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# Disease Progression in CMT related to *MPZ* Mutations: A Longitudinal Study

Running Title: 'Disease Progression in MPZ-Neuropathies'

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**Abbreviations:** Charcot Marie Tooth disease (CMT); CMT Examination Score (CMTES); CMT Infant and Toddler Scale (CMTInfS); CMT Pediatric Scale (CMTPedS); Inherited Neuropathy Consortium (INC) *Myelin Protein Zero (MPZ)*; nerve conduction study (NCS); nerve conduction velocity (NCV); Rare Diseases Clinical Research Network (RDCRN); Rasch modified CMT Examination Score (CMTES-R); standardized response mean (SRM)

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2. What is the current knowledge on the topic?

Neuropathy related to mutations in the *MPZ* gene constitute the second most common forms of Charcot Marie Tooth disease type 1 (CMT1). Few studies have examined the natural history of *MPZ*-neuropathy, which remains a barrier to clinical trials.

3. What question did this study address?

This study aimed to assess whether the Charcot Marie Tooth Examination Score (CMTES) is an effective clinical measure of progression in *MPZ*-neuropathy and to define the rate of clinical progression over a 5-year time frame. The study also aimed to determine the optimal patient population for future treatment trials in *MPZ*-neuropathy.

4. What does this study add to our knowledge?

This study demonstrates that the CMTES is an effective clinical measure of *MPZ*-neuropathy and reflects the change in clinical severity of neuropathy over a 2-year time frame. Additionally, the findings suggest that adult participants with axonal neuropathy caused by mutations in the *MPZ* gene will serve as the optimal population in future clinical trials.

5. How might this potentially impact on the practice of neurology?

The findings of this study will allow clinicians to more effectively help their patients understand the prognosis of *MPZ*-neuropathy overall, and in regard to specific genetic variants in the gene. Additionally, these results will help guide patient selection for upcoming clinical trials.

**Abstract:**

**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 Neuropathy Consortium.

**Methods:** Change in Charcot Marie Tooth Examination scores (CMTES) and Rasch modified CMTES (CMTES-R) scores 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 scores were 10.84 (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, ( $p=0.016$ ) versus 0.06 points, ( $p=0.889$ )]. Patients with a moderate baseline neuropathy severity also showed more notable change, by estimate, than those with mild or severe neuropathy [mean 2 year change of 1.14 for baseline CMTES 8-14 ( $p=0.025$ ), versus -0.03 for baseline CMTES 0-7 ( $p=0.958$ ) and 0.25 for baseline CMTES  $\geq 15$  ( $p=0.6897$ )]. The progression in patients harboring specific *MPZ* mutations was highly variable.

**Interpretation:** CMTES scores are 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.

### **Introduction:**

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<sup>1-3</sup>. *MPZ*-neuropathies are genetically heterogeneous, with over 200 different disease-causing mutations identified to date<sup>4</sup>. Genotype-phenotype correlation studies have identified 3 distinct types of *MPZ*-neuropathy, including an infantile form (clinical presentation prior to 3 years of age with severely slowed motor conduction velocities (NCV; <15 m/s), a childhood-onset (first or second decade) demyelinating neuropathy (CMT1B), and an adult-onset axonal neuropathy (CMT2I)<sup>4-10</sup>. 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<sup>8</sup>.

*MPZ* encodes the MPZ/P<sub>0</sub> protein, which is expressed almost exclusively by myelinating Schwann cells, and is the major protein in peripheral nerve myelin<sup>11</sup>. MPZ is a member of the immunoglobulin (Ig) supergene family and is a homophilic adhesion molecule that is required for the normal compaction of myelin<sup>12</sup>. 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<sup>13, 14</sup>. Some mutations result in retention of the mutant MPZ protein in the ER, whereas other MPZ mutants have abnormal interactions with wild type MPZ in the myelin sheath<sup>15</sup>.

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 multi-center Inherited Neuropathies Consortium over a 5-year period. Disease progression was assessed using the CMT Examination Score (CMTES) as well as the Rasch analysis-based weighted CMT Examination Score (CMTES-R), which are commonly employed clinical outcome assessments in CMT that have previously demonstrated responsiveness to changes in patients with CMT1A<sup>16</sup>.

### **Methods:**

#### *Standard protocol approvals, registrations, and patient consents*

All sites participating in the Inherited Neuropathy Consortium (INC) natural history study (protocol 6601) received Institutional Review Board (IRB)/Ethics Board approval for the study. All patients or their guardian signed consent forms. This trial was registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) (ID number NCT01193075).

