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Association of Allelic Polymorphism of the Proinflammatory Cytokine Gene VEGFA (rs2010963) with the Development and Severity of Immune Microthrombovasculitis

Dilfuza Saburovna Matkarimova[1,*], Khamid Yakubovich Karimov[2], Kodirjon Tukhtabaevich Boboev[2], Gulnozakhon Atabaevna Matniyazova[3]

[1]Department of Hematology, Transfusiology and Laboratory Science, Tashkent Medical Academy, Tashkent, Uzbekistan
[2]Department of Molecular Medicine and Cell Technologies, Scientific Research Institute of Hematology and Blood Transfusion of the Ministry of Health of the Republic of Uzbekistan, Tashkent, Uzbekistan
[3]Department of Faculty and Hospital Therapy, Urgench Branch of the Tashkent Medical Academy, Uzbekistan, Urgench City, Uzbekistan

Abstract Purpose of the study: To assess the role of the polymorphic variant of the VEGFA (rs2010963) in the development of a severe course of immune microthrombovasculitis (IMTV). The study included 75 patients with immune microthrombovasculitis (IMTV) (main group) aged 16 to 80 years and 73 conditionally healthy individuals (comparison group) without pathology of the hemostasis system, comparable in age and gender with the main group. Detection of polymorphic variants of genes TNF-α (rs 1800629) and VEGFA (rs2010963) was carried out by SNP-PCR. Differences in the frequency of occurrence of the mutant genotype G/G ($\chi 2 > 3.8$; $P < 0.05$) of the polymorphic variant of the VEGFA gene (rs2010963) were established depending on the severity of IMTV, which allows clinicians to determine it as a genetic predictor of severe disease.

Keywords VEGFA (rs2010963), Immune microthrombovasculitis (IMTV), Allele, Genotype, Pathogenesis, Severity of the course

1. Introduction

In everyday practice, doctors of almost all specialties encounter various forms of hemorrhagic diathesis (HD), while their late and late diagnosis leads to the use of long-term unjustified therapeutic tactics in patients, as well as the development of thrombohemorrhagic complications, which significantly reduce their quality of life [1,4,5,13].

In terms of the frequency of its occurrence among all populations and age groups of the population, polymorphism and severity of the clinical course in the general structure of HD, a special place is given to immune microthrombovasculitis (IMTV). However, the consequence of insufficient knowledge of the pathogenesis of the disease is the high frequency of its complications and relapses [3,22,23,24].

At the same time, the analysis of the research results of recent years indicates the important role of gene polymorphisms in the mechanisms of the formation of IMTV [2,7,8,9]. In addition, there are data on the influence of functional polymorphisms of a number of genes on the nature of the course, which determine the possibility of predicting the outcome of this disease [11,12,14]. Meanwhile, the presence of conflicting results of the currently existing studies on the contribution of various genetic polymorphisms in the genesis of these diseases, and thus the unknown and unresolved many aspects of these major problems determine their relevance [14].

The results of studies by foreign authors have shown an assessment of the relationship of genetic polymorphisms with the development of a severe course of IMTV [10,17]. In particular, Chinese researchers Liu Desong, Lu Fang, Zhai Songhui, Wei Liu, Ma Shi, Chen Xiuying et al. (2010) found that polymorphisms of the genes of the renin-angiotensin system (ACE-I / D, M235T and T174M) are significantly associated with the severity of the BMI course ($p = 0.045$ and $p = 0.026$) [10]. Mahsa M. Amoli et al. (2014) and López-Mejías R. et al. determined that polymorphism of the IL 1 β gene (rs16944) is important in the development of severe renal manifestations in IMTI [16,17].

* Corresponding author:
alteam2201@gmail.com (Dilfuza Saburovna Matkarimova)
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Along with this, in recent years, researchers have increasingly emphasized the weighty role of allelic polymorphisms of the endothelial vascular growth factor gene (VEGFA) in both the development and severity of IMTB [2,18].

Taking these facts into account, we studied the possible association of the VEGF A gene polymorphism (rs2010963) with the risk of developing immune microthrombovasculitis (Schonlein-Genoch purpura) and the severity of its clinical course.

