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The rs4712527 Polymorphism in the *CDKAL1* Gene: A Protective Factor for Proliferative Diabetic Retinopathy Progress in Type 2 Diabetes

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Abstract

Purpose: Diabetic retinopathy (DR) is one of the chronic retinal disorders linked to diabetes and remains the leading cause of blindness in working-age people. Many studies have demonstrated the existence of associations between type 2 diabetes mellitus (T2DM) and variants in the cyclin-dependent kinase 5 regulatory subunit–associated protein 1-like 1 (*CDKAL1*) gene. Here, we performed a case-control study in the *CDKAL1* gene (rs4712527 polymorphism) to investigate the potential association between this single-nucleotide polymorphism (SNP) and DR risk. **Methods:** Two hundred thirty-one patients with T2DM (126 patients with proliferative diabetic retinopathy [PDR] and 105 patients without diabetic retinopathy [WDR]), who assisted at the Centro Privado de Ojos Romagosa, Fundación VER, were studied. An independent cohort of 98 patients (56 with PDR and 42 with WDR) from the Hospital Nacional de Clínicas was taken for replication. A complete ophthalmological examination included an external examination of the eye and adnexa, pupil responsiveness, and slit-lamp biomicroscopic examination. Genotyping of rs4712527 was carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The odds ratio (OR) and 95% CI were calculated by unconditional logistic regression adjusted for diabetes duration, body mass index, insulin therapy, HbA_{1c}, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, and systolic and diastolic blood pressure. **Results:** Analysis from the rs4712527 SNP in the Centro Privado de Ojos Romagosa, Fundación VER, cohort was found to be associated with decreased risk of PDR both before and after adjustment, under the codominant (adjusted OR = 0.16 [95% CI, 0.06-0.44]; $P = 4e\text{-}04$), dominant (adjusted OR = 0.17 [95% CI, 0.07-0.43]; $P = 1e\text{-}04$), overdominant (adjusted OR = 0.20 [95% CI, 0.08-0.52]; $P = 5e\text{-}04$), and log-additive (adjusted OR = 0.28 [95% CI, 0.13-0.59]; $P = 4e\text{-}04$) models. In the combined analysis including both cohorts, the rs4712527 was nominally involved as a protective factor in the development of DR. **Conclusions:** Our findings suggest that the rs4712527 in the *CDKAL1* gene might be involved in the protection to develop PDR in T2DM.

Keywords

CDKAL1 gene, polymorphism, rs4712527, diabetic retinopathy, type 2 diabetes

Introduction

There is compelling evidence that the individual risk of developing type 2 diabetes mellitus (T2DM) is strongly influenced by genetic factors.[1] Recent reports on genome-wide association studies (GWASs) have effectively revealed a number of loci linked with propensity to T2DM.[2] The heritability for diabetic retinopathy (DR) was estimated at 18%, while for proliferative diabetic retinopathy (PDR), it would be 52%.[3,4]

DR is a complication of diabetes due to sustained high blood sugar levels, which causes progressive damage to the retina. It is one of the principal causes of blindness in the working-age population and affects around 5.3 million adults causing an estimated 12 000 to 24 000 new cases of blindness each year.[5]

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The number of adults with diabetes worldwide is estimated to increase by 42% in developed countries like United States and 170% in developing countries like Argentina. Visual impairment in DR is largely attributed to microvascular changes induced by ischemic retinopathy.[6] These ischemic changes stimulate increased microvascular permeability and neovascular proliferation in the retina leading to diabetic macular edema and PDR, 2 major retinopathy stages in diabetes that induce blindness.[7]

Clinically, the first changes in the diabetic retina include microaneurysms, hemorrhages, hard exudates, cotton wool spots, venous beading, and intraretinal microvascular abnormalities. The growth of new abnormal blood vessels often leading to vitreous and preretinal hemorrhage and tractional retinal detachment is the main feature of PDR.[8]

