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THE C936T POLYMORPHISM OF THE VEGFA GENE AS A GENETIC PREDICTOR OF THE DEVELOPMENT AND PROGRESSION OF CHRONIC VENOUS INSUFFICIENCY OF THE LOWER EXTREMITIES

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ABSTRACT

Background. Vascular endothelial growth factor A (VEGFA) is a key regulator of angiogenesis and of the integrity and tone of the venous wall. The functional C936T (rs3025039) polymorphism, located in the 3' untranslated region of the gene, may modify susceptibility to chronic venous insufficiency (CVI), but data in different populations remain inconsistent.

Aim. To evaluate the contribution of the VEGFA C936T polymorphism to the risk of developing and progressing CVI of the lower extremities in an ethnically defined cohort from the Fergana Valley of Uzbekistan.

Materials and methods. A case-control study included 98 patients with CVI (CEAP classes C3-C6; 45 with C3-C4 and 53 with C5-C6) and 87 healthy controls of comparable age and sex. The C936T polymorphism was genotyped by polymerase chain reaction. Allele and genotype frequencies were compared using the Pearson chi-square test, and the strength of association was expressed as the odds ratio (OR) with a 95% confidence interval (CI).

Results. The unfavorable T allele was significantly more frequent in patients than in controls (22.4% vs 13.2%; OR=1.9; 95% CI 1.1-3.28; p=0.025), while the C/C genotype showed a protective effect (62.3% vs 77.0%; OR=0.5; 95% CI 0.26-0.93; p=0.05). The heterozygous C/T genotype (OR=1.8; 95% CI 0.92-3.58) and the homozygous T/T genotype (OR=2.2; 95% CI 0.56-8.36) were more frequent in patients. The association was strongest in the C3-C4 subgroup, in which the T allele reached OR=2.3 (95% CI 1.19-4.26; p=0.025) and the C/C genotype was protective (OR=0.4; 95% CI 0.19-0.88; p=0.025).

Conclusion. The C936T polymorphism of the VEGFA gene is a genetic predictor of CVI: the T allele and the C/T and T/T genotypes are associated with increased risk through promotion of endothelial dysfunction, whereas the C/C genotype is protective. Genotyping of this variant may support early, genetically informed prediction and prevention of CVI.

Keywords: chronic venous insufficiency; VEGFA; C936T; rs3025039; vascular endothelial growth factor; endothelial dysfunction; genetic predictor; CEAP.

INTRODUCTION

Chronic venous disorders are among the most frequent diseases in many countries of the world, affecting from a third to a half of the adult population, with women particularly affected. Over the past four decades the prevalence of chronic venous insufficiency has risen sharply, and if this trend continues more than 30% of the population of industrialized countries is projected to suffer from various forms of the disease. Chronic venous insufficiency remains one of the principal causes of disability among the working-age population, and the high incidence together with the rising cost of care places a heavy financial burden on health-

care systems. Demographic and predisposing factors that increase the risk include age, female sex, the use of oral contraceptives and estrogen therapy, pregnancy, overweight and obesity, leg trauma, and a positive family history, the last of which points to a substantial hereditary component.

Chronic venous insufficiency (CVI) of the lower extremities is one of the most common vascular disorders worldwide and a major contributor to the loss of working capacity and quality of life. Despite advances in diagnosis and treatment, the proportion of patients with severe, complicated forms continues to grow, and effective tools for the early prediction of disease development and progression remain limited [1, 2]. The aetiology and pathophysiology of CVI are complex and multifactorial, integrating genetic, proteomic and cellular mechanisms that lead to alterations of venous structure and function. Accordingly, the search for reliable molecular predictors that could underpin personalized, predictive medicine has become an actively pursued objective.

The pathophysiology of CVI begins with reduced shear stress secondary to venous stasis and retrograde flow, which converts the endothelium to an inflammatory, prothrombotic phenotype. Endothelial dysfunction, hypoxia of the venous wall and the subsequent inflammatory cascade drive the remodeling of the extracellular matrix, dilatation of the venous wall, valvular incompetence and increased permeability [3, 4]. Among the molecules activated in this setting, vascular endothelial growth factor A (VEGFA) occupies a central position, linking angiogenesis, endothelial activation and matrix remodeling. The plasma level of VEGFA is higher in patients with chronic venous disease and rises in proportion to the severity of CVI, reaching its highest values in patients with trophic ulcers [5].

