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# Investigating the relationship between VEGF gene C936T-rs3025039 polymorphism and type 2 diabetes

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**Abstract**

Type 2 diabetes (T2D), formerly known as adult-onset diabetes, is characterized by high blood sugar, insulin resistance, and relative lack of insulin. Diabetic retinopathy, a secondary microvascular complication of diabetes mellitus, is the leading cause of blindness. There is extensive evidence that the pathologic ocular angiogenesis in diabetic retinopathy is regulated by the vascular endothelial growth factor-A (VEGF-A). The single nucleotide polymorphism (SNP) C936T (rs3025039) of the VEGF gene has been investigated in relation to cancer, endometriosis, and age-related macular degeneration. The relationship between the rs3025039 VEGF gene polymorphism and the risk of type 2 diabetic retinopathy in 80 DT2 patients was examined. No significant association was found between polymorphism C936T and type 2 diabetes. It is recommended that this study be repeated on a larger population.

## INTRODUCTION

Angiogenesis, the formation and maintenance of blood vessel structures, is essential for the physiological functions of tissues and is important for the progression of diseases such as cancer and inflammation (1). Diabetic retinopathy, a secondary microvascular complication of diabetes mellitus, is the leading cause of blindness in the United States among individuals aged 20 to 64 years (2). Two major retinal problems cause most of the diabetes-related vision loss: diabetic macular edema and complications from abnormal retinal blood vessel growth, angiogenesis. Secondary to angiogenesis, increased retinal blood flow is of pathogenic importance in the progression of diabetic retinopathy. In recent decades, a variety of signaling molecules, such as VEGF-VEGFRs, ephrin-Eph receptors, angiopoietin-Tie, and the Delta-Notch system, have been identified as playing important roles in angiogenesis (3). Among these, vascular endothelial growth factors (VEGFs) and receptors (VEGFRs) regulate both vasculogenesis, the development of blood vessels from precursor cells during early embryogenesis, and angiogenesis (4). Diseases associated with VEGFA include microvascular complications of diabetes 1 and POEMS syndrome. Located at chromosome 6p21.3, it is involved in angiogenesis modulation under physiologic and pathologic conditions (5). There is extensive evidence that pathologic ocular angiogenesis in diabetic retinopathy is regulated by vascular endothelial growth factor-A (VEGF-A). Miller et al. showed that the level of VEGF-A in ocular tissue correlates with new vessel formation. Additionally, the role of VEGF-A in increasing vascular permeability plays a role in the development of macular edema in diabetic retinopathy (6). VEGF-induced leakage is probably mediated by a variety of factors, including leukocyte-mediated endothelial injury, fenestrae formation, dissolution of tight junctions, and transcellular bulk flow (7). The amount and duration of VEGF exposure required for blood-retina barrier breakdown may be less than required for neovascularization. By necessity, neovascularization may be preceded by an increase in vascular permeability. Hypoxia is a key regulator of VEGF-induced ocular neovascularization through the production of hypoxia-inducible factor-1. The single nucleotide polymorphism (SNP) C936T (rs3025039) of the VEGF gene has been investigated in relation to cancer endometriosis and age-related macular degeneration (8-9). Studies have shown that the CC genotype of C936T polymorphism in the 3' untranslated region (3' -UTR) of the VEGF gene was associated with an increased serum VEGF level compared with the CT and TT genotypes. In the current study, the relationship between rs3025039 VEGF gene polymorphism and the risk of type 2 diabetic retinopathy was examined.

## METHODS AND MATERIALS

The sample was composed of two groups: 80 people with type 2 diabetes mellitus and 80 people as a control group. Blood (4 ml) was collected from

each individual, and the genomic DNA obtained from total peripheral blood was extracted using the salting-out technique (27). Polymorphisms were identified through polymerase chain reaction followed by enzymatic digestion by restriction enzyme. PCR reactions with a final volume of 50 µl were performed. The sequences of the applied primers for the investigation of polymorphism C936T of the VEGF gene were as follows: Forward: 5'AAG GAAGAGGAGACTCTGCGCAGAGC3' and reverse: 5'TAAATGTATGTA T G T G G G T G G G T G T G T C T A G A G 3 ' (GI:559098479 NM_001287044.1), and a fragment of 208 base pairs was generated. After the PCR products were enzymatically digested with NlaIII and for the visualization of the fragments, electrophoresis was performed on 2% agarose gel. Statistical analyses were conducted using the IBM SPSS software version 20 (IBM, Armonk, NY, USA). A value of $p<0.05$ was considered to indicate a statistically significant difference.

