The G308A Polymorphism of the Tumor Necrosis Factor-alpha Gene and the Inflammatory Mechanism of Chronic Venous Insufficiency of the Lower Extremities

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ABSTRACT

Background. Tumor necrosis factor-alpha (TNF-alpha) is a multifunctional pro-inflammatory cytokine implicated in the chronic inflammation that drives venous wall remodeling and trophic complications in chronic venous insufficiency (CVI). The functional promoter polymorphism G308A (rs1800629) may modify susceptibility to the disease and its complicated forms.

Aim. To evaluate the association of the TNF-alpha G308A polymorphism with 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 G308A 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 A allele was more frequent in patients than in controls (10.2% vs 4.6%; OR=2.4; 95% CI 1.03-5.39; p=0.05), and the heterozygous G/A genotype was associated with disease (20.4% vs 9.2%; OR=2.5; 95% CI 1.07-5.97; p=0.05), while the G/G genotype showed a protective tendency (OR=0.4; 95% CI 0.17-0.93). The rare A/A genotype was not detected in either group. The association was consistent across severity subgroups, with the A allele reaching OR=2.3 in C3-C4 and OR=2.4 in C5-C6 patients, and the G/A genotype reaching OR=2.5 and OR=2.6, respectively.

Conclusion. The G308A polymorphism of the TNF-alpha gene is associated with CVI of the lower extremities. Carriage of the unfavorable A allele and the G/A genotype increases the risk of disease development and progression, reflecting an amplified inflammatory process and trophic tissue changes, and may serve as a genetic predictor for early, personalized prevention.

Keywords: chronic venous insufficiency; TNF-alpha; G308A; rs1800629; pro-inflammatory cytokine; inflammation; venous trophic ulcer; genetic predictor

1. Introduction

Chronic venous insufficiency (CVI) of the lower extremities is one of the most widespread vascular disorders and a leading cause of reduced quality of life and disability among the working-age population. Its prevalence has risen steadily over recent decades, and the proportion of patients with severe, complicated forms - including venous trophic ulcers - continues to grow, imposing a substantial socio-economic burden on health-care systems [1, 2]. The aetiology and pathophysiology of CVI are

complex and multifactorial, and chronic inflammation is now recognized as a central mechanism linking venous hypertension and stasis to progressive structural damage of the venous wall and surrounding tissues.

The inflammatory cascade in CVI is initiated by a reduction in shear stress caused by venous stasis and retrograde flow, which converts the endothelium to an inflammatory, prothrombotic phenotype. Activated endothelial cells express adhesion molecules that recruit leukocytes, monocytes and macrophages into the venous wall and surrounding soft tissue; these cells release pro-inflammatory cytokines and growth factors that drive extracellular matrix remodeling, valvular incompetence and, ultimately, trophic skin changes and ulceration [3, 4]. Among the cytokines involved, tumor necrosis factor-alpha (TNF-alpha) occupies a key position. Elevated levels of pro-inflammatory cytokines, including interleukin-1, interleukin-6 and TNF-alpha, have been documented in patients with CVI, and these mediators modulate wound healing and the integrity of the extracellular matrix [5, 6].

TNF-alpha is a multifunctional pro-inflammatory cytokine that participates in the pathogenesis of both acute and chronic inflammatory diseases. It is able to interact with other cytokines and to stimulate the secretion of interleukins and chemokines, to activate leukocytes and to enhance the production of further cytokines. TNF-alpha triggers inflammation through the generation of reactive oxygen species and influences the production of nitric oxide synthase, reducing the bioavailability of nitric oxide and thereby promoting endothelial dysfunction [7, 8]. In the context of venous trophic ulcers, TNF-alpha has been identified in intracapillary monocytes; in vitro it stimulates the proliferation of dermal fibroblasts but inhibits the proliferation of keratinocytes and induces cell-adhesion molecules, contributing to impaired wound healing. The study of this cytokine therefore allows the genetic predisposition to the progression of inflammatory and necrotic processes to be assessed.

