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Comparing Objective Perimetry, Matrix Perimetry, and Regional Retinal Thickness in Mild Diabetic Macular Edema

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Purpose: To compare per-region macular sensitivity and delay from objective perimetry with Matrix perimetry and retinal thickness in mild diabetic macular edema (DMO).

Methods: Thirty-three patients with type 2 diabetes (T2D) aged 59.2 ± 10.5 years participated in a longitudinal study. Macular thickness, sensitivities and delays from the objectiveFIELD Analyzer (OFA), and Matrix perimeter sensitivities were mapped onto a common spatial layout to compute per-region correlations between structure/function measures. A generalized linear mixed-effects logistic regression model determined which variables contributed to clinical diagnosis of DMO.

Results: For OFA, the mean sensitivity differences compared with normal in patients with T2D were negative and the mean delay differences positive, indicating lowered sensitivities and prolonged delays, both increasing with diabetes duration. Shorter diabetes duration could produce either localized peripheral hypersensitivities or shorter delays. Functional change could occur when retinal thickness was stable. Peripheral macular thickness correlated with central and peripheral OFA sensitivity and delay, all $P < 0.0012$ in DMO and a median of $P = 0.001$ without DMO; this was not true for Matrix sensitivities. The logistic model determined that peripheral thickness, OFA sensitivity ($P = 0.043$), and time in the study ($P = 0.001$) contribute independently to the odds of DMO versus no DMO.

Conclusions: Mean sensitivities decreased and mean delays increased with duration of diabetes. Peripheral macular thickness correlated significantly with central and peripheral macular OFA sensitivity and delay. Peripheral macular thickness and functional measures may provide sensitive prognostic data.

Translational Relevance: Functional loss can precede structural change in DMO, so including such functional assessment for deciding on treatment may be beneficial.

Introduction

Globally, 285 million people had diabetes in 2010. Over one-third of patients with diabetes have some form of diabetic retinopathy (DR); a third of these are considered to have vision-threatening DR, either proliferative or severe nonproliferative, or diabetic macular edema (DMO).[1] In Australia alone, 72,000 of the 1.73 million patients with diabetes had DMO in 2015, predicted to increase by 42% by 2030.[2] The indirect cost of vision loss associated with DMO in 2015 alone was estimated to be $2.07 billion.[2] Despite improved diagnosis and management, diabetes remains an escalating global health challenge.[3]

The key factor in preventing vision-threatening diabetic eye disease is early diagnosis of DR and DMO and timely treatment.[4] Functional methods such as conventional perimetry and best-corrected visual acuity (BCVA) may not have the sensitivity to pick

up early pathology.[5] Microperimeters[6] and multifocal pupillographic objective perimetry (mfPOP),[7,8] as exemplified by the objectiveFIELD Analyzer (OFA), appear to identify early focal lesions. Reduced OFA central macular sensitivity, with some peripheral hypersensitivity, and asymmetry in local delay deviations between eyes correlate with the severity of type 2 diabetes (T2D).[7] Focal OFA visual field defects have been reported in patients with early T2D with no DR,[9] and defects measured by Matrix perimetry also precede diabetic structural change.[10,11] Interestingly, BCVA was more strongly predicted by off-axis retinal thickness than central thickness in one of those studies.[10] Peripheral macular thickness has also been reported to be a more useful disease indicator than central thickness in advanced age-related macular degeneration (AMD).[5] Taken together, these data might suggest that less-affected retina around developing central lesions may provide better prognostic information than more damaged central macula itself.

The present study was conducted to compare OFA data with macular thickness, OFA sensitivity and delay, and Matrix perimetry data, all at the same 44 central and peripheral macular locations/eye. Patients without DMO were included for comparison.

Methods

Participants

Patients with T2D with and without mild DMO were recruited through treating physicians, ophthalmologists, and optometrists. Informed written consent was obtained from the participants after explanation of the nature and possible consequences of the study, and the research was conducted in compliance with Australian National University Human Experimentation Ethics Committee (approval number ETH.2010/194) and Human Research Ethics Committee (ACT Health approval number ETH.7.07.667). Thirty-three patients with T2D were tested for up to 2.93 years, providing 128 data sets. The diagnosis of DMO was based on clinical evaluation and the optical coherence tomography (OCT) reports; specifically, two or more Early Treatment Diabetic Retinopathy Study (ETDRS) grid regions from posterior pole scans had to be flagged. Our primary interest here included relationships between the different structural and functional test methods at 44 matched visual field/retinal locations per eye (see below). Normative OFA data were based on a study of 133 participants each tested twice, 2 weeks apart.[12] Patients with other retinal, neurologic, or other disorders that would potentially affect pupillary function were excluded. We also excluded patients with BCVA less than 6/12 OU, colorblindness, or treatment with retinal laser, intravitreal anti–vascular endothelial growth factor (VEGF) injections, or vitreoretinal surgery. If patients received treatment during the study period, further follow-up was discontinued.

Ophthalmic Examination

Other eye tests followed the OFA testing on the same day. Matrix 10-2 perimetry was done on each visit (Carl Zeiss Meditec, Inc., Dublin, CA, USA). We measured BCVA with an ETDRS Chart 2, corneal curvature with an auto-refractometer (ARK-1s NIDEK Co. Ltd.; Hiroishi-cho, Gamagori, AICHI, Japan), intraocular pressure with Goldmann applanation tonometry, and pachymetry (Pachmate DGH 55; DGH Technology, Inc., Exton, PA 19341, USA). Both pupils were then dilated with 1% tropicamide eye drops for the rest of the eye examination. We evaluated anterior and posterior segments under a slit-lamp (BQ 900; Haag Streit, 3098 Koeniz, Switzerland). An 8 × 8 grid macular thickness scan and a retinal nerve fiber layer analysis were done with OCT (Spectralis; Heidelberg Engineering GmbH, Max-Jarecki-Straße, Germany). ETDRS-compliant fundus photographs were taken (Canon Digital Retinal Camera CR-2; Canon Inc., Tokyo, Japan). Systemic blood pressure and glycosylated hemoglobin (HbA1c) values were recorded.

