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Original research article

Association of 18bp insertion/deletion polymorphism, at −2549 position of *VEGF* gene, with diabetic vascular complications in type 2 diabetes mellitus

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ABSTRACT

Purpose: Diabetes mellitus type 2 (T2DM) and its vascular complications are a serious world health problem. For this reason it is important to look for new diabetes complication risk factors. The aim of this study was to determine whether 18-bp insertion/deletion (I/D) polymorphism at −2549 position of the vascular endothelial growth factor (VEGF) gene is associated with diabetic vascular complications (DVC).

Material and methods: Caucasian subjects (n = 100) with T2DM were recruited for this study. Genotyping of the *VEGF* gene I/D polymorphism was done by the polymerase chain reaction (PCR) method. The results were correlated with laboratory and clinical data.

Results: In our population heterozygous of the *VEGF* gene polymorphism was observed most frequently (57%). DVC were observed in 53 patients. Heterozygous T2DM patients significantly more often suffered from heart failure (HF) and stroke (p = 0.05). Amongst all the DVC, D allele of the *VEGF* polymorphism had a significantly increased risk of diabetic retinopathy (DR) (OR = 1.31; p = 0.033) irrespective of the duration of diabetes, BMI, the glycemia control expressed by HbA1c, renal function, lipid values or applied treatment. The studied polymorphism did not correlate with coronary heart disease, peripheral vascular disease, cardiovascular death, diabetic kidney disease or applied treatment.

Conclusions: The multivariate logistic regression analysis showed that the D allele in the promoter region of the *VEGF* gene is an independent risk factor of DR irrespective of other laboratory and clinical variables in T2DM patients. Our study suggests that I/D allele in the studied gene is associated with HF and strokes.

1. Introduction

Diabetes mellitus (DM) is one of the most dangerous civilization diseases, causing not only ill-health, but also social and economic problems with significant impact on the quality of life. It has become known as a 'global epidemic' of the 21 st century. The incidence of the disease is still increasing and is often associated with severe complications related to an increased risk of cardiovascular complications and death among diabetics [1,2]. The latest recommendations (2016) of the European Society of Cardiology (ESC) categorizes people with diabetes as a group with very high risk of developing cardiovascular disease, regardless of age, gender, cholesterol levels, hypertension or smoking status [3]. Currently, risk assessment scales for diabetic complications are widely used by clinicians who implement the recommendations of scientific societies to avoid potentially damaging complications due to diabetes including stroke, coronary disease, myocardial infarction, atherosclerotic peripheral vascular disease or retinopathy. In most cases, the risk of developing complications can be reduced by following the principles of a healthy diet or targeted drug therapy associated with the optimization of blood sugar levels and lipids, undertaking a recommended amount of daily physical activity for weight control or the effective treatment of comorbid diabetes mellitus type 2 (T2DM) disorders such as hypertension [3,4]. Clinical observations provide evidence that not all people suffering from T2DM develop diabetic

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angiopathy, which is a major cause of morbidity and mortality during the course of T2DM [4,5]. In clinical practice, there is a group of patients without complications, despite having diabetes for many years or not having the best glycemic control. The molecular mechanisms underlying the long-term effects of high glucose concentrations in the first years after T2DM diagnosis are still being studied and are thought to depend on metabolic memory conditioned by epigenetic processes [6]. The prognostic diversity among all individuals with diabetes indicates a probable heterogenous nature of metabolic disorders in diabetes that may be associated with genetic susceptibility [7]. The current state of knowledge on the pathogenesis of T2DM indicates the important role of genetic factors, which in combination with environmental conditions determine the clinical course of T2DM and related complications [8]. Medical genetics is concentrated on the search for and identification of specific genes that contribute to the increased risk of developing the disease. An important part of the analyses of genetic research is searching for associations between the clinical data and the specific genetic variants in different parts of the human genome. These studies can be carried out by analyzing the frequency of polymorphisms in the range of previously described candidate genes that play a potentially important role in the development of pathophysiological mechanisms associated with the establishment of the disease [9,10].