The TNF-alpha gene is located on the short arm of chromosome 6 at locus 6p21.3, within the major histocompatibility complex class III region. The single-nucleotide polymorphism G308A (rs1800629) is a functional promoter variant in which guanine (G) is replaced by adenine (A); the A allele is associated with higher transcriptional activity and increased cytokine production. Because elevated TNF-alpha activity amplifies the inflammatory response, promotes endothelial dysfunction and impairs the healing of venous ulcers, this polymorphism is a plausible candidate predictor of CVI and of its complicated forms [9, 10]. Studies in other populations have reported associations between pro-inflammatory gene polymorphisms, including TNF-alpha, and the severity of chronic venous disease, although the findings are not fully consistent across ethnic groups [11, 12].

The influence of cytokine gene polymorphism on the development of venous trophic ulcers - a late and disabling indicator of CVI - is of particular interest. Venous trophic ulcers account for the majority of leg ulcers and remain unhealed for prolonged periods because of sustained inflammation. Prolonged inflammation in the venous ulcer recruits leukocytes and releases inflammatory modulators that adversely affect the surrounding tissue, and previous studies have reported elevated cytokine concentrations in patients with venous trophic ulcers, suggesting that CVI generates an inflammatory state responsible for poor wound healing. Because TNF-alpha influences the growth and viability of the cell types found in healing skin and the integrity of the extracellular matrix, genetic variation that modulates its expression is a plausible determinant of the trophic complications of CVI.

Despite the recognized importance of inflammation in the pathogenesis of CVI, no definitive combination of inflammatory gene variants has been validated as a predictor of the disease in the population of Uzbekistan, and few studies have examined genetic interactions that could substantially increase the risk of CVI. The aim of the present study was therefore to evaluate the association of the TNF-alpha G308A polymorphism with 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.

2. 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 disease duration was 5.6 +/- 4.1 years in C3-C4 patients and 11.8 +/- 4.6 years in C5-C6 patients. The diagnosis of CVI was verified according to the CEAP 2020 criteria on the basis of clinical examination and duplex Doppler ultrasonography. 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 TNF-alpha G308A (rs1800629) 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, expressed as absolute numbers and percentages, 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.

3. Results

3.1. Allele and genotype distribution

The distribution of the G308A genotypes conformed to the Hardy-Weinberg equilibrium in both the patient and the control groups. The G and A allele frequencies were 0.90/0.10 in the main group and 0.95/0.05 in controls. Statistical analysis revealed a reduction in the frequency of the wild-type G allele in patients (OR=0.4; 95% CI 0.19-0.97; chi-square=4.1; p=0.05) and a tendency towards a higher frequency of the mutant A allele. The frequency of the unfavorable A allele in patients was approximately 2.4 times higher than in controls (OR=2.4; 95% CI 1.03-5.39; chi-square=4.1; p=0.05) (Figure 1, Table 1).