Multifocal Pupillographic Objective Perimetry

Presentation of stimuli and monitoring of pupillary diameter with infrared video cameras were undertaken using a prototype mfPOP device, the OFA (Konan Medical USA, Irvine, CA, USA), cleared by the US Food and Drug Administration. The OFA presents dichoptic multifocal stimuli to both eyes, measuring direct and consensual responses from each eye concurrently.[15] Figure 1 gives the stimulus layout. The patients fixated a red plus-symbol at the center of the viewing field. Fixation and blinks were monitored online, and data obtained during those periods were removed. The responses measured are the constriction amplitude (sensitivity) and time to peak (delay). The OFA stimuli and OCT 8 × 8 retinal thickness grid both overlapped the boundaries of the Matrix 10-2 stimulus layout (Fig. 2), permitting accurate mapping of all the data onto equivalents of the 44 regions of the 10-2 pattern using a published method.[16] We have similarly mapped the OCT 8 × 8 thickness grid onto the Matrix pattern

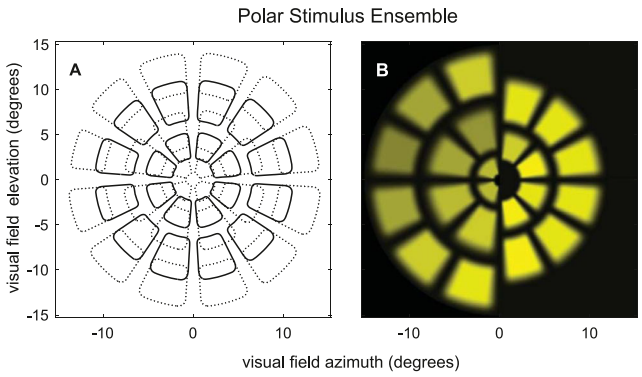

Figure 1. Multifocal stimuli arranged in a dartboard layout consisting of 44 test regions per eye. During OFA testing, stimuli of the shape and brightness indicated were presented at these locations as pseudo-randomly presented transient onset stimuli, duration 33 ms. (A) Potentially overlapping stimuli are never presented simultaneously. The mean per-region presentation interval was 4 seconds. Stimuli were presented in nine segments of 40 seconds' duration, and thus each region was tested 90 times. The stimulus array tested the central 30° (extending to ±15° eccentricity) of the visual field, with the five interleaved rings of *yellow* stimuli. No stimuli encroach upon the horizontal and vertical midlines. (B) Illustration of luminance balancing.[13] To aid viewing, only half of the regions in each of two subsets of rings are shown, remaining regions as in A. The objective of luminance balancing is to make response amplitudes homogeneous across the entire field. Stimulus luminance varied from 134 to 288 cd/m^2 on a 10-cd/m^2 background.[14] Vergence deficits were corrected before testing.

to allow quantitative region-by-region comparisons of structure and function in AMD.[5] In all, 128 data sets of macular thickness, sensitivities and delays from the OFA, and Matrix perimeter sensitivities were mapped onto a common spatial layout to compute per-region correlations between structure/function measures.

Analysis

Data analysis was performed using MATLAB (2016b; The MathWorks, Natick, MA, USA). The response waveforms for each of the 44 OFA test regions per eye were extracted from raw pupillary responses using multiple regression, with blinks removed from the pupil data before regression.[17] Responses for each retinal region were fitted to a log-normal function,[18] allowing per-region estimation of sensitivity and delay. The regressive method also generates standard errors for each sensitivity and delay, and to reduce the effects of age and pupil size, the mean diameters were standardized to 3.5 mm.[15] Thus, we were examining relative pupil constriction-amplitudes (as decibel sensitivity) rather than absolute diameter, as is common.[19,20]

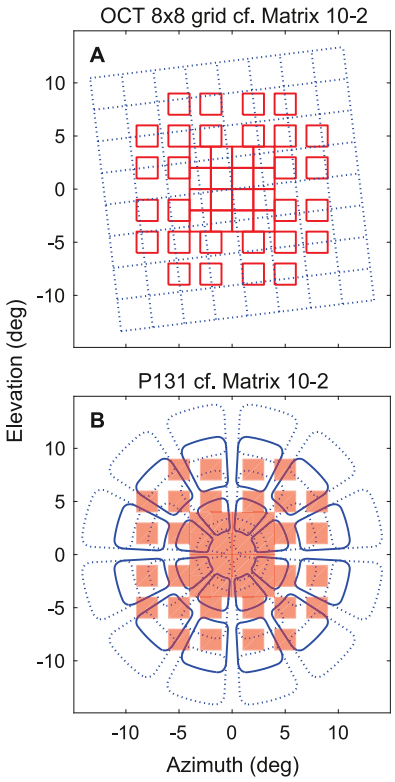

Figure 2. Correspondences of the structural and functional measures. (A) Overlap of the Spectralis OCT 8 × 8 macular thickness grid of 3 × 3° regions, set at its standard 7° tilt, with the Matrix 10-2 grid of forty-four 2 × 2° square stimuli. The thickness map was flipped to fit projection onto the visual field. (B) Overlap of the 44 stimulus regions of the OFA test (extending to ±15° eccentricity of visual field) with the 44 regions of Matrix 10-2.

Scatterplots and Principal Curves

Data for mapped 10-2 Matrix-equivalent regions for the OFA and OCT, as well as the Matrix data, now all on the same grid, were used to generate scatterplots for each data type onto the others. We examined trends using principal curve analysis (Pcurve, of the Princurve package of R version 2.15.0; Free Software Foundation, Boston, MA, USA). Several perimeters have been shown to have quite nonlinearly related data using this method.[21–23] We selected conservative parameters for the method.[24]

Correlations

An issue for any analysis of DMO is that it can be distributed asymmetrically across the retina, posing a problem for comparing different eyes. To deal with this issue, we averaged results from small groups of field regions beginning with the OCT thickness data. We then sorted those group-averaged retinal thickness scores and applied that sort order to equivalent OFA and Matrix scores. Thus, we were examining the OCT

data from the thickest groups of regions with the OFA and Matrix data from the same regions, wherever they occurred across the field/retina. We then computed correlations between these sorted scores. Correlation matrices and their *P* values were calculated using the *corrcoeff* function of MATLAB.

Logistic Regression

We investigated which variables were predictive of clinically diagnosed DMO as opposed to diabetes with no DMO. We compared the data from the central and peripheral macula/field. Obviously, the main predictor variable for DMO should be retinal thickness. The question was, would there be any independent variable(s) that had power to predict DMO compared to diabetes without DMO? We fitted a generalized linear mixed-effects logistic regression model (*fitglme* in MATLAB) to account for the effects of multiple comparisons, eyes, and repeats being nested within participants.

Results

Demographics

A total of 33 patients with T2D, 17 men (51.5%), were included for analysis. The mean age at presentation was 59.2 ± 10.5 years, and 19 patients had DMO in at least one eye. The demographic details of the participants are shown in Table 1. Across patients, the maximum OCT central thickness was less than 350 µm, and the total volumes were within normal limits.