VEGFA is both a critical regulator of angiogenesis and a key factor that maintains the integrity and functionality of the vascular wall. It is a selective mitogen for endothelial cells, promoting their proliferation, migration and differentiation, and it mediates the growth of new vessels by binding to the cell-surface receptors VEGFR1 and VEGFR2. Increased VEGFA expression appears to play a significant role in the pathogenesis of CVI: it raises the permeability of the venous wall, thereby contributing to oedema, and reduces venous wall tone, which may lead to venous dilatation, blood stasis and the subsequent development of venous hypertension. VEGFA also stimulates endothelial cells to express the adhesion molecules ICAM-1, VCAM-1 and E-selectin, promoting leukocyte adhesion and tissue infiltration, and influences extracellular matrix remodeling through the regulation of matrix metalloproteinases and their tissue inhibitors [6, 7].

The VEGFA gene is located on the short arm of chromosome 6 (6p21.3) and comprises eight exons separated by seven introns. The single-nucleotide polymorphism C936T (rs3025039), located in the 3' untranslated region (3'UTR) of the gene, is considered the most clinically significant variant and corresponds to a substitution of cytosine (C) by thymine (T) at position 936. Because changes in VEGFA expression activate nitric oxide synthase, which can damage the structural integrity of the venous wall through free-radical injury and reduce venous tone, this functional variant is a plausible candidate predictor of CVI [4, 8]. Polymorphisms in the VEGFA gene are recognized risk factors for impaired wound healing and venous ulceration, and a previous study in a large cohort reported that variation in the 5'UTR region of the gene influences genetic predisposition to chronic venous pathology [9, 10].

Nevertheless, the available data on VEGFA polymorphisms and CVI remain inconsistent, reflecting the ethnic heterogeneity of the studied samples and population differences in allele and genotype frequencies. No definitive set of VEGFA variants has yet been validated as a predictor of CVI in the population of Uzbekistan. The aim of the present study was therefore to evaluate the contribution of the VEGFA C936T polymorphism to the risk of developing and progressing CVI of the lower extremities in an ethnically defined cohort from the Fergana Valley of Uzbekistan, and to assess its potential as a genetic predictor suitable for early, personalized prevention.

MATERIALS AND METHODS

2.1. Study design and participants

A case-control study was conducted between 2020 and 2022 at the clinics of the Andijan State Medical Institute and the Andijan regional branch of the Republican Scientific Centre of Emergency Medical Care. The main group comprised 98 patients with CVI of the lower extremities who were permanent residents of the Fergana Valley. According to the 2020 CEAP classification, patients were divided into two subgroups: 45 patients with moderate CVI (CEAP classes C3-C4) and 53 patients with severe CVI (CEAP classes C5-C6). The control group included 87 conventionally healthy, unrelated individuals without clinical manifestations of CVI and without a personal or family history of venous disease, comparable to the patients in age and sex ($p>0.05$).

Inclusion criteria were clinical manifestations corresponding to CEAP classes C3-C6 and age over 18 years; exclusion criteria were CEAP classes C0-C2, oncological or endocrine disease, confirmed diabetes mellitus, decompensated chronic somatic disease, pregnancy or lactation, and acute infectious disease. Women predominated in the cohort (72.5%), and the mean age of patients was 62.7 +/- 1.3 years. The diagnosis of CVI was verified according to the CEAP 2020 criteria on the basis of clinical examination and duplex Doppler ultrasonography of the deep and superficial veins. Informed consent was obtained from every participant.

2.2. Molecular-genetic methods

Genomic DNA was extracted from peripheral blood lymphocytes. Five millilitres of venous blood were collected into EDTA vacutainers and stored at -70 degrees Celsius until analysis. DNA was isolated using a commercial extraction kit; its concentration and purity were verified spectrophotometrically at A260/280 (ratio 1.7-1.8), confirming suitability for amplification without additional purification. The VEGFA C936T (rs3025039) polymorphism was genotyped by real-time and standard polymerase chain reaction using standard commercial detection kits and validated amplification protocols, with electrophoretic verification of products where required. Genetic analysis followed the GRIPS recommendations.

