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RESEARCH ARTICLE

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Explanations for less small fibre neuropathy in South Asian versus European subjects with type 2 diabetes in the UK

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Abstract

Background: Low foot ulcer risk in South Asian, compared with European, people with type 2 diabetes in the UK has been attributed to their lower levels of neuropathy. We have undertaken a detailed study of corneal nerve morphology and neuropathy risk factors, to establish the basis of preserved small nerve fibre function in South Asians versus Europeans.

Methods: In a cross-sectional, population-based study, age- and sex-matched South Asians (n = 77) and Europeans (n = 78) with type 2 diabetes underwent neuropathy assessment using corneal confocal microscopy, symptoms, signs, quantitative sensory testing, electrophysiology and autonomic function testing. Multivariable linear regression analyses determined factors accounting for ethnic differences in small fibre damage.

Results: Corneal nerve fibre length (22.0 ± 7.9 vs. 19.3 ± 6.3 mm/mm^2; P = 0.037), corneal nerve branch density (geometric mean (range): 60.0 (4.7-246.2) vs. 46.0 (3.1-129.2) no./mm^2; P = 0.021) and heart rate variability (geometric mean (range): 7.9 (1.4-27.7) vs. 6.5 (1.5-22.0); P = 0.044), were significantly higher in South Asians vs. Europeans. All other neuropathy measures did not differ, except for better sural nerve amplitude in South Asians (geometric mean (range): 10.0 (1.3-43.0) vs. 7.2 (1.0-30.0); P = 0.006). Variables with the greatest impact on attenuating the P value for age- and HbA_{1C}-adjusted ethnic difference in corneal nerve fibre length (P = 0.032) were pack-years smoked (P = 0.13), BMI (P = 0.062) and triglyceride levels (P = 0.062).

Conclusions: South Asians have better preserved small nerve fibre integrity than equivalent Europeans; furthermore, classic, modifiable risk factors for coronary heart disease are the main contributors to these ethnic differences. We suggest that improved autonomic neurogenic control of cutaneous blood flow in Asians may contribute to their protection against foot ulcers.

KEYWORDS

corneal nerves, ethnicity, foot ulcer, neuropathy, South Asian

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1 | INTRODUCTION

Lower limb amputation, as a consequence of foot ulceration, is a major cause of morbidity and is associated with a high mortality in type 2 diabetes mellitus, highlighting the importance of defining mechanisms and risk factors to enable targeted interventions for improving outcomes. It is therefore fascinating that migrant populations of South Asian descent to the UK, with their considerably elevated risk of type 2 diabetes and ischemic heart disease[1,2], have substantially lower risks of foot ulceration and amputation (3- and 4-fold lower, respectively) compared with Europeans[3-5]. Furthermore, recently the DISTANCE study showed that Asians in general, and South Asians in particular, in the US had a markedly reduced risk of lower extremity amputation[6]. The underlying basis for this protection, however, is not clearly understood and demonstrates differing pathophysiologies of diabetes according to ethnicity[7].

Peripheral neuropathy *per se* is one of the strongest risk factors for diabetic foot ulceration and amputation[7-11]. We have previously shown that lower levels of clinical neuropathy in Asians accounted for approximately half of their reduced risk of foot ulceration[5]. Furthermore, in a population-based sample of UK primary care patients with type 2 diabetes we have also demonstrated better large and small fibre function in South Asians vs. Europeans[12]. Better peripheral vascular function and lower smoking rates in South Asians, rather than differences in glycaemic control and hyperlipidaemia largely accounted for this difference.

It is established that small fibre damage precedes large fibre damage in diabetic neuropathy[13,14]. Furthermore, small fibre damage results in loss of pain sensation and autonomic control of cutaneous blood flow[15,16]; both are permissive factors for foot ulceration. Perhaps of relevance, we have previously shown that South Asians without 'clinical neuropathy' maintained a 50% increased risk of painful neuropathy-like symptoms compared with similar Europeans[17]; this could be suggestive of a pathology other than neuropathy in the legs accounting for increased pain in South Asians but may also indicate a greater prevalence of small fibre damage in the European cohort.

Our recent study indicated that South Asian populations with type 2 diabetes have some protection against the development of small fibre neuropathy[12]. There is an increasing body of evidence supporting the use of CCM to study small fibre neuropathy, including autonomic dysfunction[18-20]. We hypothesized, therefore, that ethnic differences in small nerve fibre function would be supported by differences in corneal nerve fibre morphology and intra-epidermal nerve fibre density in South Asians compared with Europeans. Furthermore, we aimed to determine which risk factors explained these ethnic differences in small nerve fibre structure and hence the South Asian protection against foot ulceration.

