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A case–control study of single-nucleotide variants in microRNA biogenesis genes (AGO1 and GEMIN4) in people with primary immune thrombocytopenia

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Abstract

Background The role of microRNA (miRNA) is to regulate the translation of genes involved in a variety of diseases.

Aim of the work This study investigated the relation between the *rs636832* and *rs2740348* single-nucleotide polymorphisms (SNPs) of the *AGO1* gene and the *GEMIN4* gene of miRNA biogenesis genes and the risk for primary ITP and the response to therapy.

Patients and methods In this case–control study, 100 patients with immune thrombocytopenic purpura from the clinical hematology department and outpatient clinic were compared to 100 control subjects. Patients' blood samples are taken, and DNA extraction and PCR amplification of rs636832 A/G of AGO1 and rs2740348 G/C of GEMIN4 were done on sera and compared to the clinical status of the patients.

Results According to statistical analysis, the genotype and allele frequencies of both variants did not differ significantly between cases and controls. The GG genotype was found to be more prevalent in the chronic phase of ITP than the AA and AG genotypes, with a significance level of p 0.05. The GG genotype was found to be more prevalent in the chronic phase of ITP than the AA and AG genotypes, with a significance level of p 0.05. In addition, the GG genotype was more prevalent in ITP patients who did not respond to treatment compared to the AA and AG genotypes, although this difference was not statistically significant. Concerning rs2740348: the CC genotype was more common than the GC genotype in ITP cases that did not respond to treatment and needed a second line of therapy like splenectomy or TPO-RA, but this did not reach statistical significance.

Conclusion The rs636832 and rs2740348 SNPs did not appear to be risk factors for ITP; however, the rs636832:GG genotype was more prevalent in older patients, who tend to have the chronic phase of the disease. Although this distinction was not statistically significant, they were less responsive to therapy than the AA and AG genotypes. Concerning rs2740348: the CC genotype was more common than the GC genotype in ITP cases that did not respond to treatment and needed a second line of therapy like splenectomy or TPO-RA, but this did not reach statistical significance.

Keywords Primary, ITP, miRNAs, And linkage disequilibrium

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Background

Immune thrombocytopenic purpura is one of the more common bleeding disorders, characterized by isolated thrombocytopenia with a platelet count of 100×10^9/L. Clinically, patients usually present with mucocutaneous bleeding, and the diagnosis is made after the exclusion of other causes of thrombocytopenia. The classical treatment is a combination of steroids, immune suppressive therapy, and thrombopoietin analogues [1].

Primary ITP pathogenesis is multifactorial, such as a previous viral infection that leads to the production of antiplatelet antibodies, a loss of immune tolerance, T cell cytotoxicity, and a change in the way megakaryocyte function [2].

The behavior pattern of ITP is heterogeneous, ranging from no symptoms to severe bleeding and from prolonged remission to frequent relapses [3]. Up until now, there have been many theories to explain this difference. In the past decade, scientists' interest in investigating the epigenetic mechanisms of common diseases has increased, and a lot of research has been conducted on the role of microRNA (miRNA) in various diseases [4].

MicroRNAs (miRNAs) are small noncoding RNAs (19–24 nucleotides) that regulate gene expression by binding to the messenger RNA of their target gene (mRNA). microRNAs' primary function is to regulate translation [4].

According to the human genome, there are approximately 1900 miRNA genes. MiRNA biogenesis is a complex process that starts in the cell nucleus and consists of two major steps. Most miRNA genes are transcribed by RNA polymerase II into large pri-miRNAs containing stem–loop structures with double strands of miRNA. One strand of the miRNA duplexes binds to the Argonaute protein and forms the miRNA-induced silencing complex (miRISC), which then converts to mature miRNA [5].

MiRNA biogenesis machinery genes encode proteins such as AGO1–4, GEMIN3, and GEMIN4 that participate in mRNA blockage or cropping of target mRNA. The AGO1 gene is found on chromosome 1p34.3 and encodes a member of the argonaute family of proteins, which are associated with small RNAs and play important roles in RNA interference (RNAi) and RNA silencing. The 6th intron of the AGO1 gene is the location of the rs636832 SNP. Its expression level influences the progression and prognosis of Graves' disease. In addition, it has been linked to a reduced risk of chronic hepatitis B and lung cancer. The GEMIN 4 gene is found on chromosome 17p13.3 and encodes the Gemin4 protein, which is a component of the RISC complex. GEMIN proteins in miRNA ribonucleoprotein particles play a role in miRNA precursor processing by interacting with key components of the RNA-induced silencing complex [6–8].