2.3. Statistical analysis

Statistical analysis was performed with Statistica 10.0 and the OpenEpi v2.3 software package. Conformity of genotype distributions to the Hardy-Weinberg equilibrium was tested using the GenePop online program. Allele and genotype frequencies were compared between groups using the Pearson chi-square test, with the Yates correction for continuity applied to 2x2 tables when the expected count in any cell was five or fewer. The strength of association was expressed as the odds ratio (OR) with a 95% confidence interval (CI): OR=1 indicated

absence of association, OR>1 was interpreted as an increased-risk factor, and OR<1 as a protective factor. A two-sided p-value below 0.05 was considered statistically significant.

RESULTS

3.1. Allele and genotype distribution

The distribution of the C936T genotypes conformed to the Hardy-Weinberg equilibrium in both the patient and the control groups. The C and T allele frequencies were 0.78/0.22 in the main group and 0.87/0.13 in controls. Statistical analysis revealed a reduction in the frequency of the wild-type C allele in patients, indicating a protective effect of this allele against the development of CVI (OR=0.5; 95% CI 0.3-0.91; chi-square=5.3; p=0.025). Conversely, the unfavorable T allele was significantly more frequent in patients, increasing the risk of CVI by approximately 1.9 times relative to controls (OR=1.9; 95% CI 1.1-3.28; chi-square=5.3; p=0.025) (Figure 1, Table 1).

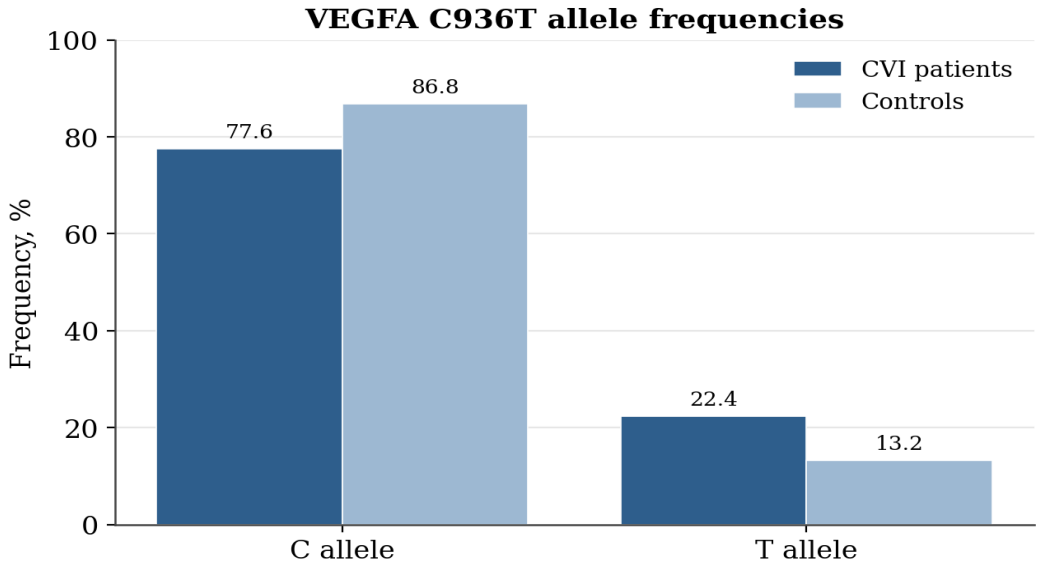

Figure 1. Frequency of the VEGFA C936T alleles in patients with CVI (n=98) and controls (n=87).

