Received: 5 May 2022 | Revised: 8 September 2022 | Accepted: 17 September 2022

DOI: 10.1111/pedi.13419

COMPLICATIONS

ISPAD International Society for Pediatric and Adolescent Diabetes WILEY

Corneal nerve and nerve conduction abnormalities in children with type 1 diabetes

Danièle Pacaud[1,2,3] | Kenneth G. Romanchuk[1,2] | Heidi Virtanen[1,2,3] | Maryam Ferdousi[4,5] | Alberto Nettel-Aguirre[1,6] | Jean K. Mah[1,2,3] | Mitra Tavakoli[7,8] | Douglas W. Zochodne[9] | Rayaz A. Malik[4,5,10]

[1]Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada

[2]Pediatric Diabetes, Pediatric Neurology and Pediatric Ophthalmology divisions, Alberta Children's Hospital, Calgary, Alberta, Canada

[3]Alberta Children's Hospital Research Institute, Calgary, Alberta, Canada

[4]Division of Cardiovascular Sciences, Cardiac Centre, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK

[5]NIHR/Wellcome Trust Clinical Research Facility, Manchester, UK

[6]Centre for Health and Social Analytics, NIASRA, University of Wollongong, Wollongong, New South Wales, Australia

[7]Centre for Endocrinology and Diabetes, Institute of Human Development, University of Manchester, Manchester, UK

[8]Exeter Centre of Excellence for Diabetes Research, NIHR Exeter Clinical Research Facility, University of Exeter Medical School, Exeter, UK

[9]Division of Neurology, Department of Medicine and the Neuroscience and Mental Health Institute, University of Alberta, Edmonton, Alberta, Canada

[10]Department of Medicine, Weill Cornell Medicine-Qatar, Doha, Qatar

Correspondence
Danièle Pacaud, Alberta Children's Hospital, 28 Oki Drive NW, Calgary, AB T3B 6A8, Canada.
Email: daniele.pacaud@albertahealthservices.ca

Funding information
Juvenile Diabetes Research Foundation International; University of Calgary Lions Sight Center Fund Award; University of Calgary; JDRF International, Grant/Award Number: 17-2008-1032

Abstract

Objective: In vivo corneal confocal microscopy (CCM) is a novel, rapid, and non-invasive technique that identifies early small fiber damage and can predict the progression and development of clinical neuropathy in adults with type 1 diabetes. However, its usefulness in children is not well established. This study compared corneal confocal microscopy with neuropathic symptoms, signs, and objective measures of neuropathy for the diagnosis of diabetic neuropathy in children with type 1 diabetes.

Research design and methods: A total of 83 children with type 1 diabetes and 83 healthy participants of similar age underwent assessment of neuropathy symptoms, signs, nerve conduction studies, quantitative sensory and autonomic function testing, and in vivo CCM.

Results: Only of 3/83 (4%) children with type 1 diabetes had subclinical neuropathy. However, corneal nerve fiber density ($p = 0.001$), branch density ($p = 0.006$), fiber length ($p = 0.002$), tibial motor nerve amplitude and conduction velocity, and sural sensory nerve amplitude and conduction velocity (all $p < 0.004$) were lower in participants with type 1 diabetes than in the controls. Vibration, cooling, and warm perception thresholds and deep breathing heart rate variability were not found to be different (all $p > 0.05$) between children with type 1 diabetes and healthy controls. Multivariate regression analysis identified a possible association between body mass index and decreased corneal nerves.

Conclusions: Decreased corneal nerves and abnormal nerve conduction were found in children with type 1 diabetes. CCM may allow rapid objective detection of subclinical diabetic neuropathy in children and adolescents with type 1 diabetes.

KEYWORDS
adolescents, children, corneal confocal microscopy, diabetic neuropathy, neurophysiology, type 1 diabetes

1 | INTRODUCTION

Clinical practice guidelines for children with type 1 diabetes[1–3] recommend annual screening by assessing the symptoms and signs of diabetic neuropathy at puberty and after 5 years of diabetes. Generally, children and adolescents do not have signs or symptoms of diabetic polyneuropathy[4–6] and screening tools recommended for adults, such as clinical examination and the Semmes-Weinstein microfilament, are unreliable, especially in children.[7,8] Abnormalities in motor and sensory nerve conduction studies (NSC) have been reported in ~20%–34.5% of children with type 1 diabetes.[9–12] Due to the absence of clinical symptoms and signs, lack of good pediatric normative data, and technical challenges in performing quantitative sensory testing (QST)[12] and other neurophysiological assessments, the diagnosis of early diabetic neuropathy is challenging in children.[13]

Although diabetic neuropathy is known to primarily affect large nerve fibers, small fiber impairment is also being observed, especially early during the subclinical phase.[12] In vivo corneal confocal microscopy (CCM) is a rapid, non-invasive technique that identifies early small fiber damage and can predict the progression[14] and development[15,16] of clinical neuropathy in adults with type 1 diabetes. It correlates with intra-epidermal nerve fiber density, autonomic neuropathy and nerve conduction studies with various definition of diabetic peripheral neuropathy.[17] CCM is a reliable and reproducible technique for children with diabetes.[18,19] Corneal nerve loss has been reported[20–22] in small cohorts of children with type 1 diabetes. Previous pediatric studies have assessed relatively small cohorts or have not compared CCM against the currently accepted gold-standard measures of neuropathy.

This study aimed to compare CCM against classic measurements of diabetic neuropathy, which include a combination of clinical symptoms, signs, QST, autonomic function testing (AFT), and NCS, in a large cohort of children with type 1 diabetes and healthy controls.