There are studies demonstrating the importance of vascular endothelial growth factor (VEGF) gene polymorphism in the pathogenesis of diabetic kidney disease (DN) [11,12]. The role of single nucleotide polymorphisms has gained significant importance since the first publication of results of the Genome-Wide Association Study (GWAS) in 2007. This study consisted of screening the human genome to find relationships of the genetic variants to the most common diseases [13,14]. Since the 1990s, when Adamis et al. published the discovery that there is an increased expression of *VEGF* in the tissues of the eye in patients with diabetic retinopathy, more than 500 studies on the role of VEGF in developing vascular complications among diabetes have been published around the world [15,16]. VEGF is a signal protein produced by cells that stimulates cell proliferation and increases the permeability of the endothelial cells of veins, arteries, and lymphatic vessels [17]. In glomeruli, VEGF is produced by podocytes, and its actions on endothelial cells, podocytes and renal medulla have been described [18–20]. Human DN is associated with diminished VEGF levels and experience in the oncological setting has taught us that VEGF blocking therapy can cause adverse renal effects in patients [20]. Total glomerular VEGF levels decrease as DN progresses in humans, and is therefore considered to provide an endogenous “protective” signal that prevents apoptosis of vascular wall cells and hence the progression of DN. Also, VEGF has been identified as a key mediator of the progression to advanced diabetic retinopathy (DR). Hyperglycemia is a key component in the development of DR and is thought to lead to alteration of biochemical pathways in the retina, resulting in inflammation and oxidative stress. Cytokines such as VEGF are upregulated as part of this response [21]. For many years, scientists tried to determine the exact mechanism responsible for the increased levels of VEGF in patients with diabetes. One possibility is that *VEGF* gene polymorphism leads to an increase in its expression. Confirmation of this association could in the future be used as a genetic marker for determining the degree of risk of vascular complications in T2DM.

The aim of the present study was to determine whether an 18bp insertion/deletion polymorphism at −2549 position of the *VEGF* gene exerts influence on the development of diabetic vascular complications (DVC).

2. Material and methods

We recruited 100 adult patients (58 women and 42 men) with T2DM for the study. These were all outpatients of the Nephrology Clinic and Department of Dialysis and Nephrology at St. Queen Jadwiga Clinical District Hospital No 2 in Rzeszów (Poland). Molecular analyses were performed in the Department of Genetics at the University of Rzeszów (Poland). Informed consent was obtained from all patients who participated in the study. The patients were recruited for 12 months. The observation period was two years.

Excluded from the study were patients with: T1DM, neoplastic diseases, hematologic diseases, systemic connective tissue diseases, glomerulonephritis, urinary tract infections and acute inflammatory diseases, nephrolithiasis, and those after nephrectomy and pregnancy.

Every patient was interviewed to obtain the individual’s complete clinical history including general information, duration of diabetes, diseases and symptoms associated with vascular complications and treatments that could potentially affect the condition of blood vessels. DVC was defined as: coronary disease confirmed by coronary revascularization or hospitalization for unstable angina, myocardial infarction, heart failure due to coronary artery disease, previous stroke or transient ischemic attack (TIA), chronic kidney disease with eGFR < 15 ml/min/1.73m^2 (CKD G5), atherosclerotic peripheral vascular disease confirmed in an ultrasound of arteries, or angiography, or a previous history of angioplasty, or following limb amputation due to atherosclerosis, diabetic retinopathy, or cardiovascular death.

Additionally, body mass index (BMI), based on the Queteleta formula was performed. Kidney function was assessed by: calculation glomerular filtration rate (eGFR) based on the CKD-EPI equation, albuminuria calculated as the albumin to creatinine ratio in a single urine sample (UACR) expressed in mg/g, and the function of kidney tubules was assessed by examining the urine neutrophil gelatinase-associated lipocalin (NGAL) to creatinine ratio in a single urine sample (UNCR) expressed in μg/g. The CKD stage was defined according to KDIGO 2012 [22]. Diabetic nephropathy (DN) was diagnosed when the patient had persistent albuminuria ≥300 mg/24 h in the absence of haematuria or infection. All patients with DN were undergoing maintenance hemodialysis.

In addition, all patients were evaluated for glycated hemoglobin (HbA_{1c}) and lipidograms. For good glycemic control all patients received a diabetic diet, while some patients were treated with insulin and metformin. In the patients with hypertension and without contraindications such as hyperkalemia, eGFR < 30 ml/min, or renal artery stenosis, we used renin-angiotensin-aldosterone system inhibitors (RAAS-I) to treat arterial hypertension. Most patients received aspirin in a cardioprotective dose. Pharmacological treatment of hyperlipidemia was based mainly on diet and statins.

We assumed that the distribution of the alleles in the Caucasian patients was comparable to that described in other studies of this gene polymorphism [23,25,26].

