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ARTICLE

Retinal vascular fractals predict long-term microvascular complications in type 1 diabetes mellitus: the Danish Cohort of Pediatric Diabetes 1987 (DCPD1987)

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Abstract
Aims/hypothesis Fractal analysis of the retinal vasculature provides a global measure of the complexity and density of retinal vessels summarised as a single variable: the fractal dimension. We investigated fractal dimensions as long-term predictors of microvasculopathy in type 1 diabetes.
Methods We included 180 patients with type 1 diabetes in a 16 year follow-up study. In baseline retinal photographs (from 1995), all vessels in a zone 0.5–2.0 disc diameters from the disc margin were traced using Singapore Institute Vessel Assessment-Fractal image analysis software. Artefacts were removed by a certified grader, and fractal dimensions were calculated using the box-counting method. At follow-up (in 2011), diabetic neuropathy, nephropathy and proliferative retinopathy were assessed and related to baseline fractal dimensions in multiple regressions adjusted for sex and baseline age, diabetes duration, HbA_{1c}, BP, BMI, vibration perception threshold, albuminuria, retinopathy and vessel diameters.
Results Mean baseline age and diabetes duration were 21.0 and 13.4 years, respectively, and of patients 50.0% were males. The mean fractal dimension was 1.3817. The 16 year incidences of neuropathy, nephropathy and proliferative retinopathy were 10.8%, 8.0% and 27.9%, respectively. Multiple regression analyses showed a lower fractal dimension to significantly predict incident neuropathy (OR 1.17 per 0.01 fractal dimension decrease [95% CI 1.01, 1.36]), nephropathy (OR 1.40 per 0.01 fractal dimension decrease [95% CI 1.10, 1.79]) and proliferative retinopathy (OR 1.22 per 0.01 fractal dimension decrease [95% CI 1.09, 1.37]).
Conclusions/interpretation The retinal vascular fractal dimension is a shared biomarker of diabetic microvasculopathy, thus indicating a possible common pathogenic pathway. Retinal fractal analysis therefore is a potential tool for risk stratification in type 1 diabetes.

Keywords Biomarkers · Childhood diabetes · Complications · Type 1 diabetes mellitus

Abbreviations

ACR	Albumin:creatinine ratio
AER	Albumin excretion rate
CRAE	Central retinal arteriolar equivalent
CRVE	Central retinal venular equivalent
DN	Diabetic nephropathy
DPN	Diabetic peripheral neuropathy
DR	Diabetic retinopathy
ETDRS	Early Treatment Diabetic Retinopathy Study
PDR	Proliferative diabetic retinopathy
VPT	Vibration perception threshold

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Introduction

Microvascular complications remain common in type 1 diabetes [1, 2]. Identifying early biomarkers of diabetic peripheral neuropathy (DPN), diabetic nephropathy (DN) and diabetic retinopathy (DR) will allow the earlier implementation of treatment and support the development of new treatment modalities.

Measurements of vessel calibres in retinal photos could potentially deliver such biomarkers [3–7], but more global variables are available, for example retinal vascular fractal dimensions. The fractal dimension summarises the complexity and density of branching structures characterised by self-similarity and a similar level of complexity despite changes in magnification (for example snowflakes, tree branches, lightning). Correspondingly, the retinal vascular tree has features of a fractal [8]. The fractal dimension increases with increasing structural complexity.

Few studies on retinal vascular fractal dimensions and microvascular complications have been performed. Higher fractal dimensions have been linked to early signs of DR [9] and proliferative diabetic retinopathy (PDR) [10]. However, one study reported lower fractal dimensions in patients with PDR after pan-retinal photocoagulation and in patients with DPN, while a trend towards associations with DN was seen [11]. The latter observation is supported by a study examining the likelihood of microalbuminuria in DN [12]. All studies were of cross-sectional design.

Only a single prospective study exists. This study examined retinal vascular fractal dimensions and incident DR [13]. However, the follow-up period was short (mean 2.9 years) and incident cases had mild non-proliferative DR. Thus, the predictive value of fractal dimensions on severe DR remains unknown.

We carried out a prospective study to examine whether retinal vascular fractal dimensions can predict long-term microvascular complications in type 1 diabetes. We aimed to investigate the predictive value of retinal vascular fractal dimensions on the 16 year incidence of DPN, DN and PDR in a young population-based Danish cohort.