2 | METHODS

2.1 | Study participants

This study was approved by the University of Calgary Conjoint Health Research Ethics Board. Children aged 8–18 years with at least 5 years of type 1 diabetes and healthy control participants of the same age were invited to participate. The lower age limit was chosen based on the duration of diabetes required and the ability to have the child tolerate the different procedures. Participants were recruited from the Alberta Children's Hospital Diabetes Clinic. Healthy control participants were recruited through advertisement posters set in general pediatric or pediatric ophthalmology clinics, and through word of mouth from siblings and friends of children with diabetes. Exclusion criteria were as follows: known history of corneal abnormality, trauma or surgery, any other cause of neuropathy, uncontrolled hypothyroidism, celiac disease, or any other serious chronic illnesses. After informed consent was obtained from caregivers and assent from the participants, demographic, clinical, and biochemical (HbA1c, urinary albumin-creatinine ratio [ACR], and lipid profile) data were collected through questionnaires and chart reviews. Height measurements using a wall-mounted stadiometer were recorded as the average of three measurements taken to the nearest 0.1 cm while the subject was standing without shoes or head garment and with head, buttocks and feet touching the wall. Weight was measured to the nearest 0.1 kg without shoes and in light clothing. Body mass index z-scores were calculated using the WHO growth charts for Canada.[23]

2.2 | Assessment of neuropathy

Diabetic neuropathy was defined as the presence of symptoms and signs of peripheral neuropathy, or either symptoms or signs of abnormal NCS, QST, or AFT.[24] Subclinical neuropathy was defined as the absence of symptoms but the presence of at least two abnormalities in the QST, AFT, or NCS.[24]

a. Symptoms and signs of peripheral neuropathy: Symptoms were assessed using the neuropathy symptom score (NSS) from a list of 18 motor, sensory, and autonomic symptoms. An NSS ≥1 was considered abnormal. The neuropathy disability score (NDS) was obtained from the assessment of vibration, pin prick, temperature, and deep tendon reflexes in both legs to produce a score ranging from 0 to 10 and has been validated in children with type 1 diabetes.[25]
b. Nerve conductions studies: Nerve conduction studies (NCS) were performed on the non-dominant leg using a Sierra Wave EMG machine (Cadwell Laboratories Inc.) with surface stimulation and recording electrodes while maintaining the skin temperature above 31°C.[26] Sural sensory nerve action potential amplitude (μV), conduction velocity (m/s), tibial and peroneal (fibular) motor nerve

action potential amplitudes (mV), distal motor latencies (ms), and conduction velocities (m/s) were evaluated and defined as abnormal if the values were outside the 2SD of healthy controls.

c. QST was performed using a computer-assisted sensory examination (CASE IV).[27] This system uses quantified and reproducible stimuli with a forced choice or step algorithm to evaluate vibration and cold sensations. Individuals are asked if they perceive difference between two stimuli vibration or cold sensation and the lowest difference they identify is termed "Just Noticeable Differences" or JND. The smallest difference perceived by a human is equal to one JND; hence, a higher JND indicates a less-sensitive individual. Abnormalities were defined when the patient's response was greater than the 97.5th percentile for responses from the healthy control group (i.e., vibration >17.2 JND, cooling >16.4 JND and warming >20 JND).

d. Autonomic neuropathy: (i) AFT was performed using a Sierra Wave machine (Cadwell Laboratories Inc.) to measure the heart rate response to deep breathing (HRV-DB). The participant was asked to inhale and exhale deeply eight times in a row in the supine position while following the rhythm of a "breathing cue". Two eight-cycle breathing series were completed, interspersed with a 5-min period of normal breathing. For each patient, the difference between the highest and lowest heart rates for five consecutive, artifact-free cycles in each eight-cycle series was calculated. (ii) Using an appropriate cuff size for the child size, blood pressure was measured after at least 5 min of lying supine in a quiet environment and then repeated 1 and 5 min after standing up. Orthostatic systolic blood pressure change >20 mmHg was considered abnormal.[28]

2.3 | Assessment of diabetic retinopathy

A complete eye examination was performed by a pediatric ophthalmologist for all participants, including standard seven-field (EDTRS) color fundus photography, which was interpreted and graded by an independent masked retinal ophthalmologist.

2.4 | Assessment of diabetic nephropathy

Microalbuminuria normal was < 20 mcg/min and ACR <30 mg/mmol. The presence of microalbuminuria was defined as two consecutive abnormal first morning ACR values or timed collections obtained at 3-to 4-month intervals over a 6-to 12-month period, as per the Diabetes Canada Clinical Practice Guidelines.[1]

2.5 | Corneal confocal microscopy

Images from the sub-basal nerve plexus of the cornea were obtained using established methodology[29] with a Heidelberg Tomograph II laser-scanning confocal microscope equipped with a Rostock cornea module (HRT II-RCM) to provide 2D images of 0.3 mm × 0.3 mm dimension (384 × 384 pixel). A topical anesthetic (Alcaine® Proparacaine hydrochloride 0.5% by Alcon) followed by a high-viscous eye gel (Tear Gel® by Alcon) was instilled in each eye. The participants were asked to fixate on an external target while the microscope objective lens covered by a one-time disposable sterile Heidelberg Tomocap was positioned to touch the participant cornea to obtain at least eight satisfactory images of the sub-basal nerve plexus from the central cornea per eye.[30]

2.6 | Image analysis

Three corneal nerve parameters were quantified: (i) corneal nerve fiber density (CNFD) (no./mm^2), the total number of major nerves/mm^2 of corneal tissue; (ii) corneal nerve branch density (CNBD), the number of branches emanating from all major nerve trunks/mm^2 of corneal tissue (no./mm^2); and (iii) corneal nerve fiber length (CNFL), the total length of all nerve fibers and branches within the area of corneal tissue (mm/mm^2). Seven to 16 images per patient were examined using automated analysis (ACCMetrics V2, M.A. Dabbah, Imaging Science, University of Manchester, Manchester, UK), and the average was tabulated for each patient. Corneal nerve parameter values <2 SD from our healthy control means were considered abnormal: CNFD <15.9 no./mm^2, CNBD <12.1 no./mm^2, and CNFL <11.2 mm/mm^2.