Summary Data from Each Test Modality

As a preliminary analysis, we plotted the difference between each patient's OFA data and age- and

Table 1. Demographic Characteristics of Patients

					Retinopathy Grading			Blood Pressure		Visual Acuity (LogMAR)		Intraocular Pressure	
Participant	Age, y	Gender	Diabetes Duration, y	Diabetes Macular Edema	OS	OD	HbA1c	Systolic	Diastolic	OS	OD	OS	OD
mf87150	44	F	2.5	No	1	1	—	142	114	0	0	18	16
mf87156	57	F	3	No	1	1	7	136	79	0.4	0	13	13
mf87159	55	M	0.5	No	1	1	7.1	140	98	0	0	22	21.5
mf87168	79	M	11	No	1	1	7	120	68	−0.1	−0.1	18	17
mf87170	52	F	2	No	1	1	7.6	142.5	88	0	0.1	16.5	17.5
mf87171	65	F	12	No	1	1	7.3	141.3	79.3	0	0	20.3	19.7
mf87173	70	M	10	No	1	1	5.7	121.5	81.5	0	0	19.5	19.5
mf87175	60	M	0.5	No	1	1	6.3	127	86	0	0	14	14
mf87176	70	F	9.5	No	2	2	7.2	131	79	−0.1	−0.1	22	25
mf87178	45	F	5	No	1	1	8.1	147	92	0	0.1	21	21
mf87179	62	M	17	No	1	1	5.7	100	63	0	0	13	12
mf87180	69	M	5	No	1	1	5.8	127	73	0	0	16.5	16.5
mf87182	70	M	2	No	1	1	—	147	80	0.2	0.1	16	16
mf87184	60	F	34	No	1	1	6.2	126	76	0.1	0.1	19	20
mf87151	60	F	18.3	Yes	3	3	6.5	147.5	63	0	0.1	13	14
mf87152	63	M	30.5	Yes	5	5	8.4	149	85	0.3	0.2	12	12
mf87153	37	M	23	Yes	4	4	8.1	114	77	0.1	0.1	17	18
mf87154	52	M	1	Yes	4	1	8.5	140	90	0	0	14	14
mf87155	66	F	10	Yes	3	4	8.4	144	84	0.9	0.2	12	12
mf87157	62	F	8	Yes	3	1	6.7	119.8	80	0	0	18.8	18.3
mf87158	63	M	11	Yes	1	3	6.9	142.2	85.3	0	−0.1	16.8	16.8
mf87160	58	F	27	Yes	5	5	6.7	150	85	0.1	0.2	23	22
mf87161	27	M	20	Yes	5	4	9.5	137	71	−0.1	−0.1	13	14
mf87162	55	M	9	Yes	4	4	9	116	79	0.1	−0.1	13	13
mf87163	62	F	13	Yes	5	5	9.8	149.5	94.5	0.3	0.2	13.5	12.5
mf87164	67	M	4	Yes	2	1	6.6	136.7	92	0	0	18	18.3
mf87167	67	F	22.8	Yes	4	4	9.2	141.7	77.3	0.1	0	20.7	20.7
mf87169	54	M	10	Yes	3	1	8.3	129.3	78.3	0	0.1	18	16.3
mf87172	65	F	1	Yes	3	3	6.6	105.8	59.5	0	0	18.3	18.3
mf87174	55	F	17	Yes	4	4	9.1	137	83.7	−0.1	0	18.7	19
mf87177	67	M	13	Yes	1	2	6.3	143	85.8	0	0	16	15.8
mf87183	67	M	20	Yes	3	3	8.9	127	74	−0.1	0	18	18
mf87190	48	F	5	Yes	3	3	10.2	101	67	−0.1	0	12	12

Grading of DR: 0 = normal; 1 = diabetic with no signs (normal retina); 2 = diabetic with pathology not consistent with retinopathy; 3 = minimal nonproliferative diabetic retinopathy (NPDR); 4 = mild NPDR; 5 = moderate NPDR, corresponding to ETDRS grades 10, 20, 35, 43, and 47. Diagnosis of diabetes macular edema is based on clinical evaluation supported by the OCT reports. OD: oculus dextrus or right eye; OS: oculus sinister or left eye.

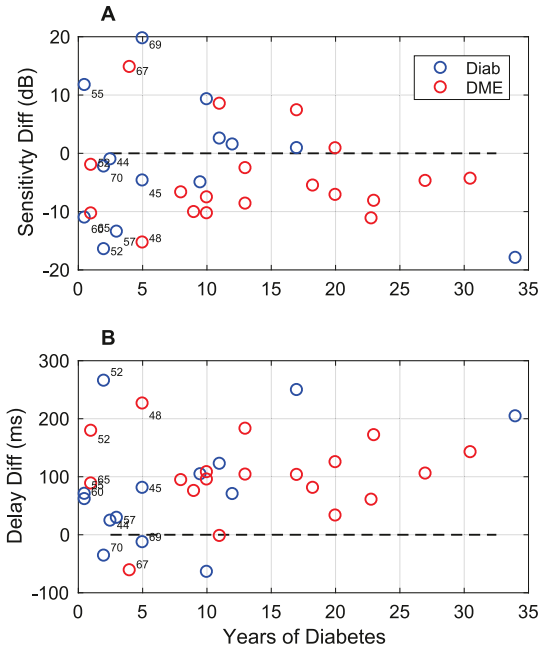

Figure 3. OFA data showing the difference compared to normal in mean sensitivity and delays for the patients with diabetes, with (*blue symbols*) and without (*red symbols*) DMO. Some data points are labeled with the patient's age so that these patients can be identified in both plots. (A) Mean sensitivity differences are largely negative, indicating lower sensitivity compared to normal. The proportion of patients with hyposensitivity increases with the duration of diabetes. In the earlier stages, some patients show retinal hypersensitivity. (B) Positive mean delay differences indicate that in most patients, time to peak was prolonged relative to normal. The proportion of patients with prolonged delays increases with the duration of diabetes. Some early patients showed also faster than normal responses. The early hypersensitivity and faster responses may indicate hyperactivity.

sex-matched normative data. We took the means across test regions, eyes, and repeats of their per-region sensitivities and plotted them on their duration of diabetes (Fig. 3A). Negative values indicate hyposensitivity and positive values hypersensitivities, both relative to normal. In early disease, some patients show retinal hypersensitivity. We labeled some data points with the participants' ages to allow them to be identified across the plots. The duration of diabetes was more important than age of patients in determining retinal sensitivity changes and sign.

The mean delay differences were positive, indicating prolonged per-region delays for the patients compared to controls (Fig. 3B). Negative delays, which indicated faster than normal responses, were more common in the patients with shorter diabetes duration. Thus, faster than normal responses are associated with hypersensitive responses. The observed delays are not simple neuropathy of the iris, however, because large differences are observed between regions, and both signs of response can occur in one retina (see Fig. 4 and Discussion).

Note that ±10-dB changes in sensitivity represent a 100-fold difference (Matrix reported changes up to 30 dB, 1000×, e.g., Fig. 5). Normal response delays are around 500 ms (depending on age and sex), so ±100 ms represents a ±20% change. A linear model showed OFA sensitivity was related to delay at −5.00 ± 1.27 dB/ms ($P = 0.002$). We also compared the per-eye mean sensitivity and delay differences with the ETDRS scores for each eye. Linear models indicated DR did not significantly determine either the sensitivity or delays (Supplementary Fig. S1).

Comparing Data Types

This is well illustrated in Figure 4, which shows a pair of data sets from a participant (mf87168) taken 17 months apart. The OCT data of Figure 4i (denoting the four panels of the top row, i) appear little changed between the two visits. The OFA sensitivity data (Fig. 4ii) are remarkably consistent centrally and show some hypersensitivity peripherally. The delay data (Fig. 4iii) seem to show worsening delay in oculus sinister or left eye (OS) on the second visit, especially in the inferotemporal field, which may be correlated with a slight increase in thickness. The Matrix fields (Fig. 4iv, v) are less consistent, especially for oculus dextrus or right eye (OD). The total deviations (Fig. 4iv) show some peripheral hypersensitivity (the pattern deviations, Fig. 4v, would not be expected to show that), which roughly matches the OFA sensitivities. Many of these features were found in the fields of other participants, which are supplied in the Supplementary Figure S2.