Methods

Study population This study formed part of a paediatric cohort study of Danish children with type 1 diabetes, initiated in 1987–1989 (n=720): the Danish Cohort of Pediatric Diabetes 1987. Clinical characteristics of this cohort have been reported elsewhere [14–17]. Of this cohort, 339 participants were included in a baseline examination in 1995, where they also had fundus photographs taken. These 339 participants were thus eligible for 16 year follow-up in 2011 and for fractal analysis. Of these, 15 (4.4%) were excluded from follow-up because of missing baseline retinal photographs, 13 (3.8%) had died and 63 (18.6%) were unreachable because they had emigrated or could not be contacted owing to research protection.

Of the remaining 248 patients, 185 (74.6%) participated in follow-up in 2011 and 63 (25.4%) declined to take part. A dropout analysis of the reduction in the cohort from 1995 to 2011 was described previously [14]. In brief, the 185 participants at follow-up had significantly lower levels of HbA_{1c}, higher values of vibration perception threshold (VPT) and were older than the ones who dropped out. The groups did not differ in terms of sex, age at onset, diabetes duration, BP, BMI, presence of albuminuria, or level of DR at baseline.

The study was performed in accordance with the criteria of the Helsinki II Declaration and was approved by the local scientific ethics committee. All patients gave written informed consent at both baseline and follow-up examinations.

Baseline examinations and measurements of the retinal vascular fractal dimension In 1995, participants underwent an interview in which information on sex, age and diabetes duration was obtained. Further, data on BP, BMI, HbA_{1c}, mean albumin excretion rate (AER) and VPT were assessed using previously described methods [15]. Baseline micro- and macroalbuminuria were defined as mean AERs of 20–200 μg/min and >200 μg/min, respectively, in at least two timed overnight urine samples. After pupillary dilation, colour retinal photographs were taken with 40°–60° retinal cameras, in accordance with European Diabetes Study Group (EURODIAB) recommendations [18]. Film slides were later digitalised using a DigitDia 5000 Filmscanner (Reflecta, Rottenburg, Germany). The disc–nasal field of the right eye was used for analyses of retinal vascular fractal dimensions. If the image of the right eye was ungradable, then the left eye was used for the analyses.

For the assessment of baseline DR and to rule out PDR, all photos were graded by the same certified grader using a modified Early Treatment of Diabetic Retinopathy Study (ETDRS) protocol and allowing for non-standard photography in 1995, as previously described [14].

A semiautomated computer program was used to assess the retinal vascular fractal dimension (Singapore Institute Vessel Assessment-Fractal version 1.0, School of Computing, National University of Singapore, Singapore) by a standardised protocol. The optic disc was automatically detected by the software, all vessels were identified and those coursing through a zone 0.5–2.0 disc diameters from the disc margin were used for the analyses. The program provided skeletonised line tracing of the vasculature and, by comparing this to the original retinal colour image, any artefacts misidentified as vessels were removed by a certified grader. Examples of artefacts erroneously included in the line tracing are choroid vessels, pigment abnormalities and photograph halos. The retinal vascular fractal dimension was calculated

from the refined line tracing by the box-counting method of the program, an established method for structures that are not perfectly self-similar, such as the retinal vasculature [19–21]. Figure 1 shows examples of retinal colour images and the corresponding refined line tracing for two of the participants in this study. The average time used for fractal analysis of each image in this study was 10 min.

The image used for fractal analysis was further analysed using different image analysis software (IVAN, Department of Ophthalmology Visual Science, University of Wisconsin, Madison, WI, USA) to assess the retinal arteriolar and venular calibres. These were summarised using the 'Big-6 formula' into the central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE), respectively [22].

Outcome measures in 2011 Participants underwent examinations between 1 January and 1 November 2011. DPN was assessed with a hand-held biothesiometer (Bio-Medical Instrument, Newbury, OH, USA). Three measurements on the apex of the right first toe were made and the VPT was calculated as the mean of the last two. Participants with a VPT of >25 V were classified as having DPN.

At follow-up, all participants were asked to hand in spot urine samples unless they were menstruating or had ongoing infections; in those circumstances, they were asked to hand in samples at a later time. A spot urine albumin:creatinine ratio (ACR) was calculated from albumin and creatinine analyses performed on an Abbott Architect analyzer (Abbott, Deerfield, IL, USA) using immunoturbidimetric and enzymatic assays, respectively. Participants with macroalbuminuria (ACR ≥300 mg/g), with a history of kidney transplantation or who had received dialysis were all classified as having DN.

Participants had mydriatic colour retinal photographs taken by the same trained and certified operator using a 3D OCT-2000 Spectral Domain optical coherence tomography system (Topcon, Tokyo, Japan). Seven 45° fields were taken for each eye, in accordance with ETDRS standards [23]. A modified ETDRS scale was used for assessment of PDR, as previously described [14]. Participants with ETDRS levels of 61 or above in the eye included in the fractal analyses were classified as having PDR.

