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ORIGINAL ARTICLE

Retinal main vessel calibers and systemic markers for long-term development of proliferative diabetic retinopathy

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Abstract

Purpose: To evaluate if retinal vascular calibers and systemic risk factors in patients with no or minimal diabetic retinopathy (DR) can predict risk of long-term progression to proliferative diabetic retinopathy (PDR).

Methods: This was a matched case–control study of patients with diabetes having no or minimal DR at baseline with (cases) or without (controls) subsequent development of PDR. We collected six-field, 45-degree retinal images, demographic and clinical data from the Funen Diabetes Database.

Results: We included 52 eyes from 39 cases and 107 eyes from 89 controls matched on sex, age, type of diabetes, time from first to last screening episode and baseline DR level. Cases had higher HbA1c (73 vs. 55 mmoL/moL; $p<0.001$), triglycerides (1.32 vs. 1.16 mmoL/L; $p=0.02$) and longer duration of diabetes (19 vs. 14 years; $p=0.01$), but the groups did not differ in calibers of retinal arterioles (229 vs. 227 μm; $p=0.49$), venules (289 vs. 290 μm; $p=0.83$) or the arterio-to-venule ratio (0.78 vs. 0.77; $p=0.86$).In a multivariable logistic regression model with cluster robust standard error, HbA1c (OR 1.54 per 10 mmoL/moL; 95%-CI: 1.15–2.07; $p=0.004$), triglyceride (OR 1.39 per 1 mmoL/L; 95%-CI: 1.03–1.86; $p=0.03$) and duration of diabetes (OR 1.09 per year; 95%-CI: 1.03–1.16; $p=0.003$) were independent risk factors for PDR.

Conclusion: Retinal vascular calibers did not predict long-term development of PDR in contrast to well-established risk factors like HbA1c, triglyceride and duration of diabetes.

KEYWORDS
proliferative diabetic retinopathy, prediction, retinal vascular calibers, risk factor

1 | INTRODUCTION

Proliferative diabetic retinopathy (PDR) is a vision-threatening complication of diabetes and is among the leading causes of preventable blindness (Carstensen et al., 2020). Early identification of patients at risk of PDR would be important in order to address modifiable risk factors (Yau et al., 2012) such as haemoglobin-A1c (HbA1c), blood lipids and blood pressure. In the last two decades, structural analysis of the retinal vascularity (Cheung et al., 2015) has been suggested as new clinical predictive measures for incidence and progression of diabetic retinopathy (DR).

An objective measure of retinal vascular calibre has been developed along with improvements in imaging the retina allowing evaluation of vessel calibre in DR (Ikram et al., 2013) and other vascular diseases (Ikram, de Jong, Bos, et al., 2006; Ikram, de Jong, van Dijk, et al., 2006; Ikram, Witteman, et al., 2006; Wong et al., 2001). Retinal venular diameter has been shown to predict DR progression and incident PDR, while retinal arteriolar narrowing has been seen to predict DR onset, but not more advanced DR stages (Wong, 2011).

The purpose of this study was to investigate the association of retinal vascular calibers and systemic factors with long-term PDR development in a population with diabetes but no or minimal DR at baseline.

2 | METHODS

2.1 | Design

This was a matched case–control study that investigated retinal vascular calibers and systemic markers in eyes with no or minimal DR at baseline with (cases) or without (controls) subsequent development of PDR.

2.2 | Database

The Funen Diabetes Database (FDDB) is a regional database containing demographic and clinical data from the Danish DR screening program for patients with diabetes mellitus that resides on Funen in Denmark (Adelborg et al., 2020).

We collected essential demographic information such as age and sex as well as clinical data including International Clinical Diabetic Retinopathy (ICDR) level, type of diabetes, screening dates, body mass index, systolic and diastolic blood pressure. Additionally, we obtained blood test results for low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride, serum-creatinine and HbA1c as well as medication prescriptions for insulin.

