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Genes of the Integrin Family of Platelet Receptors (ITGA2 (C807T) and ITGβ3 (T1565C)): Features of Distribution and Analysis of the Role in Immune Thrombocytopenia

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Abstract The aim of the study was to analyze the distribution features and the role of platelet receptor integrin genes (ITGA2 (C807T) and ITGβ3 (T1565C)) in immune thrombocytopenia. The study involved 162 adults (median age 53.3±1.4 years), including 82 patients with CLD (1st main group: - 38 with chronic viral hepatitis (CH) and 44 with liver cirrhosis (LC)), who were treated in the therapy department of the Khorezm Regional Multidisciplinary Medical Center (KhRMMC) in the period from 2020 to 2023, and 80 conditionally healthy volunteers (mean age 51.8±1.7 years). SNP genotyping of the TLR4 (-728 GC) and TLR4 (-2272 AG) genes was performed. DNA was extracted from whole blood using the DNA-sorb-B reagent kit (Russia). Genotyping was performed by PCR using a universal reagent kit (Litech, Russia), according to the manufacturer's instructions. For mathematical calculations of the obtained results, the OpenEpi 2009, Version 9.2 statistical software package was used. The distribution of observed and expected genotype frequencies of the studied polymorphic gene was compared in accordance with the Hardy-Weinberg equilibrium (HWE) (P> 0.05), comparative analysis of SNP of the TLR4 (-728 GC) and TLR4 (-2272 AG) genes between the groups of patients and healthy individuals (case-control) was carried out by calculating the $\chi 2$ criterion, reliability (P), the odds ratio (OR) and the confidence interval (95% CI). The differences identified were considered reliable at $P \leq 0.05$. Taking into account the obtained results, it can be concluded that SNPs of the ITGA2 (C807T) and ITGβ3 (T1565C) genes are not involved in the mechanisms of increasing the risk of immune thrombocytopenia (ITP) development. However, the mutant allele and genotype of the ITGβ3 (T1565C) gene SNP are statistically significantly associated with a severe decrease in the number of platelets in immune thrombocytopenia (ITP).

Keywords Immune thrombocytopenia, Platelets, Integrins, ITGA2 (C807T), ITGβ3 (T1565C), Risk of development, Severity of thrombocytopenia

1. Introduction

The problem of immune thrombocytopenia (ITP) due to its widespread prevalence and severity of hemorrhagic complications has been of particular interest to scientists for centuries [1]. Studies on the epidemiological characteristics of ITP have shown that the prevalence rates of the disease vary significantly from 4.5 to 20 per 100 thousand people, but the incidence of the disease among the group of hemorrhagic diatheses averages 25%, with half of them having thrombocytopenia accompanied by hemorrhagic manifestations [2,3].

An analysis of the periods of study of immune thrombocytopenia shows their importance for the formation of modern views in terminology, understanding the mechanisms of their development, on the basis of which significant progress has been achieved in the diagnosis and treatment of these complex diseases [4,5]. Meanwhile, the question of the causes and conditions that contribute to the development of immune thrombocytopenia remains controversial today.

The complexity of the etiological and mechanisms of immune thrombocytopenia allows us to define it as a multifactorial pathology, an important role in the formation of which is given to both exogenous and endogenous development factors, the impact of which leads to the formation of antiplatelet antibodies leading to premature destruction of platelets in immune thrombocytopenia [6,7].

As a result of numerous studies, data has been accumulated to date on the high significance of the genetic component in the risk of developing immune thrombocytopenia, which largely determines the severity of its clinical course and the development of formidable complications [8,9].

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It is known that a large number of genes are involved in platelet differentiation [10,11,12]. Mutations in one of these genes can potentially lead to thrombocytopenia due to decreased formation or shortened lifespan of platelets [13].

