MEDICAL SCIENCE MONITOR

CLINICAL RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2018; 24: 8891-8898
DOI: 10.12659/MSM.909803

Received: 2018.03.05
Accepted: 2018.06.29
Published: 2018.12.08

The Association of *CEP135* rs4865047 and *NPY2R* rs1902491 Single Nucleotide Polymorphisms (SNPs) with Rapid Progression of Proliferative Diabetic Retinopathy in Patients with Type 1 Diabetes Mellitus

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF 1 Irmante Derkac
ADEF 1 Ingrida Januleviciene
DEF 2 Ramune Sepetiene
EF 3 Rasa Valiauga
ABCDEF 4,5 Dzilda Velickiene

1 Lithuanian University of Health Sciences, Eye Clinic of Kaunas Medical Academy, Kaunas, Lithuania
2 Lithuanian University of Health Sciences, Kaunas, Lithuania
3 Waisman Center, University of Wisconsin-Madison, Madison, WI, U.S.A.
4 Department of Endocrinology, Lithuanian University of Health Sciences, Kaunas, Lithuania
5 Institute of Endocrinology, Kaunas, Lithuania

Corresponding Author: Irmante Derkac, e-mail: irmantederkac@gmail.com
Source of support: Departmental sources

Background: Diabetic retinopathy has a varied prevalence, severity, and rate of progression. The aim of this study was to determine whether the single nucleotide polymorphisms (SNPs) of the gene encoding a 135-kD centrosomal protein *CEP135* rs4865047 and the gene encoding the type 2 NPY protein *NPY2R* rs1902491 were associated with the development of rapidly progressive proliferative diabetic retinopathy in patients with type 1 diabetes mellitus.

Material/Methods: Patients with rapidly progressive proliferative diabetic retinopathy (n=48) were included in the study group. The control group (n=84) consisted of diabetes mellitus patients who had no proliferative diabetic retinopathy up to 15 years of diabetes duration. The reference group (n=90) included non-diabetic individuals who matched the study group by age and gender. The SNPs in the three groups were analyzed using real-time polymerase chain reaction (PCR) amplification.

Results: The analysis of the distribution of genotypes in *CEP135* rs4865047 and *NPY2R* rs1902491 detected significant differences only in the single nucleotide polymorphism rs4865047 genotype between the case and control group in comparison to the reference group. The co-dominant model showed that *CEP135* rs4865047 was significantly associated with patients with rapidly progressive proliferative diabetic retinopathy (OR 7.2, 95% CI, 2.28–22.74, p=0.001). No significant association was found for the *NPY2R* SNP rs1902491 genotype.

Conclusions: Our study reports a significant association of the *CEP135* single nucleotide polymorphism rs4865047 genotype with rapidly progressive proliferative diabetic retinopathy and the control group. No significant association was found of the *NPY2R* single nucleotide polymorphism rs1902491 genotype.

MeSH Keywords: Diabetes Mellitus, Type 1 • Diabetic Retinopathy • Genome-Wide Association Study

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/909803

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Background

As the worldwide prevalence of diabetes mellitus continues to rise, diabetic retinopathy represents a leading cause of loss of vision loss in many developed countries [1]. Diabetic retinopathy consists of an early non-proliferative stage characterized by microaneurysms, micro-hemorrhages, retinal vascular leakage with exudate accumulation, and in the more advanced, proliferative stage, or proliferative diabetic retinopathy, visual loss can occur from the proliferation of new retinal vessels [2]. Diabetic retinopathy has a complex pathogenesis that involves the vasculature of the inner retina and breakdown of the blood-retinal barrier. In recent decades, extensive research has determined that diabetic retinopathy is not only a vascular disease but also has a neurodegenerative component [3].

There are many known risk factors that increase the development and progression of diabetic retinopathy, including the duration of diabetes, poor glycemic control, high blood pressure, high cholesterol, pregnancy, elevated levels of glycated hemoglobin (HbA1c), tobacco use, the accumulation of advanced glycation end-products, oxidative stress, epigenetic changes, and genetic factors [1,4–9]. However, these risk factors cannot fully explain why some individuals with diabetes develop more rapidly progressive proliferative diabetic retinopathy while others do not. However, a previously published study showed that retinopathy develops in approximately 10% of patients with type 1 diabetes even with good metabolic control, whereas up to 40% of patients with type 1 diabetes remain free of retinopathy despite poor metabolic control [10]. The identification of genetic or molecular factors for the development of diabetic complications may lead to improved diagnosis, screening, or treatment to prevent complication such as diabetic retinopathy.

