Disease Progression in Charcot–Marie–Tooth Disease Related to *MPZ* Mutations: A Longitudinal Study

Vera Fridman, MD,[1] Stefan Sillau, PhD,[1] Jacob Bockhorst, BA,[1] Kaitlin Smith, MS, CGC,[1] Isabella Moroni, MD,[2] Emanuela Pagliano, MD,[2] Chiara Pisciotta, MD, PhD,[3] Guiseppe Piscosquito, MD,[3,4] Matilde Laurá, MD, PhD,[5] Francesco Muntoni, MD, FRCPCH,[6] Chelsea Bacon, BS,[7] Shawna Feely, MS, CGC,[7,8] Tiffany Grider, MS, CGC,[7] Laurie Gutmann, MD,[7] Rosemary Shy, MD,[7,8] Janel Wilcox, MS, CGC,[7] David N. Herrmann, MD,[9] Jun Li, MD, PhD,[8,10] Sindhu Ramchandren, MD, MS,[8,11,12] Charlotte J. Sumner, MD,[13] Thomas E. Lloyd, MD, PhD,[13] John Day, MD, PhD,[14] Carly E. Siskind, MS, CGC,[14] Sabrina W. Yum, MD,[15,16] Reza Sadjadi, MD,[17] Richard S. Finkel, MD,[18] Steven S. Scherer, MD, PhD,[15] Davide Pareyson, MD,[3] Mary M. Reilly, MD, FCRP,[5] and Michael E. Shy, MD,[7,8] the Inherited Neuropathies Consortium–Rare Diseases Clinical Research Network

Objective: The paucity of longitudinal natural history studies in *MPZ* neuropathy remains a barrier to clinical trials. We have completed a longitudinal natural history study in patients with *MPZ* neuropathies across 13 sites of the Inherited Neuropathies Consortium.

Methods: Change in Charcot–Marie–Tooth Examination Score (CMTES) and Rasch modified CMTES (CMTES-R) were evaluated using longitudinal regression over a 5-year period in subjects with *MPZ* neuropathy. Data from 139 patients with *MPZ* neuropathy were examined.

Results: The average baseline CMTES and CMTES-R were 10.84 (standard deviation [SD] = 6.0, range = 0–28) and 14.60 (SD = 7.56, range = 0–32), respectively. A mixed regression model showed significant change in CMTES at years 2–5 (mean change from baseline of 0.87 points at 2 years, $p = 0.008$). Subgroup analysis revealed greater change in CMTES at 2 years in subjects with axonal as compared to demyelinating neuropathy (mean change of 1.30 points [$p = 0.016$] vs 0.06 points [$p = 0.889$]). Patients with a moderate baseline neuropathy severity also showed more

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.26518

Received Jun 13, 2022, and in revised form Aug 29, 2022. Accepted for publication Sep 23, 2022.

Address correspondence to Dr Fridman, Department of Neurology, University of Colorado Denver, 12631 East 17th Avenue, Mail Stop B185, Aurora, CO 80045. E-mail: vera.fridman@cuanschutz.edu

From the [1]Department of Neurology, University of Colorado Denver, Aurora, CO; [2]Department of Child Neurology, Scientific Institute for Research and Health Care Foundation Carlo Besta Neurological Institute, Milan, Italy; [3]Department of Clinical Neurosciences, Scientific Institute for Research and Health Care Foundation Carlo Besta Neurological Institute, Milan, Italy; [4]Maugeri Scientific Clinical Institutes, Neurorehabilitation Unit, Scientific Institute of Telese Terme, Telese Terme, Italy; [5]Centre for Neuromuscular Diseases, University College London Queen Square Institute of Neurology, London, UK; [6]Dubowitz Neuromuscular Centre, University College London Institute of Child Health and Great Ormond Street Hospital, London, UK; [7]Department of Neurology, University of Iowa Hospitals and Clinics, Iowa City, IA; [8]Department of Neurology, Wayne State University, Detroit, MI; [9]Department of Neurology, University of Rochester, Rochester, NY; [10]Department of Neurology, Vanderbilt University, Nashville, TN; [11]Department of Neurology, University of Michigan, Ann Arbor, MI; [12]PRA Health Sciences, Raleigh, NC; [13]Departments of Neurology and Neuroscience, John Hopkins University School of Medicine, Baltimore, MD; [14]Department of Neurology, Stanford University, Stanford, CA; [15]Department of Neurology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [16]Department of Neurology, Children's Hospital of Philadelphia, Philadelphia, PA; [17]Department of Neurology, Massachusetts General Hospital, Boston, MA; and [18]Department of Neurology, Nemours Children's Hospital, Orlando, FL

Additional supporting information can be found in the online version of this article.

notable change, by estimate, than those with mild or severe neuropathy (mean 2-year change of 1.14 for baseline CMTES 8–14 [$p = 0.025$] vs −0.03 for baseline CMTES 0–7 [$p = 0.958$] and 0.25 for baseline CMTES ≥ 15 [$p = 0.6897$]). The progression in patients harboring specific *MPZ* mutations was highly variable.
Interpretation: CMTES is sensitive to change over time in adult patients with axonal but not demyelinating forms of *MPZ* neuropathy. Change in CMTES was greatest in patients with moderate baseline disease severity. These findings will inform future clinical trials of *MPZ* neuropathies.

ANN NEUROL 2023;93:563–576

Dominant mutations in the myelin protein zero (*MPZ*) gene account for 5% of all of forms of genetically confirmed cases of Charcot–Marie–Tooth disease (CMT), and 10% of all genetically confirmed demyelinating forms of CMT.[1–3] *MPZ* neuropathies are genetically heterogeneous, with >200 different disease-causing mutations identified to date.[4] Genotype–phenotype correlation studies have identified 3 distinct types of *MPZ* neuropathy, including an infantile form with clinical presentation prior to 3 years of age and severely slowed motor nerve conduction velocities (NCVs; <15 m/s), a childhood onset (first or second decade) demyelinating neuropathy (CMT1B), and an adult onset axonal neuropathy (CMT2I).[4–10] The clinical presentation of *MPZ* neuropathies is similar to other forms of CMT, with foot deformities, distal muscle weakness and atrophy, and length-dependent sensory loss. Some mutations are associated with additional clinical features such as tonic pupils, dysphagia, hearing loss, and neuropathic pain.[8]

MPZ encodes the MPZ/P_0 protein, which is expressed almost exclusively by myelinating Schwann cells, and is the major protein in peripheral nerve myelin.[11] MPZ is a member of the immunoglobulin supergene family and is a homophilic adhesion molecule that is required for the normal compaction of myelin.[12] The proposed biological mechanisms by which mutations in *MPZ* result in neuropathy are diverse and include gain-of-function (including dominant negative effects) and loss-of-function mechanisms.[13,14] Some mutations result in retention of the mutant MPZ protein in the endoplasmic reticulum, whereas other MPZ mutants have abnormal interactions with wild-type MPZ in the myelin sheath.[15]

The striking biological and phenotypic heterogeneity of *MPZ* neuropathies are a challenge in developing candidate therapies, gathering accurate natural history data, and designing effective clinical trials. In this study, we have evaluated the natural history of *MPZ* neuropathies within the multicenter Inherited Neuropathies Consortium (INC) over a 5-year period. Disease progression was assessed using the Charcot–Marie–Tooth Examination Score (CMTES) as well as the Rasch analysis-based weighted CMTES (CMTES-R), which are commonly employed clinical outcome assessments in CMT that have previously demonstrated responsiveness to chang in patients with CMT1A.[16]

Patients and Methods

Standard Protocol Approvals, Registrations, and Patient Consents

All sites participating in the INC natural history study (protocol 6601) received institutional review board/ethics board approval for the study. All patients or their guardians signed consent forms. This trial was registered at www.clinicaltrials.gov (ID number NCT01193075).