#### *Patients:*

Patients with *MPZ* mutations were recruited from the INC, which is a member of the NIH Rare Diseases Clinical Research Network (RDCRN; [rarediseasesnetwork.org/](http://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)<sup>17, 18</sup>. CMTES scores were then converted into their Rasch-modified scores (CMTES-R),

with total scores of 32<sup>18</sup>. 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+<sup>16</sup>. Patients were evaluated at yearly intervals, and the CMTES scores were 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 scores over time were 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 vs demyelinating, and baseline CMTES scores 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 score was an explanatory variable. T and F tests were performed on linear combinations of the model parameters. The performance of the CMTES-R vs CMTES was evaluated by comparing mean

change from baseline to 2 years to the 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  $\geq 0.80$  reflect low, moderate, and high responsiveness, respectively<sup>19</sup>. 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 vs demyelinating neuropathy status was determined according to the recorded ulnar motor nerve conduction velocity (NCV) at their first visit, with demyelinating neuropathy defined by velocities less than or equal to 38 m/s, and axonal neuropathy defined as an ulnar motor NCV greater than 38 m/s<sup>20</sup>. 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-square/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 ANOVA 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 as in Fridman et al.<sup>16</sup>



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 scores were available for 136 patients, and complete CMTNS scores were available for 65 participants (**TABLE 2**). 74 (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); with 1 participant had missing age data). 68 participants (49%) were 50 years of age or older, and 18 (13%) were under 21 years of age. 122 (88%) identified as white, 2 (1.5%) identified as Hispanic and ethnicity data was missing for 9 participants. 36 participants (31.3%) reported a delay in ambulation as defined by walking later than 15 months of age; data was 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 was only available for 28 patients (111 missing), of which 4 (14%) were reported to have abnormalities.

134 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 less than or equal to 15 m/s (table 2). 9 of the patients designated as having demyelinating

neuropathy, had an ulnar CMAP of  $<2$  mV, however their ulnar NCV 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,  $p$  value  $< 0.0001$ ), (70% vs 32%  $\geq 50$  years,  $p < 0.0001$ ), (3.3% vs 21.9%  $< 21$  years,  $p = 0.018$ ), more likely to be white (97% vs 79%,  $p = 0.003$ ), 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%,  $p < 0.0001$ ) and 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 scores (mean of 9.6 vs 11.8,  $p$  value = 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 score was 10.8 (SD = 6.0, range 0 – 28) and the average CMTES-R score was 14.6 (SD = 7.6, range 0 - 32), indicating a moderate range of severity. Baseline CMTES scores were 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) and lower MNCV ( $p = 0.012$ ), not walking before 15 months ( $p = 0.036$ ) and 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 was 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% 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

(FIGURES 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 value < 0.0001). However, a linear approximation of the time trend was questionable (likelihood ratio test p value = 0.03; AIC increase of 2.4). CMTES-R scores 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 (FIGURE 3). As compared to the CMTES-R, CMTES had a higher SRM for change from baseline to 2 years (0.37 versus 0.30). Progression of the CMTNS was not examined due to the limited sample size.

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 was 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. 62 (47%) were male and 69 (53%) were female. Mean age was 46.3 years (SD = 18.1, range: 3.9 to 76.4). 60 (46%) were classified as axonal and 71 (54%) as demyelinating. Mean baseline CMTES was 10.8 (SD=6.1, range = 0 to 28); 1 observation was missing. Neither gender nor age significantly modified CMTES progression from baseline to follow up year 2 (p values = 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 non-significant (p=0.057).

By estimate, CMTES progression decreased, as age increased, however, the overall test for progression at any age was non-significant (p value = 0.062). Female patients increased by an estimated 1.11 units (95% CI: (0.01, 2.22), p value = 0.049) from baseline to 2 year follow up, whereas the change for male patients was non-significant (estimate = 0.64, 95% CI: (-0.29, 1.57), p value = 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<sup>8</sup>. 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 non-significant (estimate = 0.06, 95% CI: (-0.76, 0.87), p value = 0.889, SRM = 0.03) (**FIGURE 3**). To reiterate, the difference in progression between axonal and demyelinating participants was non-significant (p value = 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 (**FIGURE 4**). Data was 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 value = 0.025, SRM = 0.49). The mild group was estimated to decrease non-significantly by -0.03 (95% CI: (-1.36, 1.30), p value = 0.958, SRM = -0.02), while the severe group increased non-significantly by 0.25 (95% CI: (-1.09, 1.59), p value = 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 non-significant, however (p = 0.281). Furthermore, none of the pair-wise comparisons were statistically significant.

