Received: 26 May 2023 | Revised: 10 September 2023 | Accepted: 18 September 2023

DOI: 10.1002/hsr2.1605

ORIGINAL RESEARCH

Health Science Reports Open Access WILEY

Aldose reductase (−106) C/T gene polymorphism and associated risk factors with proliferative diabetic retinopathy in Palestine: A cross sectional study

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Abstract

Background and Aims: Genetic variants play a crucial role in the development of diabetic retinopathy (DR). Therefore, our study aimed to investigate the relationship between aldose reductase (*ALR2*) (C106T) polymorphism with proliferative DR and associated risk factors in Palestinian type 2 diabetic patients.

Methods: A cross sectional study was conducted at St John Eye Hospital-East Jerusalem in 2020–2021 on patients with DR. All subjects had fundus examination by ophthalmologists and classified according to the severity of retinopathy. Genomic DNA was extracted from whole blood samples and genotyped by amplicon based next generation sequencing.

Results: A total of 155 patients were included, of them, 103 (66.5%) were diagnosed with non-proliferative DR (NPDR) and 52 (33.5%) with proliferative DR (PDR). The PDR group had a significantly lower median age (59.5 [IQR: 13.3]) compared to the NPDR group (62 [IQR: 11.5]) ($p = 0.04$). Additionally, the duration of diabetes was higher in the PDR group (20 [IQR: 9]) compared to the NPDR group (15 [IQR: 10]) ($p < 0.001$). Conversely, the mean value of diastolic blood pressure was significantly lower in the PDR group (79.2 ± 11.1) compared to the NPDR group (83.4 ± 10.3) ($p = 0.02$). Logistic regression analysis, revealed that the odds for patients with dyslipidemia to develop PDR were 2.74 times higher than those with NPDR (95% CI: 1.08–6.98) ($p = 0.034$). Furthermore, the probability of a patient with ≥20 years of diabetes to develop PDR was seven times higher than other patients (95% CI: 1.98–27.91) ($p = 0.003$). The genotypes distribution of *ALR2* gene and its allele frequency showed no statistical differences between the two groups ($p > 0.05$).

Conclusions: The present study showed that duration of diabetes and dyslipidemia were strong indicators for PDR progression, while *ALR2* (C106T) polymorphism was not associated with severity of DR.

KEYWORDS
ALR2 polymorphism, dyslipidemia, Palestine, retinopathy, type 2 diabetes

1 | INTRODUCTION

Diabetic retinopathy (DR) is a chronic microvascular complication of diabetes mellitus affecting small retinal vessels, arterioles, capillaries, and venules.[1] A recent meta-analysis including 59 population-based studies revealed that, in 2020, the global prevalence of DR was 22.27% (95% confidence interval [CI]: 19.73%–25.03%),[2] with 103.12 million adults estimated to have DR and expected to increase to 160.50 million by the year 2045. The highest prevalence was in Africa (35.90%), and the lowest was in South and Central America (13.37%).[3] In Palestine, DM has increased from the fifth-ranking cause of all deaths in 2018 (7.5%) to the second-ranking cause of all deaths (14.6%) in 2020. The new reported DM cases in 2020 were 4420 cases with an incidence rate of 160.4 per 100,000 populations.[4–6]

DR is divided into two main stages: non-proliferative (NPDR) and proliferative (PDR). NPDR is graded as mild, moderate, and severe; it represents the early stage of DR leading to macular edema and blurry vision. PDR represents the advanced stage of DR, characterized by neovascularization on the surface of the retina, which can form scar tissue, vitreous hemorrhage, or anterior retinal hemorrhage and eventually lead to permanent vision loss.[7] The major risk factors contributing to the development of DR are diabetes duration, poor glycemic control, as indicated by high HbA1c levels, and hypertension.[8] Other risk factors such as nephropathy, dyslipidemia, smoking, and a higher body mass index (BMI) are also reported to cause a substantial variation in the development and severity of DR.[9] Therefore, the early evaluation of the patient's condition and the proper diagnosis of DR stages are crucial to timely intervention to delay disease progression and improve the quality of DR patient's life. However, several studies reported a significant genetic susceptibility to the development of DR. In this context, several candidate genes have been implicated in the pathogenesis of DR, including aldose reductase (*AKR1B1*, also known as *ALR2*), vascular endothelial growth factor, erythropoietin (EPO), and receptor for AGEs (RAGE).[10] Aldose reductase is widely expressed in different human tissues including the kidney, nerve, heart, and retinal capillary pericytes.[11] It is the key enzyme that reduces glucose to sorbitol in the polyol pathway using NADPH as a cofactor.[12] Under chronic hyperglycemia, this pathway is highly activated, producing metabolic imbalances and oxidative stress that affect the nervous, renal, vascular, and ocular systems.[13] The human *ALR2* gene is located on chromosome 7q35 and consists of 10 exons encoding to 316 amino acid protein (~36 kDa weight).[14] Several studies reported that a common polymorphism in the promoter region of the *ALR2* gene at nucleotide C (−106)T (rs759853),- is implicated in the susceptibility to DR.[15] Other studies revealed that the level of erythrocyte aldose reductase is an independent risk factor for active PDR and may affect the prognosis of DR.[16] To the best of our knowledge, there is a lack of scientific research investigating the influence of the *ALR2* C106T polymorphism on the severity of DR in Palestine. Thus, the present study aimed to identify the risk factors of PDR among Palestinian T2DM patients and to investigate the association of C106T polymorphism of the *ALR2* gene with DR severity.

