ONLINE FIRST

Observational Study of Spinal Muscular Atrophy Type 2 and 3

Functional Outcomes Over 1 Year

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Objective: To characterize the short-term course of spinal muscular atrophy (SMA) in a genetically and clinically well-defined cohort of patients with SMA.

Design: A comprehensive multicenter, longitudinal, observational study.

Setting: The Pediatric Neuromuscular Clinical Research Network for SMA, a consortium of clinical investigators at 3 clinical sites.

Participants: Sixty-five participants with SMA types 2 and 3, aged 20 months to 45 years, were prospectively evaluated.

Intervention: We collected demographic and medical history information and determined the *SMN2* copy number.

Main Outcome Measures: Clinical outcomes included measures of motor function (Gross Motor Function Measure and expanded Hammersmith Functional Motor Scale), pulmonary function (forced vital capacity), and muscle strength (myometry). Participants were evaluated every 2 months for the initial 6 months and every 3 months for the subsequent 6 months. We evaluated change over 12 months for all clinical outcomes and examined potential correlates of change over time including age, sex, SMA type, ambulatory status, *SMN2* copy number, medication use, and baseline function.

Results: There were no significant changes over 12 months in motor function, pulmonary function, and muscle strength measures. There was evidence of motor function gain in ambulatory patients, especially in those children younger than 5 years. Scoliosis surgery during the observation period led to a subsequent decline in motor function.

Conclusions: Our results confirm previous clinical reports suggesting that SMA types 2 and 3 represent chronic phenotypes that have relatively stable clinical courses. We did not detect any measurable clinical disease progression in SMA types 2 and 3 over 12 months, suggesting that clinical trials will have to be designed to measure improvement rather than stabilization of disease progression.

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SPINAL MUSCULAR ATROPHY (SMA) is the leading genetic cause of death in infancy, with an estimated incidence of 1 in 6000 to 1 in 10 000 live births.[1-4] Most patients have a homozygous *SMN1* deletion of exon 7, making diagnostic confirmation readily available.[5,6] *SMN2* is an inverted duplication that differs from *SMN1* by 5 nucleotides, the only critical difference being an 840C>T transition in exon 7 that alters splicing.[7] The resulting messenger RNA lacks exon 7 (Δ7*SMN2* messenger RNA) and produces a protein with reduced stability. However, the expressed SMN2 is partially able to rescue the phenotype.[8] The clinical severity is inversely related to *SMN2* copy number.[9,10] This observation has been replicated in transgenic mice by knocking out the *SMN* gene and introducing a human *SMN2* transgene.[11] The homozygous *SMN1* mutation affects motor neurons in the spinal cord and ultimately

leads to muscle atrophy and weakness. In all but the most severe infantile forms of SMA, there is histological and electrophysiological evidence of reinnervation that partially compensates for functional loss.[12,13]

Spinal muscular atrophy leads to predominantly proximal muscle atrophy and weakness often leading to secondary scoliosis, joint contractures, and restrictive lung disease.[12] In the more severe SMA phenotypes, noninvasive respiratory support is often needed to compensate for the presence of respiratory muscle weakness.[14,15] The continuous clinical spectrum of SMA has been divided into 3 types based on the age at onset and highest motor milestone achieved.[16,17] Patients with SMA type 1 (SMA 1) become symptomatic in infancy and never achieve the ability to sit. Even with proactive respiratory and nutritional management, they typically have a shortened life expectancy.[15,18] Patients with SMA type 2 (SMA 2) can sit but never walk independently. Patients with SMA type 3 (SMA 3) have a normal life expectancy and can walk but have varying degrees of disability.[12] Both patients with SMA 2 and 3 may lose motor milestones over time.[18,19]

The timing of motor neuron loss in SMA before or after birth is incompletely understood.[20,21] It is also unclear if SMA is a developmental or a neurodegenerative disease.[13,22,23] Similar to an observation in amyotrophic lateral sclerosis, a late-onset motor neuron disease, the motor unit loss in SMA appears to precede the clinical disease onset.[24,25] Electrophysiological studies suggest that there is active motor unit loss in the preclinical and early subacute phase of SMA, followed by a chronic phase with relative stability over time.[25] Unlike most adult neurodegenerative diseases, SMA appears to exhibit slow disease progression.[18,19,26] Young children may even gain motor milestones early in their course.[27] Yet decline in muscle strength and motor function eventually occurs in SMA 2 and 3.[18,28]