2.7 | Statistical analysis

All analyses were performed using the IBM SPSS Statistics (version 25). Descriptive statistics are presented as mean and standard deviation for numerical/continuous variables, as the distribution of these variables did not contradict the assumption of a normal distribution. Categorical variables were presented as numbers and percentages.

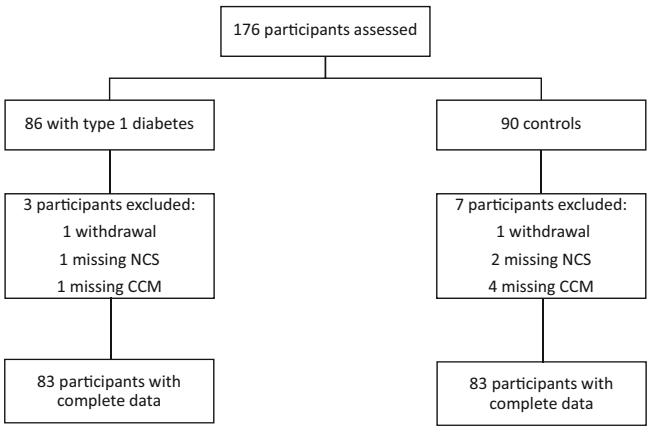

FIGURE 1 Participants recruitment flow chart. CCM, corneal confocal microscopy; NCS, nerve conduction studies. Although five participants were excluded due to missing CCM assessments, only one was secondary to the participant being unable to tolerate the procedure

TABLE 1 Participant characteristics

	Type 1 diabetes	Healthy Controls
Number	83	83
Age (years)	14.78 (2.34)	13.40 (3.03)
Age range	8.40–18.27	8.22–18.74
Sex (F/M) (%)	43 Female/40 male (51.8/48.2)	51 Female/32 male (61.4/38.6)
Ethnicity	White—74 (89.2%)	White—63 (75.9%)
	Asian—5 (6.0%)	Asian—15 (18.1%)
	Black—1 (1.2%)	Other—4 (4.8%)
	First Nations—1 (1.2%)	Missing—1 (1.2%)
	Hispanic—1 (1.2%)	
	Other—1 (1.2%)	
Age at diagnosis (years)	5.90 (3.00)	N/A
Type 1 diabetes duration (years)	8.87(2.68)	N/A
HbA1c[a] (%)	8.95 (1.76)	N/A
HbA1c[a] (mmol/mol)	74 (19.2)	N/A
Smoking (yes)	4/83	1/83
Height-Z	0.16 (0.99)	0.41 (0.95)
Height (%)	54.68 (29.20)	61.16 (28.20)
Weigh-Z	0.54 (0.80)	0.48 (0.84)
Weight (%)	66.95 (23.79)	63.91 (24.23)
BMI	21.66 (3.13)	20.37 (3.56)
BMI-Z	0.60 (0.79)	0.38 (0.84)
BMI %	68.49 (24.02)	60.85 (24.92)
Blood pressure percentile abnormalities (≥95%)		
Systolic (abnormal/total)	2/83	2/83
Diastolic (abnormal/total)	1/83	0/83
Microalbuminuria		
Microalbumin (mcg/min)[a]	16.58 (40.76)	N/A
U Albumin/creatinine ratio (mg/mmol)[a]	1.95 (5.93)	N/A
Microalbuminuria[a]	4 Positive (all intermittent)	N/A
Lipids		
Total cholesterol (mmol/L)	4.16 (0.94)	N/A
Triglycerides (mmol/L)	0.90 (0.77)	N/A
LDL (mmol/L)	2.16 (0.64)	N/A
HDL (mmol/L)	1.58 (0.36)	N/A
Ratio total:HDL	2.71 (0.68)	N/A
Retinopathy[b]	14/83	2/83[c]
Neuropathy[d]		
Clinical neuropathy	0	0
Subclinical neuropathy	3	1

Note: Data presented as mean (SD) or number (percentages).

[a]HbA1c upper limit of normal for individuals without diabetes 6.1%. Microalbuminuria normal was < 20 mcg/min and U Albumin/creatinine ratio was <30 mg/mmol. The presence of microalbuminuria was defined as two consecutive abnormal first morning ACR values or timed collections obtained at 3- to 4-month intervals over a 6- to 12-month period, as per the Diabetes Canada Clinical Practice Guidelines.

[b]All participants with retinopathy had non-proliferative retinopathy.

[c]Likely false positives (incidental findings) as one showed solitary splinter hemorrhage and another showed solitary dot retinal hemorrhage, which when isolated were considered normal findings. However, the interpreter categorized these changes as non-proliferative diabetic retinopathy, indicating his masked status to the participant's group. Further, these two children, did not have any medical antecedents excluding them from the study and did not have any other abnormalities on the complete neuropathy testing done in the control subjects of the study (including symptoms, signs or any of the nerve conductions studies, quantitative sensory testing, and autonomic function testing).

