护理杂志
Journal of Nursing
Volume 70 Number 9
ISSN: 0047-262X

Effects Of Polymorphisms RS1801133 Of The MTHFR Gene And RS2010963 Of The VEGF-A Gene On The Risk Of Lower Extremity Varicose Veins And Its Complications

Yariyev Alisher[1], Ermatov Nizom[2], Shaykhova Guli[2], Sherkuzieva Guzal[3], Bakhritdinov Shakhobiddin[2], Ortiqov Bobomurod[4] and Xasanova Muxarrama[5]

[1]DSc, Syrdarya Branch of The Republican Scientific Center for Emergency Medical Care of the Ministry of Health of The Republic of Uzbekistan.
[2]Professor, Department of Hygiene of Children, Adolescents and Nutrition, Tashkent Medical Academy, Forobiy-32, 100109, Tashkent, Uzbekistan.
[3]Associate Professor, Department of Environmental Hygiene, Tashkent Medical Academy, Forobiy-32, 100109, Tashkent, Uzbekistan.
[4]PhD, Senior Teacher, Department of Hygiene of Children, Adolescents and Nutrition, Tashkent Medical Academy, Forobiy-32, 100109, Tashkent, Uzbekistan.
[5]PhD, Department of Forensic Medicine and Medical Law, Tashkent Medical Academy, Forobiy-32, 100109, Tashkent, Uzbekistan.

Abstract

The study showed that the wild homozygous C/C genotype showed protective properties in relation to the development of varicose vein ($\chi 2>3.84$; $p<0.05$; RR=0.1; 95%CI:0.06-0.25; OR=0.1; 95%CI: 0.03-0.15), while the heterozygous C/G genotype and the mutant homozygous G/G genotype acted as genetic markers of the risk of thrombotic complications in patients with VLVL ($\chi 2>3.84$; $p<0.05$; RR=5.6; 95% CI:2.6- 11.9; OR=8.2, 95%CI:3.3-20.3) and ($\chi 2>3.84$; $p<0.05$; RR=13.6; 95%CI:5.4-34.1; OR=23.6; 95%CI:8.2-67.4). In general, according to researchers, the rs2010963 polymorphism of the VEGF-A gene is a predictor of various pathologies, including vascular ones [68, p. 481-485; 11, p. 433-438]. However, the role of this polymorphic locus in the occurrence of LVLE and venous thrombosis has not been sufficiently studied [50, p. 115-117], in connection with which this study was conducted.

Keywords: *Polymorphisms, MTHFR Gene, VEGF-A Gene, Varicose Veins.*

INTRODUCTION

Varicose veins of the lower extremities are a peripheral vascular disease and represent a serious health problem worldwide. It is more common among the elderly and leads to vascular occlusion, especially of the lower extremities [1]. The rs1801133 (C677T (Ala222Val) polymorphism of the methylenetetrahydrofolate reductase (MTHFR) gene has been associated with various diseases (vascular, cancer, neurological, diabetes, psoriasis, etc.) and the epidemiology of this rs1801133 polymorphism varies depending on geography and ethnicity. In the United States, approximately 20-40% of Hispanics are heterozygous for MTHFR rs1801133, this polymorphism is less common in US blacks (~2%), and in North America, Europe and Australia, approximately 8-20% of the population is homozygous for MTHFR rs1801133 [2, 3]. The MTHFR gene, the enzyme methylenetetrahydrofolate reductase, plays an important role in the processing of amino acids, the building blocks of proteins. Methylenetetrahydrofolate reductase converts 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. It is the main form of folic acid found in the blood and is required for the multi-step process of converting the amino acid homocysteine to another amino acid, methionine [4]. The body uses methionine to make proteins and other important

compounds. The 5, 10-methylenetetrahydrofolate reductase (MTHFR) locus is mapped on chromosome 1 at the end of the short arm [5]. The MTHFR gene mutation that causes the rs1801133 polymorphism is located in exon 4, which leads to the conversion of valine to alanine at codon 222, which is a common polymorphism that reduces the activity of these enzymes [6]. According to the literature data, the polymorphism of the MTHFR rs1801133 locus gene is closely associated with the occurrence of a number of diseases [7]. In the studies of some authors, the MTHFR rs1801133 mutation was associated with the occurrence of deep vein thrombosis, and the T/T genotype is a genetic risk factor for deep vein thrombosis [8]. Some authors showed in their studies the association of the polymorphic locus rs1801133 of the MTHFR gene with deep vein thrombosis [9, 10], while others reported no significant association between MTHFR rs1801133 and deep vein thrombosis [11]. Due to the lack of data on the effect of MTHFR gene polymorphism on the clinical course and the occurrence of complications in lower extremity LVLL, in our study we decided to investigate the role of the MTHFR gene in the development of LVLL and its complication, deep vein thrombosis of the lower extremities, venous thrombosis.

