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Measuring peripheral nerve involvement in Friedreich's ataxia

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Abstract

Objective: Experimental therapies under development for Friedreich's Ataxia (FRDA) require validated biomarkers. In-vivo reflectance confocal microscopy (RCM) of skin is a noninvasive way to quantify Meissner's corpuscle (MC) density and has emerged as a sensitive measure of sensory polyneuropathies. We conducted a prospective, cross-sectional study evaluating RCM of MCs and conventional peripheral nerve measures as candidate peripheral nerve markers in FRDA. **Methods**: Sixteen individuals with FRDA and 16 age- and gender-matched controls underwent RCM of MC density and morphology, skin biopsies for epidermal nerve fiber density (ENFD), nerve conduction studies (NCS), and quantitative sensory testing (QST) including touch, vibration, and cooling thresholds. **Results**: MC densities were measurable in all participants with FRDA, and were lower at digit V (hand), thenar eminence, and arch (foot) compared to controls. By contrast, sensory NCS showed floor effects and were obtainable in only 13% of FRDA participants. QST thresholds for touch, vibration, and cooling were higher at the hand and foot in FRDA than controls. Reductions in ENFDs were present in more severely affected individuals with FRDA (Friedreich's Ataxia Rating Scale (FARS) >60) compared to matched controls, although skin biopsies were not well tolerated in children. MC densities, ENFDs, and touch and vibration thresholds were associated with clinical disease severity (FARS and modified FARS) and duration since symptom onset. **Interpretation**: MC density, ENFD, and QST thresholds provide structural and physiologic markers of sensory involvement in FRDA. Longitudinal evaluation is needed to determine whether these measures can identify changes associated with disease progression or treatment.

Introduction

Friedreich's ataxia (FRDA) is an autosomal recessive disorder caused by abnormal expansion of an intronic GAA repeat, which interferes with transcription of frataxin, a mitochondrial protein.[1] FRDA usually manifests in mid to late childhood and is a multisystem disorder that affects both the central (CNS) and peripheral nervous systems (PNS) causing progressive ataxia, sensory and motor dysfunction, and variable occurrence of cardiomyopathy, diabetes, optic atrophy, scoliosis, and hearing deficits.[1,2]

There is no FDA-approved therapy for FRDA, but experimental agents are in development.[3] Clinical rating scales have been used as trial endpoints in FRDA including the Friedreich's Ataxia Rating Scale (FARS), the Scale for the Assessment and Rating of Ataxia (SARA), and the International Cooperative Ataxia Rating Scale (ICARS). However, these measures show floor affects and have limited sensitivity to disease progression over 12 months.[4–8] Furthermore, there are no validated, objective central or peripheral nervous system biomarkers of disease progression for use in clinical trials as intermediate endpoints.[9]

The predominant pathological involvement in the PNS in FRDA occurs at the dorsal root ganglion resulting in a progressive sensory neuronopathy, affecting large more so than small diameter sensory fibers, with more limited peripheral motor involvement.[10–13] Nerve conduction studies (NCS), quantitative sensory testing (QST), and skin biopsy assessment of epidermal nerve fiber density (ENFD) are commonly used and validated measures of peripheral nerve involvement in other hereditary and acquired polyneuropathies.[14–18] In FRDA, sensory nerve action potentials are commonly unobtainable or low in amplitude while motor conduction studies are often normal or only mildly abnormal, raising concerns for floor and ceiling effects, respectively.[19–22] ENFDs appear to be reduced in FRDA, although studies to date have been small and limited to more severely affected individuals than may be ideal for inclusion in clinical trials.[12,23,24] Further systematic evaluation of these peripheral nerve measures in individuals with FRDA is necessary to determine their potential utility as PNS markers for clinical trial application.

Meissner's corpuscle (MC) densities, the main touch-pressure sensory receptor in glabrous skin (hands and feet), are also reduced in FRDA, but have not been widely measured, as glabrous skin biopsies are invasive and not suitable for serial monitoring.[23–25] In-vivo reflectance confocal microscopy (RCM) of skin is a noninvasive way to quantify MC density, which has recently emerged as a sensitive measure of sensory polyneuropathies and has potential utility as a marker of large sensory nerve fiber involvement in FRDA.[26–29]

This prospective study, involving individuals with FRDA and age- and gender-matched healthy controls, aimed to evaluate the potential utility of conventional peripheral nerve markers and in-vivo RCM of MC densities as measures of PNS structure and function in FRDA to determine which might prove useful as biomarkers and warrant further investigation in a longitudinal study.

