A LONGITUDINAL STUDY OF CMT1A USING RASCH ANALYSIS BASED CMT NEUROPATHY AND EXAMINATION SCORES

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MM Reilly and ME Shy were involved in the study conception, collected data, and edited drafts of the manuscript.

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Abstract
Objective: To evaluate the sensitivity of Rasch analysis-based, weighted Charcot-Marie-Tooth Neuropathy and Examination Scores (CMTNS-R and CMTES-R) to clinical progression in patients with CMT1A.

Methods: Subjects with CMT1A from 18 sites of the Inherited Neuropathies Consortium were evaluated between 2009 and 2018. Weighted CMTNS and CMTES modified category responses were developed using Rasch analysis of the standard scores. Change from baseline for CMTNS-R and CMTES-R was estimated using longitudinal regression models.

Results: Baseline CMTNS-R and CMTES-R scores were available for 517 and 1177 subjects, respectively. Mean age of subjects with available CMTES-R scores was 41 years (+/- 18, range 4-87), and 56% were female. Follow up CMTES-R assessments at 1, 2, and 3 years were available for 377, 321 and 244 patients. A mixed regression model showed significant change in CMTES-R at years 2 through 6 as compared to baseline [mean change from baseline of 0.59 points at two years (p=0.0004; n=321)]. Compared to the original CMTES, the CMTES-R revealed a 55% improvement in the standardized response mean (mean change/SD change) at two years (0.17 versus 0.11). Change in CMTES-R at two years was greatest in mildly to moderately affected subjects (1.48 points mean change, CI 0.99-1.97, p<0.0001, for baseline CMTES-R 0-9).

Conclusion: The CMTES-R demonstrates change over time in patients with CMT1A and is more sensitive than the original CMTES. The CMTES-R was most sensitive to change in patients with mild to moderate baseline disease severity, and failed to capture progression in patients with severe CMT1A.

Introduction:

Charcot-Marie-Tooth Disease Type 1A (CTM1A) is the most common form of hereditary neuropathy, accounting for more than 50% of all cases (Reilly, Murphy, & Laura, 2011).

CMT1A is caused by a duplication encompassing the *PMP22* gene, and most commonly manifests in the first two decades of life with foot deformities, length dependent sensory loss, varying degrees of distal extremity weakness, and gait difficulty. No treatment for CMT1A is currently available, however, an increasing number of candidate therapies that reduce *PMP22* expression have emerged from pre-clinical studies, paving the way for clinical trials [1].

The paucity of clinical outcome assessments (COA) that are sensitive enough to capture the slow clinical progression has been a major impediment to previous clinical trials in CMT1A. The most commonly employed outcome measure in CMT1A studies to date has been the Charcot-Marie-Tooth Neuropathy Score (CMTNS), which was developed to quantify clinical severity and measure disease progression in patients with CMT [2]. The CMTNS is a validated, composite score that is based on patients' symptoms, physical findings and electrophysiology. The CMT Examination Score (CMTES) is a sub score of the total CMTNS and is based only on patients' symptoms and physical findings, excluding electrophysiology. Importantly, two randomized controlled trials of ascorbic acid in CMT1A have underscored the limitations of the CMTNS, as no meaningful clinical progression was detected in the placebo groups in either of these studies over a two year period [3, 4]. To minimize floor and ceiling effects, the CMTNS was revised yielding the CMTNS version 2 (CMTNSv2) [5]. A follow up Rasch analysis of the CMTNSv2, however, revealed that the revised score continued to cluster impairment scores from CMT1A patients in the middle range of severity, thus failing to adequately discriminate mildly and severely affected patients from those in the middle [6]. To make the CMTNS more linear, and thereby ensure that smaller differences in clinical change could be detected, Rasch-modified CMTNS scores (CMTNS-R) were developed [6].

The Rasch model is a mathematical framework developed to analyze rating scales with the goal of determining how well individual items of the scale contribute to the measurement of a derived trait (in the case of the CMTNS, the severity of neuropathy). In contrast to the original CMTNSv2, in which all items contribute identically to the overall score, the CMTNS-R variably weights category responses to provide a more accurate estimate of disease severity, as we have previously published [6]. For example the Rasch model predicted that motor items would be more representative of increased disability and therefore deserve higher scores compared to sensory items. Reapplying Rasch analysis using the weighted scale showed significant improvement in psychometric properties of the outcome measure, mainly less noise (fitting), better reliability, and reduced floor/ceiling effect [6]. The CMTNS-R is therefore predicted to be more sensitive in detecting change in patients with CMT1A, but it has not yet been evaluated in longitudinal studies.

The Inherited Neuropathies Consortium (INC) was created in part to perform natural history studies in the varied forms of CMT. The INC has included up to 20 sites, currently includes 16 actively enrolling sites in the US, Europe, and Australia, and has been evaluating patients since 2009. In the current study, we have performed a longitudinal study of CMT1A in patients enrolled at 18 centers of the INC over a seven year period using the CMTNS-R and CMTES-R. Because repeated nerve conduction

studies are uncomfortable and often costly for patients, the CMTES is increasingly employed in place of the CMTNS in our clinics. We have therefore focused our analysis on the CMTES-R, and evaluated its potential use as an independent outcome measure in CMT1A.