PURPOSE OF THE RESEARCH

To study effects of polymorphisms rs1801133 of the MTHFR gene, rs2010963 of the VEGF-A gene on the risk of lower extremity varicose veins and its complications.

MATERIALS AND METHODS

In our work, one of the studied genes was MTHFR (rs1801133) the prevalence of this gene depends on the geographical distribution and ethnicity throughout the world [12].

The results of our study showed that the frequency of the rs1801133 polymorphism in the MTHFR gene among patients with VLVL and venous thrombosis is quite high. Some studies have confirmed a weak association between an increased risk of venous thromboembolism and the rs1801133 polymorphism of the MTHFR gene, but this association was more often population-based and was not found in North America, which may be due to high dietary intake of riboflavin and folic acid. In Turkish and Iranian populations, the prevalence of this gene was 49.6% and 67.0%, respectively [13]. According to the results of Li A. et al. (2020) the rs1801133 polymorphism of the MTHFR gene was expressed in patients with carotid atherosclerosis [14]. According to the authors, genetic polymorphisms rs1801133 and A1298C of the MTHFR gene were associated with cerebral venous sinus thrombosis [15]. In addition, these loci have been associated with increased susceptibility to the development of venous thromboembolic disease, which includes deep vein thrombosis and pulmonary embolism [16].

A total of 316 people were involved in the study, which were included in the following groups: the main group of patients with a hereditary predisposition to VLLE and phlebothrombosis (n=161), from which subgroups were identified; a group of patients with varicose veins (VVVD) (n=111), a group of patients with venous thrombosis (n=50) and a control group - 155 conditionally healthy subjects.

RESULTS AND DISCUSSION

As a result of the study of the distribution frequency of alleles and genotypes of the rs1801133 polymorphism in the MTHFR gene for differences in their distribution in the main group of patients with a hereditary predisposition to VLVD and phlebothrombosis and the control sample presented in Table 1 and Figure 1, the C allele prevailed in the control group, and its frequency was 63.7% vs. 77.7% ($\chi 2>3.84$; $p<0.05$; OR=0.5; 95% CI: 0.35-0.71), and the T allele prevailed in the group of

patients with VLVL and venous thrombosis, its frequency was 36.3% vs. 22.3%, respectively ($\chi2>3.84$; $p<0.05$; OR=2; 95%CI:1.40-2.83). Thus, according to the data obtained, it can be seen that in the main group there was a predominance of the T frequency, while in the control group, the C allele prevailed, the detection frequency of which was higher ($\chi2> 3.84$; $p<0.05$; OR=2.0; 95% CI: 1.40-2.83) (Table 1).

The distribution frequency of the C/C, C/T, T/T genotypes of the rs1801133 polymorphism in the MTHFR gene in the main group of patients and controls was 48.4%, 30.4%, and 21.1% versus 62.6%, 30.3%, and 7.1%, respectively.

The wild C/C genotype was statistically significantly less frequently detected in the main group, in which its proportion was 48.4%, versus 62.6% in the control group ($\chi2>3.84$; $p<0.05$; OR=0.6; 95%CI: 0.36-0.88).

Table 1: Association between the rs1801133 polymorphism in the MTHFR gene in the main group of patients with VLVL and in the control group

Study Groups	Alleles and genotypes	Statistical difference			
		Odds ratio		χ^2	p-value
		OR	95% CI:		
Main group[(n=161)	C	0.5	0.35–0.71	15.1	0.0001*
	T	2.0	1.40–2.83		
	C/C	0.6	0.36–0.88	6.4	0.01*
	C/T	1.3	0.79–2.14	1.0	0.31
	T/T	3.8	1.83–8.08	13.7	0.0002*

Note: * - statistically significant

The incidence of the heterozygous C/T genotype was almost at the same level among the patients of the main group and in the control group, amounting to 30.4% versus 30.3%, respectively ($\chi2=1$; $p>0.05$; OR=1.3; 95% CI: 0.79-2.14).

The proportion of the unfavorable T/T genotype was significantly higher in the main group of patients compared to the control group, 21.1% vs. 7.1%, respectively ($\chi2>3.84$; $p<0.05$; OR=3.8; 95%CI:1.83-8.08) (Table. 1).