The frequency and severity of DR are very heterogeneous between different ethnic groups, despite presenting the same risk factors such as diabetes duration and glycemic control. In addition, there are patients with many years of disease evolution and good glycemic control with PDR and patients with poor metabolic control who never present severe DR. As mentioned above, genetic factors may explain this apparent paradox of retinal complications in diabetic patients. Evidence also suggests a genetic control on the development and progression of retinopathy in diabetic patients.[3] Consequently, genetic factors are one of the pillars where DR and other diabetic complications are studied.[9] Some works report the study of candidate genetic variants in the presence or progression of DR.[10,11] These reports show significant interaction between genes, environment, and behavior in the development of vascular complications in patients with T2DM, in addition to the ethnic component. However, genetic association studies have attempted to unravel the genetic risk factors for diabetic microvascular complications for DR, showing dissimilar results between different groups, having no data from the Argentine population.

Candidate genes are believed to be important in the pathogenesis of diabetes[12-14] or DR,[15-22] including the vitamin D receptor, melatonin receptor 1B, solute carrier family 30 member 8, peroxisome proliferator-activated receptors, adiponectin, fat mass and obesity associated, hypoxia-inducible factor 1-alpha, insulin-like growth factor 1, integrin alpha M, vascular endothelial growth factor B, PHD finger protein 21A, N-acetyltransferase 1, aldo-keto reductase family 1, member B1, and cyclin-dependent kinase 5 regulatory subunit–associated protein 1-like 1 (*CDKAL1*) genes.

Ultimately, CDKAL1, whose function is not fully known, is believed to encode a methylthiotransferase that catalyzes the 2-methylthio (ms2) modifications of various substrates, including the addition of ms2 to N6-threonylcabamoyladenoside at position 37 of transfer RNA Lys (UUU).[23] Individuals carrying single-nucleotide polymorphisms (SNPs) of the CDKAL1 gene associated with T2DM could have an alteration in insulin secretion and an increased risk of T2DM[24,25] and diabetic complications.[26] In turn, some studies have considered SNPs as a protective factor for T2DM and renal complications.[27] Meanwhile, Fu et al[28] suggested the possible role of the rs4712527 polymorphism (localized in one intron), with Peng et al[29] and Liu et al[26] showing an association between the rs7756992 and the rs10946398, respectively, with DR in a Chinese population with type 2 diabetes.[29]

Different GWASs have shown an association of T2DM or its complications with the CDKAL1 gene. In addition, many SNPs in the CDKAL1 gene have been shown to be associated with the development of DR or diabetic nephropathy (DN) as risk or protective factors. The purpose of this study is to determine whether another variant in the CDKAL1 gene, the rs4712527 SNP, could be associated with the presence of PDR in Argentinean patients with T2DM.

Methods

Patients

Patients from Centro Privado de Ojos Romagosa, Fundación VER. Patients older than 21 years with T2DM were recruited between July 2014 and July 2015 from a private eye clinic (Centro Privado de Ojos Romagosa, Fundación VER**)** located in the city of Córdoba, Argentina. Two hundred thirty-one patients with T2DM (126 patients with PDR and 105 patients without diabetic retinopathy [WDR]) were studied.

Patients from Hospital Nacional de Clínicas (replication study). A total of 98 patients with DR (56 with PDR and 42 with WDR) were recruited from the public Hospital Nacional de Clinicas from the city of Córdoba and were included in the replication study, since both cohorts have a comparable ethnic background so that no major differences in minor allele frequency exist. The age, gender, and body mass index (BMI) distribution was comparable in both cohorts.

Clinical Evaluation

T2DM was diagnosed according to criteria of the American Diabetes Association (2005).[30] Diabetic patients were defined as those having a self-reported previous history of physician-diagnosed T2DM treated with insulin or oral hypoglycemic agents. Patients had to show a diagnosis of diabetes of at least 5 years. The duration of diabetes was defined as the interval between the first diagnosis of diabetes and the time of enrollment in the present study. All patients completed a questionnaire that revealed basic information (age and sex), mainly Spanish and Italian ancestors (Caucasian origin), smoking habits, health status (such as the use of insulin and oral hypoglycemic therapy and any history of other systemic diseases), and family history of diseases. By study design, all recruited patients had normal renal function. A complete ophthalmological examination included an external examination of the eye and adnexa, pupil responsiveness, and slit-lamp biomicroscopic examination. Intraocular pressure measurements were performed prior to pupil dilation using a Goldmann applanation tonometer (Haag-Streit AT 900, Koeniz, Switzerland). The