Scatterplots and Principal Curve Analysis

The scatterplots and fitting of principal curves (Pcurve) were performed separately for patients with and without DMO to identify any differences (Figs. 5A, 5B). Each panel in Figure 5A contains 1848 data points, and Figure 5B has 3784. This means our perception tends to be biased toward outliers. To reduce this effect, we made the dots translucent so that more saturated parts of the plots indicate where the bulk of the data is, and that drives the Pcurve outcomes. We observed a quantized distribution of the values measured by the Matrix perimeter (Figs. 5A, 5Bii, v, vii, viii, ix, xi), as was expected.[22,23] With increasing delay, the OFA and Matrix sensitivity tended to drop (Fig. 5i, ii). The trends for OFA sensitivity and OCT macular thickness were interesting. For the DMO group, the Pcurve data suggest

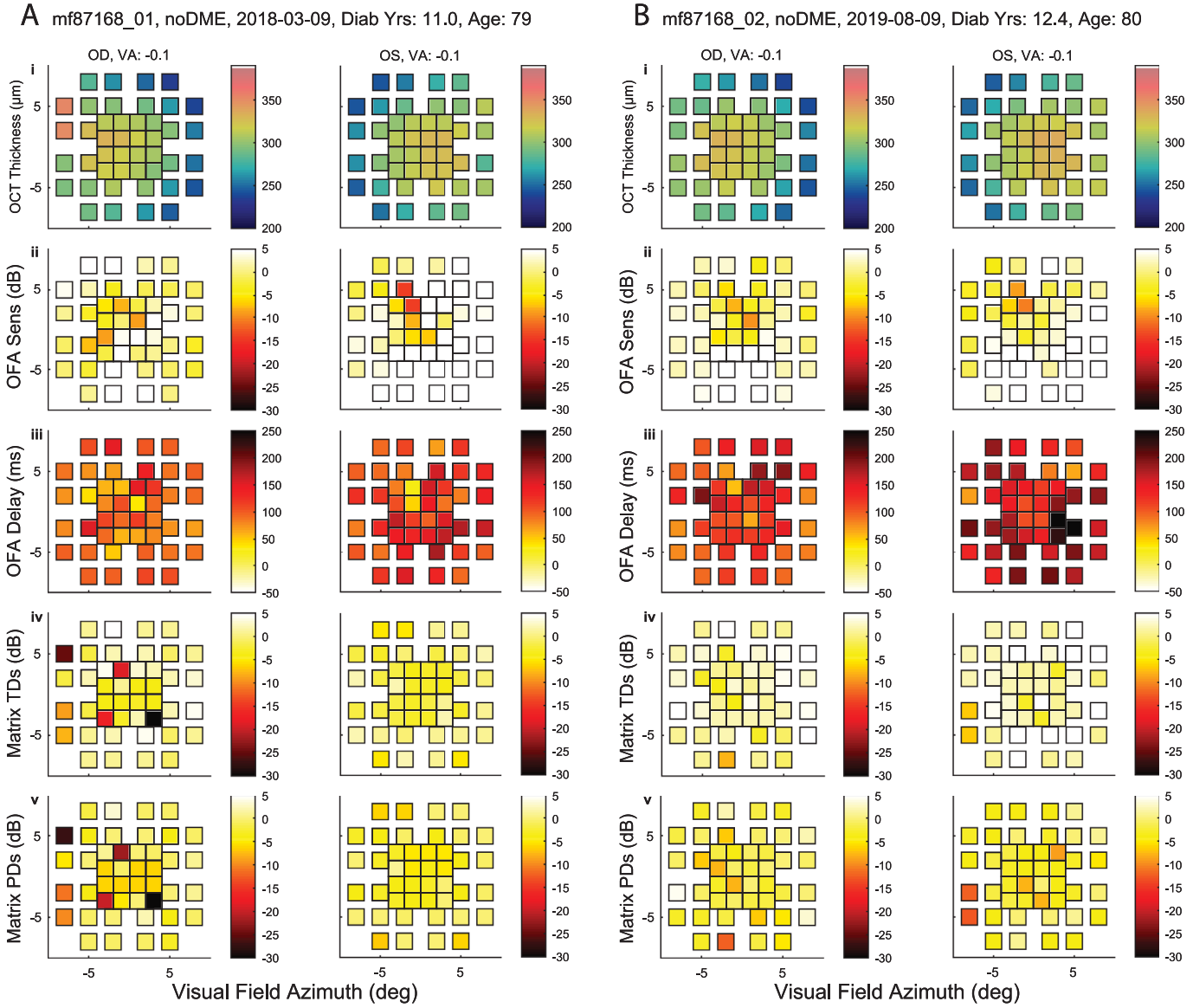

Figure 4. Illustration of data mapped to the 10-2 format for two visits 17 months apart (A, B) from a patient with diabetes whose initial age was 79 years. The pairs of columns in A (initial visit) and B (17 months later) are for OD and OS. From top to bottom, the rows of data show OCT retinal thickness, OFA sensitivities and delays, and Matrix total deviations (TDs) and pattern deviations (PDs). The rows are also identified by the numerals i to v. (i) The OCT data are very consistent between visits. (ii) The OFA sensitivities (OFA Sens) are consistent between visits with some superior loss and peripheral hypersensitivity. (iii) Delays appear to have grown on the second visit, especially OS. (iv, v) The Matrix TD and PD data are variable between visits, with the TDs showing some peripheral hypersensitivity, especially on visit 2. In this case, the OFA showed more consistent sensitivity results than the Matrix 10-2 perimeter. See all 64 data sets in the Supplementary Figure S2.

that thinner than normal retina is associated with hypersensitivity, moving to depressed sensitivity for thicker retina (Fig. 5Bvi, xi). For diabetes without DMO, the trend seemed to be reversed, with thicker retina being associated with hypersensitivity (Fig. 5Avi, xi). Decreasing macular thickness seemed to trend toward longer delays (Fig. 5iii, x). The Matrix and OFA sensitivities are quite correlated, although the PCurve is biased by the bulk of the data being at smaller values (Fig. 5v, viii).

Analysis by Retinal Location in Patients with DMO

For patients with DMO, we clustered the 44 macular regions/eye into eight groups, four central and four peripheral (Fig. 6). Within these groups, we computed the means of the per-region OCT thicknesses (relative to normative data), OFA sensitivities and delays, and Matrix sensitivities, to yield eight scores per eye/participant/visit, eight for each testing modality.