Thus, it was found that the carriage of a homozygous mutant T/T genotype increases the risk of developing LVLV by almost 4 times ($\chi2>3.84$; $p<0.05$; OR=3.8; 95% CI: 1.83-8.08).

A study of a subgroup of patients with VLVL without thrombotic complications showed that the proportion of allele C in the control sample, which was 63.1%, was lower than among patients with VLVL, among whom it was 77.7% ($\chi2>3.84$; $p<0.05$; OR=0.5; 95%CI: 0.33-0.72).

At the same time, the unfavorable T allele prevailed in the subgroup of patients relative to the control group, where its frequency was 36.9% versus 22.3%, respectively ($\chi2>3.84$; $p<0.05$; OR=2; 95% CI: 1.40-3.00) (Table 2).

The C/C, C/T, T/T genotypes of the rs1801133 polymorphism of the MTHFR gene among patients with VLVL and in the control group was distributed as follows: 49.5%, 27.0% and 23.4% versus 62%, 30% and 7%, respectively.

The proportion of the wild C/C genotype was significantly lower among patients with uncomplicated LVLD, amounting to 49.5%, relative to conditionally healthy individuals in the control group, where its proportion was 62.6% ($\chi2> 3.84$; $p<0.05$; OR=0.6; 95% CI :0.36-0.96).

In the study of the distribution of the C/T genotype, no statistically significant differences were found - there was an insignificant excess in the frequency of detection of this genotype among conditionally healthy patients, compared with the group of patients with VLVL, where they were detected in 27.0% and 30.3%, respectively ($\chi2 = 0.2$; $p>0.05$; OR=1.1; 95%CI: 0.64-1.98).

The proportion of the mutant T/T genotype was statistically significantly higher among LVL patients without venous thrombosis, amounting to 23.4% versus 7.1% in the control sample ($\chi2>3.84$; $p<0.05$; OR=4.2; 95% CI: 1.91-9.08) (Table. 2).

Table 2: Association between the rs1801133 polymorphism in the MTHFR gene in groups of patients with VLVL and in the control group

Study Groups	Alleles and genotypes	Statistical difference			
		Odds ratio		χ2	p-value
		OR	95% CI:		
Varicose disease (n=111)	C	0.5	0.33–0.72	13.7*	0.0002*
	T	2.0	1.40–3.00		
	C/C	0.6	0.36-0.96	4.5*	0.03*
	C/T	1.1	0.64–1.98	0.2*	0.68
	T/T	4.2	1.91–9.08	14.1*	0.0002*

Note: the same as in Table. 1.

Thus, in the course of the study of the distribution frequency of alleles and genotypes of the rs1801133 polymorphism in the MTHFR gene for differences in the group of patients with uncomplicated venous thrombosis of the form of VLVD and the control sample, it was found that the homozygous T/T genotype can more than fourfold increase the risk of structural changes vein walls and development of VLLE ($\chi2>3.84$; $p<0.05$; OR=4.2; 95%CI:1.91-9.08).

When studying the distribution frequency of alleles and genotypes of the rs1801133 polymorphism in the MTHFR gene for differences in the studied groups, it was found that the C allele was less frequently detected among patients with venous thrombosis, compared with apparently healthy individuals in the control sample, where they were detected in 65.0% and 77.7% of cases, respectively ($\chi2=6.5$; $p>0.01$; RR=0.6; 95%CI:0.33-0.87; OR=0.5), which indicates the protective nature of this allele.

At the same time, T allele carriers were more common among patients with venous thrombosis, in contrast to conditionally healthy subjects - 35.0% versus 22.3%, respectively ($\chi2>3.84$; $p<0.05$; OR=1.9; 95% CI: 1.15-3.07).

The proportion of wild C/C genotype among patients with thrombotic complications was 46.0%, which was significantly lower than in the control group, where its proportion was 62.6% ($\chi2>3.84$; $p<0.05$; OR=0.5; 95% CI :0.27-0.97).

The heterozygous C/T genotype in patients with venous thrombosis was found statistically insignificantly more often than in the control group, where they were detected in 38.0% and 30.3% of cases, respectively ($\chi2=2.2$; $p>0.01$; OR=1.7; 95% CI:0.85 -3.43).

The proportion of the mutant homozygous T/T genotype significantly predominated in the group of patients with venous thrombosis, amounting to 16.0% versus 7.1% of detected cases in the control group ($\chi2>3.84$; $p<0.05$; OR=3.1; 95% CI: 1.11-8.49) (Table .3).