Genetic polymorphisms in genes encoding microRNA machinery may result in abnormal miRNA expression, affecting the expression level of target genes and becoming a risk factor for cancer and autoimmune diseases. GEMIN4 is associated with an increased risk of breast cancer but a lower risk of prostate cancer [9].

A growing role for atypical microRNA expression in the etiology and progression of infectious thrombocytopenia has been demonstrated. The SNPs of miRNA- and miRNA biogenesis-related genes, such as AGO1 and GEMIN4, are associated with a variety of immunological and malignant conditions [10].

This study used polymerase chain reaction to find SNP loci in classical miRNA machinery genes (rs636832 in AGO1 and rs2740348 in GEMIN4) to study the genetic impact of these variants in miRNA machinery genes on susceptibility to immune thrombocytopenic purpura (ITP) in the Egyptian population.

Subjects and methods

Study population

This was a case–control study conducted on 100 adult Egyptian patients who were diagnosed with primary ITP and were selected from cases referred to the Hematology Outpatient Clinic, as well as 100 healthy controls of matched age, sex, and race. All cases of ITP were chosen based on the diagnostic criteria for ITP set by the American Society of Hematology (ASH) in 2011 [11].

Each patient received a comprehensive medical history, a complete physical examination, and baseline tests. These tests consisted of a complete blood count, coagulation profile, chemistry profile (random blood sugar, liver, and kidney function tests), immunological screening (ANA, C3, and C4), viral screening (HCV Ab, HBsAg, CMV IgM, EBV IgM, and HIV Ab), Helicobacter pylori antigen, and abdominal ultrasound. Patients with secondary ITP resulting from viral infections (HBV, HCV, or HIV), drug induction, H. pylori, or autoimmune diseases such as systemic lupus erythematosus (SLE) were excluded from the study. Thyroid disease patients are excluded from participating. In total, 100 healthy volunteers of the same age, gender, and race comprised the control group. These volunteers were selected because they did not have a history of ITP, immunological diseases, or other diseases. All participants in the study provided a signed, informed consent form. The Ain Shams University's Ethical Committee authorized the study. In addition, the study complied with the Declaration of Helsinki (ethical principles for medical research involving human subjects) and its 2004 amendments.

Each group of cases and controls consisted of 87 (87%) females and 13 (13%) males. The ages of the cases ranged from 18 to 67, with a mean and standard deviation of 33.8 + 14.4 years. The age of controls ranged from 18 to 68 years, with a mean value ± SD of 31.55 ± 11.88 years ($p = 0.230$).

Adults with ITP were sampled at different stages of the disease: 31 (31% of them) were just diagnosed, 16 (16%) were still sick, and 53 (53%) had the disease for a long time. The disease duration ranged from 0.08 to 15 years, with a mean and standard deviation of 2.36 ± 3.2 years. The starting age of the disease ranged from 6 to 66 years, with a mean value ± SD of 31.06 ± 14.83 years.

The ITP cases had bleeding manifestations that were either cutaneous or non-cutaneous. Cutaneous bleeding in the form of petechia and purpura was present in nearly all (98%) of the ITP cases, while ecchymosis was present in only 41% of the cases. Non-cutaneous bleeding was present in the form of bleeding from the nose in 40 cases (40%), mouth bleeding in 38 cases (38%), and abnormal vaginal bleeding in 33 cases (33%). Bleeding is classified as grade 1 in 38 cases (38%), grade 2 in 15 cases (15%), and grade 3 in 47 cases (47%). Severe ITP was present in 47 (47%) of the ITP patients.

All cases started with a first line of treatment (a corticosteroid). The second line of treatment was azathioprine (immunosuppressive) in 34 cases (34%), romiplostim (a TRO agonist) in three cases, and splenectomy in 10 cases, of which three (30%) resulted in refractory ITP. In a one-year follow-up, complete recovery was observed in 18 cases (18%), while steroid dependence was noticed in 80 cases (80%), with one case continuing only on azathioprine and one case resulting in death.

Methodology

Extraction of genomic DNA

Three ml of venous blood was collected on 5% ethylene diamine tetraacetic acid (EDTA) by sterile venipuncture using a sterile vacutainer tube. Samples are stored at − 20 °C until DNA extraction. According to the manufacturer's protocol, the Gene JET Whole Blood Genomic DNA Purification Mini Kit (Cat. No. K0781) extracted genomic DNA from a peripheral blood sample.