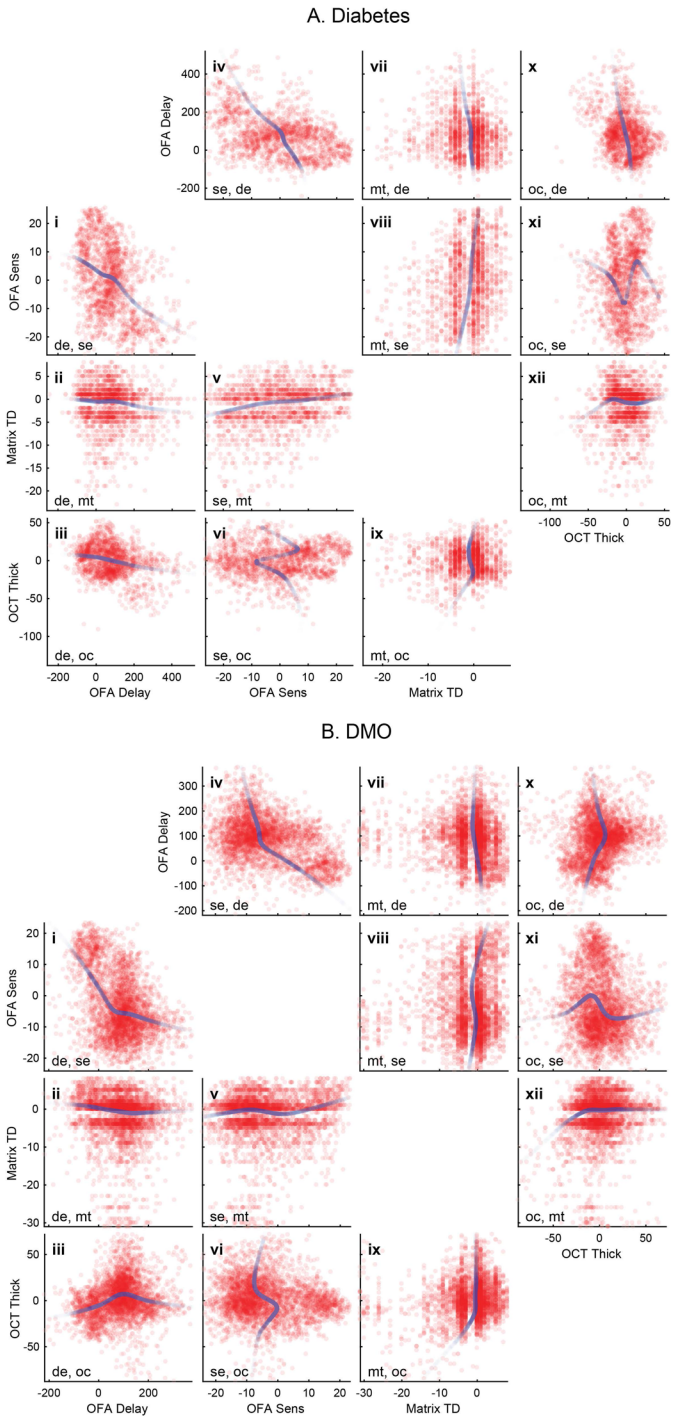

Figure 5. Scatterplots and principal curves. The mapping of all four data types onto the 10-2 pattern allowed scatterplots to be drawn, in which each dot represents a visual field region for each pair of data types. The individual dots are *translucent* to allow the density of overlap to be seen. The *blue dots* are the principal curves (PCurves) illustrating the main trends. A and B each show 12 plots for the patients with diabetes and DMO, and the two are presented together to permit easy comparisons. Each column of A and B is for one of the four data types from *left* to *right*: OFA delay (de), OFA sensitivity (Sens, se), Matrix total deviations (TD, mt), and OCT thickness (Thick, oc). At *bottom left* of each panel is a two-part mnemonic to help recall the meaning of the x and y axes of a given panel. Thus, "de, oc" (in A, Biii) indicates *O*CT thickness plotted on OFA *de*lay. The rows of A and B are in the same order as the columns, and thus the panels of A and B each have the form of a correlation matrix, and so all the unique information is captured by either the *lower* or *upper triangular part* of A or B, but showing both allows pairs of plots with transposed axes to be seen. Plots of a variable on itself on the diagonals are not shown. Each panel of A contains 1848 dots = 44 (regions) × 2 (eyes) × 21 (tests), and B has 3784 dots = 44 × 2 × 43. In case of the OCT data, (21 + 43) × 8 × 8 = 8192 8 × 8 grid data contributed to the 5632 OCT points, here using the mapping of Figure 2A.

We next sorted the OCT thickness scores for each eye/participant/repeat and then sorted the sets of eight scores from the other testing modalities following the thickness order. In this way, sensitivity and delay data were ordered by thinnest to thickest groups of OCT regions, allowing cross-modality comparisons regardless of where the focus of edema occurred.

Correlations between scores for each modality are shown in Table 2. We computed the mean of the two scores corresponding to the two thickest groups and calculated the correlations on those means. OCT thickness was significantly inversely correlated with OFA sensitivity and delay but not Matrix sensitivity. The significant correlations (all $P < 0.0032$) are in bold. Matrix sensitivity was marginally correlated with OFA delay: that correlation of -0.169 was at $P = 0.120$.

As we have reported in AMD, different responses can be happening in the central and peripheral macular regions.[5,25,26] Therefore, in the next analysis, the scores for each data type were separately sorted within four the central and four peripheral retina groups according to the OCT thickness in those groups (Fig. 6).

First, we analyzed the data from the patients with DMO (Table 3). Again, the means of the scores

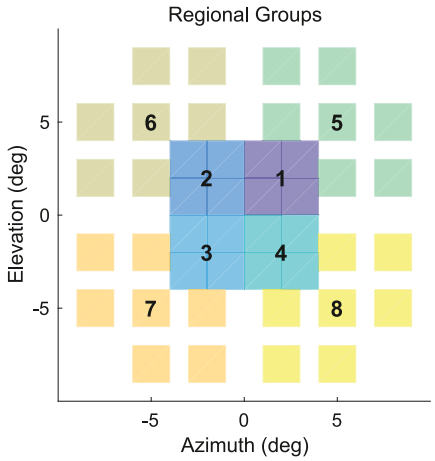

Figure 6. The macular regional groups. The macular regions were grouped into eight groups, denoted here by color coding and number. Groups 1 to 4 represent central macula, and groups 5 to 8 represent peripheral macula.

Table 2. Correlation Coefficients for Data From DMO Patients' Macular Region Groups Sorted by OCT Thickness

Characteristic	OCT	Odelay	Osens	Matrix
OCT	1	**0.315**	**−0.317**	*−0.095*
Odelay	**0.315**	1	**−0.585**	−0.169
Osens	**−0.317**	**−0.585**	1	*0.135*
Matrix	*−0.095*	−0.169	*0.135*	1

Correlations are between for data from the thickest two of eight groups for each eye of the patients with DMO. OCT represents thickness, and Matrix represents Matrix total deviations. The significant correlations are shown in bold, those that are marginally significant are in normal font, and nonsignificant correlations are italicized. Odelay, OFA delay; Osens, OFA sensitivity.

for the two thickest groups were used to calculate the correlations. The significant correlations (all $P < 0.0012$) are in bold. The correlation of −0.206 between central-Matrix and peripheral-OFA delay was at $P = 0.057$, and that between central-Matrix and central-OFA delay was −0.210 ($P = 0.052$). The main results can be seen in the first and fifth columns showing the correlation between *peripheral* and *central OCT thickness* (pOCT and cOCT) and the other variables. cOCT was not correlated with any of the other variables, while pOCT was correlated with central and peripheral OFA delay and inversely correlated with sensitivity data. Central Matrix data (column 8) were marginally inversely correlated with peripheral OFA delay. Interestingly, central and peripheral OCT data were moderately correlated with each other ($r = 0.636$), while central and peripheral data for both OFA and Matrix were well correlated, all $r > 0.9$.

