BRITISH MEDICAL JOURNAL

British Medical Journal
Volume 2, No 1., 2022
Internet address: http://ejournals.id/index.php/bmj
E-mail: info@ejournals.id
Published by British Medical Journal
Issued Bimonthly
3 knoll drive. London. N14 5LU United Kingdom
+44 7542 987055
Chief Editor
Dr. Fiona Egea

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British Medical Journal Volume-2, No 1
10.5281/zenodo.6347139

The role of VEGF-A gene polymorphism in development and progression of PDR in men of Uzbek population with type 2 diabetes

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Abstracts
The research has shown that C634G polymorphism of the VEGF-A gene is associated with duration of the disease, presence of hypertension, level of blood pressure in the morning and in the evening =130 mmHg, TG= 1.7 mmol/l and LDL =2.5 mmol/l. Consequently, C634G polymorphism of the VEGF-A gene is one of the factors for predicting the risk of proliferative diabetic retinopathy in men with type 2 diabetes in Uzbek population.
Keywords: proliferative diabetic retinopathy, genotype, VEGF-A gene.

The aim of the study was to study the development of PDR polymorphism in the VEGF-A -C634G gene in patients with type 2 diabetes mellitus in the Uzbek population.

Materials and methods. We have studied the clinical, hemodynamic and laboratory features of diabetic retinopathy in male patients with type 2 diabetes of the Uzbek population and the formation of a group of increased risk of proliferative retinopathy.

All patients completed the appropriate questionnaires and performed standard clinical and anamnestic examinations. At this stage, all men underwent anamnesis collection, anthropometric, general clinical and genetic studies, as well as the formation of a sample depending on the severity of DR.

Results and discussion. The study showed that the C634G polymorphism of the VEGF-A gene is associated with the duration of the disease, the presence of hypertonia, the level of blood pressure in the morning and evening = 130 mm Hg, TG = 1.7 mmol / L and LDL = 2.5 mmol / l. Consequently, the C634G polymorphism of the VEGF-A gene is one of the factors for predicting the risk of proliferative diabetic retinopathy in men with type 2 diabetes in the Uzbek population.
Key words: proliferative diabetic retinopathy, genotype, VEGF-A gene.

Diabetes mellitus (DM) and its complications, the scope of which keeps growing every year, cause great human suffering and huge economic costs. According to the International Diabetes Federation (IDF), in 2017 there were 425 million (8.8%) patients with diabetes mellitus in the world. According to the preliminary forecasts, by 2040 the number of patients with DM will increase to 642 million people (10.4%) [3].

In Uzbekistan, 5 per cent of the population have DM, but only slightly more than 230000 people (0.8%) are officially registered. Vascular complications of diabetes are the main cause of premature disability and death, including blindness in patients with diabetes. In this regard, the modern medicine is faced with the urgent task: to improve methods for predicting, diagnosing and treating diabetes and its complications.

According to N. M. Alikhanov, in Uzbekistan in 2007 DR was registered in 41.0% of patients with DM (47.9% of DM1 and 40.1% of DM2), in 2010 – in 51.3% (46.8% of DM1 and 51.0% of DM2), in 2016 – in 34.9%. Blindness developed in 0.59, 0.32 and 0.4% of patients, respectively. The greatest prevalence of DR was found among patients aged 50-59 years [1].

Diabetic retinopathy is the most common vascular complication of diabetes mellitus, characterized by the increased vascular permeability, impaired hemostasis, increased tissue ischemia and neoangiogenesis [4;5].

Abhary S., Hewitt A., Burdon K., Craig J. A systematic meta-analysis of genetic association studies for diabetic retinopathy//Diabetes. – 2009 is currently, a whole list of the so-called candidate

genes is being studied, which, according to researchers, may be involved in development of vascular complications in DM. A number of authors believe that genetic factors play a significant role in development of DR. According to Hallman D. et al 2005, a certain inherited component plays a role in the development of DR, which has no correlation with either glycemic control or duration of diabetes.

One of the main genetic factors in DR development is the intense expression of the VEGF gene located on the short haul of chromosome 6 (6p21. 3). VEGF is one of the members of the family of structurally related proteins: VEGF - A, VEGF-B, VEGF-C (also called VEGF-2), VEGF-D, and placental growth factor (PlGF). Aiello L., Wong J-S et.al 2000. the mediator of signals from VEGF to the vascular endothelium are 3 tyrosine kinase signaling receptors (VEGF receptor (VEGFR)-1, -2, and-3). Awata T., Inoue K., Kurihara S. et al. 2002, of them, the most interesting is VEGF-A, which plays a key role in the pathogenesis of microangiopathies, regulating the proliferation of vascular endothelial cells in various tissues.

