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Retinal Vascular Fractals Correlate With Early Neurodegeneration in Patients With Type 2 Diabetes Mellitus

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Purpose. To investigate the correlation between the retinal vascular fractal dimension (Fd) and neurodegenerative changes in patients with no or mild diabetic retinopathy (DR).

Methods. In this cross-sectional study we examined 103 patients with type 2 diabetes mellitus (T2DM) with no or mild DR. In a randomly selected eye of each patient, Fd was calculated using SIVA-Fractal, a specialized semiautomatic software. Retinal neurodegeneration was evaluated by Topcon 3D OCT-2000 spectral-domain optical coherence tomography (OCT) and by a RETI-scan multifocal ERG (mf-ERG) system in rings one to six. Level of DR was determined by a single trained grader in seven-field fundus photos according to the Early Treatment Diabetic Retinopathy Study (ETDRS) scale.

Results. Mean age and duration of T2DM were 62.3 and 11.6 years, respectively; 46.6% were men. Mean Fd was 1.413 (range, 1.278–1.509) and ETDRS levels were 10 (42.7%), 20 (35.0%), and 35 (22.3%), respectively. Fd correlated inversely with mf-ERG implicit time of ring one ($r = -0.25$, $P = 0.01$) and present diabetic neuropathy ($P = 0.02$), and positively with OCT ganglion cell layer (GCL) thickness ($r = 0.20$, $P = 0.04$). In a multivariable linear regression model, Fd was associated with mf-ERG implicit time of ring one (coefficient -0.0021/ms, $P = 0.040$) and the presence of diabetic neuropathy (coefficient -0.0209 for neuropathy present versus absent, $P = 0.041$).

Conclusions. In patients with T2DM and no or minimal DR, independent correlations were found between early vascular and neurogenic changes. Thus, retinal vascular fractal analysis might be considered as a tool to identify patients with early neurodegenerative retinal changes.

Keywords: diabetic retinopathy, neurodegeneration, retinal vascular fractal, retinal vasculature, image analysis

The prevalence of patients with type 2 diabetes mellitus (T2DM) is rising.[1] Diabetic retinopathy (DR) is a potentially sight-threatening complication. Identification of subtle changes in the early stages of DR is important to identify patients who are most likely to develop long-term proliferative DR (PDR) and diabetic macular edema.[2]

For many years DR has been described as a microvascular disease, but several studies now indicate evidence of a neurodegenerative factor with impact on the development of the disease.[3–5] Even in patients with no or minimal DR there is evidence of retinal neurodegeneration with glial activation and apoptosis in the ganglion cell layer (GCL).[6–8] In addition to the structural changes, there is also evidence of functional changes as measured by multifocal ERG (mf-ERG).[9] The structural composition of the neuroretina can be measured by the thickness of macular GCL by optical coherence tomography (OCT).[8] These advanced techniques allow the examination of subtle neurodegenerative changes and provide valuable knowledge about the pathophysiology in early DR.

The availability of computer-based software allows us to assess the geometric features of the retinal blood vessels. Retinal vascular fractal analysis quantifies the fractal pattern of the retinal vascular tree.[10] Fractal patterns, a common phenomenon in nature, are also seen in branching structures, such as frost crystals, tree branches, and lightning. Fractal structures are characterized by a self-similar pattern that is unaffected by difference in magnification. That is, under different magnification, a smaller part of the whole will have the same structure as the larger part.

Retinal vascular fractal analysis is a validated method to describe the density and complexity of the retinal vascular tree in one parameter: the retinal vascular fractal dimension (Fd).[11] Fd is defined as a noninteger unit between 1 and 2 that increases correspondingly to the density of the retinal vascular tree.

In previous studies, Fd was correlated to and predictive of microvascular complications in type 1 diabetes patients.[12,13] However, as far as we know, the relation between the Fd and

FIGURE 1. The fractal pattern of the retinal vascular tree and a disc-centered cropped Optos 200Tx image.

neurodegenerative changes has never been examined in diabetes.

The aim of the present study was to associate the Fd with retinal neurodegeneration and diabetic neuropathy in a cross-sectional study of patients with T2DM and no or minimal DR.

METHODS

Study Population

We examined 105 patients with T2DM between 1 April 2013 and 1 July 2014 at Odense University Hospital, Odense, Denmark. The patients were recruited from the local DR screening clinic. Only T2DM patients with no or minimal DR were invited. Further inclusion criteria were age between 45 and 75 years and duration of T2DM more than 5 years. Patients previously diagnosed with glaucoma were excluded, due to possible existing neurodegenerative changes.