137 patients had available *MPZ* variant data. Variant details and clinical features are described in **SUPPLEMENTAL TABLE 1**. 32 patients had a participating family member making up 12 families total, however segregation data was not available for all patients. 44 unique variants were described, 1 of which appears to be novel and is classified as of uncertain significance. 39 variants are classified as pathogenic or likely pathogenic<sup>21</sup>. CMTES score data was 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, nine with CMTES scores 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 scores were reported in the p.Gly137Ser and p.Val102fs variants.

Progression on the CMTES varied amongst participants with different *MPZ* variants, as well as between patients with the same variant and patients from the same family (**FIGURE 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 scores 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 demonstrated progression in CMTES scores over time with an estimated change of 0.87 points ( $p = 0.008$ ) from baseline to 2 years 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 ( $p=0.016$ ), versus a non-significant 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 versus 0.03), suggesting a higher level of responsiveness. It should be emphasized, however, that the difference between the 2 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 scores and age ( $p=0.001$ ), however, there was no evidence that the responsiveness of the CMTES differed according to age or gender<sup>16</sup>. 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)<sup>16, 22</sup>. 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 scores than for the CMTES-R (0.37 versus 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<sup>22</sup>. 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<sup>16</sup>. 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.

While there were fewer patients with axonal neuropathy in our cohort, they drove the overall change observed in CMTES scores. 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<sup>9, 23-25</sup>. 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 (i.e. dysmyelination), and axonal forms arising secondary to disruptions in *MPZ*-mediated signal transduction and Schwann cell-axonal interactions<sup>8, 26, 27</sup>. 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 (CMTPedS) and CMT Infant and Toddler Scale (CMTInfS), as progression in this patient population is likely to be greatest in childhood<sup>28, 29</sup>.

The finding that only participants with moderate neuropathy (CMTES scores of 8-14) showed progression in CMTES scores over 2 years also has important implications for future clinical trials. 2-year progression on the CMTES in the moderate group was 1.14, ( $p$  value = 0.025) with an SRM of 0.49. In contrast, the mild group actually showed a non-significant decrease in scores

over 2 years, while the severe group showed a non-significant 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<sup>16</sup>. 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.<sup>8</sup>, although fewer patients in our cohort reported delayed milestones (**TABLE 3**). 1 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 (gnomAD), 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<sup>21</sup>. 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. While 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<sup>8</sup>. 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<sup>8</sup>.

Several missense variants in *MPZ* have specifically been reported to cause a late-onset, severe, axonal neuropathy with rapid progression. These variants tend to congregate around codon 70 in the extracellular domain: p.Pro70Leu, p.Asp75Val, and p.Pro70Ser<sup>30,31</sup>. In contrast, the 9 subjects in our cohort with the p.Pro70Ser variant demonstrated baseline CMTES scores 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 clarify 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<sup>32</sup>.

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 scores. We have addressed this by using longitudinal regression models to evaluate change in CMTES scores, 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-FOM, 10-meter walk test, or the Overall Neuropathy Limitations Scale (ONLS)<sup>19, 33, 34</sup>, many of which were not available when we began our study. For similar reasons we did not evaluate emerging objective biomarkers such as MRI of intramuscular fat accumulation or plasma neurofilament light chain concentrations, which will need to be evaluated in future studies<sup>35</sup>.

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. While 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<sup>4</sup>. 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<sup>35, 36</sup>. 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<sup>28, 29, 37</sup>.

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**Author Contributions:**

VF, SS, SSS, MMR, and MES contributed to conception and design of the study; VF, SS, KS, CB, JD, SF, RSF, TG, LG, DNH, ML, JL, TEL, IM, FM, EP, CP, GP, SR, RS, RRS, CES, CJS, JW, SWY, and DP contributed to acquisition and analysis of data; VF, SS, JB, KS, DP, SSS, MMR, and MES contributed to drafting the text or preparing the figures.

**Potential Conflicts of Interest:**

VF, SS, JB, KS, IM, EP, CP, GP, ML, FM, CB, SF, TG, LG, RS, JW, DNH, JL, CJS, TEL, JD, CEK, SWY, RS, RSF, SSS, DP, MMR, and MES have no potential conflicts of interest to report. SR is currently employed by a CRO (PRA Health Sciences) that works with pharmaceutical companies. Istituto Neurologico Carlo Besta receives donations for research from Pfizer, LAM Therapeutics, and Acceleron Pharma Inc.