2 | MATERIALS AND METHODS

2.1 | Study subjects

This is a sub-study of a population-based bi-ethnic cohort of people with type 2 diabetes (n = 360), half European and half of South Asian descent, recruited from eight primary care registers in Manchester, UK[12]. Participants were randomly selected from within ethnicity, gender and age stratified groups to ensure matching for these variables; study participants and non-participants were similar for age and gender. European and South Asian groups were considered representative ethnic samples of primary care patients with type 2 diabetes in Greater Manchester[12]. All participants consented that they may be re-contacted for follow-up.

Approximately 4 years (2007-2012) after their initial visit each participant was invited to re-attend The Wellcome Trust Clinical Research Facility, Manchester, via a postal invitation, for a single follow-up visit. Exclusion criteria at follow-up were major lower limb amputation (distal symmetrical polyneuropathy assessment required testing in both feet), psychiatric disorder, HIV, hepatitis, or terminal illness. Of the 360 original subjects, 81 did not respond to the postal invitation and were untraceable; a further 45 had moved away from the health authority and were untraceable; 22 responded negatively and did not wish to participate further, 28 responded positively, were eligible, but later changed their minds and decided not to participate, 1 subject responded positively but was ineligible due to the development of a significant psychiatric disorder and 28 had died. No-one was excluded due to lower limb amputation. One-hundred and fifty-five individuals re-consented to participate in the follow-up study (response rate of 43%), with an equal ethnic split (South Asian n = 77, European n = 78). Differences between responders (n = 155) and non-responders (n = 205) were not significant for ethnicity, age, diabetes duration, BMI or HbA_{1c}; however, a higher proportion of men re-consented (consenters - M: F 95:59; non-consenters - M: F 96:110; P = 0.005). This study was approved by North Manchester Research Ethics Committee and by the University of Manchester Research Ethics Committee and written informed consent was obtained according to the declaration of Helsinki.

2.2 | Clinical assessments

All subjects completed a questionnaire including detailed past medical history, current medication and lifestyle factors, including smoking behaviour. Self-assigned ethnicity was checked and cross-validated with the country of birth. All South Asians were first generation migrants from India, Pakistan or Bangladesh. Height, weight, waist and hip circumference were measured once. Triplicate resting blood pressure readings were averaged using a standard protocol[12]. Blood samples were assessed in central laboratories for HbA_{1c}, total cholesterol, HDL-cholesterol, triglycerides and fibrinogen.

2.3 | Complications assessment

Retinopathy and nephropathy were defined as a history of physician diagnosed disease. Cardiovascular Disease (CVD) was defined as either a physician diagnosed myocardial infarction, angina, or a positive response to the Rose angina questionnaire[7,21]. Peripheral arterial disease was defined as a previous history of physician diagnosed claudication confirmed by a lower limb arteriogram or a positive response to the Edinburgh claudication questionnaire[22]. Transcutaneous partial pressure of oxygen ($TcPO_2$) was measured on the dorsum of the left foot[12].

2.4 | Assessment of neuropathy

Neurological symptoms of muscle weakness, sensation and autonomic neuropathy were evaluated using the Neuropathy Symptoms and Change validated questionnaire score[23] and the McGill pain analogue score was used to assess the severity of painful neuropathic symptoms. Neurological signs were assessed using the modified neuropathy disability score, evaluating vibration, pinprick and temperature perception and ankle reflexes[12]. Vibration Perception Threshold was measured on the tip of the right and left hallux using a Neurothesiometer (Horwell, Scientific Laboratory Supplies, Wilford, Nottingham, UK); mean readings were used for analysis. Electrophysiological assessments of peroneal and sural nerves in the right lower limb were performed by a Neurophysiologist using a Dantec "Keypoint" electromyography system (Dantec Dynamics Ltd, Bristol, UK) with surface electrodes at standardised points (skin temperature $\geq$ 32°C).

2.5 | Small fibre function

Cold sensation (Aδ fibre) and warm sensation (C fibre) thresholds were determined with the MEDOC TSA II (Medoc Ltd., Ramat Yishai 30095, Israel) using the method of limits on the dorsum of the left foot, as previously described[20]. Changes to heart rate variability in response to deep breathing (HRV-DB) was determined using the CASE IV system and ECG monitor, averaging two separate cycles of deep breathing[12]. Peripheral cholinergic function (sudomotor function) was assessed using the Neuropad, on the plantar skin surface of both feet[24].