2. Main body

2.1. The Purpose of Our Research

To assess the role of the polymorphic variant of the VEGFA (rs2010963) in the development of a severe course of immune microthrombovasculitis (IMTV).

2.2. Material and Methods of Study

The study included 75 adult unrelated patients of the Uzbek ethnic group aged 16 to 80 years (median age 42.1 ± 3.9 years), who constituted the main group with an established diagnosis of immune microthrombovasculitis (Schönlein-Henoch purpura) according to the modern EULAR classification criteria. PRINTO and PreS (2010) [7]. All patients were observed in the consultative and diagnostic polyclinic of the Republican Specialized Scientific and Practical Medical Center of Hematology (RSNPMCG) in the period from 2012 to 2018 yy. The control group consisted of 73 healthy unrelated persons of uzbek nationality, who had no history of inflammatory, allergic, systemic and renal diseases that matched age and sex with the examined group of patients. Written informed consent was obtained from all patients, as well as from individuals of the control group, to conduct the study.

Detection of the VEGFA gene polymorphism (rs2010963) was carried out by SNP-PCR on a programmable thermal cycler of the company "Applied Biosystems" 2720 (USA), using test systems of the company of the RPC "Litech" (Russia). The specificity and the number of amplified fragments were checked by agarose gel electrophoresis.

Statistical analysis of the results was carried out using the statistical software package "OpenEpi, Version 9.3".

2.3. Results of the Study

Table 1. Frequency of allele distribution and genotype distribution of VEGFA gene polymorphism (rs2010963) in patient and control groups

Groups		n	Frequency of allele distribution				Frequency of genotype distribution					
			C		G		C/C		C/G		G/G	
			n	%	n	%	n	%	n	%	n	%
The main group of IMTV, n=75:		75	103	68.7	47	31.3	33	44.0	37	49.3	5	6.7
Subgroup	"A", n=41	41	56	68.3	26	31.7	17	41.4	22	53.7	2	4.9
	"B", n=34	34	47	69.1	21	30.9	16	47.1	15	44.1	3	8.8
Control group, n=73		73	126	86.3	20	13.7	55	75.3	16	21.9	2	2.7

Table 2. Association between the VEGF A gene polymorphism (rs2010963) and the risk of developing IMTV

Groups		Alleles and genotypes	Statistical difference					
			Relative risk		Odds ratio		χ^2	p-value
			RR	95% CI:	OR	95% CI:		
The main group of IMTV, n=75:		C	0.64	0.52– 0.79	0.35	0.19– 0.62	13.14	0.0003*
		G	1.56	1.26– 1.93	2.87	1.60– 5.16		
		C/C	0.54	0.39– 0.74	0.26	0.13– 0.52	15.1	0.0001*
		C/G	1.86	1.35– 2.57	3.85	1.86– 7.98	13.8	0.0002*
		G/G	1.90	1.11– 3.27	4.17	0.76– 22.7	3.11	0.08
Subgroup	"A", n=41	C	0.54	0.39– 0.76	0.34	0.18– 0.66	10.57	0.001*
		G	1.84	1.32– 2.57	2.93	1.51– 5.67		
		C/C	0.41	0.25– 0.68	0.23	0.10–0.53	12.9	0.0003*
		C/G	2.45	1.49– 4.03	4.45	1.9– 10.33	12.8	0.0003*
		G/G	2.12	0.73– 6.14	3.24	0.42– 24.73	1.41	0.23
	"B", n=34	C	0.53	0.36– 0.78	0.36	0.18– 0.71	8.84	0.003*
		G	1.89	1.28– 2.77	2.81	1.40– 5.66		
		C/C	0.45	0.26– 0.77	0.29	0.12– 0.69	8.31	0.004*
		C/G	2.15	1.22– 3.77	3.22	1.31– 7.91	6.82	0.009*
		G/G	2.66	1.15– 6.14	5.16	0.79– 33.58	3.50	0.061

Analysis of the results of assessing the distribution of allelic and genotypic variants of the VEGFA gene polymorphism (rs2010963) among the studied groups showed some peculiarities. Thus, a significant increase in the proportion of carriers of unfavorable allele G from 13.7% to 31.3% ($\chi2$=13.14; P=0.0003; OR=2.87; 95%CI 1.60-5.16) was registered among the main group patients. Approximately the same values of G allele carrier were observed in both the "A" subgroup of patients in the remission stage (31.7%; $\chi2$=10.57; P=0.001; OR=2.93; 95% CI 1.51-5.67) and the "B" subgroup of patients in the remission stage (30.9%%; $\chi2$=8.84; P=0 003; OR=2.81; 95% CI 1.40-5.66), which were also significantly different from those in the healthy controls (13.7%) (Table 1), indicating the association of this allele with an increased risk of IMTV.