The study was performed in accordance with the principles of the Helsinki II Declaration. Approval from the regional scientific ethical committees for Southern Denmark was obtained before patient examinations, and all patients gave written informed consent.

Study Examinations

The patients provided a full medical history, including information about age, sex, diabetes duration, and presence of complications, such as diabetic nephropathy and neuropathy. Diabetic neuropathy was defined by hypoesthesia in the peripheral part of the extremities as questioned by the examiner or as self-reported by the patient (for those already diagnosed). The HbA1c, triglyceride, cholesterol, creatinine, and glomerular filtration rate were measured from a blood sample obtained at the time of the ophthalmic examination. A urine sample was also retrieved so as to reveal any presence of albuminuria. Additionally, the albumin-creatinine ratio was calculated. Blood pressure was measured one time using an Omron M4 monitor (Omron Matsusaka Co., Matsusaka, Japan)

with the patient at rest in a seated position. Body mass index was calculated from measurement of height and weight (lightly clothed).

Retinal Analysis

Pupils were dilated with tropicamide (10 mg/mL) and phenylephrine (10%). A Topcon TRC-NW6S (Topcon, Tokyo, Japan) was used to acquire 30-degree seven-field color fundus photographs of both eyes according to the modified Early Treatment Diabetic Retinopathy Study (ETDRS) seven standard fields. Level of DR was determined by a single certified grader (UF) from the seven-field color fundus photographs using the ETDRS scale.[14]

Retinal vascular fractal analysis was evaluated from a disc-centered cropped Optos 200Tx image (Optos plc, Dunfermline, Scotland, UK) (Fig. 1). In a randomly selected eye of each patient, Fd was calculated by a specialized semiautomatic software: SIVA-Fractal (Singapore Institute Vessel Assessment-Fractal, version 1.0; School of Computing, National University of Singapore, Singapore).[15] The software automatically detected the optic disc and traced vessels in a zone 0.5 to 2.0 disc diameter from the disc margin (Fig. 2). A trained grader (KP) then compared the automated vessel tracing with the color fundus photographs and removed any artifacts traced as vessels. The box-counting method by SIVA-fractal was used to assess the Fd.[16–18] Fractal analysis is a validated and reliable method to describe the retinal vasculature and studies have previously shown excellent intra- and intergrader reliability.[11,15]

Topcon 3D OCT-2000 spectral-domain OCT (Topcon) was used to measure the thickness of the GCL in the macula region. The thickness of the retinal nerve fiber layer (RNFL) at the optic disc and the thickness of retina from the internal limiting membrane to the RPE in the macula region were also measured. Thicknesses of the different layers were measured automatically by the Topcon 3D OCT-software (Topcon OCT Viewer, Version 6.7; Topcon). Each scan was centered and without or less than 10% cropping. The image quality of each scan was as high as possible and the OCT B-scan equal color
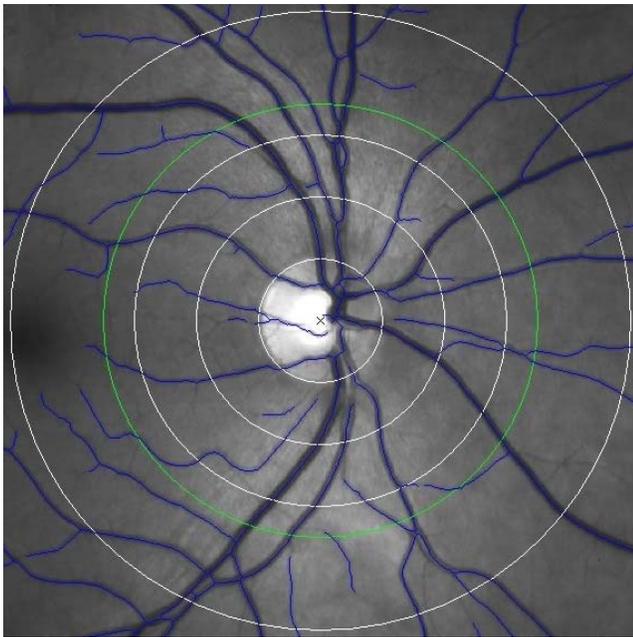
Figure 2. The SIVA-fractal analysis with an image of the grid.

density. The OCT en face image was well aligned to the underlying fundus image and without saccades through macula and optic nerve.