Patients

Patients with *MPZ* mutations were recruited from the INC, which is a member of the National Institutes of Health Rare Diseases Clinical Research Network (rarediseasesnetwork.org/). Data were collected as part of the INC natural history study (protocol 6601) between February 2009 and July 2020 from a total of 13 sites within the INC (Table 1). Patients were examined by clinical investigators who had received training and were certified in the proper use of the CMTES, a validated 7-item, 28-point composite score based on patients' symptoms (3 items), and examination findings (4 items).[17,18] CMTES was then converted into CMTES-R, with a total score of 32.[18] As previously defined in a cohort of patients with CMT1A, CMTES subgroups were defined as follows: mild, 0–7; moderate, 8–14; and severe, 15+.[16] Patients were evaluated at yearly intervals, and the CMTES was recorded at each visit.

Patients were diagnosed with *MPZ* neuropathies based on clinical evidence of sensory and/or motor peripheral neuropathy (including length-dependent sensory loss, weakness, and atrophy of the distal musculature and decreased deep tendon reflexes), nerve conduction studies (NCS), and confirmatory genetic testing for a mutation in the *MPZ* gene. A Clinical Laboratory Improvement Amendments-certified laboratory in the United States or an equivalent certified testing facility outside of the United States performed all genetic testing.

Statistical Methods

Only patients with mutations in the *MPZ* gene were included. The CMTES and CMTES-R over time were

TABLE 1. Number of Patients per INC Site

INC Site	Subjects Contributed, n
C. Besta Neurological Institute	42
National Hospital for Neurology and Neurosurgery	33
University of Iowa	28
University of Pennsylvania	10
University of Rochester	7
Wayne State University	7
John Hopkins University	3
Stanford University	3
University of Michigan	2
Children's Hospital of Philadelphia	1
Harvard/Massachusetts General Hospital	1
Nemours Children's Hospital	1
Vanderbilt University Medical Center	1

INC = Inherited Neuropathies Consortium.

analyzed with longitudinal regression. Unstructured residual correlation matrices were used to maximize flexibility. The sample was determined on the bases of available cases and the ability to fit the longitudinal regression model. The covariance matrices of longitudinal models account for missing response data, so long as the missingness is not "not at random". Site to site variance was accounted for with random intercepts. The fixed effect of time was considered both as a categorical variable and as a continuous variable with a linear effect on the response. The categorical and linear time models were compared to each other using model fit statistics and plots. Gender, age, axonal versus demyelinating, and baseline CMTES were examined for interaction effects with categorical time for baseline and the first 2 years of follow-up. Change from baseline was used as the model outcome when baseline CMTES was an explanatory variable. *T* and *F* tests were performed on linear combinations of the model parameters. The performance of the CMTES-R versus CMTES was evaluated by comparing mean change from baseline to 2 years to the standard deviation (SD) of the change, the standardized response mean (SRM = mean change/SD change), for the first 2-year change. SRM values of 0.20 to 0.49, 0.50 to 0.79, and ≥0.80 reflect low, moderate, and high responsiveness, respectively.[19] Estimated annual mean score and changes and their SDs were obtained from the model parameters. The main outcomes were progression of CMTES and CMTES-R from baseline to follow-up year 2.

Axonal versus demyelinating neuropathy status was determined according to the recorded ulnar motor NCV at their first visit, with demyelinating neuropathy defined by velocities ≤ 38 m/s, and axonal neuropathy defined as an ulnar motor NCV > 38 m/s.[20] If ulnar NCV was not available, axonal versus demyelinating status was determined based on the examiners' specification. Demographics and baseline characteristics were compared between the axonal and demyelinating groups with chi-squared/Fisher exact association tests for categorical variables, and with *t* tests for continuous and scale variables. Associations between baseline CMTES and demographics and baseline characteristics were tested with *t* tests and analysis of variance type models for categorical predictors, and with linear regression for continuous and scale predictors.

Specific mutations to either amino acids or nucleotides were studied descriptively. For each participant, both the first available 2-year change in CMTES was calculated, and a regression line for all CMTES observations on a participant was fit. Spaghetti plots of CMTES were generated.

Thresholds for mild, moderate, and severe CMTES were established by applying a discriminant analysis for established CMTES categories. The same methods and data were previously used in Fridman et al.[16]

Univariate alpha was set to 0.05. Statistical analysis was performed with SAS 9.4.

Results

The INC evaluated patients with *MPZ* neuropathies between February 2009 and July 2020. Data from 139 participants with at least 1 CMTES/CMTES-R observation done either at their initial visit or during a subsequent visit in the first 5 years of follow-up were included (Table 2). Baseline CMTES and CMTES-R were available for 136 patients, and complete Charcot–Marie–Tooth Neuropathy Score (CMTNS) scores were available for 65 participants (see Table 2). Seventy-four (53%) of the participants were female, and 65 (47%) were male. The mean age of the participants was 46 years (SD = 18, range = 4–76); 1 participant had missing age data. Sixty-eight participants (49%) were 50 years of age or older, and 18 (13%) were younger than 21 years. One hundred twenty-two (88%) identified as White, 2 (1.5%) identified as Hispanic, and ethnicity data were missing for

TABLE 2. Demographics and Baseline Statistics

Patients at Baseline, n	**139**
Age, yr, mean ± SD	46.0 ± 18.2
Gender, M/F	65 (46.8%)/74 (53.2%)
Race, White, yes/no	122 (87.8%)/17 (12.2%)
Walked before 15 mo, yes/no	79 (68.7%)/36 (31.3%)
Demyelinating/axonal	73 (54.5%)/61 (45.5%)
Wheelchair use, yes/no	21 (15.4%)/115 (84.6%)
Walking support needed, yes/no	42 (30.7%)/95 (69.3%)
Ulnar MNCV categories, m/s	
≤15	29 (28.7%)
(15–37]	14 (13.9%)
(38–45]	20 (19.8%)
>45	38 (37.6%)
Foot deformity, yes/no	93 (68.9%)/42 (31.1%)
Pupil abnormalities, yes/no	4 (14.3%)/24 (85.7%)
Hearing loss, yes/no	30 (24.6%)/92 (75.4%)
Hip dysplasia, yes/no	0 (0.0%)/129 (100.0%)

F = female; M = male; MNCV = motor nerve conduction velocity; SD = standard deviation.

9 participants. Thirty-six participants (31.3%) reported a delay in ambulation as defined by walking later than 15 months of age; data were missing for 24 patients. Most patients (115, 84.6%) remained ambulatory, with 42 participants (31%) requiring assistive devices for walking (including ankle foot orthotics and canes/walking sticks) and 21 (15%) requiring a wheelchair (data for walking assistance and wheelchair use were missing for 2 and 3 participants, respectively). Foot deformities were present in 93 patients (69%), and hearing loss in 30 patients (25%). (Foot deformity and hearing loss data were missing for 4 and 17 patients, respectively.) Data for pupillary abnormalities were only available for 28 patients (111 missing), of whom 4 (14%) were reported to have abnormalities.

One hundred thirty-four participants had available NCS data or a designation of having either demyelinating or axonal neuropathy. Of these participants, the majority (73, 54%) had a demyelinating neuropathy, with 29 of 101 patients with available NCS data (29%) having an ulnar motor NCV of ≤15 m/s (see Table 2). Nine of the patients designated as having demyelinating neuropathy had an ulnar compound motor action potential of <2 mV; however, their ulnar NCVs were severely slowed (<15 m/s), suggesting that axonal loss was not the cause of NCV slowing in the majority of these participants. As compared to those with demyelinating neuropathy, patients with axonal neuropathy were older (mean of 55 vs 38 years, $p < 0.0001$; 70% vs 32% ≥50 years, $p < 0.0001$; 3.3% vs 21.9% <21 years, $p = 0.018$), were more likely to be White (97% vs 79%, $p = 0.003$), were less likely to have foot deformity (56% vs 80%, $p = 0.003$), were more likely to have walked by 15 months of age (96% vs 48%, $p < 0.0001$), reported difficulty with walking at a later age (47 vs 13 months, $p < 0.0001$), were less likely to require a wheelchair (6.8% vs 20.6%, $p = 0.025$), and had a lower baseline average CMTES (mean of 9.6 vs 11.8, $p = 0.036$). There were no statistically significant differences between the axonal and demyelinating groups for any of the other demographic variables (Table 3).