[d]Clinical neuropathy was defined as the presence of symptoms and signs of peripheral neuropathy, or either symptoms or signs with abnormal testing from NCS, quantitative sensory testing, or autonomic testing; subclinical neuropathy was defined as absence of symptoms but presence of at least two abnormalities in the other tests of neuropathy.

TABLE 2 Comparison of corneal confocal microscopy and other neuropathy assessments between participants with type 1 diabetes and healthy controls

	Type 1 diabetes	Control	Mean difference (95% Confidence Interval)	*t* test sig. (2-tailed)[a]
CNFD (no./mm^2)	24.6 (5.8)	27.8 (7.1)	2.4 (0.4–4.4)	0.002
CNBD (no./mm^2)	30.8 (13.6)	37.8 (17.4)	6.6 (1.8–11.3)	0.006
CNFL (mm/mm^2)	15.2 (2.9)	16.7 (3.4)	1.3 (0.3–2.2)	0.003
Neuropathy assessment				
Tibial motor nerve amplitude (mV)	46.9 (4.5)	51.4 (3.9)	4.4 (3.1–5.7)	<0.001
Tibial motor nerve conduction velocity (m/s)	46.2 (3.8)	50.3 (4.1)	4.1 (2.8–5.4)	<0.001
Sural sensory nerve amplitude (μV)	17.9 (7.1)	23.2 (9.9)	5.3 (2.7–7.9)	<0.001
Sural sensory nerve conduction velocity (m/s)	45.9 (4.9)	48.3 (5.2)	2.4 (0.9–4.0)	0.004
Vibration JND	11.8 (2.3)	11.1 (3.0)	−0.7 (−1.6–0.1)	0.138
Cooling JND	8.8 (3.7)	9.4 (3.3)	0.6 (−0.5–1.6)	0.223
Warming JND	12.8 (4.0)	13.4 (3.2)	0.6 (−0.5–1.8)	0.495
Deep breathing HR variability max/min ratio	1.62 (0.19)	1.65 (0.22)	0.0 (−0.0–0.1)	0.700
Orthostatic blood pressure change >20 mmHg (abnormal/total)	0/83	3/83		

Note: Data presented as mean (SD).
Abbreviation: JND, just noticeable difference.
[a]*t* test or Mann–Whitney *U* test, as applicable.

TABLE 3 Multiple regression analysis examining the relationship between clinical risks factors for diabetic neuropathy and corneal nerve fiber length (CNFL), corneal nerve fiber density (CNFD), and corneal nerve branch density (CNBD) in participants with type 1 diabetes

Dependent variable	Variables in the model	*B*	95% CI	*p* value
CNFL $R^2 = 0.141$ $p = 0.131$	Age	−0.019	−0.339 to 0.302	0.908
	Diabetes duration	0.040	−0.225 to 0.306	0.762
	HbA1c	−0.360	−0.854 to 0.134	0.150
	BMI percentile	0.033	0.002–0.064	0.038
	SBP percentile	−0.018	−0.045 to 0.009	0.183
	LDL cholesterol	−0.758	−1.914 to 0.398	0.195
CNFD $R^2 = 0.088$ $p = 0.428$	Age	0.202	−0.447 to 0.851	0.536
	Diabetes duration	0.096	−0.442 to 0.634	0.722
	HbA1c	−0.813	−1.813 to0.188	0.109
	BMI percentile	0.046	−0.017 to 0.109	0.146
	SBP percentile	−0.012	−0.067 to 0.043	0.672
	LDL cholesterol	−1.147	−3.487 to 1.194	0.331
CNBD $R^2 = 0.112$ $p = 0.261$	Age	0.703	−0.801 to 2.208	0.354
	Diabetes duration	−0.218	−1.466 to 1.029	0.728
	HbA1c	−1.368	−3.687 to 0.950	0.243
	BMI percentile	0.158	0.012–0.304	0.034
	SBP percentile	−0.072	−0.200 to 0.056	0.263
	LDL cholesterol	−2.077	−7.503 to 3.350	0.447

CNFD, CNBD, and CNFL means between the controls and participants with T1D, and nerve conduction parameters between participants with and without diabetic neuropathy were compared using a two-tailed, independent samples Student's *t* test with a Bonferroni correction of 0.004. a priori power calculations estimated that with 100 participants per group, we would have 80% power to detect differences between groups of 7.6 no./mm^2 for CNFD, based on an SD of 19.6, 4.9 no./mm^2 for CNBD, based on a SD of 12.8 no./mm^2 and 1.8 mm/mm^2 for CNFL based on SD of 4.7 mm/mm^2. As exploratory analysis, based on the work from Cozzini et al.,[28] age, duration of diabetes, HbA1c, body mass index (BMI), systolic blood pressure (SBP) and LDL were chosen as independent variables for multiple regression analysis using CNFL, CNFD and CNBD each as the dependent variable; for this exploratory analysis, significance level of 0.05 was used.

3 | RESULTS

One hundred and seventy-six participants consented to participate. After excluding those unable to complete all assessments, 83 participants remained in each group (Figure 1). Only one participant was excluded secondary to being unable to tolerate the CCM procedure. The participants' characteristics are presented in Table 1. Participants with diabetes had a mean age of 14.8 years (SD 2.3), duration of diabetes of 8.9 years (SD 2.7) and an HbA1c of 8.95% (SD 1.76).