The RETI-scan mf-ERG system (Roland Consult; Brandenburg a.d., Havel, Germany) in rings one to six was used based on the International Society for Clinical Electrophysiology of Vision standard for clinical multifocal electroretinography (2011 edition).[19] The examined retinal area with the macula in the center corresponded to 46.4 degrees and was arranged in 103 hexagons. The mf-ERG amplitude and implicit time of rings one to six, with ring one being the central area, was calculated by the RETI-scan software.

Statistical Methods

Statistical analyses were performed with STATA 13 (StataCorp LP, College Station, TX, USA), and a *P* less than 0.05 was considered statistically significant. Categorical data are presented as percentages and continuous data are presented as
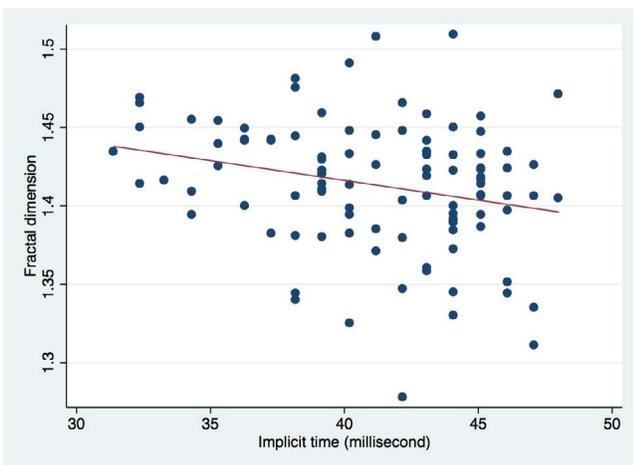

Figure 3. Correlation between implicit time of ring one and Fd among 103 patients with T2DM and minimal or no DR ($r = -0.25$, $P = 0.011$).
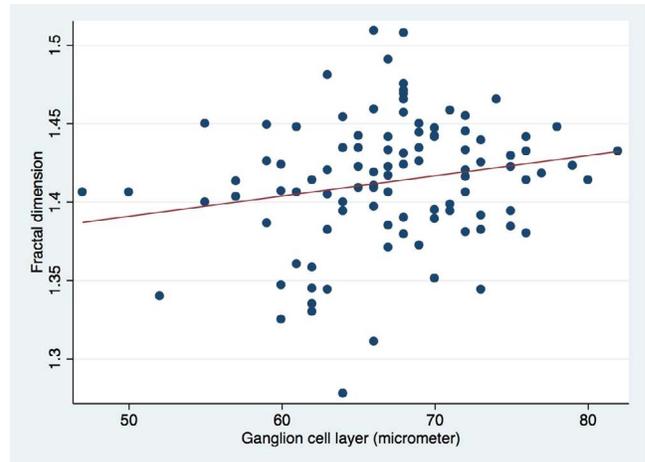

Figure 4. Correlation between GCL thickness in the macula and Fd among 103 patients with T2DM and minimal or no DR ($r = 0.20$, $P = 0.041$).

means (with range). To study possible associations between two continuous variables, Pearson's *r* correlation coefficient was used. Kruskal-Wallis equality of proportions rank test was used to test for differences in continuous data between groups. Associations from univariate analysis with *P* less than 0.05 were subsequently used in a multivariable linear regression model adjusted for potential confounders with Fd as the dependent variable. Multivariable linear regression model was used to identify significant independent factors with correlation to the Fd.

RESULTS

A total of 105 patients were examined. It was not possible to perform fractal analysis in one patient (due to asteroid hyalosis in both eyes), and one patient had missing data from the mf-ERG and OCT examination. For the remaining 103 patients, the mean age and duration of T2DM were 62.3 and 11.6 years, respectively; 46.6% were men. Mean Fd was 1.413 (range, 1.278–1.509), and ETDRS levels were 10/DR absent (42.7%), 20/microaneurysms only (35.0%), and 35/mild nonproliferative DR (22.3%), respectively (Table 1).