The average baseline CMTES was 10.8 (SD = 6.0, range = 0–28) and the average CMTES-R was 14.6 (SD = 7.6, range = 0–32), indicating a moderate range of severity. Baseline CMTES was associated with older age ($p = 0.001$), non-White race ($p = 0.018$), designation of demyelinating as opposed to axonal neuropathy (p value = 0.036), lower motor NCV ($p = 0.012$), not walking before 15 months ($p = 0.036$), younger age of difficulty walking ($p = 0.018$), likelihood of requiring walking support ($p < 0.0001$) or a wheelchair ($p < 0.0001$), and having hearing loss ($p = 0.013$).

Longitudinal CMTES and CMTES-R data were available for 67, 44, 38, 34, and 31 participants at years 1 through 5, respectively. The overall time effect was strongly statistically significant (p value <0.0001), indicating that the CMTES progresses over time. From baseline to the year 2 follow-up, the average CMTES increased by an estimated 0.87 points (95% confidence interval [CI] = 0.23–1.50, $p = 0.008$), and a significant change as compared to baseline was also evident at years 3, 4, and 5 (Figs 1 and 2). The linear model estimated an average CMTES increase of 0.73 units per 2 years (95% CI = 0.46–1.00, $p < 0.0001$). However, a linear approximation of the time trend was questionable (likelihood ratio test $p = 0.03$, Akaike information criterion increase of 2.4). CMTES-R increased from baseline to 2 years by an estimate of 0.96 points (95% CI = 0.10–1.82, $p = 0.030$), and a significant change as compared to baseline was present at years 3, 4, and 5 (Fig 3). As compared to the CMTES-R, CMTES had a higher SRM for change from baseline to 2 years (0.37 vs 0.30). Progression of the CMTNS was not examined due to the limited sample size.

TABLE 3. Clinical Characteristics of Axonal versus Demyelinating *MPZ* Neuropathy

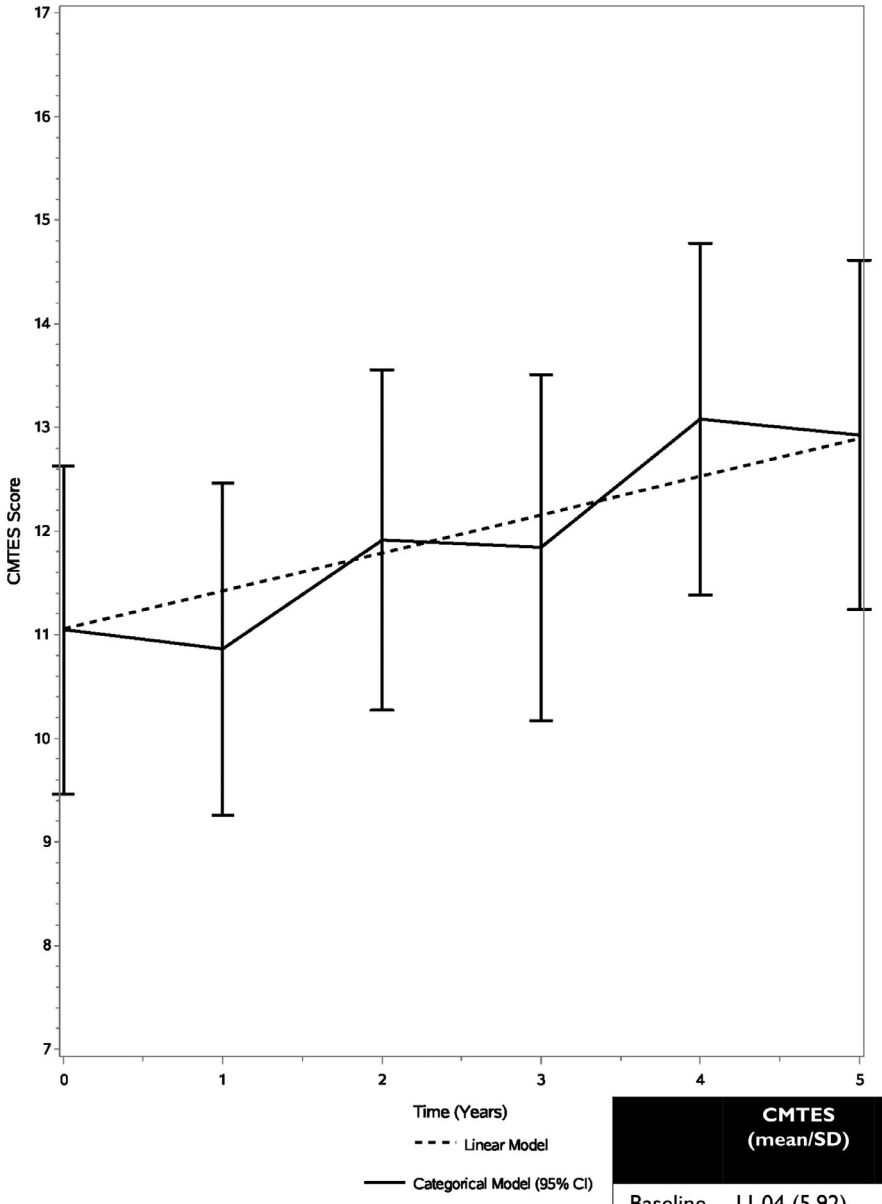
Characteristic	Axonal	Demyelinating	*p*
Patients, n	61	73	
Age, yr, mean ± SD	55.16 ± 13.13	38.26 ± 18.54	<0.0001
Gender, M/F	33 (54.1%)/28 (45.9%)	29 (39.7%)/44 (60.3%)	0.0966
Age at difficulty walking, mo, mean ± SD	46.83 ± 11.95	12.73 ± 17.49	<0.0001
Walked before 15 mo, yes/no	48 (96.0%)/2 (4.0%)	30 (48.4%)/32 (51.6%)	<0.0001
Walking support needed, yes/no	18 (30.0%)/42 (70.0%)	23 (31.5%)/50 (68.5%)	0.8515
Wheelchair use, yes/no	4 (6.8%)/55 (93.2%)	15 (20.5%)/58 (79.5%)	0. 0251
Foot deformity, yes/no	34 (55.7%)/27 (44.3%)	56 (80.0%)/14 (20.0%)	0.0028
Pupil abnormalities, yes/no	1 (10.0%)/9 (90.0%)	3 (16.7%)/15 (83.3%)	1.0000
Hearing loss, yes/no	12 (22.2%)/42 (77.8%)	18 (28.6%)/45 (71.4%)	0.4330
AFO(s) needed, yes/no	16 (26.2%)/45 (73.8%)	25 (40.3%)/37 (59.7%)	0.0974
Hip dysplasia, yes/no	0 (0.0%)/60 (100.0%)	0 (0.0%)/66 (100.0%)	-
Baseline CMTES-R, mean ± SD	13.34 ± 6.58	15.56 ± 8.30	0.0915
Baseline CMTES, mean ± SD	9.62 ± 4.73	11.79 ± 6.87	0.0361

AFO = ankle–foot orthosis; CMTES = Charcot–Marie–Tooth Examination Score; CMTES-R = Rasch modified CMTES; F = female; M = male.

The type of neuropathy (demyelinating vs axonal), gender, and age were examined as effect modifiers for the progression of CMTES over 2 years of follow-up. Complete data were used for consistency; 131 patients were included in the analysis: 130 at baseline, 63 at follow-up year 1, and 42 at follow-up year 2. Sixty-two (47%) were male, and 69 (53%) were female. Mean age was 46.3 years (SD = 18.1, range = 3.9–76.4). Sixty (46%) were classified as axonal and 71 (54%) as demyelinating. Mean baseline CMTES was 10.8 (SD = 6.1, range = 0–28); 1 observation was missing. Neither gender nor age significantly modified CMTES progression form baseline to follow-up year 2 ($p = 0.500$ and 0.972, respectively). The test for type of neuropathy as a modifier of CMTES progression to follow-up year 2 was also nonsignificant ($p = 0.057$).

