MODERN MEDICINE AND PRACTICE
https://inovatus.es/index.php/ejmmp

EUROPEAN JOURNAL OF MODERNMEDICINEAND PRACTICE
Vol. 4 No. 5 (May - 2024) EJMMP ISSN: 2795-921X

MOLECULAR-GENETIC BASES FOR THE DEVELOPMENT OF PATHOLOGIES OF THE PLATE OF HEMOSTASIS

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Abstract: Objective: to assess the features of the prevalence and the contribution of polymorphic variants of TNF-α genes (rs1800629) in the formation of immune thrombocytopenia (ITP) and GP IIb (T2622G) in the development of dysaggregation thrombocytopathy (DTP).

Material and methods: the study included 89 patients with ITP and 71 patients with disaggregation thrombocytopathy (median age - 41 ± 1.7) for comparison of the group, 48 apparently healthy donors served as control without pathology of the hemostasis system (median age - 42 ± 1.4). Detection of TNF-α (rs1800629) and GP IIb (T2622G) gene polymorphisms was performed by SNP-PCR.

Results: carriage of heterozygous G / A genotype of rs1800629 polymorphism of TNF-α gene associated with a high risk of developing ITP, whereas the homozygous G / G genotype acts as a protective genotype in the pathogenesis of ITP. At the same time, the heterozygous T / G genotype of the T2622G polymorphism of the GPIIb gene in the main group and the hereditary dysaggregation thrombocytopathy (HDTP) subgroup are not statistically significantly associated with the development of the disease.

Conclusions: the results of molecular genetic studies can be used by clinicians in screening and predicting ITP and HDTP.

Key words: gene polymorphism, rs1800629 TNF-α, GPIIb (T2622G), immune thrombocytopenia (ITP), dysaggregation thrombocytopathy (DTP). allele, genotype, pathogenesis.

1. INTRODUCTION

The increasing interest of modern researchers is involved in the study of the mechanisms of the formation of pathologies of the platelet link of hemostasis, such as immune thrombocytopenia ITP - a primary decrease in the number of platelets (up to $100x10^9$/l and less) and disaggregation thrombocytopathy (RTP - decreased platelet aggregation function), which are united by a common for these diseases - hemorrhagic syndrome characterized by an increased risk of bleeding [1,3,9,11].

The studies carried out to date to study these pathologies have expanded the understanding of many aspects of their development. However, many mechanisms of their formation are still little known.

In recent years, the growing interest of both domestic and foreign scientists in order to uncover unclear aspects of the pathogenetic mechanisms of ITP and disaggregation thrombocytopathy is manifested in the study of the role of genetic polymorphisms in the pathogenesis of these diseases. Today, according to modern literary data, it is known that genetic factors play an important role in the genesis of ITP and DTP [5,6,7,10].

Today it is known that a large number of genes are involved in platelet differentiation, mutations in one of these genes can potentially lead to thrombocytopenia and thrombocytopathy due to decreased formation, shortened life expectancy and impaired platelet function [5,6,7].

In recent years, in the development of these pathologies, foreign researchers increasingly emphasize the significant role of such genetic polymorphisms as tumor necrosis factor (TNFa) and platelet glycoprotein (GP IIb).

At the same time, the existing data on the study of the relationship of TNFa with the formation of ITP, as well as GP IIb with the development of DTP, have ambiguous conclusions [1,2,4,8,12]. In this regard, additional studies to study the contribution of the rs1800629 polymorphism of the TNF-α gene to the risk of developing ITP and GP IIb (T2622G) in the development of DTP seems to be interesting and necessary.

2. OBJECTIVE: To assess the features of the prevalence and the contribution of polymorphic variants of TNF-α genes (rs1800629) in the formation of immune thrombocytopenia (ITP) and GP IIb (T2622G) in the development of dysaggregative thrombocytopathy (DTP).

3. DATA AND RESEARCH METHODOLOGY: The study included 89 patients with ITP (median age - 41 ± 1.7) and 71 patients with DTP (median age - 31.4 ± 1.18), who were on outpatient and inpatient treatment at the Republican Specialized Scientific and Practical Medical Center of Hematology (RSNPMCH) in the period from 2016 to 2018. All subjects were divided into 2 groups: 1st main patients and 2nd group of comparative control (conditionally healthy donors). Each of the main group is subdivided into two subgroups: for patients with ITP ("A" - 49 patients with ITP with hemorrhagic manifestations, and "B" - 40 patients with ITP without hemorrhagic manifestations); for patients with DTP (HDTP - 39 patients with a hereditary form of dysaggregation thrombocytopathy and ADTP - 32 patients with an acquired form of dysaggregation thrombocytopathy). The diagnosis of ITP and DTP was verified based on the recommendations of international experts (2009) [6].