Similarly, we have analyzed the data for the diabetics without DMO (Table 4). Significant correlations are bolded and had a median P value of 0.001 (least significant $P = 0.048$). The correlation between pOCT thickness and central-OFA delay of −0.284 had a P value of 0.065.

Independent Determinants of DMO versus No DMO

We used a mixed-effects logistic regression to determine which central and peripheral functional data can contribute to a clinical diagnosis of DMO by comparing the patients with and without DMO (Table 5). Surprisingly, peripheral, but not central, OCT thickness as well as central OFA sensitivity both contributed independently to the odds of patients having DMO. Being male and each repeat visit (time in the study) also significantly increased the odds of DMO.

We investigated what happened if we removed pOCT, leaving no OCT data. Central OFA sensitivity remained significant ($P = 0.045$). In both models, increased OFA sensitivity relative to normal increased the odds for each dB by about 1/0.85 = 18% per dB. We also did a logistic regression on DMO versus none using the data points of Figures 3A, 3B. DMO was determined by years of diabetes (YoD) ($P = 0.01$) and an interaction between YoD and delay ($P = 0.02$).

Discussion

It has been challenging to find a suitable diagnostic tool to monitor functional changes prior to the clinically diagnosable DMO, or even early DMO, and potentially provide better management. So far, treatment is administered mainly based on macular structural changes (OCT) and visual acuity. Here there was no correlation with acuity as vision was normal. A study on enhancing risk assessment in patients with DR found that the prediction of subsequent intervention was best when combining structural and functional (electroretinography) information.[27] We correlated functional assessment using OFA and Matrix perimetry with structural changes. We recruited patients with T2D without or with mild DMO, so their macular thickness was normal or near normal, to examine if functional damage could precede major structural change. The need to introduce mapping between device reporting methods (Fig. 2) illustrates a flaw in current standards. In an attempt to improve the situation, we have introduced the OFA M18 test. M18 has stimuli that are the size of each ETDRS grid region split in two, thus providing simple structure–function comparisons between M18 and OCT data. M18 concurrently tests both eyes in 80 seconds.[28]

Although DR is traditionally regarded as a microvascular disease, growing evidence indicates that the degeneration of retinal neurons also occurs before clinical signs of DR, and the typical microvascular alterations of DR may be a subsequent event,[29,30] which is also demonstrated by animal models.[31] In early diabetes, the On-sustained retinal ganglion cells (RGCs) receive excessive excitation under dark- and light-adapted conditions.[31,32] We observed that the mean sensitivity difference compared to normal in the patients with diabetes, both with and without DMO, was reduced, particularly later in the disease. It appeared that in earlier diabetes, there was an initial stage of hypersensitivity, which gradually progressed to

Table 3. Patients With DMO Comparing Central Versus Peripheral Group Data

	Peripheral Groups				Central Groups			
Characteristic	OCT	Odelay	Osens	Matrix	OCT	Odelay	Osens	Matrix
Peripheral								
OCT	1	**0.366**	**−0.345**	*−0.016*	**0.636**	**0.449**	**−0.343**	*−0.004*
Odelay	**0.366**	1	**−0.616**	*−0.160*	*0.081*	**0.916**	**−0.650**	*−0.153*
Osens	**−0.345**	**−0.616**	1	*0.177*	*−0.110*	**−0.611**	**0.941**	*0.125*
Matrix	*−0.016*	*−0.160*	*0.177*	1	*−0.018*	−0.210	*0.100*	**0.938**
Central								
OCT	**0.636**	0.081	*−0.110*	*−0.018*	1	*0.152*	*−0.118*	*0.090*
Odelay	**0.449**	**0.916**	**−0.611**	−0.210	*0.152*	1	**−0.622**	−0.206
Osens	**−0.343**	**−0.650**	**0.941**	*0.100*	*−0.118*	**−0.622**	1	*0.067*
Matrix	*−0.004*	*−0.153*	*0.125*	**0.938**	*0.090*	−0.206	*0.067*	1

Matrix represents Matrix total deviations. The significant correlations are shown in bold, those that are marginally significant are in normal font, and nonsignificant correlations are italicized.

Table 4. Patients With Diabetes Without DMO Comparing Central Versus Peripheral Group Data

	Peripheral Groups				Central Groups			
Characteristic	OCT	Odelay	Osens	Matrix	OCT	Odelay	Osens	Matrix
Peripheral								
OCT	1	**−0.359**	*0.012*	*0.045*	**0.818**	−0.284	*−0.014*	*−0.015*
Odelay	**−0.359**	1	**−0.502**	*−0.131*	**−0.348**	**0.959**	**−0.546**	*0.011*
Osens	*0.012*	**−0.502**	1	**0.379**	*0.102*	**−0.443**	**0.967**	*0.232*
Matrix	*0.045*	*−0.131*	**0.379**	1	*0.220*	*−0.162*	**0.342**	**0.872**
Central								
OCT	**0.818**	**−0.348**	*0.102*	*0.220*	1	**−0.307**	0.130	*0.151*
Odelay	−0.284	**0.959**	**−0.443**	*−0.162*	**−0.307**	1	**−0.490**	*−0.039*
Osens	*−0.014*	**−0.546**	**0.967**	**0.342**	*0.130*	**−0.490**	1	*0.179*
Matrix	*−0.015*	*0.011*	*0.232*	**0.872**	*0.151*	*−0.039*	*0.179*	1

Matrix represents Matrix total deviations. The significant correlations are shown in bold, those that are marginally significant are in normal font, and nonsignificant correlations are italicized.

hyposensitivity with increasing duration and severity of the disease (Figs. 3, 5). Similarly, delays could be quicker than normal earlier in the disease. These signs of apparent hyperactivity appear at different times in different parts of the field. We have already reported similar findings with OFA in early T2D without DMO,[7,8,10] indicating that several stages of functional change may be identified before structural change.[7] Here progression of delays could precede structural change (cf. Fig. 4, rows i and iii). Detailed analysis of progression in this study is given elsewhere. This hyperactivity in early stages is also supported by animal experiments suggesting increased activity of ON-type RGCs occurs before massive RGC apoptosis.[33]

OFA peripheral hypersensitivity was also shown to agree with multifocal visual evoked potentials when tested on the same patients with diabetes.[8] Those hypersensitivities were observed on electroencephalogram electrodes recording the extrastriate cortex,[8] which is known to synapse with the pretectal olivary nucleus.[34] Just like OFA, multifocal electroretinogram (mfERG) delays not only precede the onset and progression of retinopathy but also predict the location of future retinopathy up to 3 years before the visible changes occur.[35]