Materials and methods

In total, VEGF-A genotyping was performed in 132 patients with type 2 diabetes with and without diabetic retinopathy, of whom 69 patients were diagnosed with proliferative DR.

All men underwent a standard clinical examination.

Evaluation of carbohydrate metabolism included determination of glucose in capillary blood in the fasted state and 2 hours after eating, using the kits of Cypress Diagnostics (Belgium). Glycated hemoglobin (HbA1c) was determined by the colorimetric thiobarbitur method.

The study of the blood lipid spectrum was performed using the kits of Cypress Diagnostics (Belgium) on a spectrophotometer "Hospitex Diagnostics" (Italy). OHS and TG in blood serum were determined by a standard enzymatic method, high density lipoprotein cholesterol in the supernatant-after precipitation of lipoproteins of other classes with dextran sulfate.

The following values were used as target levels of lipid spectrum indicators: for total cholesterol <4.5 mmol/l; for LDL <2.5 mmol/l; for HDL >1.0 mmol/l; for TG <1.7 mmol / l.

Blood creatinine was determined using Mindray BS-200 biochemical analyzer using the immuno-enzyme method. Errera F., Canani L., Silva M. et al. Functional vascular endothelial growth factor -634G>C SNP is associated with proliferative diabetic retinopathy: a case-control study in a Brazilian population of European ancestry//Diabetes Care - 2007 is a glomerular filtration rate (GFR) was estimated using an equation developed by the Collaboration on epidemiology of chronic kidney disease (CKD-EPI). We used an online calculator for the calculation https://www.msdmanuals.com/medical-calculators/GFR_CKD_EPI-ru.htm. For men, the following coefficients are used: Gender = 1; alpha = -0.411; Kappa = 0.9.

Molecular genetic research of the VEGF-A gene was conducted in the Genomics laboratory of the Institute of Bioorganic chemistry of the Russian Academy of Sciences.

The obtained data was processed using the Statistica 6.0 application software package (StatSoft, USA). The odds ratio (OR) and 95% confidence interval (95% CI) were calculated using logistic regression (online calculator http://medstatistic.ru/calculators.html). The reliability of differences in indicators was assessed using the nonparametric criterion $\chi 2$ (Pearson's criterion). Quantitative indicators for normal distribution are presented as M±SD. Differences between the groups were considered statistically significant at $p<0.05$.

Results and discussion

The distribution of VEGF-A genotypes in the studied group was dominated by the genotypes CG (47.8%) and GG (40.6%). The SS genotype (11.6%) was significantly less common.

The groups were comparable in age. The duration of type 2 diabetes in patients with proliferative diabetic retinopathy (PDR) ranged from 7 to 34 years, with an average of 16.5±6.8 years (table 1.).

The duration of type 2 diabetes in the groups with genotypes GG, SG and SS ranged from 7 to 30 years (at an average of 14.3±5.5 years), 8 to 34 years (at an average of 18.1±7.9 years) and 10 to 26 years (at an average of 17.1±4.6 years), respectively.

Table 1.

Comparative characteristics of patients with PDR depending on the poly-morphism of the VEGF-A gene based on the results of ANOVA and pairwise analysis

Indicators	GG, n=28	CG, n=33	CC, n=8	ANOVA; p
Age, years	60,0±9,7	60,3±7,0	60,3±5,6	0,99
Duration of the disease, years	14,3±5,5	18,1±7,9*	17,1±4,6	0,08
BMI	28,6±3,1	29,0±4,4	30,5±3,8	0,47
Fasting glucose, mmol/l	10,2±3,4	11,3±2,9	10,8±2,4	0,38
After 2 hours, mmol / l	13,8±4,0	14,1±2,3	14,0±2,8	0,93
HbA1c, %	9,1±1,8	9,5±2,1	9,3±1,0	0,71
SBP, day, Mmhg	142,0±30,6	158,3±27,9*	155,0±35,9	0,11
DBP day, Mmhg	83,6±8,0	91,7±10,1#	86,3±7,3	**0,003**
SBP, day, Mmhg	137,7±27,2	153,6±25,2*	141,3±25,9	0,06
DBP day, Mmhg	82,7±8,1	90,2±9,8#	83,8±8,8	**0,006**
Heart rate, beats per minute.	78,9±9,7	81,2±10,5	79,8±5,7	0,66
Creatinine, mcmole/l	131,5±60,3	126,1±28,9	123,5±17,6	0,85
GFR, ml/min/1.73m^2	76,7±38,9	69,1±16,3	72,1±9,01	0,56
TC, mmol / l	5,4±1,3	6,5±2,1	6,1±1,2	0,05
Thyreoglobulin, mmol / l	2,7±1,9	3,4±2,1	3,2±1,6	0,39
HDL, mmol / l	0,95±0,37	0,95±0,35	0,99±0,31	0,96
LDL, mmol / l	3,2±1,08	4,1±1,6*	3,9±1,4	**0,04**
Fibrinogen, g / l	4,6±1,8	5,2±2,3	4,2±1,6	0,34
Hematocrit, %	48,6±10,4	47,1±8,3	44,9±7,7	0,58
Thrombotest, dg	5,5±0,7	5,8±0,6	5,3±0,7	0,08
Blood phagocytic activity, %	11,8±2,2	13,2±4,6	10,7±2,1	0,13
PTI, %	84,9±10,2	90,9±11,9	88,1±6,7	0,10