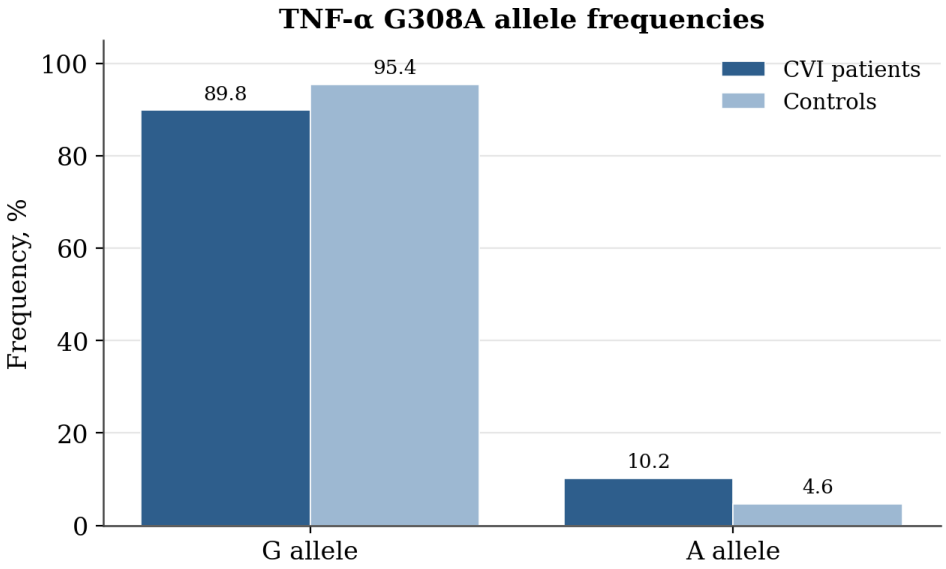

Figure 1. Frequency of the TNF-alpha G308A alleles in patients with CVI (n=98) and controls (n=87).

The wild-type G/G genotype was less frequent in patients than in controls (79.6% vs 90.8%; OR=0.4; 95% CI 0.17-0.93), indicating a protective tendency. By contrast, the unfavorable heterozygous G/A genotype was markedly more frequent among patients (20.4% vs 9.2%), increasing the risk of CVI by approximately 2.5 times (OR=2.5; 95% CI 1.07-5.97; chi-square=4.5; p=0.05). The rare mutant A/A genotype was not detected in either the patient or the control group, which may reflect its low population frequency and the modest sample size (Figure 2).

Analysis of heterozygosity supported these findings. The observed heterozygosity (Hobs) of the G308A locus was higher in patients than in controls (0.20 vs 0.09), and the relative deviation of observed from expected heterozygosity was positive in both groups (D=0.11 in the main group and D=0.05 in controls), with a relatively high level of heterozygosity at this locus. The representativeness of the data and the conformity of the genotype distribution to the Hardy-Weinberg equilibrium confirm that the observed excess of heterozygotes among patients reflects a genuine association of the G/A genotype with disease rather than a sampling artefact.

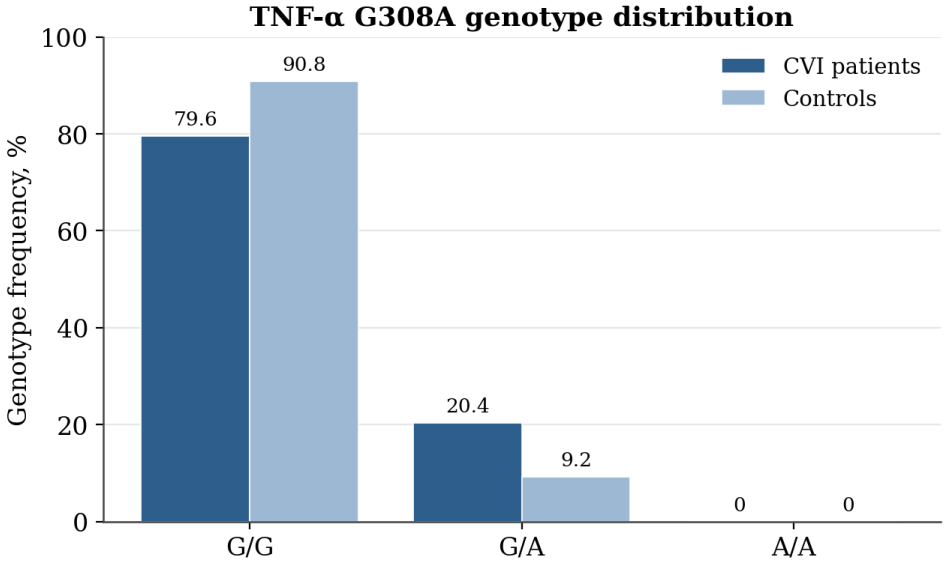

Figure 2. Distribution of TNF-alpha G308A genotypes in patients with CVI (n=98) and controls (n=87); the A/A genotype was not detected.