By estimate, CMTES progression decreased as age increased; however, the overall test for progression at any age was nonsignificant ($p = 0.062$). Female patients increased by an estimated 1.11 units (95% CI = 0.01–2.22, $p = 0.049$) from baseline to 2-year follow-up, whereas the change for male patients was nonsignificant (estimate = 0.64, 95% CI = −0.29 to 1.57, $p = 0.170$). Based on sample size, participants were divided into axonal and demyelinating neuropathy groups, as opposed to the 3 phenotypic groups previously described in *MPZ* neuropathies.[8] Axonal patients increased on the CMTES by an estimated 1.30 points (95% CI = 0.27–2.33, p value = 0.016, SRM = 0.51) from baseline to 2-year follow-up, whereas the change for demyelinating patients was nonsignificant (estimate = 0.06, 95% CI = −0.76 to 0.87, p value = 0.889, SRM = 0.03; see Fig 3). To reiterate, the difference in progression between axonal and demyelinating participants was nonsignificant ($p = 0.057$), possibly because the test for effect modification was underpowered.

Baseline CMTES, classified as mild (0–7), moderate (8–14), and severe (15+), was also considered as an effect modifier of CMTES progression. Change scores from baseline to follow-up years 1 and 2 were fit to a longitudinal regression model (Fig 4). Data were available for 83 patients: 21 mild, 40 moderate, and 22 severe. There were 66 available observations at follow-up year 1 and 44 at follow-up year 2. Only the moderate group showed statistically significant progression from baseline to follow-up year 2 (estimate = 1.14, 95% CI = 0.16–2.12, $p = 0.025$, SRM = 0.49). The mild group was estimated to decrease nonsignificantly by −0.03 (95% CI = −1.36 to 1.30, $p = 0.958$, SRM = −0.02), whereas the severe group increased nonsignificantly by 0.25 (95%

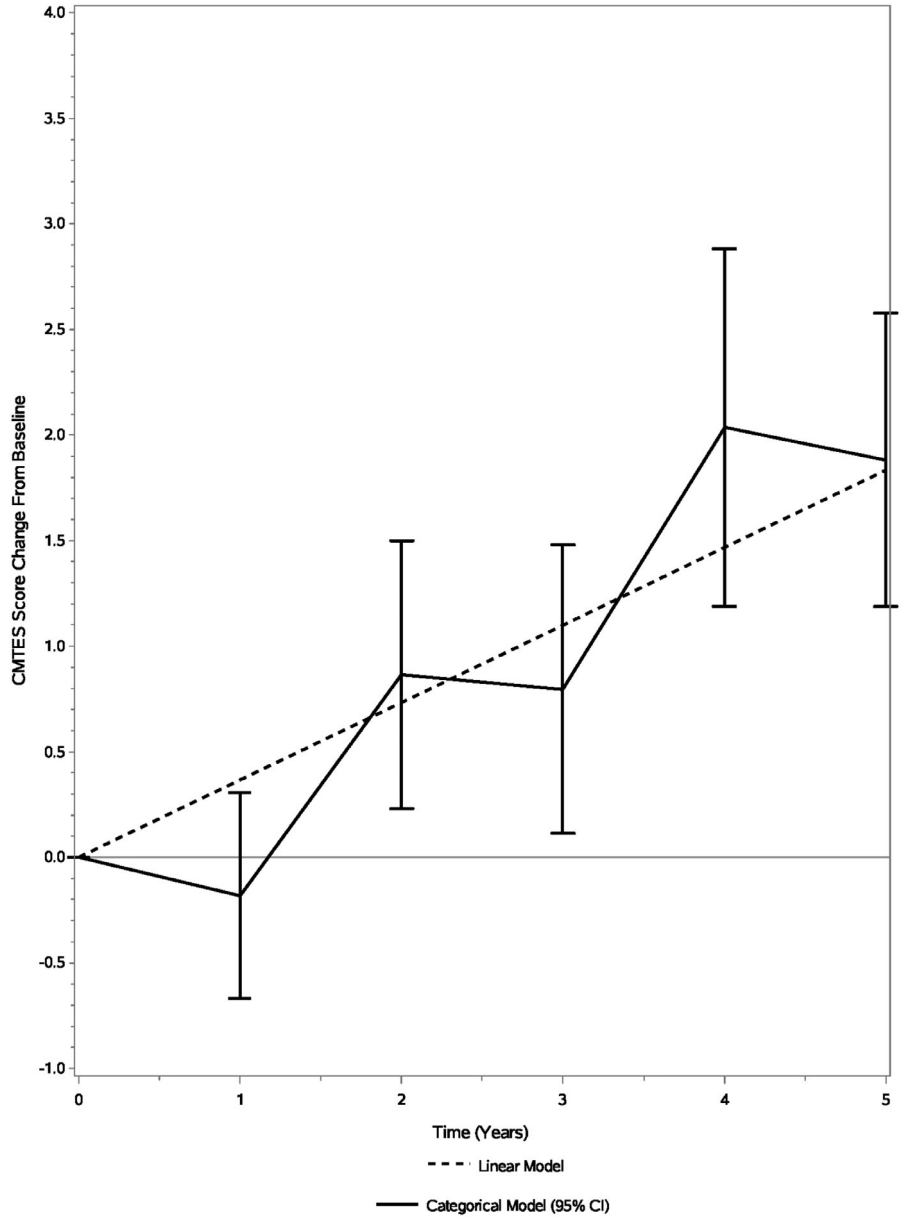


	Available CMTES
Baseline	136
1 Year	67
2 Years	44
3 Years	38
4 Years	34
5 Years	31

	CMTES (mean/SD)	95% Confidence Interval
Baseline	11.04 (5.92)	9.46-12.63
Year 1	10.86 (5.87)	9.26-12.47
Year 2	11.91 (5.97)	10.27-13.55
Year 3	11.84 (6.32)	10.17-13.51
Year 4	13.08 (6.10)	11.38-14.78
Year 5	12.93 (6.34)	11.24-14.61

FIGURE 1: Charcot–Marie–Tooth Examination Score (CMTES) over a 5-year period. CMTES increases gradually over subsequent years with relatively linear progression. Bars are 95% confidence intervals (CIs). SD = standard deviation.

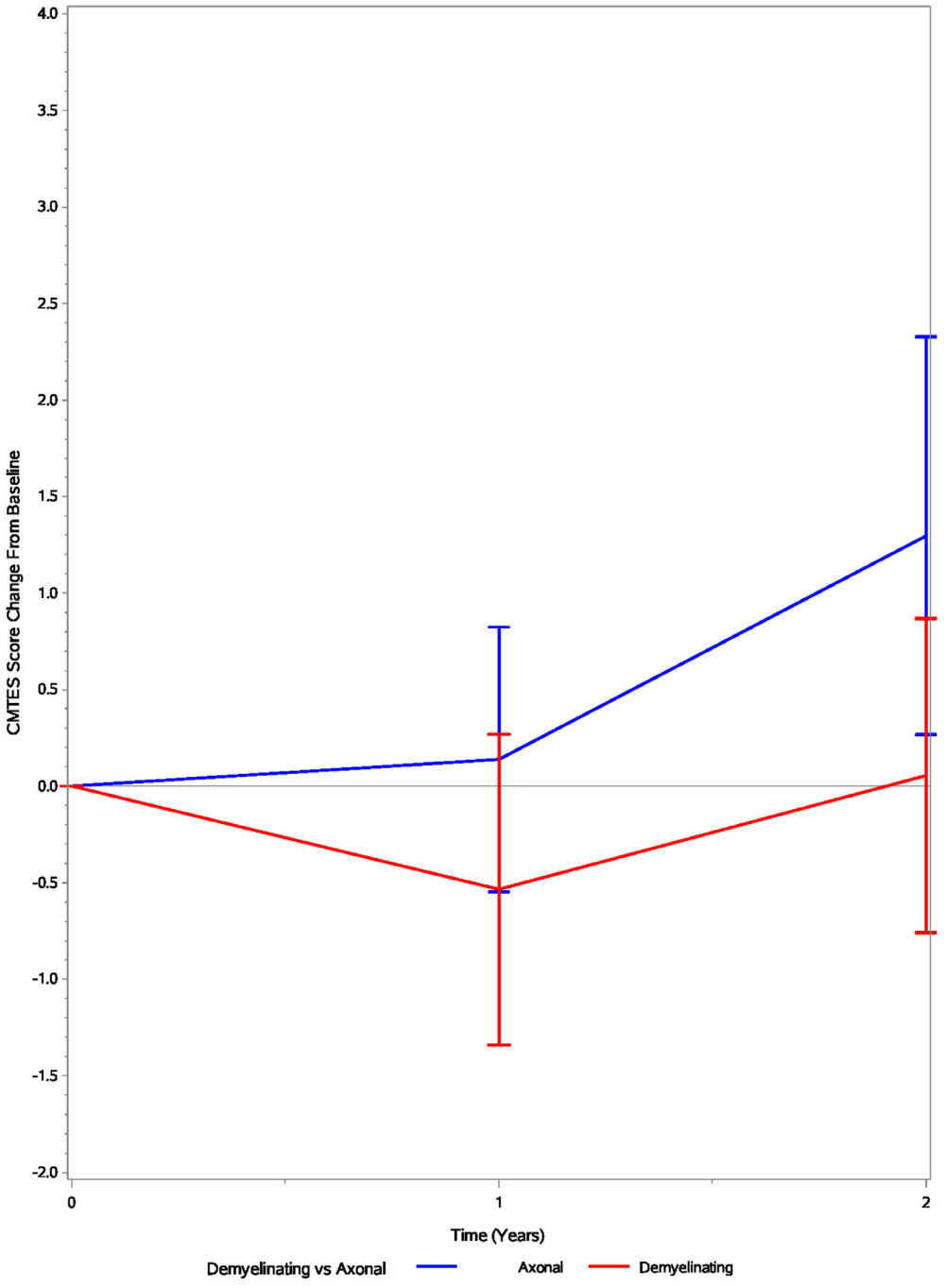
CI = −1.09 to 1.59, $p = 0.690$, SRM = 0.11). The overall test for baseline CMTES category modifying the progression of CMTES from baseline to follow-up year 2 was nonsignificant, however ($p = 0.281$). Furthermore, none of the pairwise comparisons was statistically significant.