Methods

Study design and participants

Sixteen individuals with FRDA and 16 age- and gender-matched unaffected individuals were prospectively recruited from the Neuromuscular Clinic at the University of Rochester and through referrals from the Friedreich's Ataxia Research Alliance to participate in an observational study. All subjects provided informed consent under a Research Subjects Review Board approved protocol. Written consent was obtained from participants aged 18–40 years. Parental consent was obtained for participants aged 6–17 years along with verbal assent from participants aged 7–12 years and written assent from participants aged 13–17 years.

Males and females aged 6–40 years with genetically confirmed FRDA (homozygosity for GAA repeat expansion or heterozygosity for a GAA repeat expansion and a point mutation on the other allele in the frataxin gene) with a GAA repeat ≥400 on the shorter allele were eligible to participate. Unaffected controls included individuals aged 6–40 years without signs or symptoms of peripheral neuropathy. Exclusion criteria for both groups included a history of another systemic condition or neurotoxin exposure that predisposes to peripheral neuropathy or signs or symptoms of a compression mononeuropathy.

Procedures

Participants underwent evaluation of peripheral nerve markers including RCM of MC density and morphometry (cross-sectional diameter and area), skin biopsies for epidermal nerve fiber density (ENFD), nerve conduction studies, and quantitative sensory testing (QST) including touch pressure, vibration, and cooling thresholds. Additionally, the Friedreich's Ataxia Rating Scale (FARS) was assessed for all participants with FRDA.

Friedreich's ataxia functional rating scale (FARS)

Individuals with FRDA underwent FARS assessments as a clinical measure of disease severity. The FARS is a disease specific functional outcome measure scored out of 159 with higher scores indicating worse disease.[4,6,30,31] It is comprised of subscales for general ataxia, activities of daily living, neurologic examination, and upright stability. The neurologic examination subsection is further subdivided into bulbar function, upper limb coordination, lower limb coordination, and peripheral nervous system subscore (i.e., atrophy, strength, vibratory and position sense, and deep tendon reflexes). The modified FARS excludes the peripheral nervous system subscore in order to better reflect patient-identified functional impairments.[32]

In-vivo RCM of MC density and morphometry

In-vivo RCM of MCs was performed on the palmar surface of the distal phalanx of digit V, the midpoint of the thenar eminence (TE), and midpoint of the medial sole (arch) of the foot on the nondominant side.[26–29] MC imaging was performed by a trained microscopist using the Vivascope 3000 (Caliber I.D. Inc., Rochester, NY). At each imaging site, matrices of 0.75 mm × 0.75 mm images were acquired in progressively deeper horizontal planes through the dermis. The most superficial matrix of

images was at a plane through the top of the dermal papillae, where the most superficial MCs were visualized. Thirty-one additional matrices of images were acquired, each in a plane 7 μm deeper than the prior, for a total sampling depth of 217 μm below the basal layer of the epidermis. At the digit V and TE imaging sites, each matrix consisted of 15 evenly spaced 0.75 mm × 0.75 mm images in a 5 × 3 grid within a 10 mm × 17 mm area. At the arch, each matrix consisted of 30 evenly spaced 0.75 mm × 0.75 mm images in a 6 × 5 grid within an 18 mm × 22 mm area. Imaging site specific templates were adhered to subjects using predefined anatomical landmarks in order to standardize imaging locations. MCs were counted by a blinded rater, according to previously described techniques, using Image J open source software.[27–29] Double counting was avoided by comparing images at sequential depths. MC density for each imaging site was expressed as MCs/mm^2 based on a total area of 8.44 mm^2 at digit V and TE, and 16.88 mm^2 at the arch. The largest cross-sectional (Feret) diameter (μm) and area (μm^2) of each MC was measured using Image J and the mean MC cross-sectional diameter and area were determined for each imaging site. Image acquisition took about 12 min per site and image processing and MC quantitation took about 15 min per site. The reliability of MC counts with RCM has previously been evaluated in health controls and individuals with peripheral neuropathy with an intra-rater intraclass correlation coefficient (ICC) of 0.98 and an inter-rater ICC of 0.91.[28]

Quantitative sensory testing (QST)

Monofilament touch-pressure sensory thresholds were assessed at each RCM imaging site (distal phalanx of digit V, thenar eminence, and arch of the foot) as well as the dorsal surfaces of the nondominant great toe and index finger using a series of nine monofilaments (WR TestWorks #5611) of logarithmic increasing bending force (−3, −2, −1, … 5 ln grams) at 5/6th of the extended lengths. A two-Alternative Forced-Choice Stepping Algorithm was used to determine the threshold at each site as previously described.[33] Briefly, 10 pairs of randomly assigned stimulus events (a stimulus and a null stimulus) were used for each level of stimulus force tested (each monofilament). After each pair of stimulus events, the subject indicated whether the touch was felt at "one" or at "two." The stimulus force resulting in 7 or 8 out of 10 correct choices was declared the threshold. Greater than eight correct choices prompted testing with the next weaker monofilament and few than seven correct choices prompted testing with the next stronger monofilament. The nine levels of stimulus forces were able to define 19 threshold levels.[33]

Vibration detection thresholds and cooling detection thresholds, expressed as just noticeable differences (JND), were assessed using the Case IV system (WR Medical Electronics Company, Maplewood, Minnesota) and a 4-2-1 algorithm.[15] Timed vibration sensation thresholds using a 128 Hz tuning fork were also determined.[26,34] Vibration thresholds were assessed at the first interphalangeal joints of the great toe and index finger and cooling thresholds were assessed over the dorsal surfaces of the hand and foot, on the nondominant side.