In 2008, a study on the heritability of diabetic retinopathy showed that the estimated heritability was 27% for 15 years of diabetes mellitus duration and 50% for proliferative diabetic retinopathy [11]. Studies have been undertaken to determine the genetic basis of diabetic retinopathy by using candidate gene and gene linkage approaches. The aldose reductase gene (*AKR1B1)* has been shown to have the most significant number of polymorphisms associated with diabetic retinopathy, with polymorphisms in the genes for endothelial nitric oxide synthase (*NOS3*), vascular endothelial growth factor (*VEGF)*, αβ1 integrin (*ITGA2)*, and intercellular cell adhesion molecule 1 (*ICAM1*) are also associated with diabetic retinopathy [12]. However, although an association with several gene polymorphisms and diabetic retinopathy have been made, the genetics of diabetic retinopathy remain poorly understood [13]. A previous genome-wide association study (GWAS) replicated and evaluated several novel genetic *loci* that may be associated with severe or rapidly progressive proliferative diabetic retinopathy [14]. This study identified the rs4865047 single nucleotide polymorphism (SNP) in an intron of the gene *CEP135*, encoding a 135-kD centrosomal protein CEP135, and the rs1902491 SNP of the gene *NPY2R* gene encoding the type 2 NPY protein [14]. The *CEP135* gene is expressed in the retina, and previously published studies have shown that a truncating mutation of *CEP135* can alter the number of neural progenitor cells, which may be the cause of reduced neurogenesis [15,16].

Therefore, the aim of this study was to determine whether *CEP135* rs4865047 and *NPY2R* rs1902491 were associated with the development of rapidly progressive proliferative diabetic retinopathy in patients with type 1 diabetes mellitus. Three groups were studied, including the study group, consisting of patients with type 1 diabetes for more than 15 years who had rapidly progressive proliferative diabetic retinopathy, the control group of patients with type 1 diabetes for more than 15 years had no proliferative diabetic retinopathy up to 15 years of diabetes mellitus duration who matched the study group by diabetes mellitus duration and gender, and a reference group of non-diabetic individuals, who matched the study group by age and gender.

Material and Methods

Ethical approval and patient consent

The study included patients with type 1 diabetes mellitus patients who underwent ophthalmologic consultations at the Department of Eye and Endocrinology Clinics of the Lithuanian University of Health Sciences (LUHS), Kaunas, Lithuania from 2013 to 2017. Ethical approval was obtained from the Ethics Committee of the Lithuanian Health Science University (No. BE-2-16). Three groups were studied: the study group, consisting of patients with type 1 diabetes (for >15 years) who had proliferative diabetic retinopathy up to 15 years of diabetes duration; the control group, consisting of patients with type 1 diabetes (for >15 years) who had no proliferative diabetic retinopathy up to 15 years of diabetes mellitus duration; and a reference group of non-diabetic individuals. The single nucleotide polymorphisms (SNPs) of the gene encoding a 135-kD centrosomal protein *CEP135* rs4865047 and the gene encoding the type 2 NPY protein *NPY2R* rs1902491 were evaluated from venous blood samples obtained from all study participants. All subjects who met the evaluation criteria and who participated in the study provided written informed consent.

Evaluation of diabetic retinopathy in patients with type 1 diabetes mellitus

Patients with type 1 diabetes underwent an examination of the optic fundus was performed using indirect slit-lamp biomicroscopy and fundus color photography using a Nidek AFC-230 auto

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

fundus camera (Nidek Co. Ltd., Japan) to detect the presence and grade of diabetic retinopathy, following dilatation of the pupils. The severity of diabetic retinopathy was graded according to the International Clinical Diabetic Retinopathy Disease Severity Scale approved by the American Academy of Ophthalmology, using standardized seven-field mydriatic fundus color photographs [18]. The patients were divided into five groups according to the imaging findings: i) no diabetic retinopathy; ii) mild diabetic retinopathy with microaneurysms only; iii) moderate nonproliferative diabetic retinopathy – more than just microaneurysms but less than severe nonproliferative diabetic retinopathy; iv) severe nonproliferative retinopathy, with more than 20 intraretinal hemorrhages in all four retinal quadrants and/or definite venous beading in more than two retinal quadrants and/or prominent intraretinal microaneurysms in more than one quadrant and; v) proliferative diabetic retinopathy with neovascularization that required treatment. The severity of diabetic retinopathy was based on the level of diabetic retinopathy of the worse eye.