	Change from Baseline	95% Confidence Interval	P-value
Year 1	-0.18	-0.67, 0.31	0.4625
Year 2	0.87	0.23, 1.50	0.0084
Year 3	0.80	0.11, 1.48	0.0230
Year 4	2.04	1.19, 2.88	<0.0001
Year 5	1.88	1.19, 2.58	<0.0001

FIGURE 2: Charcot–Marie–Tooth Examination Score (CMTES) change from baseline over a 5-year period. Bars are 95% confidence intervals (CIs).

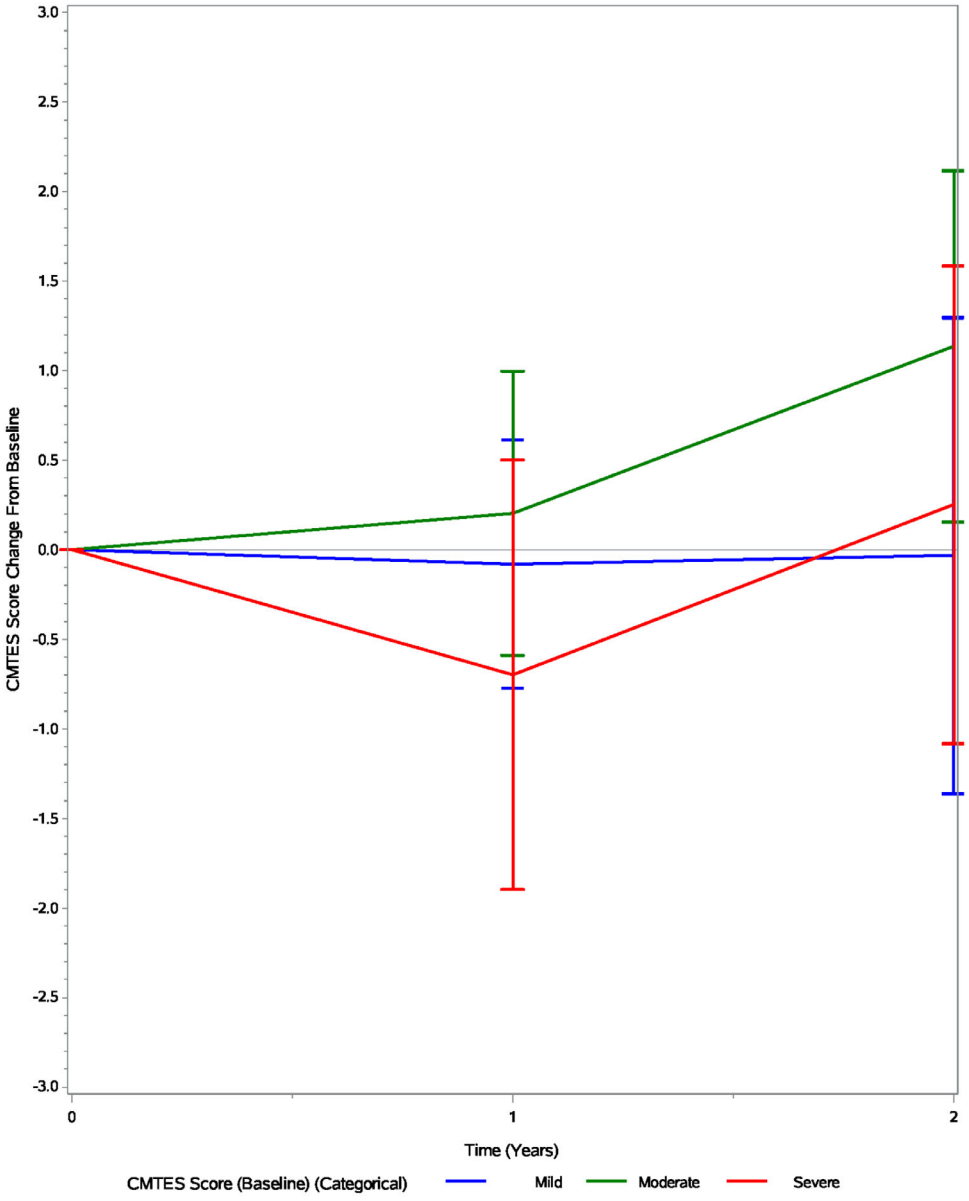
One hundred thirty-seven patients had available *MPZ* variant data. Variant details and clinical features are described in Table S1. Thirty-two patients had a participating family member, making up 12 families total; however, segregation data were not available for all patients. Forty-four unique variants were described,



	Mean change from baseline at 2 years	P-value	95% Confidence Interval at 2 years	Mean/SD (SRM)
Axonal	1.30	0.0156	0.27, 2.33	0.51
Demyelinating	0.06	0.8891	-0.76, 0.87	0.03

FIGURE 3: Categorical model showing change in Charcot–Marie–Tooth Examination Score (CMTES) based on axonal versus demyelinating disease type over a 2-year period. Red represents demyelinating model; blue represents axonal model. Bars are 95% confidence intervals. SD = standard deviation; SRM = standardized response mean.

1 of which appears to be novel and is classified as of uncertain significance. Thirty-nine variants are classified as pathogenic or likely pathogenic.[21] CMTES data were available for 60 patients with 32 unique variants. The most common variant in our cohort was p.Pro70Ser, which was reported in 13 total participants, 9 with



CMTES	Mean change from baseline at 2 years	P-value	95% Confidence Interval at 2 years	SRM
Mild 0-7	-0.03	0.96	-1.36, 1.30	-0.02
Moderate 8-14	1.14	0.02	0.16, 2.12	0.49
Severe ≥15	0.25	0.69	-1.09, 1.59	0.11

FIGURE 4: Categorical model showing change in Charcot–Marie–Tooth Examination Score (CMTES) based on disease severity at baseline over a 2-year period. Red = mild (CMTES 0–7), green = moderate (CMTES 8–14), blue = severe (CMTES ≥15). Bars are 95% confidence intervals. SRM = standardized response mean.

CMTES available. All 13 patients had their initial visit in adulthood (42–76 years of age) and presented with an axonal neuropathy. Of the 10 with available data, all walked before 15 months. The highest baseline CMTES was reported in the p.Gly137Ser and p.Val102fs variants.

