A longitudinal and cross-sectional study of plasma neurofilament light chain concentration in Charcot-Marie-Tooth disease

Alexander Martin Rossor1 | Mahima Kapoor1 | Henny Wellington2,3 |
Emily Spaulding4,5 | James N. Sleigh1,3 | Robert W. Burgess4,5 | Matilde Laura1 |
Henrik Zetterberg2,3,6,7 | Alexa Bacha8 | Xingyao Wu8 | Amanda Heslegrave2,3 |
Michael E. Shy8 | Mary M. Reilly1

1Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, London, UK
2Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK
3UK Dementia Research Institute at UCL, London, UK
4The Jackson Laboratory, Bar Harbor, Maine, USA
5Graduate School of Biomedical Science and Engineering, University of Maine, Orono, Maine, USA
6Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden
7Clinical Neurochemistry Laboratory, The Sahlgrenska University Hospital, Mölndal, Sweden
8Department of Neurology, Carver College of Medicine, University of Iowa, Iowa City, Iowa, USA

Correspondence
Dr. Alexander Martin Rossor, Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, Queen Square, London WC1N 3BG, UK.
Email: a.rossor@ucl.ac.uk

Funding information
British Medical Association (Vera Down); H2020 European Research Council, Grant/ Award Numbers: #681712, 860197; NIHR Biomedical Research Centre, University College London Hospital Biomedical Research Centre (Therapeutic Innovation Call for Neuroscience Theme); Swedish Research Council, Grant/Award Number: #2018-02532; the National Institutes of Neurological Diseases and Stroke and Office of Rare Diseases, Grant/Award Numbers: 1UOINS109403-01, R21RO03034, U54NS065712; INC, the Charcot-Marie-Tooth Association (CMTA); Muscular Dystrophy Association, Grant/Award Number: MDA10281; Medical Research Council, Grant/Award Number: MRC/ MR/S005021/1; Wellcome Trust; the UK Dementia Research

Abstract
Advances in genetic technology and small molecule drug development have paved the way for clinical trials in Charcot-Marie-Tooth disease (CMT); however, the current FDA-approved clinical trial outcome measures are insensitive to detect a meaningful clinical response. There is, therefore, a need to identify sensitive outcome measures or clinically relevant biomarkers. The aim of this study was to further evaluate plasma neurofilament light chain (NFL) as a disease biomarker in CMT. Plasma NFL was measured using SIMOA technology in both a cross-sectional study of a US cohort of CMT patients and longitudinally over 6 years in a UK CMT cohort. In addition, plasma NFL was measured longitudinally in two mouse models of CMT2D. Plasma concentrations of NFL were increased in a US cohort of patients with CMT1B, CMT1X and CMT2A but not CMT2E compared with controls. In a separate UK cohort, over a 6-year interval, there was no significant change in plasma NFL concentration in CMT1A or HSN1, but a small but significant reduction in patients with CMT1X. Plasma NFL was increased in wild type compared to GARSC201R mice. There was no significant difference in plasma NFL in GARS<sup>p278KY</sup> compared to wild type mice. In patients with CMT1A, the small difference in cross-sectional NFL concentration vs
healthy controls and the lack of change over time suggests that plasma NFL may lack sufficient sensitivity to detect a clinically meaningful treatment response in adulthood.

**KEYWORDS**

biomarkers, Charcot-Marie-Tooth disease, neurofilament

# INTRODUCTION

Charcot-Marie-Tooth disease (CMT) is one of the commonest inherited neurological diseases with a population prevalence of 1 in 3000. With increased understanding of the genetic aetiology of CMT combined with advances in genetic therapies and small molecule drug development, the field is now entering an era where there are a number of promising therapies in the pipeline.