With advances in medical care, individuals with SMA 2 and 3 often have a normal life expectancy but remain severely disabled physically.[18,19,29] As a result of our increased understanding of the etiology and pathogenesis of SMA, several new treatments are now on the horizon of clinical investigation. Recent and carefully collected observational data can help in designing clinical trials. We now report the results of a prospective multicenter study to follow the clinical evolution of SMA.

METHODS

SETTING

The study was carried out by the Pediatric Neuromuscular Clinical Research Network for SMA, a consortium of clinical investigators at 3 clinical sites: Columbia University (Clinical Coordinating Center and Molecular Genetics Core), The Children's Hospital of Philadelphia and University of Pennsylvania, and Harvard University. The data management and statistical analyses were performed at the Muscle Study Group Coordination and Biostatistics Centers at the University of Rochester, Rochester, New York.

PARTICIPANTS

We included 65 patients with *SMN1*-associated SMA 2 or 3. Participants of any age were included, as long as they had been diagnosed with SMA before age 19 years. Exclusion criteria were unstable medical conditions that would preclude participation, significant respiratory compromise at baseline that would interfere with safe travel to the site of evaluation, and patient's location beyond a reasonable driving distance in the opinion of the site investigator. Participants were enrolled between May 25, 2005, and September 10, 2007. The diagnosis of SMA type for each subject was made by the principal investigator at each study site following generally accepted criteria of maximal motor function: patients with SMA 2 sat independently but never walked independently, and patients with SMA 3 achieved independent ambulation. No subject evolved from SMA 2 to SMA 3 during the course of the study. To avoid selection bias, all patients seen in the neuromuscular clinics who fulfilled the inclusion and exclusion criteria were offered enrollment. Additional recruitment efforts included a study Web site and interactions with family groups. The study was approved by the institutional review board at each site and all parents or participants provided signed informed consent or assent.

VISIT SCHEDULE

Participants were evaluated at baseline and at months 2, 4, 6, 9, and 12. The outcome measures summarized next were administered at each visit on a single day. For most participants, the order of outcome measure testing was the same across all visits, with the goal of testing motor function early on, followed by strength testing. Also, the same evaluators, in general, performed the evaluations for all participants at a given site over time. We established excellent interrater reliability between primary and backup evaluators.

OUTCOME MEASURES

Motor Function

We used 3 scales that have been used previously in SMA clinical research: the Hammersmith Functional Motor Scale (HFMS), the expanded Hammersmith Functional Motor Scale (HFMSE), and the Gross Motor Function Measure (GMFM). The HFMS is a 20-item scale that was specifically developed to measure function in patients with SMA 2.[30] Each item is scored on a 0 to 2 scale and the total score ranges from 0 to 40. The GMFM is another standardized instrument originally designed to measure change in gross motor function over time in children with cerebral palsy and later validated for SMA.[31] The 88 items of the GMFM are scored on a 4-point (0-3) ordinal scale. The scale is divided into 5 domains (lying and rolling; sitting; crawling and kneeling; standing; and walking, running, and jumping), and each domain score is expressed as a percentage of the maximum score for that domain. The total score is obtained by averaging the percentages across the 5 domains and ranges from 0 to 100.

The HFMSE is an expanded version of the HFMS that adds 13 items from the GMFM to capture aspects of ambulation, rescaled to the same 0 to 2 metric on which the HFMS items are scored. The HFMSE thus captures a wider range of functional abilities and has demonstrated reliability and validity in patients with SMA 2 and 3.[32] The HFMSE contains 33 items and has a total score that ranges from 0 to 66. After obtaining the GMFM and HFMS scores, we computed the sum of the HFMS and the 13 additional GMFM items to obtain the HFMSE score. In many participants, the HFMSE score is identical to the HFMS score because they could not perform any of the more challenging 13 tasks that distinguish the HFMS from the HFMSE.