2 | METHODS

2.1 | Patients' selection and classification

This cross sectional study was conducted from March 2020 to August 2021 at St John Eye Hospital in East Jerusalem. We used purposive sampling technique to select the study participants who met specific criteria. The inclusion criteria included T2DM individuals of either gender, aged over 40 years, had diabetes duration of more than 5 years and had been diagnosed with DR based on slit lamp examination and fundoscopy. Hence, patients with T1DM or other types of diabetes, those with ocular diseases unrelated to DR or with non-diabetic retinopathy were excluded. T2DM diagnosis was based on the World Health Organization criteria: Fasting blood glucose ≥126 mg/dL and/or currently on use of antidiabetic medication.[17] Retinopathy was defined by the presence of characteristic changes in the retina, which encompassed several classic retinal lesions of DR which include: microaneurysms, hemorrhages, venous beading (consisting of alternating areas of venous dilation and constriction), intraretinal microvascular abnormalities, hard exudates (lipids deposits), cotton-wool spots (resulting from ischemic retina and accumulation of axoplasmic debris within adjacent bundles of ganglion cell axons), and retinal neovascularization.

All DR cases were classified as PDR when neovascularization and/or vitreous/preretinal hemorrhage were observed. Mild NPDR was indicated by the presence of microaneurysms only, while moderate NPDR was characterized by microaneurysms and other signs (e.g., dot and blot hemorrhages, hard exudates, cotton wool spots). Severe NPDR was characterized by the presence of moderate NPDR along with any of the following: intraretinal hemorrhages (≥20 in each quadrant), definite venous beading (in 2 quadrants), intraretinal microvascular abnormalities (in 1 quadrant), and no signs of proliferative retinopathy. Based on fundus examination, the study subjects were divided into two groups: PDR and NPDR groups according to the guidelines of International Classification of Diabetic Retinopathy and Diabetic Macular Edema. The non-proliferative group included cases of mild, moderate, and severe NPDR. Personal and clinical data including age, gender, height, weight, duration of diabetes, and family history of diabetes, smoking, history of cardiovascular disease (CVD), and dyslipidemia were obtained. BMI was calculated as kilograms divided by the square of height in meters. Blood pressure was measured in sitting position with a mercury sphygmomanometer. Blood samples were collected from all subjects in EDTA tubes for DNA extraction. This study adhered to the ethical principles of the Declaration of Helsinki. The

study protocol was approved by St John Eye Hospital Group Jerusalem Institutional Review Board (Ref: IRB/2/2020). Informed consent was obtained from all participants before blood sampling. Confidentiality and privacy of participants were strictly maintained, and data were handled and analyzed anonymously.

2.2 | DNA extraction and genotyping by amplicon based next generation sequencing

Genomic DNA was extracted from whole blood (200 µL) using genomic QIAamp DNA Blood Mini kit (Qiagen) according to the manufacturer's instructions. The DNA concentration was measured by a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific). A concentration of 50−100 ng/µL was used in each reaction. Amplification of DNA samples was done by PCR using the newly designed forward (AR-F:GGAGCCTTCTGATTGGTTGC) and reverse primer (AR-R:TTCCCACCAGATACAGCAGC targeting 161 bp fragment of *ALR2* gene. All primers were modified with over hanged Illumina adapter sequences at the 5′ ends of the forward (5′-CGTCGGCAGCGTCAGATGTGTATAAGAGACA-3′) and reverse primers (5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGA-3′). The PCR assay was carried out using 2 µL of DNA sample, 0.3 µL of forward and reverse primers (10 pmol each), 12.5 µL of PCR master mix (PCRBIO HS Taq Mix Red), and 9.6 µL of double distilled water. The PCR amplification condition was as follows: initial denaturation of DNA at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, an annealing at 63°C for 30 s, and extension at 72°C for 30 s and final extension at 72°C for 6 min.