Electrophysiologic studies

Nerve conduction studies (NCS) were performed in all participants with FRDA and in healthy controls 18 years and older. Under the requirements of the RSRB approved protocol, NCS were optional for children with FRDA, and were not conducted in healthy controls under age 18 years. Amplitudes (uV) and conduction velocities (CV) (m/s) were measured from sural, ulnar, and radial sensory nerves and peroneal and ulnar motor nerves in limbs ipsilateral to the RCM imaging, according to standard approaches.[35] All studies were performed by a technologist certified by the American Association of Electrodiagnostic Technicians and skin temperatures were maintained above 32°C.

Skin biopsy (epidermal nerve fiber density)

A 3-mm skin biopsy was obtained from the lateral distal thigh and the lateral distal leg ipsilateral to the RCM imaging in participants with FRDA and control participants 18 years and older. As with NCS, under the RSRB approved protocol, skin biopsies were optional for children with FRDA, and were not obtained for control children under age 18 years. Biopsies were processed and immunostained in the Cutaneous Innervation Laboratory at University Rochester with monoclonal antibodies to the panaxonal marker, protein gene product 9.5, and ENFDs based on nerve fibers crossing the dermal-epidermal junction were quantitated by a blinded observer according to the guidelines of the European Federation for Neurological Sciences.[16,17,36,37]

Statistical analysis

Demographic and clinical characteristics were compared between groups in a pairwise fashion using the Wilcoxon rank sum test or Fisher's exact test as appropriate.

Analysis of covariance (ANCOVA) based on ranks was used to compare MC density, diameter, and area between individuals with FRDA and unaffected controls at each imaging site with a two-tailed significance level of 5%.[38]

Models for hand digit V and thenar eminence were adjusted for age, sex, and hand surface area and those for the arch were adjusted for age, sex, and foot surface area.[26]

Nerve conduction study outcomes, ENFDs, monofilament touch-pressure thresholds, vibration thresholds and cooling thresholds were compared between individuals with FRDA and unaffected controls using ANCOVA based on ranks with a significance level of 5% (two-tailed).[38] Given prior evidence that ENFDs may be affected at more advanced stages of the disease, comparisons of ENFDs between the two groups were also conducted among individuals with moderate to severe FRDA (FARS > 60) and matched controls using ANCOVA based on ranks with a significance level of 5% (two-tailed).[23,24] Covariates in these ANCOVA models included age and sex.

Associations between peripheral nerve markers (MC densities, diameters, and areas at each imaging site, nerve conduction study measures, ENFDs, touch-pressure thresholds, vibration thresholds, and cooling thresholds) and total FARS, modified FARS, duration since self-reported symptom onset and GAA repeat length were examined in the FRDA cohort using Spearman's rank correlation coefficients and a two-tailed significance level of 5% in this exploratory study. Associations between GAA repeat length, FARS (total and modified), and duration of symptoms were also examined in the same manner.

Results

Demographic and clinical features

Sixteen individuals with FRDA and 16 healthy control subjects were enrolled. Control and FRDA participants were matched for age and gender with a mean age of 18 years and gender distribution of 9/7 (F/M) in both groups (Table 1). The distribution of height, weight, hand area, and foot area were comparable between the two groups. Among participants with FRDA, the mean (std. dev.) duration from self-reported symptom onset to baseline visit was 6.8 (6.5) years, the mean short allele repeat number was 662.3 (224.9), and the mean baseline total FARS was 70.9 (31.8). All patients with FRDA had a hemoglobin A1c level less than 5.6 within 1 year prior to enrollment.

MC densities, diameters, and areas

MC densities (MC/mm^2) were lower in individuals with FRDA compared to control subjects at digit V, thenar eminence, and arch (mean (SD); digit V: 11.56 (4.02) vs. 2.81 (2.62), $P < 0.0001$; thenar eminence: 6.36 (2.15) vs. 1.58 (1.7), $P < 0.0001$; arch: 3.02 (1.42) vs. 1.37 (1.25), $P = 0.001$; Table 2 and Fig. 1). MC densities were measurable in all participants with FRDA. Mean MC diameters and areas were also smaller in individuals with FRDA compared to control subjects at digit V, thenar eminence and arch.