2.1. Determination of VEGF genotypes

High molecular weight genomic DNA was isolated from 400 μl whole blood using a MagCore® HF16 Automated Nucleic Acid Extractor (RBC Bioscience). The I/D polymorphism was determined using the primers whose sequences were shown in the study by Amle et al. [27]: forward 5’-GCTGAGGATGGGGCTGACTAGGTA-3’and reverse 5’-GTTT CTGACCTGGCTATTTCCAGG-3’. Amplification was carried out in a final volume of 20 μl containing 4 μl 5 × PCR Buffer, 0.6 μl of 10 mM dNTP mix, 1.4 μl of 25 mM MgCl2, 1.2 μl (12 pmol) each of forward and reverse primers, 0.5 U of Taq DNA polymerase (Promega), 50 ng of purified DNA and Nuclease Free Water to make the volume up to 20 μl. The PCR was performed in the Labcycler (SensoQuest) under the following conditions: initial denaturation at 95 °C for 2 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 67.1 °C for 30 s, extension at 72 °C for 1 min, with final extension at 72 °C for 5 min. The amplification products were separated by electrophoresis through 2% agarose gel stained with Midori Green Stain (Nippon Genetics). For the *VEGF* I/D polymorphism two bands were observed, 211 bp (D allele) and 229 bp (I allele). Representative results of gel electrophoresis are shown in Fig. 1.

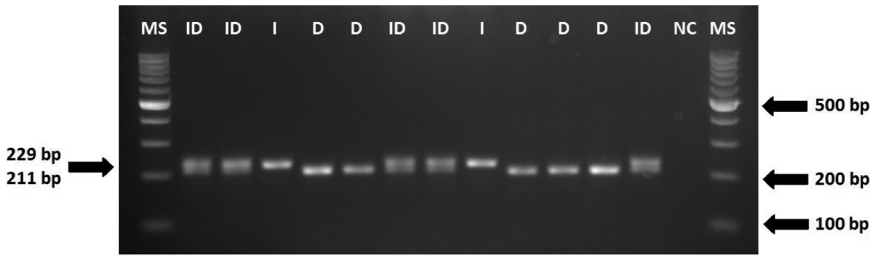

Fig. 1. Representative results of gel electrophoresis.
All of the bands from unedited gel were used in the final image. The gel was photographed using a G:BOX imaging system (Syngene).
Lane 1, 15 – DNA molecular mass marker (100-bp ladder).
Lane 2, 3, 7, 8, 13 – I/D genotype (two bands at 229 bp and 211 bp).
Lane 4, 9 – I genotype (one band at 229 bp).
Lane 5, 6, 10, 11, 12 – D genotype (one band at 211 bp).
Lane 14 – negative control (bands not observed).

2.2. Ethical issues

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Bioethical Committee of the Medical Faculty of Rzeszow University (number: 12/06/2015).

2.3. Statistical analysis

Categorical variables were presented as numbers followed by percentages in brackets. Differences between categorical variables were evaluated using Pearson's Chi-square test. Continuous variables were presented as medians followed by interquartile ranges (IQR) in brackets. The Shapiro-Wilk test was used for continuous variables' distribution assessment. Due to non-normal distribution, continuous variables were compared using Mann Whitney's *U* test for two groups, or the Kruskal-Wallis one-way analysis of variance with additional post-hoc comparisons for three or more groups. A correction for multiple testing was applied. Multivariate analyses were made using Generalized Linear Models (GLZ). Hardy Weinberg equilibrium was tested for allele frequencies using an online calculator: http://www.oege.org/software/hwe-mr-calc.shtml. The Statistica 12.5 PL package (Statsoft, Tulsa, USA) was used for other analyses. P values < 0.05 were considered as statistically significant.

3. Results

The study group consisted of T2DM patients over the age of 18 who chose to participate. The average age was 65.7 ± 11.4 years, the youngest patient was 31 years old and the oldest was 91 years. The mean duration of diabetes in the whole group of patients was 11.1 ± 9.3 years. The distribution of 18bp insertion/deletion polymorphism in the patients was according to the predicted Hardy-Weinberg's distribution (df = 2; Chi2 = 1.76).

Clinical characteristics and genotype analysis of the patients in the test study group are presented in Table 1. Homozygous DD patients had significantly higher LDL cholesterol levels in comparison with both ID and II patients (p = 0.04) (Table 1, Fig. 2).

Patients in the study group were treated, inter alia, with metformin (n = 57), insulin (n = 28), statin (n = 54), a cardioprotective dose of aspirin (n = 38), and in the treatment of hypertension they received RAAS-I (n = 55) (Table 1).