2.6 | Intra epidermal nerve fibre density

Skin biopsies were taken in a sub-group of 49 of the 155 patients; this sub-group was matched for ethnicity (24 South Asians vs 25 Europeans), age (61.3 ± 7.9 vs 62.5 ± 7.8 years), gender (15 men: 9 women vs 20 men: 5 women) and HbA_{1C} (8.0 ± 1.3 vs 7.8 ± 1.4%). A 3-mm punch skin biopsy was taken from the dorsum of the foot and stained with protein gene product 9.5 to identify and count intra-epidermal nerve fibres and establish intra epidermal nerve fibre density, *i.e.* the number of fibres per millimetre of basement membrane[25].

2.7 | Corneal confocal microscopy

Participants underwent examination with the Heidelberg Retina Tomograph (HRT III) in vivo corneal confocal microscope (IVCCM) using our established protocol[26]. Several scans of the entire depth of the cornea were recorded to provide en face two dimensional images with a lateral resolution of approximately 2 μm/pixel and final image size of 400 x 400 pixels of the sub-basal nerve plexus of the cornea. Five images per patient from the centre of the cornea were selected and examined in a masked and randomised fashion[27]. Four corneal nerve parameters were quantified: (i) Corneal nerve fibre density - number of major nerves/mm^2 of corneal tissue; (ii) Corneal nerve branch density - number of branches emanating from all major nerve trunks/mm^2 of corneal tissue; (iii) Corneal nerve fibre length - length of all nerve fibres and branches (mm/mm^2) within the area of corneal tissue; (iv) tortuosity – a mathematically derived index of the curvilinear deviation from the midline of each main nerve fibre[28].

2.8 | Statistical analysis

Corneal nerve fibre length was selected as the primary corneal nerve outcome to assess small nerve fibre structure, as it combines data for both branch and fibre length. We estimated that 160 participants (80 in each ethnic group) would be sufficient with 85% power to detect a difference in corneal nerve fibre length of at least one-third of SD at the 5% significance level, making allowances for missing data. Neuropathy measures were compared between the ethnic groups as continuous variables using simple means. Several comparison tests have been carried out and hence the results of these tests should be interpreted as showing an indication of a possible difference.

Normally distributed data were tested using Student's *t* test, non-normally distributed data were either log transformed before analysis or the Mann-Whitney test was used. Categorical variables were compared as simple proportions and tested using chi-square test for significance.

Independent statistically significant variables for the neuropathy outcomes were found using a backward stepwise selection procedure in the multivariable regression models. Ethnicity and other neuropathy risk factors (duration of diabetes, HbA_{1C}, triglycerides, BMI) were considered for selection.

Multivariable linear regression models were constructed to investigate the effect of potential confounders previously shown to be associated with neuropathy, including glycaemic control, height, smoking, measures of hypertension, obesity and hyperlipidaemia and determine which factors could account for any ethnic differences in neuropathy measures.

Sensitivity analyses were performed to examine the data further. Generalised additive models (GAMs) were used to investigate if any of the covariates required a more complex relationship than linear with the outcome. These analyses were performed in R version 3.1. Heckman selection models were used to determine if there was selection bias that affected the outcome models and was implemented in Stata 13. All other analyses were performed using SPSS 20 or 22 (IBM).

3 | RESULTS

We studied 155 people with type 2 diabetes (4.9 ± 0.6 years post original visit) comprising 77 South Asians and 78 Europeans. South Asians were younger (P = 0.047) and had a (close to significantly) longer duration of diabetes (P = 0.063) than Europeans (Table 1). South Asians were shorter (P = 0.046) and had a lower BMI (P < 0.001). There was no significant difference in the prevalence of clinically evident CVD or retinopathy and nephropathy. There was no difference in systolic and diastolic blood pressure; antihypertensive therapy use was lower in South Asians (P = 0.044). Total cholesterol and HDL-cholesterol were comparable but triglyceride levels (P = 0.001) were lower in South Asians; a lower percentage of South Asians used statins but the difference was not significant (p = 0.074). South Asians had a higher HbA_{1c} (P = 0.016).