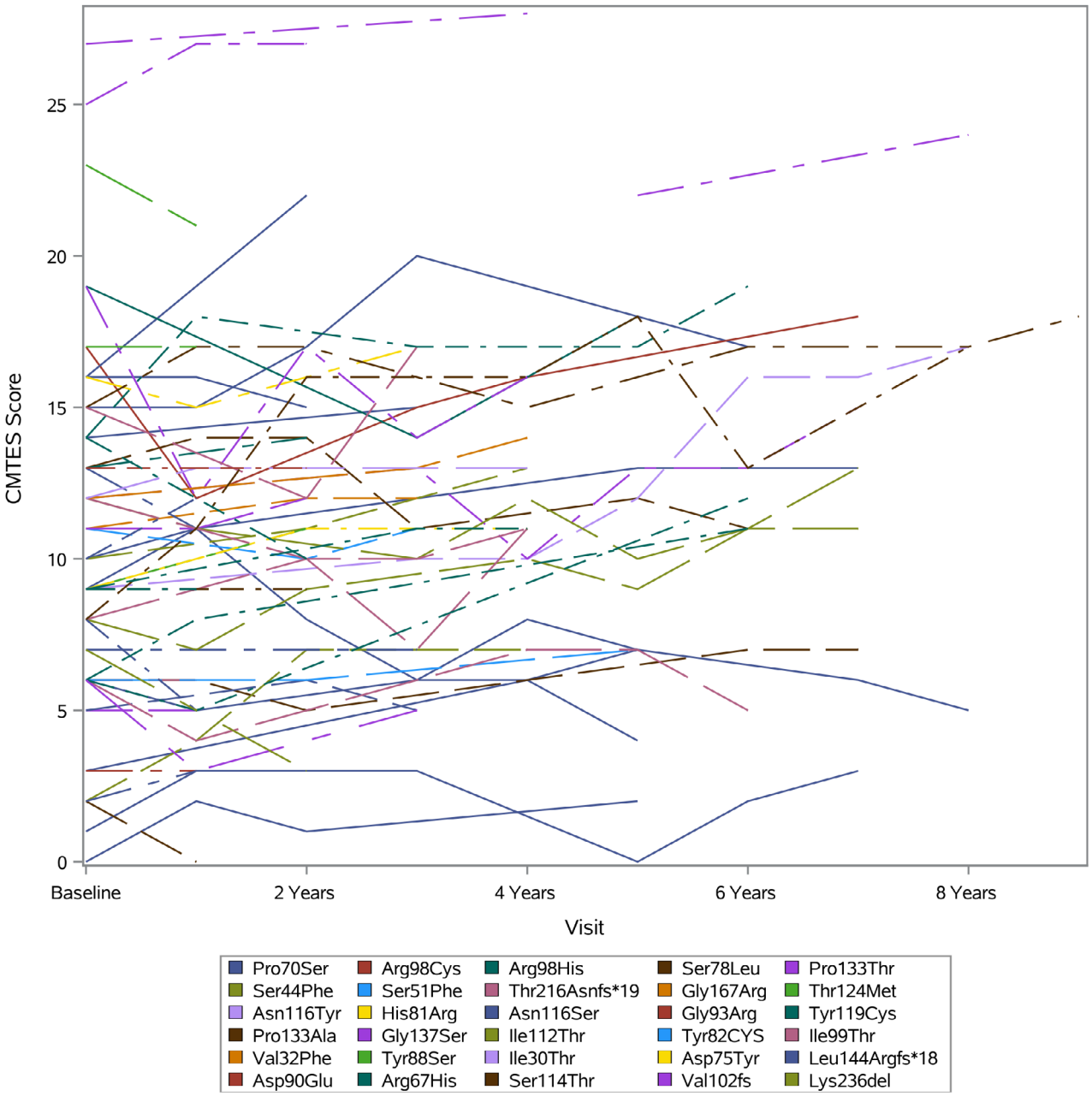


FIGURE 5: Charcot–Marie–Tooth Examination Score (CMTES) over 8 years for individual participants.

Progression on the CMTES varied among participants with different *MPZ* variants, as well as between patients with the same variant and patients from the same family (Fig 5). For example, in the 9 participants with the p.Pro70Ser variant, the 2-year change in CMTES showed an increase in 6 patients, remained stable in 1 patient, and decreased in 2 patients. Despite this variable progression, axonal or demyelinating phenotypes were largely consistent for individual mutations. Variants associated with the greatest 2-year change included p.Ser140Thr and p.Pro70Ser. In contrast, participants with the p.Arg98His and p.Lys236del variants showed a notable decrease in CMTES over 2 years.

Discussion

To our knowledge, this is the largest longitudinal study of *MPZ* neuropathies to date, and is the first study to evaluate mutation-specific natural history in this patient population. Our findings demonstrate progression in CMTES over time, with an estimated change of 0.87 points ($p = 0.008$) from baseline to 2-year follow-up, confirming that this clinical outcome assessment will be useful for future clinical trials. Importantly, by examining this large cohort, we were able to detect a qualitative difference in the 2-year change in the CMTES between participants with axonal versus demyelinating neuropathy (1.30-point

increase for axonal [$p = 0.016$] vs a nonsignificant increase of 0.06 points for demyelinating [$p = 0.889$]). The 2-year change in the axonal group was also associated with a higher SRM (0.51 vs 0.03), suggesting a higher level of responsiveness. It should be emphasized, however, that the difference between the two groups did not meet statistical significance ($p = 0.057$), potentially owing to a lack of power. Additionally, CMTES was only sensitive to change in participants with moderate neuropathy (as defined by the baseline CMTES), and did not reliably capture progression in patients on the phenotypic extremes. Finally, the mutation-specific natural history in this cohort showed highly variable rates of progression among patients with different *MPZ* mutations, as well as among patients with shared genetic variants, underscoring the heterogeneity in the clinical progression of these disorders.

In keeping with prior studies, we observed an association between baseline CMTES and age ($p = 0.001$); however, there was no evidence that the responsiveness of the CMTES differed according to age or gender.[16] Baseline scores were also associated with a younger age of walking difficulty ($p = 0.018$), suggesting that earlier gait difficulty in *MPZ* neuropathy may portend a more severe neuropathy in later life. The 2-year change in CMTES that we observed in *MPZ* neuropathy is greater than that previously observed in CMT1A (0.4 points) and smaller than that seen in patients with CMT2A (0.97 points).[16,22] These change scores are consistent with the known differences in the clinical severity of these disorders, which together comprise the most common forms of AD hereditary neuropathy.

In contrast to our observations in CMT1A, we found that the SRM associated with the 2-year change in *MPZ* neuropathy was slightly higher for unweighted CMTES than for the CMTES-R (0.37 vs 0.30, respectively), suggesting that the use of the CMTES-R introduces a greater variability in scores. This observation parallels prior findings in CMT2A (*MFN2* mutations) reported by Pipis et al, which demonstrated a lower 2-year SRM for the CMTES-R than for the unweighted CMTES.[22] The CMTNS-R was developed based on a psychometric analysis using CMT1A data in an effort to allow for the detection of smaller differences in clinical severity and did result in an improved SRM in the CMT1A population.[16] Our current findings suggest that the CMTES-R could be further refined for use in more rapidly progressive neuropathies such as *MPZ* neuropathy and CMT2A.