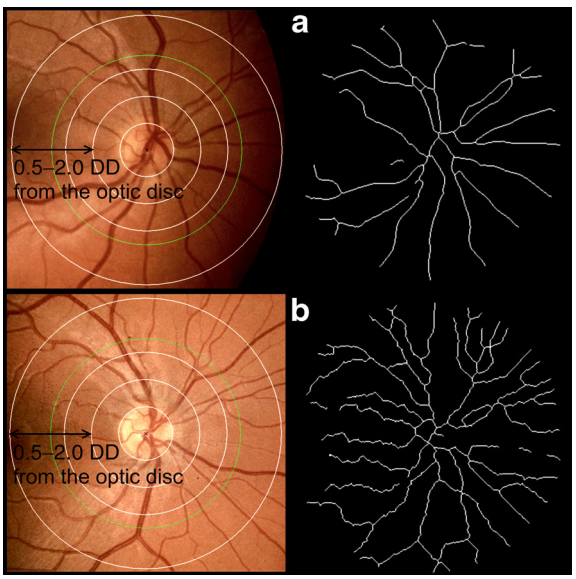

Fig. 1 Line tracings and zones. The retinal vascular fractal dimension is calculated from the line tracing in the marked zone 0.5–2.0 disc diameters (DD) from the disc margin; magnification ×1.84. The disc diameter is 1,800 μm in humans. (**a**) Cropped retinal image and line tracing of an eye in the tenth percentile with a fractal dimension of 1.3180 and a less complex vascular pattern. At follow-up, this participant had developed peripheral neuropathy, nephropathy and proliferative retinopathy. (**b**) Cropped retinal image and line tracing of an eye in the 90th percentile with a fractal dimension of 1.4678 and a more complex vascular pattern. At follow-up, this participant had neither peripheral neuropathy, nephropathy nor proliferative retinopathy

Statistical methods Categorical data are presented as percentages, and continuous data are presented as means ± SD. We used the Mann–Whitney *U* test to assess differences between two groups, Cuzick's test to identify trends among several groups and the Spearman rank correlation to test for associations between continuous variables. Multiple logistic regression analyses with backwards selection were performed to estimate ORs for incident DPN, DN and PDR. Models were adjusted for sex and baseline age, diabetes duration, HbA_{1c}, systolic and diastolic BP, BMI, VPT, mean AER, level of retinopathy, CRAE, and CRVE.

All participants with a VPT of <25 V at baseline were considered at risk of incident DPN, and all participants without PDR at baseline were considered at risk of incident PDR. Patients with a baseline mean AER of >200 μg/min (indicating overt DN) were excluded from renal function analyses at follow-up.

For estimates of ORs, 95% CIs were considered statistically significant when they did not cross 1.0. Findings with a p value of <0.05 were considered statistically significant.

All statistical calculations were performed using Stata 11.1 (StataCorp, College Station, TX, USA).

Results

Study population characteristics Of the 185 participants, fractal analysis was not possible in either eye of five patients (2.7%) because more than 25% of the vessels were untraceable within the predefined grid, in accordance with the standardised protocol. No significant differences in baseline characteristics between participants with and without gradable photos were found with respect to sex, baseline age, diabetes duration, HbA_{1c}, BP, BMI, VPT, mean AER and level of

retinopathy (data not shown). None of the five participants with non-gradable baseline photos for retinal vascular fractals had developed DPN, DN or PDR at follow-up.

A total of 180 patients with at least one gradable photo were included in the remaining analyses. For those participants, mean age and diabetes duration at baseline were 21.0 and 13.4 years, respectively, and 50.0% were males. The mean retinal vascular fractal dimension was 1.3817 (range 1.1932–1.5164).

Relationship between retinal vascular fractal dimension and clinical baseline characteristics Table 1 shows that a lower retinal fractal dimension was significantly related to higher levels of both albuminuria and retinopathy at baseline, while there were no differences in fractal dimensions between males and females. When testing for rank correlations between retinal vascular fractal dimensions and the remaining baseline variables (i.e. age, diabetes duration, HbA_{1c}, systolic and diastolic BP, BMI, VPT, CRAE and CRVE), no significant associations were found (data not shown).

Associations between retinal vascular calibres and incident DPN DPN was evaluated at follow-up in 157 patients who had a VPT of <25 V at baseline in 1995 and gradable baseline photos. Of these, 10.8% (n=17) had developed DPN in the intervening 16 years.