**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.

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**Figure Legends:****FIGURE 1 – CMTES scores over 5 years**

**Legend:** CMTES scores over a 5-year period. CMTES scores increase gradually over subsequent years with relatively linear progression. Bars are 95% confidence intervals.

**FIGURE 2 – CMTES change from baseline over 5 years**

**Legend:** Change in CMTES scores from baseline over a 5-year period. Bars are the 95% confidence intervals.

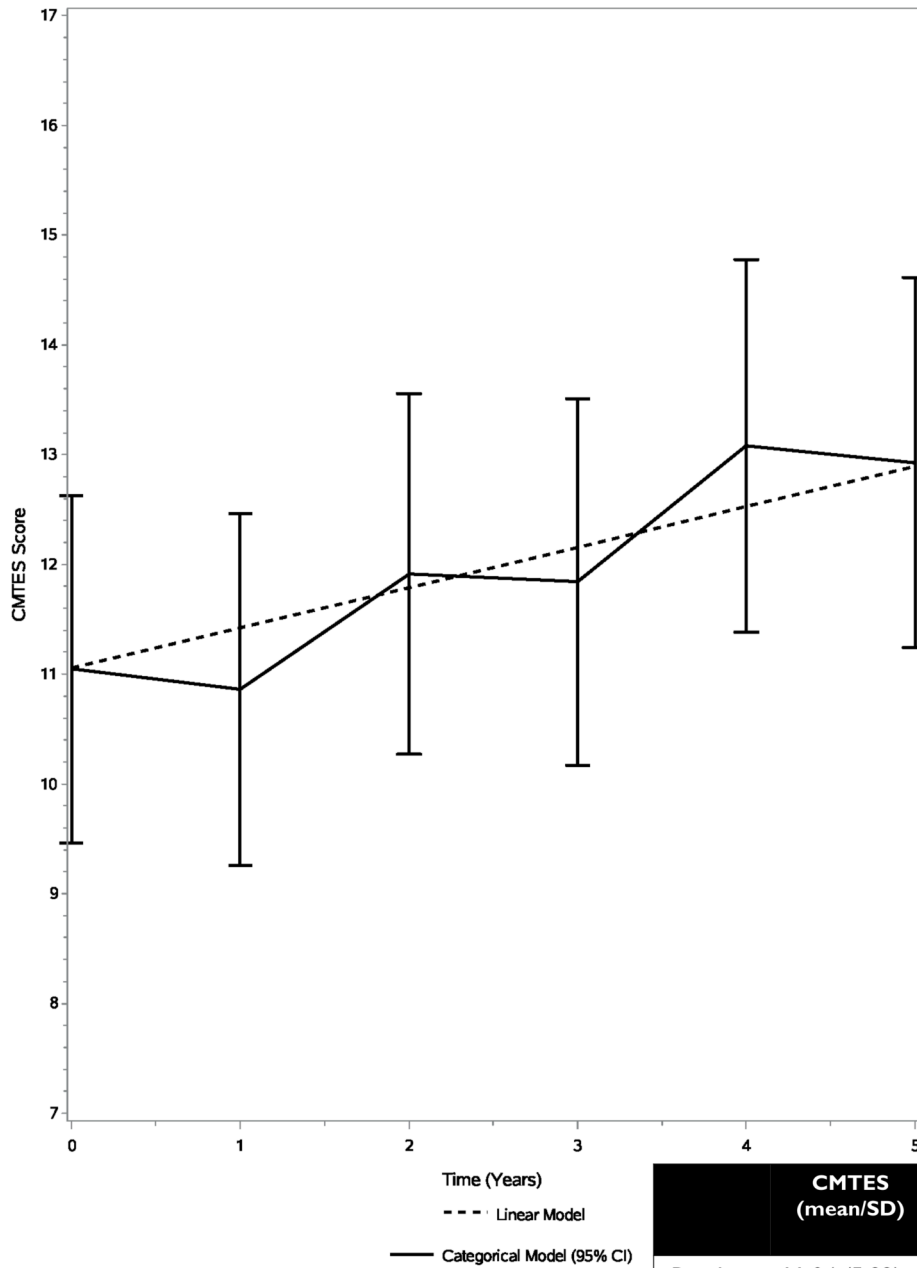
**FIGURE 3 – Influence of axonal vs. demyelinating disease type on change from baseline to 2 years**

**Legend:** Categorical model showing change in CMTES based on axonal vs. demyelinating disease type over a 2-year period. Red = demyelinating model. Blue = axonal model. Bars are the 95% confidence intervals.