The frequency of the ancestral C/C genotype was significantly lower among patients than among controls (62.3% vs 77.0%), confirming a protective effect of this genotype against CVI (OR=0.5; 95% CI 0.26-0.93; chi-square=4.7; p=0.05). The frequency of the unfavorable heterozygous C/T genotype was higher in patients than in controls (30.6% vs 19.5%), increasing the risk of CVI by approximately 1.8 times (OR=1.8; 95% CI 0.92-3.58; chi-square=3.0; p=0.1). A tendency towards a higher frequency of the mutant T/T genotype was also observed (7.1% vs 3.5%; OR=2.2; 95% CI 0.56-8.36), reflecting an additional, smaller increment in risk (Figure 2).

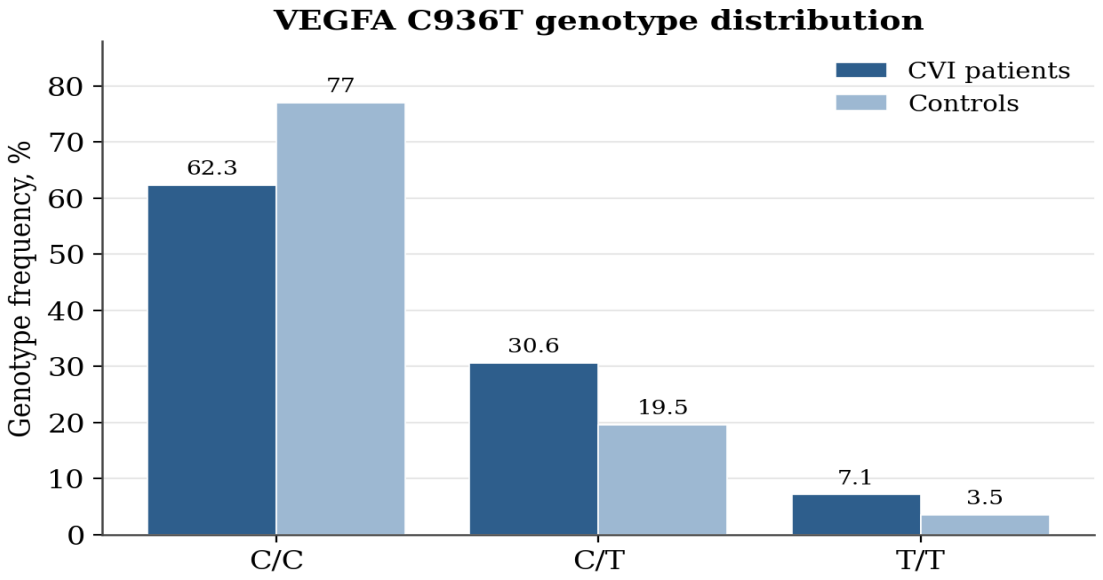

Figure 2. Distribution of VEGFA C936T genotypes in patients with CVI (n=98) and controls (n=87).

Table 1. Distribution and association of the VEGFA C936T polymorphism in the main group and controls

Allele / genotype	Patients, %	Controls, %	OR (95% CI)	p
C allele	77.6	86.8	0.5 (0.30-0.91)	0.025
T allele	22.4	13.2	1.9 (1.10-3.28)	0.025
C/C	62.3	77.0	0.5 (0.26-0.93)	0.05
C/T	30.6	19.5	1.8 (0.92-3.58)	0.1
T/T	7.1	3.5	2.2 (0.56-8.36)	0.3

OR - odds ratio; CI - confidence interval. Statistically significant associations ($p<0.05$) were obtained for the C and T alleles and the C/C genotype.

3.2. Association by CEAP severity

Analysis by severity subgroup revealed that the association was strongest in patients with moderate disease (CEAP C3-C4). In this subgroup the C and T allele frequencies were 74.4% and 25.6% versus 86.8% and 13.2% in controls. Detection of the unfavorable T allele increased the risk of CVI by approximately 2.3 times (OR=2.3; 95% CI 1.19-4.26; chi-square=6.3; p=0.025), while the C allele exerted a protective effect (OR=0.4; 95% CI 0.23-0.84; p=0.025). The C/C genotype was significantly less frequent among C3-C4 patients than among controls (57.8% vs 77.0%; OR=0.4; 95% CI 0.19-0.88; chi-square=5.3; p=0.025), confirming its protective role, whereas the C/T genotype raised the risk approximately 2.1 times (33.3% vs 19.5%; OR=2.1; 95% CI 0.92-4.62) and the T/T genotype showed a tendency towards a 2.7-fold increase (8.9% vs 3.5%; OR=2.7; 95% CI 0.62-12.12) (Figure 3, Table 2).

