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THE VEGFA G634C (RS2010963) POLYMORPHISM AND IMPAIRED ANGIOGENESIS IN THE PATHOGENESIS OF DIABETIC FOOT SYNDROME IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

O. T. Dadabaev
Andijan State Medical Institute, Ministry of Health of the Republic of Uzbekistan, Andijan, Uzbekistan
Corresponding author: O. T. Dadabaev
E-mail: dadabaev.o@agmi.uz

ABSTRACT

Background. Vascular endothelial growth factor A (VEGFA) is the principal regulator of angiogenesis, a process that is critically impaired in the diabetic foot syndrome (DFS). The functional G634C (rs2010963) polymorphism in the VEGFA gene modulates circulating VEGF levels, but its contribution to DFS susceptibility and to the distinct clinical forms of the disease has not been characterized in Central Asian patients.

Aim. To determine the distribution of the VEGFA G634C polymorphism in patients with DFS and controls, and to assess its association with DFS and with the neuropathic and neuroischemic forms of the disease.

Materials and methods. The G634C polymorphism was genotyped by polymerase chain reaction in 96 patients with type 2 diabetes and DFS (35 neuropathic, 61 neuroischemic) and in 83 control subjects without diabetes. Allele and genotype frequencies were compared using the χ^2 test and the odds ratio (OR) with 95 % confidence intervals (CI); conformity to Hardy–Weinberg equilibrium was verified.

Results. Genotype distributions conformed to Hardy–Weinberg equilibrium in both groups. The minor C allele was more frequent in DFS patients than in controls (24.5 % vs 10.8 %; OR = 2.7, 95 % CI 1.5–4.74). The heterozygous G/C genotype (32.3 % vs 16.9 %; OR = 2.4, 95 % CI 1.16–4.76) and the homozygous C/C genotype (8.3 % vs 2.4 %; OR = 3.7, 95 % CI 0.83–16.25) were associated with increased DFS risk, whereas the G allele and G/G genotype were protective. The risk markers were expressed in both clinical forms, and the heterozygous G/C genotype was somewhat more frequent in the neuroischemic form, in which it accompanied a marked reduction of tissue oxygenation.

Conclusion. The C allele and the C-bearing genotypes of the VEGFA G634C polymorphism are associated with susceptibility to DFS in patients with type 2 diabetes, supporting a role for genetically determined impairment of angiogenesis in the pathogenesis of the syndrome.

Keywords: VEGFA; rs2010963; G634C polymorphism; angiogenesis; diabetic foot syndrome; type 2 diabetes mellitus; single-nucleotide polymorphism.

INTRODUCTION

The diabetic foot syndrome (DFS) is one of the most serious chronic complications of diabetes mellitus and the leading cause of non-traumatic lower-limb amputation [1, 2]. It is classically characterized by a triad of neuropathy, ischaemia and infection, and a substantial proportion of patients ultimately require amputation because of progressive, non-healing purulent-

necrotic lesions [1]. A central mechanism underlying these chronic wounds is the failure of reparative angiogenesis: the formation of new capillaries from the existing vasculature, which is indispensable for the delivery of oxygen and nutrients to healing tissue [3].

Angiogenesis is a tightly regulated, multistage process driven by the interaction of cytokines, integrins and growth factors, among which vascular endothelial growth factor A (VEGFA) occupies a central position [3, 4]. Hypoxia is the principal physiological trigger of angiogenesis: it induces hypoxia-inducible factor-1α, which activates VEGFA and its receptors and thereby stimulates the proliferation and migration of endothelial cells [4]. Under the conditions of chronic hyperglycaemia, however, endothelial injury and dysregulated VEGF signalling disturb this balance. In diabetic retinopathy, VEGFA is pathologically overexpressed, producing excessive neovascularization; in the diabetic foot, by contrast, a relative deficiency of VEGFA at the wound impairs angiogenesis and delays healing, contributing to the chronicity of ulcers [5, 6].

Wound repair proceeds through overlapping phases of haemostasis, inflammation, proliferation and remodelling, and angiogenesis is integral to the proliferative phase, when new capillaries restore perfusion to the regenerating tissue [3, 4]. VEGFA acts at several points in this sequence: it directly stimulates the proliferation and migration of endothelial cells, stabilizes newly formed vessels, and participates in both the inflammatory and proliferative stages of healing. It is produced by a variety of cells at the wound, including endothelial cells, vascular smooth-muscle cells, keratinocytes and macrophages, and signals predominantly through the receptors VEGFR-1 and VEGFR-2 [3, 5]. Beyond its angiogenic action, VEGFA also exerts neuroprotective effects on peripheral nerve elements, which is of particular relevance in a complication that combines vascular and neural injury [4]. A deficiency, excess or dysregulation of any component of this finely balanced system can therefore produce the aberrant angiogenesis and impaired healing observed in the diabetic foot [3, 5].