Pulmonary Function

In children older than 5 years and in a few sufficiently cooperative children younger than 5 years, we measured forced expiratory vital capacity (FVC) as percentage of predicted for age and height or a surrogate for height (see later) using a spirometer (KoKo spirometer; nSpire Health Inc, Longmont, Colorado) with incentive visual reinforcement displayed on the computer screen.[33] We recorded 3 consecutive attempts for each participant, and the maximum result was taken as the measure of FVC. Previous studies in SMA have found that FVC is reliable.[34]

Muscle Strength

In cooperative children, we performed myometry using a handheld dynamometer to measure elbow flexion, knee extension, and knee flexion strength. Myometry was not included in our study protocol at the onset of the study, so this measure was introduced after the first visit in several participants.

ANTHROPOMETRICS

We measured standing height or, if not feasible, supine height by adding measured head, trunk, and leg segments. When height could not be measured (eg, because of severe scoliosis or contractures), we measured ulna length from the olecranon to the styloid process and calculated estimated height based on published data as a surrogate height measure.[35]

CONCURRENT MEDICATIONS AND MEDICAL EVENTS

Given that there is no known effective treatment for SMA, we did not exclude participants taking drugs or supplements intended as treatment for SMA. The use of concurrent medications was recorded at each visit. We also collected information on the timing of hospitalizations for spine surgery, respiratory infections, or other intercurrent events.

GENETIC TESTING

Confirmation of the *SMN1* exon 7/8 common deletion was carried out by polymerase chain reaction (PCR) amplification and restriction fragment length polymorphism analysis of DNA using primers flanking *SMN1* and *SMN2* exon 7. Digestion of the PCR product with Dra I followed by agarose gel electrophoresis from subjects with SMA lacking *SMN1* exon 7 results in a loss of a fragment without a Dra I restriction site and a smaller fragment on gel electrophoresis that easily distinguishes the *SMN1* and *SMN2* genes. Using *SMN2*-specific primers and primers for the control gene *GAPDH*, we quantified *SMN2* copy number by real-time PCR using a light cycler to detect intensity of fluorescence in real time. Crossing points for each gene were determined and compared with *GAPDH* and then with the controls with a known *SMN2* copy number to determine the relative *SMN2*:*GAPDH* ratio. High specificity of the PCR product was ensured by performing a melting curve analysis to help distinguish between specific and nonspecific by-products, eg, primer dimers. Each sample was run in duplicate.

EVALUATOR TRAINING AND MEASUREMENT QUALITY

Dedicated evaluators and backup evaluators collected the outcome data at the clinical sites. Evaluators were trained at an initial meeting prior to study onset and had follow-up meetings and conference calls to maintain uniformity of evaluation procedures across sites. We developed a study manual for all procedures that was available to the evaluators in print and online. For pulmonary function measures, flow volume curves were uploaded into the central data management system and checked for technical limitations and poor patient effort by a single pulmonologist (A.C.) for all sites. When the curve was deemed of insufficient quality, the FVC result was not included in the data set because it was not thought to reflect the underlying physiologic event.

QUALITY CONTROL

The quality of study operations was enhanced by a comprehensive Web-based data entry and management system developed specifically for the Pediatric Neuromuscular Clinical Research Network SMA study. Data were entered at each site and managed centrally by the Muscle Study Group Coordination Center. The system identified out-of-range values and missing data at the time of entry. Further extensive data checking was accomplished after data entry. Regular queries were issued for missing or implausible data.