DVC were observed in 53 patients. Across the whole study population there were 7% cardiovascular deaths. In the study group, DVC appeared with higher frequency in the elderly (p < 0001), with greater wrinkles: UNCR (p = 0.04), total cholesterol (p = 0.02) and LDL cholesterol (p = 0.016). In univariate analysis, heterozygous T2DM patients suffered from heart failure and stroke significantly more often (p = 0.05). Table 2 shows the incidence of all DVC according to the polymorphism of the *VEGF* gene in univariate analysis.

The multivariate logistic regression analysis showed that in addition to the patients' age ($p = 0.02$) and eGFR ($p = 0.02$), the D allele of the *VEGF* polymorphism had a significantly increased risk of diabetic retinopathy (beta+/-SE = 0.27 ± 0.12; OR = 1.31; $p = 0.03$) irrespective of the duration of diabetes, BMI of patients, the degree of diabetes compensation expressed by HbA_{1c}, serum creatinine, eGFR, UACR and UNCR as well as lipidogram values and applied treatment, between all DVC (Table 4).

In the T2DM population heterozygotes were characterized by a higher serum creatinine but these results did not affect the eGFR CKD-EPI. Among the study population, 16 individuals had eGFR CKD-EPI < 15 ml/min and required hemodialysis, while 54 cases had eGFR CKD-EPI > 60 ml/min/1.73m^2. Table 3 shows the study population according to the polymorphism of the *VEGF* gene and the CKD stage defined according to KDIGO 2012.

Table 1
Clinical characteristics and genotype analysis of the study group.

	Genotypes			*p*-value
	D/D (n = 21)	I/D (n = 57)	I/I (n = 22)	
Gender [n (%)]				
man	6 (15.0)	28 (70.0)	6 (15.0)	0.10
woman	15 (25.0)	29 (48.3)	16 (26.7)	
Age (years)	65.6 ± 9.4 (59–73)	65.9 ± 12.1 (59–74)	65.3 ± 11.8 (57–73)	0.97**
Duration of diabetes (years)	11.9 ± 9.5 (3.0–20.0)	9.92 ± 8.4 (4.0–16.0)	12.2 ± 8.9 (5.0–18.0)	0.47
BMI (kg/m^2)	32.4 ± 4.5 (29.5–33.9)	30.5 ± 6.3 (24.8–34.2)	30.8 ± 6.6 (28.2–35.0)	0.48
Hypertension [n (%)]	20 (95.2)	48 (84.2)	19 (86.4)	0,37
HbA_{1c} (%)	7.5 ± 1.9 (6.1–7.9)	6.7 ± 1.2 (5.9–7.4)	7.5 ± 2.3 (6.2–8.1)	0.15**
Serum creatinine (mmol/l)	106.8 ± 70.7 (61.9–97.2)	194.5 ± 203.3 (70.7–203.3)	141.4 ± 194.48 (61.9–97.2)	0.078*
eGFR CKD-EPI (ml/min/1.73 m^2)	70.3 ± 28.1 (55.0–94.0)	56.8 ± 35.5 (24.0–93.0)	70.2 ± 33.7 (47.0–98.0)	0.15*
UACR (mg/g)	96.4 ± 134.0 (4.3–276.5)	118.9 ± 166.3 (5.0–301.0)	96.9 ± 170.0 (5.1–97.1)	0.46
UNCR (μg/g)	19.8 ± 17.7 (7.2–31.1)	20.3 ± 19.0 (7.5–29.8)	26.1 ± 27.5 (6.1–31.6)	0.96
Total cholesterol (mmol/l)	4.3 ± 1.8 (0.9–6.6)	4.0 ± 1.3 (1.3–7.0)	4.4 ± 1.3 (2.3–7.0)	0.11*
HDL cholesterol (mmol/l)	1.4 ± 0.4 (0.8–2.5)	1.3 ± 0,4 (0.8–2.7)	1.3 ± 0.5 (0.7–2.7)	0.4*
LDL cholesterol (mmol/l)	4.0 ± 1.3 (1.3–7.0)	2.6 ± 1.1 (1.0-4.3)	2.3 ± 0.9 (0.9–4.0)	0.04*
Triglycerides (mmol/l)	1.8 ± 0.9 (0.5–1.8)	1.6 ± 0.8 (0.6–4.3)	1.7 ± 0.9 (0.6–4.1)	0.55*
Treatment:	16 (16.2)	35 (35.4)	12 (12.1)	0.38
Metformin [n (%)]	7 (35.0)	15 (26.3)	5 (23.8)	0,68
Insulin [n (%)]	11 (11.2)	32 (32.7)	10 (10.2)	0.79
Statin [n (%)]	8 (40.0)	23 (40.3)	6 (28.6)	0.61
Aspirin [n (%)]	14 (14.3)	29 (29.6)	11 (11.2)	0.32
RAAS-I [n (%)]				