Although there were fewer patients with axonal neuropathy in our cohort, they drove the overall change observed in CMTES. This finding is also consistent with the prior observations that demyelinating *MPZ* neuropathy progresses less rapidly beyond adolescence, whereas axonal forms can progress rapidly in adulthood, with some patients losing ambulation within years of disease onset.[9,23–25] The differences in natural history between axonal and demyelinating *MPZ* neuropathy may be related to the varied pathophysiology of these conditions, with childhood onset demyelinating forms more commonly resulting from developmental defects in myelin (ie, dysmyelination), and axonal forms arising secondary to disruptions in *MPZ*-mediated signal transduction and Schwann cell–axonal interactions.[8,26,27] These data suggest that clinical trials in the adult population with *MPZ* neuropathy employing the CMTES may be most informative in participants with axonal neuropathy. Additionally, our findings underscore the importance of ongoing natural history studies in children with demyelinating CMT1B using age-appropriate scales such as the CMT Pediatric Scale and CMT Infant and Toddler Scale, as progression in this patient population is likely to be greatest in childhood.[28,29]

The finding that only participants with moderate neuropathy (CMTES of 8–14) showed progression in CMTES over 2 years also has important implications for future clinical trials. Two-year progression on the CMTES in the moderate group was 1.14, ($p = 0.025$), with an SRM of 0.49. In contrast, the mild group actually showed a nonsignificant decrease in scores over 2 years, whereas the severe group showed a nonsignificant increase. The lack of responsiveness of the CMTES in severely affected patients mirrors our prior findings in CMT1A, underscoring that the CMTES is insensitive to the possible progression in those with severe neuropathy. In contrast, the lack of significant progression in patients with initial scores in the mild range of severity in this cohort differs from our observations in CMT1A, as mildly affected patients with CMT1A did have significant progression over 2 years on the CMTES-R.[16] Given that the severity categories employed in the current study were defined in a CMT1A population, further examining these categories in conjunction with other measures of neuropathy severity will be needed to determine their relevance in *MPZ* neuropathy.

Genotype–phenotype correlations in our cohort were consistent with those defined by Sanmaneechai et al,[8] although fewer patients in our cohort reported delayed milestones (see Table 3). One new variant with an unknown classification was also identified: p.Pro105Ser, for which segregation analysis was not available. This variant has not been reported in the Genome Aggregation Database, implying it is not present in the general population. The expected amino acid residues are highly

conserved throughout species, and the pathogenic variant p.Pro105Thr affects the same codon.[21] Further characterization of this novel variant will be needed to determine their likelihood of pathogenicity.

The progression in CMTES among patients with differing *MPZ* mutations was highly variable, with the greatest progression over 2 years observed in a patient with the p.Ser140Thr variant and a patient with the p.Pro70Ser variant, both with axonal neuropathy. Although the majority of individual *MPZ* variants did consistently manifest with either axonal or demyelinating phenotypes, as previously observed by Sanmaneechai et al, patients with the same *MPZ* variant demonstrated variable 2-year change on the CMTES, as specifically highlighted by the 13 participants with the p.Pro70Ser variant.[8] Our findings are purely descriptive and cannot be extrapolated to other patients with shared variants in *MPZ*; however, it does appear that the natural history associated with individual *MPZ* mutations is complex and heterogeneous. These findings align with prior observations that specific cellular disruptions resulting from *MPZ* mutants do not correlate with specific clinical phenotypes.[8]

Several missense variants in *MPZ* have been specifically reported to cause a late onset, severe, axonal neuropathy with rapid progression. These variants tend to congregate around codon 70 in the extracellular and domain: p.Pro70Leu, p.Asp75Val, and p.Pro70Ser.[30,31] In contrast, the 9 subjects in our cohort with the p.Pro70Ser variant demonstrated baseline CMTES in the mild to moderate range and showed variable progression on the CMTES that was not distinct from the other variants examined. The difference in the observed disease progression could be related to the possibility that genetic testing was selectively offered to patients with more severe clinical phenotypes in the past. The broader use of genetic testing more recently may have led to the identification of milder and more slowly progressing *MPZ* neuropathies. The natural history of specific *MPZ* variants will therefore likely continue to become clarified as more affected individuals are identified. Given the clinical heterogeneity of *MPZ* neuropathy, as well as its varied gain-of-function mechanisms, future treatments will likely need to be personalized, with focus on allele-specific approaches.[32]

Our study is limited by sample size as well as by missing data, as not all patients returned every year and some had missing baseline CMTES/CMTES-R. We have addressed this by using longitudinal regression models to evaluate change in CMTES, allowing for missing years between visits. Our determination of axonal versus demyelinating neuropathy also relied on ulnar NCV, rather than more complete electrophysiological data. Sample size also limited our comparison of change in CMTES in the axonal and demyelinating groups beyond 2 years of follow-up, precluding a comparison of previously described phenotypic groups in *MPZ* neuropathy, including early onset, childhood (classic CMT1B) onset, and adult onset patients. Finally, our study focused on the CMTES as the measure of progression, and did not examine other established measures of neuropathy, such as the CMT functional outcome measure, 10m walk test, or the Overall Neuropathy Limitations Scale,[19,33,34] many of which were not available when we began our study. For similar reasons, we did not evaluate emerging objective biomarkers such as magnetic resonance imaging (MRI) of intramuscular fat accumulation or plasma neurofilament light chain concentrations, which will need to be evaluated in future studies.[35]

Taken together, our results suggest that the CMTES will serve as a valuable tool for clinical trials in axonal forms of *MPZ* neuropathy. Based on our findings, a 2-year double-blinded, randomized, placebo-controlled trial in this population (powered to evaluate complete arrest in disease progression with 80% power and alpha of 0.05) would require 62 subjects in each arm. Although this is still an ambitious number given the rarity of *MPZ* neuropathy, the recent rise in gene testing has led to a rapid identification of new *MPZ* cases, with 76 new mutations reported between 2005 and 2018, the majority of which are axonal.[4] Additionally, the CMTES would not be used in isolation, but rather alongside other biomarkers, such as quantitative calf muscle fat accumulation on MRI (which shows significant 1-year change in CMT1A with an SRM of 0.83), allowing for further reductions in sample size.[35,36] Importantly, we have shown that the CMTES is not an effective clinical outcome assessment for adult patients with early onset, demyelinating forms of *MPZ* neuropathy. These patients should therefore be evaluated in early life using pediatric clinical outcome assessments, such as the CMT pediatric and infant scores, and the recently developed pediatric CMT-specific quality of life outcome measure.[28,29,37]

Acknowledgments

This research was supported by the National Institute for Health Research University College London Hospitals Biomedical Research Centre. The Inherited Neuropathies Consortium (2U54NS065712-07) is a part of the Rare Diseases Clinical Research Network, an initiative of the Office of Rare Diseases Research, National Center for Advancing Translation Sciences (NCATS). The INC is funded through a collaboration between NCATS and the NIH National Institute of Neurological Disorders and

Stroke (NINDS). The INC also receives support from the Muscular Dystrophy Association and Charcot-Marie-Tooth Association. This work was partially supported by the Italian Ministry of Health (RRC to DP). The funders had no role in the study design, data collection, data analysis, data interpretation, or writing of the manuscript. V.F. reports funding support from National Institute of Diabetes and Digestive and Kidney Diseases (5K23DK118202-02). D. N.H. reports grant support through U54 NS065712, NINDS, 5U01NS109403-03, Charcot-Marie-Tooth Association, 1R01DK115687-03, Muscular Dystrophy Association, Friedreich's Ataxia Research Alliance, Voyager Pharmaceuticals, and Acceleron Pharma. C.J.S. reports funding support from Roche and Argenx. D.P. reports grant support from Telethon-UILDM, AFM-Telethon, and the Charcot-Marie-Tooth Association. S.S.S. reports grant support from U54 NS0657, the Charcot-Marie-Tooth Association, and the Muscular Dystrophy Association. M.M.R. reports grant support from U54 NS0657, the Muscular Dystrophy Association, Charcot-Marie-Tooth Association, Medical Research Council, and Wellcome trust. M.E.S. reports grant support from U54 NS0657, the Muscular Dystrophy Association, and the Charcot-Marie-Tooth Association.

We thank all the patients who participated in the INC and their families, without whom this study would not be possible; and the people working at INC sites who contributed to this study, especially J. Blake, B. Burgos, D. Calabrese, V. Chaudhry, D. Cornblath, K. Eichinger, T. Estilow, C. Gandioli, A. Hamilton, A. M. Glanzman, A. Hoke, A. Kelley, L. Medne, M. Menezes, J. Mountain, S. Murphy, J. Olsen, A. Rossor, O. Sanmaneechai, P. Saveri, A. Sorey, M. Skorupinska, J. Sowden, and A. Swenson.