2.3 | Study population

We included patients of all ages with type 1 or 2 diabetes who attended the Danish DR screening program from 2003 to 2019 (Larsen et al., 2017).

The cases had to be registered in the FDDB with one or two eyes having ICDR-level 0 (No DR) or 1 (microaneurysms and/or dot haemorrhages only) and develop PDR during the observation period from 2003 to 2019. When these criteria were fulfilled for one or both eyes, the local image database was retrospectively checked for availability of the initial retinal images with ICDR-level 0 or 1 and the agreement between the screening dates/grades in the image database and FDDB was ascertained. The patients were excluded when initial fundus images were unavailable (Figure 1: flowchart).

The first screening episode registered served as baseline for the study population from which we matched cases with controls (Figure 2). Case patients were individually matched to control patients with the same baseline level of DR, who did not progress beyond ICDR-level 0 (baseline DR-level 0) or ICDR-level 1 (baseline DR-level 1). Further matching included age, sex, type of diabetes and time from first to last screening (observation time). The study was designed for each case to be matched with three controls, but we only managed to match 11 of the case patients to a third suitable control patient that fulfilled the criteria for matching.

2.4 | Assessment of DR

All mydriatic digital fundus photos were obtained as six field, 45-degree images with Topcon TRC-50X (Topcon).

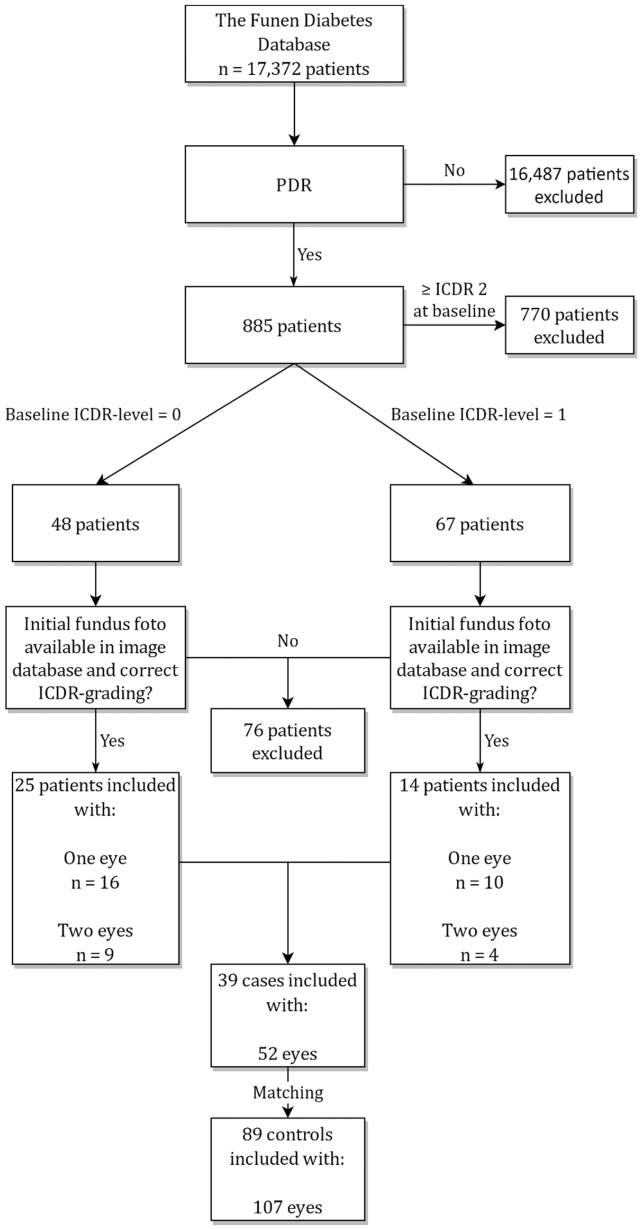

FIGURE 1 Flowchart for identification of case patients from the Funen Diabetes Database. ICDR, International Clinical Diabetic Retinopathy scale; PDR, proliferative diabetic retinopathy.