Developing successful treatments in preclinical models of CMT is only part of the journey in delivering therapies to patients. For a treatment to be adopted in routine clinical practice, it will need to show efficacy in clinical trials. CMT provides particular difficulties when it comes to designing clinical trials. CMT is usually a lifelong disease, and even in the rapidly progressive forms, the rate of progression is slower than for other diseases such as amyotrophic lateral sclerosis. It is widely assumed that a successful treatment would be one that stops the progression of the disease, and therefore, clinical trials need to be designed with outcome measures that are able to detect a slowing in the rate of progression. A number of CMT-specific clinical outcome measures have been designed that have been validated or are undergoing validation, including the CMT neuropathy score, CMT Functional Outcome Measure, CMT Health Index and CMT Peds. In addition, biomarkers of disease progression, such as nerve and muscle MRI are also being developed as outcome measures for clinical trials.

Neurofilaments are the most abundant cytoskeletal proteins in neurons of both the central and peripheral nervous systems. It has been shown that plasma neurofilament light chain concentration (NFL) is increased in several neurological diseases, including CMT, where it also correlates with disease severity. To be able to use a blood biomarker such as NFL in clinical trials, it is important to know how plasma concentrations vary over time. In this study, we replicate our previous cross-sectional work in another cohort of CMT patients, investigate the change in plasma NFL over time in patients with CMT and in two mouse models of the disease.

# METHODS

## GARS mouse models

The generation and characterisation of the GARS mouse models have been described previously. All experimental procedures were conducted in accordance with animal care protocols approved by the Institutional Animal Care and Use Committee at The Jackson Laboratory. Blood samples were obtained from 5, 7, 9 and 11-week-old wild-type and GARS mice (n = 3-7 per age group) using a lancet puncture of the submandibular vein.

## Blood sampling and sample collection and storage

All participants were evaluated in outpatient clinics, and blood samples were taken and processed within 1 hour. Blood was collected into EDTA-containing tubes and centrifuged at 20°C at 3500 rpm for 10 minutes. Plasma was then aliquoted and stored at −80°C.

## Standard protocol approvals, registrations, and patient consents

This study was approved by The National Hospital for Neurology and Neurosurgery Research Ethics Committee/Central London REC 3 09/H0716, and written informed consent was obtained from all participants in the study.
Institutional Review Board approval was also obtained from the University of Iowa, and written informed assent/consent was provided by participants under a protocol approved by the ethics board of the NIH Rare Diseases Clinical Research Network (Protocol INC6601).

### 2.5 Simoa plasma NFL measurements

Plasma NFL concentration was measured using two highly correlated methods, employing the same antibodies: the in-house Simoa NFL assay that has been described in detail previously, and the commercially available NF-Light assay (Quanterix, Billerica, MA). Samples were analysed ‘blind’ and in duplicate using one batch of reagents. For the UK samples, an aliquot of the original baseline sample was analysed in the same batch as the 6-year follow up sample.

### 2.6 Statistical analysis

Statistical analysis was performed using SPSS version 27.00 (IBM, New York, USA) and GraphPad Prism 9.0 (GraphPad Inc., California, USA). Correlations were assessed using Spearman’s correlation coefficient. Two-tailed paired t-tests were used to compare differences in plasma NFL concentration in patients with CMT at baseline and after 6 years. One-way ANOVA with post hoc Dunnett’s two-tailed t-test was used to compare differences in age and plasma NFL between CMT subtypes and controls in the Iowa cohort.

### 3 RESULTS

There has been recent interest in the potential use of plasma NFL as a biomarker of disease progression in CMT for use in clinical trials. We, therefore, sought to examine plasma NFL concentration in a cross-sectional cohort of CMT patients and longitudinally in a further cohort of patients with CMT and in two established mouse models of the disease.