Table 1. Distribution and association of the TNF-alpha G308A polymorphism in the main group and controls

Allele / genotype	Patients, %	Controls, %	OR (95% CI)	p
G allele	89.8	95.4	0.4 (0.19-0.97)	0.05
A allele	10.2	4.6	2.4 (1.03-5.39)	0.05
G/G	79.6	90.8	0.4 (0.17-0.93)	0.05
G/A	20.4	9.2	2.5 (1.07-5.97)	0.05
A/A	0	0	not detected	-

OR - odds ratio; CI - confidence interval. Statistically significant associations (p=0.05) were obtained for the G and A alleles and for the G/G and G/A genotypes.

3.2. Association by CEAP severity

Analysis by severity subgroup showed a consistent association across both moderate and severe disease. In patients with CVI C3-C4, the favourable G allele was less frequent than in controls (90.0% vs 95.4%; OR=0.4; 95% CI 0.17-1.14; chi-square=2.9; p=0.1), indicating a possible protective effect, while the unfavorable A allele was more frequent (10.0% vs 4.6%) and raised the risk of CVI approximately 2.3 times (OR=2.3; 95% CI 0.88-6.06). The heterozygous G/A genotype was more frequent in these patients (20.0% vs 9.2%), increasing the relative risk of moderate CVI approximately 2.5 times (OR=2.5; 95% CI 0.90-6.77) (Figure 3, Table 2).

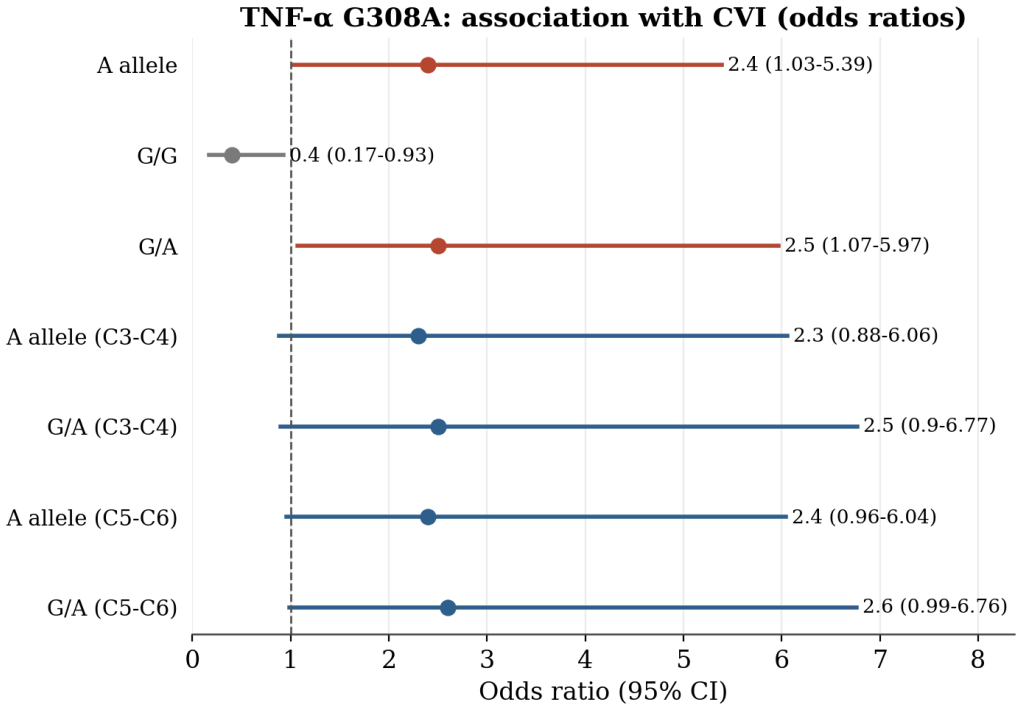

Figure 3. Odds ratios (with 95% confidence intervals) for the association of TNF-alpha G308A alleles and genotypes with CVI in the whole cohort and by severity subgroup; the dashed line marks OR=1.