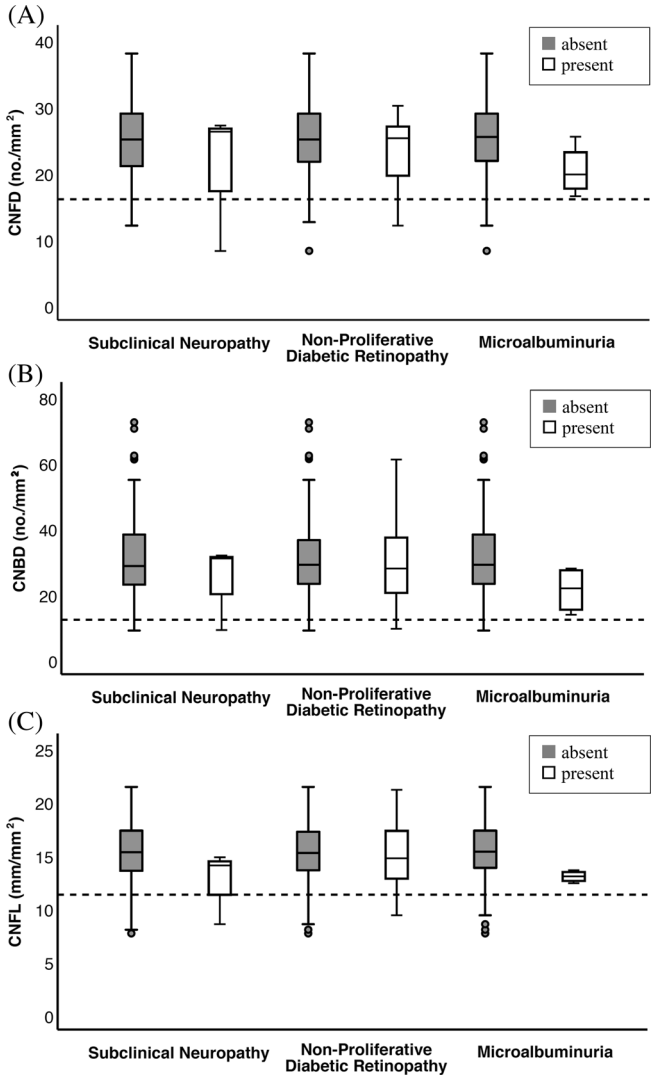

FIGURE 2 Boxplots of automated corneal confocal microscopy parameters in relation to presence (white box) or absence (gray box) of microvascular complications in children with type 1 diabetes: (a) CNFD, (b) CNBD, (c) CNFL. Dashed lines indicate the lower 2.5 percentile lower limit of the normal cohort. CNBD, corneal nerve branch density; CNFD, corneal nerve fiber density; CNFL, corneal nerve fiber length. Subclinical neuropathy defined as absence of symptoms but presence of at least two abnormalities in the other tests of neuropathy. Retinopathy assessed using standard seven-fields color fundus photography interpreted by an independent masked ophthalmologist. Presence of microalbuminuria defined as two consecutive abnormal first morning albumin to creatinine ratio (ACR) or timed collections obtained at 3- to 4-month intervals over a 6- to 12-month period, as per Diabetes Canada Clinical Practice Guidelines.[1] Statistical analysis was not possible due to the small number of participants affected by subclinical neuropathy ($n = 3$), non-proliferative retinopathy ($n = 14$) and microalbuminuria ($n = 4$)

None of the participants with type 1 diabetes or the healthy controls had clinical neuropathy. Three participants with type 1 diabetes had subclinical neuropathy: two had two abnormal NCS in two different nerves and an abnormal vibration perception threshold; one individual had five abnormal NCS parameters in three different nerves and an orthostatic drop in blood pressure >20 mmHg upon standing. One control participant had an orthostatic drop in blood pressure >20 mmHg upon standing and an abnormal warm perception threshold.

Table 2 presents the differences in each neuropathy parameter between subjects with type 1 diabetes and control subjects. CNFD (95% CI 0.4–4.4, $p = 0.001$), CNFL (95% CI 0.3–2.2, $p = 0.002$) and CNBD (95% CI 1.8–11.3, $p = 0.006$) were significantly lower in participants with type 1 diabetes than in healthy control participants. Tibial motor nerve amplitude (95% CI 3.1–5.7, $p < 0.001$), tibial motor nerve conduction velocity (95% CI 2.8–5.4, $p < 0.001$), sural sensory nerve amplitude (95% CI 2.7–7.9, $p < 0.001$), and sural nerve conduction velocity (95% CI 0.9–4.0, $p < 0.004$) were all significantly lower in participants with type 1 diabetes than in controls. Vibration (95% CI −1.6 to 0.1, $p = 0.138$), cooling (95% CI −0.5 to 1.6, $p = 0.223$), and warm (95% CI −0.5 to 1.8, $p = 0.495$) perception thresholds and deep breathing heart rate variability ($p = 0.700$) did not significantly differ between the two groups.

Seven, five, and four participants with diabetes had CNFL, CNFD, and CNBD values below the 2.5th percentile of the control participants. The results of the multiple regression models for the association of each corneal nerve parameter (CNFL, CNFD, and CNBD) with the clinical and biochemical risk factors for neuropathy are presented in Table 3. BMI correlated with CNFL and CNBD, but not with CNFD, while age, duration of diabetes, HbA1c, BMI, systolic blood pressure (SBP), and LDL did not correlate with CNFL, CNFD, and CNBD.

Boxplots for CNFL, CNFD, and CNBD in patients with type 1 diabetes with and without subclinical neuropathy, retinopathy, or microalbuminuria are shown in Figure 2. Statistical analysis was not possible because of the small number of patients affected by subclinical neuropathy ($n = 3$), non-proliferative retinopathy ($n = 14$), and microalbuminuria ($n = 4$). However, CCM parameters appear lower in those with subclinical neuropathy as well as those with microalbuminuria but not in those with retinopathy.