Clinical assessment of patients with type 1 diabetes mellitus

Patients with type 1 diabetes underwent clinical examination. Diabetic nephropathy was defined by the presence of two occasions of urinary albumin levels >30 mg/day in specimens over the course of four months in 24-hour urine samples. Normal albumin excretion was considered as <30 mg/day, microalbuminuria as 30–300 mg/day, and macroalbuminuria as >300 mg/day. Dyslipidemia was assessed from each patient's medical history of the use of lipid-lowering medications. Hypertension was diagnosed if the mean of two blood pressure measurements was >140/90 mmHg or if the patient was being treated with antihypertensive medications.

Determination of the average of time for the onset of development of proliferative diabetic retinopathy

Following a review of patients being treated proliferative diabetic retinopathy at the Eye and Endocrinology Clinics of the Lithuanian University of Health Sciences (LUHS), 225 consecutive patients with type 1 diabetes mellitus and proliferative diabetic retinopathy were identified. The median time for the development of proliferative diabetic retinopathy after the onset of type 1 diabetes mellitus was 15 years. A previously reported study that included patients with diabetes Lithuania conducted between 1996–2002 estimated that the average of time for the onset of development of proliferative diabetic retinopathy was 14±1.8 years [17].

Calculation of the sample size of the study group and control group: an initial pilot study

The minor allele frequency (MAF) in the general population for *CEP 135* rs4865047 is T=0.1837/920 (*https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/*). To evaluate MAF in the study and control groups we conducted a pilot study. Our study found that *CEP135* rs4865047 MAF in the study group was 20%, while in the control group 4%. Using a calculator for sample size estimation (*http://osse.bii.a-star.edu.sg/calculation1.php*), we evaluated that the targeted sample size is 48 subjects in the study group and 95 subjects in the control group.

Inclusion criteria for the study group (type 1 diabetes and proliferative diabetic retinopathy developed <15 years from diabetes onset) and the control group (type 1 diabetes and no proliferative diabetic retinopathy up to 15 years of diabetes duration)

Patients were referred to the study group if proliferative diabetic retinopathy developed <15 years from diabetes, and 84 control group patients with type 1 diabetes mellitus who had no proliferative diabetic retinopathy up to 15 years of diabetes duration. Each patient in the study group was matched to the patients in the control group by gender and duration of type 1 diabetes.

The inclusion criteria for patients in the study group (n=48) and the control group (n=84) included: the patient signed an informed consent to take part in the study; age ≥18 years; type 1 diabetes mellitus for >15 years; confirmed the status of diabetic retinopathy on fundus color photography. The following additional criteria were required for patient inclusion into the study group: the finding of proliferative diabetic retinopathy confirmed on fundus color photography; treatment for confirmed proliferative diabetic retinopathy with neovascularization or scars from laser photocoagulation.

The reference group with no history of diabetes mellitus

The reference group consisted of 90 healthy individuals with no history of diabetes mellitus, with a median age 42 years (range, 20–67 years) matched according to their age and gender with the study group. The reference group was recruited from a randomized cluster sampling of local population. One cluster consisted of students and staff volunteers aged 23–67 years from the LUHS; the second cluster consisted of volunteers aged 41–87 years selected by their physician from the Kaunas Dainavos outpatient clinic; and the third cluster consisted of healthy women aged 18–40 years who were attending a local gynecological birth control clinic in the city of Kaunas. The median age and gender of the reference group and the study group did not differ significantly (p=0.69 and p=0.86, respectively). However, there was a significantly higher prevalence of hypertension in the study group compared with the reference group (p=0.01). All individuals who volunteered to be in this group completed questionnaires on the state of their health.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 1. Clinical characteristics of the study group (with type 1 diabetes and proliferative diabetic retinopathy) and the control group (with diabetes and no proliferative diabetic retinopathy up to 15 years of diabetes).