Note: the data are presented as M±SD; * - differences relative to the data of patients with the GG genotype are significant (*- $p<0.05$, ● - $p<0.01$, # - $p<0.001$).

The single-factor analysis of variance (ANOVA) did not reveal a significant difference in duration of the diabetes mellitus between the groups. However, pairwise analysis of the data showed that duration of SD in the Geno-type CG carriers is significantly higher than in GG carriers of.

There was no statistically significant difference in BMI between the groups. Indicators of carbohydrate metabolism by genotypes also did not differ significantly: fasting glycemia (GG-10.2±3.4 mmol/l, CG - 11.3±2.9 mmol/l and CC-10.8±2.4 mmol/l), after 2 years (GG - 13.8±4.0 mmol/l, CG - 14.1±2.3 mmol/l and CC-14.0±2.8 mmol/l) and HbA1c (GG - 9.1±1.8 %, CG - 9.5±2.1% and CC - 9.3±1.0%).

Analysis of hemodynamic indicators revealed the following: the level of SBP both during the day and at night did not differ significantly, while the DBP day and night had a significant difference in groups. In pairwise analysis, the levels of SBP and DBP in individuals with the CG genotype were significantly higher than in GG carriers. The heart rate indicator in the groups was comparable.

The creatinine index is slightly lower in carriers of the CC (123.5±17.6 mmol/l) and CG (126.1±28.9 mmol/l) genotypes compared with the data of the group with the GG genotype (131.5±60.3 mmol/l). Glomerular filtration rate is lower in patients with the CG (69.1±16.3 ml/min/1, 73m2) and CC (72.1±9.01 ml/min/1,73m2) genotypes than with the GG (76.7±38, ml/min/1, 73m2) genotype.

10.5281/zenodo.6347139

When analyzing lipid metabolism indicators, a statistically high LDL content (4.1±1.6 mmol/l) was observed in carriers of the CG genotype, compared with the values in patients with the GG (3.2±1.08 mmol/l) and CC (3.9±1.4 mmol/l) genotypes.

Such indicators of blood clotting as fibrinogen, hematocrit, thrombotest, fibrinolytic activity and PTI did not depend on the genotype carriership.

Next, we conducted a comparative frequency analysis of occurrence of certain anamnestic and clinical indicators depending on the VEGF-A gene carriership (Table 2.).

Analysis of the VEGF-A gene genotypes distribution depending on the disease duration showed that the CG (60.6%) and CC (75.0%) genotypes carriers are dominated by persons with a disease duration of ≥16 years, as compared to the GG carriers (28.6%; $\chi 2$=8.62; p=0.01). There were no statistically significant differences between the groups depending on the age and polymorphism of the VEGF-A gene.

In the group of GG (32.1%) and CC (50.0%) genotype carriers, the patients with BMI >30 kg/m2 are not reliably found, but more often than for the CG (30.3% χ 2=1.15; p=0.56) genotype.

Levels of SAD in the morning and in the evening ≥130 mmHg were statistically more common among carriers of the CG (90.9% 69.7%, respectively) and CC (88.0% and 63.0%, respectively) genotypes compared to the GG genotype (53.6% $\chi 2$=12.1; p=0.002 and 32.1% $\chi 2$=8.88; p=0.01, respectively).

Table 2.