4 | DISCUSSION

This is the most comprehensive study to date, comparing corneal confocal microscopic nerve findings with extensive neuropathy assessments in children with type 1 diabetes and normal controls. There were abnormalities in the NCS and CCM but no abnormalities in QST or AFT in children with type 1 diabetes when compared to similarly aged healthy controls. As CCM is gaining recognition for assessing

diabetic neuropathy in adults, it is important to report these findings in children.

A significant reduction in corneal nerve fibers has been reported in children with type 1 diabetes without retinopathy or microalbuminuria.[20] Similarly, in our cohort of children with a relatively short duration of type 1 diabetes, corneal nerves were lower in children without diabetic retinopathy or microalbuminuria. In adults, corneal nerve loss is associated with age, diabetes duration, weight, height, BMI, HbA1c, total cholesterol, LDL cholesterol, and triglycerides.[31–33] We did not find a similar association, which may be due to the age of our population, short duration of diabetes and the relatively normal lipid levels. Recently, Cozzini et al.[21] showed that corneal nerve loss was associated with age, HbA1c and systolic blood pressure in 150 children with type 1 diabetes without clinical symptoms or signs of neuropathy. Of note, they did not assess NCS, QST, or AFT and thus did not report a correlation with CCM. In the present study, an exploratory multivariate analysis showed an association between lower corneal nerve parameters and BMI, but no correlation with age, HbA1c and blood pressure as previously shown by Cozzini et al.[21] This may be due to a differing genetic background, lifestyle and metabolic control in European compared to North American youth.[34,35]

Symptoms, signs, sensory perception thresholds, and autonomic function were not abnormal in this cohort of children, despite current pediatric guidelines[1–3] endorsing the assessment of symptoms, signs, vibration perception, and monofilament testing to identify early diabetic neuropathy in children. Our findings are in contrast to previously reported studies showing autonomic dysfunction in children and adolescents with type 1 diabetes.[35–37] This may be explained by differences in methods used to evaluate QST and AFT as well as differences in the population studied. Nevertheless, when combined with a retinopathy screen, CCM may be a practical screening test to reliably identify signs of neuropathy earlier than traditional clinical assessments. It is well tolerated by the children in this age group and can be completed in a few minutes by trained personnel. We had already shown that CCM had good reproducibility with excellent intra-individual and inter-individual variability in pediatric subjects.[18]

Strengths of our study included use of stringent and widely accepted criteria for the assessment of diabetic neuropathy that is, the San Antonio criteria of the American Diabetes Association and American Academy of Neurology,[24] as well as inclusion of a healthy control group assessed with the same methodology. Use of a 2SD cut-off from this control group to define normal from abnormal makes the comparison more robust. A limitation of this study is the small number of participants with subclinical neuropathy and lack of overt neuropathy. This likely explains the inability to reproduce the findings from adult cohorts, including individuals with overt neuropathy.[14–16,38] However, a low frequency of overt diabetic neuropathy is expected in children with T1DM. Secondly, as we used the simplified protocol to test for nerve conduction studies and did not include testing of the upper limb. This may have resulted an underestimation of cases with subclinical neuropathy as up to 20% of children with diabetes can have abnormal NCS of the upper limbs.[39] Thirdly, while several measures were used to classify the subjects as having diabetic neuropathy, these did not include intra-epidermal nerve fiber density, the gold standard measure to identify early small fiber damage. Previously, we showed comparable corneal and intra-epidermal nerve fiber loss in adults with diabetic neuropathy,[40] suggesting that IENFD may also be reduced in children with corneal nerve loss. However, skin biopsy is an invasive procedure and cannot be justified in children with subclinical diabetic neuropathy.

In conclusion, we show that both decreased corneal nerves and abnormal nerve conduction studies can identify early evidence of diabetic neuropathy in children without diabetic retinopathy or microalbuminuria. Longitudinal studies will explore whether corneal nerve loss has the same predictive value for developing clinical neuropathy as in adults with type 1 diabetes. CCM has the potential to be a rapid, well-tolerated, and reproducible test for identifying early subclinical neuropathy in children with type 1 diabetes.

AUTHOR CONTRIBUTIONS

Danièle Pacaud, Kenneth G. Romanchuk, Jean K. Mah, Alberto Nettel-Aguirre, Douglas W. Zochodne, and Rayaz A. Malik designed the study. Danièle Pacaud, Heidi Virtanen, Maryam Ferdousi, Kenneth G. Romanchuk, Mitra Tavakoli, and Jean K. Mah participated in data acquisition. Danièle Pacaud, Heidi Virtanen, Maryam Ferdousi, Kenneth G. Romanchuk, Jean K. Mah, Alberto Nettel-Aguirre, Mitra Tavakoli, Douglas W. Zochodne and Rayaz A. Malik participated in data interpretation, reviewed the manuscript for scholarly content and accuracy, and gave approval for the final draft. Danièle Pacaud is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

ACKNOWLEDGMENTS

We are grateful to the families and patients who agreed to participate in this study. We would also like to acknowledge Dr. William Astle and Dr. Linda Cooper for performing the eye examination of some patients during the initial visit; the ophthalmic photographer Brad Uphill for taking the fundus photographs; Dr. Amin Kherani for interpreting the retinal photographs in a masked manner; the orthoptists Christine Millar, Charlene Penner, and Heather Sandusky for performing the corneal confocal microscopy; Valerie Hebert for performing the neurophysiology tests; and Andrei Nastase for help with coordination of the study. This project was funded by grants from the JDRF International (Grant: 17-2008-1032) and the University of Calgary Lions Sight Center Fund Award.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/pedi.13419.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