Characteristics	Control Group	Study Group	p-Value
N	84	48	–
Women	64 (72.7%)	35 (72.9%)	0.89
Age median (range) (yrs)	36 (18–73)	39 (20–63)	0.65
Duration of DM (yrs)	23.4±6.7	24.3±6.5	0.41
Age of onset of DM (yrs)	15.89±12.1	15.96±8.9	0.77
PDR onset from DM onset	20.9±6.1	10.5±2.9	<0.001
HbA1c (%)	8.4±1.5	8.7±1.6	0.93
Nephropathy	21 (25%)	26 (54.2%)	0.002
Hypertension	28 (34.1%)	29 (64.4%)	0.04
Dyslipidemia	13 (15.3%)	15 (31.9%)	0.02

Results are expressed as the mean (±SD), median, range, or number (%). N – number; DM – diabetes mellitus; PDR – proliferative diabetic retinopathy; HbA1c – glycated hemoglobin.

DNA extraction and genotyping

Blood samples were collected in vacuum tubes containing ethylenediaminetetraacetic (EDTA) for DNA extraction. Analysis of the gene polymorphisms of *CEP 135* rs4865047 and *NPY2R* rs1902491 were performed for all the study participants in the three groups, the study group, the control group, and the reference group, at the Laboratory of Molecular Cardiology at the Institute of Cardiology of the LUHS. DNA was extracted from the venous blood samples using the TaqMan® Predesigned Single Nucleotide Polymorphism (SNP) Genotyping Assay, according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA) with the 7900HT Fast Real-Time PCR System. Allelic discrimination was performed using the Applied Biosystems software.

Statistical analysis

The Kolmogorov-Smirnov test was used to analyze data with a normal distribution. Non-parametric unpaired continuous values were compared by the Mann-Whitney test. The odds ratio (OR) of allelic association with a 95% confidence interval (CI) were used for each minor allele of *CEP135* rs4865047 and *NPY2R* rs1902491 to determine the odds for development of rapidly progressive proliferative diabetic retinopathy. The association of genotypes with rapidly progressive proliferative diabetic retinopathy was assessed using logistic regression models adjusted for gender, age, and hypertension. The OR and 95% CI for *CEP135* rs4865047 polymorphism were calculated using codominant associations (wild-type homozygous versus heterozygous) because there was no minor homozygous allele detected in this study. For *NPY2R* rs1902491 all inheritance models were assessed: recessive (wild-type homozygous and heterozygous versus minor allele homozygous), dominant (wild-type homozygous versus heterozygous and minor allele homozygous), codominant (wild-type homozygous versus heterozygous; wild-type homozygous versus minor allele homozygous) and additive inheritance models. Hardy-Weinberg analysis was performed to compare the observed and expected frequencies of polymorphisms (rs4865047and rs1902491) using the chi-squared (χ^2) test in all groups. Akaike information criterion (AIC) was used to choose the inheritance model that best fitted the data. The model with the lowest AIC value was judged to show the best fit. Data analysis was performed by using SPSS statistical software version 24.0 for Windows. A p-value <0.05 was considered to be statistically significant.

Results

Patients with rapidly progresive proliferative diabetic retinopathy (n=48) were included in the study group. The control group (n=84) included patients with no proliferative diabetic retinopathy up to 15 years of diabetes. The reference group (n=90) included non-diabetic individuals who matched the study group by age and gender. The study and control groups did not differ significantly regarding gender (p=0.89) and age (p=0.65). Also, the duration of type 1 diabetes mellitus and the level of glycated hemoglobin (HbA1c) did not differ between the study and control groups (p=0.41 and p=0.93, respectively). The demographic and clinical characteristics of all study participants are shown in Table 1.

There were significantly increased frequencies of hypertension, dyslipidemia, and nephropathy in the study group, compared with the control group (p<0.05). Patient age at onset of

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 2. The genotype distributions of *CEP135* rs4865047 and *NPY2R* rs1902491 in the study group and the control group and the reference group (without diabetes).