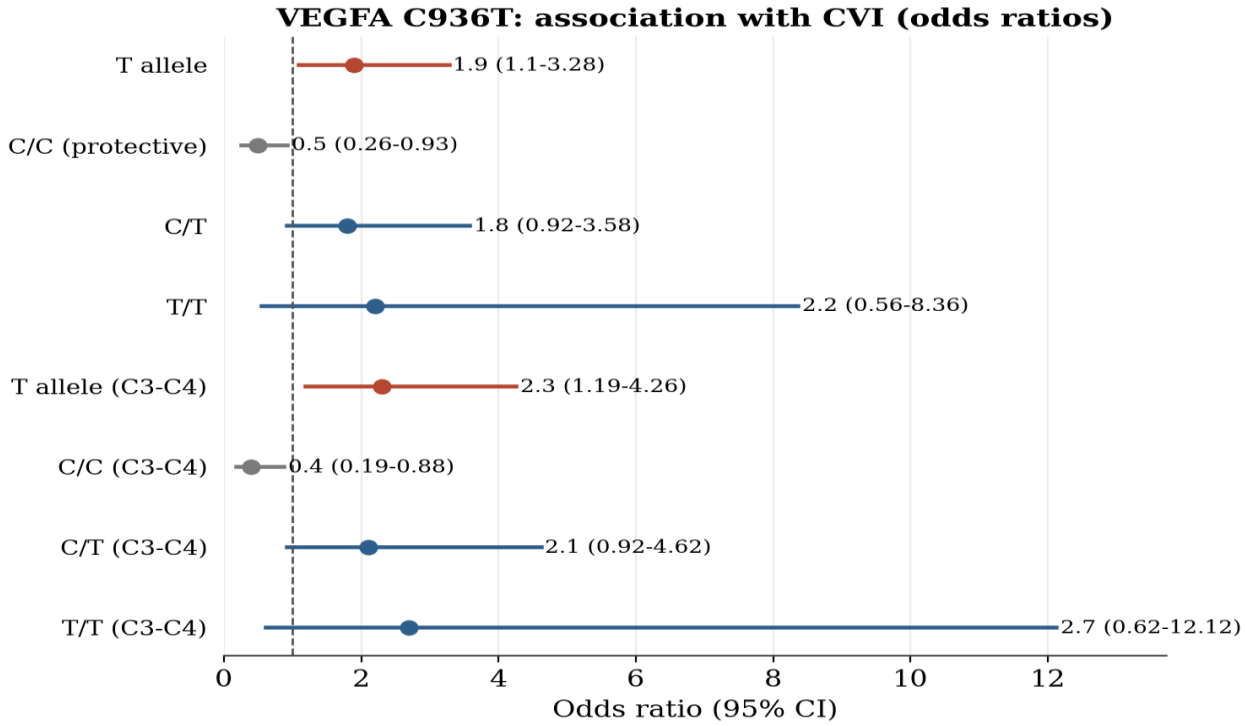

Figure 3. Odds ratios (with 95% confidence intervals) for the association of VEGFA C936T alleles and genotypes with CVI in the whole cohort and in the C3-C4 subgroup; the dashed line marks OR=1.

In patients with severe disease (CEAP C5-C6), the differences in allele frequencies relative to controls did not reach statistical significance (C allele 80.2% vs 86.8%; OR=0.6; 95% CI 0.32-1.17), although a tendency towards a higher frequency of the T allele was observed (19.8% vs 13.2%; OR=1.6; 95% CI 0.85-3.09). The C/C genotype was less frequent (66.0% vs 77.0%; OR=0.6; 95% CI 0.27-1.23), the C/T genotype was more frequent (28.3% vs 19.5%; OR=1.6; 95% CI 0.73-3.6), and the T/T genotype showed a weak tendency towards increased risk (5.7% vs 3.5%; OR=1.7; 95% CI 0.33-8.51) (Figure 4).