All PCR products were cleaned using the Agencourt AMPure XP system (X1, cat. no. A63881; Beckman Coulter Genomics) and subjected to a second round of amplification for barcoding using Nextera XT Index Kit (Illumina) as described previously.[18] Samples were sequenced on a NextSeq. 500/550 machine using the 150-cycle Mid Output Kit (Illumina, Inc.). The obtained DNA sequences were analyzed using the Galaxy program (https://usegalaxy.org/). Two virtual probe sequences were used to identify the AR −106C>T polymorphism: (GCA**C**CCCCAGC) for the C allele (in bold) and (GCA**T**CCCCAGC) for the T allele (In bold).

2.3 | Statistical analysis

The statistical analysis was conducted using the SPSS package, version 26.0 (SPSS, Inc.), and the R environment v.4.1.3. We utilized packages inherent to the R environment. Genotypic and allelic associations were assessed using logistic regression analysis, with the CC as a reference genotype and C as a reference allele. Three genetic models (codominant, dominant, and recessive) were evaluated using the "SNPassoc" package. We utilized Q−Q plots and histograms to evaluate the appropriateness of the normality assumption for continuous variables. Normally distributed data were expressed as means and standard deviations (SD), while non-normally distributed data were presented as medians and interquartile ranges (IQR). Independent sample *t*-tests and Wilcoxon rank-sum tests were used for comparing groups means and medians of continuous variables, respectively. The χ^2 test was employed for categorical variables. To identify potential independent risk factors associated with PDR, variables that showed a significant association with PDR ($p < 0.05$) in the univariate analysis or were deemed clinically important were included in the multiple logistic regression model. Subsequently, the model was adjusted for age, gender, and smoking. The results of the logistic regression were presented as odds ratios (OR) with corresponding 95% CI and *p*-values. We further explored the impact of genetic polymorphism on baseline variables within each study group (PDR and NPDR). For this purpose, we compared these variables across different genotypes. The genotypes were classified based on the dominant model (CC vs. CT + TT). All tests were two-tailed, and a *p*-value < 0.05 was considered statistically significant.

3 | RESULTS

3.1 | Demographic and clinical characteristics of the studied populations

A total of 155 patients diagnosed with DR were included in this study. The median age was 60 years, with a range between 27 and 83 years. Among them, 83 were males accounting for 53.5% of the total. The median age for males was 58 [IQR: 14.5], and it was 61.5 [IQR: 10] for females ($p = 0.01$). Based on the severity of DR, the study participants were categorized into two groups: the NPDR group included 103 (66.5%) patients and the PDR group included 52 (33.5%) patients. Baseline characteristics of the NPDR and PDR patients are shown in Table 1. No significant differences were found between the two groups regarding sex, family history of DM, history of CVD, history of dyslipidemia, and diabetic macular edema. Comparison of quantitative characteristics that is, systolic blood pressure, and BMI showed no significant differences between the two groups, while the HbA1c levels were significantly higher in the PDR (9 [IQR: 2.2] compared to the NPDR group (8 [IQR: 3]) ($p = 0.04$).

In the PDR group, it was observed that the duration of diabetes was significantly higher (20 [IQR: 9]) compared to the NPDR group (15 [IQR: 10]) ($p < 0.001$). Conversely, in the NPDR group, the median age at the time of sampling was significantly higher (62 [IQR: 11.5]) compared to the PDR group (59.5 [IQR: 13.3]) ($p = 0.04$). Additionally, the mean value of DBP was higher in the NPDR group (83.4 ± 10.3) compared to the PDR group (79.2 ± 11.1) ($p = 0.02$) (Table 1).

3.2 | Association of *ALR2* genotypes with risk of PDR

The genotypes distribution of *ALR2* gene and its allele frequency showed no statistical differences among the two groups ($p > 0.05$) (Table 2). There was no evidence of an association of *ALR2* genotypes with risk of PDR under the three genetic models (codominant, dominant, and recessive) (Supporting Information: Table S1). The

comparisons of mean values of quantitative characteristics stratified by *ALR2* genotypes (CC vs. CT + TT) between NPDR and PDR patients are shown in Table 3. Among PDR group, the mean value of DBP (mean ± SD) was higher in CC carriers (82.9 ± 8.3) compared to (CT + TT) carriers (73.3 ± 12.5) ($p = 0.002$) (Table 3).