examination also included a dilated indirect ophthalmoscopic examination (including evaluation of the posterior segment and vitreous, optic nerve, retinal vasculature, peripheral retina, and macula) performed by 1 of the 2 retinal specialists (J.D.L. or A.L.G.) and dilated fundus photography. Seven fields of 30° color fundus photographs with stereoscopic images of the optic disc and macula were taken through the dilated pupils of each patient, using a digital fundus camera (Zeiss Visucam Pro, Oberkochen, Germany). Optical coherence tomography (OCT) imaging (Cirrus high-resolution spectral domain-OCT system; Carl Zeiss Meditec, Inc, Dublin, California) was also obtained at the time of enrollment. Two retinal specialists (J.D.L. and A.L.G.) determined again the presence or absence of DR and classified the pathology in a masked manner. Our study groups were classified into 2 extreme forms of patients with T2DM according to severity of retinal affection, following the gold standard grading system for defining severity of DR, the Early Treatment Diabetic Retinopathy Study (ETDRS).[31] One group included patients showing no signs of DR at all, and the other group comprised patients with retinal neovascularization with or without previous treatment for PDR.

Laboratory Assay

Patients came to the clinical laboratory after a 12-hour fasting overnight and venous blood samples were obtained. The fasting venous blood samples were collected for the measurement of biochemical parameters, which were studied as routine follow-up assays for diabetic patients. These measurements included fasting glucose, glycosylated hemoglobin (HbA_{1c}), total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C), determined by an automated system with reagents for routine biomarkers with a Roche COBAS 6000 (Roche Diagnostics, Rotkreuz, Switzerland) analyzer.

Genotyping

DNA preparation. Patients' DNA was obtained from peripheral blood using the Wizard Genomic DNA Purification Kit (Promega, Madison, Wisconsin), according to the manufacturer's instructions. One hundred fifty microliters of anticoagulated blood were lysed with cell lysis solution and nucleic lysis solution. The sample was then centrifuged and the supernatant was discarded. Different solutions provided by the manufacturer (RNAse solution, protein precipitation solution) were used for DNA extraction. In addition, isopropanol and ethanol 70% were used for DNA precipitation. Finally, the extracted DNA was suspended again in DNA rehydration solution.

Single-Nucleotide Polymorphism

The rs4712527 SNP was studied in the CDKAL1 gene,[28] which was analyzed with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay using specific primers in a Biometra UNO II cycler (Göttingen, Germany).

PCR was performed using 80 ng of patients' DNA: 3 μL of 5X enzyme buffer; 200 mM of each dGTP, dATP, dCTP, and dTTP; 10 μM of each forward 5′ GGGTGTGAGGATTGAAGTCGG 3′ and reverse 5′ AAACATTAGCCCCATCTCCCT 3′ primer; 0.6 U of Go-Taq polymerase (Promega); and water qs 15 μL. The cycling parameters were as follows: 94°C for 2 minutes and then 35 cycles of 94°C for 50 seconds, 60°C for 50 seconds and 72°C for 60 seconds, and a final extension at 72°C for 5 minutes.

The amplification product (expected 343 bp) was checked by electrophoresis on agarose gels (1.5% wt/vol) and visualized by irradiation with ultraviolet (UV) light after staining with ethidium bromide. Afterward, PCR products were digested with the restriction endonuclease *Alu I* and were then separated by agarose gel electrophoresis (2.5% wt/vol) prestained with ethidium bromide and visualized by UV light irradiation. For the A allele, the expected sizes were 189 + 26 + 128 bp, while for the G allele, the sizes were 189 + 154 bp. The accuracy of each PCR-RFLP was confirmed by sequencing 10 selected samples.