When analyzing the literature on the involvement of the genetic component in the development and severity of immune thrombocytopenia, numerous studies were found to study the role of SNPs of cytokine genes. However, the results of the analysis of the influence of platelet receptor integrin genes in increasing the risk of immune thrombocytopenia and its severity are few [14,15].

The molecular pathogenesis of immune thrombocytopenia has not been fully studied, and no single process can be considered as an unambiguous mechanism for the development of this pathology [16].

In turn, conducting molecular genetic analyses of platelet receptor integrin genes (ITGA2 (C807T) and ITGβ3 (T1565C)) in immune thrombocytopenia will improve the understanding of the pathogenetic mechanisms of the disease, as well as develop criteria for predicting the formation, development, clinical course and outcomes of the disease, which will ultimately lead to broad opportunities for improving early diagnosis, the effectiveness of treatment results and the quality of life of patients with immune thrombocytopenia.

2. Main Body

2.1. The Purpose of Our Research

To analyze the distribution features and role of platelet receptor integrin genes (ITGA2 (C807T) and ITGβ3 (T1565C)) in immune thrombocytopenia.

2.2. Material and Methods of Study

The study involved 179 adults (median age 38.4±1.8 years), including 91 patients with immune thrombocytopenia (the 1st main group of patients) and 88 healthy volunteers without blood system pathology (the 5th control comparison group). Of the subjects, 48% were female and 52% were male.

The 1st main group of patients with immune thrombocytopenia (n=91) was divided into three groups depending on the platelet count:

the 2nd group of immune thrombocytopenia with a platelet level of > 50 x 10^9/l (n=36);

the 3rd group of immune thrombocytopenia with a platelet level of 30-50 x 10^9/l (n=32) and

the 4th group of immune thrombocytopenia with a platelet level of <30 x 10^9/l (n=23).

All patients included in the study were selected randomly as they applied to the Termez Regional Multidisciplinary Medical Center (TRMMC, Republic of Uzbekistan, Termez) in the period from 2021 to 2023.

In this study, all examined individuals underwent molecular genetic testing to study the SNP characteristics of the ITGA2 (C807T) and ITGβ3 (T1565C) genes. Genomic DNA was isolated from venous blood leukocytes using the Ribo-Preb kit (Russia). Allelic variants of the ITGA2 (C807T) and ITGβ3 (T1565C) genes were determined using restriction analysis of amplification products of genomic regions. DNA fragment amplification was performed using polymerase chain reaction (PCR) with specific oligonucleotide primers, with an annealing temperature of 600C (Rotor Gene Q, (Quagen, Germany), using “Syntol” test systems (Russia). Genotypes were visualized using electrophoretic separation of restriction products.

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Comparative analysis of allele and genotype frequencies of ITGA2 (C807T) and ITGβ3 (T1565C) gene polymorphisms was performed using Pearson's $\chi 2$ criterion, odds ratio (OR) and confidence interval (95% CI). The ratio of genotype frequencies of the studied genes was analyzed for compliance with the Hardy-Weinberg equilibrium. Differences were assessed as statistically significant at $P \leq 0.05$. Statistical calculations of the obtained results were performed using the OpenEpi 2009, Version 9.2 statistical software package.

2.3. Results of the Study

An assessment of the distribution of SNPs of the genetic markers ITGA2 (C807T) and ITGβ3 (T1565C) for compliance with RHT showed the absence of statistically significant differences between the observed (Ho) and expected (He) frequencies of genotypes in the groups of patients with immune thrombocytopenia and controls (RHT, $p>0.05$).

An assessment of the distribution of SNP genetic markers ITGA2 (C807T) and ITGβ3 (T1565C) for compliance with the Hardy-Weinberg equilibrium showed the absence of statistically significant differences between the observed (Ho) and expected (He) genotype frequencies in the groups of patients with immune thrombocytopenia and controls ($p>0.05$).