Methods:

Standard protocol approvals, registrations, and patient consents

All sites participating in the 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 (ID number NCT01193075).

Patients

Subjects with CMT1A were recruited from the INC, which is a member of the NIH Rare Diseases Clinical Research Network (RDCRN; http://www.rarediseasesnetwork.org/). Data were collected as part of the INC natural history study (protocol 6601) between February 2009 and September 2018 from a total of 18 sites within the INC. Subjects were examined by clinical investigators who had received training and were certified in the proper use of the CMTNSv2, a validated 9 item, 36 point composite score based on patients' symptoms (3 items), examination findings (4 items) and electrophysiology (2 items), with scores of 0-10, 11-20, and >21 indicating mild, moderate and severe disease, respectively (Murphy et al., 2011; Shy et al., 2005). The CMTES is a sub score of the total CMTNSv2 that includes 7 items based on patients' symptoms and examination findings, and excludes the electrophysiology, with a maximum total score of 28 points. Patients were evaluated at yearly intervals and the CMTNSv2 and CMTES were recorded at each visit. If patients did not undergo nerve conduction studies during a particular visit, only the CMTES was obtained. CMTNSv2 and CMTES were then converted into their Rasch-modified scores (CMTNS-R and CMTES-R), with total scores of 40 and 32, respectively).

Patients were diagnosed with CMT1A on the basis of 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, and confirmatory genetic testing for a *PMP22* duplication. Standard methods were used for all electrophysiology. A Clinical Laboratory Improvement Amendments (CLIA) certified laboratory in the USA or an equivalent certified testing facility outside of the USA performed all genetic testing. Participants were required to have a duplication in the *PMP-22* gene to be enrolled in the INC database with a diagnosis of CMT1A, however, detailed descriptions of duplication length was not available for all subjects.

Statistical Methods:

Only patients with a *PMP22* duplication were included. Data were mostly analyzed on an available case basis. When fitting longitudinal models the number of follow up times was limited so there would be adequate sample size for all groups and all times. Because the model incorporates longitudinal correlation between repeated measures on a subject, every subject with at least one measurement contributes information to estimating the model parameters. We assume data missingness is not "not at random", and keep the covariance structures of the longitudinal models as free as possible, so the longitudinal models fit to available case data should be valid. Most results were obtained from the model parameter estimates, unless otherwise stated. Alpha was set to 0.05 by default.

Summary statistics for CMTES, CMTNS, CMTES-R, and CMTNS-R were obtained by time point, along with baseline demographic data. Change over time was estimated using longitudinal mixed model regression. Interaction effects for variables including gender, age, and baseline category of severity status were studied.

Severity categories for CMTES-R were determined by using the established categories for CMTNS (mild, moderate, and severe) and CMTES-R as a discriminator variable. A standard discriminant analysis was applied and evaluated with cross validation. The classification ranges in CMTES-R were then categorized as mild, moderate, and severe. The effects of baseline CMTES-R category on longitudinal change were then analyzed with mixed model regression. Change scores from baseline were the response, so to be included in the model it was necessary for a subject to have a measurement at baseline and at a minimum of one follow up time.

The performance of the CMTES-R versus CMTES and the CMTNS-R versus CMTNS was evaluated by comparing mean change from baseline to two years to the standard deviation of the change, the standardized response mean (SRM = mean change/SD change), for the first two year change. SRM values of 0.20-0.49, 0.50-0.79 and ≥0.80 reflect low, moderate and high responsiveness, respectively [7]. Estimated annual mean score and changes, and their standard deviations were obtained from the model parameters.

SRMs were also obtained on the two year change scores for the components of the scales. The components were not modeled with regression, so it was necessary for each observation to have data at baseline and 2 years of follow up.

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.

Results:
The INC evaluated subjects with CMT1A between February 2009 and September 2018. Baseline CMTNS-R and CMTES-R scores were available for 517 and 1177 subjects, respectively (CMTNS-R and CMTES-R baseline data was missing for 754 and 94 subjects) (**TABLE 1**). Among the 1209 subjects who had at least one CMTES-R

observation, either at baseline or during the first six years of follow up, the mean age was 41 years (+/- 18, range 4-87 years), and 233 (19%) were less than 21 years of age. 678 (56%) were female and 530 (44%) were male (gender data was missing for one subject). Follow up assessments at 1, 2, and 3 years were available for 377, 321 and 244 patients, respectively (**TABLE 1**). A mixed regression model, fit with the sample described in TABLE 1, showed significant change in CMTES-R at years 2 through 6 as compared to baseline [mean change from baseline of 0.59 points at two years (p=0.0004; n=321)] (**FIGURE 1**). Consistent increases in CMTES-R were found at two year intervals [mean change of 0.59 points (95% CI 0.27-0.92, p=0.0004) from 0 (baseline) to 2 years, 0.72 points (95% CI 0.35-1.09, p=0.0002) from 1 year to 3 years, and 0.84 points (95% CI 0.46-1.22, p<0.0001) from 2 years to 4 years]. Figure 1 also compares the categorical time model to a simplified linear time model, and shows that the deviation of the two was marginally statistically non-significant (likelihood ratio test p value = 0.07). Goodness of fit tests suggests the tradeoff between model fit and parsimony is about equal. The linear model estimates a change in mean CMTES-R of 0.59 (95% CI 0.44-0.74, p<0.0001) per 2 years. Comparing the CMTES-R to the original CMTES on the same data set revealed a 55% improvement in the SRM (mean change/SD change) for the CMTES-R at two years (0.17 versus 0.11) (**FIGURE 2**).