Author Contributions

V.F., S.S., S.S.S., M.M.R., and M.E.S. contributed to conception and design of the study; V.F., S.S., K.S., C.B., J.D., S.F., R.S.F., T.G., L.G., D.N.H., M.L., J.L., T.E.L., I.M., F.M., E.P., C.P., G.P., S.R., R.Sh., R.Sa., C.E.S., C.J.S., J.W., S.W.Y., and D.P. contributed to acquisition and analysis of data; V.F., S.S., J.B., K.S., D.P., S.S.S., M.M.R., and M.E.S. contributed to drafting the text or preparing the figures.

Potential Conflicts of Interest

S.R. is currently employed by a CRO (PRA Health Sciences), which works with pharmaceutical companies. Carlo Besta Neurological Institute receives donations for research from Pfizer, LAM Therapeutics, and Acceleron Pharma. The other authors have no potential conflicts of interest to report.

Data Availability Statement

Data not provided in the article because of space limitations as well as the study protocol and statistical analysis will be shared at the request of any qualified investigator for purposes of replicating procedures and results.

References

1. Saporta AS, Sottile SL, Miller LJ. et al. Charcot-Marie-tooth disease subtypes and genetic testing strategies. Ann Neurol 2011;69:22–33.
2. Murphy SM, Laura M, Fawcett K, et al. Charcot-Marie-tooth disease: frequency of genetic subtypes and guidelines for genetic testing. J Neurol Neurosurg Psychiatry 2012;83:706–710.
3. Nelis E, Van Broeckhoven C, De Jonghe P, et al. Estimation of the mutation frequencies in Charcot-Marie-tooth disease type 1 and hereditary neuropathy with liability to pressure palsies: a European collaborative study. Eur J Hum Genet 1996;4:25–33.
4. Callegari I, Gemelli C, Geroldi A, et al. Mutation update for myelin protein zero-related neuropathies and the increasing role of variants causing a late-onset phenotype. J Neurol 2019;266:2629–2645.
5. Shy ME, Jani A, Krajewski K, et al. Phenotypic clustering in MPZ mutations. Brain 2004;127:371–384.
6. Krajewski KM, Lewis RA, Fuerst DR, et al. Neurological dysfunction and axonal degeneration in Charcot-Marie-tooth disease type 1A. Brain 2000;123(pt 7):1516–1527.
7. Thomas PK, Marques W Jr, Davis MB, et al. The phenotypic manifestations of chromosome 17p11.2 duplication. Brain 1997;120(pt 3): 465–478.
8. Sanmaneechai O, Feely S, Scherer SS, et al. Genotype-phenotype characteristics and baseline natural history of heritable neuropathies caused by mutations in the *MPZ* gene. Brain 2015;138:3180–3192.
9. Bird TD, Kraft GH, Lipe HP, et al. Clinical and pathological phenotype of the original family with Charcot-Marie-tooth type 1B: a 20-year study. Ann Neurol 1997;41:463–469.
10. Brennan KM, Bai Y, Shy ME. Demyelinating CMT—what's known, what's new and what's in store. Neurosci Lett 2015;596:14–26.
11. Hayasaka K, Himoro M, Sato W, et al. Charcot-Marie-tooth neuropathy type 1B is associated with mutations of the myelin P0 gene. Nat Genet 1993;5:31–34.
12. Volpi VG, Touvier T, D'Antonio M. Endoplasmic reticulum protein quality control failure in myelin disorders. Front Mol Neurosci 2016; 9:162.
13. Grandis M, Vigo T, Passalacqua M, et al. Different cellular and molecular mechanisms for early and late-onset myelin protein zero mutations. Hum Mol Genet 2008;17:1877–1889.
14. Xu W, Shy M, Kamholz J, et al. Mutations in the cytoplasmic domain of P0 reveal a role for PKC-mediated phosphorylation in adhesion and myelination. J Cell Biol 2001;155:439–446.
15. Wrabetz L, D'Antonio M, Pennuto M, et al. Different intracellular pathomechanisms produce diverse myelin protein zero neuropathies in transgenic mice. J Neurosci 2006;26:2358–2368.
16. Fridman V, Sillau S, Acsadi G, et al. A longitudinal study of CMT1A using Rasch analysis based CMT neuropathy and examination scores. Neurology 2020;94:e884–e896.
17. Shy ME, Blake J, Krajewski K, et al. Reliability and validity of the CMT neuropathy score as a measure of disability. Neurology 2005; 64:1209–1214.
18. Sadjadi R, Reilly MM, Shy ME, et al. Psychometrics evaluation of Charcot-Marie-tooth neuropathy score (CMTNSv2) second version, using Rasch analysis. J Peripher Nerv Syst 2014;19:192–196.

19. Piscosquito G, Reilly MM, Schenone A, et al. Responsiveness of clinical outcome measures in Charcot-Marie-tooth disease. Eur J Neurol 2015;22:1556–1563.

20. Harding AE, Thomas PK. The clinical features of hereditary motor and sensory neuropathy types I and II. Brain 1980;103:259–280.

21. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17: 405–424.

22. Pipis M, Feely SME, Polke JM, et al. Natural history of Charcot-Marie-tooth disease type 2A: a large international multicentre study. Brain 2020;143:3589–3602.

23. Bai Y, Ianokova E, Pu Q, et al. Effect of an R69C mutation in the myelin protein zero gene on myelination and ion channel subtypes. Arch Neurol 2006;63:1787–1794.

24. Dacci P, Taroni F, Bella ED, et al. Myelin protein zero Arg36Gly mutation with very late onset and rapidly progressive painful neuropathy. J Peripher Nerv Syst 2012;17:422–425.

25. Liu L, Li X, Zi X, et al. Two novel MPZ mutations in Chinese CMT patients. J Peripher Nerv Syst 2013;18:256–260.

26. De Jonghe P, Timmerman V, Ceuterick C, et al. The Thr124Met mutation in the peripheral myelin protein zero (MPZ) gene is associated with a clinically distinct Charcot-Marie-tooth phenotype. Brain 1999;122(2):281–290.

27. Li J, Bai Y, Ianakova E, et al. Major myelin protein gene (P0) mutation causes a novel form of axonal degeneration. J Comp Neurol 2006;498:252–265.

28. Mandarakas MR, Menezes MP, Rose KJ, et al. Development and validation of the Charcot-Marie-tooth disease infant scale. Brain 2018; 141:3319–3330.

29. Burns J, Ouvrier R, Estilow T, et al. Validation of the Charcot-Marie-tooth disease pediatric scale as an outcome measure of disability. Ann Neurol 2012;71:642–652.

30. Laura M, Milani M, Morbin M, et al. Rapid progression of late onset axonal Charcot-Marie-Tooth disease associated with a novel MPZ mutation in the extracellular domain. J Neurol Neurosurg Psychiatry 2007;78:1263–1266.

31. Benedetti S, Previtali SC, Coviello S, et al. Analyzing histopathological features of rare Charcot-Marie-Tooth neuropathies to unravel their pathogenesis. Arch Neurol 2010;67:1498–1505.

32. Fratta P, Ornaghi F, Dati G, et al. A nonsense mutation in myelin protein zero causes congenital hypomyelination neuropathy through altered P0 membrane targeting and gain of abnormal function. Hum Mol Genet 2019;28:124–132.

33. Eichinger K, Burns J, Cornett K, et al. The Charcot-Marie-Tooth functional outcome measure (CMT-FOM). Neurology 2018;91:e1381–e1384.

34. Graham RC, Hughes RA. A modified peripheral neuropathy scale: the overall neuropathy limitations scale. J Neurol Neurosurg Psychiatry 2006;77:973–976.

35. Morrow JM, Evans MRB, Grider T, et al. Validation of MRC Centre MRI calf muscle fat fraction protocol as an outcome measure in CMT1A. Neurology 2018;91:e1125–e1129.

36. Morrow JM, Sinclair CD, Fischmann A, et al. MRI biomarker assessment of neuromuscular disease progression: a prospective observational cohort study. Lancet Neurol 2016;15:65–77.

37. Ramchandren S, Wu TT, Finkel RS, et al. Development and validation of the pediatric Charcot-Marie-Tooth disease quality of life outcome measure. Ann Neurol 2021;89:369–379.