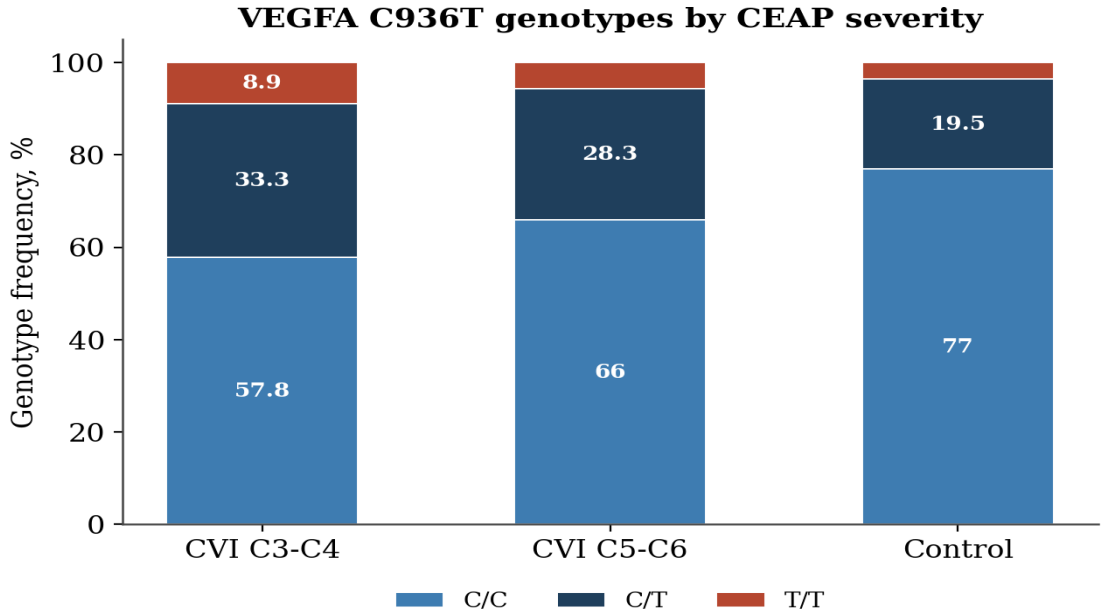

Figure 4. Distribution of VEGFA C936T genotypes across CEAP severity subgroups (C3-C4, C5-C6) and controls.

Table 2. Association of the VEGFA C936T polymorphism with CVI by CEAP severity subgroup

Subgroup	Variant	Patients, %	Controls, %	OR	95% CI
CVI C3-C4 (n=45)	T allele	25.6	13.2	2.3	1.19-4.26
	C/C	57.8	77.0	0.4	0.19-0.88
	C/T	33.3	19.5	2.1	0.92-4.62
	T/T	8.9	3.5	2.7	0.62-12.12
CVI C5-C6 (n=53)	T allele	19.8	13.2	1.6	0.85-3.09
	C/T	28.3	19.5	1.6	0.73-3.60
	T/T	5.7	3.5	1.7	0.33-8.51

Taken together, the relative risk of CVI rose with detection of the minor T allele and the C/T and T/T genotypes in both severity subgroups, with the strongest and statistically significant associations in patients with moderate (C3-C4) disease, indicating that VEGFA C936T variation is most informative at earlier clinical stages.

DISCUSSION

The present study demonstrates that the C936T polymorphism of the VEGFA gene is associated with CVI in an ethnically defined cohort from the Fergana Valley of Uzbekistan. The minor T allele was significantly more frequent in patients and increased the risk of disease by approximately 1.9 times, whereas the ancestral C allele and the C/C genotype exerted a protective effect. These findings are consistent with the established role of VEGFA in maintaining the integrity of the venous wall: increased VEGFA expression activates nitric oxide synthase, reduces venous tone and renders the vascular wall more susceptible to injury by reactive oxygen species, thereby promoting endothelial dysfunction and predisposing to venous dilatation and stasis [4, 6].