Inter-rater reliability of MC density, area, and diameter were assessed in a subset of images from participants with FRDA using a second blinded rater. The inter-rater interclass correlation coefficients for MC density, area, and diameter were 0.89, 0.88, and 0.69, respectively.

Table 1. Clinical and demographic characteristics of participants.

	Controls	FRDA
N	16	16
Age (years)	17.9 (8.6)	18.0 (8.0)
Gender (F/M)	9/7	9/7
Height (cm)	164 (17.0)	161 (19.0)
Weight (kg)	58.1 (17.0)	52.3 (19.5)
Hand area (cm^2)	74.9 (13.1)	74.1 (17.0)
Foot area (cm^2)	219.6 (44.9)	185.9 (34.4)
Symptom duration (years)		6.8 (6.5)
Age at symptom onset (years)		11.6 (5.6)
Shorter GAA repeat number		662.3 (224.9)
Average GAA repeat number		817.4 (176.3)
FARS		70.9 (31.8)
Modified FARS		45.5 (19.6)

Data are mean (standard deviation). FARS, Friedreich's Ataxia Rating Scale; FRDA, Friedreich's Ataxia.

Quantitative sensory testing

Monofilament touch pressure thresholds (digit V, digit II, thenar eminence, arch, and great toe) and QST vibration and cooling thresholds (digit II and great toe) were higher at all sites in individuals with FRDA compared to controls subjects (Table 2). Timed vibration sensation in seconds at the great toe and at digit II were decreased in individuals with FRDA compared to control subjects. QST thresholds were measurable in all individuals with FRDA.

Nerve conduction studies

Sensory nerve conduction studies were only obtainable in 13% of individuals with FRDA and therefore statistical analyses were not performed on these peripheral nerve measures. Peroneal and ulnar motor CV were slowed and peroneal compound muscle action potential (CMAP) amplitudes were reduced in individuals with FRDA compared to control subjects (Table 2). However, ulnar CMAP amplitudes did not differ between the two groups.

Table 2. Comparison of neuropathy measures between individuals with FRDA and matched controls.

	Controls	FRDA	*P*-value
MC Imaging			
MC density*, digit V (MC/mm^2)	11.56 (4.02)	2.81 (2.62)	<0.0001
MC density*, TE (MC/mm^2)	6.36 (2.15)	1.58 (1.7)	<0.0001
MC density*, arch (MC/mm^2)	3.02 (1.42)	1.37 (1.25)	0.001
MC Diameter*, digit V (μm)	43.43 (4.46)	37.99 (4.37)	0.004
MC Diameter*, TE (μm)	43.91 (3.8)	37.14 (6.09)	0.001
MC Diameter*, arch (μm)	47.79 (4.38)	41.45 (4.46)	0.003
MC Area*, digit V (μm^2)	1149.24 (250.58)	858.32 (209.59)	0.002
MC Area*, TE (μm^2)	1202.0 (230.9)	822.89 (296.43)	<0.0001
MC Area*, arch (μm^2)	1432.23 (291.22)	1068.59 (251.58)	0.007
ENFD (fibers/mm)[1,2]			
ENFD, thigh	23.38 (6.44)	14.29 (7.78)	0.11
ENFD*, thigh, FARS > 60	21.88 (6.2)	12.0 (8.03)	0.04
ENFD, leg	18.25 (2.9)	9.93 (6.95)	0.28
Motor NCS			
Ulnar CMAP amplitude (uV)	12.33 (2.06)	9.96 (2.0)	0.10
Ulnar motor CV* (m/s)	58.88 (3.25)	53.89 (5.81)	0.03
Peroneal CMAP amplitude* (uV)	6.3 (1.16)	3.79 (1.73)	0.003
Peroneal Motor CV* (m/s)	48.70 (3.84)	40.76 (7.18)	0.005
QST			
MF threshold*, digit V (JND)	1.06 (0.25)	6.69 (3.24)	<0.0001
MF threshold*, digit II (JND)	2.69 (1.67)	8.63 (2.53)	<0.0001
MF threshold*, TE (JND)	1.44 (0.89)	8.0 (3.2)	<0.0001
MF threshold*, arch (JND)	1.94 (1.06)	9.13 (3.18)	<0.0001
MF threshold*, toe (JND)	3.69 (1.66)	10.31 (3.59)	<0.0001
Timed vibration*, digit II (sec)	28.11 (5.66)	19.83 (5.67)	0.002
Timed vibration*, toe (sec)	20.80 (3.53)	11.69 (6.74)	<0.0001
QST Vibration*, digit II (JND)	7.7 (2.46)	12.5 (3.15)	<0.0001
QST Vibration*, toe (JND)	10.54 (2.53)	18.56 (4.32)	<0.0001
QST Cooling*, digit II (JND)	4.6 (1.3)	7.43 (3.71)	<0.0001
QST Cooling*, toe (JND)	6.38 (2.1)	10.9 (4.5)	<0.0001

Data are mean (standard deviation). CMAP, compound muscle action potential amplitude; CV, conduction velocity; ENFD, epidermal nerve fiber density; FARS, Friedreich's Ataxia Rating Scale; JND, just noticeable difference; MC, Meissner's corpuscle; MF, monofilament; TE, thenar eminence.