**FIGURE 4 - Influence of baseline disease severity**

**Legend:** Categorical model showing change in 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  $\geq 15$ ). Bars are the 95% confidence intervals.

**FIGURE 5 – CMTES scores over 8 years for individual participants**

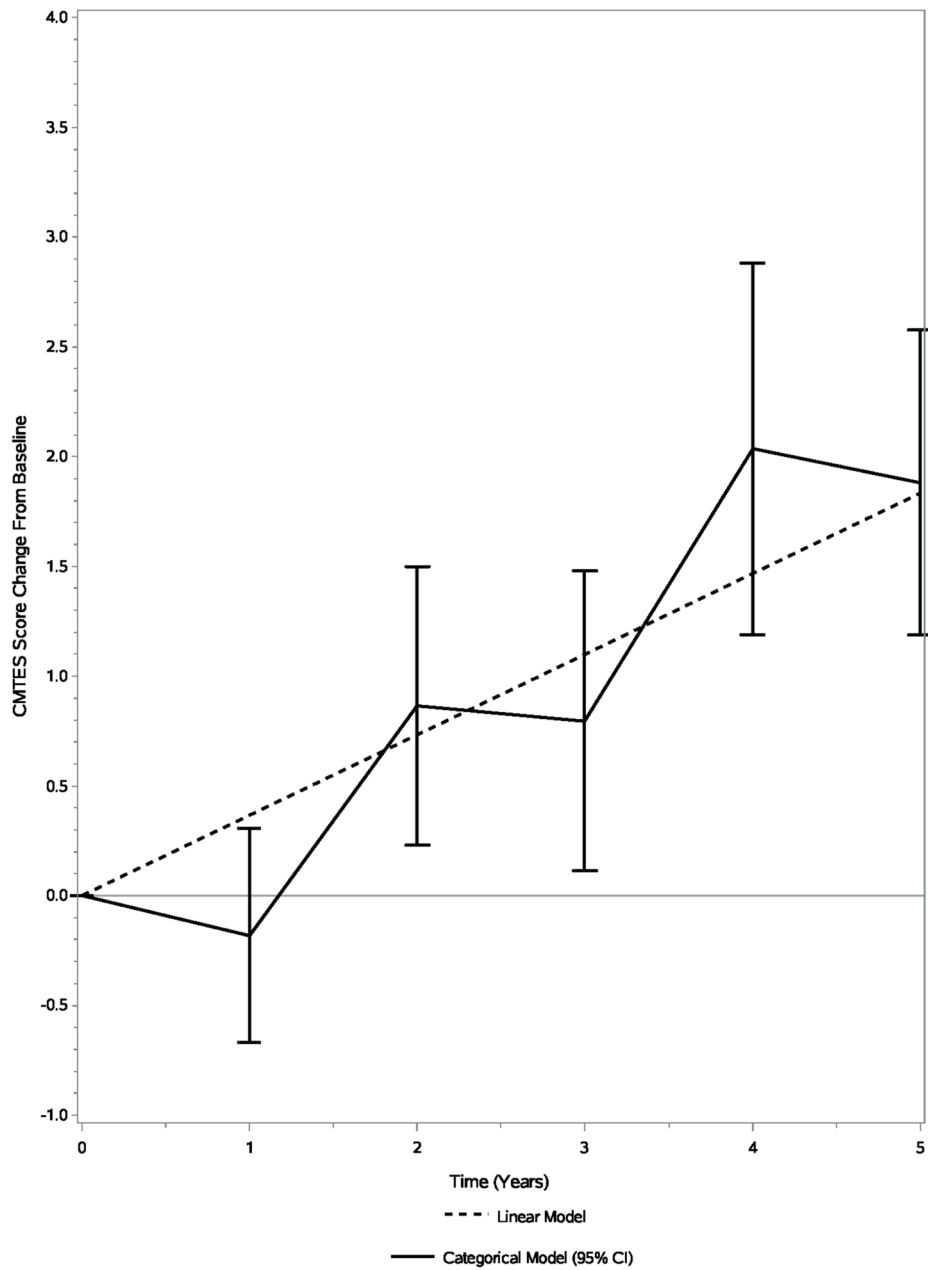


Available CMTES	
Baseline	136
1 Year	67
2 Years	44
3 Years	38
4 Years	34
5 Years	31

