

# The Role of the Vegfa C936t Polymorphism in the Development of Chronic Venous Insufficiency of the Legs And Its Analysis

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**Annotation.** In 98 patients, an analysis of the genetic risk factor for the development of chronic venous insufficiency was carried out by studying the frequency of distribution of alleles and genotypes of the C936T polymorphism in the VEGFA gene. In both groups, the actual distribution of C807T polymorphism genotypes corresponded to those expected under the Hardy-Weinberg equilibrium ( $p < 0.05$ ). A significant relationship was established between the risk of developing chronic venous insufficiency and the distribution of predisposing/protective variants of the C807T polymorphism genotypes in the VEGFA gene. These data allow us to suggest testing this locus to predict the risk of occurrence and progression of chronic venous insufficiency.

**Key words:** chronic venous insufficiency, vascular endothelial growth factor, genetic polymorphism, VEGFA.

**Actuality of the problem.** Chronic venous disease (CVD) is a persistent, progressive and often underdiagnosed condition, widely represented in the general population, with enormous socioeconomic, physical and psychological impact [1,4]. CVD entails a wide range of venous abnormalities in which blood return is severely impaired. In the pathophysiology of CVD, the interaction between genetics and environmental factors is responsible for the increase in regional venous pressure, which leads to significant changes in the entire structure and functioning of the venous system [7]. Currently, special attention is paid to the connection between the development of this disease and the human genome, since chronic venous insufficiency (CVI) is characterized by high prevalence, frequent relapses and rejuvenation of the disease. Genetic disorders in the regulation of the synthesis of components of the three-dimensional extracellular matrix structure form the basis of the pathogenesis of varicose veins. Studying the role of growth factors, in particular the importance of vascular endothelial growth factor, a protein responsible for the growth and proliferation of endothelial cells and vessels and thus for angiogenesis, may become a promising direction [5,7,9]. Related to this is the interest shown in the possible role of polymorphic variants of the VEGFA gene in the development of such CVI [6,10]. Vascular endothelial growth factor A (VEGFA) is a physiological and pathological regulator of angiogenesis that plays a key role in maintaining vascular reactivity and integrity. Today, there is a concept about the polygenic nature of CVI, which shows the presence of not one, but several genetic variants that independently or jointly provoke the risk of developing this type of disease [2, 4, 8]. All of the above justifies the need for a fundamentally new approach when studying the causes of genetic predisposition to CVI [10,11]

**Aim of the research.** Assessment of the contribution of the C936T polymorphism of the VEGFA gene to the development of chronic venous insufficiency and its role in the progression of CVI.

**Materials and methods of the research.** We observed 98 patients aged from 20 to 78 years with chronic venous insufficiency, including, in accordance with the CEAP classification, 45 patients with moderate severity of CVI (class C3-C4) and 53 patients with severe CVI. (class C5-C6), who were undergoing inpatient treatment in the department of cardiovascular surgery of the clinics of the Andijan State Medical Institute. The control group consisted of 87 healthy individuals. Diagnosis of CVI was carried out in accordance with currently accepted recommendations and was established on the basis of the clinical picture and the results of Doppler ultrasound (USDG) of the vessels of the lower extremities.

In all patients with CVI and in the control group, the frequency distribution of detection of C936T polymorphisms of the VEGFA gene was determined. For this purpose, blood was collected into vacuum tubes with EDTA (K3 EDTA, 5 ml).

The selected biomaterial was studied using the Real-TimePCR method. DNA from peripheral blood lymphocytes was isolated using the AmpliPrime RIBO-prep kit (Interlabservice LLC, Russia). Detection of polymorphism was carried out using test systems from NPF Litech LLC (Russia), amplification was carried out using a Real-time thermal cycler “RotorGene Q” (Quagen, Germany).