Of particular interest for future clinical trials for CMT1A was the subgroup analysis evaluating sensitivity to change of the CMTES-R in patients with varying baseline disease severity. This evaluation required us to define "mild", "moderate" and "severe" disease categories for the CMTES-R as was previously done with the CMTNS (Shy et al., 2005). Based on this analysis, CMTES-R subgroups were defined as: mild 0-9, moderate 10-18, and severe >19. Change in CMTES-R at two years was numerically greater in mildly affected subjects as compared to moderately affected subjects: 1.48 points mean change (CI 0.99-1.97, p<0.0001) for subjects with baseline CMTES-R 0-9 versus 0.79 points mean change (CI 0.28-1.29, p=0.002) for those with baseline scores of 10-18 (**FIGURE 3).** The difference between the two changes did not reach statistical significance, (-0.70, 95% CI -1.40-0.00, p=0.05); however the SRM in the CMTES-R at two years was higher in mildly than in moderately affected patients (0.55 versus 0.22), suggesting that CMTES-R scores may be more sensitive in detecting clinical progression in those with milder disease.

Severely affected subjects (CMTES-R >19) demonstrated a decrease in CMTES-R at one year (-0.83 points, CI -1.34- -0.32, p=0.002) and at two years (-0.77 points, CI -1.50- -0.05, p=0.037). There was a small estimated decrease for moderately affected subjects at one year, but it was not statistically significant (-0.11, CI -0.49-0.27, p = 0.56). The baseline data in the severe group ranged from 19 to 32, with 22 as the median and 27 as the 95% percentile. The trajectories of individual subjects were examined and were quite varied, with some increasing and some decreasing during the initial follow up years, and a slow drift towards higher values over the 6 year follow up period. The maximum possible value of the CMTES-R scale (total of 32 points) was not reached by the vast majority of subjects, suggesting that there was not a true ceiling effect. Participants with milder disease severity in our cohort were younger (mean age of 30 years in the mild group, 43 years in the moderate group, and 54 years in the severe group, p<0.0001). Adjusting for age at baseline revealed the same patterns of

progression on the CMTES-R in the three groups, with the mild group progressing by 2.82 point more than the severe group in the first two years (p=<0.0001).

Sample sizes limited the analysis of the CMTNS-R and change over time was therefore only evaluated over a four-year timeframe. Baseline CMTNS-R scores were available for 517 subjects, and 548 subjects had at least one CMTNS-R observation at baseline or between one and four years of follow up (**TABLE 1**). A significant change in the scores (as compared to year 0/baseline) was present at years 2 through 4 (mean change of 2.3 points, CI 0.92-3.74, p=0.002 at 2 years (n=27)). A mixed model found a mean CMTNS-R change of 1.61 points from baseline to 2 years (95% CI 0.54-2.67, p=0.004), 1.36 points from 1 year to 3 years (95% CI -0.25 – 2.96, p=0.0959), and 0.50 points from 2 years to 4 years (95% CI -1.08 – 2.08, p=0.53). (**FIGURE 4**). Similarly to the CMTES, comparing CMTNS-R to original CMTNS revealed an improvement in the SRM for the CMTNS-R at two years (0.51 versus 0.43).

The individual items on the CMTES that showed the most progression at two years included "motor symptoms legs" (change of 0.14 points, p=<0.0001), and "motor symptoms arms" (change of 0.09 points, p=0.023). These two items also demonstrated the highest SRM (0.25, and 0.13 respectively). The remaining items showed a trend towards progression with the exception of "pin prick sensitivity" and "strength arms" (**FIGURE 5**). Evaluation of the two electrophysiological items on the complete CMTNS showed the most change in the ulnar CMAP (change of 0.3 points, p=0.04), however this finding must be interpreted with caution given the low sample size (n=27).

We also analyzed the potential effects of age and gender. As expected, subjects less than 26 years of age had lower CMTES-R scores at baseline than those greater than or equal to 26 years of age (8.3 for age < 26, N=292 subjects, and 14.8 for age >= 26, N=916 subjects, p <0.0001). However, there was no age group effect on rate of change at any individual time point (p=0.75 for baseline to 2 years). There was also no evidence of a mean baseline difference in CMTES-R between genders (p=0.32), or that gender affected the rate of change in CMTES-R (p=0.54 for baseline to 2 years, and p=0.70 for all times).

Discussion:

This is the largest longitudinal study of patients with CMT1A to date, and the first longitudinal study to evaluate the CMTNS-R and CMTES-R. Our results show that the CMTES-R demonstrates change over time in subjects with CMT1A, and that Rasch-modified scores show improved sensitivity to change as compared to the raw CMTES scores (55% improvement in the SRM at two years). We found no evidence that the responsiveness of the CMTES-R differs according to age or sex. In regards to individual items on the CMTNS, our findings corroborate prior observations that pin prick sensitivity is the least responsive [8, 9].