Groups (n)	Genotype rs4865047	Genotype rs1902491	Genotype frequency, n (%)	
			rs4865047	rs1902491
Study group (n=47; 48)	CC	AA	33 (70.2%)	16 (33.3%)
	CT	AG	14 (28.8%)	27 (56.3%)
	–	GG	–	5 (10.4%)
p-value	–	–	**0.001**	0.31
HWE p-value	–	–	0.58	0.36
Control group (n=84)	CC	AA	51 (60.7%)	29 (34.1)
	CT	AG	33 (39.3%)	41 (48.2)
	–	GG	–	14 (16.7)
p-value	–	–	**<0.001**	0.78
HWE p-value	–	–	0.03	0.99
Reference group (n=90)	CC	AA	83 (92.2%)	27 (30.0%)
	CT	AG	7 (7.8%)	44 (48.9%)
	–	GG	–	19 (21.1%)

Study group: one patient with missing value for rs4865047 (n=1) was excluded from analysis. Results are expressed as the number (%). HWE – Hardy-Weinberg equilibrium; TT – homozygous carriers of minor alleles; CT – heterozygous carriers of minor alleles. p<0.05 (χ^2 test); p-value versus reference group.

type 1 diabetes mellitus did not significantly differ between the study group and the control group (15.96 years *vs.* 15.89 years) (p=0.77).

The single nucleotide polymorphisms (SNPs) of the gene encoding a 135-kD centrosomal protein *CEP135* rs4865047 and the gene encoding the type 2 NPY protein *NPY2R* rs1902491 were evaluated from venous blood samples obtained from all study participants. The analysis of the distribution of genotypes in *CEP135* rs4865047 and *NPY2R* rs1902491 detected significant differences in the *CEP135* rs4865047 genotype frequencies between the study group and the control group when compared with subjects in the reference group (Table 2). There were no significant differences in the distribution of *NPY2R* rs1902491 genotype frequencies between the study group and the control group when compared with subjects in the reference group (p>0.05) (Table 2).

The aim of this study was to investigate the associations between the SNPs pf patients with type 1 diabetes mellitus in the study group and the control group, using inheritance models (Table 3). The models were adjusted by age, gender, and hypertension in the study and control groups in relation to the reference group. Although this study did not detect homozygous carriers of minor alleles (TT) for *CEP135* rs4865047 and did not evaluate other inheritance models, the codominant model for the SNP rs4865047 association with the study group showed that heterozygous carriers of minor alleles (CT) had a sevenfold higher odds ratio (OR) for rapidly progressive proliferative diabetic retinopathy when compared with wild-type homozygous carriers (OR 7.2, 95% CI, 2.28–22.74, p=0.001) and when compared to the reference group. The additive, recessive, and codominant models and their associations were evaluated for SNP rs1902491 with the rapidly progressive proliferative diabetic retinopathy group compared with the reference group. The results showed the possible impact of decreasing the OR for the rapidly progressive proliferative diabetic retinopathy group, but no significant association was found (Table 3). The minor allele frequency (MAF) of *CEP135* rs4865047 was significantly higher in the study and control groups when compared with the reference group (p=0.001), while MAF of rs1902491 did not differ significantly between the groups (p>0.05) (Table 4).

Discussion

This study aimed to determine whether the single nucleotide polymorphisms (SNPs) of the gene encoding a 135-kD centrosomal protein *CEP135* rs4865047 and the gene encoding the type 2 NPY protein *NPY2R* rs1902491 were associated with the development of rapidly progressive proliferative diabetic retinopathy in patients with type 1 diabetes mellitus. The three groups included the study group of patients with type 1 diabetes (for >15 years) who had rapidly progressive proliferative diabetic retinopathy,

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 3. The risk prediction of *CEP135* SNP (rs4865047) and *NPY2R* SNP (rs1902491) genotype polymorphisms in the study group (with type 1 diabetes and proliferative diabetic retinopathy) and the control group (with type 1 diabetes).