The polymorphism of the ITGA2 gene (C807T) was distributed according to the principle of dominance of the main allele and genotype, the maximum frequency of which was observed in the group of patients with immune thrombocytopenia with a mild decrease in platelets. The recessive allele C (19.3%) was most often recorded in the group of healthy people, while the frequency of heterozygote C/T (28.1%) was higher among patients with immune thrombocytopenia with moderate thrombocytopenia, and the mutant genotype T/T among patients with immune thrombocytopenia with severe thrombocytopenia (8.7%) (see Table 1).

Table 1. Distribution of ITGA2 (C807T) gene polymorphism in healthy groups and patients with immune thrombocytopenia

Group		Alleles, (n/%)				Genotypes, (n/%)					
№	Name	C		T		C/C		C/T		T/T	
		n	%	n	%	n	%	n	%	n	%
1	Main group of ITP, n=91	153	84.1	29	15.9	66	72.5	21	23.1	4	4.4
2	ITP with thrombosis (>50 x 10^9/l), n=36	62	86.1	10	13.9	27	75.0	8	22.2	1	2.8
3	ITP with thrombosis (30-50x10^9/l), n=32	53	82.8	11	17.2	22	68.8	9	28.1	1	3.1
4	ITP with thrombosis (<30x10^9/l), n=23	38	82.6	8	17.4	17	73.9	4	17.4	2	8.7
5	Control group, n=88	142	80.7	34	19.3	59	67.0	24	27.3	5	5.7

Table 2. Distribution of ITGB3 gene polymorphism (T1565C) in groups of healthy individuals and patients with immune thrombocytopenia

Group		Alleles, (n/%)				Genotypes, (n/%)					
№	Name	T		C		T/T		T/C		C/C	
		n	%	n	%	n	%	n	%	n	%
1	Main group of ITP, n=91	155	85.2	27	14.8	68	74.7	19	20.9	4	4.4
2	ITP with thrombosis (>50 x 10^9/l), n=36	66	91.7	6	8.3	30	83.3	6	16.7	0	0.0
3	ITP with thrombosis (30-50x10^9/l), n=32	54	84.4	10	15.6	23	71.9	8	25.0	1	31
4	ITP with thrombosis (<30x10^9/l), n=23	35	76.1	11	23.9	15	65.3	5	21.7	3	13.0
5	Control group, n=88	159	90.3	17	9.7	73	82.9	13	14.8	2	2.3

Meanwhile, the differences in the distribution of the ITGA2 (C807T) marker in the main and control groups were not statistically significant for the attenuated T allele (15.9% versus 19.3%; χ^2=0.7; P=0.5; OR=0.8; CI: 0.46-1.37), as well as the C/T (23.1% versus 27.3%; χ^2=0.4; P=0.6; OR=0.8; CI:0.41-1.57) and T/T (4.4% versus 5.7%; χ^2=0.2; P=0.7; OR=0.8; CI:0.2-2.93) genotypes. Statistically insignificant differences in the distribution of the polymorphic gene ITGA2 (C807T) show that the studied marker does not have an independent effect on the risk of developing immune thrombocytopenia.

The differences in the distribution of the ITGA2 (C807T) marker in the ITP group with moderate thrombocytopenia (2nd group) compared to the control group were also not significantly significant for the attenuated T allele (13.9% vs. 19.3%; χ2=1.0; P=0.4; OR=0.7; CI: 0.31 - 1.44), as well as the C/T (22.2% vs. 27.3%; χ2=0.3; P=0.6; OR=0.8; CI: 0.31 - 1.9) and T/T (2.8% vs. 5.7%; χ2=0.5; P=0.5; OR=0.5; CI: 0.06 - 4.02) genotypes.