3.3 | Risk factors for development of PDR

Univariate logistic regression adjusted for age and gender revealed three major risk factors for PDR: low DBP, duration of diabetes, and history of dyslipidemia while smoking status was marginally significant ($p = 0.08$). Multiple logistic regression adjusted for age, gender, and smoking status revealed that the odds for patients with dyslipidemia to get PDR was 2.74 times higher than NPDR patients (95% CI: 1.08–6.98, $p = 0.03$) (Table 4). Moreover, the probability of a patient with ≥20 years of diabetes to develop PDR was seven times higher than other patients with a diabetes duration of 5–10 years (95% CI: 1.98–27.91, $p = 0.003$) (Table 4).

TABLE 1 Clinical characteristics of NPDR and PDR patients.

	NPDR ($n = 103$)	PDR ($n = 52$)	p Value
Age (years)	62 [11.5]	59.5 [13.3]	**0.04**
Duration of DM (years)	15 [10]	20 [9]	**<0.001**
SBP (mmHg)	145.8 ± 16.6	144.2 ± 16.1	0.57[a]
DBP (mmHg)	83.4 ± 10.3	79.2 ± 11.1	**0.02**[a]
BMI (kg/m^2)	29.4 [8.2]	28.1 [9.5]	0.92
HbA1c (%)	8 [3]	9 [2.2]	**0.04**
Females *n*, %	47–45.6	25–48.1	0.77
Males *n*, %	56–54.4	27–51.9	0.77
Family history of DM *n*, %	87–84.5	47–90.4	0.31
CVD *n*, %	38–36.9	24–46.2	0.27
Dyslipidemia *n*, %	66–64.1	39–75	0.17
Diabetic macular edema *n*, %	68–66	37–71.2	0.52
Current smokers *n*, %	14–13.6	14–26.9	**0.04**

Note: Continuous variables are expressed as median [interquartile range] or mean ± standard deviation. For categorical variables, Pearson's χ^2 test was used. Significant *p*-values are in bold.

Abbreviations: BMI, body mass index; CVD, cardiovascular diseases; DBP, diastolic blood pressure; DM, diabetes mellitus; HbA1c, hemoglobin A1c; NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; SBP, systolic blood pressure.

[a]*p* Values were obtained by the Wilcoxon rank-sum test or independent sample *t*-test.

4 | DISCUSSION

The risk of DR is mainly attributed to poor glycemic control and diabetes duration. A recent cross-sectional study conducted on the northern provinces of Palestine revealed that the Prevalence of DR was 30% with 12.2% proliferative DR. The same study reported that hypertension, uncontrolled T2DM, and duration of T2DM were the major risk factors for developing DR and the duration of diabetes was a strong predictor to PDR.[19] Our study showed that the probability of a patient with ≥20 years of diabetes to develop PDR was seven times higher than other patients with a diabetes duration of 5-10 years. The glycemic control as indicated by the median of the HbA1c level was also associated with PDR. Moreover, our results demonstrated that patients with dyslipidemia had a 2.74 times higher likelihood of developing PDR compared to patients with NPDR. A study conducted on mice model revealed that oxidative stress from sustained dyslipidemia lead to retinal dysfunction.[20] Several studies have also demonstrated that individuals with metabolic syndrome are more likely to develop retinopathy in the absence of diabetes.[21,22] However, identification of genetic risk factors of DR may help in understanding the complex complication of T2DM. It was reported that C106T polymorphism in the promoter region of *ALR2* gene doubled the gene transcription activity and thus involved in the pathogenesis of diabetic microvascular complications.[23] On the other hand, overexpression of *ALR2* within the conditions of hyperglycemia and insulin deficiency make the lens more susceptible to disruptions in signaling through the extracellular signal-regulated kinase (ERK) and c-Jun N-terminal (JNK) pathways, ultimately affecting the balance between cell growth and apoptosis which is crucial for lens transparency and homeostasis.[24]

TABLE 2 Distribution of (−106)C/T (rs759853) genotypes and allele frequencies among NPDR and PDR patients.