All fundus images were re-assessed for correct ICDR-grading by a certified grader according to the ICDR guidelines (Wilkinson et al., 2003) before enrolment in the study.

2.5 | Retinal vascular calibre analysis

The semi-automatic software VAMPIRE (version 3.2; Vessel Assessment and Measurement Platform for Images of the Retina; The Vampire Group; Edinburgh United Kingdom) was used to evaluate the retinal vascular calibers according to the VAMPIRE grading protocol. The method is described in details elsewhere (Emanuele Trucco et al., 2015; Perez-Rovira et al., 2011) but will be briefly outlined below.

The software automatically detected retinal vessels and landmarks including the optic disc and the macula, which enabled the creation of a grid around the margin

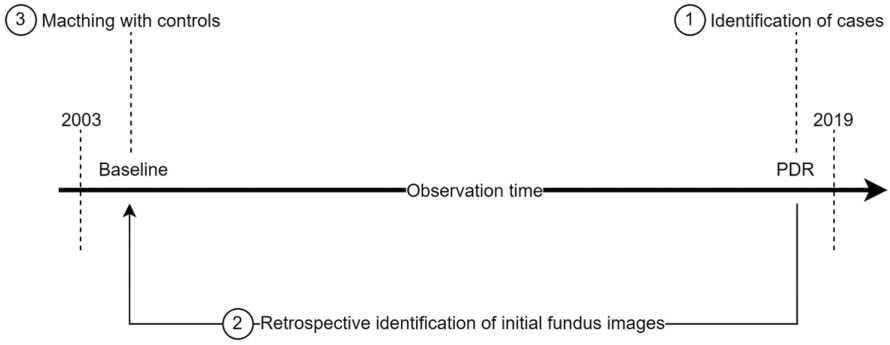

FIGURE 2 A timeline displaying the formation of the study population starting with (1) identification of patients with proliferative diabetic retinopathy in the Funen Diabetes Database from year 2003 to 2019 followed (2) by retrospective identification of initial fundus images and finally (3) matching of cases to controls with the first screening episode as baseline. PDR, proliferative diabetic retinopathy.

TABLE 1 Clinical and demographic differences at the first screening episode for 39 case patients with subsequent progression PDR and 89 control patients with no or minimal (ICDR-level 1) progression in diabetic retinopathy. The patients in both groups had ICDR-level 0 or 1 at baseline.

	Cases	Controls	Total	*p*-Value
Individuals, *n*	39	89	128	
Eyes, *n*	52	107	159	
Baseline DR-level, *n* (%) DR-level 0	33 (63.5%)	63 (59.9%)	96 (60.4%)	
Demographics				
Sex, *n* (%) female	21 (53.8%)	47 (52.2%)	68 (52.7%)	0.87
Age, years (IQR)	42.0 (24.0–60.0)	39.5 (25.0–60.0)	40.0 (25.0–60.0)	0.93
Diabetes type				
1	25 (64.1%)	57 (63.3%)	82 (63.6%)	0.93
2	14 (35.9%)	33 (36.7%)	47 (36.4%)	
Observation time, years (IQR)	10.8 (6.5–11.8)	10.4 (7.2–11.6)	10.5 (7.0–11.7)	0.81
Diabetes duration, years (IQR)	19.0 (12.0–29.0)	14.0 (10.0–21.0)	6.0 (2.0–13.0)	0.01
Para clinical measures				
Body Mass Index, kg/m^2	28.8 (23.9–33.8)	26.7 (24.2–30.1)	27.7 (24.0–31.4)	0.13
Systolic blood pressure, mmHg (IQR)	130 (120–140)	130 (118–137)	130 (120–140)	0.13
Diastolic blood pressure, mmHg (IQR)	80 (74–85)	75 (70–80)	77 (70–82)	0.20
Blood samples				
HbA1c, mmoL/moL (IQR)	73 (60–90)	55 (46–65)	57 (50–75)	<0.001
Serum-creatinine, μmoL/L (IQR)	83.0 (76.0–92.0)	78.5 (66.0–91.0)	80.0 (68.5–91.5)	0.16
LDL, mmoL/L (IQR)	2.45 (2.10–2.90)	2.36 (1.90–2.80)	2.40 (1.90–2.80)	0.73
HDL, mmoL/L (IQR)	1.49 (1.13–2.00)	1.35 (1.08–1.87)	1.39 (1.09–1.88)	0.30
Triglyceride, mmoL/L (IQR)	1.32 (1.03–2.40)	1.16 (0.76–1.69)	1.21 (0.90–1.93)	0.02
Medications				
Insulin, *n* (%) yes	34 (87.2%)	70 (77.8%)	104 (80.6%)	0.21
Retinal geometry				
CRAE, μm (IQR)	229 (211–250)	227 (210–247)	228 (211–248)	0.49
CRVE, μm (IQR)	289 (272–318)	290 (268–312)	290 (270–315)	0.83
AVR	0.78 (0.74–0.85)	0.77 (0.72–0.85)	0.77 (0.72–0.85)	0.86