SNPs	Model	Study group				Control group			
		OR	95% CI	p-Value	AIC	OR	95% CI	p-Value	AIC
rs4865047	Codominant								
	CT	7.2	2.28–22.74	**0.001**	149.26	8.2	3.2–21.5	**<0.001**	219.33
rs1902491	Dominant	1.17	0.5–2.76	0.71	161.73	0.91	0.47–1.75	0.78	244.14
	Recessive	0.52	0.17–1.61	0.26	160.48	0.81	0.38-1.73	0.56	244.02
	Additive	0.89	0.5–1.48	0.69	161.71	0.89	0.58–1.38	0.62	244.99
	Codominant								
	AG	1.39	0.57–3.39	0.46	161.94	0.9	0.446–1.81	0.76	245.97
	GG	0.64	0.18–2.29	0.49	161.94	0.8	0.32–1.64	0.38	245.97

Adjusted by age, gender, and hypertension. SNP – single nucleotide polymorphism; OR – odds ratio; AIC – Akaike information criterion; TT – homozygous carriers of minor alleles; CT – heterozygous carriers of minor alleles.

Table 4. Minor allele frequency of *CEP135* and *NPY2R* SNPs and associations between the study group (with type 1 diabetes and proliferative diabetic retinopathy) and the control group (with type 1 diabetes) compared with the reference group (without diabetes).

SNPs	Study group		Control group		Reference group	
	MAF	OR (95% CI)	MAF	OR (95% CI)	MAF	OR (95% CI)
rs4865047	0.149*	4.325 (1.681–11.130) p=0.001	0.198**	6.089 (2.619–14.157) p<0.0001	0.039	1.00
rs1902491	0.394	0.776 (0.467–1.288) p=0.326	0.419	0.860 (0.564–1.312) p=0.484	0.456	1.00

MAF – minor allele frequency; OR – odds ratio; CI – confidence interval; SNP – single nucleotide polymorphism. * 0.003 *vs.* MAF in the reference group. ** 0.0001 *vs.* MAF in the reference group.

the control group of patients with type 1 diabetes (for >15 years) who had no proliferative diabetic retinopathy up to 15 years of diabetes mellitus duration, and a reference group of non-diabetic individuals. The findings showed an association between *CEP135* rs4865047 with the rapidly progressive proliferative diabetic retinopathy in the study and the control group in comparison to the reference group. No significant association was found between *NPY2R* rs1902491 and rapidly progressive proliferative diabetic retinopathy in the study group or in the controls in the Lithuanian cohort of patients with type 1 diabetes.

CEP135 rs4865047 and *NPY2R* rs1902491 were nominally associated with severe diabetic retinopathy from the single nucleotide polymorphism (SNP) candidates of a recently reported investigation of patients with type 1 diabetes mellitus, which replicated SNPs of a previous genome-wide association study (GWAS) for the complication of progressive proliferative diabetic retinopathy [14]. In this previous study, the strongest association with severe diabetic retinopathy was at rs4865047, within the intronic region of the SNP of the *CEP135* gene [14]. The strongest association from the sub-analysis (excluding patients with end-stage diabetic retinopathy) was at rs1902491, a SNP that is sited upstream of the *NPY2R* gene. Both the *CEP135* and the *NPY2R* genes are located on chromosome 4 [14].

Among clinical factors that have been identified as increasing the risk of developing diabetic retinopathy, glycated hemoglobin (HbA1c), arterial hypertension, and dyslipidemia are considered to be important and also to be involved in the progression of proliferative diabetic retinopathy [1]. The findings of the present study support the previously published data, as patients with rapidly progressive proliferative diabetic retinopathy had the highest prevalence of hypertension, nephropathy, and dyslipidemia. Proliferative diabetic retinopathy developed significantly more rapidly in the study group than in the control group, while the age of onset of type 1 diabetes mellitus, levels of HbA1c, and the duration of diabetes did not differ between the study group and the control group.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

The gene *CEP135* is expressed in the retina and is involved in centriole adhesion and cell replication. Reducing *CEP135* expression in cells via RNA interference has been shown to result in disorganization of interphase and mitotic spindles, leading to the hypothesis that *CEP135* has a role in maintaining the structure and organization of the centrosome and of microtubules [15]. *CEP135* interacts with SMAD family member 9 (SMAD9), which is involved in transforming growth factor-β (TGF-β) signaling. During the development of proliferative diabetic retinopathy, TGF-β is significantly upregulated in the aqueous humor and vitreous body [19]. SNP rs4865047 is also 50.5 kb downstream of the exocyst complex component 1 gene, *EXOC1*, and the exocyst complex is required for the insulin-stimulated transport of the glucose transporter type 4 (Glut4) to the plasma membrane [14].