BMI – body mass index, HbA1c – glycated hemoglobin A1c, UACR- urinary albumin-creatynin ratio, UNCR - urinary neutrophil gelatinase associated lipocalin-creatynin ratio, eGFR CKD-EPI – estimated glomerular filtration rate using the CKD-EPI formula, RAAS-I – renin-angiotensin-aldosteron system inhibitors; x ± SD – arithmetic interval ± standard deviation. Type of statistical test: **ANOVA test, *Kruskal-Wallis test, without a marker – Chi2 test.

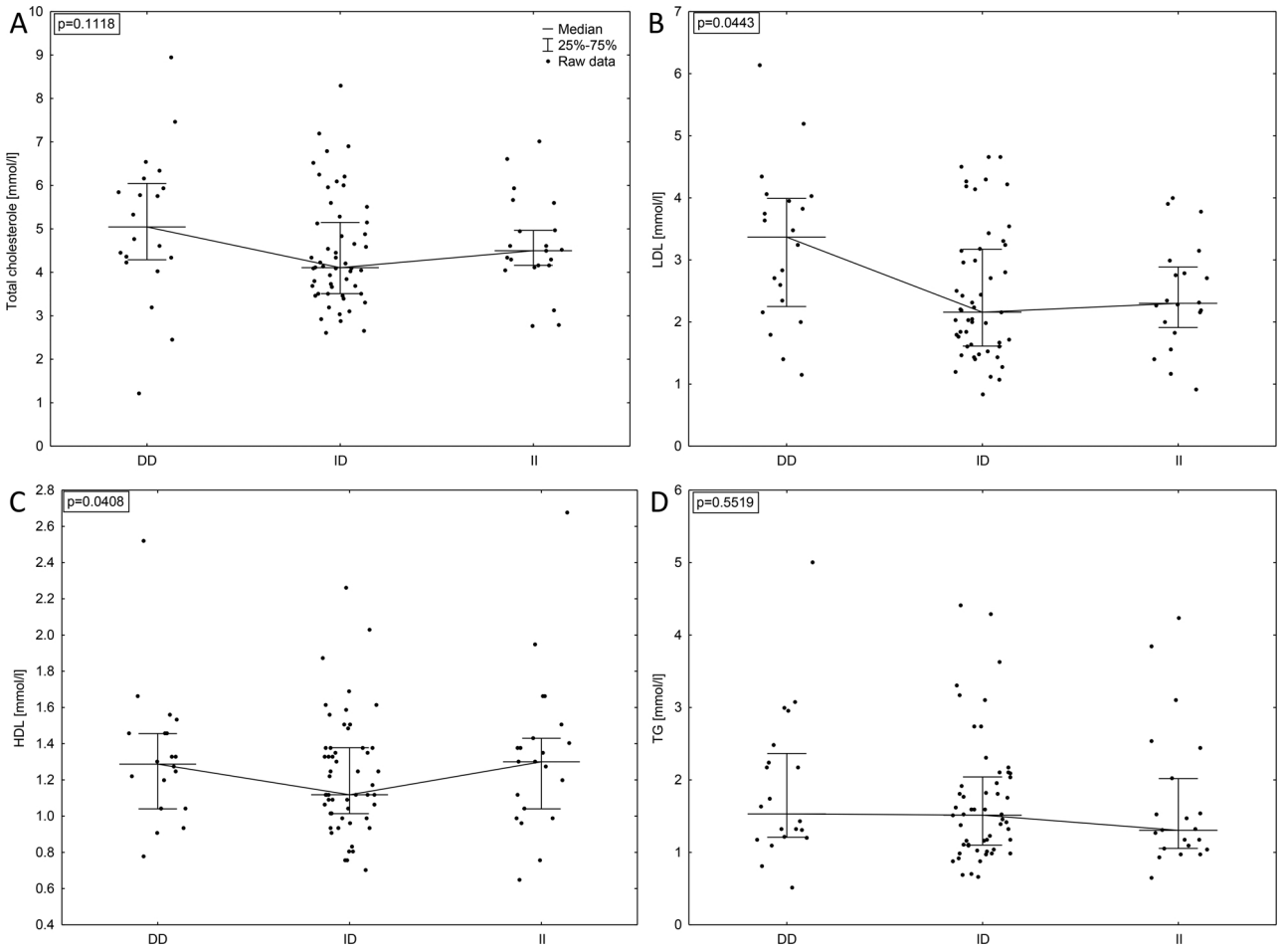

Fig. 2. Correlations between lipidogram and *VEGF* gene polymorphism.