Statistical Analysis

SNPStats software (http://bioinfo.iconcologia.net/SNPstats) was used and a multiple logistic regression model (codominant, dominant, recessive, overdominant, and log-additive) was made to obtain odds ratios (ORs), considering a significant difference P value <.05, with a 95% CI.[32] The genotypic and allelic frequencies observed for the different variants were compared with those expected according to the Hardy-Weinberg Law. Biochemical and clinical parameters were analyzed using the t test and χ^2, respectively, using the Infostat program.[33]

Results

Centro Privado de Ojos Romagosa, Fundación VER cohort

The population studied consisted of 231 patients with T2DM. As shown in Table 1, the compared cohorts in our study had no significant differences in terms of age, smoking habits, presence of arterial hypertension, and the biochemical parameters. On the contrary, there is a significant difference in the proportion of men and women in the sample, with the number of women greater in the WDR group than in the PDR group. Also, there are significant differences in the duration and time of onset of diabetes and also in glycosylated hemoglobin levels as in the presence of insulin treatment, all these parameters being higher in the PDR group. Finally, the BMI was significantly higher in the WDR group compared to the PDR group. The genetic and allelic frequencies for rs4712527 in the CDKAL1 gene were in Hardy-Weinberg equilibrium in both cohorts (Table 2). A multiple logistic regression model was used (codominant, dominant, recessive, overdominant, and log-additive) and variables were adjusted by diabetes duration,

Table 1. Clinical, Lifestyle, and Biochemical Parameters Analyzed in the Populations Studied (Average).[a]

	Centro Privado de Ojos Romagosa, Fundación VER Cohort			Hospital Nacional de Clínicas Cohort		
	WDR (n = 105)	PDR (n = 126)	*P* Value	WDR (n = 42)	PDR (n = 56)	*P* Value
Sex (F/M)	81/24	36/90	<.0001[b]	31/11	17/39	<.0001[b]
Age (years)	67.29 (8.42)	67.07 (6.36)	.9001	67.95 (9.03)	66.84 (6.30)	.4965
Known diabetes duration (years)	10.34 (6.68)	19.52 (8.82)	<.0001[b]	10.95 (7.14)	19.05 (8.34)	<.0001[b]
Age of onset (years)	56.94 (9.04)	47.55 (10.84)	.0001[b]	57.00 (9.56)	47.79 (10.31)	<.0001[b]
Hypertension (yes/no)	81/24	108/18	.0926	30/12	47/9	.1356
Smoker (yes/no)	27/78	39/87	.3802	11/31	20/36	.3157
BMI (kg/m^2)	31.69 (5.97)	29.24 (3.58)	.04[b]	32.24 (6.24)	29.58 (3.55)	.016[b]
Insulin therapy (yes/no)	6/99	84/42	<.0001[b]	2/40	38/18	<.0001[b]
FPG (mg %)	120.46 (35.87)	129.52 (41.03)	.3144	121.02 (36.65)	134.71 (43.40)	.1023
HbA_{1c} (%)	7.02 (1.13)	7.85 (1.24)	.0035[b]	6.98 (1.19)	7.90 (1.26)	.0004[b]
Total cholesterol (mg/dL)	175.94 (39.31)	186.64 (32.54)	.1992	167.81 (39.33)	186.52 (31.07)	.0099[b]
Triglycerides (mg/dL)	154.14 (67.49)	148.00 (51.58)	.6552	167.88 (79.57)	149.79 (58.31)	.2173
HDL cholesterol (mg/dL)	48.37 (11.85)	45.53 (11.82)	.3019	48.40 (11.99)	45.09 (11.77)	.1750
LDL cholesterol (mg/dL)	108.60 (40.30)	113.21 (41.96)	.6292	108.02 (45.07)	111.70 (40.32)	.6724
Systolic blood pressure (mm Hg)	126.57 (8.97)	130.48 (11.99)	.119	127.14 (10.19)	130.36 (11.28)	.149
Diastolic blood pressure (mm Hg)	76.86 (10.68)	76.90 (11.28)	.9851	76.57 (10.02)	77.32 (11.68)	.579

Abbreviations: BMI, body mass index; FPG, fasting plasma glucose; HbA_{1c}, glycosylated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PDR, proliferative diabetic retinopathy; WDR, without diabetic retinopathy.
[a]The data are expressed as mean (SD) or number of patients. Differences between both groups of patients (with PDR and WDR) were compared using *t* test or χ^2 test.
[b]Statistically significant.