DNA amplification

For DNA amplification, use the TaqMan® Genotyping Master Mix provided by Thermo Fisher Scientific Company (Cat. No. 4371353). PCRs were carried out in 20 L of final volume using 3 L of extracted DNA, 10 L of genotyping master mix, 6.5 L of dissolving water, and 0.5 L of SNP.

SNP genotyping assay

SNP TaqMan ready-made assay supplied by Thermo Fisher Scientific Company (Cat. No. 4351379) The TaqMan assay, or 5′-nuclease allelic discrimination assay, is a PCR-based method for determining the type of single-nucleotide polymorphisms (SNPs). With two fluorescent probes that are specific to each allele, the area around the SNP is amplified. The 3′ end of the probes has a substance that stops the fluorescence from happening. Because there are two probes, it is possible to find both alleles in a single tube. Since probes are part of the PCR, genotypes can be found without any processing after the PCR, which is not possible with most other genotyping methods. The context sequence for:

- rs636832 A/G of AGO1 [VIC/FAM] was: TCTGATTCCAGAACATATCACTCCT**[A/G]**AAAGAAAGCCTGTATTCATTAGCAG.
- rs2740348 G/C of GEMIN4 [VIC/FAM] was: AGCAGCCTCAACACCAAGTCTGGCT**[C/G]**TCGGAAGAGGGCCCTGTTACTCCCC.

The PCRs were carried out in 20 L of the final volume, which consisted of 3 L of extracted DNA, 10 L of the Genotyping Master Mix, 6.5 L of distilled water, and 0.5 L of a ready-made SNP TaqMan assay.

An Applied Biosystems Step One real-time PCR thermal cycler was used with the serial number 271001759. The PCR amplification process started with a 30-s pre-PCR reading at 60 °C, followed by a 10-min hold at 95 °C. Then, for 1 min, 50 amplification cycles of denaturation at 95 °C and annealing/extension at 60 °C were performed. Finally, a 30-s post-PCR was performed at 60 °C. Allelic and genotypic identification is performed using the plotted fluorescence signals as they flow:

Homozygous for G allele of rs636832 A/G with FAM-dye fluorescence.

Heterozygous for A and G alleles of rs636832 A/G with VIC/FAM-dye fluorescence.

10% of the samples are genotyped twice, and the results are read by two examiners who do not know what the results are. This is done to make sure the quality is good.

Statistical analysis

Hardy–Weinberg equilibrium was determined using a goodness-of-fit 2 test to compare the observed and expected genotype frequencies. SPSS Inc., Chicago, IL, used IBM SPSS Advanced Statistics version 20 to analyze the data. The statistical information was presented as a mean and standard deviation. The frequency and percentage of qualitative data were expressed. The relationship between qualitative variables is analyzed using

the Chi-square or Fisher's exact test. For quantitative, non-normally distributed data, a Student's t-test is used to compare two groups. Using the Kruskal–Wallis test, a comparison was made between the three groups (non-parametric ANOVA). For risk estimation, an odds ratio (OR) with a 95% confidence interval (CI) was used. Every test was two-tailed. A p value of 0.05 was deemed statistically significant. Linked disequilibrium (LD) was computed using the methods described in the cited source.

Results

Genotype and allele frequencies among cases and controls

The observed genotypes and allele frequencies for both SNPs were found to be congruent with expectations under Hardy–Weinberg equilibrium in both cases and controls with a p value greater than 0.05. Logistic regression analysis revealed that the *rs636832 A/G* and *rs2740348 G/C* genotypes were not associated with ITP risk (Table 1).

An LD analysis of the two SNPs revealed that there was a modest LD between *rs636832 A/G* and *rs2740348 G/C* among ITP cases with D' = 0.41, $r^2 = 0.04$, and p value = 0.007, and that this was more frequent in the ITP cases than in the control group, which showed D' = 0.17, $r^2 = 0.006$, and *p* value = 0.298.

Genotypes and ITP characteristics

For AGO1 rs636832 A/G, the association between mean age at onset of ITP and genotyping showed a statistically significant difference in the recessive model, with the GG genotype being associated with a later age at onset of disease ($p = 0.022$; Table 2).

Regarding the phase of the disease, chronic ITP was more prevalent in GG genotypes (*p* value = 0.044) compared to AA and AG genotypes. The GG genotype was more obvious in ITP cases that showed no response to treatment and cases that required a second line of treatment in the form of TPO-RA agonists or splenectomy compared to AA and AG genotypes, but this was not statistically significant (Table 3).