In univariate analysis, we found a negative correlation between Fd and mf-ERG implicit time of ring one ($r = -0.25$, $P = 0.01$) (Fig. 3, Table 2). Fd was positively correlated to the GCL thickness of the macula region ($r = 0.20$, $P = 0.04$) (Fig. 4), and a lower Fd was associated with the presence of diabetic neuropathy ($P = 0.02$) (Fig. 5). There were no statistically significant correlations between Fd and age, sex, duration of diabetes, blood pressure, body mass index, nephropathy, or any other OCT and mf-ERG parameters.

In a multivariable linear regression model adjusted for age, sex, diabetes duration, HbA1c, systolic and diastolic blood pressure, and presence of DR, Fd was associated inversely with mf-ERG implicit time of ring one (coefficient −0.0021 per millisecond increase of implicit time, $P = 0.040$) and diabetic neuropathy (coefficient −0.0209 for neuropathy being present versus absent, $P = 0.041$) (Table 2). In separate multivariable regression analysis in patients with no DR (n = 44) and in patients with mild DR (n = 59), respectively, there was no statistically significant correlation between Fd and any of the covariates tested in Table 2.

In clinical terms, each millisecond prolongation of the implicit time of ring one in mf-ERG was associated with a

TABLE 1. Clinical Characteristics of the Examined Patients With T2DM

Characteristic	Mean	Range
Age, y	62.3	47.9–70.3
Sex, % male	46.6	
Duration of diabetes, y	11.6	6–26
HbA1c, mmol/mol	54.3	36–99
Systolic blood pressure, mm Hg	134	98–177
Diastolic blood pressure, mm Hg	80	58–102
Body mass index, kg/m^2	31	22–53
Mf-ERG implicit time of ring one, ms	41.2	31.4–48
Thickness of GCL in macula, μm	67.2	47–82
DR present, %	57.3	
Diabetic neuropathy present, %	20	
Fd	1.413	1.278–1.509

FIGURE 5. Correlation between neuropathy being present or absent and Fd among 103 patients with T2DM and minimal or no DR ($P = 0.016$).

decrease in Fd of 0.0021 and the presence of diabetic neuropathy was correlated with a decrease in Fd of 0.0209.

In univariate analysis, mf-ERG amplitude of rings one to six was positively correlated to the GCL thickness of the macula region ($r = 0.21$–0.26, $P = 0.01$–0.03) and mf-ERG implicit time of ring three was positively correlated to the GCL thickness of the macula region ($r = 0.24$, $P = 0.01$) (data not shown). However, we found no statistically significant correlations between mf-ERG implicit time of the other rings and GCL thickness of the macula region.

DISCUSSION

In this study of T2DM patients with no or early DR, we found that the Fd correlated independently and inversely with the implicit time of ring one mf-ERG as well as the presence of diabetic neuropathy. Fd was associated with the thickness of the macular GCL in univariate analysis.

Previous studies that included patients with T2DM have investigated the correlation between Fd and vascular parameters, such as retinal blood flow[20] and the development of PDR.[21] Most clinical studies measuring Fd have included patients with type 1 diabetes mellitus,[12,13,22–24] patients with increased levels of HbA1c,[25,26] or examined data from patients with diabetes who developed DR over time.[27–29]

In a cross-sectional study, Grauslund et al.[12] found an independent correlation between lower Fd and the presence of inactive PDR as well as neuropathy. Broe et al.[13] expanded
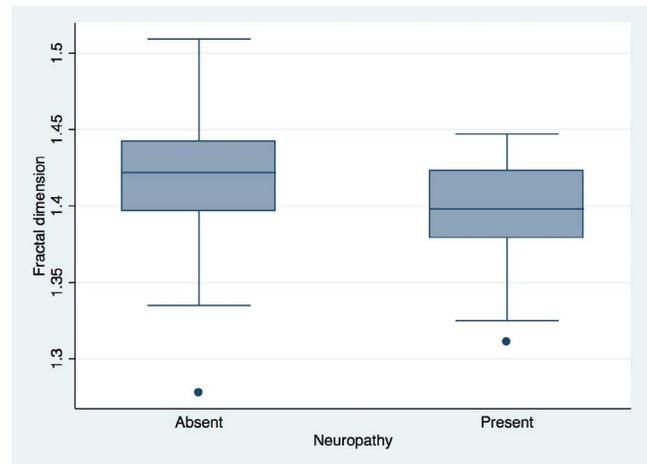
these findings in a 16-year prospective study in which lower Fd independently was able to predict all types of microvascular impairment (inactive PDR, nephropathy, and neuropathy). These findings support our results as we also found a significant correlation between a lower Fd and the presence of diabetic neuropathy.