It has previously been shown that a truncating mutation of *CEP135* is associated with autosomal recessive primary microcephaly [16]. The polarity of cell division can be affected in neural progenitor cells, leading to an altered number of neural progenitor cells, which may be the cause of reduced neurogenesis [15]. Also, a previous study has shown that diabetes mellitus affects the entire neurovascular unit of the retina, not only the microvasculature [20]. Recent study findings suggest that dysfunction of the neuroretina may precede the characteristic vascular findings of diabetes [21]. Therefore, abnormalities in *CEP135* gene regulation may be associated with early decreased levels of neurogenesis in patients with rapidly progressive proliferative diabetic retinopathy.

Neuropeptide Y (NPY) is a sympathetic co-transmitter preferentially released during intense or prolonged stress, which causes vasoconstriction and vascular smooth muscle cell proliferation. Neuropeptide Y receptor subtype 2 (NPY2R) is a G protein-coupled receptor for NPY, a neurotransmitter released by endothelial cells that are implicated in ischemic angiogenesis [22]. There are genetic, biological, and functional data supporting a role for neuropeptide Y signaling in the development of diabetic retinopathy [23].

In the present study, we estimated that the rs4865047 CT genotype frequency, the inheritance models, and minor allele frequency (MAF) (allele T) in the study group and control group was significantly different compared with the reference group, indicating that this variant may confer harmful effect on diabetic retinopathy in patients with type 1 diabetes. Conversely, the CC genotype could have a protective effect, as has been shown in a study by Grassi et al., the Genetics of Kidney in Diabetes (GoKinD), and the Epidemiology of Diabetes Intervention and Control Trial (EDIC), where the CC genotype showed an odds ratio (OR) with a decreasing effect on severe diabetic retinopathy (OR 0.65, 0.87, and 0.44, respectively) [14]. However, the SNP rs1902491 genotype frequencies, the inheritance models, and MAF did not differ between the study group, the control group, and the reference group in our study. We found no significant differences in the analyzed SNPs between the study group and control group, but this could be caused by a relatively small sample size.

The strength of our study was that all subjects with diabetic subjects had type 1 diabetes mellitus and the study compared patients with diabetes and rapidly progressive proliferative diabetic retinopathy, controls with diabetes and no proliferative diabetic retinopathy up to 15 years of DM duration, and healthy subjects, where all participants were of the same ethnicity, which may have minimized study heterogeneity and exposure to environmental confounders. Additionally, all patients in the rapidly progressive proliferative diabetic retinopathy group previously had laser treatment. The main limitation of our study was a relatively small sample size of the participants. From the pilot study to determine the study population size required, when analyzing SNP rs4865047 the participant numbers in the study group and the control group were adequate. However, the patient sample size for evaluation of SNP rs1902491 should have included at least 200 participants, but this number of patients and the requirements of the study inclusion criteria limited the size of the study groups.

Previous extensive candidate gene studies have been performed and numerous genetic variants associated with diabetic retinopathy have been identified in several genes. However, much of this data remains controversial. The patients with type 1 diabetes with the SNP variants identified in the present study may have a more rapid progression of proliferative diabetic retinopathy than other diabetic patients. Therefore, although other factors in addition to analyzed SNPs may contribute to rapidly progressive proliferative diabetic retinopathy, the confirmation of the findings of the present study in other populations with a larger sample size of participants would be important to further validate the findings and their clinical implications.

Conclusions

To our knowledge, this was the first study to evaluate rapidly progressive proliferative diabetic retinopathy in type 1 diabetes and its associations with the single nucleotide polymorphisms (SNPs) *CEP135* rs4865047 and *NPY2R* rs1902491 genotypes in study and control groups. The main finding of the study was the significant association between the *CEP135* SNP rs4865047 genotype and rapidly progressive proliferative diabetic retinopathy, but with no significant relationship found for the *NPY2R* SNP rs1902491 genotype.

