

SUPPLEMENTARY DATA

Additional Statistical Analysis Details.

To determine the threshold for abnormal change in corneal nerve fibre length (CNFL), termed rapid corneal nerve fiber loss (RCNFL), the 5th percentile of healthy controls was selected *a priori* to achieve a sample size of 11. Although the 2.5th percentile is commonly used to establish abnormality in clinical measures, this would have limited power as the 2.5th percentile would have resulted in a group size of five participants (**Supplemental Table 1**).

There were three different analytical approaches that could have been used to determine the number of participants with RCNFL. Shown in **Table 2** are a comparison of these three approaches using GLM slope for all groups, a mixed effects model for all groups, and a hybrid analysis as described in our primary analysis in the manuscript. This hybrid analysis used a mixed effects model for the non-diabetes controls and GLM slope for diabetes participants. Using GLM for all participants lowers the threshold for RCNFL to -12.3% and as such, the T1D and T2D populations are not different from the non-diabetes controls.

The use of *in vivo* corneal confocal microscopy allows for the measurement of corneal nerve fiber length (CNFL), corneal nerve branch density (CNBD) and corneal nerve fiber density (CNFD). Data on the annual change of CNFL, CNBD and CNFD and a reference distribution for annual change in each parameter is shown in **Table 3**.

Linear regression models were used to determine the association between a 1% loss of corneal nerve fiber density (CNFD) and corneal nerve brand density (CNBD) and change in clinical, neurological and electrophysiological variables in participants in diabetes (**Table 4 & 5**). Changes in both of these parameters were associated with an increase in DSP signs ($p < 0.01$) but not A_{1c} ($p > 0.05$).

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Supplementary Table 1. Percentile annual change in CNFL for the 204 non-diabetes controls and the associated change in CNFL to establish the threshold for Rapid Corneal Nerve Fiber Loss. The percentage of the 399 type 1 diabetes participants and 191 type 2 diabetes participants that meet the criteria for Rapid Corneal Nerve Fiber Loss (RCNFL) at the different percentiles.

Percentile of non-diabetes controls n=204	Sample Size at percentile (n)	Change in CNFL (%)	Type 1 Diabetes with RCNFL (%) n=399	Type 2 Diabetes with RCNFL (%) n=191
2.5 th	5	-6.6	60 (15.0%)	29 (15.2%)
5 th	11	-5.9	64 (16.0%)	37 (19.4%)
7.5 th	15	-4.6	76 (19.0%)	44(23.0%)

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Supplementary Table 2. Comparison of the number of participants with Rapid Corneal Nerve Fiber Loss at the 5th Percentile of Non-Diabetes Controls using different analysis models.

Characteristic	Non-diabetes controls n=204	RCNFL Threshold	Type 1 Diabetes n=399	Type 2 Diabetes n=191	P-value
Primary Analysis*	11 (5.4%)	-5.9%	64 (16.0%)	37 (19.4%)	<0.001
Mixed Effects Model in the diabetes participants	11 (5.4%)	-5.9%	52 (13.0%)	22 (11.5%)	<0.001

*Primary analysis denotes the analysis that was used in the manuscript, which analyzed the non-diabetes controls using the mixed-effects model and the diabetes participants analyzed using the GLM slope. The RCNFL threshold is the slope of the 5th percentile of healthy controls. GLM, general linear model.

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Supplementary Table 3. The annual change in *in vivo* corneal confocal parameters in 204 non-diabetes controls, 399 type 1 diabetes participants and 191 type 2 diabetes participants. For each parameter, the 5th percentile change in non-diabetes controls is shown along with the number of diabetes participants that exceed this threshold.

Characteristic	Non-diabetes controls n=204	Type 1 Diabetes n=399	Type 2 Diabetes n=191
CNFL (mm/mm²)	-0.1 ± 3.3	-0.2 ± 12.0	-0.2 ± 9.0
CNFL 5th Percentile	-5.9	64 (16.0%)	37 (19.4%)
CNBD (branches/mm²)	-0.03 ± 10.0	8.5 ± 72.9	0.3 ± 28.1
CNBD 5th Percentile	-16.3	52 (13.0%)	36 (18.8%)
CNFD (fibers/mm²)	-1.97 ± 6.36	0.4 ± 21.4	1.4 ± 16.1
CNFD 5th Percentile	-14.4	36 (9.0%)	15 (7.8%)

Data presented as mean±SD. CNFL, corneal nerve fiber length; CNFD, corneal nerve fiber density; CNBD, corneal nerve branch density.

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Supplementary Table 4. The estimated change in clinical, neurological and electrophysiological variables associated with a 1% per year loss of corneal nerve fiber density in the 590 participants with diabetes.

Characteristic	Parameter Estimate	Lower CL	Upper CL	P-value
A _{1c}	-0.74	-4.22	2.73	0.67
BMI (kg/m ²)	0.88	-1.39	3.15	0.45
DSP Signs	4.18	2.08	6.27	<0.001
DSP Symptoms	3.55	1.01	6.08	0.006
Small Nerve Fiber Measures				
CNBD (branches/mm ²)	-1.39	-1.58	-1.21	<0.001
Cooling Detection (°C)	-0.65	-1.86	0.55	0.23
Large Nerve Fiber Measures				
Vibration Perception (V)	0.15	-0.15	0.45	0.33
Sural CV (m/s)	0.78	0.03	1.53	0.040
Sural AMP (mV)	0.36	-0.42	1.13	0.36
Peroneal AMP (mV)	-1.54	-4.72	1.64	0.34
Peroneal CV (m/s)	-0.59	-1.94	0.77	0.39
Peroneal F-wave (ms)	0.27	-0.30	0.84	0.35

The regression parameter estimate shows the percent change in CNFD associated with a one-unit change in the given variable. CNFD, corneal nerve fiber density; CNBD, corneal nerve branch density.

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Supplementary Table 5. The estimated change in clinical, neurological and electrophysiological variables associated with a 1% per year loss of corneal nerve branch density in the 590 participants with diabetes.

Characteristic	Parameter Estimate	Lower CL	Upper CL	P-value
A_{1c}	-0.93	-11.14	9.29	0.86
BMI (kg/m²)	-3.25	-10.98	4.47	0.40
DSP Signs	10.32	3.40	17.24	0.0035
DSP Symptoms	6.19	-1.94	14.32	0.13
Small Nerve Fiber Measures				
CNFD (Fibers/mm²)	-9.03	-10.43	-7.63	<0.001
Cooling Detection (°C)	-2.03	-6.24	-2.19	0.34
Large Nerve Fiber Measures				
Vibration Perception (V)	0.10	-0.94	1.13	0.86
Sural CV (m/s)	2.00	-0.41	4.41	0.10
Sural AMP (mV)	3.55	1.11	6.00	0.0045
Peroneal AMP (mV)	-8.61	-18.91	1.69	0.10
Peroneal CV (m/s)	-3.59	-7.97	0.79	0.11
Peroneal F-wave (ms)	0.22	-1.80	2.24	0.83

The regression parameter estimate shows the percent change in CNFD associated with a one-unit change in the given variable. CNFD, corneal nerve fiber density; CNBD, corneal nerve branch density.