Table 2
Associations between 18bp I/D polymorphism at − 2549 position of the *VEGF* gene and vascular complications in the univariate analysis.

	Genotypes			Genotypes comparison *p*-value
	D/D (*n* = 21)	I/D (*n* = 57)	I/I (*n* = 22)	
All vascular complications [n (%)]	9 (16.7)	34 (62.9)	11 (20.4)	0.38
Coronary disease [n (%)]	4 (13.3)	21 (70.0)	5 (16.7)	0.22
Myocardial infarction [n (%)]	3 (11.6)	20 (76.8)	3 (11.6)	0.23
Heart failure [n (%)]	3 (14)	20 (35)	3 (14)	0,05
Stroke or TIA [n (%)]	3 (27.3)	8 (72.7)	0 (0)	0.05
CKD G5 [n (%)]	2 (9.5)	12 (21.1)	2 (9.1)	0.28
Atherosclerotic peripheral vascular disease [n (%)]	7 (15.2)	28 (60.9)	11 (23.9)	0.37
Diabetic retinopathy [n (%)]	6 (15.8)	28 (73.7)	4 (10.5)	0,21
Cardiovascular death [n (%)]	1 (4.8)	4 (7.0)	2 (9.0)	0.23

CKD G5 – G5 stage of chronic kidney disease, TIA - transient ischemic attack; Type of statistical test: Chi^2 test.

Twenty five of the study subjects had albuminuria above 300 mg/g and this group of patients met the criteria for the diagnosis of DKD. No significant correlation was observed between the UACR ($p = 0.68$), UNCR ($p = 0.96$), serum creatinine ($p = 0.079$) and eGFR CKD-EPI values ($p = 0.43$) and the study *VEGF* gene polymorphism (Fig. 3).

Table 3
The chronic kidney disease stages defined according to KDIGO 2012 and genotype analysis of patients.

	Genotypes		
CKD stage	D/D (*n* = 21)	I/D (*n* = 57)	I/I (*n* = 22)
G1-G2 [n (%)]	15 (27.8)	25 (46.3)	14 (25.9)
G3 [n (%)]	3 (13.6)	14 (63.6)	5 (22.7)
G4 [n (%)]	1 (16.7)	4 (66.7)	1 (16.7)
G5 [n (%)]	2 (12.5)	12 (75)	2 (12.5)
UACR A1 [n(%)]	13 (24.1)	28 (51.9)	13 (24.1)
UACR A2 [n(%)]	2 (14.3)	10 (71.4)	2 (14.3)
UACR A3 [n(%)]	5 (20.0)	16 (64.0)	4 (16)

4. Discussion

Vascular complications are the leading cause of death among diabetics [23]. The VEGF is currently considered to be the main factor in regulating angiogenesis [20,21,24]. There are evidences that VEGF is a risk factor for different diabetes-associated microvascular and macrovascular complications in T2DM patients [1,4,5]. In recent years, several polymorphisms in the *VEGF* gene have been extensively studied in both type 1 and type 2 diabetes [27–29]. In the present study, we examined the relationship between 18bp insertion/deletion polymorphism at −2549 position of the *VEGF* gene and different DVC in T2DM patients. Based on previous studies, we know that the polymorphism studied in our work is a functional polymorphism. Recent studies have shown that 18bp insertion/deletion polymorphism at

Table 4
Multivariate analysis of DD genotype 18bp polymorphism, at − 2549 position of *VEGF* gene against ID and II polymorphism association and selected T2DM vascular complications.

Vascular complications	beta + /-SE (OR)	*p* - value
CKD G5	−0.14 ± 0.13 (0.87)	0.38
Diabetic retinopathy	0.27 ± 0.12 (1,31)	0.03
Coronary disease	0.03 ± 0.11 (1.03)	0.7
Stroke or TIA	−0.12 ± 0.13 (0.89)	0.36
Atherosclerotic peripheral vascular disease	0.06 ± 0.13 (1.06)	0.64
Heart failure	−0.14 ± 0.11 (0.87)	0.25
Cardiovascular death	−0.08 ± 0.13 (0.93)	0.54

CKD G5 – G5 stage of chronic kidney disease, TIA – transient ischemic attack.

position −2549 of the *VEGF* gene may provide genetic information about the production of the VEGF protein. Heterozygotes I/D and homozygotes D/D are characterized by a higher transcriptional activity of the *VEGF* gene and by an increase in VEGF protein production [23]. The results presented by Buraczynska et al. [25] and Bleda et al. [26] indicate that there are higher serum VEGF levels in patients with D/D and I/D genotypes.