The biological activity of VEGFA depends in part on the expression level of its gene, which is influenced by single-nucleotide polymorphisms (SNPs) in regulatory regions. Among the most studied is the G634C variant (rs2010963), located in the 5′-untranslated region of the gene. The genotype at this locus has been associated with differences in circulating VEGF concentration and in lipopolysaccharide-stimulated VEGF production by monocytes, with the C-bearing genotypes generally linked to altered protein expression [7, 8]. Because the magnitude and direction of these associations vary between populations as a result of ethnic heterogeneity and differing allele frequencies, population-specific data are required to interpret the role of the polymorphism in disease [1, 9].

Several case-control studies have examined VEGFA polymorphisms in DFS in different ethnic groups, but their results are inconsistent. In an Iranian population, a lower frequency of a particular VEGFA allele exerted a protective effect, attributed to enhanced angiogenesis in carriers [10]. In a Chinese Han population, carriers of a specific VEGF variant showed reduced susceptibility to DFS [6]. An Indonesian study reported that certain VEGF alleles acted as protective factors against DFS [11], while a Ukrainian study found no association between another VEGFA polymorphism and DFS [9]. These divergent findings underline both the biological plausibility of a VEGFA contribution to DFS and the need for additional population-

specific investigation. To date, the G634C polymorphism has not been characterized in the diabetic population of Uzbekistan. The present study therefore determined its distribution in patients with DFS and controls, and assessed its association with the disease and with its neuropathic and neuroischemic forms.

MATERIALS AND METHODS

2.1. Participants. The study used a case-control design. The case group comprised 96 patients with type 2 diabetes mellitus and clinically established DFS examined at the clinics of the Andijan State Medical Institute and the Andijan Regional Endocrinology Dispensary between 2020 and 2022; 35 had the neuropathic form and 61 the neuroischemic form, classified according to International Working Group on the Diabetic Foot criteria. The control group comprised 83 subjects without clinical manifestations of diabetes at examination or in their history and without a family history of diabetes. The groups were matched for age, sex and ethnicity. All participants gave written informed consent.

2.2. DNA extraction and genotyping. Venous blood (5 mL) was collected into EDTA tubes and stored at –70 °C. Genomic DNA was isolated from peripheral-blood leukocytes by a modified phenol–chloroform extraction method, and DNA concentration and purity were assessed spectrophotometrically (NanoDrop 2000) at A260/280, with values of 1.7–1.8 confirming suitability for amplification. The G634C polymorphism (rs2010963) of the VEGFA gene was genotyped by polymerase chain reaction (PCR) using a commercial detection kit (Sintol, Moscow) on Applied Biosystems 2720, Corbett Research CG1-96 and Rotor-Gene Q thermocyclers, with optimized amplification programmes. PCR products were separated by electrophoresis in 1–2 % agarose gel containing ethidium bromide and visualized under an ultraviolet transilluminator. Genotyping was performed at the Department of Molecular Medicine and Cell Technologies and the Laboratory of Medical Genetics of the Republican Specialized Scientific-Practical Medical Centre of Haematology, following GRIPS recommendations.

2.3. Statistical analysis. Allele frequencies were calculated by the standard gene-counting method, and observed and expected heterozygosity were used to test conformity to Hardy–Weinberg equilibrium with the GenePop programme. Differences in the distribution of alleles and genotypes between groups were assessed with the Pearson χ^2 test (with Yates' correction for 2×2 tables where any expected frequency was ≤ 5). The strength of association was expressed as the odds ratio (OR) with a 95 % confidence interval (CI); OR > 1 was interpreted as an increased-risk factor and OR < 1 as a reduced-risk factor. A two-sided p value below 0.05 was considered significant. Calculations used StatSoft Statistica 10.0 and Open Epi version 2.3.