[1]The first six participants enrolled only underwent a skin biopsy at the thigh, after which point the protocol was amended to include a distal leg skin biopsy. As a result, subgroup analysis of ENFD at the leg between individuals with FRDA with FARS > 60 and matched controls was not performed due to a small sample size.

*Statistically significant at $P < 0.05$ (two-tailed).

Epidermal nerve fiber densities

In the overall FRDA cohort, differences from matched controls in ENFD at the thigh and leg (lower in those with FRDA), did not reach statistical significance. However, thigh ENFD was significantly lower in individuals with FRDA (FARS > 60) as compared to matched controls (Table 2). Comparative ENFD analyses were limited, per RSRB approved protocol, since for control subjects, only those greater than 17 years of age underwent skin biopsies. Additionally, two participants with FRDA less than 18 years of age chose not to undergo skin biopsies.

Associations between peripheral nerve markers and FARS, modified FARS, and duration since symptom onset

The FARS, modified FARS, and duration since symptom onset were associated with MC densities at all sites (digit V, thenar eminence, and arch), but not with MC diameters or areas (Table 3 and Fig. 2). The FARS, modified FARS, and duration since symptom onset were also associated with ENFD at the thigh and leg, monofilament touch pressure thresholds at digit V, arch and great toe and QST vibration thresholds at the great toe. Monofilament touch pressure thresholds at digit II and the thenar

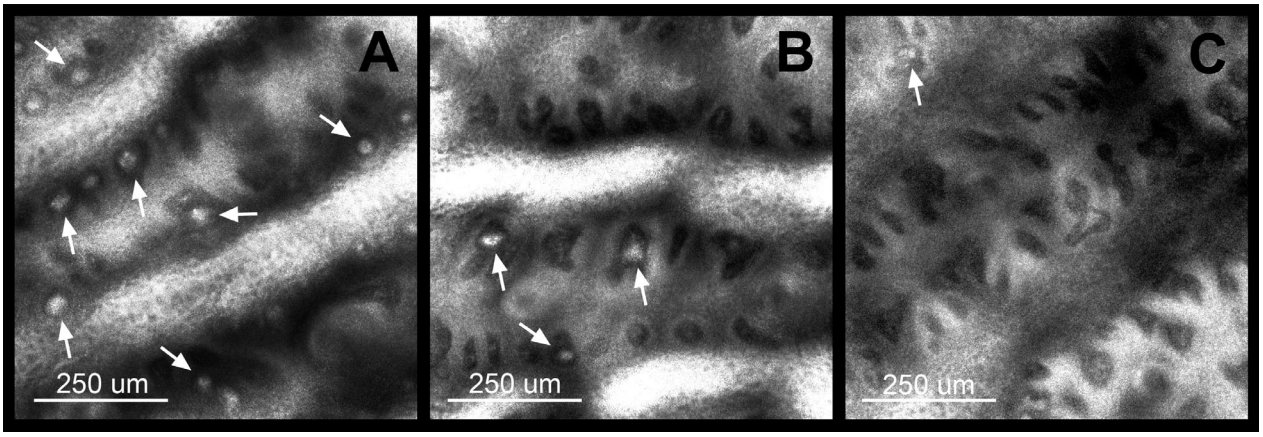

Figure 1. In-vivo reflectance confocal microscopy images of Meissner's corpuscles. Representative 0.75 mm × 0.75 mm images obtained from the palmar surface of the distal phalanx of digit V using in-vivo reflectance confocal microscopy illustrating progressively lower Meissner's corpuscle density between (A) a control subject, (B) a subject with Friedreich's Ataxia with less severe clinical manifestations (FARS < 60), and (C) a subject with Friedreich's Ataxia with more severe clinical manifestations (FARS > 60). White arrows highlight Meissner's corpuscles.

eminence were associated with FARS and modified FARS, but not duration since symptom onset. Peroneal motor CV was associated with FARS and modified FARS, but not duration since symptom onset. There was a non-significant trend towards an association between peroneal CMAP amplitude and duration since symptom onset ($P = 0.06$). Associations were not found with the additional motor nerve conduction measurements, timed vibration sensation, QST cooling thresholds, or QST vibration thresholds at digit II.