Table 3: Association between the rs1801133 polymorphism in the MTHFR gene in groups of patients with venous thrombosis and in the control group

Study Groups	Alleles and genotypes	Statistical difference			
		Odds ratio		χ^2	p-value
		OR	95% CI:		
Varicose veins of the lower extremities with venous thrombosis (n=50)	C	0.5	0.33–0.87	6.5*	0.01*
	T	1.9	1.15–3.07		
	C/C	0.5	0.27–0.97	4.3*	0.04*
	C/T	1.7	0.85–3.43	2.2	0.1
	T/T	3.1	1.11–8.49	5.0*	0.02*

Note: the same as in Table. 1.

Thus, in the course of the study, the frequency of distribution of alleles and genotypes of the rs1801133 polymorphism of the MTHFR gene for differences in the group of patients with LVL and the control sample, it was found that the homozygous T/T genotype increases the risk of developing both LVL and its complication by venous thrombosis.

Study of the influence of the rs2010963 polymorphism of the VEGF-A gene

The urgency of the problem of VBNC is due to the rather wide prevalence of the disease, especially among the older age group and, in particular, among the female representatives.

Investigation of the role of growth factors, in particular the value of the vascular endothelial growth factor, a protein responsible for the growth and proliferation of endothelial cells and blood vessels, and thus for angiogenesis, can become a promising direction [17,18,19]. This is the reason for the interest shown in the possible role of polymorphic variants of the VEGF gene in the development of such vascular pathology as VVLE and its complications in the form of phlebothrombosis [20, 21]. The distribution frequency of alleles and genotypes of the rs2010963 polymorphism in the VEGF-A gene was studied, and a search was made for an association between differences in their detection and the incidence of VVLE and their complications in the form of varicose vein.

The analysis showed that the frequency of allele C was significantly lower in the main group of patients, including patients with uncomplicated LVL and patients with LVL complicated by venous thrombosis, where the incidence of this allele was 54.3%, compared with the control sample, where it was 85.5% ($\chi2>3.84$; $p<0.05$; RR=0.5; 95%CI: 0.45-0.60; OR=0.2; 95%CI: 0.14-0.30) (Table 3.7).

The proportion of the prevalence of the G allele in the main group of patients was 45.6%, which was significantly higher compared with conditionally healthy persons in the control group, where its prevalence was only 14.5%, which indicates the presence of an associative relationship between the detection of this allele and the presence of VBNC ($\chi2>3.84$; $p<0.05$; RR=1.9; 95% CI: 1.68-2.21; OR=5.0; 95% CI: 3.37-7.27) (Table 4).

Table 4: Association between the rs2010963 polymorphism in the VEGF-A gene in the main group of patients and in the control group

Study Groups	Alleles and genotypes	Statistical difference					
		Relative risk		Odds ratio		χ^2	p-value
		RR	95% CI:	OR	95% CI:		
Main group (n=161)	C	0.5	0.45 - 0.60	0.2	0.14 - 0.30	72.4	0.000001*
	G	1.9	1.68 - 2.21	5.0	3.37 - 7.27		
	C/C	0.4	0.33 - 0.54	0.2	0.10 - 0.26	57.3	0.000001*
	C/G	2.2	1.69 - 2.86	4.7	2.79 - 7.92	35.9	0.000001*
	G/G	2.9	2.22 - 3.70	2.9	2.22 - 3.70	45.2	0.000001*

The proportion of the wild genotype C/C polymorphism rs2010963 in the VEGF-A gene in the main group, where its prevalence was 31.6%, was lower than in the control group, in which its proportion was 74.2% ($\chi 2>3.84$; $p<0.05$; RR=0.4; 95%CI: 0.33-0.54; OR=0.2; 95%CI: 0.10-0.26) (Table 4).

The concentration of the heterozygous genotype among patients of the main group, C/G, was 45.3%, which exceeded its occurrence in the control group, where it was found in 22.6% of cases ($\chi 2> 3.84$; $p<0.05$; RR=2.2; 95% CI: 1.69- 2.86; OR=4.7; 95%CI: 2.79-7.92).

The proportion of detection of the mutant homozygous genotype G/G among patients of the main group was at the level of 23.0% and was greater than the percentage of cases of detection of this genotype in the control sample, which was 3.2% ($\chi 2>3.84$; $p<0.05$; RR=2.9; 95 %CI:2.22-3.70; OR=2.9; 95%CI:2.22-3.70) (Table 4).