As seen in Table 2, a lower retinal vascular fractal dimension was significantly associated with incident DPN (OR 1.17 per 0.01 decrease in fractal dimension [95% CI 1.01, 1.36]) in the full multiple logistic regression model adjusted for sex and baseline age, diabetes duration, HbA_{1c}, BP, BMI, VPT, mean AER, level of retinopathy, CRAE and CRVE. The same association was seen in models after adjusting for fewer baseline variables.

Table 1 Baseline characteristics (1995) in relation to the retinal vascular fractal dimension for 180 participants from the DCPD1987 study

Clinical characteristic	Retinal fractal dimension		
	n	Mean±SD	*p* value[a]
Sex			
Male	90	1.3807±0.061	0.90
Female	90	1.3828±0.049	
Albuminuria			
None	149	1.3866±0.052	<0.01
Micro	13	1.3473±0.059	
Macro	5	1.3277±0.096	
Retinopathy			
ETDRS 10	84	1.3913±0.052	<0.04
ETDRS 20	65	1.3790±0.055	
ETDRS 35	29	1.3691±0.063	
ETDRS 43	6	1.3399±0.044	
ETDRS 65	1	1.3693	

Data are means±SD

[a] Mann–Whitney *U* tests were used for comparison between two groups, and Cuzick's tests were used for comparison between several groups

Associations of retinal vascular calibres and DN A total of 163 participants with gradable baseline photos and no macroalbuminuria in 1995 handed in urine samples at follow-up. Of these, 8.0% (n=13) had developed macroalbuminuria by 2011, had had a kidney transplant or had received dialysis by the time of follow-up.

A lower fractal dimension was significantly associated with the 16 year incidence of DN in all multiple logistic regression analyses carried out for this outcome, as seen in Table 2 (OR 1.40 per 0.01 decrease in fractal dimension in the full model [95% CI 1.10, 1.79]).

Associations between retinal vascular calibres and incident PDR Of the 180 participants with gradable baseline photos, one had PDR in 1995 and was therefore excluded from this analysis. Of the 179 patients at risk, 27.9% (n=50) progressed to PDR on the eye chosen for vessel analyses during the 16 year period. As shown in Table 2, the full multiple logistic regression found lower retinal vascular fractal dimension to be significantly associated with the 16 year incidence of PDR (OR 1.22 per 0.01 decrease in fractal dimension [95% CI 1.09, 1.37]), when adjusting for sex and baseline age, diabetes duration, HbA_{1c}, BP, BMI, VPT, mean AER, level of retinopathy, CRAE and CRVE. The same association was seen in models that adjusted for fewer baseline variables.

Discussion

A lower retinal vascular fractal dimension was consistently associated with the 16 year incidence of DPN, DN and PDR in this prospective study of young Danish patients with type 1 diabetes. It was also associated with higher levels of albuminuria and retinopathy at baseline. These findings are consistent with existing cross-sectional studies [11, 12], but not with the single prospective report on incident retinopathy prediction [13]. However, for the latter study, the follow-up period was relatively short (mean 2.9 years) and all incident cases had mild retinopathy (ETDRS of ≤31). Therefore, no conclusions can be made on whether the retinal vascular fractal dimension can predict more severe levels of DR based on this study. Furthermore, the dynamic nature of DR creates misclassification issues because it is common for a patient to have minor signs of DR (ETDRS of ≤31) at one examination and none at the next, and vice versa.

The pathophysiological mechanisms underlying the findings in the present study are unclear, but endothelial dysfunction has previously been speculated to be the common link

Table 2 Associations between retinal vascular fractal dimensions and 16 year incidences of microvascular complications among 180 young patients with type 1 diabetes from the DCPD1987 study

Model	Change in fractal dimension	DPN			DN			PDR		
		OR	95% CI	*p* value[a]	OR	95% CI	*p* value[a]	OR	95% CI	*p* value[a]
1	−0.01	1.16	1.04, 1,29	<0.01	1.24	1.10, 1.39	<0.01	1.18	1.10, 1.27	<0.01
2	−0.01	1.19	1.06, 1.33	<0.01	1.27	1.11, 1.45	<0.01	1.21	1.11, 1.31	<0.01
3	−0.01	1.16	1.02, 1.32	<0.03	1.28	1.09, 1.50	<0.01	1.23	1.11, 1.35	<0.01
4	−0.01	1.18	1.02, 1.36	<0.03	1.23	1.05, 1.44	<0.02	1.24	1.11, 1.39	<0.01
5	−0.01	1.17	1.01, 1.36	<0.05	1.40	1.10, 1.79	<0.01	1.22	1.09, 1.37	<0.01

Model 1: univariate logistic regression, using only the retinal vascular fractal dimension as a predictor

Model 2: multiple logistic regressions, adjusted for sex and baseline (1995) age and duration

Model 3: same as Model 2, with additional adjustments for baseline (1995) HbA_{1c}, systolic and diastolic BP and BMI.