Peripheral nerve markers, total FARS, and modified FARS did not correlate with the repeat length of the shorter GAA expansion or the average GAA repeat length of the two alleles. However, duration since symptom onset inversely correlated with short ($r = -0.66$, $P = 0.006$) and average ($r = -0.61$, $P = 0.01$) repeat lengths, and correlated with total ($r = 0.76$, $P = 0.001$) and modified ($r = 0.80$, $P < 0.0001$) FARS scores.

Discussion

We undertook a prospective, cross-sectional, matched case–control study evaluating in-vivo reflectance confocal microscopy imaging of Meissner's corpuscles and several peripheral nerve measures as candidate markers of PNS involvement in Friedreich's ataxia. Among the measures evaluated, our data provide evidence for the potential utility of MC densities via in-vivo RCM, ENFD, and QST thresholds as sensory markers in FRDA. This compliments prior studies supporting MC imaging via RCM as a noninvasive and objective measure of sensory neuropathies.[26–28,39]

FRDA is a mitochondrial disorder caused by the abnormal expansion of an intronic GAA repeat, which interferes with transcription of frataxin, a mitochondrial protein involved in iron-sulfur cluster synthesis.[1] The exact pathogenesis remains unclear. However, tissue specific somatic repeat instability and selective tissue vulnerability likely contribute to the characteristic selective dysfunction of the central and peripheral nervous systems, heart, and pancreas.[40,41] The predominant pathological involvement in the PNS occurs at the dorsal root ganglion resulting in a sensory neuronopathy.[10] Histological studies demonstrate peripheral sensory nerve hypoplasia with superimposed degeneration of predominantly large sensory nerve axons and associated mechanoreceptors, including Meissner's corpuscles.[11–13,23,25,42,43] Small unmyelinated epidermal nerve fibers are also reduced, but to a lesser extent.[12,24] There is also anterior horn cell involvement, although progressive sensory dysfunction is the hallmark PNS manifestation in FRDA.[11].

Despite clear PNS involvement in FRDA, validated peripheral nerve markers to monitor disease progression or response to therapy are lacking.[9] Several previous studies have investigated peripheral nerve measures including NCS, QST, and ENFD in individuals with FRDA.[19–22,24,44] However, inferences from these studies are variously limited by small sample sizes, a lack of control groups, and limited outcome assessments. Here, we systematically evaluated multiple peripheral nerve measures alongside clinical severity assessments in a matched case–control study.

Our data provide evidence for the feasibility and potential utility of several complementary (small and large

Table 3. Correlation between neuropathy measures, clinical disease severity measures, and duration since symptom onset.

	FARS	Modified FARS	Symptom duration
MC imaging			
MC Density*, digit V	−0.55 (0.03)	−0.58 (0.02)	−0.54 (0.03)
MC Density*, TE	−0.66 (0.006)	−0.67 (0.004)	−0.59 (0.02)
MC Density*, arch	−0.69 (0.003)	−0.72 (0.002)	−0.81 (<0.0001)
MC Diameter, digit V	−0.32 (0.26)	−0.3 (0.3)	0.08 (0.79)
MC Diameter, TE	−0.21 (0.46)	−0.22 (0.46)	−0.06 (0.85)
MC Diameter, arch	−0.32 (0.27)	−0.26 (0.37)	0.18 (0.54)
MC Area, digit V	−0.4 (0.16)	−0.38 (0.19)	−0.02 (0.96)
MC Area, TE	−0.29 (0.31)	−0.31 (0.29)	−0.15 (0.61)
MC Area, arch	−0.27 (0.35)	−0.22 (0.45)	0.18 (0.54)
ENFD			
ENFD thigh*	−0.77 (0.001)	−0.76 (0.002)	−0.55 (0.04)
ENFD leg*	−0.76 (0.01)	−0.78 (0.008)	−0.67 (0.03)
Motor NCS			
Ulnar motor amplitude	0.32 (0.24)	0.37 (0.17)	0.37 (0.18)
Ulnar motor CV	−0.37 (0.18)	−0.36 (0.18)	−0.13 (0.65)
Peroneal Motor amplitude	−0.38 (0.17)	−0.41 (0.13)	−0.50 (0.06)
Peroneal Motor CV*	−0.66 (0.008)	−0.65 (0.008)	−0.39 (0.16)
QST			
MF threshold*, digit V	0.74 (0.001)	0.77 (0.001)	0.65 (0.007)
MF threshold*, digit II	0.55 (0.03)	0.59 (0.02)	0.37 (0.16)
MF threshold*, TE	0.63 (0.009)	0.65 (0.007)	0.45 (0.08)
MF threshold*, arch	0.75 (0.001)	0.77 (0.001)	0.73 (0.001)
MF threshold*, toe	0.65 (0.006)	0.64 (0.007)	0.73 (0.001)
Timed vibration, digit II	−0.27 (0.32)	−0.28 (0.30)	−0.27 (0.31)
Timed vibration, toe	−0.42 (0.11)	−0.42 (0.11)	−0.43 (0.1)
QST Vibration, digit II	0.43 (0.09)	0.41 (0.12)	0.42 (0.11)
QST Vibration*, toe	0.64 (0.008)	0.66 (0.006)	0.66 (0.005)
QST Cooling, digit II	0.32 (0.23)	0.32 (0.23)	0.31 (0.24)
QST Cooling, toe	0.31 (0.24)	0.30 (0.28)	0.15 (0.6)