Table 2. Genotypic and Allelic Distribution and Hardy-Weinberg Equilibrium in the Populations Studied.

	Genotype			Alleles		
CDKAL1 (rs4712527)	Wt/Wt	Wt/Mut	Mut/Mut	Wt	Mut	H-W Equilibrium (*P*)
Centro Privado de Ojos Romagosa, Fundación VER cohort	21	78	132	120	342	.085
Hospital Nacional de Clínicas cohort	6	24	68	36	160	.070

Abbreviations: CDKAL1, cyclin-dependent kinase 5 regulatory subunit–associated protein 1-like 1; H-W, Hardy-Weinberg; Mut, mutant; Wt, Wild type.

BMI, insulin therapy, HbA_{1c}, total cholesterol, HDL-C, LDL-C, triglycerides, systolic blood pressure, and diastolic blood pressure to obtain the ORs. Table 3 shows the results obtained for rs4712527 in the CDKAL1 gene. It seems that the presence of G variant for A>G polymorphism (rs4712527) in the CDKAL1 gene is a protective factor for the development of retinal neovascularization in retinas of diabetic patients under the codominant (OR = 0.31 [95% CI, 0.17-0.57]; P = 3e-04), dominant (OR = 0.34 [95% CI, 0.19-0.58]; P = 1e-04), overdominant (OR = 0.35 [95% CI, 0.20-0.64]; P = 4e-04), and log-additive models (OR = 0.49 [95% CI, 0.33-0.75]; P = 6e-04). This result was demonstrated after the genetic data were adjusted to the biochemical and clinical parameters (diabetes duration, BMI, insulin therapy, HbA_{1c}, total cholesterol, HDL-C, LDL-C, triglycerides, systolic blood pressure, and diastolic blood pressure); the OR, 95% CI, and *P*, respectively, were as follows: the codominant (OR = 0.16 [95% CI, 0.06-0.44]; P = 4e-04), dominant (OR = 0.17 [95% CI, 0.07-0.43]; P = 1e-04), overdominant (OR = 0.20 [95% CI, 0.08-0.52]; P = 5e-04), and log-additive models (OR = 0.28 [95% CI, 0.13-0.59]; P = 4e-04; Table 3).

Hospital Nacional de Clínicas Cohort

The clinical, lifestyle, and biochemical parameters and genotypic and allelic distribution in the Hospital Nacional de Clínicas cohort were similar to those that came from the Centro Privado de Ojos Romagosa, Fundación VER cohort (Table 1). The adjusted genetic data with biochemical and clinical parameters include diabetes duration, BMI, insulin therapy, HbA_{1c}, total cholesterol, HDL-C, LDL-C, triglycerides, systolic blood pressure, and diastolic blood pressure; the OR, 95% CI, and *P*, respectively, were as follows: codominant model (OR = 0.13 [95% CI, 0.02-0.83]; P = 0.0053), dominant model (OR = 0.10 [95% CI, 0.02-0.60]; P = .0059), recessive model (OR = 0.01 [95% CI, 0.00-0.54]; P = .024), and log-additive model (OR = 0.10 [95% CI, 0.02-0.47]; P = .015).