**Table 1.** Genotype and allele frequency of C936T (rs3025039)

| Genotype | DM2 | Control | Group | *p*-value |
|---|---|---|---|---|
| CC | 67 | 54 | CCxCT | 0.211 |
| CT | 10 | 21 | CTxTT | 0.989 |
| TT | 3 | 5 | CCxTT | 0.485 |
| Allele | | | | |
| C | 77 | 75 | CC+CTxTT | 0.541 |
| T | 13 | 26 | TT+CTxCC | 0.084 |

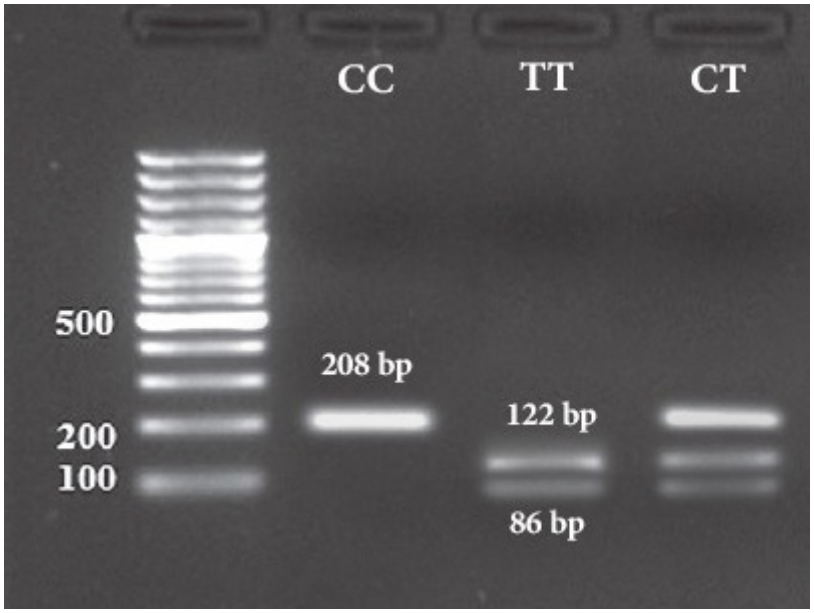


**Fig. 1.** CC genotype 208 bp band, TT genotype 122+86 bp and CT heterozygote 208+122+86 bp

## RESULTS

The study group included 40 men and 40 women with type 2 diabetes and an average age of 53 years; the control group included 40 healthy men and 40 healthy women with a mean age of 42 years. Of the 80 people with type 2 diabetes, 67 individuals had genotype CC, 10 had genotype CT, and 3 had genotype TT. In the healthy group, 54 individuals had genotype CC, 21 had genotype CT, and 5 had genotype TT. The prevalence of allele C was 77 in the diabetic group and 75 in the control group, while the prevalence of allele T was 13 in the diabetic group and 26 in the healthy group. There was no significant association between SNP C936T of the VEGF gene polymorphism and type 2 diabetes (Table 1).

## DISCUSSION

Type 2 diabetes (T2D), formerly known as adult-onset diabetes, is characterized by high blood sugar, insulin resistance, and a relative lack of insulin (10). Common symptoms include increased thirst, frequent urination, and unexplained weight loss. Type 2 diabetes (T2D) is the result of interaction between environmental factors and a strong hereditary component (11). Very few T2D risk genes were identified using candidate gene and linkage-based

studies, but the advent of genome-wide association studies has led to the identification of multiple genes, including several that were not previously known to play any role in T2D (12). Diabetic retinopathy, a secondary microvascular complication of diabetes mellitus, is a leading cause of blindness (13). All persons with diabetes are at risk of developing retinal complications. As reported by epidemiological studies, the major risk factors for diabetic retinopathy include duration of diabetes, hypertension, hyperlipidemia, and most importantly, hyperglycemia (14-15). There is extensive evidence that pathologic ocular angiogenesis in diabetic retinopathy is regulated by vascular endothelial growth factor-A (VEGF-A). Simo et al. (2002) found that both free IGF1 and VEGF were increased in the vitreous fluid of diabetic patients with proliferative diabetic retinopathy. The elevation of IGF1 was unrelated to the elevation of VEGF in these patients. The authors felt that their results supported the concept that VEGF was directly involved in the pathogenesis of proliferative diabetic retinopathy, whereas the precise role of free IGF1 remained to be established. Funatsu et al. (2002) investigated the relationship between diabetic macular edema and the levels of VEGF and interleukin-6 (IL6; 147620) in aqueous humor and plasma (16). They found that aqueous levels of VEGF and IL6 correlated significantly with the severity of macular edema and that aqueous levels were significantly higher than plasma levels. In addition, the aqueous level of VEGF correlated significantly with that of IL6 (17). The authors concluded that both VEGF and IL6 are produced together in intraocular tissue and that both are involved in the pathogenesis of diabetic macular edema. Awata et al. (2002) identified 7 polymorphisms of the VEGF gene in the promoter region and 5-prime and 3-prime untranslated regions. The genotype distribution of one of these (-634G-C; rs2010963) differed significantly between type 2 diabetes patients without retinopathy and those with any retinopathy, and the C allele was significantly associated with the presence of retinopathy. In the current study, the relationship between rs3025039 VEGF gene polymorphism and the risk of type 2 diabetic was examined in 90 DM2 patients and 90 healthy control individuals (18-19). The results showed no significant association between SNP C936T of the VEGF gene polymorphism and type 2 diabetes (Table 1). These results are consistent with the results of Ghisleni et al. (2015) who studied the relationship between type 2 diabetes and the genotype and allele frequency of SNP C936T of the VEGF gene polymorphism; they found no significant association between polymorphism C936T and type 2 diabetes. It is recommended that this study be repeated on a larger population as well as between diabetic macular edema patients and that the results be compared with those of previous studies.

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