A complete response is defined by a platelet count of more than 100 109/L and the absence of bleeding. A mean response is defined by a platelet count of 30–100 109/L, or at least double the baseline count. No response is defined by a platelet count of less than 30 109/L, or less than double the baseline count [12].

Table 1 Genotypes and allele frequencies among cases and controls

	Cases ($n = 100$) NO. and %	Controls ($n = 100$) NO. and %	OR (95% CI)	X^2	*p*-value*
AGO1 rs636832 A/G					
Genotypes			–	0.702	0.704
AA	5	3			
AG	22	20	1.515 (0.320–7.169)		
GG	73	77	1.758 (0.406–7.621)		
Dominant model					
AA	5	3		0.521	0.470
AG + GG	95	97	1.702 (0.396–7.321)		
Recessive model					
AA + AG	27	23	1.238 (0.652–2.352)	0.427	0.514
GG	73	77			
Alleles			1.275 (0.729–2.230)	0.504	0.478
A	16	13			
G	84	87			
GEMIN4 rs2740348 G/C					
Genotypes			1.515 (0.320–7.169)	0.072	0.788
GG	0	0			
GC	8	7.00			
CC	92	93.00			
Alleles			1.149 (0.409–3.230)	0.000	1.00
G	4	3.5			
C	96	96.5			

*p-value ≤ 0.05 is statistically significant

Table 2 Genotypes and age of onset and duration of disease

	Age at onset Mean ± SD (years)	*p*-value *	Duration of disease Mean ± SD (years)	*p*-value *
AGO1rs636832 A/G				
AA	24.000 ± 8.775	0.301	0.374 ± 0.217	0.301
AG	25.864 ± 12.021		2.087 ± 3.412	
GG	33.11 ± 15.496		2.581 ± 3.229	
Recessive model				
AA + AG	25.519 ± 11.362	0.022*	1.770 ± 3.142	0.264
GG	33.110 ± 15.496		2.581 ± 3.229	
GEMIN4 rs2740348 G/C				
GC	34.375 ± 18.5	0.513	1.334 ± 1.768	0.348
CC	30.772 ± 14.56		2.451 ± 3.297	

*p-value ≤ 0.05 is statistically significant

For GEMIN4 rs2740348 G/C, the CC genotype was seen more often than the GC genotype in ITP cases that did not respond to treatment and needed a second line of treatment like splenectomy or TPO-RA, but this did not reach statistical significance (Table 4).

Tables 3 and 4 show that there was no link between the patient's genotype and their sex, family history, or how ITP showed up in their bodies.

Discussion

The development of ITP may be explained by dysfunction in the immune system because of genetic abnormalities. However, the actual mechanism of ITP is still obscure [13]. miRNAs are expressed in the cells of the innate and adaptive immune systems and have a role in the regulation of both cell development and function. Zheng et al. study focused on the role of miRNA in T and B cell differentiation in the thymus and bone marrow [14]. During

Table 3 *rs636832 A/G* genotypes frequencies and ITP characteristics

	***rs636832 A/G* genotypes**						***p*-value ***	**Recessive model**				***p*-value ***
	AA		**AG**		**GG**			**AA + AG**		**GG**		
	N (5)	**%**	**N (22)**	**%**	**N (73)**	**%**		**N (27)**	**%**	**N (73)**	**%**	
Sex												
Male	1	20	3	13.64	9	12.33	0.881	4	14.81	9	12.33	0.743
Female	4	80	19	86.36	64	87.67		23	85.19	64	87.67	
Phase												
Newly diagnosed	2	40	8	36.36	21	28.77	**0.044***	10	37.04	21	28.77	0.304
Persistent	3	60	3	13.64	10	13.70		6	22.22	10	13.70	
Chronic	0	0	11	50	42	57.53		11	40.74	42	57.53	
Cutaneous bleeding												
Petechia and Purpura	5	100	21	95.45	72	98.63	0.613	26	96.30	72	98.63	0.459
Ecchymosis	1	20	13	59.09	29	39.73	0.155	14	51.85	29	39.73	0.277
Non-cutaneous bleeding												
Epistaxis	2	40	9	40.91	29	39.73	0.993	11	40.74	29	39.73	0.927
Vaginal Bleeding	2	40	7	31.82	24	32.88	0.939	9	33.33	24	32.88	0.966
Bleeding/gums	2	40	8	36.36	28	38.36	0.981	10	37.04	28	38.36	0.904
Grade of bleeding												
1	2	40	8	36.36	28	38.36	0.718	10	37.04	28	38.36	0.833
2	0	0	5	22.73	10	13.70		5	18.52	10	13.70	
3	3	60	9	40.91	35	47.95		12	44.44	35	47.95	
Treatment modalities												
Corticosteroid	4	80	18	81.82	55	75.34	0.808	22	81.84	55	75.34	0.517
Immunosuppressive	3	60	5	22.73	26	35.62	0.242	8	29.63	26	35.62	0.575
TPO-RA agonist	0	0	0	0	3	4.11	0.564	0	0	3	4.11	0.285
Splenectomy	0	0	2	9.09	8	10.96	0.722	2	7.41	8	10.96	0.599
Treatment Response												
No response	0	0	0	0	5	6.85	0.378	0	0	5	6.85	0.163
Response	5	100	22	100	68	93.15		27	100	68	93.15	
Death	0	0	0	0	1	1.37	0.830	0	0	1	1.37	0.541