Analysis Including Centro Privado de Ojos Romagosa, Fundación VER, and Hospital Nacional de Clínicas Patients

The analysis of the contribution of the rs4712527 polymorphism with the risk of developing DR in 329 patients (both

Table 3. Association of rs4712527 Polymorphism in the CDKAL1 Gene With the Presence of PDR.[a]

CDKAL1 (rs4712527)		Centro Privado de Ojos Romagosa, Fundación VER Cohort						Hospital Nacional de Clínicas Cohort					
Model	Genotype	WDR, n (%)	PDR, n (%)	OR (95% CI)	*P* Value	Adjusted OR (95% CI)[b]	*P* Value[b]	WDR, n (%)	PDR, n (%)	OR (95% CI)	*P* Value	Adjusted OR (95% CI)[b]	*P* Value [b]
Codominant	A/A	51 (48)	93 (73.8)	1.00	3.00E-04	1.00	.0015	22 (52.4)	46 (82.1)	1.00	.0039	1.00	.0053
	A/G	42 (40)	24 (19.1)	0.31 (0.17-0.57)		0.22 (0.09-0.54)		15 (35.7)	9 (16.1)	0.29 (0.11-0.76)		0.13 (0.02-0.83)	
	G/G	12 (11.4)	9 (7.1)	0.41 (0.16-1.04)		0.31 (0.07-1.48)		5 (11.9)	1 (1.8)	0.10 (0.01-0.87)		0.00 (0.00-0.28)	
Dominant	A/A	51 (48.6)	93 (73.8)	1.00	1.00E-04	1.00	3.00E-04	22 (52.4)	46 (82.1)	1.00	.0015	1.00	.0059
	A/G-G/G	54 (51.4)	33 (26.2)	0.34 (0.19-0.58)		0.23 (0.10-0.54)		20 (47.6)	10 (17.9)	0.24 (0.10-0.60)		0.10 (0.02-0.60)	
Recessive	A/A-A/G	93 (88.6)	117 (92.9)	1.00	.26	1.00	.3	37 (88.1)	55 (98.2)	1.00	.035	1.00	.024
	G/G	12 (11.4)	9 (7.1)	0.60 (0.24-1.48)		0.46 (0.10-2.05)		5 (11.9)	1 (1.8)	0.13 (0.02-1.20)		0.01 (0.00-0.54)	
Overdominant	A/A-G/G	63 (60)	102 (81)	1.00	4.00E-04	1.00	.001	27 (64.3)	47 (83.9)	1.00	.026	1.00	.049
	A/G	42 (40)	24 (19.1)	0.35 (0.20-0.64)		0.24 (0.10-0.59)		15 (35.7)	9 (16.1)	0.34 (0.13-0.89)		0.20 (0.04-1.05)	
Log-additive	–	–	–	0.49 (0.33-0.75)	6.00E-04	0.37 (0.20-0.71)	.0015	–	–	0.34 (0.13-0.64)	9.00E-04	0.10 (0.02-0.47)	.0015

Abbreviations: BMI, body mass index; CDKAL1, cyclin-dependent kinase 5 regulatory subunit–associated protein 1-like 1; OR, odds ratio; PDR, proliferative diabetic retinopathy; WDR, without diabetic retinopathy.

[a]*P* calculated from χ^2 test.

[b]Adjusted for known diabetes duration, BMI, insulin therapy, HbA_{1c}, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, systolic blood pressure, and diastolic blood pressure.

cohorts), corrected by biochemical and clinical parameters (diabetes duration, BMI, insulin therapy, HbA_{1c}, total cholesterol, HDL-C, LDL-C, triglycerides, systolic blood pressure, and diastolic blood pressure); the OR, 95% CI, and *P*, respectively, were as follows: in the codominant model (OR = 0.16 [95% CI, 0.07-0.37]; $P \leq .0001$), dominant model (OR = 0.15 [95% CI, 0.07-0.34]; $P \leq .0001$), overdominant model (OR = 0.20 [95% CI, 0.09-0.45]; $P = 1e\text{-}04$), and log-additive model (OR = 0.22 [95% CI, 0.11-0.44]; $P \leq .0001$).

Conclusions

With the increasing prevalence of DM and the increasing life span of people with diabetes, DR is expected to be the leading global cause of vision loss in many countries, including many South American countries, such as Argentina. According to information supplied by the International Federation of Diabetes, it is estimated that the number of diabetics in the world (currently around 382 million) will rise to 553 million by 2030 (Prevalence of Diabetes, www.idf.org), and with this, retinal complications. With these data in mind, it is necessary to determine the risk factors that may contribute to the development of DR and, most importantly, to the progression to PDR, the most devastating form of the disease in the eyes.