REFERENCES

1. Diabetes Canada Clinical Practice Guidelines Expert Committee, Wherrett DK, Ho J, et al. Type 1 diabetes in children and adolescents. *Can J Diabetes*. 2018;42((Suppl 1)):S234-S246. doi:10.1016/j.jcjd.2017.10.036
2. Donaghue KC, Marcovecchio ML, Wadwa RP, et al. ISPAD clinical practice consensus guidelines 2018: microvascular and macrovascular complications in children and adolescents. *Pediatr Diabetes*. 2018;19-((Suppl 27)):262-274. doi:10.1111/pedi.12742
3. American Diabetes Association. Children and adolescents: standards of medical Care in Diabetes 2021. *Diabetes Care*. 2021;44(1):S180-S199. doi:10.2337/dc21-S013
4. Karsidag S, Morali S, Sargin M, Salman S, Karsidag K, Us O. The electrophysiological findings of subclinical neuropathy in patients with recently diagnosed type 1 diabetes mellitus. *Diabetes Res Clin Pract*. 2005;67(3):211-219.
5. Verrotti A, Loiacono G, Mohn A, Chiarelli F. New insights in diabetic autonomic neuropathy in children and adolescents. *Eur J Endocrinol*. 2009;161(6):811-818. doi:10.1530/EJE-09-0710
6. Bao XH, Wong V, Wang Q, Low LC. Prevalence of peripheral neuropathy with insulin-dependent diabetes mellitus. *Pediatr Neurol*. 1999;20(3):204-209.
7. Nelson D, Mah JK, Adams C, et al. Comparison of conventional and non-invasive techniques for the early idenfification of diabetic neuropathy in children and adolescents with type 1 diabetes. *Pediatr Diabetes*. 2006;7:305-310.
8. Hirschfeld G, von Glischinski M, Blankenburg M, Zernikow B. Screening for peripheral neuropathies in children with diabetes: a systematic review. *Pediatrics*. 2014;133(5):e1324-e1330. doi:10.1542/peds.2013-3645
9. Hajas G, Kissova V, Tirpakova A. A 10-yr follow-up study for the detection of peripheral neuropathy in young patients with type 1 diabetes. *Pediatr Diabetes*. 2016;17(8):632-641. doi:10.1111/pedi.12382
10. Ghaemi N, Hasanabadi H, Ashrafzadeh F, Sarvari S, Rahimi H, Hashemian S. Peripheral neuropathy in children and adolescents with insulin-dependent diabetes mellitus. *Iran J Child Neurol*. 2018;12(2):83-90.
11. Turkyilmaz H, Guzel O, Edizer S, Unalp A. Evaluation of polyneuropathy and associated risk factors in children with type 1 diabetes mellitus. *Turk J Med Sci*. 2017;47(3):942-946. doi:10.3906/sag-1601-183
12. Galosi E, Hu X, Michael N, Nyengaard JR, Truini A, Karlsson P. Redefining distal symmetrical polyneuropathy features in type 1 diabetes: a systematic review. *Acta Diabetol*. 2022;59(1):1-19. doi:10.1007/s00592-021-01767-x
13. Louraki M, Karayianni C, Kanaka-Gantenbein C, Katsalouli M, Karavanaki K. Peripheral neuropathy in children with type 1 diabetes. *Diabetes Metab*. 2012;38(4):281-289. doi:10.1016/j.diabet.2012.02.006
14. Lewis EJH, Lovblom LE, Ferdousi M, et al. Rapid corneal nerve fiber loss: a marker of diabetic neuropathy onset and progression. *Diabetes Care*. 2020;43(8):1829-1835. doi:10.2337/dc19-0951
15. Pritchard N, Edwards K, Russell AW, Perkins BA, Malik RA, Efron N. Corneal confocal microscopy predicts 4-year incident peripheral neuropathy in type 1 diabetes. *Diabetes Care*. 2015;38(4):671-675. doi:10.2337/dc14-2114
16. Tavakoli M, Ferdousi M, Petropoulos IN, et al. Normative values for corneal nerve morphology assessed using corneal confocal microscopy: a multinational normative data set. *Diabetes Care*. 2015;38(5):838-843. doi:10.2337/dc14-2311
17. Petropoulos IN, Ponirakis G, Khan A, et al. Corneal confocal microscopy: ready for prime time. *Clin Exp Optom: J Aust Optometr Assoc*. 2020;103(3):265-277. doi:10.1111/cxo.12887
18. Pacaud D, Romanchuk KG, Tavakoli M, et al. The reliability and reproducibility of corneal confocal microscopy in children. *Invest Ophthalmol Vis Sci*. 2015;56(9):5636-5640. doi:10.1167/iovs.15-16995
19. Sellers EA, Clark I, Tavakoli M, Dean HJ, McGavock J, Malik RA. The acceptability and feasibility of corneal confocal microscopy to detect early diabetic neuropathy in children: a pilot study. *Diabet Med*. 2013;30(5):630-631. doi:10.1111/dme.12125
20. Gad H, Al-Jarrah B, Saraswathi S, et al. Corneal nerve loss in children with type 1 diabetes mellitus without retinopathy or microalbuminuria. *J Diabetes Investig*. 2020;11(6):1594-1601. doi:10.1111/jdi.13313
21. Cozzini T, Piona C, Marchini G, et al. In vivo confocal microscopy study of corneal nerve alterations in children and youths with type 1 diabetes. *Pediatr Diabetes*. 2021;22(5):780-786. doi:10.1111/pedi.13219
22. Deak EA, Szalai E, Toth N, Malik RA, Berta A, Csutak A. Longitudinal changes in corneal cell and nerve fiber morphology in young patients with type 1 diabetes with and without diabetic retinopathy: a 2-year follow-up study. *Invest Ophthalmol Vis Sci*. 2019;60(2):830-837. doi:10.1167/iovs.18-24516
23. Canadian Paediatric Society, College of Family Physicians of Canada, Community Health Nurses of Canada, Dietitians of Canada and Canadian, Pediatric Endocrine Group WHO growth charts for Canada. Accessed May 3, 2022. https://cpeg-gcep.net/content/who-growth-charts-canada
24. England JD, Gronseth GS, Franklin G, et al. Distal symmetric polyneuropathy: a definition for clinical research: report of the American Academy of Neurology, the American Association of Electrodiagnostic Medicine, and the American Academy of physical medicine and rehabilitation. *Neurology*. 2005;64(2):199-207.
25. Weintrob N, Amitay I, Lilos P, Shalitin S, Lazar L, Josefsberg Z. Bedside neuropathy disability score compared to quantitative sensory testing for measurement of diabetic neuropathy in children, adolescents, and young adults with type 1 diabetes. *J Diabetes Complications*. 2007;21(1):13-19.
26. Kimura J. *Electrodiagnosis in Diseases of Nerve and Muscle: Principle and Practice*. Davis; 1989.
27. Dyck PJ, Larson TS, O'Brien PC, Velosa JA. Patterns of quantitative sensation testing of hypoesthesia and hyperalgesia are predictive of diabetic polyneuropathy: a study of three cohorts. Nerve growth factor study group. *Diabetes Care*. 2000;23(4):510-517.
28. Freeman R, Wieling W, Axelrod FB, et al. Consensus statement on the definition of orthostatic hypotension, neurally mediated syncope and the postural tachycardia syndrome. *Clin Auton Res*. 2011;21(2):69-72. doi:10.1007/s10286-011-0119-5
29. Tavakoli M, Malik RA. Corneal confocal microscopy: a novel noninvasive technique to quantify small fibre pathology in peripheral neuropathies. *J Vis Exp*. 2011;47:2194. doi:10.3791/2194
30. Vagenas D, Pritchard N, Edwards K, et al. Optimal image sample size for corneal nerve morphometry. *Optom Vis Sci*. 2012;89(5):812-817. doi:10.1097/OPX.0b013e31824ee8c9
31. Petropoulos IN, Ponirakis G, Ferdousi M, et al. Corneal confocal microscopy: a biomarker for diabetic peripheral neuropathy. *Clin Ther*. 2021;43:1457-1475. doi:10.1016/j.clinthera.2021.04.003
32. Ferdousi M, Kalteniece A, Azmi S, et al. Diagnosis of neuropathy and risk factors for corneal nerve loss in type 1 and type 2 diabetes: a corneal confocal microscopy study. *Diabetes Care*. 2021;44(1):150-156. doi:10.2337/dc20-1482
33. Wu T, Ahmed A, Bril V, et al. Variables associated with corneal confocal microscopy parameters in healthy volunteers: implications for diabetic neuropathy screening. *Diabet Med*. 2012;29(9):e297-e303. doi:10.1111/j.1464-5491.2012.03678.x
34. Hermann JM, Miller KM, Hofer SE, et al. The transatlantic HbA1c gap: differences in glycaemic control across the lifespan between people included in the US T1D exchange registry and those included in the German/Austrian DPV Registry. *Diabet Med*. 2020;37(5):848-855. doi:10.1111/dme.14148
35. Franceschi R, Mozzillo E, Di Candia F, et al. A systematic review of the prevalence, risk factors and screening tools for autonomic and