TABLE 1 Demographic and clinical characteristics of ethnic groups

	South Asians	Europeans	P-value
n (M/F)	77 (51/26)	78 (45/33)	0.27
Age (years)	61.2 ± 9.7	64.0 ± 8.0	0.047
Duration diabetes (years)	11.9 (4.2-35.2)	10.3 (4.8-30.9)	0.063
Height (m)	1.63 ± 0.10	1.66 ± 0.09	0.046
Weight (kg)	80.1 ± 14.0	93.0 ± 17.3	<0.001
BMI (kg/m^2)	30.2 ± 5.2	33.6 ± 5.4	<0.001
Waist (cm)	103.6 ± 11.2	112.6 ± 12.1	<0.001
Hips (cm)	103.0 ± 13.1	113.2 ± 12.3	<0.001
Waist-to-hip ratio	0.99 (0.82-2.23)	0.99 (0.84-1.42)	0.78
HbA_{1c} (%) HbA_{1c} (mmol/Mol)	7.8 (5.8-14.2) 62 (40-132)	7.3 (5.6-12.6) 57 (38-114)	0.016
Total cholesterol (mmol/l)	3.8 (2.1-7.3)	4.0 (1.9-7.7)	0.34
HDL-cholesterol (mmol/l)	1.1 ± 0.2	1.1 ± 0.4	0.49
Triglycerides (mmol/l)	1.7 (0.6-5.2)	2.2 (0.8-7.9)	0.001
Fibrinogen (g/l)	3.6 (2.1-7.6)	3.7 (1.9-5.6)	0.42
Retinopathy (%)	22/64 (34)	21/52 (29)	0.48
Nephropathy (%)	9/62 (15)	5/75 (7)	0.13
Myocardial infarction (%)	12/69 (17)	12/75 (16)	0.82
Angina (%)	19/64 (30)	15/74 (20)	0.20
Coronary artery bypass graft (%)	13/70 (19)	11/74 (15)	0.55
Resting heart rate (bpm)	75.7 ± 14.3	74.9 ± 12.6	0.72
Systolic blood pressure (mmHg)	132.8 ± 16.2	135.8 ± 16.6	0.26
Diastolic blood pressure (mmHg)	72.2 ± 9.8	70.4 ± 9.3	0.27
$TcPO_2$ (mmHg)	62.0 ± 9.9	57.7 ± 12.6	0.019
Peripheral arterial disease (%)	5/58 (9)	15/74 (20)	0.064
Peripheral vascular surgery	2/67 (3)	6/69 (9)	0.27
Pack-years smoked (n)*	0, 0-14.9 (0-78)	10.5, 0-31 (0-99)	0.006
Insulin therapy (%)	14/69 (20)	20/67 (30)	0.20
Oral antidiabetic drugs (%)	64/69 (93)	62/68 (91)	0.73
Antihypertensive drugs (%)	45/69 (65)	54/67 (81)	0.044
Statin therapy (%)	53/68 (78)	59/66 (89)	0.074

Data are % prevalence, means ± SD, or geometric means (range).

*Data presented as median, IQR (range).

$TcPO_2$, transcutaneous partial pressure of oxygen.

3.1 | Clinical signs and symptoms

There was no difference in neuropathy disability score or vibration perception threshold between Europeans and South Asians (Table 2) and both groups had evidence of mild neuropathy. Painful diabetic neuropathy (significant signs plus at least one positive sensory symptom) did not differ between the groups, however painful neuropathy-like symptoms in the absence of clinical neuropathy (no signs but at least one positive sensory symptom) were more prevalent in South Asians (P = 0.018).

3.2 | Neurophysiology

Peroneal nerve conduction velocity, amplitude and F wave latency did not differ significantly between groups. Sural nerve conduction velocity was comparable but South Asians had a significantly higher sural nerve amplitude (μA) than Europeans (geometric mean (range): 10.0 (1.3-43.0) vs 7.2 (1.0-30.0), P = 0.006) (Table 2).

3.3 | Small nerve fibre function

Thermal threshold assessments: cold sensation (median, IQR: 25.9, 23.1-27.7 vs. 25.6, 22.9-27.2), warm sensation (41.6 ± 4.2 vs. 42.0 ± 3.8), cold-induced pain (median, IQR: 1.9, 0-9.4 vs. 1.3, 0-5.9) and heat-induced pain (median, IQR: 49.1, 46.8-50.0 vs. 49.0, 47.4-49.9) did not differ between South Asian and European participants (Table 2). There was no significant difference for the Neuropad response (%) (median, IQR: 68.8, 30.6-95.0 vs. 55.0, 15.0-87.5) between South Asians and Europeans. Heart rate variability to deep breathing (HRV-DB) (beats/min) (geometric mean (range): 7.9 (1.4-27.7) vs 6.5 (1.5-22.0), P = 0.044) was significantly higher in South Asians compared with Europeans.