#### 3.1 Plasma NFL concentration is increased in patients with CMT1B, CMT1X and CMT2A, but not CMT2E, compared with controls

We have previously demonstrated an increase in plasma NFL concentration in UK patients with CMT1A, CMT1X and HSN1. We, therefore, sought to see if we could replicate this finding in an independent cohort of patients with CMT from the United States of America. The cohort of patients from Iowa comprised 18 patients with CMT1B, 18 with CMT1X, 4 with CMT2A and 9 with CMT2E and 25 controls (Table 1). There was no significant difference in the age of the patients with each type of CMT and controls (One-way ANOVA, \( P < .931 \)) or the sex ratio (Chi-square, \( P = .53 \)). Plasma NFL concentration was significantly increased in patients with CMT1B (ANOVA \( P < .0001 \), Dunnett’s two-tailed t-test, \( P < .0001 \)), CMT1X (\( P = .001 \)) and CMT2A (\( P = .048 \)) compared with controls but not in patients with CMT2E (\( P = .939 \)) (Figure 1A and Table 1). In contrast to our previous study in a UK cohort, there was no correlation between plasma NFL and the weighted CMTES and CMTNS for any of the CMT subtypes included in the study (Figure 1B and Table 1). There was a significant correlation between plasma NFL in patients with CMT1B and the ulnar nerve Conduction Velocity (CV) (Spearman Rho = 0.876, \( P < .0001 \)) and Ulnar Compound Muscle Action Potential (Rho = 0.682, \( P = .015 \)) but not for patients with CMT1X or CMT2A (Table 1). There was no correlation between age of onset and plasma NFL in the CMT1B cohort (Pearson correlation coefficient, \( r = 0.44, P = .11 \)).

#### 3.2 Plasma NFL changes with time in two mouse models of CMT

In order for plasma NFL to be of use as a biomarker of disease progression in CMT, it is necessary to know if the concentration changes with time. We have previously shown that in a mouse model of CMTX, the concentration of plasma NFL rises rapidly between 2 and 3 months before falling by a third at 1 year. We, therefore, measured plasma NFL at 5, 7, 9 and 11 weeks in two mouse models of CMT2D (Figure 2). The GARS\textsuperscript{201R} mouse is a milder model with

### TABLE 1 Plasma NFL concentration in a US (Iowa) cohort of patients with CMT

| Number of patients | Age (mean, 95% CI) | Median [NFL] pg/ml (range) | *CMTES/[NFL] Spearman correlation co-efficient | *CMTNS/[NFL] Spearman correlation co-efficient | Ulnar CV/[NFL] correlation co-efficient | Ulnar CMAP/[NFL] correlation co-efficient |
|-------------------|-------------------|-----------------------------|-----------------------------------------------|-----------------------------------------------|----------------------------------------|----------------------------------------|
| CMT1B             | 18                | 49.9 (37.5-60.9)            | 25.5 (6.7-52.4)                                | 0.329, \( P = .183 \)                         | 0.208, \( P = .517 \)                   | 0.876, \( P < .0001 \)               | 0.682, \( P = .015 \) |
| CMT1X             | 18                | 47.4 (39.5-55.3)            | 18.3 (11.2-26.5)                                | -0.144, \( P = .568 \)                       | -0.043, \( P = .907 \)                  | 0.258, \( P = .471 \)                | -0.322, \( P = .364 \) |
| CMT2A             | 4                 | 42 (28.8-55.2)              | 19.7 (15.6-23.7)                                | -0.8, \( P = .2 \)                           | -0.896, \( P = .104 \)                  | -0.5, \( P = .667 \)                 | -0.5, \( P = .667 \) |
| CMT2E             | 9                 | 46.9 (35.9-57.8)            | 5.58 (3.84-17.6)                                | n/a                                          | n/a                                    | n/a                                   | n/a                                   |
| Controls          | 25                | 49 (44.2-53.8)              | 7.54 (4.52-15.8)                                | n/a                                          | n/a                                    | n/a                                   | n/a                                   |