Note: Continuous variables are presented as medians with interquartile ranges. Categorical variables are presented as numbers with percentages. Case and control differences are calculated at the individual level except for CRAE, CRVE and AVR that are calculated at eye level. Observation time was defined as time from first to last screening or until reach of PDR. Diabetes duration refers to duration of diabetes from debut to last screening episode.

Abbreviations: AVR, arteriole-to-venule ratio; CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; DR, diabetic retinopathy; HbA1c, glycosylated haemoglobin; HDL, high-density lipoprotein cholesterol; ICDR, International Clinical Diabetic Retinopathy scale; LDL, low-density lipoprotein cholesterol; PDR, proliferative diabetic retinopathy.

of the optic disc with circular zones A (0.0–0.5 disc diameters from the optic disc margin), B (0.5–1.0) and C (0.5–2.0). The automatic detection was not always on point and manual corrections were made. Retinal vessels were deleted when the vessel type was unclear, two vessels graded as one or if a choroid layer vessel was misinterpreted as a retinal vessel by the software.

The central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE) represents a summarization of the six largest arterioles and venules

coursing through zone B. These are automatically calculated in pixels by the VAMPIRE software and has previously been validated by other researchers (Lupascu et al., 2013; McGrory et al., 2018; Project, 2021). We applied conversion from pixels to absolute measurements in microns to ease the interpretation, in which we calculated the conversion factor based on the assumption of an average optic disc diameter of 1800 µm in adult humans (Jonas et al., 1988) and the mean optic disc diameter for all included eyes.

2.6 | Image quality

Every image was manually assessed for quality to exclude ungradable images. Image quality was finally assessed using a binary white and black vessel map as an expression of the software's availability to track the retinal vascularity. Eyes were excluded when the software was unable to track a single quadrant or the majority of the retinal vascularity. No images were excluded due to image quality, which can be related to the fact that patients had no to minimal (ICDR-level 1) when the fundus images were captured.

2.7 | Reliability of retinal vascular analysis

A random sample of 38 (25%) retinal images were included in an intergrader agreement analysis performed between two trained and independent VAMPIRE analysers. The consistency of agreement between the independent analysers was 90% and 94% for CRAE and CRVE, respectively.

2.8 | Statistics

Descriptive statistics are presented as medians with interquartile ranges (continuous data) and numbers with percentages (categorical data). We used the Mann–Whitney *U* test for continuous independent samples and the Chi-square test for categorical independent samples.

We constructed two logistic regression models to test for association between clinical variables (independent) and PDR (dependent): a uni- and a multivariable logistic regression model adjusted for age, sex, HbA1c, blood pressure, duration of diabetes, LDL and triglyceride. The models were performed as mixed-effects regressions with cluster robust standard error to account for the symmetry introduced given that some patients were included with two eyes. We accounted for clustering to address the potential non-independence of observations within each cluster.