TABLE 2 Measures of neuropathy signs, symptoms, nerve fibre function, corneal nerve fibre structure and intra epidermal nerve fibre density by ethnicity

Neuropathy variables	South Asian	European	P-value*
Neuropathy signs			
Neuropathy disability score (0-10)**	2, 1-6 (0-10)	3, 1.8-6 (0-10)	0.50
Vibration perception threshold (volts)	12.2 (2.5-40.7)	14.2 (3.7-50.0)	0.16
Neuropathy symptoms			
Neuropathy symptoms and change (0-38)	4, 2-11 (0-23)	4, 2-9 (0-23)	0.57
McGill dimension of pain (0-5)**	1, 0-2 (0-4)	1, 0-2 (0-5)	0.77
McGill visual analogue score (0-10)**	4, 0-6.3 (0-10)	3, 0-6 (0-10)	0.19
McGill Total pain rating index (0-60)**	8, 1-19 (0-41)	7.5, 2-16.5 (0-49)	0.92
Painful neuropathy (%)	30/75 (40)	36/77 (47)	0.40
Painful symptoms, without neuropathy (%)	31/75 (41)	18/77 (23)	0.018
Functional measures			
Sural nerve conduction velocity (m/s)	45.3 ± 7.1	45.2 ± 6.8	0.95
Sural amplitude (μV)	10.0 (1.3-43.0)	7.2 (1.0-30.0)	0.006
Peroneal nerve conduction velocity (m/s)	44.7 ± 5.5	44.4 ± 6.0	0.74
Peroneal nerve amplitude (mV)**	3.3, 1.6-5.3 (0.1-43.0)	2.6, 1.7-4.5 (0.1-7.4)	0.25
Peroneal nerve F wave latency (m/s)	51.0 ± 7.2	53.0 ± 5.9	0.091
Heart rate variability deep breathing (bpm)	7.9 (1.4-27.7)	6.5 (1.5-22.0)	0.044
Neuropad colour change (%)**	68.8, 30.6-95.0 (0-100)	55.0, 15.0-87.5 (0-100)	0.11
Cold sensation threshold (°C)**	25.9, 23.1-27.7 (1.4-30.2)	25.6, 22.9-27.2 (0-30.3)	0.30
Warm sensation threshold (°C)	41.6 ± 4.2	42.0 ± 3.8	0.53
Cold induced pain (°C)**	1.9, 0-9.4 (0-23.0)	1.3, 0-5.9 (0-21.6)	0.31
Heat induced pain (°C)**	49.1, 46.8-50.0 (42.7-50.0)	49.0, 47.4-49.9 (44.4-50.0)	0.80
Corneal confocal morphometry			
Corneal nerve fibre length (mm/mm^2)	22.0 ± 7.9	19.3 ± 6.3	0.037
Corneal nerve fibre density (no/mm^2)	24.7 ± 8.2	25.1 ± 7.4	0.76
Corneal nerve branch density (no/mm^2)	60.0 (4.7-246.2)	46.0 (3.1-129.2)	0.021
Corneal nerve fibre tortuosity coefficient	18.7 ± 4.7	20.1 ± 6.2	0.16
Intra-epidermal nerve fibre density (no/mm^2)	2.9 (0.5-7.5)	2.1 (0.2-6.5)	0.14

Data are % prevalence, means ± SD, or geometric means (range).

*P value for ethnic difference.

**Data presented as median, IQR (range).

3.4 | Small nerve fibre structure

Intra epidermal nerve fibre density was slightly higher in South Asian participants than in European participants, though the difference was not statistically significant (Table 2). Corneal nerve fibre density and tortuosity were comparable between the ethnic groups. However, both corneal nerve fibre length (mm/mm^2) (22.0 ± 7.9 vs. 19.3 ± 6.3; P = 0.037) and corneal nerve branch density (no./mm^2) (geometric mean (range): 60.0 (4.7-246.2) vs. 46.0 (3.1-129.2); P = 0.021) were significantly higher in South Asians than Europeans (Table 2).

Multivariable stepwise regression analyses established that ethnicity (P = 0.032), age (P = 0.004) and HbA_{1C} (P < 0.001) were independent statistically significant risk factors for corneal nerve fibre length (Table 3). The exact same variables were risk factors for corneal nerve branch density (ethnicity P = 0.026, age P = 0.007, HbA_{1C} P = 0.011). The independent statistically significant risk factors for sural nerve amplitude were ethnicity (P = 0.006), age (P < 0.001) and duration of diabetes (P = 0.003). Age (P < 0.001) and hypertensive therapy (P = 0.003) were independently significant for heart rate deep breathing but ethnicity was not statistically significant (Table 4).

3.5 | Explanations for ethnic differences in corneal nerve fibre structure

Multivariable analyses were used to explore the role of neuropathy risk factors, i.e. glycaemic control, height, smoking, vascular function, measures of hypertension, obesity and hyperlipidaemia as potential explanations for the ethnic difference in corneal nerve fibre length (Table 3). The variables with the greatest impact on attenuating the P value for the age- and HbA_{1C}-adjusted ethnic difference in corneal nerve fibre length (P = 0.032) were BMI (P = 0.062), triglyceride levels (P = 0.062) and pack-years smoked (P = 0.13). There was no appreciable impact of diabetes duration, or vascular factors (blood pressure, $TcPO_2$) on the corneal nerve fibre length model by ethnicity.