RESULTS

3.1. Hardy–Weinberg equilibrium. In both the case and control groups the observed genotype distribution of the G634C polymorphism conformed to Hardy–Weinberg equilibrium, with no significant deviation between observed and expected genotype frequencies. In the case group the major G and minor C allele frequencies were 0.76 and 0.24, against 0.89 and 0.11 in controls; the relative deviation of observed from expected heterozygosity was negative in both

groups (D = –0.13), indicating a slight excess of heterozygotes and confirming the quality of genotyping and the homogeneity of the samples.

3.2. Association with DFS overall. The minor C allele was significantly more frequent in DFS patients than in controls (24.5 % vs 10.8 %), corresponding to a 2.7-fold increase in the odds of DFS (χ^2 = 11.1, p = 0.01, OR = 2.7, 95 % CI 1.5–4.74), while the G allele was correspondingly protective (OR = 0.4, 95 % CI 0.21–0.67). At the genotype level, the protective G/G genotype was less frequent in patients than in controls (59.4 % vs 80.7 %; OR = 0.3, 95 % CI 0.18–0.68), whereas the heterozygous G/C genotype was more frequent and conferred increased risk (32.3 % vs 16.9 %; OR = 2.4, 95 % CI 1.16–4.76). The homozygous C/C genotype showed a non-significant trend towards higher risk (8.3 % vs 2.4 %; OR = 3.7, 95 % CI 0.83–16.25). The allele and genotype frequencies are summarized in Table 1 and Figure 1, and the corresponding risk estimates are shown in Figure 2.

Table 1. Distribution of the VEGFA G634C alleles and genotypes in DFS patients and controls.

Allele / genotype	DFS group (n = 96)	Control (n = 83)	χ^2	p	OR (95 % CI)
G allele	75.5 %	89.2 %	11.1	0.01	0.4 (0.21–0.67)
C allele	24.5 %	10.8 %	11.1	0.01	2.7 (1.5–4.74)
G/G	59.4 %	80.7 %	9.5	0.01	0.3 (0.18–0.68)
G/C	32.3 %	16.9 %	5.6	0.025	2.4 (1.16–4.76)
C/C	8.3 %	2.4 %	3.0	0.1	3.7 (0.83–16.25)

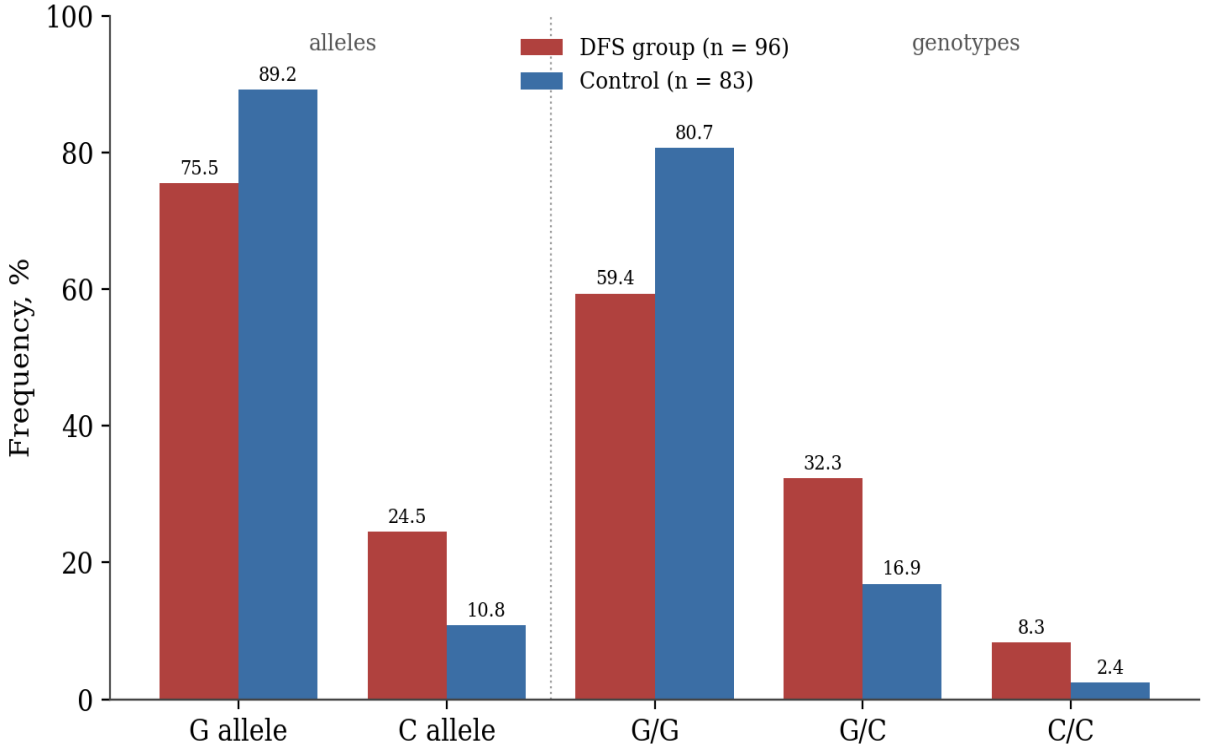

Figure 1. Allele and genotype frequencies of the VEGFA G634C polymorphism in DFS patients and controls.