Conditionally healthy donors without pathology of the hemostatic system were used as a comparative control (median age - 42.0 ± 1.4).

Detection of TNF-α (rs1800629) and GP IIb (T2622G) gene polymorphisms was performed by SNP-PCR on a programmable thermal cycler from Applied Bio systems 2720 (USA), using test systems of the company "Litekh" (Russia), according to the manufacturer's instructions. Mathematical analysis of the results was carried out using the statistical software package "Open Epi, Version 9.3".

4. DATA ANALYSIS

The observed frequency (Hobs) of genotypes of the studied polymorphisms revealed in the study in the general group of patients with ITP and DTP, as well as in the control group, corresponded to the expected distribution (Hexp) according to the Hardy-Weinberg equilibrium (P> 0.05).

5. RESULTS AND DISCUSSION

The results of the frequency distribution of the G allele of the rs 1800629 polymorphism of the TNF-α gene in the main ITP group were as follows: in the "A" subgroup - 83.7%; in "B" subgroup 83.8%, and in the control group this indicator was slightly higher (92.3%). The frequency of the A allele of the studied gene in the study group averaged 16.3%, and in the control group this indicator was much lower (7.4%).

These results indicate that in the main group the G allele ($\chi 2 = 6.31$; P = 0.012; OR = 0.41; 95% CI = 0.20-0.84) occurs somewhat less frequently than in the control group, while allele A, on the contrary, was more often observed in the main group ($\chi 2 = 6.31$; P = 0.012; OR = 2.43; 95% CI = 1.20-4.95). Analysis of the frequency distribution of the genotypes of the rs1800629 polymorphism of the TNF-α gene showed that homozygotes for the mutant allele A/A were identified in the main group (1.1%; $\chi 2 = 1.12$; P = 0.29), the

frequency of genotypes G/A (30.3% versus 14.8%) exceeded that in the control group (14.8%), while the frequency of the G/G genotype in the main and control was 68.5% versus 85.2% (table 1).

Table 1. Frequency of distribution of alleles and genotypes of gene polymorphism TNF-α (rs1800629) in the control group and in ITP patients

Group	n	Allele frequency				Genotype distribution frequency					
		G		A		G/G		G/A		A/A	
		n	%	n	%	n	%	n	%	n	%
ITP main group	89	149	83.7	29	16.3	61	68.5	27	30.3	1	1.1
"A" - subgroup	49	82	83.7	16	16.3	34	69.4	14	28.6	1	2.0
"B" - subgroup	40	67	83.8	13	16.2	27	67.5	13	32.5	0	0
Control group	81	150	92.3	12	7.4	69	85.2	12	14.8	0	0

According to the data given in table 1., it is obvious that, the differences in the frequency of occurrence of the A allele ($\chi2 = 6.31$; P = 0.012; OR = 2.43; 95% CI = 1.20-4.95, respectively) and the G / A genotype (OR = 2.55; $\chi2 = 5.98$; P = 0.014; 95% CI = 1.19-5.45) rs 1800629 polymorphism of the TNF-α gene between the main (ITP) and the control group are statistically significant.

In subgroups "A" and "B", the analysis of the difference in the frequency of the allele A of the rs 1800629 polymorphism of the TNF-α gene showed a statistically significant increase in more than 2.44 ($\chi2 = 5.05$; P = 0.024; OR = 2.44; 95 % CI = 1.10-5.40) and 2.43 times ($\chi2 = 4.52$; P = 0.03; OR = 2.43; 95% CI = 1.05-5.59), respectively. The frequency of the G/A genotype (rs 1800629) of the TNF-α gene also increased statistically significantly by more than 2.37 (OR = 2.37; $\chi2 = 3.86$; P = 0.049; 95% CI = 0.99-5, 67) and 2.77 times (OR = 2.77; $\chi2 = 5.11$; P = 0.02; 95% CI = 1.12-6.82), respectively, in subgroups "A" and "B" of patients with ITP. Consequently, the risk of developing ITP in the presence of this polymorphism of the studied gene, in general, statistically significantly increased by 2.43 (A) and 2.55 (G> A) times.

Based on the foregoing, it is obvious that against the background of a significant decrease in the carriage of the protective homozygous G / G genotype in the main group in patients with ITP diseases, there is an increase in the proportion of carriers of the heterozygous G/A genotype by about 1.7 times - up to 25.3%, which in turn indicates the presence of a reliable association between the carriage of the heterozygous G/A genotype of the rs 1800629 polymorphism of the TNF-α gene with the development of ITP.