Abbreviations: CI, confidence interval; CMAP, compound muscle action potential; CMT, Charcot-Marie-Tooth disease; *CMTES, Rasch modified (weighted) CMT subtypes and controls in the Iowa cohort. Was used to compare differences in age and plasma NFL between 6 years. One-way ANOVA with post hoc Dunnett’s two-tailed plasma NFL concentration in patients with CMT at baseline and after 2.5 years. The concentration of plasma NFL rises rapidly between 2 and 3 months before falling by a third at 1 year. We, therefore, measured plasma NFL at 5, 7, 9 and 11 weeks in two mouse models of CMT2D (Figure 2). The GARS\textsuperscript{201R} mouse is a milder model with...
normal life expectancy in contrast to the GARS\textsuperscript{P278KY} mouse, which has a background-dependent reduced life expectancy of less than 6 months.\textsuperscript{22} Plasma NFL was increased in wild type compared with GARS\textsuperscript{C201R} mice at 5, 7 and 11 weeks, although the difference only reached statistical significance at 11 weeks (Mann-Whitney U-test, $P = .01$, Figure 2A). Plasma NFL concentration was also increased in wild type compared with GARS\textsuperscript{C201R} mice at 11 weeks in an unrelated colony in the United Kingdom (see Figure S1). Plasma NFL was increased in the GARS\textsuperscript{P278KY} mouse compared with wild type at 5 weeks, although this did not reach significance. There was no difference in plasma NFL at 7, 9 and 11 weeks consistent with the early axon loss in these mice, followed by very slow progress after 6-8 weeks of age (Figure 2B).\textsuperscript{22}

3.3 | Plasma NFL is stable in CMT1A and HSN but not CMT1X over a 6-year period

Repeat blood samples were collected from 27 patients with CMT after a 6-year time interval (CMT1A = 10, CMT1X = 6, HSN1 = 6, SPTLC2 = 2, CMT2A = 1, CMT4C = 1, CMT4B1 = 1). The mean increase in the weighted CMTES over this time period was $+2.3$. Unlike the US cohort, there was a significant correlation between 6-year follow up plasma NFL and weighted CMTES (Spearman Rho = 0.53, $P = .004$) (Figure 3A). There was no significant difference in plasma NFL over 6 years for all CMT patients, (mean change $= -2.44$ pg/mL, SD = 11.5, $P = .52$, Figure 4A) and HSN1 (mean change $= -1.06$ pg/mL, SD = 1.18, $P = .21$, Figure 4C) but a significant reduction in CMT1X (mean change $= -3.28$ pg/mL, SD = 2.13, $P = .01$, Figure 4B). Plotting the 6-year change in plasma NFL against the 6-year change in the weighted CMTES showed no significant correlation (CMT1A, Spearman Rho = $-0.2$, $P = .6$; CMT1X Rho = $-0.7247$, $P = .12$; HSN1 Rho = 0.25, $P = .63$) (Figure 3B-D).

4 | DISCUSSION

Clinical trials in CMT require the development and validation of clinical outcome measures and biomarkers that are sufficiently sensitive to detect a modest reduction in the rate of progression. This study
provides further evidence on plasma NFL as a biomarker in CMT. In a post hoc analysis of the Phase 3 study of Patisiran (APOLLO) in patients with hereditary transthyretin-mediated (hATTR) amyloidosis, there was a significant reduction in plasma NFL in those patients randomised to Patisiran compared with placebo, validating the use of plasma NFL as a biomarker for this subtype of inherited peripheral neuropathy. Nevertheless, the concentration of plasma NFL in hATTR patients prior to treatment (69.4 pg/mL) was significantly higher than in our cohort of patients with CMT (18.6 pg/mL).25

In our previous single UK centre study, we demonstrated that plasma NFL was increased in several forms of CMT compared with age and sex-matched controls.21 The current study replicates those findings in a separate cohort from the Iowa group in the United States. In this cohort, plasma NFL was increased in all the subtypes of CMT examined except for CMT2E due to mutations in the neurofilament light chain (NEFL). Interestingly, the concentrations in this group were lower than controls, although this did not reach statistical significance. The reasons for this are not clear, but the finding is concordant with a previous report demonstrating reduced NFL expression in cutaneous nerve fibres of patients with CMT2E.26 An alternative explanation may be due to alteration of the NFL epitope recognised by the antibody used in the Simoa analysis as a result of neurofilament aggregations induced by the point mutation.27