Two-way mixed-effects model was used to estimate intraclass correlation coefficients for intergrader agreement on individual measurements for retinal vascular calibers.

Statistical significance was considered with *p*-values under 0.05 and when 1.0 was not included in the 95%-confidence intervals (CI). We performed statistical analyses using STATA17 (StataCorp).

2.9 | Permissions

Approval was given by The Danish Data Agency (journal number: 22/9016) and the study was performed according to the Declaration of Helsinki.

3 | RESULTS

Table 1 shows the baseline characteristics of the 128 individuals included in the study as 39 cases and 89 controls.

Cases had longer duration of diabetes (19 vs. 14 years; $p=0.01$) as well as higher levels of HbA1c (73 vs. 55 mmoL/moL; $p<0.001$) and triglyceride (1.32 vs. 1.16 mmoL/L; $p=0.02$) compared to controls. Cases and controls did not differ according to calibers of retinal arterioles (229 vs. 227 µm; p=0.49), venules (289 vs. 290 µm; $p=0.83$) or the arterio-to-venule ratio (0.78 vs. 0.77; $p=0.86$). Likewise, no differences were found in sex (53.8 vs. 52.2% female; $p=0.87$), age (median 42 vs. 40 years; $p=0.93$), rate of type 1 diabetes (64.1 vs. 63.3%; $p=0.93$), observation time (10.8 vs. 10.4 years; $p=0.81$), body mass index (28.8 vs 26.7 kg/m^2; $p=0.13$), serum-creatinine (83.0 vs. 78.5 µmoL/L; $p=0.16$), LDL (2.45 vs. 2.36 mmoL/L; $p=0.73$), HDL (1.49 vs. 1.35 mmoL/L; $p=0.30$), use of insulin (87.2 vs. 77.8%; $p=0.21$) as well as in systolic (130 vs. 130 mmHg; $p=0.13$) and diastolic blood pressure (80 vs. 75 mmHg; $p=0.20$) between the two groups.

Table 2 shows if independent parameters are associated with PDR in a uni- and a multivariable logistic regression model of 52 case eyes and 107 control eyes. Development of PDR was predicted by HbA1c (OR 1.54 per 10 mmoL/moL; 95%-CI: 1.15–2.07; $p=0.004$), triglyceride (OR 1.39 per 1 mmoL/L; 95%-CI: 1.03–1.86; $p=0.03$) and duration of diabetes (OR 1.09 per 1 year; 95%-CI: 1.03–1.16; $p=0.003$). No other clinical or demographic markers were predictive for upcoming PDR.

4 | DISCUSSION

In this case–control study of patients with no or minimal DR at baseline, long-term development of PDR was predicted by HbA1c, triglycerides, and duration of diabetes, but not by retinal vascular changes.

Previous studies reported that increased CRVE (Klein et al., 2004; Roy et al., 2011; Wong, 2011) predicts incident PDR, whereas CRAE (Cheung et al., 2008; Rogers et al., 2008; Wong, 2011) appears to predict incidence of more mild stages of DR in patients with type 1 diabetes. The findings in the current study did not support these previous findings for CRVE as an early predictor for PDR (64.1% had type 1 diabetes). We were unable to reliably comment on CRAE as a predictor for incident DR due to our selection criteria, in which some patients had mild DR at baseline.

While Klein et al. (Klein et al., 2004) found that larger CRVE was associated with increased 4-, 10- and 14-year PDR incidence in patients with type 1 diabetes, our data suggest otherwise. The difference is likely to be due to the fact that, our cohort consisted of patients with no to mild DR while Klein et al. included

TABLE 2 Uni- and multivariable logistic regression models with odds ratios for association between independent variables and dependent variable PDR. The models include 52 eyes from case patients with subsequent incidence of PDR and 107 eyes from control patients.