STATISTICAL ANALYSIS

Data were included for all participants who were enrolled in the observational cohort study at least 12 months prior to the cutoff date of June 1, 2009, and had at least 1 postbaseline evaluation. Only measurements up to 12 months postbaseline were included in the analyses. For each of the outcome measures, we performed formal analyses of the change over time using 2 different strategies: (1) a repeated-measures analysis of covariance model and (2) a mixed-effects linear regression model. The repeated-measures analysis of covariance model included SMA type as a covariate and time (categorical) as the independent variable of interest; this model describes the change in outcome from baseline to each separate visit, with month 12 being the visit of primary interest. The mixed-effects linear regression model included SMA type as a covariate and time (continuous) as the independent variable of interest; this model assumes a linear relationship between the outcome and time and allows subject-specific slopes and intercepts, with the average slope being of primary interest. Unstructured covariance patterns were assumed for both models. These models use maximum likelihood to estimate the parameters of interest using available data from all subjects, dealing with the problem of missing data in an appropriate way under the "missing at random" assumption. If a participant had scoliosis surgery during the 1-year follow-up period, observations obtained after the surgery were excluded from the analyses; the impact that such surgery had on the observed outcomes is summarized separately. The muscle strength outcomes were analyzed using only the mixed-effects linear regression model because of the irregularity in the timing of the visits for these outcomes, which were introduced partway into the study.

We examined potential baseline correlates of change over time by adding appropriate main effect and interaction terms to the mixed-effects linear regression model. The following baseline variables were considered (separately): age (3-12 and ≥13 years), sex, SMA type (2 or 3), ambulatory status (not walking or walking), *SMN2* copy number (2-3 or 4), HFMS score (<20 or ≥20), FVC (<70% or ≥70%), and use of agents to treat SMA (yes or no).

Intrarater reliability of the outcome measures was assessed using data from the baseline and month 2 visits. This was quantified by intraclass correlation coefficients, computed using 1-way random-effects analysis of variance models.

Table 1. Baseline Characteristics for Participants With SMA 2 and SMA 3

Variable	%		
	SMA 2 (n= 35)	SMA 3 (n=30)	Overall (n=65)
Age, y, mean (SD)	9.6 (7.6)	13.2 (10.3)	11.2 (9.1)
Age ≤12 y	77	57	68
Age <5 y	31	23	28
Male	40	50	45
Race			
White	69	80	74
Black	0	3	2
Mixed	23	10	17
Asian	6	3	5
Unknown	3	3	3
Center			
Columbia	46	50	48
Harvard	26	27	26
CHOP	29	23	26
Height, cm, mean (SD)	116.3 (23.5)	138.9 (29.3)	126.9 (28.5)
Weight, kg, mean (SD)	24.9 (13.1)	40.3 (23.9)	32.1 (20.3)
SMN2 copy number			
3	100	53	78
4	0	43	20
5	0	3	2
Walking	0	73	34
Sitting	77	100	88
BiPAP use	29	0	15
HFMS score, mean (SD)	9.0 (8.9)	33.5 (10.2)	20.1 (15.5)
HFMSE score, mean (SD)	10.9 (10.6)	45.9 (14.7)	26.5 (21.5)
GMFM score, mean (SD)	17.3 (13.3)	70.6 (25.2)	41.0 (33.0)
FVC, % of predicted, mean (SD)	46.1 (22.6)	96.9 (17.3)	70.5 (32.5)
Muscle strength, kg, mean (SD)			
Elbow flexion	2.5 (1.4)	11.4 (8.7)	6.8 (7.5)
Knee extension	0.8 (0.8)	3.8 (2.9)	2.3 (2.6)
Knee flexion	2.5 (1.4)	8.3 (6.1)	5.3 (5.2)

Abbreviations: BiPAP, bilevel positive airway pressure; CHOP, Children's Hospital of Philadelphia; FVC, forced vital capacity; GMFM, Gross Motor Function Measure; HFMSE, expanded Hammersmith Functional Motor Scale; HFMS, Hammersmith Functional Motor Scale; SMA 2, spinal muscular atrophy type 2; SMA 3, spinal muscular atrophy type 3.

RESULTS

RECRUITMENT AND RETENTION

We enrolled 71 participants with SMA 2 and 3 , but 3 of these (all younger than 2 years) were not evaluated with the functional assessments and 3 others did not have post-baseline evaluations (2 prematurely withdrew from follow-up and 1 had scoliosis surgery shortly after enrollment). Data from the remaining 65 participants (35 SMA 2 and 30 SMA 3) were included in the analyses. There were no deaths over the 12-month follow-up period. Of the 65 participants, 51 had a month 12 visit. Among the 14 participants without month 12 observations were 4 who missed the month 12 visit but remained in the study, 3 who had scoliosis surgery prior to the month 12 visit, and 7 who prematurely withdrew from follow-up. Reasons for premature withdrawal from follow-up included participation in a clinical trial at another institution (n=2), difficulties in coping with the burden of research visits in terms of time and travel (n=4), and family illness (n=1).