	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

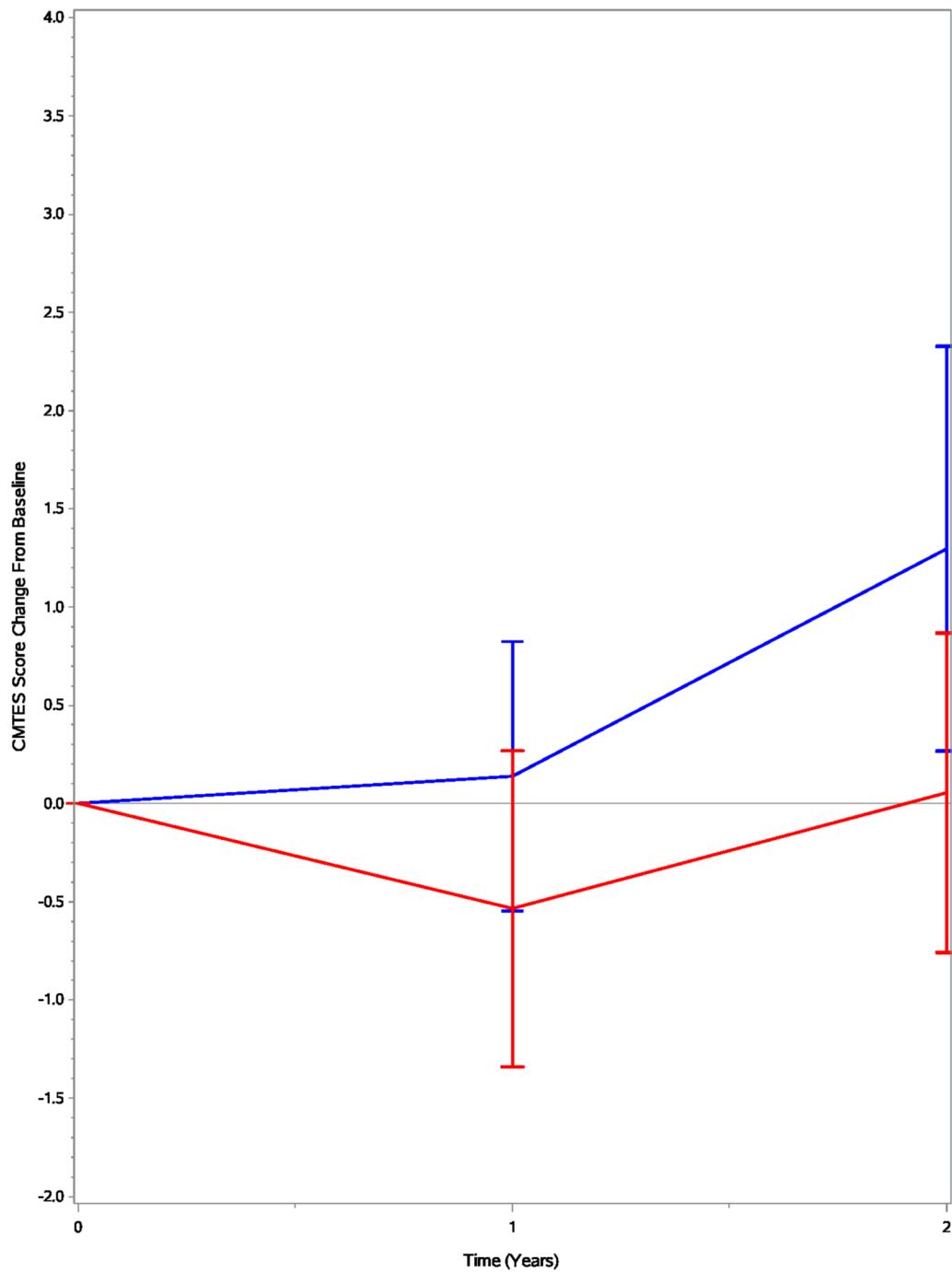
ANA\_26518\_Figure 1-CMTES scores over 5 years.tif