We also studied the prevalence of alleles and genotypes of the rs2010963 polymorphism of the VEGF-A gene in 111 patients with uncomplicated LVLS compared with a group of conditionally healthy subjects (Table 5).

Table 5: Association between alleles and genotypes of the rs2010963 polymorphism of the VEGF-A gene in groups of patients with uncomplicated VVLE and in the control group

Study Groups	Alleles and genotypes	Statistical difference					
		Relative risk		Odds ratio		χ^2	p-value
		RR	95% CI:	OR	95% CI:		
Varicose disease (n=111)	C	0.5	0.44 - 0.64	0.3	0.19 - 0.43	37.0	0.000001*
	G	1.9	1.57 - 2.27	3.5	2.32 - 5.34		
	C/C	0.4	0.32 - 0.58	0.2	0.13 - 0.37	33.7	0.000000
	C/G	2.2	1.63 - 3.01	4.1	2.33 - 7.04	25.9	0.000001*
	G/G	2.8	1.92 - 3.95	8.0	2.75 - 23.42	18.5	0.000018*

It was found that the proportion of the detected C-allele of the polymorphic locus rs2010963 of the VEGF-A gene in the main group was 62.6%, which was less than in the control group, where its detection rate was 85.5% ($\chi 2>3.84$; $p<0.05$; RR= 0.5; 95%CI:0.45-0.60; OR=0.2; 95%CI:0.14-0.30).

Allele G, on the contrary, prevailed among patients with uncomplicated LVLD, where it occurred in 37.4% of cases, which was more common than in the control group (14.5%) ($\chi 2>3.84$; $p<0.05$; RR=1.93; 95% CI: 1.68-2.21; OR=5.0; 95%CI: 3.37-7.27).

The C/C, C/G, and G/G genotypes of the polymorphic locus rs2010963 of the VEGF-A gene were distributed as follows: in the group of patients with LVLV they were 38.7%, 47.5%, and 13.5% versus 74.2%, 22.6%, and 3.2% in the control group.

The concentration of the wild C/C genotype in the control group was 74.2%, which is statistically significantly higher than among patients with uncomplicated LVLD, among whom the frequency of its detection was 38.7%, which indicates that this genotype has protective properties in relation to the development of an uncomplicated form VBNC ($\chi 2>3.84$; $p<0.05$; RR=0.4; 95%CI: 0.32-0.58; OR=0.2; 95%CI: 0.13-0.37).

The proportion of the heterozygous C/G genotype among patients with VLVL was at the level of 47.5%, which is significantly higher ($\chi 2>3.84$; $p<0.05$; RR=2.2; 95% CI: 1.63-3.01; OR=4.1; 95% CI: 2.33- 7.04) than among conditionally healthy individuals in the control sample, where its concentration was 22.6%.

The homozygous G/G genotype in the group of patients with LVLV was detected with a frequency of 13.2%, which was significantly higher compared to the control sample, where its

concentration was only 3.2%, which indicates its association with the development of uncomplicated LVLV ($\chi 2 > 3.84$; $p<0.05$; RR=2.8; 95%CI:1.92-3.95; OR=8.0; 95%CI:2.75-23.42).

We also studied the prevalence of alleles and genotypes of the rs2010963 polymorphic locus of the VEGF-A gene among patients with VLLE complicated by venous thrombosis and in the control group (Table 6).

The concentration of the C-allele in the group of patients with varicose vein was at the level of 36.0%, which is statistically significantly less than among apparently healthy individuals in the control group, where its concentration was 85.5% ($\chi 2>3.84$; $p<0.05$; RR=0.2; 95 %CI:0.14-0.29; OR=0.1; 95%CI:0.06-0.16).

The proportion of the G-allele, on the contrary, statistically significantly prevailed among patients with venous thrombosis and amounted to 64.0%, which was higher than in the control sample, where its occurrence was 14.5% ($\chi 2>3.84$; $p<0.05$; RR=4.9; 95% CI :3.48-6.93; OR=10.5; 95%CI: 6.25-17.54).

The C/C, C/G and G/G genotypes of the rs2010963 polymorphism of the VEGF-A gene were distributed in the group of patients with varicose vein in the following ratio: 16.0%, 40.0% and 44.0%, and among the conditionally healthy individuals of the control group as 74.2%, 22.6% and 3.2% (Table 6).