To further investigate the effect of smoking, we compared corneal nerve fibre length in never smokers, although numbers were small. The ethnic difference did not remain in this sub-group (age and HbA_{1C} -adjusted estimated marginal means were 20.7 (17.9-23.5 [95% CI]) mm/mm^2 (South Asian n = 30) and 21.7 (18.0-25.3) mm/mm^2 (European n = 18); P = 0.68). South Asian ex-smokers, however, retained their high corneal nerve fibre length (21.2 (18.4–24.0) mm/mm^2) whereas

TABLE 3 Multivariable regression analysis and attenuation information for corneal nerve fibre length.

Variables	Parameter estimates (95% CI)	P value
Ethnicity (South Asian compared to European)	2.593 (0.225, 4.961)	0.032
Age (per year)	-0.198 (-0.331, -0.065)	0.004
HbA_{1c} (per unit)	-1.531 (-2.380, -0.682)	<0.001

Difference in corneal nerve fibre length based on ethnicity only (unadjusted), the difference based on ethnicity after adjusting for age and HbA_{1c}, and the difference based on ethnicity after adjusting for age, HbA_{1c} and one additional specified variable	Estimated marginal means for ethnicity (95% CI)		
Adjustment variables	**South Asian**	**European**	**P value for ethnicity**
Unadjusted	22.0 (20.2-23.7)	19.3 (17.6-21.0)	0.037
Adjustment for age and HbA_{1c}:			
Age (p = 0.004) + HbA_{1c} (p < 0.001)	21.9 (20.2-23.6)	19.3 (17.7-20.9)	0.032
Adjustment for age, HbA_{1c} and variable listed:			
Duration of diabetes (*p* = 0.51)	22.2 (20.4-23.9)	19.3 (17.6-21.0)	0.023
BMI (*p* = 0.33)	22.0 (20.1-23.8)	19.5 (17.7-21.3)	0.062
Triglycerides (p = 0.68)	21.8 (20.0-23.5)	19.4 (17.7-21.0)	0.062
Diastolic BP (*p* = 0.99)	21.9 (20.2-23.6)	19.3 (17.7-20.9)	0.033
$TcPO_2$ (*p* = 0.47)	21.9 (20.2-23.7)	19.2 (17.6-20.9)	0.027
Pack-years smoked (*p* = 0.39)	21.0 (19.1-22.9)	18.9 (17.1-20.7)	0.13
Hypertensive therapy (*p* = 0.62)	21.7 (19.9-23.5)	18.7 (16.8-20.6)	0.021

Statistically significant variables chosen in the backward step-wise selection procedure for corneal nerve fibre length. This procedure includes all neuropathy risk factors in the regression model and then removes the least significant variable, runs the regression model again and repeats these steps until only variables significant at the 5% level remain in the model.

P-value in parentheses shows significance of adjustment factor in the regression model.

$TcPO_2$, transcutaneous partial pressure of oxygen.

N = 130 for unadjusted, *N* = 125-129 for all other analyses except pack years smoked where *N* = 106, hypertensive where *N* = 113 and smoking status where N = 113

European ex-smokers had lower corneal nerve fibre length (17.8 (15.8-19.9) mm/mm^2, *P* = 0.053), despite the fact that pack-years smoked were not significantly different between these two sub-groups (*P* = 0.55).

The analysis for corneal nerve branch density by ethnicity showed similar results to corneal nerve fibre length. Variables with the greatest impact accounting for the ethnic difference in corneal nerve branch density estimated marginal means were pack-years smoked and BMI, changing the age- and HbA_{1C}-adjusted means from 59.3 (50.7-68.8) [95% CI] no/mm^2 (South Asian) and 46.1 (39.1-53.8) no/mm^2 (European), *P* = 0.026 to 54.8 (45.6-65.1) no./mm^2 (South Asian) and 45.0 (37.3-53.7) no./mm^2 (European), *P* = 0.13 for pack years smoked and to 58.0 (49.4-67.6) no./mm^2 (South Asian) and 47.1 (39.9-55.1) no./mm^2 (European), *P* = 0.076, for BMI.