The study of differences between the group of patients with moderate immune thrombocytopenia (3rd group) and the control group in the distribution of the ITGA2 (C807T) marker also did not reveal any significant differences, where the weakened T allele (17.2% versus 19.3%; χ2=0.1; P=0.8; OR=0.9; CI: 0.41 - 1.83) and T/T genotype (3.1% versus 5.7%; χ2=0.3; P=0.6; OR=0.5; CI: 0.06 - 2.57) were recorded less frequently than in the control, and the C/T genotype was not much more common (28.1% versus 27.3%; χ2<3.84; P=0.95; OR=1.0; CI: 0.42 - 2.57).

In the distribution of the ITGA2 (C807T) marker in the immune thrombocytopenia group with severe thrombocytopenia (4th group) compared to the control group, no reliable significant differences were again found for the attenuated T allele (17.4% vs. 19.3%; χ^2=0.1; P=0.8; OR=0.9; CI:0.38-2.05) and T/T genotype (8.7% vs. 5.7%; χ^2=0.3; P=0.6; OR=1.6; CI:0.29-8.62), as well as for the C/T heterozygote (17.4% vs. 27.3%; χ^2=0.9; P=0.4; OR=0.6; CI:0.18-1.8). Moreover, the statistically insignificant differences identified in the loci of the polymorphic gene ITGA2 (C807T) between the 4th and 5th groups also indicate the absence of independent participation of the studied marker in the risk of immune thrombocytopenia with severe thrombocytopenia.

Unlike the previous genetic polymorphism ITGA2 (C807T), noticeable changes were found in the distribution of the genetic marker ITGβ3 (T1565C) between the groups with immune thrombocytopenia and healthy subjects. The changes were characterized by a decrease in the frequencies of favorable loci and a simultaneous increase in unfavorable variants in the main group with immune thrombocytopenia, as well as in groups with moderate and severe thrombocytopenia in comparison with similar ones in the healthy group. However, in the group with moderate thrombocytopenia in relation to healthy subjects, the main loci were encountered more often, and weakened ones less often with a complete absence of carriers of the mutant homozygote C/C (Table 2).

Unlike the previous genetic polymorphism ITGA2 (C807T), noticeable changes were found in the distribution of the genetic marker ITGβ3 (T1565C) between the groups with immune thrombocytopenia and healthy subjects. The changes were characterized by a decrease in the frequencies of favorable loci and a simultaneous increase in unfavorable variants in the main group with immune thrombocytopenia, as well as in groups with moderate and severe thrombocytopenia

compared to similar ones in the healthy group. However, in the group with moderate thrombocytopenia, in relation to healthy subjects, the main loci were encountered more often, and weakened ones less often with a complete absence of carriers of the mutant homozygote C/C (Table 2).

Assessing the significance of the studied marker ITGβ3 (T1565C) according to functional features, it was established that there is no statistically significant association with the risk of immune thrombocytopenia. This conclusion was supported by the presence of statistically insignificant differences between the frequencies of the attenuated C allele (14.8% vs. 9.7%; χ^2=2.2; P=0.2; OR=1.6; CI:0.86-3.09), as well as the T/C (20.9% vs. 14.8%; χ^2=1.1; P=0.3; OR=1.5; CI:0.7-3.3) and C/C (4.4% vs. 2.3%; χ^2=0.6; P=0.5; OR=2.0; CI:0.36-10.75) genotypes between the main group with immune thrombocytopenia and healthy individuals.

Even less noticeable differences between the frequencies of alleles and genotypes for the polymorphic gene ITGβ3 (T1565C) were established when comparing the groups with immune thrombocytopenia with moderate thrombocytopenia and healthy subjects. Thus, in immune thrombocytopenia with moderate thrombocytopenia, in relation to healthy subjects, the frequency of the weakened allele C decreased without reaching unity (8.3% versus 9.7%; χ^2=0.1; P=0.8; OR=0.9; CI:0.32-2.25), and the proportion of the T/C genotype was 1.2 times higher (16.7% versus 14.8%; χ^2=0.1; P=0.8; OR=1.2; CI:0.4-3.32).