Analyzing the severity of differences in the distribution of the frequencies of alleles and genotypes of the GPIIb gene polymorphism (T2622G) in the main group of DTP, an insignificant increase in the frequency of the G allele by 1.27 times was found ($\chi2 = 0.80$; P = 0.37; OR = 1.27; 95% CI: 0.75- 2.14) than in the control group.

Along with this, the frequency of occurrence of heterozygotes for the GPIIb (T2622G) polymorphism in the group of patients was less than 1-fold lower ($\chi2 = 0.12$; P = 0.72; OR = 0.86; 95% CI: 0.38-1.98).

At the same time, the frequency of the homozygous genotype G / G exceeded the values in the control by 1.68 times, but the differences did not reach statistical significance ($\chi2 = 0.98$; P = 0.32; OR = 1.68; 95% CI: 0.60-4.73) (table 2).

Table 2. Frequency distribution of alleles and genotypes of the T2622G polymorphism of the GPIIb gene in patient and control groups

№	Group	n	Allele frequency				Genotype distribution frequency					
			T		G		T/T		T/G		G/G	
			n	%	n	%	n	%	n	%	n	%
1	Main group	71	76	53,5	66	46,5	24	33,8	28	39,4	19	26,8
a	HDTP	39	38	48,7	40	51,3	11	28,2	16	41,0	12	30,8
b	ADTP	32	38	59,4	26	40,6	13	40,6	12	37,5	7	21,9
2	Control group	48	57	59,4	39	40,6	17	35,4	23	47,9	8	16,7

Further analysis of the results showed that in the group of HDTP patients, the share of the G allele was recorded less in relation to the control group (48.7% versus 59.4%), while the share of the T allele was higher (51.3% versus 40.6%).

In ADTP patients, the proportion of alleles G (59.4% versus 59.4%) and T (40.6% versus 40.6%) practically did not differ from those in the control.

The results of studying the distribution of genotypes made it possible to determine a lower registration of the heterozygous genotype T/G than in the control group by 1.08 times in patients with HDTP ($\chi2 = 0.02$; P = 0.89; OR = 1.08; 95% CI: 0.40-2.90) and less than 1 time in ADTP patients ($\chi2 = 0.56$; P = 0.45; OR = 0.68; 95% CI: 0.25-1.86).

A more pronounced increase in the frequency of the mutant genotype G/G among the studied groups of patients with DTP was observed only in patients with HDTP, which turned out to be 2.32 times higher than in the control ($\chi2 = 2.01$; P = 0.16; OR = 2.32; 95% CI: 0.72-7.49).

A more pronounced increase in the frequency of the mutant genotype G/G among the studied groups of patients with DTP was observed only in patients with HDTP, which turned out to be 2.32 times higher than in the control ($\chi2 = 2.01$; P = 0.16; OR = 2.32; 95% CI: 0.72-7.49). Despite the higher registration of the frequency of the mutant genotype in the group of patients with HDTP, the difference was insignificant.

Thus, summarizing the above data from the study of the features of TNF-α gene polymorphism (rs1800629) in the control group and in the group of ITP patients, we can conclude that, there is a statistically significant high association between the carriage of the unfavorable allele A and the G/A genotype of the TNF-α gene polymorphism (rs1800629) and the development of ITP. In this connection, the carriage of the minor allele A and the unfavorable genotype G/A of the TNF-α gene polymorphism (rs1800629) can be considered as a prognostic ally unfavorable marker, contributing to the high risk of developing ITP among people of Uzbek nationality.

Moreover, the obtained results of the study on the study of the peculiarities of the distribution of allele frequencies and genotypes of genetic polymorphism GPIIb (T2622G) in road traffic accident patients and conventionally healthy persons of Uzbek nationality showed the absence of a statistically significant association of the unfavorable G allele ($\chi2 = 1.80$; P = 0.37) and the mutant G/G genotype ($\chi2 = 0.98$; P = 0.32) with an increased risk of hereditary and acquired forms of road traffic accidents. At the same time, a tendency towards the development of the disease was found in patients with a hereditary form of dysaggregation thrombocytopathy ($\chi2 = 2.01$; P = 0.16).

Conclusions:

1. Carriage of the minor allele A and the heterozygous G/A genotype of the rs1800629 polymorphism of the TNF-α gene is associated with a high risk of developing ITP, which allows clinicians to use these results in screening and predicting ITP.
2. There was a tendency in patients with HDTP to increase the proportion of the G/G genotype of the GPIIb polymorphism (T2622G) compared with the control sample ($\chi 2 = 2.01$; P = 0.16; OR = 2.32; 95% CI: 0.72-7.49), these data indicate that this genotypic variant has a predisposing effect on the formation of disturbances in the regulation of aggregation and the development of HDTP in patients.

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