Of particular interest for future clinical trials for CMT1A was the subgroup analysis evaluating sensitivity to change of the CMTES-R in patients with varying baseline disease severity. This evaluation required us to define "mild", "moderate" and "severe" disease categories on the CMTES-R. Subgroup analysis employing the new severity categories (0-9, 10-18, >19) on the CMTES-R revealed that change in CMTES-R at two

years was numerically greater in mildly than in moderately affected subjects, and that the SRM for change in CMTES-R at two years was 150% higher in mildly as compared to moderately affected subjects. While the difference in change on the CMTES-R between the mild and moderately affected subjects did not reach statistical significance (p=0.05), these findings suggest that the CMTES-R may be more sensitive in detecting clinical progression in mildly affected patients with CMT1A. In contrast, severely affected subjects (CMTES-R >19) demonstrated a small improvement in the CMTES-R over the first two years of the study, even when controlling for age, and are therefore not good candidates for clinical trials using the instrument. Our findings contrast with prior findings showing that items on both the CMTNS and CMTNSv2 are most appropriate for assessing patients with moderate and severe neuropathy [6, 10]. Additional studies using the CMTES-R in conjunction with other COA and biomarkers are needed to confirm that the severity categories we have defined accurately reflect disease severity and disability, and that mildly affected patients are indeed the optimal candidates for emerging treatment trials.

Our data underscore the limitations of using the CMTES-R as the sole clinical outcome measure in CMT1A. While the CMTES-R significantly changed over a two-year time frame, the numeric change (0.59 on a 32 point scale) is small, and commensurate with the slow clinical progression experienced by patients. Even for the mildly affected CMT1A patients, the group that changed the most, a two year randomized, double blind, clinical trial evaluating a disease arresting treatment would require ~235 subjects per group to achieve a 75% effect size (alpha=0.05). Therefore, it seems appealing to combine the CMTES-R with biomarkers that correlate to it but that are themselves more sensitive to change and can serve as more sensitive primary outcome measures for emerging clinical trials in CMT1A. Fat fraction in the calf muscle on MRI is particularly promising in this regard; it has an SRM of 1.04 over 12 months, and correlates well with the CMTES [11]. The fat fraction, however, has higher responsiveness in patients with moderate to severe CMT1A, and correlating this with the CMTES-R will require additional work [12].

The low number of subjects who underwent electrophysiology prevented us from adequately evaluating the responsiveness of the CMTNS-R in CMT1A. Electrophysiology is an objective test, and indirectly measures axonal loss, but is not routinely done as part of annual visits for CMT. The INC has therefore increasingly moved towards using the CMTES in order to maximize the number of subjects contributing to our natural history data. It has previously been suggested that the CMTES may be preferable to the CMTNS in clinical trials, as it was demonstrated to show more deterioration over time with a higher SRM than the CMTNS [7]. Post-hoc analysis of an ascorbic acid trial in CMT1A also demonstrated a better outcome as measured by the CMTES [7]. Another limitation of our study is that not all patients returned every year, and that some patients had missing baseline CMTES-R scores. We have addressed this by employing longitudinal regression models to evaluate change in CMTES-R, allowing for missing years between visits.

The INC continues to evaluate novel outcome measures for CMT in an effort to develop optimal tools that capture disease severity and progression for emerging clinical trials. These include the recently developed CMT-FOM, a functional outcome measure for CMT, and the patient reported CMT Health Index (CMT HI) [13, 14]. Emerging COA and biomarkers for CMT1A have to be correlated with clinical assessments that have been used in natural history studies in order to ensure clinical relevance. In addition to serving as a measure of clinical progression, the CMTES-R will therefore also have an important role in the evaluation of these novel measures and biomarkers in patients with CMT1A. It is worth noting that while our study did include younger patients with CMT1A (233 patients under 21 years of age, range 4-83 years), outcome measures designed specifically for children (CMTPedS) and infants (CMTInfS) with CMT have been developed within the INC and remain optimal measures for clinical trials in pediatric subjects with CMT[15, 16].

In conclusion, the Rasch analysis-based CMTES-R demonstrates change over time in patients with CMT1A and is more sensitive than the original, unweighted CMTES. We found that the CMTES-R was most sensitive to change in patients with mild to moderate baseline disease severity, and failed to capture progression in patients with severe CMT1A. While significant, the actual degree of change in CMTES-R remains small, underscoring the importance of its use in conjunction with other objective and patient reported outcome measures in future clinical trials.

Legends

Table I. Demographics and CMT Scores.

Figure 1. Change in CMTES-R over 6 years. Change in CMTES-R scores from baseline over a 6 year period. CMTES-R scores increase gradually over subsequent years with relatively linear progression. The linear model estimates a change in mean CMTES-R of 0.59 (95% CI 0.44-0.74, p<0.0001) over 2 years.

Figure 2. Change in CMTES over 6 years. Change in CMTES scores from baseline over a 6 year period. CMTES scores increase gradually over subsequent years with relatively linear progression.