It is often assumed that the rate of axonal degeneration in genetic peripheral neuropathy such as CMT is constant. This is an important assumption to test, because if the rates of axonal degeneration

| CMT subtype | Mean age (years) | Number of participants | Median baseline | Median 6-y follow up | Mean nfl change (95% CI) | 2-sided paired t test | Mean change in *CMTES |
|-------------|-----------------|------------------------|-----------------|---------------------|--------------------------|---------------------|-----------------------|
| CMT1A       | 46.0            | 10                     | 19.03 (9.47-31.4) | 14.72 (6.46-32.4)   | -2.44 (-10.67-5.80)      | P = .52             | +3.56                 |
| CMT1X       | 48.0            | 6                      | 18.98 (8.79-28.7) | 14.54 (7.14-25.3)   | -3.28 (-10.04-5.51)      | P = .01             | -0.67                 |
| HSN1 (SPTLC1) | 42.3           | 6                      | 18.0 (13.0-24.4)  | 17.9 (12.3-23.9)    | -0.69 (-1.93-0.55)       | P = .21             | +3.167                |
| HSN1 (SPTLC2) | 50              | 2                      | 12.9 (7.90-18.0)  | 9.68 (5.84-13.5)    | -3.24 (-18.3-11.8)       |                    | +3.5                  |
| CMT2A       | 20              | 1                      | 24.9             | 13.8                | -11.1                    | n/a                 | +1                    |
| CMT4C       | 49              | 1                      | 48.7             | 27.1                | -21.6                    | n/a                 | -5                    |
| CMT4B1      | 32              | 1                      | 16.5             | 18.2                | 1.67                     | n/a                 | -1                    |
| All CMT     | 46.2            | 27                     | 18.6 (7.90-48.7)  | 15.6 (5.84-38.9)    | -3.17 (-6.37-0.02)       | P = .05             | -2.3                  |

Abbreviations: CI, confidence interval; CMT, Charcot-Marie-Tooth disease; *CMTES, Rasch modified (weighted) CMT Examination Score; HSN, hereditary sensory neuropathy.
change with time, it may affect the timing of, or the ability to detect a significant alteration in NFL concentration in a clinical trial. To explore this further, we examined plasma NFL in two different mouse models of CMT2D, which are known to show progressive neurodegeneration over the examination period. In the more severe GARS<sup>P278KY</sup> mouse, plasma NFL was highest at 5 weeks before falling to normal levels suggesting an early window of opportunity for treatment. Plasma NFL for the GARS<sup>Z201R</sup> mouse was similar to the baseline wild type concentration; however, the wild type mice showed significantly increased plasma NFL at multiple later time points. The cause for this difference is unknown but raises concern about the suitability of plasma NFL as a biomarker of axonal degeneration for trials in mouse models of this subtype of the disease. We originally speculated that the elevated plasma NFL was due to haemolysis of samples, which can result in spuriously elevated NFL concentrations (NFL is expressed in red blood cells); however, the replication of this result in a separate colony at a different time point would argue against this, although it remains a possibility.

In this study, we were also able to collect paired blood samples on 27 patients from our original CMT cohort after a 6-year interval. Our analysis shows no statistically significant change in plasma NFL over 6 years for patients with either CMT1A or HSN1, although there was a trend towards a reduction in CMT1A, but this was not significant. On the other hand, there was a statistically significant, albeit small, reduction in plasma NFL in patients with CMT1X over 6 years, although the number of patients was small (n = 6). Large cross-sectional studies of patients with different CMT subtypes and ages spanning all decades will be invaluable in identifying the age of maximal axonal degeneration and the window of opportunity for maximum therapeutic effect.