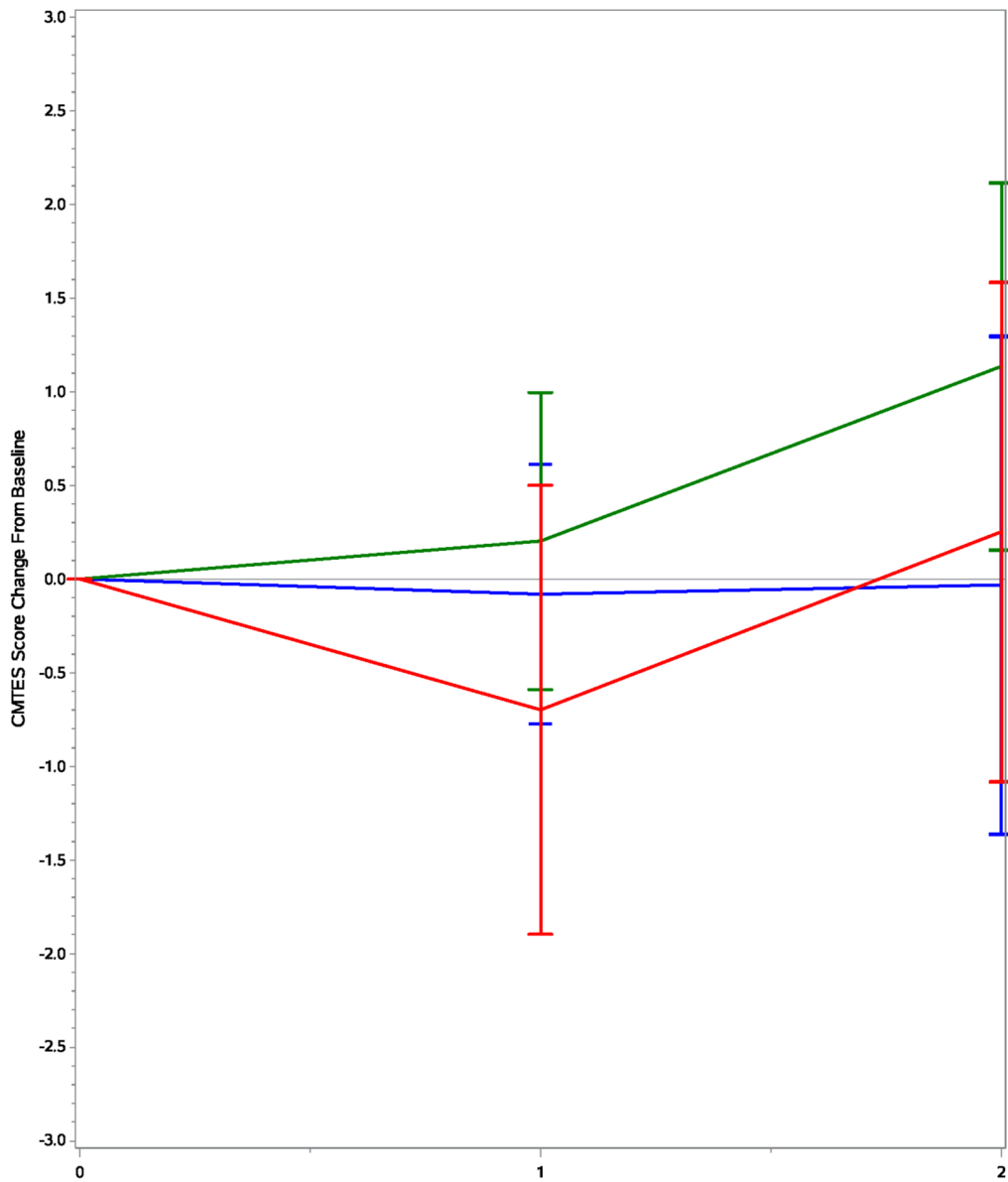
	<b>Change from Baseline</b>	<b>95% Confidence Interval</b>	<b>P-value</b>
Year 1	-0.18	-0.67, 0.31	0.4625
Year 2	0.87	0.23, 1.50	0.0084
Year 3	0.80	0.11, 1.48	0.0230
Year 4	2.04	1.19, 2.88	<0.0001
Year 5	1.88	1.19, 2.58	<0.0001