Table 4 *rs2740348 G/C* genotypes frequencies and ITP characteristics

	***GEMIN4 rs2740348 G/C* genotype**				***p*-value ***
	GC		**CC**		
	N (8)	**%**	**N (92)**	**%**	
Sex					
Male	1	12.5	12	13.04	0.965
Female	7	87.5	80	86.96	
Phase					
Newly diagnosed	4	50	27	29.35	0.298
Persistent	0	0	16	17.39	
Chronic	4	50	49	53.26	
Cutaneous bleeding					
Petechia and Purpura	8	100	90	97.83	0.674
Ecchymosis	4	50	39	42.39	0.677
Non-cutaneous bleeding					
Epistaxis	4	50	36	39.13	0.547
Vaginal Bleeding	2	25	31	33.7	0.616
Bleeding/ gums	3	37	35	38.04	0.976
Grade of bleeding					
1	2	25	36	39.13	0.612
2	2	25	13	14.13	
3	4	50	43	46.74	
Treatment modalities					
Corticosteroid	8	100	69	75	0.107
Immunosuppressive	3	37.5	31	33.7	0.828
TPO agonist	0	0	3	3.26	0.604
Splenectomy	0	0	10	10.87	0.326
Response					
No response	0	0	5	5.34	0.499
Response	8	100	87	94.57	
Death	0	0	1	1.09	0.767

the effector phases of adaptive immunity, miRNAs contribute to the differentiation of T cells into functional lineages, class switching, germinal center formation in B cells, and activation of antigen-presenting cells (APCs) through pattern recognition pathways. MiRNAs are also directly involved in innate immunity, transduction signaling by Toll-like receptors (TLRs), and the ensuing cytokine response [14].

MiRNAs are predicted to directly regulate up to half of innate immune genes, as well as T and B cell survival and death, which is critical for preventing the uncontrolled proliferation of adaptive immune cells, which can lead to cancer or autoimmunity [15].

Recently, it was found that miRNAs are expressed in autoimmune diseases and may impact the development or prevention of autoimmunity. miRNA dysregulation is linked to autoimmune diseases that include rheumatoid arthritis, systemic lupus erythematosus, primary biliary cirrhosis, ulcerative colitis, psoriasis, idiopathic thrombocytopenic purpura, primary Sjögren's syndrome, and multiple sclerosis [16].

On the basis of their function in the development of tumors, microRNAs are divided into two categories: suppressor miRNAs (which inhibit the expression of oncogenes or genes that induce apoptosis) and oncogenic miRNAs (activating oncogenesis or inhibiting the expression of suppressor genes) [17].

The goal of this study is to find out how SNPs in the miRNA biogenesis pathway genes (rs636832 and rs2740348 in the AGO1 and Gemin4 genes, respectively) affect the development of primary ITP and how the patients would respond to treatment.

The rs636832 variant is found in the intron of AGO1, which is found on chromosome 1p35-p34. It might affect the conformation and function of proteins or the splicing of precursor miRNA. Its level of expression is linked to the number of Th17 cells, which affect the growth of different diseases and make people more likely to get cancer, autoimmune diseases, and viral infections [18].

The rs2740348 polymorphism is a G/C mutation located in the exon region of the GEMIN4 gene. The GEMIN4 protein was thought to be an essential molecule in the RNA-induced silencing complex, contributing to the maturation of miRNAs, target RNA recognition, and inhibition, but its role in cancer is still debated [19].