Figure 3. Influence of baseline disease severity on change in CMTES-R (mixed model regression). Categorical model showing change in CMTES-R based on disease severity at baseline over a 6 year period. Red=mild (CMTES-R 0-9), green=moderate (CMTES-R 10-18), blue=severe (CMTES-R >19). Bars are the 95% confidence intervals. Mildly and moderately affected patients show more disease progression than severely affected patients.

Figure 4. Change in CMTNS-R over 4 years. Categorical model showing change in CMTNS-R scores from baseline over a 4 year period. CMTES-R scores increase over subsequent years.

Figure 5. Responsiveness to change of individual CMTES items at 2 years.
Change in individual CMTES items over a two year period. Pin prick sensibility and arm strength were least responsive.

Table I. Demographics and CMT Scores.

	CMTES-R	CMTNS-R
Age, years (mean +/-SD)	41.1 +/- 18.0	43.1 +/- 16.7
Sex (M/F)	530 (44%)/678 (56%)	316 (58%)/231 (42%)
Race (white yes/no)	1100 (91%)/109 (9%)	480 (88%)/68 (12%)
Data are mean +/- SD (N)		

	Baseline	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
CMTES	9.8 +/- 4.9 (1177)	9.8 +/- 4.6 (377)	10.2 +/- 4.8 (321)	10.5 +/- 5.2 (244)	10.6 +/- 4.9 (208)	10.7 +/- 5.3 (160)	11.4 +/- 5.0 (119)
CMTES-R	13.3 +/- 6.4 (1177)	13.4 +/- 6.9 (377)	14.1 +/- 6.2 (321)	14.4 +/- 6.7 (244)	14.5 +/- 6.3 (208)	14.7 +/- 6.8 (160)	15.6 +/- 6.4 (119)
CMTNS	15.4 +/- 5.7 (517)	15.5 +/- 5.6 (54)	17.6 +/- 5.9 (32)	17.4 +/- 6.6 (18)	17.2 +/- 5.4 (17)	21.5 +/- 7.0 (15)	18.2 +/- 5.1 (11)
CMTNS-R	19.1 +/- 7.1 (517)	19.0+/- 7.0 (54)	22.1 +/- 7.5 (32)	21.8 +/- 8.3 (18)	21.1 +/- 6.9 (17)	26.4 +/- 8.4 (15)	22.2 +/- 6.7 (11)

CMTNS = Charcot-Marie-Tooth Neuropathy Score version 2
CMTNS-R = Rasch Charcot-Marie-Tooth Neuropathy Score version 2
CMTES = Charcot-Marie-Tooth Examination Score
CMTES-R = Rasch Charcot-Marie-Tooth Examination Score

Figure 1. Change in CMTES-R over 6 years.
Change determined using longitudinal mixed model regression in all subjects with at least one measurement (N=1209).
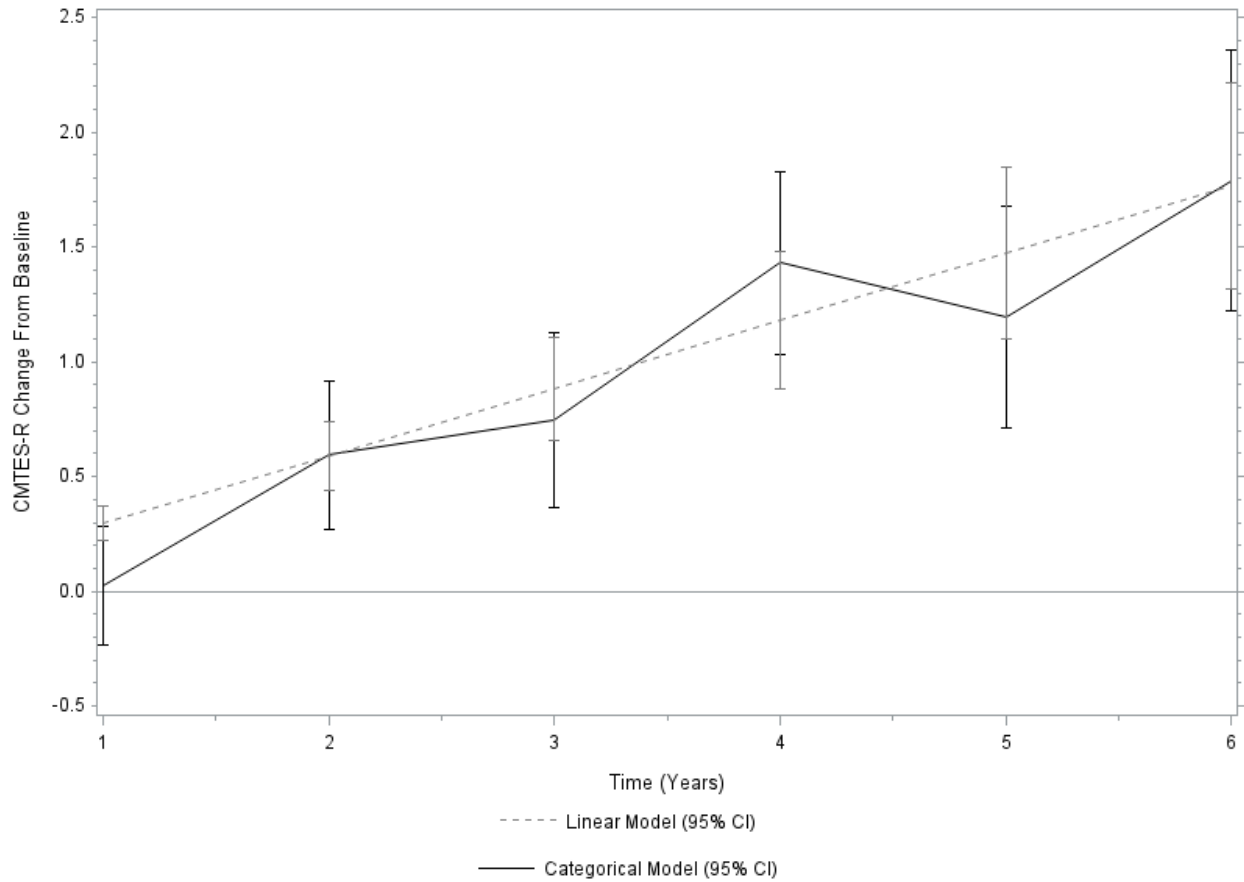
	Change from Baseline	95% Confidence Interval	P-value
Year 1	0.2	-0.23, 0.28	0.85
Year 2	0.59	0.27, 0.92	0.0004
Year 3	0.74	0.37, 1.12	0.0001
Year 4	1.43	1.03, 1.83	<0.0001
Year 5	1.20	0.71, 1.68	<0.0001
Year 6	1.79	1.22, 2.36	<0.0001