The detection of the heterozygous and mutant forms of the polymorphism alters the activity of VEGFA, which acts as a trigger for endothelial dysfunction and vascular disturbances and raises the threat of CVI development and progression. The observation that VEGFA also regulates the expression of matrix metalloproteinases and their tissue inhibitors links this variant mechanistically to extracellular matrix remodeling, providing a coherent pathophysiological pathway from genetic predisposition to structural venous damage [3, 7]. The higher plasma levels of VEGFA reported in patients with advanced CVI, and the proportional rise with disease severity and ulceration, are in keeping with this interpretation [5].

Our results agree with several reports in the literature. Increased expression of VEGFA and its receptor VEGFR2 has been documented in the varicose vein wall and is more pronounced when concomitant thrombophlebitis is present. VEGFA participates in maintaining the integrity of the vessel wall, and its elevated expression contributes to the activation of nitric oxide synthase, a reduction of vascular tone and increased susceptibility to free-radical injury [6, 7]. Polymorphisms in the VEGFA gene are recognized risk factors for impaired wound healing and venous ulceration [11, 12]. A study of a large cohort reported that the rs2010963 variant in the 5'UTR region of VEGFA influences genetic predisposition to chronic venous pathology [10], and a regional study likewise associated VEGFA variation with the development of varicose veins and phlebothrombosis [9]. Together, these data support the view

that VEGFA variation modulates the risk of CVI across populations, although the specific variants and effect sizes differ.

A notable feature of our findings is that the association was strongest and statistically significant in the C3-C4 subgroup, where the T allele reached OR=2.3 and the C/C genotype was protective (OR=0.4). This pattern suggests that VEGFA C936T is most informative at moderate clinical stages, when endothelial dysfunction and early wall remodeling predominate, and is consistent with the role of VEGFA in the initial phases of capillary morphological change and lipodermatosclerosis. In the C5-C6 subgroup the associations were weaker and did not reach significance, which may reflect both the smaller effect of a single variant at advanced stages, when multiple pathophysiological mechanisms converge, and the limited statistical power of the subgroup.

The principal limitation of this study is the modest sample size, which restricts power for the low-frequency T/T genotype and accounts for the wide confidence intervals observed for that genotype. The study also did not measure circulating VEGFA, which would have allowed the genotype to be related directly to the protein phenotype. Larger, multi-centre studies, ideally combining genotyping with plasma VEGFA measurement and with the assessment of additional genes involved in endothelial function and matrix remodeling, are required to define the relative contribution of VEGFA variation to venous pathology in this region and to support its translation into clinical risk stratification.

From a translational standpoint, the identification of the VEGFA C936T variant as a predictor of CVI has practical implications for predictive and preventive phlebology. Because more than 60% of patients with chronic venous disease have a positive family history, pre-symptomatic genotyping of at-risk relatives could enable earlier counselling and the timely introduction of preventive measures aimed at reducing venous stasis and endothelial injury. Genetic testing of a single locus is unlikely to be sufficient for individual risk prediction on its own; rather, the value of VEGFA C936T is greatest when it is interpreted together with established clinical risk factors and with other genetic markers of endothelial dysfunction and matrix remodeling. As genotyping becomes a routine component of preventive medicine, the incorporation of validated venous-disease variants into risk panels may allow asymptomatic individuals at risk to be identified and offered appropriate surveillance and venoactive therapy.

CONCLUSION

1. The C936T polymorphism of the VEGFA gene is associated with CVI of the lower extremities: the unfavorable T allele significantly increased the risk of disease (OR=1.9; p=0.025), and the C/T and T/T genotypes were more frequent in patients, whereas the C/C genotype exerted a protective effect (OR=0.5; p=0.05).
2. The association was strongest and statistically significant in patients with moderate disease (CEAP C3-C4), in whom the T allele reached OR=2.3 (p=0.025) and the C/C genotype was protective (OR=0.4; p=0.025), indicating that the variant is most informative at earlier clinical stages.
3. Genotyping of the VEGFA C936T polymorphism may serve as a genetic predictor for the early, personalized prediction and prevention of CVI and its progression, particularly in individuals with a positive family history.

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