The standardised response of the mean (SRM) is a measure of the responsiveness of an outcome measure to detect the change and is calculated by dividing the mean change by the SD of the change. In CMT1A, version 1 of the CMT Neuropathy Score has an SRM of 0.13 to detect a 50% slowing of disease progression over 24 months. This would equate to 7700 patients with CMT1A required in each arm of a placebo-controlled trial to detect a 50% change in disease progression with a significance level of 0.05% with 80% power. For calf muscle MRI fat fraction with baseline fat fraction >10%, the SRM is 2.19 over 12 months, equating to a requirement of 11 patients in each arm of a trial. We have previously demonstrated a mean difference in plasma NFL concentration between patients with CMT1A and controls of 10 pg/mL. If one uses the SD of the mean change in NFL at 6 years in this study as a measure of the intra-subject variability, one can estimate an SRM for plasma NFL in CMT1A. For a 50% drop in plasma NFL concentration (5 pg/mL) and an SD of 11.51, the estimated SRM for plasma NFL is 0.04, which is significantly worse than version 1 of the CMTNS (which has been shown to be underpowered for use as a primary outcome measure in clinical trials in CMT1A). This suggests that due to the small increase in plasma NFL in CMT1A compared with controls and the significant intrasubject variation that it is unlikely to be suitable as a primary outcome measure in CMT1A for this age group. On the other hand, plasma NFL increases with age, and it is noteworthy that the mean age of the CMT cohort was 46 years. As the rate of axonal degeneration in CMT may vary, with higher rates theoretically possible at a younger age, it remains a possibility that plasma NFL may still have a role as a biomarker for clinical trials at earlier time points.

In summary, this study provides additional data on the use of plasma NFL as a biomarker in CMT. We have replicated our previous findings of increased concentrations in patients with CMT compared with controls, and we have shown that in mouse models of the disease, concentrations can vary over the lifetime of the animal and that in humans, the change in concentration may vary according to subtype. We have also shown pilot data that NFL is unlikely to be suitable as a primary outcome measure in patients with CMT1A. We are currently exploring NFL vs a number of other clinical, plasma and MRI biomarkers in longitudinal studies of CMT1A, CMT1B, CMT2A and CMT1X.

**ACKNOWLEDGEMENTS**

HZ is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018-02532), the European Research Council (#681712), Swedish State Support for Clinical Research...
(ALFGBG-720931), the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862), the AD Strategic Fund and the Alzheimer’s Association (#ADSF-21-831376-C, #ADSF-21-831381-C and #ADSF-21-831377-C), the Olav Thon Foundation, the Erlanger-Persson Family Foundation, Stiftelsen för Gamla Tjänarinnor, Hjärrfonden, Sweden (#FO2019-0228), the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860197 (MIRIADE), and the UK Dementia Research Institute at UCL. A Multi-User Equipment Grant from Wellcome Trust funded the Simoa instruments. MMR is grateful for the Medical Research Council (MRC MR/S05021/1), the National Institutes of Neurological Diseases and Stroke and the Office of Rare Diseases (US4NS065712 and 1UOINS109403-01 and R21TROO3034), Muscular Dystrophy Association (MDA510281) and the Charcot-Marie-Tooth Association (CMTA) for their support. The INC (US4NS065712) is a part of the NCATS Rare Diseases Clinical Research Network (RDCRN). This research was also supported by the National Institute for Health Research University College London Hospitals Biomedical Research Centre. AMR is grateful to the BMA for the Vera Down Award and to the National Institute for Health Research University London Hospitals Biomedical Research Centre. Funding from NIH/NINDS R37054154 and the CMTA to RWB and NIH/NINDS F31NS100328 to ELS supported this study. JNS is in receipt of a MRC UK Career Development Award (MR/S006990/1).

CONFLICT OF INTEREST

HZ has served at scientific advisory boards for Alector, Eisai, Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, AZTherapies and CogRx, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Programme (outside submitted work).

ORCID

Alexander Martin Rossor https://orcid.org/0000-0003-4648-2896
James N. Sleigh https://orcid.org/0000-0002-3782-9045
Michael E. Shy https://orcid.org/0000-0001-8460-6971