Our recent study of AMD with OFA identified two types of patients based on peripheral macular responses: a positive type showing hypersensitivity and prolonged delay and a negative type with hyposensitivity and delay shorter than normal.[25,26] In another study, we reported that extrafoveal hypersensitivity among the AMD cases was a good prognostic biomarker for improvement with early ranibizumab therapy.[36]

Table 5. Mixed-Effects Logistic Regression Model Summary

Characteristic	Odds	Log(Odds)	SE	t-Stat	*P*	Lower	Upper
(Intercept)	0.072	−2.625	0.834	−3.149	**0.0021**	−4.276	−0.974
Visits	2.923	1.073	0.305	3.512	**0.0006**	0.468	1.677
Male	3.742	1.320	0.605	2.180	**0.0313**	0.121	2.518
pOCT	1.042	0.041	0.016	2.582	**0.0111**	0.010	0.072
pOdelay	*1.002*	*0.002*	*0.008*	*0.203*	*0.8391*	*−0.014*	*0.017*
pOFAsens	*1.094*	*0.090*	*0.075*	*1.191*	*0.2359*	*−0.059*	*0.239*
pMatrix	*1.085*	*0.081*	*0.127*	*0.642*	*0.5222*	*−0.170*	*0.332*
cOFAdelay	*0.996*	*−0.004*	*0.008*	*−0.588*	*0.5579*	*−0.020*	*0.011*
cOFAsens	0.849	−0.164	0.080	−2.044	**0.0432**	−0.323	−0.005
cMatrix	*0.869*	*−0.140*	*0.115*	*−1.220*	*0.2251*	*−0.368*	*0.087*

Matrix represents Matrix total deviations. The significant correlations are shown in bold, those that are marginally significant are in normal font, and nonsignificant correlations are italicized. Lower and upper are the 95% confidence limits of the log(odds). cOFAdelay, central OFA delay; cOFAsens, central OFA sensitivity; cMatrix, central Matrix; pMatrix, peripheral Matrix; pOdelay, peripheral OFA delay; pOFAsens, peripheral OFA sensitivity.

Principal curve analysis was first used by Artes et al.[21] and later by Wall et al.[22] to compare per-region results between different perimeters. We used it to compare macular thickness by OCT, functional assessment with Matrix, and OFA in terms of sensitivity and delay (Fig. 5). The Pcurves of Figure 5Ai, ii, iii, and v show clear trends. Supplementary Figure S1 shows regression lines that are a good match for the shallow sloping Pcurves of Figure 5ii and v. The Pcurves for Matrix sensitivity versus OCT thickness indicated the lack of correlation shown by Tables 3 and 4. There was no simple correlation between macular thickness and OFA sensitivity. Instead, sensitivity fluctuated between hyper- and hyposensitivity, possibly related to progress of the disease (cf. Figs. 3 and 5vi, xi). Taken together, the results suggested that functional change precedes structural in diabetic patients with and without DMO.

On examining the group mean scores of the macular thickness, OFA sensitivity and delay, and Matrix data (Fig. 6), we found that the central macular thickness was not correlated with any other variables, while peripheral macular thickness was inversely correlated with central and peripheral OFA sensitivity and correlated with delay (Tables 3, 4). Previously, we have reported that in AMD, BCVA was related to the off-axis macular thickness and average Matrix 10-2 sensitivity loss but not the central thickness.[10] This may be due to the fact that ganglion cell densities are higher off-axis than centrally.[37] The disorganization of the retinal inner layers (DRIL), including the RGC layer, serves as prognostic biomarker in DMO: patients without DRIL have better responses to treatment and better visual recovery.[38] Another study reported that the presence of RGC layer cysts in DMO yields poorer prognosis than those without RGC layer cysts.[39]

The reduction in the thickness of the RGC layer and inner plexiform layer, measured by OCT and microperimetry, is correlated with improved acuity following anti-VEGF treatment of DMO.[40] RGC loss is present in patients with diabetes, even without DR.[41] These studies indicate that involvement of the RGC layer in DMO is a significant pathophysiologic process that affects retinal function, as illustrated by decreased OFA sensitivity and prolonged delays in the current study.

Our mixed-effects logistic regression analysis determined that peripheral macular thickness and OFA sensitivity contributed independently to a clinical diagnosis of DMO versus no DMO. Male gender increased the odds of DMO, which could be because T2D is more common in men than in women,[42] and DR is more common among men due to genetic susceptibility and other factors.[35]

A noticeable feature of many of the OFA delay plots is just how long some of the delays are (e.g., Figs. 4, 5). This cannot be due to iris neuropathy because the isolated per-region changes reflect localized functional changes, as reported even in persons with no peripheral neuropathy.[7,9] It is possible, however, that iris neuropathy contributed to the extended per-region delays by effectively stretching them out in time. That is, if the iris itself was becoming slower, the effect would be to low-pass filter (smooth) all the responses. This cannot, however, explain retinas showing both faster and slower than normal responses (e.g., Fig. 4iii).

This study is limited by a small number of participants. Some patients were recorded to have developed DMO and DR within less than 5 years of diabetes duration, which might be inaccurate due to misreporting by the patients, or late diagnosis. DMO and

diabetes were associated with peripheral functional change, as measured by OFA but not Matrix.

Conclusions

OFA may be a useful tool for assessing retinal function, detecting altered sensitivity and delay even before detectable structural changes. Peripheral macular regional thickness was more correlated with OFA sensitivity and delay than the central macula. Thus, peripheral macular health may have higher prognostic value than central retinal.