The prognosis of patients with T2DM is highly dependent on the presence of DVC. In our study, the ID genotype was associated with the highest number of DVC (almost 60%). Interestingly, patients with II genotype had no stroke or TIA incidences. Wu et al. used meta-analysis to summarize the results from separate research studies about the correlation of different polymorphisms of the *VEGF* gene and stroke incidence [14]. However, to our knowledge, our present study is the first study to compare the 18bp insertion/deletion polymorphism, at −2549 position of the *VEGF* gene with the occurrence of strokes.

A multitude of data suggests that the endothelin system is involved in the pathophysiology of heart failure (HF) [30]. The VEGF plays a key role in angiogenesis and endothelial integrity and affects microvascular abnormalities in HF [31]. In our study, heterozygote the 18bp I/D polymorphism, at −2549 position of the *VEGF* gene more often (35% in heterozygotes vs 14% in homozygotes) suffered from HF. In the available literature, the association of −405C and −634 gene alleles of *VEGF* polymorphism has been considered as a risk factor playing a role in the clinical outcome of HF [32,33]. In our study, *VEGF* gene polymorphism does not correlate with the frequency of coronary disease and myocardial infarction occurrences. Furthermore, other studies conducted on Caucasian [37,38] and Asian [39] populations indicate that several other polymorphisms in the 9p21 region are associated with higher prevalence of coronary heart disease.

In addition, we observed that patients with DD polymorphism are characterized by a higher LDL cholesterol concentration. What is interesting is that this finding does not translate into an increased incidence of DVC in these homozygotes. VEGF's effect on lipid parameters is considered important, although yet unclear [34]. There are very few studies demonstrating the association of *VEGF* gene and lipids profile for example: *VEGF* gene transfer pro-atherogenic changes in lipoprotein profiles in animals [35]. The study by Ghazizadeh et al. demonstrated a *VEGF* genetic variant and its influence on the lipid profile [36].

Buraczynska et al. [25] showed that DD genotype and D allele in I/D polymorphism at −2549 position of the *VEGF* gene is associated with increased susceptibility to DR in Caucasian populations. A similar observation was made by Khan et al. [40] in a Pakistani population. Given that the