It should be noted that carriers of all three genotype variants, including the rarely encountered G/G genotype, were present in both subgroups of patients, as well as in the control group of healthy individuals. As an inevitable consequence of the increase in the proportion of G allele carriage in both subgroups of patients with immune microthrombovasculitis, a significant decrease in C/C genotype carriers and a significant increase in the detectability of the heterozygous C/G genotype and some increase in the homozygous G/G genotype were noted.

The protective role of the homozygous C/C genotype in the pathogenesis of immune microthrombovasculitis is indirectly indicated by the significant decrease in the proportion of carriers of this genotype in the main group of patients ($\chi2$=15. 1; P=0.0001; OR=0.26; 95% CI 0.13-0.52), as well as in patients in subgroup "A" ($\chi2$=12.9; P=0.0003; OR=0.23; 95% CI 0.10-0.53) and subgroup "B" ($\chi2$=8.31; P=0.004; OR=0.29; 95% CI 0.12-0.69) (Table 2).

In patients with IMTV against the background of a significant decrease in the carriage of the protective homozygous C/C genotype from 86.3% to 68.7% there was an increase in the proportion of carriers of the heterozygous C/G genotype from 21.9% to 49.3% ($\chi2$=13.8; P=0.0002; OR=3.85; 95%CI 1.86-7.98) both due to patients in subgroup "A" ($\chi2$=12. 8; P=0.0003; OR=4.45; 95% CI 1.91-10.33) and patients in subgroup "B" ($\chi2$=6.82; P=0.009; OR=3.22; 95% CI 1.31-7.91), clearly indicating a significant association between the heterozygous C/G genotype rs2010963 polymorphism of the VEGFA gene with the development of immune microthrombovasculitis. Determining the prognostic value (AUS) revealed that the rs2010963 polymorphism of the VEGFA gene acts as a middle classifier marker in the formation of immune microthrombovasculitis (C/G: Se, %- 52.86; Sr, %- 77.46; AUC, 65.2; OR, 3.85; $\chi2$ = 13.8; P = 0.0002).

Thus, our results suggest that functionally unfavourable G allele and heterozygous C/G genotype of rs2010963 polymorphism of VEGFA gene are significant markers of increased risk of immune microthrombovasculitis formation in persons of Uzbek nationality. Determination of prognostic value of heterozygous C/G genotype (in the main group AUS=0.65, in "A" subgroup AUS=0.67 and in "B" subgroup AUS=0.63) showed that it acts as a middle classifier marker in the formation of immune microthrombovasculitis.

Next, we analyzed the significance of the polymorphic variant of the VEGFA gene (rs2010963) in the risk of developing a severe course of IMTV. According to our data, carriage of C/C and C/G genotypes in patients with mild degree of disease severity was detected in 54.5% and 45.5% of cases, respectively; in moderate degree of disease severity their values were 53.6% and 46.4%, respectively, and in severe degree of disease severity - 24.0% and 56.0%, respectively. In addition, it should be noted that only in patients with a severe degree of severity there was registered the carriage of the mutant G/G genotype, which amounted to 20.0%.

Comparative analysis of the observed differences between the carriage of the VEGFA gene polymorphism (rs2010963) genotypes depending on IMTV severity revealed no significant differences in relation to the proportion of C/C and C/G genotypes carriage between patients with mild and moderate severity ($\chi2$<3.8; P>0.05).