The mean (SD) age of the 65 participants was 11.2 (9.1) years, 45% were male, and 74% were white. Seventy-three percent of the participants with SMA 3 were walking at baseline. For participants with SMA 3 younger than 3 years, 68% were walking at baseline, compared with 82% for those at least 3 years old. Seventy-seven percent of participants with SMA 2 had the ability to sit at baseline, compared with 100% of participants with SMA 3. Twenty-nine percent of participants with SMA 2 (and no participants with SMA 3) were using bilevel positive airway pressure at baseline. Other baseline characteristics of participants are summarized by SMA type in **Table 1**.

TEST-RETEST RELIABILITY

Intraclass correlation coefficients for test-retest reliability, using data from the baseline and month 2 visits, were very high (>0.98) for the GMFM, HFMS, HFMSE, and FVC (liters).

MOTOR FUNCTION

As expected, participants with SMA 2 and SMA 3 differed substantially with respect to baseline motor function (Table 1). There was no appreciable mean change in motor function over the 12-month follow-up period as measured by the GMFM, HFMS, and HFMSE (**Table 2** and **Table 3**; **Figure 1** and **Figure 2**).

MUSCLE STRENGTH

Participants with SMA 2 and SMA 3 differed substantially with respect to baseline muscle strength, as measured by quantitative myometry (Table 1). No significant mean changes in elbow flexion, knee extension, or knee flexion strength were detected over 12 months (**Table 4**).

PULMONARY FUNCTION

Forced expiratory vital capacity was measured in 50 of the 65 participants (77%) and differed substantially between participants with SMA 2 and SMA 3 at baseline (Table 1). The average measured value of FVC (percentage of predicted) decreased slightly over time, by approximately 2% over 12 months ($P=.23$) (Table 3). The mean annual rate of change was –1.13% (95% confidence interval [CI], –4.18% to 1.91%; $P=.46$) (Table 4; **Figure 3**).

PREDICTORS OF PROGRESSION

Over the 12-month observation period, there were no significant associations between annual rates of change in motor function (GMFM and HFMSE) or pulmonary function (percentage of predicted FVC) and age, SMA type, baseline HFMS score, or baseline percentage of predicted FVC. The mean rate of change in FVC differed between female participants (–3.51% per year; 95% CI, –7.14% to 0.12%) and male participants (3.12% per year; 95% CI, –1.68% to 7.91%) ($P=.03$).

Table 2. Change Over Time for Participants With SMA 2 and SMA 3

Variable	Mean (SD) of the Change From Baseline to Each Visit				
	Month 2	Month 4	Month 6	Month 9	Month 12
HFMS	−0.83 (2.79) [n = 23]	−0.26 (1.51) [n = 23]	0.00 (2.41) [n = 29]	−0.30 (2.01) [n = 23]	−0.31 (2.24) [n = 26]
HFMSE	−0.52 (2.92) [n = 42]	0.37 (2.98) [n = 46]	0.27 (2.72) [n = 55]	0.00 (3.12) [n = 43]	0.15 (3.22) [n = 48]
GMFM	−0.43 (2.93) [n = 42]	1.33 (4.88) [n = 46]	0.46 (4.74) [n = 55]	1.03 (4.99) [n = 43]	0.78 (5.94) [n = 47]
FVC (% of predicted)	−1.73 (8.31) [n = 30]	−0.29 (7.82) [n = 34]	0.36 (9.81) [n = 39]	0.61 (8.74) [n = 36]	−2.49 (10.60) [n = 37]

Abbreviations: FVC, forced vital capacity; GMFM, Gross Motor Function Measure; HFMSE, expanded Hammersmith Functional Motor Scale; HFMS, Hammersmith Functional Motor Scale (participants with SMA 2 only); SMA 2, spinal muscular atrophy type 2; SMA 3, spinal muscular atrophy type 3.