3.6 | Explanations for ethnic differences in HRV-DB and sural nerve amplitude

The multivariable regression analysis showed that ethnicity was not an independent statistically significant variable for HRV-DB (Table 4). Multivariable modelling of HRV-DB by ethnicity showed that after adjusting for age and hypertensive therapy, which were the independent statistically significant variables, BMI attenuated the ethnicity P-value most (Table 4). The adjusted means changed from 7.9 (6.9-8.9) beats/min. (South Asian) and 7.1 (6.1-8.2) beats/min. (European), *P* = 0.27 to 7.7 (6.7-8.7) beats/min. (South Asian) and 7.2 (6.2-8.3) beats/min. (European), *P* = 0.52. There was no appreciable impact of diabetes duration, glycaemia or triglyceride differences on the HRV-DB model by ethnicity. Pack-years smoked reduced the P-value of ethnicity in the age and hypertensive therapy adjusted model to 0.13; 8.3 (7.2-9.4) beats/min. (South Asian) and 7.1 (6.1-8.3) beats/min. (European).

BMI and hypertensive therapy appeared to have the greatest impact on the ethnic difference in sural nerve amplitude after adjusting for age and duration of the diabetes, the independent statistically significant variables for sural nerve amplitude. BMI changed the age and diabetes duration adjusted estimated marginal means for ethnicity from 10.1 (8.7-11.6) μV (South Asian) and 7.5 (6.3-8.7) μV (European), *P* = 0.006 to 9.8 (8.5-11.3) μV (South Asian) and 7.7 (6.5-9.0) μV (European), *P* = 0.030 and hypertensive therapy changed them to 10.1 (8.6-11.8) μV (South Asian) and 8.1 (6.7-9.7) μV (European), *P* = 0.068. There were no additional effects of glycaemia, vascular factors or triglycerides on the sural nerve amplitude model by ethnicity.

The GAMs analyses appeared to show little difference in including spline functions for the continuous variables in the four outcome models. These analyses suggest that it is reasonable for the covariates to be modelled as linear variables in the regression. However, other functional forms should be considered in further studies as the lack of large differences between models with more complex relationships and the simple linear terms may be due to limited sample size and power.

TABLE 4 Multivariable regression analysis and attenuation information for heart rate variability to deep breathing.

Variables	Parameter estimates* (95% CI)	P value
Age (per year)	-0.019 (-0.025, -0.012)	<0.001
Hypertensive therapy (yes compared to no)	-0.186 (-0.306, -0.066)	0.003

	Estimated marginal means for ethnicity (95% CI)		
Adjustment variables	**South Asian**	**European**	**P value for ethnicity**
Unadjusted	7.9 (6.9-9.0)	6.5 (5.6-7.5)	0.044
Adjustment for age and hypertensive therapy:			
Age (p < 0.001) + hypertensive therapy (p = 0.005)	7.9 (6.9-8.9)	7.1 (6.1-8.2)	0.27
Adjustment for age, hypertensive therapy and variable listed:			
Duration of diabetes (p = 0.36)	8.0 (7.0-9.1)	7.0 (6.0-8.2)	0.18
BMI (p = 0.15)	7.7 (6.7-8.7)	7.2 (6.2-8.3)	0.52
HbA_{1c} (p = 0.27)	7.9 (7.0-9.0)	7.0 (6.1-8.1)	0.19
Triglycerides (p = 0.80)	7.9 (6.9-8.9)	7.1 (6.1-8.2)	0.30
Diastolic BP (p = 0.072)	7.9 (7.0-8.9)	7.2 (6.2-8.2)	0.28
$TcPO_2$ (p = 0.25)	7.8 (6.9-8.8)	7.1 (6.2-8.2)	0.32
Pack-years smoked (p = 0.78)	8.3 (7.2-9.4)	7.1 (6.1-8.3)	0.13

Statistically significant variables chosen in the backward stepwise selection procedure for heart rate variability to deep breathing. This procedure includes all neuropathy risk factors in the regression model and then removes the least significant variable, runs the regression model again and repeats these steps until only variables significant at the 5% level remain in the model.

*These are on the natural logarithm scale of (HRDB plus 5) i.e. each increase in age is related to a decrease of 0.019 in the natural logarithm of (HRDB plus 5).

Difference in heart rate variability to deep breathing based on ethnicity only (unadjusted), the difference based on ethnicity after adjusting for age and HbA_{1c}, and the difference based on ethnicity after adjusting for age, HbA_{1c} and one additional specified variable.

P-value in parentheses shows significance of adjustment factor in the regression model.

$TcPO_2$, transcutaneous partial pressure of oxygen.

N = 135 for unadjusted, N = 116-118 for all other analyses except pack years smoked where N = 97 and smoking status where N = 103.

The Heckman selection models considered a number of variables as possible determinants of whether a participant was in the follow-up study and the impact this had on the outcome model. In three of the four models there was a lack of evidence that the selection model has any impact on the outcome model. For the sural nerve amplitude outcome, there did appear to be a relationship between whether a participant was included in the follow-up analyses and the outcome model. However, the three variables in the model all remained statistically significant at the 5% level and 1% level. The parameter coefficients changed minimally for ethnicity and age – both were less than 7% changed – but the duration of diabetes coefficients changed by 27%, probably due to the duration of diabetes variable being significant in the selection model. Therefore, due to the small differences with the model that did not consider selection, and for consistency with the other outcomes, the results above for sural nerve amplitude are presented without adjustment for selection bias.