Competing interests

None.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

References:

1. Yau JW, Rogers SL, Kawasaki R et al: Meta-analysis for Eye Disease (META-EYE) Study Group. A large Global prevalence and major risk factors of diabetic retinopathy. Diabetes Care, 2012; 35: 556–64
2. Fong DS, Aiello LP, Ferris FL, Klein R: Diabetic retinopathy. Diabetes Care, 2004; 27: 2540–53
3. Barber AJ: Diabetic reinopathy: Recent advances towards understanding neurodegeneration and vision loss. Sci China Life Sci, 2015; 58(6): 541–49
4. Klein R, Klein BE, Moss SE et al: The Wisconsin epidemiologic study of diabetic retinopathy. II. Prevalence and risk of diabetic retinopathy when age at diagnosis is less than 30 years. Arch Ophthalmol, 1984; 102: 520–26
5. Klein R, Klein BE, Moss SE, Cruickshanks KJ: The Wisconsin epidemiologic study of diabetic retinopathy: XVII. The 14-year incidence and progression of diabetic retinopathy and associated risk factors in type 1 diabetes. Ophthalmology, 1998; 105: 1801–15
6. Diabetes Control and Complications Trial Research Group: Effect of pregnancy on microvascular complications in the diabetes control and complications trial. The Diabetes Control and Complications Trial Research Group. Diabetes Care, 2000; 23: 1084–91
7. Pitocco D, Tesauro M, Alessandro R et al: Oxidative stress in diabetes: Implications for vascular and other complications. Int J Mol Sci, 2013; 14: 21525–50
8. Kowluru RA: Diabetic retinopathy, metabolic memory and epigenetic modifications. Vision Res, 2017; 139: 30–38
9. Warpeha KM, Chakravarthy U: Molecular genetics of microvascular disease in diabetic retinopathy. Eye, 2003; 17: 305–11
10. Zhang LY, Krzentowski G, Albert A, Lefebvre PJ: Risk of developing retinopathy in diabetes control and complications trial type 1 diabetic patients with good or poor metabolic control. Diabetes Care, 2001; 24: 1275–79
11. Hietala K, Forsblom C, Summanen P, Groop PH: Heritability of proliferative diabetic retinopathy. FinnDiane Study Group. Diabetes, 2008; 57: 2176–80
12. Abhary S, Hewitt AW, Burdon KP, Craig JE: A systematic meta-analysis of genetic association studies for diabetic retinopathy. Diabetes, 2009; 58: 2137–47
13. Ng DP: Human genetics of diabetic retinopathy: Current perspectives. J Ophthalmol, 2010; 2010: pii: 172593
14. Grassi MA, Tikhomirov A, Ramalingam S et al: Replication analysis for severe diabetic retinopathy. Invest Ophthalmol Vis Sci, 2012; 53: 2377–81
15. Ohta T, Essner R, Ryu JH et al: Characterization of Cep135, a novel coiled-coil centrosomal protein involved in microtubule organization in mammalian cells. J. Cell Biol, 2002; 156: 87–99
16. Hussain MS, Baig SM, Neuman S et al: Truncating mutation of CEP135 causes primary microcephaly and disturbed centrosomal function. Am J Hum Genet, 2012; 90: 871–78
17. Bumblyte A, Pakalnyte R, Radzeviciene L et al: Dynamics of diabetic nephropathy and other complications of type I diabetes mellitus in the period of 1996–2002 (data from 2 Kaunas outpatient polyclinics). Medicina, 2003; 39: 51–58
18. Wilkinson CP, Ferris FL, Klein RE et al: Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology, 2003; 110: 1677–82
19. Tan X, Chen C, Zhu Y et al: Proteotoxic stress desensitizes TGF-beta signaling through receptor downregulation in retinal pigment epithelial cells. Curr Mol Med, 2017; 17: 189–99
20. Gardner TW, Davila JR: The neurovascular unit and the pathophysiologic basis of diabetic retinopathy. Graefes Arch Clin Exp Ophthalmol, 2017; 255: 1–6
21. Sohn EH, van Dijk HW, Jiao C et al: Retinal neurodegeneration may precede microvascular changes characteristic of diabetic retinopathy in diabetes mellitus. Proc Natl Acad Sci USA, 2016; 113: 2655–64
22. Zukowska Z, Grant DS, Lee EW. Neuropeptide Y: A novel mechanism for ischemic angiogenesis. Trends Cardiovasc Med, 2003;13: 86–92
23. Korn JM, Kuruvilla FG, McCarroll SA et al: Integrated genotype calling and association analysis of SNPs, common copy number polymorphisms and rare CNVs. Nat Genet, 2008; 40: 1253–60

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]