Data are Spearman's rank correlation coefficient (*P*-value). CV, conduction velocity; ENFD, epidermal nerve fiber density; FARS, Friedreich's Ataxia Rating Scale; MC, Meissner's corpuscle; MF, monofilament, TE, thenar eminence.
*Statistically significant at $P < 0.05$ (two-tailed).

fiber) peripheral nerve markers in FRDA. While sensory nerve conduction studies are not likely to be useful biomarkers in FRDA due to a floor effect, MC density via RCM and monofilament touch pressure and vibration QST thresholds are feasible in children (over age 8) and adults with FRDA, appear to provide structural and physiologic markers of large sensory fiber involvement, respectively, across the spectrum of FRDA disease severity and do not appear to be limited by a significant floor effect. Similarly, ENFDs at the thigh via skin biopsy and QST cooling thresholds appear to provide complementary markers of small sensory fiber involvement in FRDA.

In addition to distinguishing affected individuals from unaffected controls, MC densities, and monofilament touch pressure and vibration QST thresholds all correlate with clinical disease severity, as measured by the FARS and modified FARS, as well as the duration since symptom onset. These findings support the concept that symptom progression and functional decline in FRDA are, in part, related to progressive structural and physiologic changes in peripheral sensory nerves and that these disease related changes may be detectable via the above-mentioned markers. Longitudinal evaluation is, however, needed to further define the course of PNS disease progression in FRDA. The present study did not find correlations between peripheral nerve markers and GAA repeat lengths. However, this was not unexpected, since correlations were also not found between clinical disease severity measures (FARS) and GAA repeat lengths, likely reflecting the relatively wide variation in duration since symptom onset in our cohort.

Our data highlight (in contrast to MC imaging and QST) the potential challenge in using ENFD as a PNS marker in clinical trials involving younger children with FRDA, due to tolerability issues with skin biopsies. Corneal nerve fiber density and morphology via corneal confocal microscopy (CCM) has recently been reported (after conduct of the present study) as a promising small fiber