Undoubtedly, many factors influence the progression of the severity of DR in different populations,[34] among which is the family association with the progression of retinal complications. On the other hand, it has been suggested that Hispanic individuals with diabetes develop more severe retinopathy earlier in life and progresses faster compared to that found in African American or European patients having the same health status.[35]

Our study groups were classified into 2 extreme forms of patients with T2DM according to severity of retinal affection, following the gold standard grading system for defining severity of DR, the ETDRS.[31] One group was circumscribed to patients showing no signs of DR at all, and the other group comprised patients with retinal neovascularization with or without previous treatment for PDR. Between the 2 groups, despite there being no age difference, we found a significant difference in the duration of the disease, as we can appreciate in the PDR group having significantly more years of DM than the group of patients without retinopathy. Although glycemic control during examination was not significantly different between the groups, the HbA_{1c} data showed a statistically significant difference between groups, with the PDR group having a worse glycemic control. Both the duration of diabetes and the high HbA_{1c} values in the PDR group should not surprise us, since it is known that diabetic complications such as PDR are associated with poor glycemic control as well as with the duration of the disease.

Another difference found between groups was sex distribution, which showed more males in the PDR group than the nonneovascularized group. These data agree with those reported by Arar et al[35] in the FIND-Eye Study, where they observed that men had more severe DR.

It is well known that T2DM is caused by a complex interaction between environmental and genetic factors, in the same way as familial predisposition in the presence of DR.[36] This fact has led researchers to develop a number of studies to become aware of driving genes and their variants associated with DR.

Our data indicate that the presence of G polymorphism in the rs4712527 of the CDKAL1 gene confers a lower risk of developing PDR in a diabetic Argentinean population. Although the presence of this polymorphism in the CDKAL1 gene has been found in diabetic patients from other populations, such as Chinese people,[26] the association with a lower development of PDR is not currently reported.

To our knowledge, this is the first report showing a positive association between rs4712527 polymorphism in the CDKAL1 gene and T2DM retinopathy. Other polymorphisms in the CDKAL1 gene have been studied by other researchers such as Liu et al,[26] Peng et al,[29] Jiang et al,[37] and Rask-Andersen et al.[38] The first mentioned author showed an association between rs10946398 variant and an increased risk of progression to DR.[26] Peng et al[29] revealed a significant association between rs7756992 and DR, with the minor allele A conferring a lower risk of DR. On the other hand, there are reports of this polymorphism and its association with another diabetic microvascular complication. Studies by Lasram et al[27] showed that rs7756992 variant in the CDKAL1 gene was associated with a reduced risk for developing DN in patients with T2DM. Although our study did not perform an analysis of the renal function of patients (since all of them had normal renal function), the possibility of renal involvement in patients with PDR is widely described.[35] The fact that they found an association of rs7756992 variant in the CDKAL1 gene as a protective marker in patients with PDR, also reported in diabetic patients with a lower possibility of DN, would strengthen the possibility of evidencing a marker of good prognosis for ocular and renal complications of T2DM.

The present study has 2 limitations. First, the size of the sample is relatively small; second, participants come only from the Argentinean population, which, being a fundamentally immigrant population, has a diverse genetic component. However, these are preliminary results and case-control studies with larger samples and multiple comparisons will ensure and provide a more comprehensive explanation of the relationship between the presence of SNPs and the severity of DR.

In conclusion, we showed that the rs4712527 in the CDKAL1 gene is associated with DR risk in an Argentinean population. The results found here need be confirmed in further studies with larger samples and different populations.

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Ethical Approval

The protocol for this study was approved by the Ethics Committee of Instituto Oulton and Clínica Romagosa, Ethics Committee of Hospital Nacional de Clínicas, and the Department of Health of Córdoba. Research was conducted according to the standards of the Helsinki Declaration.

Statement of Informed Consent

Written informed consent was obtained from all participants before their enrollment.

Declaration of Conflicting Interests

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