In a population-based cross-sectional study of 352 patients, Tham et al.[30] examined Fd in relation to retinal neurodegenerative changes in nonglaucomatous eyes. Although the study population was not solely patients with diabetes mellitus, the results were partly consistent with our findings that decreased Fd was associated with a thinner peripapillary RNFL and macular ganglion cell–inner plexiform layer thickness after adjustment for age and sex. The later result corresponds to the finding in our study using univariate analysis. However, the association between Fd and GCL thickness was not significant when adjusted for several variables in the multivariable model ($P = 0.14$). In the present study, we did not find a correlation between Fd and RNFL ($P = 0.13$). Moreover, the statistically significant correlations between Fd and neurodegenerative parameters in multivariable linear regression model were not present in separate regression analysis that included only patients with/without DR. We suspect these findings were

TABLE 2. P Values From Univariate Analysis and Regression Coefficients Derived From Multivariable Linear Regression Model With Fd as the Dependent Variable

Parameter/Variable	Univariate Analysis	Multivariable Linear Regression	
	P	Coefficient (95% CI)	P
Age, per y	0.114	−0.0009 (−0.0024 to 0.0007)	0.271
Sex, male	0.414	0.0077 (−0.0088 to 0.0242)	0.358
Duration of diabetes, per y	0.881	−0.0001 (−0.0019 to 0.0018)	0.918
HbA1c, per mmol/mol	0.229	−0.0005 (−0.0011 to 0.0002)	0.166
Systolic blood pressure, per 10 mm Hg	0.884	0.0012 (−0.0048 to 0.0072)	0.690
Diastolic blood pressure, per 10 mm Hg	0.697	−0.00004 (−0.0111 to 0.0110)	0.995
Mf-ERG implicit time of ring one, per ms	0.011*	−0.0021 (−0.0040 to −0.0001)	0.040*
Thickness of GCL in macula, per μm	0.041*	0.0010 (−0.0003 to 0.0023)	0.136
DR, present	0.626	0.0011 (−0.0159 to 0.0180)	0.898
Diabetic neuropathy, present	0.016*	−0.0209 (−0.0410 to −0.0009)	0.041*
Constant		1.4976 (1.3241 to 1.6711)	

Model includes age, sex, duration of diabetes, HbA1c, blood pressure (systolic and diastolic), DR, mf-ERG implicit time of ring one, GCL thickness in the macula, and diabetic neuropathy.
* Statistically significant ($P < 0.05$).

caused by a small sample size in each group. Nevertheless, the significant findings support our hypothesis of retinal neurodegenerative changes being present despite no or minimal visible vascular morphological changes secondary to DR.

Diabetic peripheral neuropathy (DPN) and retinal neurodegenerative changes are different in an anatomical perspective: DPN is a disease of the peripheral nervous system and retinal ganglion cells form the second cranial nerve. Hence, retinal neurodegeneration is defined as a disease located to the central nervous system.[31] Given the fact that both diseases have an early onset in patients with diabetes, we speculate that similarities in the pathophysiological pathway might be found. One of them being a neurovascular coupling and the other that they both belong to microvascular complications of T2DM. We speculate that diabetic neuropathy develops over time by some of the same mechanisms as DR, partly driven by the glycemic load. However, the significant correlation between Fd and the neurodegenerative changes in our study remained consistent despite adjustments for HbA1c and duration of diabetes. Therefore, we hypothesize that other mechanisms might play a significant role as well.

Several studies have examined the association between mf-ERG and DR. To our knowledge, no other study has examined the association between Fd and mf-ERG implicit time in patients with T2DM. A few studies found evidence that mf-ERG implicit time can predict development of DR.[9,32] They found that areas with prolonged mf-ERG implicit time in eyes without DR can predict DR lesions. The finding corresponds with our result that retinal neurodegenerative changes (mf-ERG implicit time delays) are associated with subtle changes in the retinal vasculature; a decreased Fd.

To examine the relation between functional and structural neurodegenerative parameters, we correlated the mf-ERG parameters (functional marker) to GCL thickness of the macula region (structural marker). The mf-ERG amplitude of rings one to six was positively correlated to the GCL thickness of the macula region. This indicates that patients with a strong electrical signal were more likely to have increased GCL thickness. The correlation between mf-ERG amplitude and GCL thickness of the macula region in patients with T2DM, to our knowledge, is a novel finding.