Model 4: model 3, with additional adjustments for baseline (1995) level of retinopathy, VPT and albuminuria

Model 5: model 4, with additional adjustments for CRAE and CRVE

[a] Calculated using logistic regression models

between microvascular complications in type 1 diabetes [24]. Several endothelium-generated vasoconstrictors are produced in abnormally high amounts in diabetes, the main one being endothelin-1 [25]. At the same time, impaired production of nitric oxide (a vasodilator) is observed in endothelial dysfunction [26]. Vasoconstriction may therefore contribute to the lower vessel density seen in our study. However, in early uncomplicated diabetes, both small and large blood vessels have been shown to dilate, not contract [27]. Nonetheless, dilation will increase blood flow and cause capillary hypertension, which will damage the endothelium over time. At this stage, impaired endothelium-dependent vasodilation can occur, thus reducing blood flow and leading to hypoxia. We therefore speculate that a lower fractal dimension of the retinal vasculature may be linked to vasoconstriction induced by endothelial dysfunction.

The autoregulatory response in the retinal vasculature is impaired in patients with diabetes and no retinopathy, and becomes progressively more impaired with increasing levels of retinopathy [28]. Thus, collateral formation in response to hypoxia may be altered, leading to a less complex vasculature than is normally required for optimal oxygenation. As the renal vasculature exhibits similar autoregulation, we speculate that this is likely to occur in the kidneys.

Genetics provides another perspective to these findings. It was previously suggested that subsets of patients with type 1 diabetes may have a genetic predisposition towards microvasculopathy because not all patients with high glucose levels develop complications [29]. Additionally, some patients develop complications despite having only slightly elevated glucose levels [30]. A less complex branching pattern of the microvasculature may provide a limited facility to form collaterals when metabolic demand exceeds the current oxygen supply, which will lead to hypoxia.

Studies have shown a reduction in the retinal fractal dimension with age [11, 31, 32]. If this represents a more general phenomenon for the systemic microvasculature, then some patients could be more prone to hypoxia-induced organ damage over time, simply as a result of suboptimal vascular geometry being already in place at diabetes onset.

In this study, a rather high incidence of PDR was seen. This was previously discussed in detail in another report [14] but, in brief, a high level of HbA_{1c} at baseline (mean HbA_{1c} 9.5%±1.6% [81±17 mmol/mol]) in 1995) was believed to be the main cause.

Major strengths of this study are the population-based design, long follow-up time and use of quantitative methods for assessing retinal vascular fractal dimensions by standardised protocols. Furthermore, it was possible to adjust for a broad spectrum of baseline variables, including vessel diameter and the level of microvascular complications. Few patients (n=5) had ungradable retinal photos and the remaining were all graded by the same certified and validated grader. Most participants had little or no vascular disease at baseline, which allowed a good estimate of the predictive value of the retinal fractal dimension to be made because progression to the end stage of any complication is associated with a major change in microvasculature.

A limitation is the relatively small number of participants and the low number of incident cases; nonetheless, our results were statistically significant. Intermediate measurements of the cohort would provide valuable information on the timeline of development of complications and correlating changes in retinal vasculature, but such data were not available.

In conclusion, our study has shown the retinal vascular fractal dimension to be a possible common preclinical biomarker of the three major complications seen in type 1 diabetes: DPN, DN and PDR. This indicates that diabetic microvascular

complications share a common pathogenic pathway. Fractal analysis is therefore a potentially useful non-invasive tool for early risk stratification in diabetes regarding any microvascular complication. Fractal analysis of the retinal vasculature has previously demonstrated a high level of reproducibility [33]; however, the time to process each image needs to be shortened before its clinical use can be considered.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

Contribution statement RB, JG, BSO and HBM contributed to the study concept and design. RB, MLR, UF-O, BSO and HBM contributed to data acquisition. Data analyses were performed by RB, who also wrote the initial draft of the paper. MLR, UF-O, BSO, HBM, TP and JG were involved in the interpretation of data and revised the paper critically for intellectual content. RB is the guarantor of this work and, as such, had full access to all study data and takes full responsibility for the integrity of the data, the accuracy of the data analysis, and for the decision to submit for publication. All authors approved the final version of the paper.

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