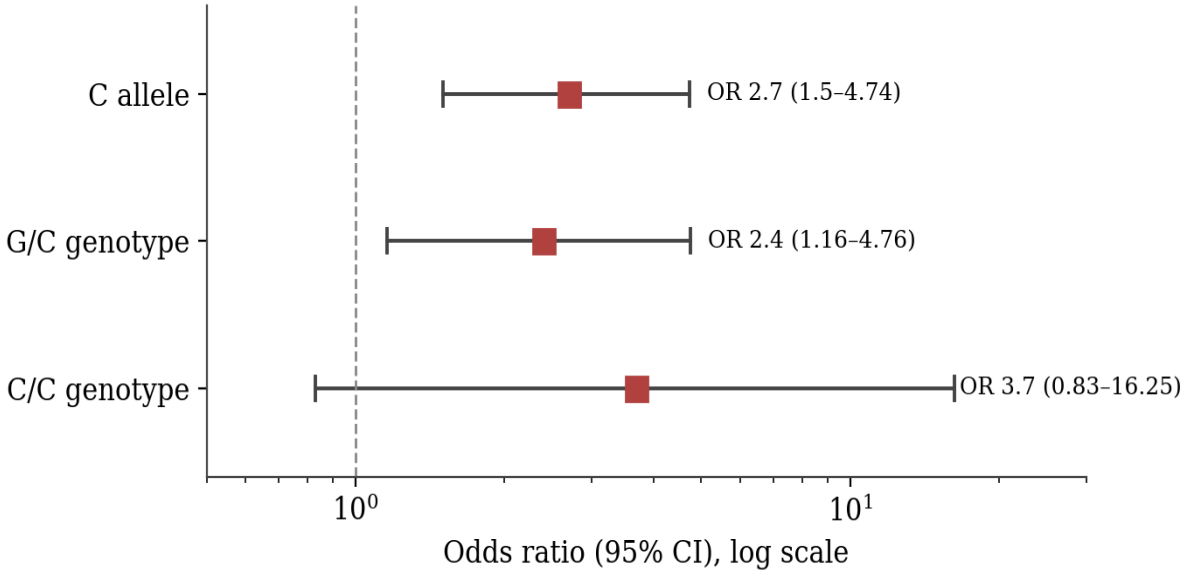

Figure 2. Odds ratios (95 % CI) for DFS risk associated with the C allele and the C-bearing genotypes of VEGFA G634C; the dashed line marks OR = 1.

3.3. Neuroischemic form versus control. In patients with the neuroischemic form the C allele frequency reached 25.4 %, against 10.8 % in controls. The heterozygous G/C genotype (34.4 %) and the homozygous C/C genotype (8.2 %) were more frequent than in controls, with a tendency towards increased risk of the neuroischemic form: the G/C genotype raised the odds approximately 2.6-fold (χ^2 = 5.9, p = 0.025, OR = 2.6, 95 % CI 1.2–5.58) and the C/C genotype approximately 3.6-fold (χ^2 = 2.5, p = 0.2, OR = 3.6, 95 % CI 0.75–17.54). Carriage of the C-bearing genotypes thus exerted a meaningful influence on the risk of the neuroischemic form relative to controls.

3.4. Neuropathic form versus control. In the neuropathic form the G allele was less frequent than in controls (77.1 % vs 89.2 %; OR = 0.4, 95 % CI 0.2–0.85) and the C allele correspondingly more frequent (22.9 % vs 10.8 %; OR = 2.4, 95 % CI 1.18–5.04). The protective G/G genotype was reduced (62.9 % vs 80.7 %; OR = 0.4, 95 % CI 0.17–0.96), while the G/C genotype (28.6 % vs 16.9 %; OR = 2.0, 95 % CI 0.78–4.96) and the C/C genotype (8.6 % vs 2.4 %; OR = 3.8, 95 % CI 0.68–21.27) showed trends towards increased risk of the neuropathic form.