This is thought to be a pilot study to learn more about the rs636832 and rs2740348 single-nucleotide polymorphisms in ITP adult patients, how they relate to clinical and laboratory data, and how they influence response to treatment. The role of epigenetic markers in ITP, like the role of genetic markers, is unknown and underappreciated.

The results of the present study revealed no difference between the genotypic and allelic frequencies of AGO1_rs636832 A/G and Gemin4_rs2740348 G/C in ITP patients and controls. In ITP cases, a minor LD (linked disequilibrium) was discovered between both variants (p value 0.05). The LD referred to a non-random association of alleles on two or more chromosomes; it was not mandatory for this association to be on the same chromosome that leads to the inheritance of SNV alleles [18].

Concerning rs636832A/G, the results of this study showed that the GG genotype of rs636832 seems to affect older ITP patients and is statistically more common in patients with chronic ITP than the AA or AG genotypes. Neither variant had a significant relationship with sex, bleeding on the skin or elsewhere, or the amount of bleeding. Compared to the AA and AG genotypes, the rs636832 GG genotype was more common in ITP cases that did not respond to treatment and needed a second

line of treatment like TPO-RA or splenectomy, but this was not statistically significant. The rs2740348 CC genotype was found more often than the GC genotype in ITP cases that did not respond to treatment and needed a second line of treatment like splenectomy or TPO-RA, but this did not reach statistical significance.

Rabab et al. discovered similar results in their study of ITP patients. They investigated the association of single-nucleotide polymorphism (SNP) with ITP and concluded that no statistically significant difference was found between SNPs in the patient group in terms of age, sex, clinical characteristics, and laboratory data, or between the severity of bleeding and the genetic association of SNPs in the patient group, as well as no statistically significant differences in the distribution of alleles and genotypes.

Previous research on autoimmune diseases and cancer found that the AA genotype and A allele were associated with a lower risk of lymphatic metastasis of gastric carcinoma, similar to the A allele, which was associated with a lower risk of lymphatic metastasis [19]. When compared to the AA genotype, the rs636832 AG or GG genotype was associated with a lower risk of lung cancer [19].

Liu et al. discovered that rs2740348 was linked to a lower risk of prostate cancer. In colon cancer, a similar protective effect was observed [10]. According to Zhao et al., no association exists in colorectal cancer. Furthermore, there was no link to lung cancer, chronic myeloid leukemia, or gastric cancer [20]. On the contrary, Yang et al. discovered that rs2740348 was linked to an increased risk of bladder cancer [11].

As Zhou et al. documented the AGO1, our bodies responded differently to chronic infections in infectious disease. A single allele, rs636832, was associated with a lower risk of chronic HBV infection. Furthermore, AG and GG increased the risk of chronic HBV infection when compared to the AA genotype [8].

Previous studies on microRNA polymorphism yielded disparate results, which could be attributed to racial differences, specific immunological and malignant disease criteria, and the presence of other natural factors and linked genotypes. Still, the role of genetic polymorphism in miRNA biogenesis processes and its effect on human body expression are debatable and under investigation [21].

Conclusion

In conclusion, the rs636832 and rs2740348 SNPs did not appear to be risk factors for ITP; however, the rs636832:GG genotype was more prevalent in older patients, who tend to have the chronic phase of the disease. Although this distinction was not statistically significant, they were less responsive to therapy than the AA and AG genotypes. Concerning rs2740348: the CC genotype was more common than the GC genotype in ITP cases that did not respond to treatment and needed a second line of therapy like splenectomy or TPO-RA, but this did not reach statistical significance.

Recommendations

There have been no studies on ITP patients to prove the association of SNPs with disease development and treatment response; future studies on a larger scale of patients are required.

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Author contributions

IAM contributed to patients interview and clinical follow up, the analysis of study data, and writing and revising the manuscript; furthermore, she is the corresponding author. All authors have read and approved the manuscript. HSM is the principal investigator who had the research point, created the study design, patients interview and clinical follow up, and contributed to the analysis of the study data. WMA contributed to the laboratory work including blood samples collection, performing polymerase chain reaction and the analysis of data.

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Availability of data and materials

Data and materials are available upon request.

Declarations

Ethical approval and consent to participate

An informed written consent was taken from all the participants to participate and to publish their data and confidentiality of information was assured, an official written administrative permission letter was obtained from dean of faculty of medicine, Ain Shams University hospital with serial number 196.

Consent for publication

Consent to publish had been obtained from the participants or in the case of children, their parent or legal guardian.

Competing interests

The authors declare no competing financial interests or conflict of interest.

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