In patients with severe CVI C5-C6, the wild-type G allele (89.6% vs 95.4%) and the G/G genotype (79.2% vs 90.8%) were detected less frequently (OR=0.4; 95% CI 0.17-1.05 and OR=0.4; 95% CI 0.15-1.01, respectively). A tendency towards a higher frequency of the unfavorable A allele (10.4% vs 4.6%) and the G/A genotype (20.8% vs 9.2%) was observed, with the relative risk of disease progression increasing approximately 2.4 times for the A

allele (OR=2.4; 95% CI 0.96-6.04) and approximately 2.6 times for the G/A genotype (OR=2.6; 95% CI 0.99-6.76) (Figure 4).

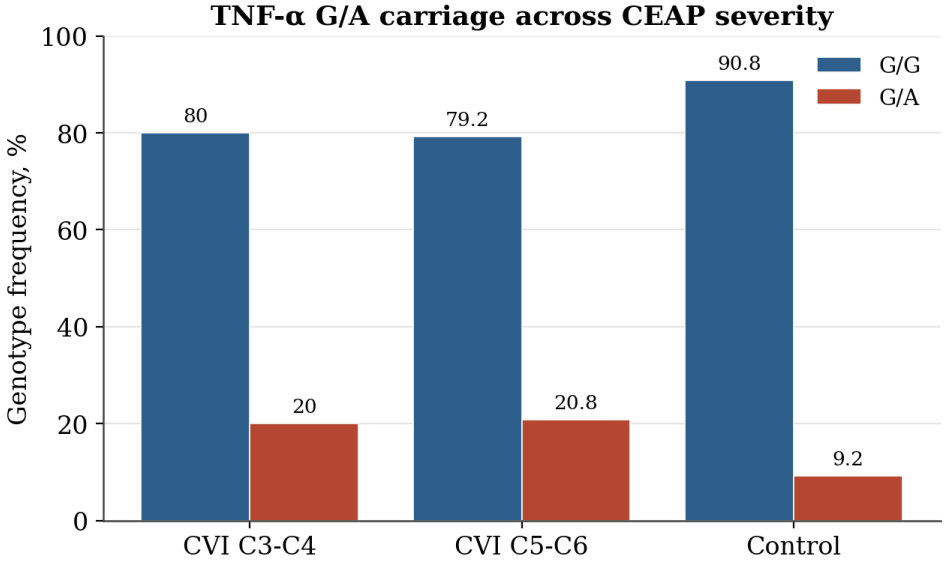

Figure 4. Carriage of the TNF-alpha G/G and G/A genotypes across CEAP severity subgroups (C3-C4, C5-C6) and controls.

Table 2. Association of the TNF-alpha G308A polymorphism with CVI by CEAP severity subgroup

Subgroup	Variant	Patients, %	Controls, %	OR	95% CI
CVI C3-C4 (n=45)	A allele	10.0	4.6	2.3	0.88-6.06
	G/G	80.0	90.8	0.4	0.15-1.11
	G/A	20.0	9.2	2.5	0.90-6.77
CVI C5-C6 (n=53)	A allele	10.4	4.6	2.4	0.96-6.04
	G/G	79.2	90.8	0.4	0.15-1.01
	G/A	20.8	9.2	2.6	0.99-6.76

Across both severity subgroups, the A/A genotype was absent, a finding likely attributable to its low frequency in this population and to the limited sample size. Taken together, the detection of the unfavorable A allele and the associated G/A genotype was consistently linked to an increased risk of CVI development and progression, with comparable effect sizes in moderate and severe disease, supporting a role for the TNF-alpha G308A variant as a marker of the inflammatory component of the disease.

4. Discussion

The present study demonstrates that the G308A polymorphism of the TNF-alpha gene is associated with CVI in an ethnically defined cohort from the Fergana Valley of Uzbekistan. Carriage of the unfavorable A allele increased the risk of disease by approximately 2.4 times, and the heterozygous G/A genotype was associated with a 2.5-fold increase, whereas the wild-type G/G genotype showed a protective tendency. The consistency of these associations across moderate (C3-C4) and severe (C5-C6) subgroups suggests that the inflammatory predisposition conferred by the A allele contributes both to the development of CVI and to its progression towards trophic complications.