Meanwhile, a comparative analysis in the frequency distribution of VEGFA gene polymorphic variant (rs2010963) genotypes between patients with mild and severe IMTV revealed a statistically significant difference with regard to C/C and G/G genotypes (for C/C genotype: $\chi2$=4.6; P=0.03; P=0.02). 6; P=0.03; for the G/G genotype: $\chi2$=4.9; P=0.02), whereas no significant difference was found in the proportion of heterozygous C/G genotype ($\chi2$=0.4; P=0.5).

The frequency of the C/C and C/G genotypes of the VEGFA gene (rs2010963) in IMTV patients with moderate and severe severity did not differ between themselves statistically significantly (for the C/C genotype: $\chi2$=4.8; P=0.03; for the C/G genotype: $\chi2$=0.5; P=0.5). However, a significant difference was found in the proportion of carriage of the G/G mutant genotype ($\chi2$=6.2; P=0.01).

Thus, the study of the association between the carriage of unfavorable genotypes of the VEGFA gene polymorphism (rs2010963) and the severity of IMTV revealed no statistically significant differences with regard to the heterozygous C/G genotype ($\chi2$<3.8; P>0.05). However, for the unfavorable G/G genotype, there was an association with the risk of developing a severe course of IMTV ($\chi2$=4.9; P=0.02 and $\chi2$=6.2; P=0.01). Consequently, the G/G genotype of the VEGFA gene polymorphism (rs2010963) is a prognostic marker of the development of a severe course of IMTV among persons of Uzbek ethnicity.

3. Conclusions

Immune microthrombovasculitis (IMTV, Schonlein-Genoch disease) is a multifactorial disease with a rather complex mechanism of development where genetic factors play a special role [1,20]. The clinical manifestations of IMTV are characterized by their

polymorphism, the severity of which depends on the nature and severity of the disease [4]. At the same time, it is known that genetic polymorphisms play an important role in the severity of the pathological process [2,3]. This is evidenced by the results of studies by a number of foreign authors evaluating the association of genetic polymorphisms with the development of a severe course of IMTV [9,12,21]. In this regard, analysis of the distribution of allelic and genotypic variants of proinflammatory cytokine genes in patients with immune microthrombovasculitis, determination of the role of carriage of unfavorable genotypes of these gene polymorphisms in the formation of course severity, their association with the severity of clinical manifestations of IMTV is of particular importance.

Our findings demonstrated that the frequency of heterozygous C/G genotype of VEGFA gene (rs2010963) in the group of patients with immune microthrombovasculitis was significantly higher compared to the control group (49.3% vs 21.9%; $\chi 2$=13.8; P=0.0002; OR=3.85; 95%CI 1.86-7.98), which in turn indicates the involvement of this polymorphism in the increased risk of IMTV development.

The results of our studies demonstrated that the frequency of heterozygous C/G genotype of VEGFA gene (rs2010963) in the group of patients with immune microthrombovasculitis is significantly higher compared to the control group (49.3% versus 21.9%; $\chi 2$=13.8; P=0.0002; OR=3.85; 95%CI 1.86-7.98), which in turn indicates the involvement of this polymorphism in increasing risk of IMTV.

The study of the association between the carriage of unfavorable genotypes of the polymorphic variant of the VEGFA gene (rs2010963) and the severity of the IMTV course revealed a significant difference with regard to the G/G mutant genotype ($\chi 2$=6.2; P=0.01). Consequently, the established differences in the frequency of G/G mutant genotype of the VEGFA gene (rs2010963) in IMTV depending on the severity allows clinicians to identify it as a genetic predictor of the development of a severe course of the disease.

The findings suggest that the VEGFA gene polymorphism (rs2010963) may be involved in the processes of endotheliocyte damage as well as in the modulation of inflammatory processes. Together with other risk factors, this polymorphism may determine the development of a severe course of the disease and, from the clinical point of view, contribute to predicting the clinical course and development of complications in patients with IMTV of Uzbek ethnicity, allowing targeted planning of therapeutic strategies.

To conclude the discussion, it should be noted that the obtained results of the study allow us to expand the current understanding of the mechanisms of the formation of a severe course of IMTV. Analysis of genetic polymorphisms and the frequency of their allelic and genotypic variants, taking into account ethnic characteristics, allows monitoring among the population to identify predisposition to the development of an unfavorable course of the disease, assess the prognosis, differential approach in the management and prevention of complications in patients with IMTV.

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