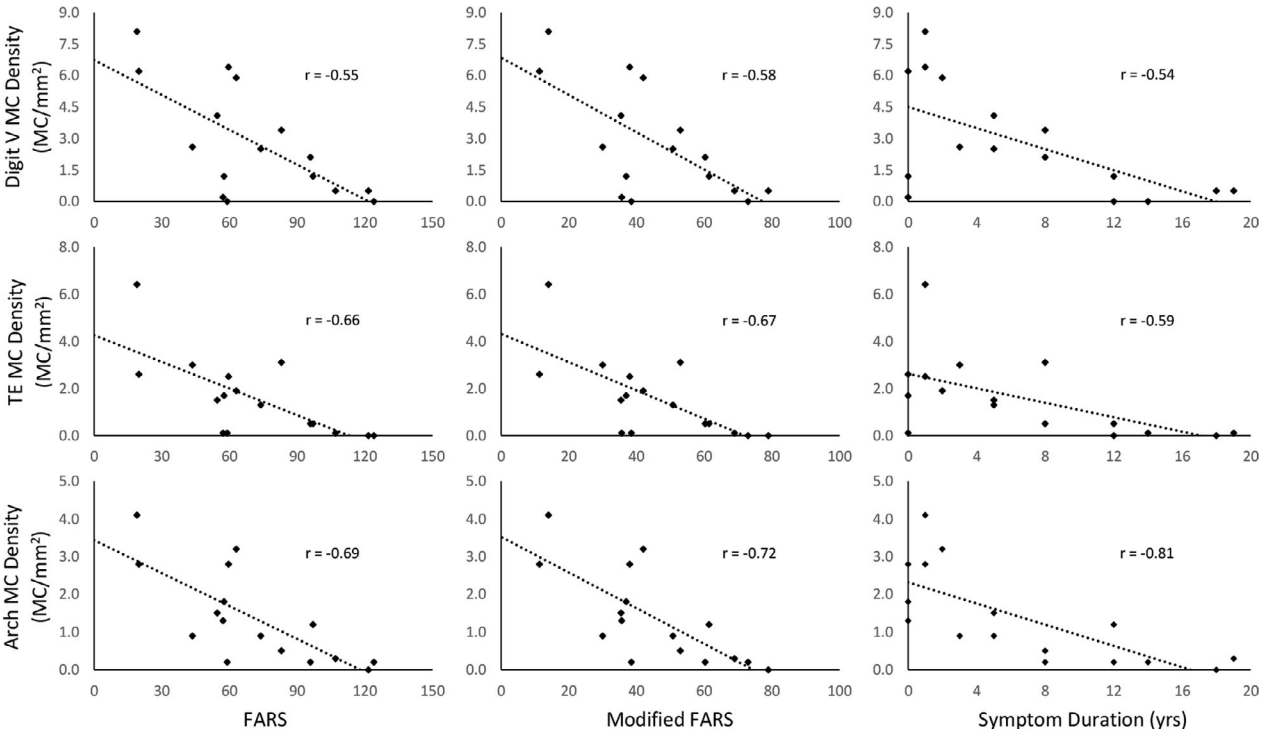

Figure 2. Correlations between Meissner's corpuscle (MC) densities (at the fingertip (Digit V), thenar eminence (TE), and arch of the foot) and Friedreich's Ataxia Rating Scale (FARS), modified FARS, and duration since symptom onset in participants with Friedreich's Ataxia. *R* values represent Spearman's rank correlation coefficients.

marker in adults with FRDA.[45] Given the tolerability concerns with skin biopsies in children, the feasibility/tolerability of CCM as a small fiber structural measure warrants further investigation in children with FRDA who might enter clinical trials. Similarly, motor nerve conduction measures were only minimally abnormal and did not consistently correlate with clinical measures of disease severity in FRDA, and are likely to be of low yield for clinical trial application.

Prior studies evaluating in-vivo RCM imaging in sensory neuropathies have focused on MC density measurements, while the present study also explored the utility of MC morphometry (as defined by cross-sectional diameter and area) as novel markers of sensory nerve involvement.[26–28] We found that RCM MC diameter and area measurements are feasible in children and adults. However, MC area measurements have high inter-rater reliable than diameter measurements. MC diameters and areas were smaller in individuals with FRDA compared to controls, but did not correlate with clinical disease severity. These findings may be explained by observations from pathological studies of sensory nerve hypoplasia in FRDA that is congenital and nonprogressive in nature.[11,13]

The present study had some limitations. While strong associations between peripheral nerve measures and clinical measures of disease severity were found, the cross-sectional design limits definitive conclusions about the responsiveness of these peripheral nerve measures to disease related changes over time. Additionally, although this is the largest match controlled study evaluating a range of conventional and novel, large and small fiber peripheral nervous system markers in FRDA to date, the findings are still limited by a relatively small sample size, particularly for skin biopsies in children, as is often the case with rare diseases.

In summary, MC density via in-vivo RCM, ENFD, and QST thresholds are feasible and provide structural and physiologic markers of sensory involvement in FRDA. Further longitudinal evaluation is needed to determine whether these measures can identify quantitative changes associated with disease progression or treatment and ultimately their potential for clinical trial application.

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Author Contributions

P.D.C., B.R., J.L., and D.N.H. contributed to conception and design of the study. P.D.C., J.M., J.E.S., K.E., and D.N.H. contributed to acquisition and analysis of data. P.D.C., B.R., J.L., and D.N.H. were responsible for drafting the manuscript.

Conflict of Interest

P.D.C., J.M., J.E.S., K.E., and D.N.H. received grant support from Friedreich's Ataxia Research Alliance and Voyager Pharmaceuticals for this work. P.D.C., J.M., and J.E.S. report no other disclosures. K.E. has done consulting for Acceleron Pharma and Ionis Pharmaceuticals and is on the scientific advisory board for Biogen. D.N.H. has done consulting for Acceleron Pharma, Flex Pharma, Narrow River Management, Guidepoint Global, GLG, Slingshot Insights, LAM Therapeutics, Inc., Voyager Therapeutics, ClearView Health Partners, MedPace, DDB Health NY, Cydan, Trinity Partners, Schlesinger, Human First Therapeutics and is on the scientific advisory board for Regenancy Pharmaceuticals. J.L. is an employee of the Critical Path Institute and the Friedreich's Ataxia Research Alliance. B.R. was an employee at Voyager Therapeutics during the conduct of this study and is now an employee at Praxis Precision Medicines. The University of Rochester co-holds a use patent for in-vivo reflectance confocal microscopy of Meissner's corpuscles in peripheral neuropathy. D.N.H. is listed as a coinventor on this patent.

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