The OpenEpi 2009, Version 2.3 application package was used as a calculation tool

**The results obtained and their discussion.** The results of calculations of deviations in the observed and expected frequencies of the distribution of alleles and genotypes of the C936T polymorphism in the VEGFA gene in the main and control groups showed that in the studied groups the actual distribution of genotypes of the C936T polymorphism corresponded to those expected at Hardy-Weinberg equilibrium (HWE) ( $p < 0.05$ ). The data obtained show the mutual combination of the studied groups for the C936T polymorphism in the VEGFA gene. In the studied groups of patients with vascular thrombosis of various locations and the control sample, there was no heterogeneity between the actual observed and theoretically expected genotype values of the C936T polymorphic variant in the VEGFA gene.

**Table № 1.**

**Expected and observed frequencies of distribution of locus genotypes for HWE (C936T polymorphism in the VEGFA gene)**

Main group					
Alleles	Allele frequency				
C	0,78				
T	0,22				
Genotypes	Genotype frequency		$\chi^2$	p	df
	observed	expected			
C/ C	0,62	0,6	0,07		
C/ T	0,31	0,35	0,5		
T/ T	0,07	0,05	0,86		
Bcero	1	1	1,43	0,225	1

Control group					
Alleles	Allele frequency				
C	0,87				
T	0,13				
Genotypes	Genotype frequency		$\chi^2$	p	df
	observed	expected			
C/ C	0,77	0,75	0,03		
C/ T	0,2	0,23	0,44		
T/ T	0,03	0,02	1,44		
Total	1	1	1,91	0,166	1

Groups	Ho	He	D*
Main group	0,31	0,35	-0,12
Control group	0,2	0,23	-0,15

Note:  $D = (H_o - H_e)/H_e$

When analyzing the C936T polymorphism in the VEGFA gene, no deviation of genotype distributions from those expected at Hardy-Weinberg equilibrium (HWE) was detected ( $\chi^2=1.43$ ,  $p=0.225$  in the main group;  $\chi^2=1.91$ ,  $p=0.166$  in the control group).

**Table № 2.**

**Differences in the frequency of allelic and genotypic variants of the C936T polymorphism in the VEGFA gene in groups of patients with severe CVI (C5-C6) and the control group**

Alleles and genotypes	Number of alleles and genotypes examined				$\chi^2$	p	RR	95%CI	OR	95%CI
	CVI (C5-C6)		Control group							
	n	%	n	%						
C	85	80,2	151	86,8	2,2	p = 0,2	0,9	0,46 - 1,85	0,6	0,32 - 1,17
T	21	19,8	23	13,2	2,2	p = 0,2	1,1	0,6 - 1,94	1,6	0,85 - 3,09
C/C	35	66,0	67	77,0	2,0	p = 0,2	0,9	0,37 - 1,99	0,6	0,27 - 1,23
C/T	15	28,3	17	19,5	1,4	p = 0,3	1,4	0,6 - 3,49	1,6	0,73 - 3,6
T/T	3	5,7	3	3,4	0,4	p = 0,6	1,6	0,32 - 8,34	1,7	0,33 - 8,51

The distribution frequency of genotypes C/C, C/T and T/T was: 66%, 28.3% and 5.7%, respectively, in the main group and 77%, 19.5% and 3.4% in the control group. As can be seen from our data, the combination of genotypes C/T and T/T of the C936T polymorphism in the VEGFA gene indicates a higher risk of developing severe forms of chronic venous insufficiency (CEAP C5-C6) (OR = 1.6 and 1.7; 95% CI=0.73 – 3.6 and 0.33 – 8.51).

Changes in VEGF activity have been observed in many diseases of the circulatory system [2,4,9]. VEGFA increases the permeability of existing blood vessels, helping to maintain inflammation, allowing leukocytes to migrate to their destination.

Thus, the results obtained during the study reliably indicate the presence of an association of carriers of the T allele and genotypes C/T and T/T of the C936T polymorphism in the VEGFA gene with the risk of developing complicated forms of CVI.

### Conclusion

1. Allele C and genotype C/C of the C936T polymorphism of the VEGFA gene are protective factors against the development of complicated forms of CVI.
2. Conducted studies in the local population confirmed the presence of an association of the heterozygous genotype C/T and the mutant homozygous genotype T/T of the C936T polymorphism of the VEGFA gene with the risk of developing complicated forms of CVI.

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