Characteristic	Increment	Univariable logistic regression odds ratios (95%-confidence interval)	*p*-Value	Multivariable logistic regression odds ratios (95%-confidence interval)	*p*-Value
Sex	Male vs. female	0.89 (0.40–1.98)	0.78	1.30 (0.45–3.73)	0.63
Age	10 years	0.97 (0.79–1.20)	0.80	0.77 (0.55–1.08)	0.13
Diabetes type	Type 1 vs. 2	1.09 (0.47–2.51)	0.20	1.84 (0.45–7.43)	0.40
Diabetes duration	1 year	1.06 (1.02–1.10)	0.001	1.09 (1.03–1.16)	0.003
Body mass index	1%-point	1.06 (0.99–1.14)	0.07	1.00 (0.89–1.13)	0.94
Systolic blood pressure	5 mmHg	1.09 (0.96–1.23)	0.17	1.18 (0.96–1.45)	0.11
Diastolic blood pressure	5 mmHg	1.20 (0.99–1.46)	0.06	1.00 (0.75–1.33)	0.98
HbA1c	10 mmoL/mol	1.51 (1.22–1.86)	<0.001	1.54 (1.15–2.07)	0.004
Serum-creatinine	1 μmoL/litre	1.01 (0.99–1.03)	0.25	1.02 (0.99–1.04)	0.19
LDL	1 mmoL/litre	1.24 (0.71–2.16)	0.45	1.06 (0.54–2.08)	0.86
HDL	1 mmoL/litre	1.57 (0.82–2.98)	0.17	1.59 (0.65–3.92)	0.31
Triglyceride	1 mmoL/litre	1.69 (1.17–2.44)	0.005	1.39 (1.03–1.86)	0.03
Insulin	Yes vs. no	2.06 (0.67–6.35)	0.21	1.48 (0.32–6.87)	0.62
CRAE	10 μm	0.99 (0.91–1.08)	0.82	1.03 (0.92–1.14)	0.64
CRVE	10 μm	0.99 (0.94–1.05)	0.91	1.01 (0.94–1.10)	0.76
AVR	1 SD	0.95 (0.68–1.33)	0.75	0.98 (0.66–1.46)	0.93

Note: The multivariable model was adjusted for sex, age, HbA1c, diastolic blood pressure, systolic blood pressure, triglyceride and duration of diabetes.
Abbreviations: AVR, arterio-to-venule ratio; CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; HbA1c, glycosylated haemoglobin; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; PDR, proliferative diabetic retinopathy; SD, standard deviation.

all patients free of PDR at baseline, meaning that we investigated our population at an earlier stage of DR. The onset of the pathophysiological mechanisms associated with changes in retinal vascular calibre may be related with higher levels of DR. This statement is consistent with CRVE becoming larger as the degree of DR increases reported by several studies (Cheung et al., 2010, 2012; Nguyen et al., 2008).

In a short-term study restricted to African Americans with type-1 diabetes, Roy et al. (Roy et al., 2011) found larger CRVE to be predictive for progression to PDR after 6 years of follow-up. Larger CRVE was found to be a more important PDR predictor in eyes with lower DR grades at baseline, but, again, the patients had higher DR grade than ours (ETDRS ≤53) at baseline (Roy et al., 2011).

In contrast to investigating progression to PDR from all baseline DR levels, Klein et al. and Roy et al. did also investigate the incidence of any DR in patients without DR at baseline, which showed no association with changes in CRAE or CRVE. This is closely comparable to our study suggesting that incident DR or PDR may be difficult to predict with CRVE long before onset of DR in patients with type 1 diabetes (Wong, 2011). In contrast to these findings of CRVE as a predictor for PDR, a long-term study from Denmark in young individuals with type 1 diabetes included with any DR at baseline (Rasmussen et al., 2017), showed that narrower CRAE was associated with 16-year progression to PDR. A pilot study of patients with type 1 and 2 diabetes having minimal DR at baseline (microaneurysms or mild haemorrhages only), found no difference in retinal arteriolar or venular diameter after 4 days of follow-up. This short timeframe is most likely inadequate to develop measurable changes in vessel diameter as a result of vascular endothelial dysfunction (Mlcak et al., 2022).