Genotype/allele	NPDR	PDR	OR (95% CI)	p Value
CC	52 (50.5%)	32 (61.5%)	Ref	-
CT	38 (36.9%)	13 (25%)	0.56 (0.26–1.2)	0.13
TT	13 (12.6%)	7 (13.5%)	0.88 (0.32–2.42)	0.80
C	146 (69.5%)	77 (74%)	Ref	-
T	64 (30.5%)	27 (26%)	0.8 (0.47–1.36)	0.41

Note: Odds ratios and *p* values were obtained by logistic regression analysis.

Abbreviations: NPDR, nonproliferative diabetic retinopathy; OR, odds ratio; PDRP, proliferative diabetic retinopathy; ref, reference.

TABLE 3 Biochemical and anthropometrical parameters of study participants based on (−106) C/T (rs759853) genotypes.

	NPDR			PDR		
	CC (*n* = 52)	CT + TT (*n* = 51)	*p* Value	CC (*n* = 32)	CT + TT (*n* = 20)	*p* Value
Age (years)	63 [10]	60 [11.5]	0.23	59 [12.3]	59.5 [13.8]	0.55
Duration of DM (years)	15 [8]	15 [10.5]	0.31	20 [8]	20 [9.5]	0.34
SBP (mmHg)	145.1 ± 17.1	146.6 ± 16.3	0.64[a]	144.8 ± 16.8	143.3 ± 15.3	0.75[a]
DBP (mmHg)	82.5 ± 11	84.3 ± 9.5	0.37[a]	82.9 ± 8.3	73.3 ± 12.5	**0.002**[a]
BMI (kg/m^2)	30.9 [8]	28.4 [6.8]	0.19	28.4 [9.1]	27.8 [9.8]	0.87
HbA1c (%)	8 [2.3]	8 [3]	0.90	9 [2]	8.9 [2.4]	0.54
CVD *n*, %	34–65.4	32–62.7	0.78	24–75	15–75	>0.99
Dyslipidemia *n*, %	19–36.5	19–37.3	0.94	17–53.1	7–35	0.20

Note: Baseline characteristics stratified based on genotypes (CC vs. CT + TT). Continuous variables are expressed as median [interquartile range] or mean ± standard deviation. For categorical variables, Pearson's χ^2 test was used. Significant *p*-values are in bold.

Abbreviations: BMI, body mass index; CVD, cardiovascular diseases; DBP, diastolic blood pressure; DM, diabetes mellitus; HbA1c, hemoglobin A1c; NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; SBP, systolic blood pressure.

[a]*p* Values were obtained by the Wilcoxon rank-sum test or independent sample *t*-test.

TABLE 4 Univariate logistic regression analysis and multiple logistic regression adjusted for age, sex, and smoking.

Univariate logistic regression adjusted for age and sex		
	OR (95% CI)	*p* Value[a]
Duration of DM (years)	1.11 (1.05–1.17)	**<0.001**
5–10 (*n* = 22)	Ref	
10–20 (*n* = 70)	1.77 (0.49–6.43)	0.39
≥20 (*n* = 63)	7.42 (1.98–27.91)	**0.003**
SBP (mmHg)	1.00 (0.98–1.02)	0.94
DBP (mmHg)	0.96 (0.93–0.99)	**0.02**
BMI (Kg/m^2)	1.02 (0.96–1.08)	0.54
HbA1c (%)	1.12 (0.96–1.32)	0.16
Family history of DM (yes)	2.03 (0.68–6.05)	0.21
CVD (yes)	1.48 (0.74–2.96)	0.26
Dyslipidemia (yes)	2.52 (1.09–5.82)	**0.03**
Current smoker (yes)	2.17 (0.90–5.12)	0.08
Multiple logistic regression[b]		
Duration of DM (years)	1.11 (1.05–1.18)	**<0.001**
DBP (mmHg)	0.97 (0.93–1.003)	0.07
Dyslipidemia (yes)	2.74 (1.08–6.98)	**0.03**

Note: Significant *p*-values are in bold.

Abbreviations: BMI, body mass index; CVD, cardiovascular diseases; DBP, diastolic blood pressure; DM, diabetes mellitus; HbA1c, hemoglobin A1c; OR, odds ratio; ref, reference; SBP, systolic blood pressure.

[a]*p* Values were obtained by univariate logistic regression adjusted for age and sex.

[b]*p* Values adjusted for age, sex, and smoking.

