimmune process in ITP.

The primary function of these T-cell immunosuppressive receptors is to prevent autoimmune disorders by limiting the activity of self-reactive T cells. An increase in T cell activation is one possible link between the etiology of autoimmune diseases and low expression of these receptors. Rarely have researchers looked at PD-1 and PD-L1 variants in ITP [16].

Tregs expressed PD-1 and PD-L1, crucial in regulating peripheral tolerance by preventing immune-mediated tissue damage and blocking effector T cells [17].

When PD-1 and PD-L1 interact, Treg production and the suppressive characteristics of Tregs can be affected. When PD-L1 and PD-L2 aren't present, activated lymphocytes can build up, and T cell-mediated autoimmunity can occur [18].

There are few studies investigating the PD-1 signaling pathway among city patients. Only very few studies have found that decreased sPD-1 levels may have a role in ITP pathogenesis, as without the inhibitory regulation of PD-1, sustained activation of T cells may cause inflammatory responses, which is the case in ITP.

Birtas et al. [19] presented the first study on the PD-1 signaling pathway in ITP. They discovered that 67 individuals with ITP, including 24 with newly diagnosed ITP and 43 with chronic ITP, had lower levels of sPD-1.

However, Birtas et al. [19] found that those with newly diagnosed ITP had a lower sPD-L1 level compared to those with chronic ITP. However, this difference was not statistically significant when compared to healthy

subjects.

According to some researchers, the pathophysiology of cITP may be influenced by the expression of immunological checkpoint molecules. Patients with cITP, who do not have full-length PD-1 but have the transmembrane domain, had lower serum soluble PD-1 (sPD-1) levels than healthy controls [19].

Regarding PD 1 correlation with platelet count:

Wu et al. [21] studied the serum levels of interferon-gamma (IFN-γ), interleukin-17 (IL-17), and sPD-1. They showed that these levels were raised in 40 untreated ITP patients compared to 30 healthy individuals. In contrast, IL-4 and transforming growth factor-β (TGF-β) were found to be lowered in these patients. Furthermore, there was a negative correlation between the platelet count and the level sPD-1.

Also, in another study by Birtas et al. [19], who measured concentrations of soluble PD-1 and soluble PD-L1 in the serum of 67 individuals diagnosed with ITP, including 24 patients with newly diagnosed ITP [ndITP] and 43 patients with chronic ITP [cITP], as well as 21 healthy controls (HCs). Researchers observed that ndITP and cITP patients had lower serum sPD-1 levels than healthy controls. There was also a favorable relationship between platelet counts and sPD-1 levels. In contrast to patients with cITP, those with ndITP had lower sPD-L1 levels.

The ability of programmed cell death protein 1 (PD-1) signaling to generate peripheral tolerance and turn off autoreactive T cells was also discovered by Han et al. [22]. Patients with ITP have reduced levels of PD-1 and PD-L1 in their CD8+ T cells. In vitro, CTLs-mediated platelet destruction in ITP was reduced by activating the PD-1 pathway through the PD-L1-Fc fusion protein. A reduction in PD-1 expression was seen in ITP patients due to PD-1 promoter hypermethylation in CD8+ T cells. In addition, CTLs from ITP patients may be resistant to low-dose decitabine if PD-1 is blocked. Consequently, they hypothesized that ITP's pathogenesis involves the abnormal PD-1/PD-L1 pathway and that improving PD-1/PD-L1 signaling would be a proper treatment strategy for the administration of ITP.

This finding confirms the critical role of the PD1 molecule in suppressing the autoimmune process in ITP, as described by Bardis et al. [8]. In addition, they discovered that PLT is positively correlated with PD-1 expression in CD4+T cells. Increased PLT count is associated with increased PD-1 expression on autoreactive helper T-cells. This explains the PD-1 molecule's critical function in preventing the immune system's destruction of PLT.

In the current study, statistically non-significant differences were revealed between the CC genotype and the (CT+TT) genotype related to SNP(rs 1386349) and SNP (rs 2227982) regarding sex and age. This agrees with Kasamatsu et al. [23], who found that neither the genotype distribution nor the allele frequencies varied significantly when comparing the control group with cITP patients overall regarding sex and age.

In the present study, statistically insignificant differences were found between CC genotype and (CT+TT) genotype related to SNP(rs 1386349) and SNP (rs 2227982) regarding response, in the same way as the results in the study done by Kasamatsu et al. [23], who found no significant difference between genotypes related to SNP(rs 1386349) and SNP (rs 2227982) and response.

Limitations of this study include a small sample size (total of 36 subjects) and its being done in a single center, so generalization of our findings needs more validated larger sample and multicenter studies. There are also not enough similar studies to give us more

information.

CONCLUSIONS:

There is no relation between PD1 polymorphisms SNP (rs 1386349) and SNP (rs 2227982) and susceptibility or the clinical features of cITP.

Conflictsof Interest

The authors report no conflicts of interest.

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None declared

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