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References

1. Lee R, Wong TY, Sabanayagam C. Epidemiology of diabetic retinopathy, diabetic macular edema and related vision loss. *Eye Vis*. 2015;2:17.
2. Deloitte Access Economics. The economic impact of diabetic macular oedema in Australia. 2015, https://www2.deloitte.com/au/en/pages/economics/articles/economic-impact-diabetic-eye-disease.html. Accessed September 5, 2018.
3. Gale R, Scanlon PH, Evans M, et al. Action on diabetic macular oedema: achieving optimal patient management in treating visual impairment due to diabetic eye disease. *Eye*. 2017;31(suppl 1):S1–S20.
4. Ranchod TM, Fine SL. Primary treatment of diabetic macular edema. *Clin Interv Aging*. 2009;4:101–107.
5. Sabeti F, Lane J, Rohan EMF, Essex RW, McKone E, Maddess T. Central versus peripheral macular structure and function and acuity in age-related macular degeneration. *Trans Vis Sci Tech*. 2021;10:1–12.
6. Laishram M, Srikanth K, Rajalakshmi AR, Nagarajan S, Ezhumalai G. Microperimetry—a new tool for assessing retinal sensitivity in macular diseases. *J Clin Diagn Res*. 2017;11:Nc08–Nc11.
7. Sabeti F, Nolan CJ, James AC, Jenkins A, Maddess T. Multifocal pupillography identifies changes in visual sensitivity according to severity of diabetic retinopathy in type 2 diabetes. *Invest Ophthalmol Vis Sci*. 2015;56:4504–4513.
8. Sabeti F, James AC, Carle CF, Essex RW, Bell A, Maddess T. Comparing multifocal pupillographic objective perimetry (mfPOP) and multifocal visual evoked potentials (mfVEP) in retinal diseases. *Sci Rep*. 2017;7:45847.
9. Bell A, James AC, Kolic M, Essex RW, Maddess T. Dichoptic multifocal pupillography reveals afferent visual field defects in early type 2 diabetes. *Invest Ophthalmol Vis Sci*. 2010;51:602–608.
10. Rai BB, Maddess T, Carle CF, et al. Comparing retinal thickness and Matrix 10-2 functional testing in diabetic macular edema. *Invest Ophthalmol Vis Sci*. 2020;61:4866.
11. Montesano G, Ometto G, Higgins BE, et al. Evidence for structural and functional damage of the inner retina in diabetes with no diabetic retinopathy. *Invest Ophthalmol Vis Sci*. 2021;62:35.
12. Carle CF, Saraç Ö, Essex RW, et al. Properties of normative data for three multifocal pupillographic objective perimetry (mfPOP) tests. *Imaging and Perimetry Society 2016*; Udine, Italy.
13. Carle CF, James AC, Kolic M, Essex RW, Maddess T. Luminance and colour variant pupil perimetry in glaucoma. *Clin Exp Ophthalmol*. 2014;42:815–824.
14. Sabeti F, Maddess T, Essex RW, Saikal A, James AC, Carle CF. Multifocal pupillography in early age-related macular degeneration. *Optom Vis Sci*. 2014;91:904–915.
15. Maddess T, Bedford SM, Goh XL, James AC. Multifocal pupillographic visual field testing in glaucoma. *Clin Exp Ophthalmol*. 2009;30:678–686.
16. Ruseckaite R, Maddess T, James AC. Frequency doubling illusion VEPs and automated perimetry in multiple sclerosis. *Documenta Ophthalmol*. 2006;113:29–41.
17. James AC, Ruseckaite R, Maddess T. Effect of temporal sparseness and dichoptic presentation on multifocal visual evoked potentials. *Vis Neurosci*. 2005;22:45–54.
18. Carle CF, James AC, Kolic M, Loh YW, Maddess T. High-resolution multifocal pupillographic objective perimetry in glaucoma. *Invest Ophthalmol Vis Sci*. 2011;52:604–610.
19. Bremner FD. Pupillometric evaluation of the dynamics of the pupillary response to a brief light

stimulus in healthy subjects. *Invest Ophthalmol Vis Sci*. 2012;53:7343–7347.
20. Maddess T. Pupil dynamics and response amplitude: only size matters. *Invest Ophthalmol Vis Sci*. 2012;53:7644.
21. Artes PH, Hutchison DM, Nicolela MT, LeBlanc RP, Chauhan BC. Threshold and variability properties of matrix frequency-doubling technology and standard automated perimetry in glaucoma. *Invest Ophthalmol Vis Sci*. 2005;46:2451–2457.
22. Wall M, Woodward KR, Doyle CK, Zamba G. The effective dynamic ranges of standard automated perimetry sizes III and V and motion and matrix perimetry. *Arch Ophthalmol*. 2010;128:570–576.
23. Fredette MJ, Giguere A, Anderson DR, Budenz DL, McSoley J. Comparison of Matrix with Humphrey Field Analyzer II with SITA. *Optom Vis Sci*. 2015;92:527–536.
24. Hastie T, Struetzle W. Principal curves. *J Am Stat Soc*. 1989;85:502–516.
25. Rai BB, Essex RW, Sabeti F, et al. Per-region visual field sensitivities and delays in AMD. *Invest Ophthalmol Vis Sci*. 2021;62:317.
26. Rai BB, Essex RW, Sabeti F, et al. An objective perimetry study of central vs. peripheral sensitivities and delays in age-related macular degeneration. *Trans Vis Sci Tech*. Accepted for publication as of November 19, 2021.
27. Brigell MG, Chiang B, Maa AY, Davis CQ. Enhancing risk assessment in patients with diabetic retinopathy by combining measures of retinal function and structure. *Transl Vis Sci Technol*. 2020;9:40.
28. Maddess T, Rai BB, Carle CF, et al. Diagnostic power of rapid ETDRS-grid matched objective perimetry in early- to late-stage AMD. *Invest Ophthalmol Vis Sci.* 2021;62:316.
29. Catalani E, Cervia D. Diabetic retinopathy: a matter of retinal ganglion cell homeostasis. *Neural Regen Res*. 2020;15:1253–1254.
30. Simó R, Stitt AW, Gardner TW. Neurodegeneration in diabetic retinopathy: does it really matter? *Diabetologia*. 2018;61:1902–1912.
31. Cui RZ, Wang L, Qiao SN, et al. ON-type retinal ganglion cells are preferentially affected in STZ-induced diabetic mice. *Invest Ophthalmol Vis Sci*. 2019;60:1644–1656.
32. Flood MD, Wellington AJ, Cruz LA, Eggers ED. Early diabetes impairs ON sustained ganglion cell light responses and adaptation without cell death or dopamine insensitivity. *Exp Eye Res*. 2020;200:108223.
33. Yu J, Wang L, Weng SJ, Yang XL, Zhang DQ, Zhong YM. Hyperactivity of ON-type retinal ganglion cells in streptozotocin-induced diabetic mice. *PLoS One*. 2013;8:e76049.
34. Gamlin PD. The pretectum: connections and oculomotor-related roles. *Prog Brain Res*. 2006;151:379–405.
35. Ozawa GY, Bearse MA, Adams AJ. Male-female differences in diabetic retinopathy? *Curr Eye Res*. 2015;40:234–246.
36. Sabeti F, Maddess T, Essex RW, James AC. Multifocal pupillography identifies ranibizumab-induced changes in retinal function for exudative age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2012;53:253–260.
37. Curcio CA, Allen KA. Topography of ganglion cells in human retina. *J Comp Neurol*. 1990;300:5–25.
38. Zur D, Iglicki M, Sala-Puigdollers A, et al. Disorganization of retinal inner layers as a biomarker in patients with diabetic macular oedema treated with dexamethasone implant. *Acta Ophthalmol*. 2020;98:e217–e223.
39. Ceklic L, Huf W, Ebneter A, Wolf S, Zinkernagel MS, Munk MR. The impact of ganglion cell layer cysts in diabetic macular oedema treated with anti-vascular endothelial growth factor. *Acta Ophthalmol*. 2019;97:e1041–e1047.
40. Xu Y, Qu Y, Suo Y, et al. Correlation of retinal layer changes with vision gain in diabetic macular edema during conbercept treatment. *BMC Ophthalmol*. 2019;19:123.
41. Ng DS, Chiang PP, Tan G, et al. Retinal ganglion cell neuronal damage in diabetes and diabetic retinopathy. *Clin Exp Ophthalmol*. 2016;44:243–250.
42. Nordstrom A, Hadrevi J, Olsson T, Franks PW, Nordstrom P. Higher prevalence of type 2 diabetes in men than in women is associated with differences in visceral fat mass. *J Clin Endocrinol Metab*. 2016;101:3740–3746.