REFERENCES

1. Foley C, Schofield I, Egolon G, Bailey G, Chinnery PF, Horvath R. Charcot-Marie-Tooth disease in Northern England. J Neurol Neurosurg Psychiatry. 2012;83(5):572-573. doi:10.1136/jnnp-2011-300285
2. Stavrou M, Sargiannidou I, Georgiou E, Kagiava A, Kleopa KA. Emerging therapies for Charcot-Marie-Tooth inherited neuropathies. Int J Mol Sci. 2021;22(11):6048. doi:10.3390/ijms22116048
3. Rossor A. Are we prepared for clinical trials in Charcot-Marie-Tooth disease? Brain Res. 2019;1729:146625. doi:10.1016/j.brainres.2019.146625
4. Johnson NE, Heatwole C, Creigh P, et al. The Charcot-Marie-Tooth Health Index: evaluation of a patient-reported outcome. Ann Neurol. 2018;84(2):225-233. doi:10.1002/ana.25282
5. Eichinger K, Burns J, Cornett K, et al. The Charcot-Marie-Tooth Functional Outcome Measure (CMT-FOM). Neurology. 2018;91(15):e1381-e1384. doi:10.1212/WNL.0000000000006323
6. Burns J, Ouvrier R, Estlows T, et al. Validation of the Charcot-Marie-Tooth disease pediatric scale as an outcome measure of disability. Ann Neurol. 2012;71(5):642-652. doi:10.1002/ana.23572
7. Mandarakas MR, Menezes MP, Rose KJ, et al. Development and validation of the Charcot-Marie-Tooth Disease Infant Scale. Brain. 2018;141(12):3319-3330. doi:10.1093/brain/awy280
8. Murphy SM, Herrmann DN, McDermott MP, et al. Reliability of the CMT neuropathy score (second version) in Charcot-Marie-Tooth disease. J Peripher Nerv Syst. 2011;16(3):191-198. doi:10.1111/j.1529-8027.2011.00350.x
9. Sadjadi R, Reilly MM, Shy ME, et al. Psychometrics evaluation of Charcot-Marie-Tooth Neuropathy Score (CMTNv2) second version, using Rasch analysis. J Peripher Nerv Syst. 2014;19(3):192-196. doi:10.1111/jnnp.12084
10. Morrow JM, Evans MRB, Gridt T, et al. Validation of MRC Centre MRI calf muscle fat fraction protocol as an outcome measure in CMT1A. Neurology. 2018;91(12):e1125-e1129. doi:10.1212/WNL.000000000006214
11. Morrow JM, Sinclair CDJ, Fischmann A, et al. MRI biomarker assessment of neuromuscular disease progression: a prospective observational cohort study. Lancet Neurol. 2016;15(1):65-77. doi:10.1016/S1474-4422(15)00242-2
12. Dortch RD, Dethage LM, Gore JC, Smith SA, Li J. Proximal nerve magnetization transfer MRI relates to disability in Charcot-Marie-Tooth diseases. Neurology. 2014;83(17):1545-1553. doi:10.1212/2000000000000919
13. Petzold A. Neurofilament phosphoforms: surrogate markers for axonal injury, degeneration and loss. J Neurol Sci. 2005;233(1-2):183-198. doi:10.1016/j.jns.2005.03.015
14. Hansson O, Janelidze S, Hall S, et al. Blood-based NfL. Neurology. 2017;88(10):930-937. doi:10.1212/200000000000038580
15. Disanto G, Barro C, Benkert P, et al. Swiss Multiple Sclerosis Cohort Study Group. Serum Neurofilament light: a biomarker of neuronal damage in multiple sclerosis. Ann Neurol. 2017;81(6):857-870. doi:10.1002/ana.24954
16. Rohrer JD, Woollacott IO, Dick KM, et al. Serum neurofilament light chain protein is a measure of disease intensity in frontotemporal dementia. Neurology. 2016;87(13):1329-1336. doi:10.1212/20000000000003154
17. Mariotto S, Farinazzo A, Magliozzi R, Alberti D, Monaco S, Ferrani S. Serum and cerebrospinal neurofilament light chain levels in patients with acquired peripheral neuropathies. J Peripher Nerv Syst. 2018;23(3):174-177. doi:10.1111/jnnp.12279
18. Lu C-H, Macdonald-Walls C, Gray E, et al. Neurofilament light chain: a prognostic biomarker in amyotrophic lateral sclerosis. Neurology. 2015;84(22):2247-2257. doi:10.1212/20000000000001642
19. Miller E, Rots D, Simrén J, et al. Plasma neurofilament light chain as a potential biomarker in Charcot-Marie-Tooth disease. Eur J Neurology. 2021;28(3):974-981. doi:10.1111/ene.14689
20. Wang H, Davison M, Wang K, et al. Transmembrane protease serine 5: a novel Schwann cell plasma marker for CMT1A. Ann Clin Transl Neuro. 2020;7(1):69-82. doi:10.1002/acn3.50965
21. Sandellius Å, Zetterberg H, Blennow K, et al. Plasma neurofilament light chain concentration in the inherited peripheral neuropathies. Neurology. 2018;90(6):e518-e524. doi:10.1212/20000000000004932
22. Seburn KL, Nangle LA, Cox GA, Schimmel P, Burgess RW. An active dominant mutation of glycy1-tRNA synthetase causes neuropathy in a Charcot-Marie-Tooth 2D mouse model. Neuron. 2006;51(6):715-726. doi:10.1016/j.neuron.2006.08.027
23. Achilli F, Bros-Facer V, Williams HP, et al. An ENU-induced mutation in mouse glycy1-tRNA synthetase (GARS) causes peripheral sensory and motor phenotypes creating a model of Charcot-Marie-Tooth type 2D peripheral neuropathy. Dis Model Mech. 2009;2(7-8):359-373. doi:10.1242/dmm.002527
24. Kagiava A, Karaiskos C, Richter J, et al. AAV9-mediated Schwann cell-targeted gene therapy rescues a model of demyelinating neuropathy. Gene Ther. 2021;28:659-675. doi:10.1038/s41434-021-00250-0
25. Ticau S, Sridharan GV, Tsour S, et al. Neurofilament light chain as a biomarker of hereditary transthyretin-mediated amyloidosis. Neurology. 2021;96(3):e412-e422. doi:10.1212/200000000000011090
26. Pisciotta C, Bai Y, Brennan KM, et al. Reduced neurofilament expression in cutaneous nerve fibers of patients with CMT2E. Neurology. 2015;85(3):228-234. doi:10.1212/WNL.0000000000001773