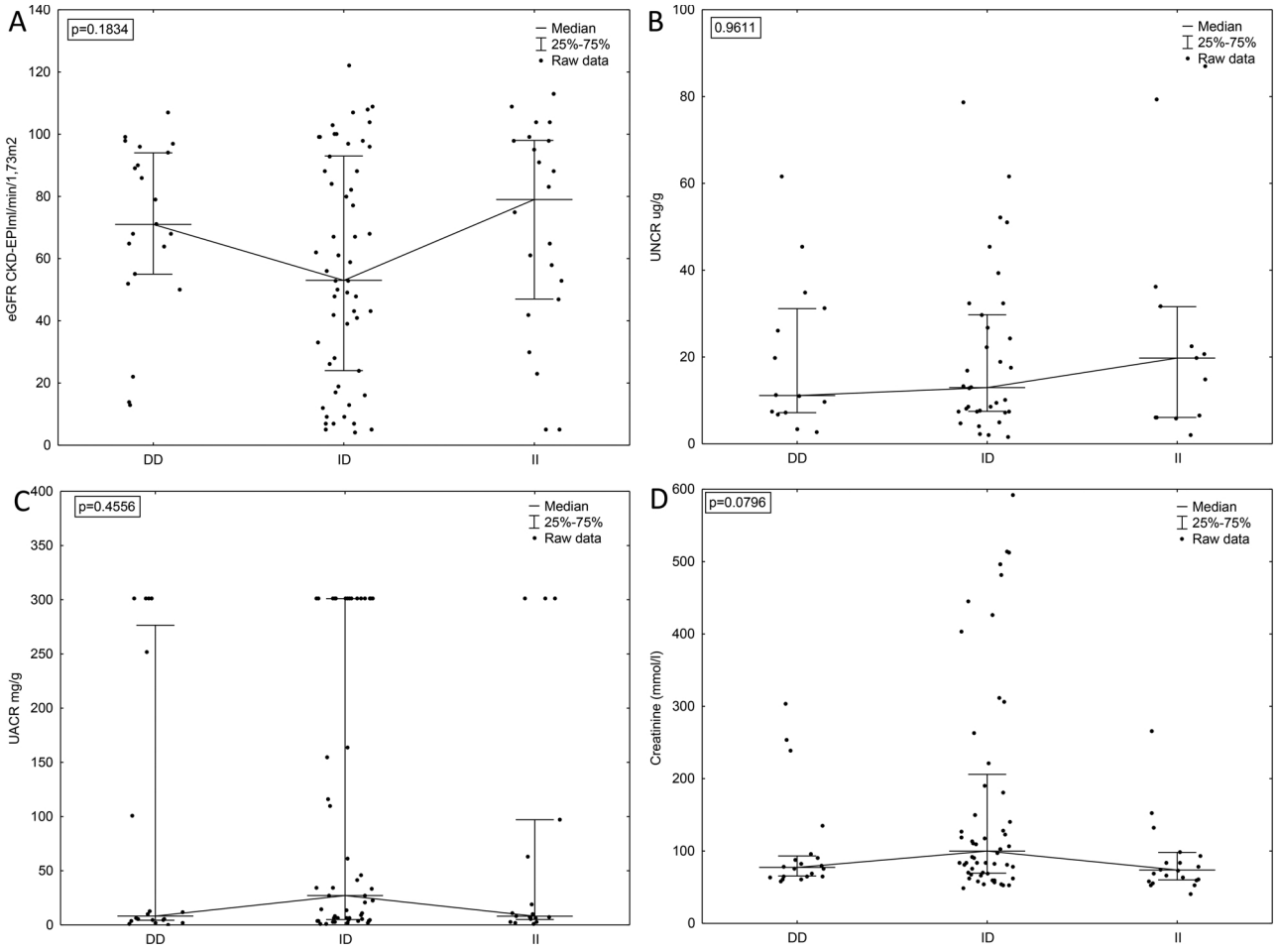

Fig. 3. Correlations between biomarkers of kidney functions and *VEGF* gene polymorphism.

deletion allele leads to a 1.95-fold increased transcriptional activity compared with the genotype containing the insertion, it is expected that homozygotic patients should be associated with protective or deleterious phenotypes while, heterozygotes should display "intermediate" states [41]. In our study we observed that the presence of the D allele predominantly predisposes to DR. This finding confirms the observations of other researchers that D allele is an independent risk factor of DR [25,40].

Amle et al. [27] showed that the DD genotype and D allele in I/D polymorphism at −2549 position of the *VEGF* gene is associated with increased susceptibility to DN in north Indian populations. However, in our study we observed that heterozygotes were characterized by a higher serum creatinine, but these results did not affect the eGFR CKD-EPI. Buraczynska et al. [25] also suggest that the polymorphism in the promoter region of the *VEGF* gene is not associated with DN in T2DM patients. In our study, we also did not observe any correlation between the type of polymorphism of the tested gene and the presence of nephropathy in T2DM patients. However, there are research papers that show positive correlations of other *VEGF* genetic polymorphisms with the presence of DKD [42].

A limitation of our study was the small size of the test group, which may in turn, limit its statistical power. For this reason, the authors intend to increase the number of participants in the study group in future. In future studies on *VEGF* gene polymorphism we should specify whether to assess the predisposition to some of the DVC in patients already in the first years after the diagnosis T2DM.

5. Conclusions

In our study, the multivariate logistic regression analysis showed that the D allele of 18bp I/D polymorphism at −2549 position of the *VEGF* gene is an independent risk factor of DR in a population with T2DM in the first years since the diagnosis of diabetes irrespective of the duration of diabetes, BMI, the glycemia control expressed by HbA_{1c}, renal function, as well as lipidogram values and applied treatment. For this reason, the population with DD and ID of *VEGF* gene polymorphism should remain under special care by the ophthalmologist following diagnosis of T2DM, especially in the elderly population and among those with reduced eGFR. Also, genotype ID seems to be a risk factor for heart failure due to coronary artery disease and stroke, although a more atherogenic lipidogram (higher LDL cholesterol) is observed in the DD homozygote. In addition, polymorphism of the promoter region of the *VEGF* gene does not affect the DN and renal function assessed using the CKD classification by KDIGO 2012 in patients with T2DM.

Conflicts of interest

The authors declare no conflict of interests.

Financial disclosure

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Funds Collection: Artur Mazur, Izabela Zawlik

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