3.5. Comparison of the two clinical forms. Direct comparison of the neuroischemic and neuropathic forms revealed only minor differences in allele and genotype distribution (Table 2, Figure 3). The heterozygous G/C genotype was somewhat more frequent in the neuroischemic form (34.4 % vs 28.6 %; OR = 1.3, 95 % CI 0.53–3.24), whereas the G/G and C/C genotypes were almost equally distributed (OR = 0.8 and 1.0, respectively). Although these differences did not reach statistical significance, the relative enrichment of the unfavourable heterozygous genotype in the neuroischemic form is noteworthy in the context of its more severe microcirculatory impairment.

Table 2. Distribution of the VEGFA G634C genotypes across the clinical forms of DFS and controls.

Genotype	Neuropathic (n = 35)	Neuroischemic (n = 61)	Control (n = 83)
G/G	62.9 %	57.4 %	80.7 %
G/C	28.6 %	34.4 %	16.9 %
C/C	8.6 %	8.2 %	2.4 %

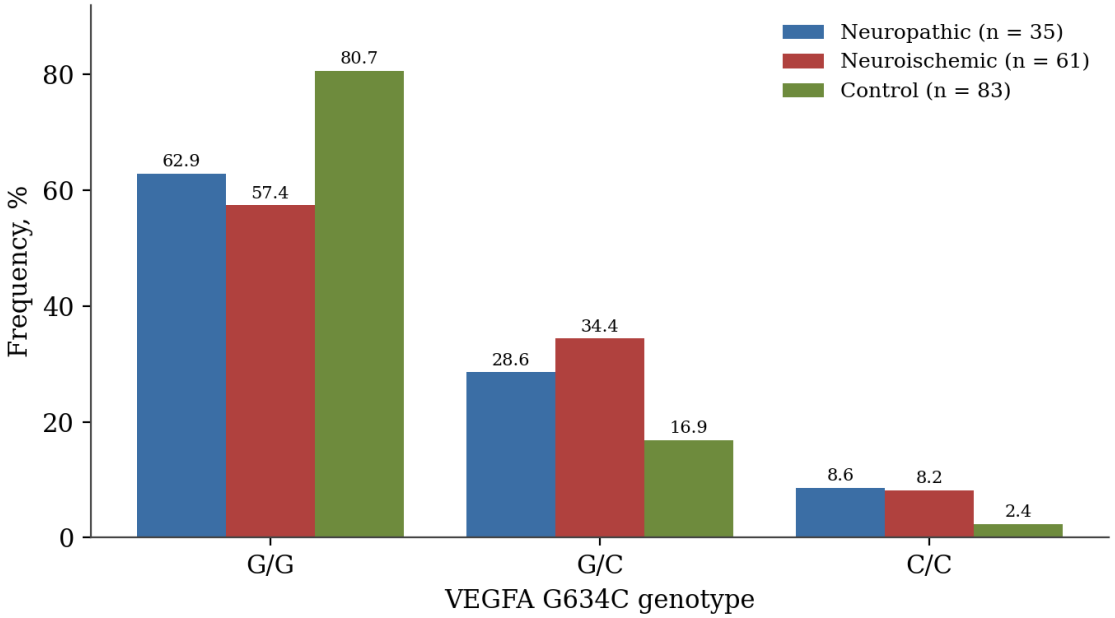

Figure 3. Genotype distribution of the VEGFA G634C polymorphism across the neuropathic and neuroischemic forms of DFS and controls.

3.6. Relationship with tissue oxygenation. The enrichment of the C-bearing genotypes in the neuroischemic form paralleled the instrumental findings of impaired perfusion. Transcutaneous oxygen tension in the neuroischemic form was markedly lower than in the neuropathic form (33.7 ± 1.9 vs 42.3 ± 1.6 mmHg), a more than 1.2-fold reduction consistent with the grosser disturbance of angiogenesis expected from reduced VEGFA activity. This convergence links the genetic findings to the clinical phenotype of deep, non-healing ulceration that characterizes the neuroischemic form.

DISCUSSION

This study shows that the C allele of the VEGFA G634C polymorphism and the C-bearing genotypes are associated with susceptibility to DFS in patients with type 2 diabetes, whereas the G allele and the G/G genotype are protective. The associations were evident both for DFS overall and within each clinical form, and the unfavourable heterozygous genotype was relatively enriched in the neuroischemic form. These findings are biologically coherent: VEGFA is the central effector of reparative angiogenesis, and genetically determined alterations in its expression would be expected to influence the capacity for wound healing in the diabetic foot [3, 4, 7].