Table 6: Association between alleles and genotypes of the rs2010963 polymorphism of the VEGF-A gene in groups of patients with venous thrombosis and in the control group

Study Groups	Alleles and genotypes	Statistical difference					
		Relative risk		Odds ratio		χ^2	p-value
		RR	95% CI:	OR	95% CI:		
Venous thrombosis (n=50)	C	0.2	0.14 - 0.29	0.1	0.06 - 0.16	94.9	0.000001*
	G	4.9	3.48 - 6.93	10.5	6.25 - 17.54		
	C/C	0.1	0.06 - 0.25	0.1	0.03 - 0.15	55.9	0.000001*
	C/G	5.6	2.63 - 11.90	8.2	3.33 - 20.26	25.6	0.000001*
	G/G	13.6	5.4-34.1	23.6	8.2 – 67.4	54.9	< 0.001

The proportion of heterozygous C/G genotype among patients with venous thrombosis was 40.0%, significantly prevailing compared to the control group, where the detection rate was 22.6% ($\chi 2>3.84$; $p<0.05$; RR=5.6; 95% CI: 2.63 -11.90; OR=8.2, 95% CI: 3.33-20.26).

The proportion of the mutant homozygous G/G genotype among patients with VLLE complicated by thrombotic complications in the form of varicose vein was 44.0%, statistically significantly predominating, compared with the control group, where it was detected only in 3.2% of cases ($\chi 2>3.84$; $p<0.05$; RR=12.5; 95% CI: 6.26-25.07; OR=23.6; 95% CI: 18.92-211.45).

The study showed that the wild homozygous C/C genotype showed protective properties in relation to the development of varicose vein ($\chi 2>3.84$; $p<0.05$; RR=0.1; 95%CI:0.06-0.25; OR=0.1; 95%CI: 0.03-0.15), while the heterozygous C/G genotype and the mutant homozygous G/G genotype acted as genetic markers of the risk of thrombotic complications in patients with VLVL ($\chi 2>3.84$; $p<0.05$; RR=5.6; 95% CI:2.6- 11.9; OR=8.2, 95%CI:3.3-20.3) and ($\chi 2>3.84$; $p<0.05$; RR=13.6; 95%CI:5.4-34.1; OR=23.6; 95%CI:8.2-67.4).

In general, according to researchers, the rs2010963 polymorphism of the VEGF-A gene is a predictor of various pathologies, including vascular ones [22]. However, the role of this polymorphic locus in the occurrence of LVLE and venous thrombosis has not been sufficiently studied [23], in connection with which this study was conducted.

At the same time, a study in a group of patients with VLVD complicated by varicose vein showed that the detection of a heterozygous C/G genotype increases the risk of developing a form of the disease complicated by venous thrombosis by more than 5.6 times ($\chi2$=3.84; p<0.05; 95% CI: 2.63-11.90), and the detection of the homozygous mutant G/G genotype was more than 13.6 times higher ($\chi2$=3.84; p<0.05; 95%CI:5.4-34.1).

CONCLUSION

According to the results the presence of a protective role of the C/C genotype ($\chi2$>3.84; p<0.05; RR=0.4; 95%CI:0.33-0.54; OR=0.2; 95%CI:0.10-0.26), in contrast to from C/G and G/G genotypes, more often detected among patients with both complicated and uncomplicated forms of LVLD ($\chi2$>3.84; p<0.05; RR=2.2; 95% CI: 1.69-2.86; OR=4.7; 95% CI:2.79-7.92) and ($\chi2$>3.84; p<0.05; 95%CI:2.22-3.70; OR=2.9; 95%CI: 2.22-3.70) are indicated.

In summary that the wild homozygous C/C genotype showed protective properties in relation to the development of varicose vein ($\chi2$>3.84; p<0.05; RR=0.1; 95%CI:0.06-0.25; OR=0.1; 95%CI: 0.03-0.15), while the heterozygous C/G genotype and the mutant homozygous G/G genotype acted as genetic markers of the risk of thrombotic complications in patients with VLVL ($\chi2$>3.84; p<0.05; RR=5.6; 95% CI:2.6- 11.9; OR=8.2, 95%CI:3.3-20.3) and ($\chi2$>3.84; p<0.05; RR=13.6; 95%CI:5.4-34.1; OR=23.6; 95%CI:8.2-67.4).

The wild C/C genotype of the rs2010963 polymorphism of the VEGF-A gene has protective properties, while the heterozygous C/G genotype and the mutant homozygous G/G genotype are markers of the risk of LVL and venous thrombosis.

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