Clinical characteristics of patients depending on polymorphism of the VEGF-A gene

Indicators	GG, n=28		CG, n=33		CC, n=8		χ^2; p
	n	%	n	%	n	%	
Age under 50 years	7	25	3	9,1	1	13	2,71; 0,26
50-59 years	10	35,7	14	42,4	2	25	1,52; 0,474
≥ 60 years	11	39,3	16	48,5	5	63	1,46; 0,48
Duration of the desease ≤10 years	9	32,1	7	21,2	1	13	1,69; 0,43
11-15 years	11	39,3	6	18,2	1	13	4,37; 0,11
≥ 16 years	8	28,6	20	60,6	6	75	**8,62; 0,01**
BMI >30 kg / m2	9	32,1	10	30,3	4	50	1,15; 0,56
Hyperglycemia HbA1c >7.0%	26	92,9	32	97,0	8	100	1,03; 0,60
SBP in the morning ≥130 mm Hg.	15	53,6	30	90,9	7	88	**12,1; 0,002**
in the evening ≥130 mm Hg.	9	32,1	23	69,7	5	63	**8,88; 0,01**
DBP in the morning ≥85 mm Hg.	14	50,0	16	48,5	7	88	4,19; 0,12
in the evening ≥85 mm Hg.	8	28,6	16	48,5	4	50	2,80; 0,24
Inheritance of the diabetes mellitus	12	42,9	7	21,2	2	25	3,48; 0,18
obesity	6	21,4	10	30,3	4	50,0	2,52; 0,28
arterial hypertension	10	35,7	20	60,6	3	38	4,15; 0,13
BMI	9	32,1	13	39,4	1	13	2,13; 0,35
Smoking	1	3,6	4	12,1	2	25	3,41; 0,18
Blindness	1	3,6	1	3,0	2	25	6,12; 0,05
Cataract	9	32,1	13	39,4	3	38	0,35; 0,84
Chronic renal disease	21	75,0	32	97,0	7	88	**6,45; 0,04**
Diabetic foot infections	9	32,1	11	33,3	2	25	0,21; 0,90
Heart attack	3	10,7	4	12,1			1,06; 0,59
Insultus	2	7,1	6	18,2	1	13	1,63; 0,44
Arterial hypertension	16	57,1	30	90,9	7	88	**10,3; 0,006**
Cholesterol =4.5 mmol/l	17	60,7	24	72,7	6	75	1,20; 0,55
Triglycerides = 1.7 mmol/l	13	46,4	30	90,9	7	88	**16,0; 0,0001**
HDL < 1.03 mmol/l	18	64,3	18	54,5	3	38	1,92; 0,38
LDL =2.5 mmol/l	5	17,9	25	75,8	6	75	**22,2; 0,0001**

Note: χ^2-Pearson criterion; p – confidence level; df =2 - number of degrees of freedom

The groups did not differ significantly in terms of the frequency of DBP in the morning and in the evening ≥85 mm Hg. Indicators of HbA1c >7.0% were observed with the same frequency in all groups.

Further analysis showed no statistically significant associations between the VEGF-A genotypes and risk factors such as heredity, smoking, hypercholesterolemia, and reduced HDL.

Chronic renal disease and AH were significantly more frequently observed among carriers of the CG (97.0% and 90.9%, respectively) and CC (88.0% and 88.0%, respectively) genotypes compared to the GG genotype (75.0% $\chi 2=6.45$; p=0.04 and 57.1% $\chi 2=10.3$; p=0.006).

Among the indicators of the lipid spectrum, the level of triglycerides =1.7 mmol/l and LDL =2.5 mmol/l was statistically more frequently registered among carriers of the CG (90.9% and 75.8%, respectively) and CC (88.0% and 75.0%, respectively) genotypes than among carriers of the GG genotype (46.4% $\chi 2=16.0$, p=0.0001 and 19.0% $\chi 2=17.9$%, p=0.0001).

The next stage of our research was a comparative analysis of clinical and anamnestic indicators among carriers of the GG genotype, relative to carriers of the CC and CG genotypes of the VEGF-A gene, in order to identify the most important factors for predicting the risk of retinopathy.

The analysis showed: such factor as duration of the disease makes a significant contribution to development of DR and is genetically associated (GG vs CC – OR 4.71; 95% CI 1.10-20.2; p=0.02 and vs CG - OR 1.81; 95% CI 1.12-2.94; p=0.01). The presence of AH was also significant for carriers of the heterozygous CG genotype (HR 3.26; 95% CI 1.16-9.17; p=0.003). In addition, for carriers of the CG heterozygous genotype, such factors as hereditary burden on the diabetes mellitus (OR 0.60; 95% CI 0.32-1.12; p=0.07) and obesity (OR 1.59; 95% CI 0.98-2.58; p=0.05) were near to startistically relaible. In carriers of the CC homozygous genotype, smoking contributes to the DR development (OR 3.67; 95% CI 1.25-10.8; p=0.05).