4 | DISCUSSION

In this detailed, primary care, population-based study, we show that South Asians with type 2 diabetes in the UK have longer corneal nerve fibres and greater corneal nerve branch densities, i.e. better preserved structure of small nerve fibres, than equivalent Europeans. Despite low levels of clinical neuropathy in both of these community-based groups, South Asians also demonstrated better cardiac autonomic function during HRV-DB testing, indicating less small fibre neuropathy, compared with Europeans, plus some preservation of electrophysiological variables, supporting our previous findings from the original cohort[12]. This is the first report of a lower prevalence of small nerve fibre structural abnormalities in South Asian compared with European people with type 2 diabetes and may provide further evidence for a potential mechanism for the lower incidence of foot ulceration and amputation in South Asians compared to Europeans. We also show that classical risk factors for coronary heart disease, *i.e.* smoking, BMI and triglyceride levels, accounted for much of the structural differences in small nerve fibres between South Asians and Europeans, with negligible effects, surprisingly, from either vascular or glycaemic factors. Although the study was sufficiently powered to determine ethnic differences in corneal morphometric measures, a significant limitation of this study is that biopsy sample numbers (and therefore intra-epidermal nerve fibre density data) lacked power to support the corneal nerve findings. A further limitation was that cardiac autonomic neuropathy was only assessed using heart rate variability on deep breathing and did not include other measures including E:I index.

Hyperglycaemia has consistently been shown to be a strong risk factor for diabetic peripheral neuropathy in epidemiological studies of people with type 1 and type 2 diabetes[29-32]. Of relevance, the large Eurodiab study showed that vascular risk factors are as important as glycaemic control in the development of diabetic neuropathy in people with type 1 diabetes[29]. In one of the few longitudinal studies assessing

the development of neuropathy in type 2 diabetes, a higher HbA_{1c} and a lower fasting and post glucose serum insulin and paradoxically, lower systolic and diastolic blood pressure were associated with the development of neuropathy[32]. Our data supports our previous findings that the lower prevalence of small fibre neuropathy in our South Asian population is not explained by favourable HbA_{1c} levels[12].

Recently elevated triglycerides have been associated with the development of neuropathy in a cohort of people predominantly with type 2 diabetes[33]. Furthermore, obesity and hyperlipidaemia have also been shown to be important risk factors for early neuropathy in prediabetes[34]. Thus our findings that lower levels of smoking and triglycerides were the major factors to explain the greater preservation of corneal nerve fibres in the South Asians supports these studies' findings. Furthermore, the lower BMI of the South Asians (body fat measure) was also the main driver of ethnic differences in sural nerve amplitude (large fibre) and heart rate deep breathing (small, autonomic fibre). Weight and triglycerides are both indicators of adiposity and the imbalanced metabolic environment in obesity[33], and therefore may be a proxy for other abnormal metabolic parameters such as oxidative stress or pro-inflammatory cytokines. One may argue that corneal nerve fibres do not represent peripheral nerve fibres regulating cutaneous blood flow, however, our recent study has shown that corneal confocal microscopy has comparable sensitivity and specificity to IENFD in the diagnosis of diabetic neuropathy[35]. Furthermore, we have also shown that a lower corneal nerve fibre length predicts incident clinical diabetic neuropathy in Type 1 diabetes[36] and worsening glucose tolerance in subjects with impaired glucose tolerance[37].

The link between CCM and CAN is an important finding and CCM has also previously been shown to correlate significantly with sudomotor and cardiac autonomic neuropathy[18-20]. Here we also show a relationship between CCM and a measure of cardiac autonomic neuropathy. Previous studies have shown stronger associations between neuropathy and CCM abnormalities due to the inclusion of patients with more severe neuropathy. However, our patient cohort was recruited from primary care and most patients had no or mild neuropathy. This may also suggest that CAN may be an early manifestation of diabetic neuropathy.

The identification of cardiovascular risk factors in attenuating the increased risk of small fibre neuropathy in Europeans vs. South Asians may provide potentially important therapeutic targets for a major end point of diabetes[38]. However, this needs to be verified in a prospective study. Furthermore, the altered pathophysiology of diabetic small fibre neuropathy, especially early neuronal changes, found here between Europeans and South Asians highlights the growing need for evidence-based medicine for treating diabetes in different ethnic groups[7].

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