Surprisingly, we also found a positive correlation between mf-ERG implicit time of ring three and GCL thickness. There were no correlations between the implicit time of the other rings and GCL thickness. Physiologically, we would have expected a negative correlation with prolonged implicit time associated with GCL atrophy. This indicates that the positive correlation between ring three implicit time and GCL thickness could be caused by mass significance due to the multiple comparisons tested.

There is a variance in quantitative measurements with spectral-domain OCT images that may affect the accuracy of GCL thickness measurement.[33] The cross-sectional design of this study prevents any prospective analysis of microvascular and neurodegenerative changes. Nonetheless, the clinical features of the T2DM patients (all with no or minimal DR) enabled the opportunity to examine and determine clinically subtle changes in early DR in an ethnically homogeneous Caucasian population.

In conclusion, Fd correlated significantly both with functional and structural neurodegenerative changes in the retina and was associated with the presence of diabetic neuropathy. We suggest that future prospective clinical studies examining novel treatment for arresting or preventing early DR include fractal analysis to identify subtle changes in the retinal vasculature.

The fractal pattern of the retinal vascular tree may give valuable information about the presence of neurodegenerative changes in patients with T2DM, even in patients with no or early stages of DR.

References

1. Ko F, Vitale S, Chou CF, Cotch MF, Saaddine J, Friedman DS. Prevalence of nonrefractive visual impairment in US adults and associated risk factors, 1999-2002 and 2005-2008. *JAMA*. 2012; 308:2361–2368.

2. Rasmussen ML, Broe R, Frydkjaer-Olsen U, et al. Microaneurysm count as a predictor of long-term progression in diabetic retinopathy in young patients with type 1 diabetes: the Danish Cohort of Pediatric Diabetes 1987 (DCPD1987). *Graefes Arch Clin Exp Ophthalmol*. 2015;253:199–205.

3. Simo R, Hernandez C. European Consortium for the Early Treatment of Diabetic Retinopathy. Neurodegeneration is an early event in diabetic retinopathy: therapeutic implications. *Br J Ophthalmol*. 2012;96:1285–1290.

4. Simo R, Hernandez C. European Consortium for the Early Treatment of Diabetic Retinopathy. Neurodegeneration in the diabetic eye: new insights and therapeutic perspectives. *Trends Endocrinol Metab*. 2014;25:23–33.

5. Abcouwer SF, Gardner TW. Diabetic retinopathy: loss of neuroretinal adaptation to the diabetic metabolic environment. *Ann N Y Acad Sci*. 2014;1311:174–190.

6. van Dijk HW, Verbraak FD, Kok PH, et al. Early neurodegeneration in the retina of type 2 diabetic patients. *Invest Ophthalmol Vis Sci*. 2012;53:2715–2719.

7. van Dijk HW, Verbraak FD, Kok PH, et al. Decreased retinal ganglion cell layer thickness in patients with type 1 diabetes. *Invest Ophthalmol Vis Sci*. 2010;51:3660–3665.

8. van Dijk HW, Verbraak FD, Stehouwer M, et al. Association of visual function and ganglion cell layer thickness in patients with diabetes mellitus type 1 and no or minimal diabetic retinopathy. *Vision Res*. 2011;51:224–228.

9. Ng JS, Bearse MA Jr, Schneck ME, Barez S, Adams AJ. Local diabetic retinopathy prediction by multifocal ERG delays over 3 years. *Invest Ophthalmol Vis Sci*. 2008;49:1622–1628.

10. Masters BR. Fractal analysis of the vascular tree in the human retina. *Annu Rev Biomed Eng*. 2004;6:427–452.

11. Cosatto VF, Liew G, Rochtchina E, et al. Retinal vascular fractal dimension measurement and its influence from imaging variation: results of two segmentation methods. *Curr Eye Res*. 2010;35:850–856.

12. Grauslund J, Green A, Kawasaki R, Hodgson L, Sjolie AK, Wong TY. Retinal vascular fractals and microvascular and macrovascular complications in type 1 diabetes. *Ophthalmology*. 2010;117:1400–1405.

13. Broe R, Rasmussen ML, Frydkjaer-Olsen U, et al. Retinal vascular fractals predict long-term microvascular complications in type 1 diabetes mellitus: the Danish Cohort of Pediatric Diabetes 1987 (DCPD1987). *Diabetologia*. 2014;57: 2215–2221.