In the present study, the genotypic and allelic distributions of *ALR2* C106T polymorphism in NPDR and PDR subjects were nonsignificant ($p > 0.05$). In line to our findings, a study conducted on Jordanian diabetic patients showed no correlation between CT/TT genotypes and the severity of DR.[2] Another study also revealed no association between *ALR2* C106T polymorphism and risk of DR in Pakistani T2DM pateints but showed association with risk of T2DM compared with healthy control group.[25] A recent meta-analysis study conducted in 2020 showed a significant association between the *ALR2* C106T polymorphism and DR susceptibility. In that study, subgroup analysis stratified by DM type and ethnicity showed increased susceptibility for DR in East Asian populations and Middle Eastern populations among T1DM not T2DM patients.[26]

Herein, comparison of clinical and biochemical data based on genotypes (CC vs. CT + TT) revealed no statistically significant differences between the two analyzed groups in the HbA1c levels, duration of diabetes, history of dyslipidemia, CVD, SBP, age, and BMI. Inconsistent to our findings, Sivenius and colleagues revealed that diabetic patients with the −106T allele were younger and had higher BMI than the subjects with the CC genotype.[27]

Furthermore, we noted that CT + TT carries had lower DBP (73.3 ± 12.5) compared to CC carriers (82.9 ± 8.3) ($p = 0.002$). However, the mean value of DBP was lower in PDR group compared to NPDR group regardless of genotypes. Univariate logistic regression adjusted for age and sex showed that the patients with PDR were more likely to have low DBP. When multiple logistic regression adjusted for age, sex, and smoking was used, the duration of diabetes and dyslipidemia remained significant risk factors for PDR but not the DBP values. A prospective cohort study conducted on type 1 diabetic patients reported that DBP ≤ 83 mmHg along with waist to hip ratio and age at diagnosis were all strong predictors for progression to

PDR.[28] However, we believe that our findings on DBP could be affected by the use of specific classes of antihypertensive medications. It was reported that the use of multiple antihypertensive therapy was associated with lowering of DBP more than SBP.[29] A population-based study on Chinese, Malay, and Indian diabetic patients showed no association between DBP and DR severity.[30] In contrast, a 6-years follow-up study conducted in a cohort of 544 patients with T2DM, revealed that DBP was a significant predictor for progression of DR.[31] These conflicting results revealed by different genetic studies with different ethnic background reflect the complexity of risk factors associated with severity of DR.

Some limitations in this study should be noted; first, the lack of information on the prevalence of hypertension among our participants and on the dosage and class of the used antihypertensive drug that might affect our findings on DBP. Second, the relatively small sample size and thus the stratification of data based on grades of DR severity were not applicable. Moreover, the functional role of the C106T polymorphism on the expression of aldose reductase gene and on the enzyme activity has not been investigated which is, however, beyond the scope of this study. A previous study reported that the mean activity of AR enzyme is significantly higher in T2DM patients compared to control group which is correlated with cataract and DR.[32]

In conclusion, this is the first study conducted among Palestinian diabetic patients investigating the relationship between *ALR2* C106T polymorphism and severity of DR, along with related risk factors. The study compared NPDR patients to those with PDR, the findings revealed that diabetes duration and dyslipidemia are strong risk factors for PDR in Palestinian T2DM patients. The polymorphism C-106T of *ARL2* gene was not associated with risk of PDR progression. Prospective and long-term follow-up studies with larger sample size are needed to verify our findings.

AUTHOR CONTRIBUTIONS

Suheir Ereqat: Conceptualization; data curation; project administration; supervision; writing—original draft. **Mohammad Abdelhafez**: Formal analysis; methodology; validation; writing—review and editing. **Salam Iriqat**: Investigation; resources; validation; writing—review and editing. **Qusai Ghaleb**: Investigation; validation; writing—review and editing. **Amjaad Abu Shams**: Investigation; validation; writing—review and editing. **Omar Abd Aldayem**: Investigation; validation; writing—review and editing. **Manal Ghattas**: Methodology; writing—review and editing. **Abdelmajeed Nasereddin**: Conceptualization; data curation; supervision; writing—review and editing. All authors have read and approved the final version of the manuscript.

ACKNOWLEDGMENTS

We are grateful to the staff at St John Eye Hospital-East Jerusalem who participated in data collection. We also thank all study participants. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, S. E., upon reasonable request. S. E., S. I. and A. N. had full access to all of the data in this study and take complete responsibility for the integrity of the data and the accuracy of the data analysis.

TRANSPARENCY STATEMENT

The lead author Suheir Ereqat affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Ereqat S, Abdelhafez M, Iriqat S, et al. Aldose reductase (−106) C/T gene polymorphism and associated risk factors with proliferative diabetic retinopathy in Palestine: a cross sectional study. *Health Sci Rep*. 2023;6:e1605. doi:10.1002/hsr2.1605