27. Fabrizi GM, Cavallaro T, Angiari C, et al. Giant axon and neurofilament accumulation in Charcot-Marie-Tooth disease type 2E. Neurology. 2004;62(8):1429-1431. doi:10.1212/01.WNL.0000120664.07186.3C

28. Sleigh JN, Mech AM, Schiavo G. Developmental demands contribute to early neuromuscular degeneration in CMT2D mice. Cell Death Dis. 2020;11(7):564. doi:10.1038/s41419-020-02798-y

29. Terasawa K, Taguchi T, Momota R, Naito I, Murakami T, Ohtsuka A. Human erythrocytes possess a cytoplasmic endoskeleton containing beta-actin and neurofilament protein. Arch Histol Cytol. 2006;69(5):329-340. doi:10.1679/aohc.69.329

30. Piscosquito G, Reilly MM, Schenone A, et al; CMT-TRAUK Group. Responsiveness of clinical outcome measures in Charcot-Marie-Tooth disease. Eur J Neurol. 2015;22(12):1556-1563. doi:10.1111/ene.12783

31. Nyberg L, Lundquist A, Nordin Adolfsson A, et al. Elevated plasma neurofilament light in aging reflects brain white-matter alterations but does not predict cognitive decline or Alzheimer’s disease. Alzheimer’s Dement (Amsterdam, Netherlands). 2020;12(1):e12050. doi:10.1002/dad2.12050

SUPPORTING INFORMATION
Additional supporting information may be found in the online version of the article at the publisher’s website.

How to cite this article: Rossor AM, Kapoor M, Wellington H, et al. A longitudinal and cross-sectional study of plasma neurofilament light chain concentration in Charcot-Marie-Tooth disease. J Peripher Nerv Syst. 2021;1-8. doi:10.1111/jns.12477