14. Early Treatment Diabetic Retinopathy Study Research Group. Grading diabetic retinopathy from stereoscopic color fundus photographs—an extension of the modified Airlie House

classification. ETDRS report number 10. *Ophthalmology*. 1991;98:786–806.

15. Thomas GN, Ong SY, Tham YC, et al. Measurement of macular fractal dimension using a computer-assisted program. *Invest Ophthalmol Vis Sci*. 2014;55:2237–2243.

16. Mainster MA. The fractal properties of retinal vessels: embryological and clinical implications. *Eye (Lond)*. 1990;4: 235–241.

17. Stosic T, Stosic BD. Multifractal analysis of human retinal vessels. *IEEE Trans Med Imaging*. 2006;25:1101–1107.

18. Macgillivray TJ, Patton N, Doubal FN, Graham C, Wardlaw JM. Fractal analysis of the retinal vascular network in fundus images. *Conf Proc IEEE Eng Med Biol Soc*. 2007;2007:6456–6459.

19. Hood DC, Bach M, Brigell M, et al. ISCEV standard for clinical multifocal electroretinography (mfERG) (2011 edition). *Doc Ophthalmol*. 2012;124:1–13.

20. Nagaoka T, Yoshida A. Relationship between retinal fractal dimensions and retinal circulation in patients with type 2 diabetes mellitus. *Curr Eye Res*. 2013;38:1148–1152.

21. Crosby-Nwaobi R, Heng LZ, Sivaprasad S. Retinal vascular calibre, geometry and progression of diabetic retinopathy in type 2 diabetes mellitus. *Ophthalmologica*. 2012;228:84–92.

22. Nybo M, Hodgson LA, Kawasaki R, Wong TY, Grauslund J. Hemoglobin is associated with retinal vascular fractals in type 1 diabetes patients. *Ophthalmic Epidemiol*. 2014;21: 327–332.

23. Lim SW, Cheung N, Wang JJ, et al. Retinal vascular fractal dimension and risk of early diabetic retinopathy: a prospective study of children and adolescents with type 1 diabetes. *Diabetes Care*. 2009;32:2081–2083.

24. Cheung N, Donaghue KC, Liew G, et al. Quantitative assessment of early diabetic retinopathy using fractal analysis. *Diabetes Care*. 2009;32:106–110.

25. Zhu P, Huang F, Lin F, et al. The relationship of retinal vessel diameters and fractal dimensions with blood pressure and cardiovascular risk factors. *PLoS One*. 2014;9:e106551.

26. Cheung CY, Lamoureux E, Ikram MK, et al. Retinal vascular geometry in Asian persons with diabetes and retinopathy. *J Diabetes Sci Technol*. 2012;6:595–605.

27. Kunicki AC, Oliveira AJ, Mendonca MB, Barbosa CT, Nogueira RA. Can the fractal dimension be applied for the early diagnosis of non-proliferative diabetic retinopathy? *Braz J Med Biol Res*. 2009;42:930–934.

28. Lee J, Zee BC, Li Q. Detection of neovascularization based on fractal and texture analysis with interaction effects in diabetic retinopathy. *PLoS One*. 2013;8:e75699.

29. Daxer A. The fractal geometry of proliferative diabetic retinopathy: implications for the diagnosis and the process of retinal vasculogenesis. *Curr Eye Res*. 1993;12:1103–1109.

30. Tham YC, Cheng CY, Zheng Y, Aung T, Wong TY, Cheung CY. Relationship between retinal vascular geometry with retinal nerve fiber layer and ganglion cell-inner plexiform layer in nonglaucomatous eyes. *Invest Ophthalmol Vis Sci*. 2013;54: 7309–7316.

31. Fix JD. *Neuroanatomy. 4th ed*. Philadelphia, PA: Lippincott Williams & Wilkins; 2007:177.

32. Harrison WW, Bearse MA Jr, Ng JS, et al. Multifocal electroretinograms predict onset of diabetic retinopathy in adult patients with diabetes. *Invest Ophthalmol Vis Sci*. 2011; 52:772–777.

33. Pierro L, Gagliardi M, Iuliano L, Ambrosi A, Bandello F. Retinal nerve fiber layer thickness reproducibility using seven different OCT instruments. *Invest Ophthalmol Vis Sci*. 2012; 53:5912–5920.