The role of VEGFA polymorphisms in DFS has been examined in several populations with heterogeneous results. A protective effect of a particular VEGFA allele was reported in an Iranian cohort, attributed to enhanced angiogenesis in carriers [10], and reduced susceptibility

was observed in carriers of a VEGF variant in a Chinese Han population [6]. An Indonesian case-control study likewise identified certain VEGF alleles as protective factors [11], and promoter polymorphisms have been associated with DFS in further work [12]. In contrast, a Ukrainian study found no association between another VEGFA polymorphism and DFS [9]. This heterogeneity reflects differences in the specific variants studied, in linkage structure and in population allele frequencies, and underscores the value of region-specific data such as those presented here [1, 13]. Our observation that the C allele increases DFS risk in the Uzbek population adds to this body of evidence and is consistent with the concept that impaired, rather than excessive, angiogenesis predominates in the diabetic foot.

The apparent paradox between diabetic retinopathy, in which VEGFA is overexpressed and drives pathological neovascularization, and the diabetic foot, in which a relative VEGFA deficiency impairs healing, is well recognized [5, 6]. The G634C genotype is one determinant of VEGF expression, and the C-bearing genotypes have been linked to altered VEGF production [7, 8]. In the diabetic foot, where hypoxia should normally up-regulate VEGFA to promote angiogenesis, a genetically constrained or dysregulated response would be expected to compromise capillary formation and to favour chronic, non-healing ulceration [3, 6, 14]. The relative enrichment of the G/C genotype in the neuroischemic form, together with the substantially lower transcutaneous oxygen tension in that group, is in keeping with a greater contribution of impaired angiogenesis to the neuroischemic phenotype.

The mechanistic context of these associations lies in diabetic endothelial dysfunction. Sustained hyperglycaemia injures the vascular endothelium through several converging pathways — the polyol pathway, accumulation of advanced glycation end-products, oxidative stress and activation of protein kinase C — leading to apoptosis of endothelial cells, reduced production of vasodilators and a pro-inflammatory, procoagulant vascular phenotype [1, 5]. Against this background, the capacity to mount an adequate angiogenic response becomes a decisive determinant of whether a foot wound heals or becomes chronic. A genotype that constrains VEGFA expression would be expected to aggravate the already impaired angiogenesis of the diabetic foot, and the phenomenon of metabolic memory — the persistence of hyperglycaemia-induced changes after glucose normalization — may further entrench this deficit. The association of the C-bearing genotypes with DFS observed here is consistent with this model and complements the clinical and instrumental evidence of impaired perfusion in affected patients.

These findings have potential clinical implications. Growth factors and cytokines are increasingly studied as therapeutic targets in diabetic wound healing, and VEGF-based and angiogenesis-modulating strategies are under active investigation [4, 10]. Identification of patients carrying high-risk VEGFA genotypes could, in principle, contribute to risk stratification and to the selection of candidates for closer surveillance and early, angiogenesis-oriented intervention. However, the contribution of any single SNP is modest, and DFS is a polygenic, multifactorial condition; the practical value of VEGFA genotyping will therefore depend on its integration with other genetic and clinical markers [1, 13]. The limitations of the present study include its single-centre design, the modest sample size — which limited the statistical power for the rarer C/C genotype — and the absence of direct measurement of

circulating or wound VEGF. Larger, multi-centre studies with functional correlates are required to confirm these associations and to define their prognostic utility.

CONCLUSION

In patients with type 2 diabetes, the C allele and the C-bearing genotypes (G/C and C/C) of the VEGFA G634C (rs2010963) polymorphism are associated with increased susceptibility to DFS, while the G allele and the G/G genotype are protective. The risk markers are expressed in both clinical forms and are relatively enriched in the neuroischemic form, in which they accompany a pronounced reduction of tissue oxygenation. These results support a role for genetically determined impairment of angiogenesis in the pathogenesis of DFS and justify the inclusion of the VEGFA G634C polymorphism in a broader panel of molecular-genetic markers for the syndrome.

DECLARATIONS

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Conflict of interest. The authors declare no conflict of interest.

Ethical approval. The study was conducted in accordance with the Declaration of Helsinki; all participants provided written informed consent.

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