Figure 2. Change in CMTES over 6 years.
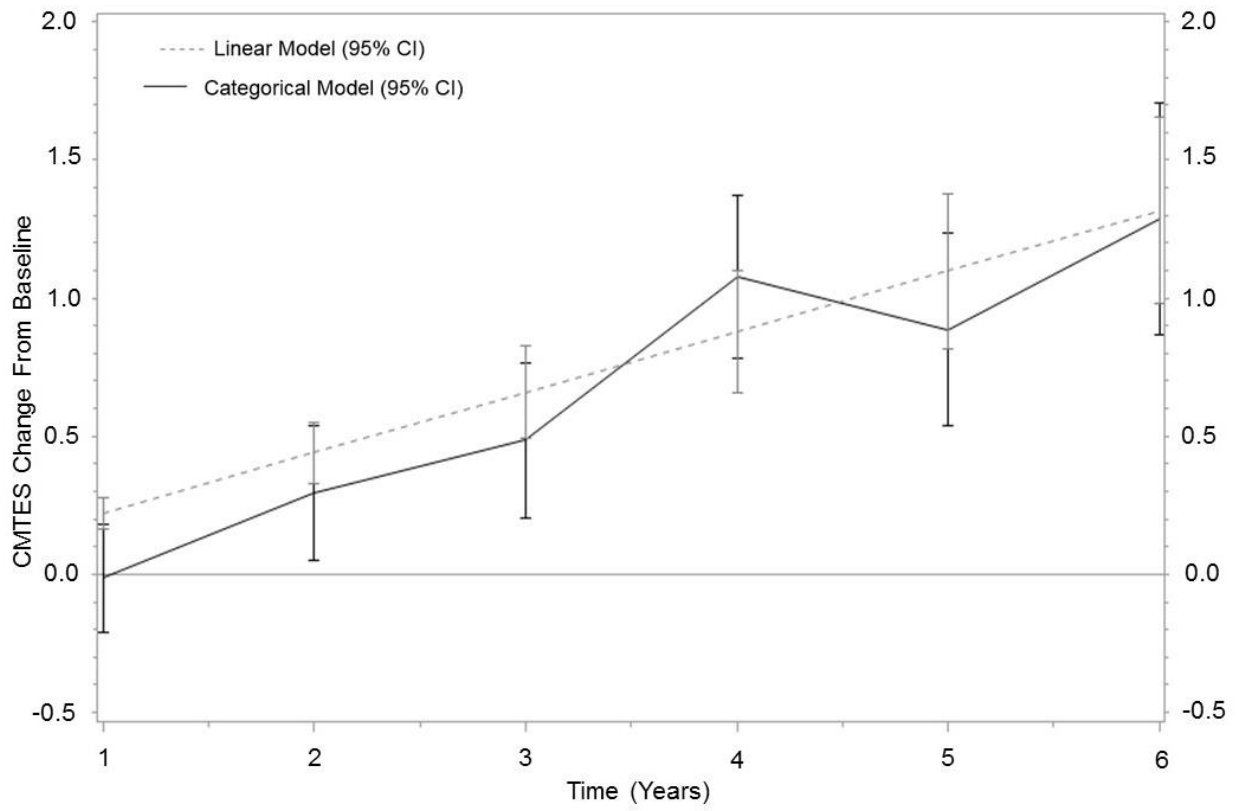
Figure 3. Influence of baseline disease severity on change in CMTES-R (mixed model regression).
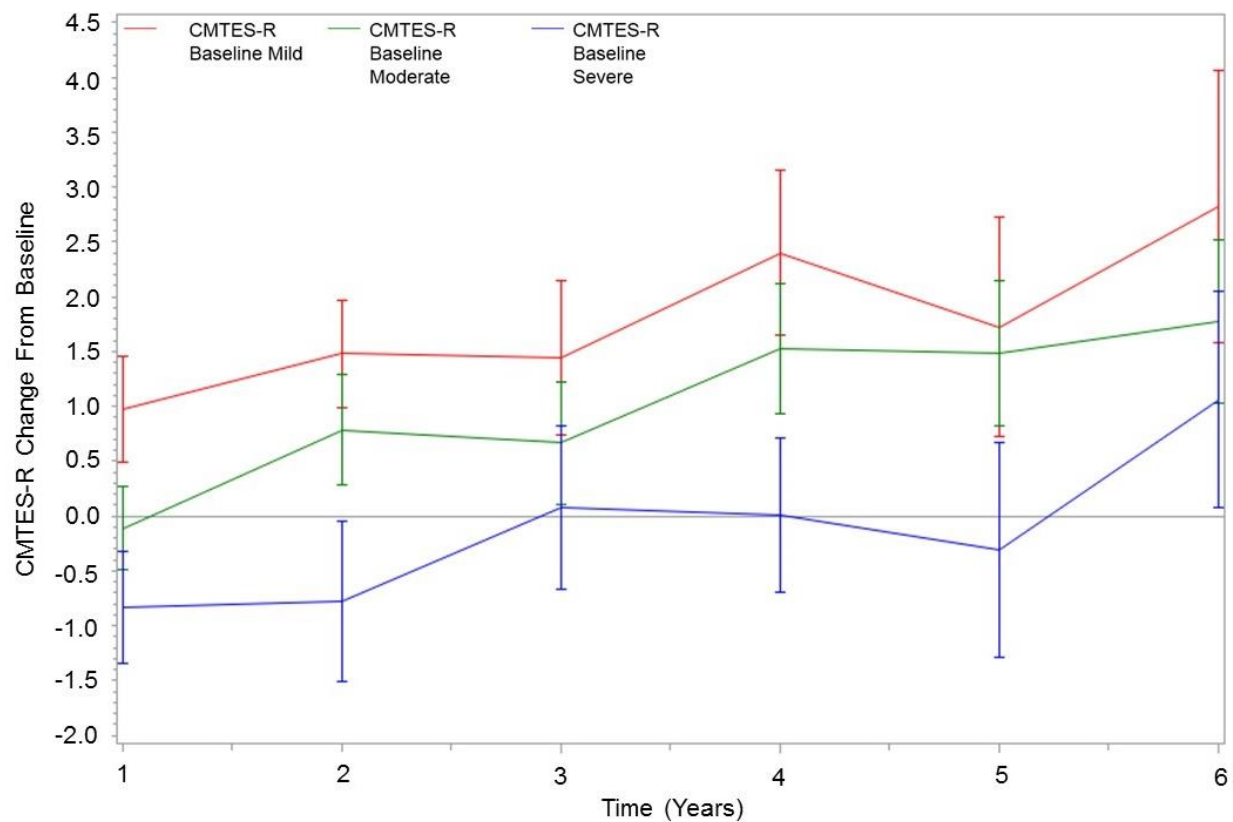
Weighted CMTES	Mean change from baseline at 2 years (CI)	P-value	SRM
0-9 (n=89)	1.48 (0.99, 1.97)	<0.0001	0.55
10-18 (n=154)	0.79 (0.28, 1.29)	0.002	0.22
>19 (n=68)	-0.77 (-1.50, -0.05)	0.037	0.22