Next, we tracked which of the hemodynamic parameters can complicate the course of retinopathy in patients with type 2 diabetes. Thus, in carriers of the CG heterozygous genotype the risk factors for DR development are SBP ≥130 mmHg in the morning (OR 3.56; 95% CI 1.26-10.1; p<0.001) and in the evening (OR 2.08; 95% CI 1.21-3.60; p=0.004). As for carriers of the CC homozygous genotype, the levels of SBP ≥130 mmHg in the morning (OR 4.46; 95% CI 0.61-32.4; p=0.08) and SBP ≥85 mmHg in the morning (OR 5.0; 95% CI 0.69-6.64; p=0.06) were near to startistically relaible.

Further, we analyzed the contribution of factors such as HbA1c level>7.0%, as well as lipid spectrum indicators: cholesterole ≥4.5 mmol/l, TG ≥1.7 mmol/l, HDL <1.03 mmol/l, and LDL ≥2.5 mmol/l, to the development of DR.

It was not possible to estimate the contribution of HbA1c>7.0% to the complicated course of DR, since 92.9% (GG genotype), 97.0% (CG genotype), and 100.0% (CC genotype) had HbA1c >7.0%.

As for the frequency of occurrence of critical levels of the lipid spectrum, in carriers of the CG heterozygous genotype, the course of retinopathy is complicated by TG ≥1.7 mmol/l (OR 4.19; 95% CI 1.46-12.0; p<0.001) and LDL ≥2.5 mmol/l (OR 3.23; 95% CI 1.74-5.99; p<0.001).

In carriers of the CC homozygous genotype, the contribution of LDL ≥2.5 mmol/l was statistical (OR 6.82; 95% CI 1.62-28.6; p=0.002) and near to statistically reliable TG ≥1.7 mmol/l (OR 5.60; 95% CI 0.77-40.9; p=0.05).

In the course of further analysis, it was found that the presence of CKD has a significant importance in complicating the course of DR in carriers of the CG heterozygous genotype (OR 4.83; 95%CI 1.16-30.6; p=0.01).

It is probably impossible to determine the contribution of factors to the development of DR among carriers of the CC homozygous genotype due to the small number of this group (n=8).

Gavrilenko T. I., Ryzhkova N. A., Parkhomenko A. N. Vascular endothelial growth factor in the clinic of internal diseases and its pathogenetic value / / Ukrainian cardiological journal. – 2011 is the VEGF-A plays an important role in the neovascularization of proliferative retinopathy in

10.5281/zenodo.6347139
DR, as well as in the sharp deterioration of the hematopoietic barrier, characterized by hyper-permeability of retinal vessels.

In the course of research conducted in Japan, one can note both the presence and absence of an association between polymorphism -634C/G and retinopathy. Similarly, the opposite results have been observed in studies conducted in ethnically more diverse societies, but most of these results indicate a link between DR and polymorphisms in the VEGF-A promoter region. Therefore, it was suggested that 634C/G VEGF-A - polymorphism can serve as a predictor of the DR development [5]

Errera F. et al. 2009 found no association between the 634G/C (rs2010963)-polymorphism of the VEGF-A gene and the presence of DR or type 2 diabetes. However, homozygous CC polymorphism -634G/C was more common in patients with PDR compared to the control group (patients with type 2 diabetes without PDR). The authors suggest that presence of the homozygous 634c VEGF-A allele is an independent risk factor for PDR in patients of European origin with type 2 diabetes.

Petrovic M. et al. 2008 found no association between PDR and 634c/G VEGF-A gene polymorphism in patients with type 2 diabetes. However, average serum VEGF-A levels were significantly higher in patients with PDR compared to the control group (diabetes mellitus without DR). Moreover, in patients with diabetes mellitus with the CC genotype compared to other genotypes (CG and GG).

Thus, as a result of studying the associative relationships between the C634G polymorphism of the VEGF-A gene, it was found that:

- in carriers of the heterozygous CG genotype, the duration of DM, hemo-dynamic parameters, and LDL level are significantly higher than in the GG carriers;
- individuals with a disease duration of ≥16 years and a SBP level of ≥130 mmHg in the morning and in the evening dominate among carriers of the CC and CG genotypes as compared to those of the GG;
- CKD and AH were significantly more frequent among carriers of CC and CG genotypes as compared to those of the GG.

Among the clinical and anamnestic indicators, C634G polymorphism of the VEGF-A gene is associated with duration of the disease, presence of hypertension, the level of SBP in the morning and in the evening ≥130 mmHg, TG≥ 1.7 mmol/l and LDL ≥2.5 mmol/l. Consequently, C634G polymorphism of the VEGF-A gene is one of the factors for predicting the risk of proliferative diabetic retinopathy in men with type 2 diabetes in Uzbek population.

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