These findings are biologically coherent. The G308A variant lies in the promoter region of the TNF-alpha gene, and the A allele is associated with increased transcriptional activity and higher cytokine production. Elevated TNF-alpha amplifies the inflammatory cascade through the generation of reactive

oxygen species, reduces the bioavailability of nitric oxide by influencing nitric oxide synthase, and thereby promotes endothelial dysfunction - a key early event in the pathophysiology of CVI [7, 8]. TNF-alpha also induces cell-adhesion molecules and promotes leukocyte infiltration of the venous wall and surrounding tissue, contributing to extracellular matrix remodeling and to the impaired healing that characterizes venous trophic ulcers [3, 5]. The association of the A allele with disease is therefore consistent with its role in sustaining the chronic inflammation that underlies progressive venous damage.

Our results are in line with reports from other populations. A study of women with severe chronic venous disease found that the distribution of TNF-alpha genotypes was related to the severity of clinical manifestations [10], and polymorphisms in inflammation-related genes have been associated with the risk of primary varicose veins [11]. Immunohistochemical studies of surgically removed varicose veins have demonstrated increased expression of ICAM-1 and TNF-alpha on the valve leaflets and in the vein wall proximal to the valve, supporting the local involvement of this cytokine in venous wall pathology. Elevated levels of pro-inflammatory cytokines, including TNF-alpha, have been documented in patients with CVI and in non-healing venous ulcers, where excessive production of inflammatory mediators adversely affects the surrounding tissue and delays wound healing [5, 6]. The diagnostic and prognostic informativeness of the G308A polymorphism, together with the consistency of its association across severity subgroups, supports its consideration as a marker of the inflammatory component of CVI.

Importantly, the risk of CVI is increased not only by the total number of unfavorable genetic variants but also by individual mutations with a substantial effect, such as the G/A genotype of the TNF-alpha G308A polymorphism. In the present cohort, the simultaneous carriage of heterozygous variants of the MMP9 and VEGFA genes was observed in all patients, and a considerable proportion of patients with detected mutations had first-degree relatives with CVI. This pattern reinforces the view that the combined assessment of several genes affecting venous wall remodeling, endothelial dysfunction and inflammation is the most patho-physiologically justified approach to risk prediction, and that genotyping should be performed for several markers rather than a single locus.

The principal limitation of this study is the modest sample size, which restricts statistical power for the low-frequency A allele and accounts for the wide confidence intervals and for the absence of the rare A/A genotype in both groups. The study also did not measure circulating TNF-alpha, which would have allowed the genotype to be related directly to the cytokine phenotype. Larger, multi-centre studies that combine genotyping with the measurement of inflammatory mediators and with the assessment of additional genes are required to define the relative contribution of the TNF-alpha G308A variant to venous pathology in this region and to support its incorporation into clinical risk stratification. From a translational perspective, pre-symptomatic genetic counselling and testing of individuals with a positive family history may enable the timely introduction of preventive measures and venoactive therapy, thereby reducing the number of complicated forms of the disease.

5. Conclusion

1. The G308A polymorphism of the TNF-alpha gene is associated with CVI of the lower extremities: the unfavorable A allele (OR=2.4; p=0.05) and the heterozygous G/A genotype (OR=2.5; p=0.05) increased the risk of disease, whereas the G/G genotype showed a protective tendency; the rare A/A genotype was not detected.
2. The association was consistent across CEAP severity subgroups, with the A allele reaching OR=2.3 in C3-C4 and OR=2.4 in C5-C6 patients and the G/A genotype reaching OR=2.5 and OR=2.6, respectively, reflecting a role of the variant in both the development and the progression of CVI through an amplified inflammatory process.

3. Genotyping of the TNF-alpha G308A polymorphism, preferably as part of a multi-gene panel together with markers of venous wall remodeling and endothelial dysfunction, may serve as a genetic predictor for the early, personalized prediction and prevention of CVI and its complicated forms.

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