ANA\_26518\_Figure 2-CMTES change from baseline over 5 years.tif



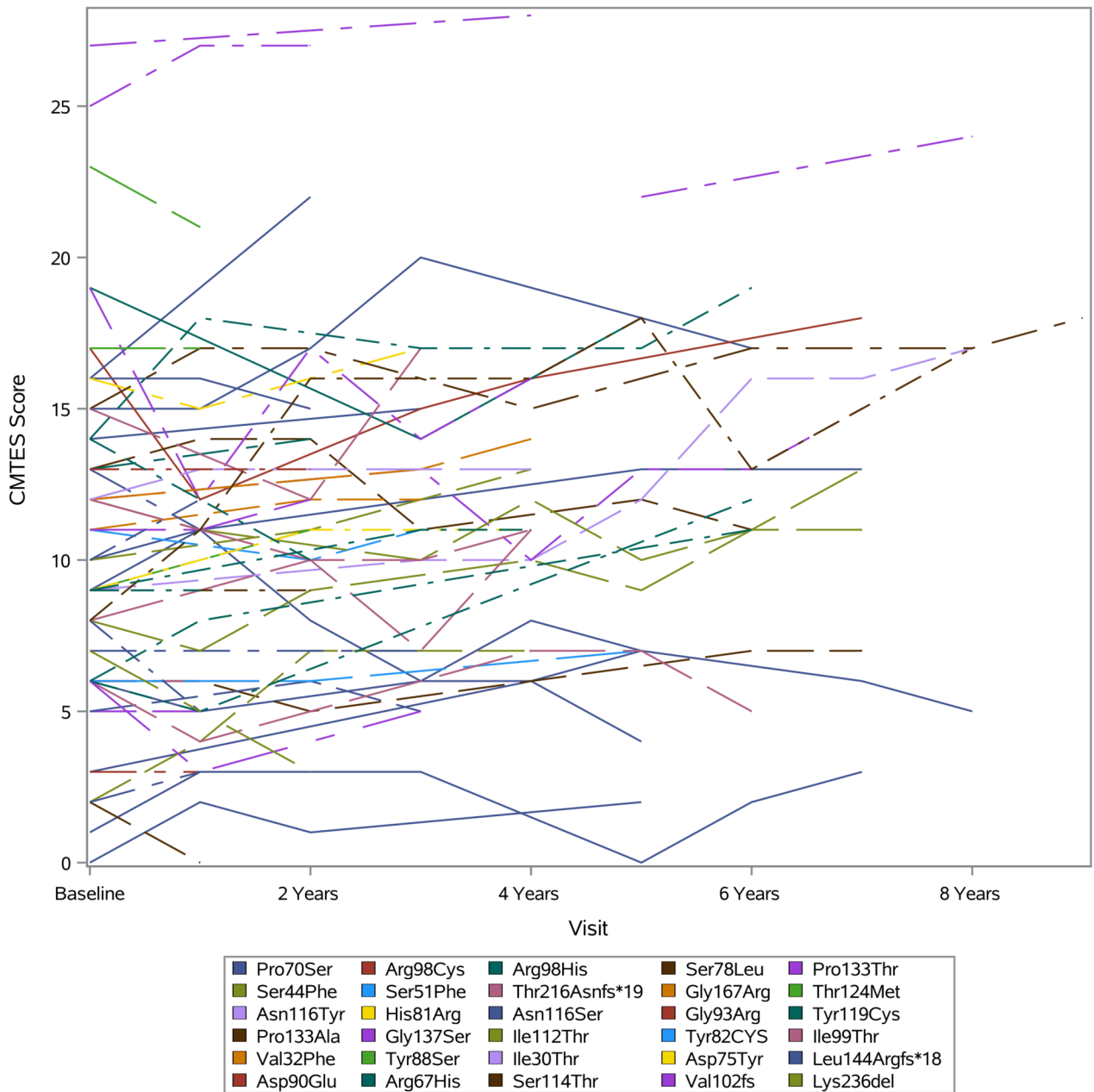
	Mean change from baseline at 2 years	P-value	95% Confidence Interval at 2 years	Mean/SD (SRM)
Axonal	1.30	0.0156	0.27, 2.33	0.51
Demyelinating	0.06	0.8891	-0.76, 0.87	0.03

ANA\_26518\_Figure 3-Influence of axonal vs. demyelinating disease type on change from baseline to 2 years.tif



CMTES Score (Baseline) (Categorical)					
			Mild	Moderate	Severe
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	

ANA\_26518\_Figure 4-Influence of baseline disease severity.tif



ANA\_26518\_Figure 5-CMTES scores over 8 years for individual participants.tiff

**TABLE 1** – Number of patients per Inherited Neuropathies Consortium (INC) site.

INC Site	Number of Subjects Contributed
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

**TABLE 2** – Demographics and Baseline Statistics.

# of Patients at Baseline	139
Age, years (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 Months (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 supports 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%)

**TABLE 3** - Clinical Characteristics of Axonal Versus Demyelinating *MPZ*-Neuropathy.

	Axonal	Demyelinating	P-value
# of Patients	61	73	
Age, years (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 difficulty walking, months (mean +/-SD)	46.83 (+/-11.95)	12.73 (+/-17.49)	<0.0001
Walked before 15 months (yes/no)	48 (96.0%) / 2 (4.0%)	30 (48.4%) / 32 (51.6%)	<0.0001
Walking supports 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