According to the genetic marker ITGβ3 (T1565C), in the group of patients with immune thrombocytopenia with moderate thrombocytopenia, the frequencies of the weakened allele C (15.6% versus 9.7%; χ^2=1.7; P=0.2; OR=1.7; CI: 0.75-3.98), genotype T/C (25.0% versus 14.8%; χ^2=1.7; P=0.2; OR=1.9; CI:0.72-5.14) and mutant genotype C/C (3.1% versus 2.3%; χ^2=0.1; P=0.8; OR=1.4; CI:0.12-15.68) showed dynamics towards an increase in their frequencies compared to healthy people, respectively, by 1.7; 1.9 and 1.4 times without reaching a significant level.

However, the results of comparing the differences in the distribution of the polymorphic marker ITGβ3 (T1565C) in immune thrombocytopenia with severe thrombocytopenia in relation to the healthy group showed a statistically significant increase in the frequencies of the attenuated allelic variant C by 2.9 times (23.9% versus 9.7%; χ^2=6.7; P=0.01; OR=2.9; CI:1.3-6.64) and the C/C genotype by 6.5 times (13.0% versus 2.3%; χ^2=4.9; P=0.05; OR=6.5; CI:1.24 -33.5) with an insignificant increase in the frequency of the T/C heterozygote by 1.6 times (21.7% versus 14.8%; χ^2=0.7; P=0.5; OR=1.6; CI:0.51-5.04).

The established statistically significant difference in the frequencies of the weakened C allele and the C/C genotype proves that these variants are statistically significantly associated with an increased risk of severe thrombocytopenia in immune thrombocytopenia by 2.9 and 6.5 times, respectively.

In addition, in the distribution of the polymorphic marker ITGβ3 (T1565C) between the groups of immune thrombocytopenia with moderate and severe thrombocytopenia, a statistically significant increase in the frequencies of the protective T allele by 3.5 times (91.7% versus 76.1%; χ^2=5.5; P=0.03; OR=3.5; CI:1.23-9.73) was found with a tendency to an increase in the frequency of the main T/T genotype by 2.7 times (83.3% versus 65.2%; χ^2=2.5; P=0.2; OR=2.7; CI:0.8-8.9) among patients with moderate thrombocytopenia. The obtained results show that among carriers of the wild T allele, the risk of developing severe thrombocytopenia is significantly reduced by 3.5 times, and among carriers of the main T/T genotype, there is a tendency for this risk to decrease by 2.7 times.

3. Conclusions

Analyzing the results of the study of the nature of the distribution of SNP of the ITGA2 gene (C807T) among patients with immune thrombocytopenia and healthy people, statistically significant differences between allelic and genotypic variants showing their participation in the risk of immune thrombocytopenia were not established.

In addition, analyzing the distribution features of the polymorphic loci of the ITGβ3 gene (T1565C) in groups of patients with immune thrombocytopenia and healthy individuals, no statistically significant association with the risk of immune thrombocytopenia and the studied marker was found. However, a reliable correlation was found between the carriage of weakened mutant alleles C and the C/C genotype with an increase in the risk of severe decrease in the number of platelets in immune thrombocytopenia by 2.9 (χ2=6.7; P=0.01) and 6.5 times (χ2=4.9; P=0.05), respectively.

In addition, the analysis revealed a statistically significant decrease in the risk of severe thrombocytopenia compared to moderate thrombocytopenia among carriers of protective T loci by 3.5 times (χ2=5.5; P=0.03) with a tendency to decrease this risk among carriers of the main T/T genotype by 2.7 times (χ2=2.5; P=0.2).

Therefore, taking into account the obtained results, it can be concluded that SNPs of the ITGA2 (C807T) and ITGβ3 (T1565C) genes are not involved in the mechanisms of increasing the risk of developing immune thrombocytopenia. However, the mutant allele and genotype of the ITGβ3 (T1565C) gene SNP are statistically significantly associated with a severe decrease in the number of platelets in immune thrombocytopenia.

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