Only a few studies examined patients with type 2 diabetes exclusively (Cheung et al., 2017; Crosby-Nwaobi et al., 2012). A short-term prospective study by Cheung et al. (Cheung et al., 2017) and a study of 30 eyes by Nwaobi et al. (Crosby-Nwaobi et al., 2012) both reported wider CRAE as predictive for incident PDR while Nwaobi et al. also found that wider CRVE was predictive for PDR in patients with type 2 diabetes who were investigated before onset of any DR.

In comparison to these studies in patients with type 2 diabetes, larger CRAE was associated with progression in DR by Klein et al. (Klein et al., 2004) while narrower CRAE was associated with PDR reported by Broe et al. in type 1 diabetes, which emphasizes that the scientific field maintains a large discrepancy for inexplicable reasons limiting the comparability. These differences include variations in sample size, age, type of diabetes, DR grade at baseline, follow-up periods and potentially in the selection of which semi-automatic computer-assisted software programs was used.

The clinical implementation of CRAE and CRVE as predictors for DR and PDR has so far been unsuccessful due to several challenges. The variety of available semi-automatic software for retinal vascular analysis are time-consuming and, therefore, difficult to implement in a busy clinical setting. Secondly, the lack of clear scientific consensus as well as the small differences of the measured values between study groups makes the clinical interpretation difficult (Crosby-Nwaobi et al., 2012; Klein et al., 2004; Rasmussen et al., 2017; Roy et al., 2011).

We matched the two groups on age, sex, baseline DR-level, type of diabetes and time from first to last screening, which we consider a strength since these are considered risk factors for PDR (Dinesen et al., 2023; Sabanayagam et al., 2019; Wong et al., 2009).

Limitations for the study include that we were unable to find suitable control patients when matching for duration of diabetes leaving the case group with longer duration of diabetes.

In order to be able to match cases and controls in regards to comparability of disease duration and thus a more equal risk-time to develop PDR, we decided to match on time in the screening program as a surrogate measure for duration of diabetes, which is a well-known risk factor for PDR (Song et al., 2018; Yau et al., 2012). We were encouraged to be able to find suitable control patients with this matching criteria and was done to minimize the risk of selecting incomparable controls, which is considered a major weakness in case–control studies.

Examination for signs of diabetic macular oedema (DME) from fundus images is standard of care in the Danish DR screening, but optical coherence tomography is not performed unless it is indicated. Therefore, DME is unfortunately poorly reported in the FDDB and thus we were not able to report the prevalence of DME as a sign of retinal endothelial vascular dysfunction.

We were furthermore unable to calibrate the retinal measurements for axial length, refractive error and corneal curvature as this information is not a part of the Danish DR screening program and is, therefore, absent in the FDDB. The arterio-to-venular ratio is, however, independent of the refraction problem, since the ratio between the two will always be constant regardless of the magnification (Liew et al., 2006).

The calibers of larger vessels in the retina were examined for alterations in relation to diabetes, but it would have been of high interest to study if alterations occur in the even smaller vessels of the retina at this early stage of diabetes. Unfortunately, we were not able to include data from optical coherence tomography angiography or fluorescein angiography due to the register-based nature of the study design that relied on data from the Danish screening program, which only includes six-field fundus imaging as standard examination. Future research should use newer methods, such as optical coherence tomography angiography, to investigate if early capillary dropout increases the risk of long-term progression to PDR.

5 | CONCLUSION

In a long-term study of patients at an early stage of diabetes with no or minimal DR, calibers of the retinal arterioles or venules were not able to predict the incidence of PDR in contrast to well-established risk factors like HbA1c, triglyceride and duration of diabetes.

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