diabetic peripheral neuropathy in children, adolescents and young adults with type 1 diabetes. *Acta Diabetol*. 2022;59(3):293-308. doi:10.1007/s00592-022-01850-x

36. Clarke CF, Piesowicz AT, Spathis GS. Pupillary size in children and adolescents with type 1 diabetes. *Diabet Med*. 1989;6:780-783.
37. Kallinikou D, Soldatou A, Tsentidis C, et al. Diabetic neuropathy in children and adolescents with type 1 diabetes mellitus: diagnosis, pathogenesis, and associated genetic markers. *Diabetes Metab Res Rev*. 2019;35(7):e3178. doi:10.1002/dmrr.3178
38. Perkins BA, Lovblom LE, Lewis EJH, et al. Corneal confocal microscopy predicts the development of diabetic neuropathy: a longitudinal diagnostic multinational consortium study. *Diabetes Care*. 2021;44(9):2107-2114. doi:10.2337/dc21-0476
39. Louraki M, Katsalouli M, Kanaka-Gantenbein C, et al. The prevalence of early subclinical somatic neuropathy in children and adolescents with type 1 diabetes mellitus and its association with the persistence of autoantibodies to glutamic acid decarboxylase (GAD) and islet antigen-2 (IA-2). *Diabetes Res Clin Pract*. 2016;117:82-90. doi:10.1016/j.diabres.2016.04.044
40. Quattrini C, Tavakoli M, Jeziorska M, et al. Surrogate markers of small fiber damage in human diabetic neuropathy. *Diabetes*. 2007;56(8):2148-2154.

How to cite this article: Pacaud D, Romanchuk KG, Virtanen H, et al. Corneal nerve and nerve conduction abnormalities in children with type 1 diabetes. *Pediatr Diabetes*. 2022;23(8):1665-1673. doi:10.1111/pedi.13419