Figure 4. Change in CMTNS-R over 4 years.

Figure 5. Responsiveness to change of individual CMTES items at 2 years.

Appendix 1: Authors

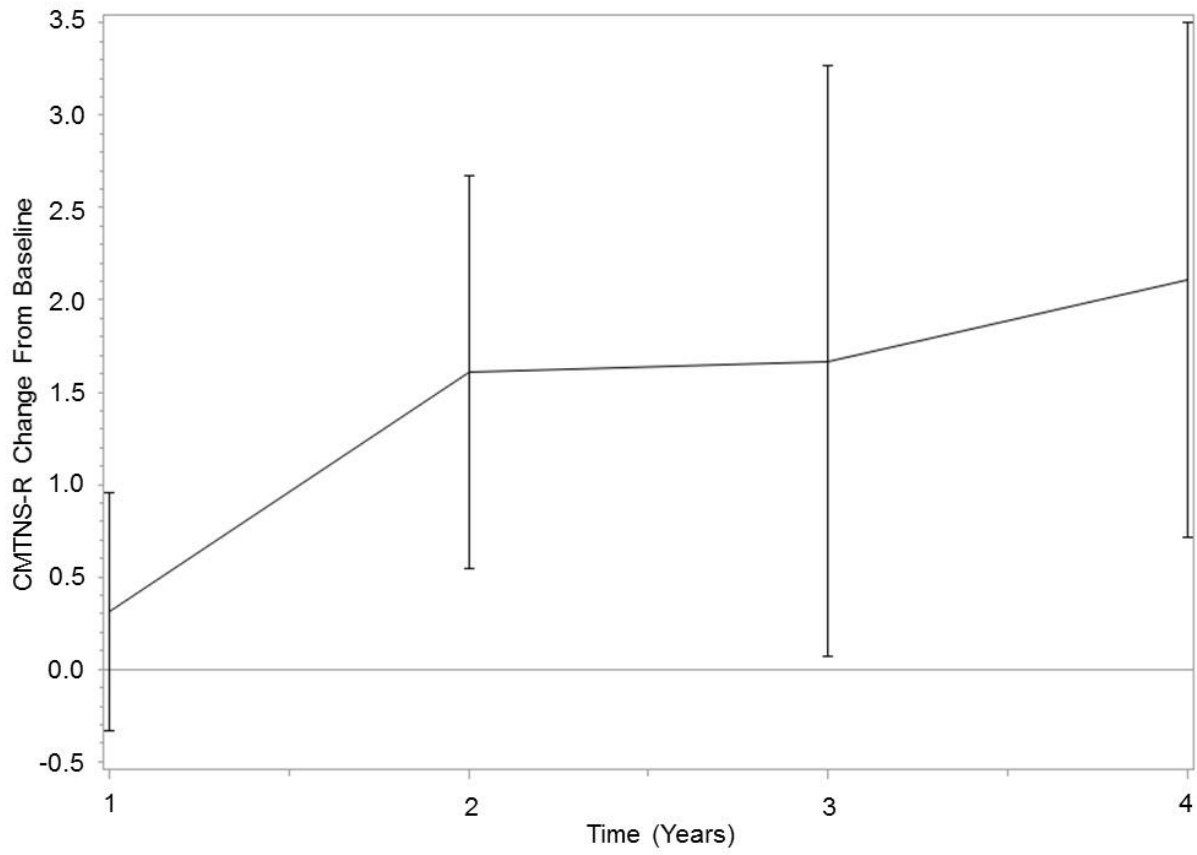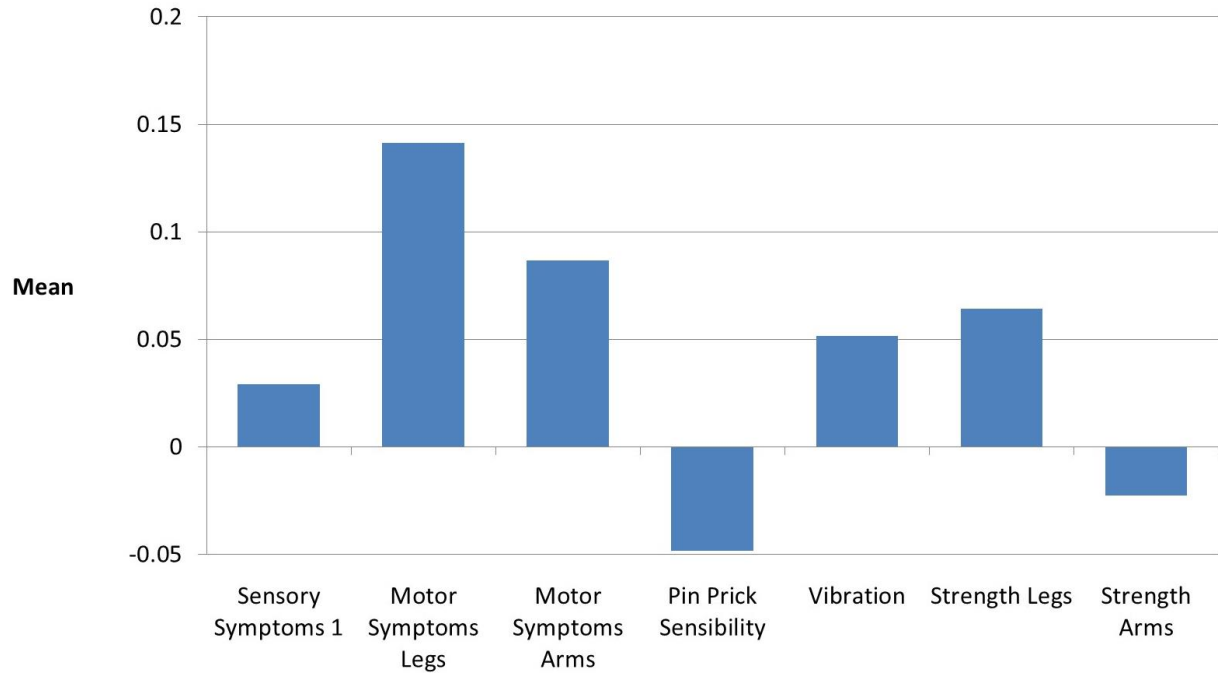| Name	Degree	Location	Role(s)	Contribution
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Appendix 2: Sites of the Inherited Neuropathies Consortium (INC)

INC Site	Number of Subjects Contributed
National Hospital for Neurology and Neurosurgery	398
University of Iowa	251
C. Besta Neurological Institute	197
Johns Hopkins University	90
University of Pennsylvania	87
University of Rochester	48
Wayne State University	33
Children's Hospital of Philadelphia	23
Stanford University	21
Harvard/Massachusetts General Hospital	12
Cedars Sinai Medical Center	11
University of Michigan	8
University of Minnesota	8
Vanderbilt University Medical Center	7
University of Miami	5
The Children's Hospital at Westmead	4
University of Washington Medical Center	3
Nemours, Orlando	3

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