Table 3. Analyses of Change Over Time for Participants With SMA 2 and SMA 3[a]

Variable	Mean Change (95% CI)	*P* Value
HFMS, mo		
6	−0.06 (−0.94 to 0.81)	.88
9	−0.39 (−1.14 to 0.37)	.30
12	−0.16 (−1.05 to 0.73)	.72
HFSME, mo		
6	0.27 (−0.43 to 0.97)	.44
9	−0.08 (−0.90 to 0.74)	.85
12	0.19 (−0.70 to 1.08)	.67
GMFM, mo		
6	0.42 (−0.80 to 1.64)	.49
9	0.39 (−1.12 to 1.90)	.61
12	0.79 (−0.99 to 2.57)	.38
FVC, % of predicted, mo		
6	0.46 (−2.34 to 3.26)	.74
9	0.57 (−2.07 to 3.20)	.67
12	−2.21 (−5.84 to 1.43)	.23

Abbreviations: CI, confidence interval; FVC, forced vital capacity; GMFM, Gross Motor Function Measure; HFMSE, expanded Hammersmith Functional Motor Scale; HFMS, Hammersmith Functional Motor Scale (participants with SMA 2 only); SMA 2, spinal muscular atrophy type 2; SMA 3, spinal muscular atrophy type 3.

[a] Mean changes, CIs, and *P* values were obtained from a repeated-measures analysis of covariance model that included SMA type as a covariate and time (categorical) as the independent variable of interest; see text for details.

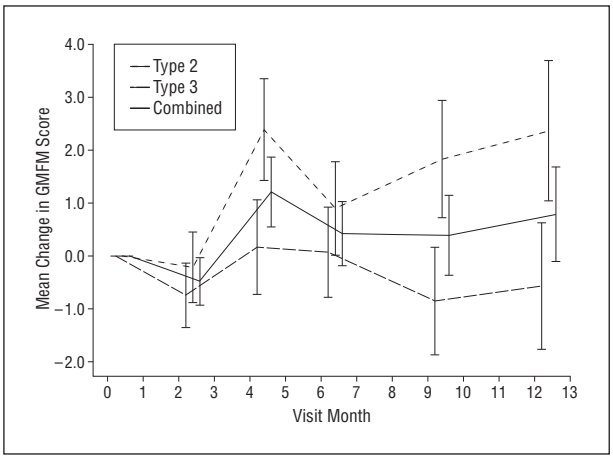

Figure 1. Mean change in Gross Motor Function Measure (GMFM) total score over time estimated using a repeated-measures analysis of covariance model. Error bars indicate 1 SE of the mean. Mean changes are plotted for spinal muscular atrophy (SMA) type 2 and SMA type 3 combined, SMA type 2 only, and SMA type 3 only.

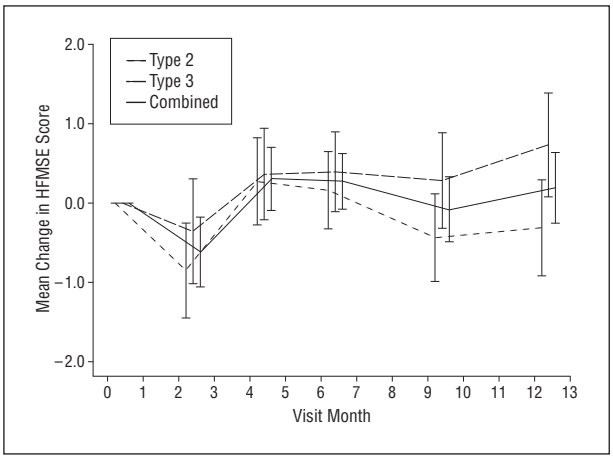

Figure 2. Mean change in expanded Hammersmith Functional Motor Scale (HFMSE) score over time estimated using a repeated-measures analysis of covariance model. Error bars indicate 1 SE of the mean. Mean changes are plotted for spinal muscular atrophy (SMA) type 2 and SMA type 3 combined, SMA type 2 only, and SMA type 3 only.

Ambulatory status at baseline was significantly associated with annual rate of change in motor function, with a significant increase in function over time in ambulatory participants compared with a slight decline in function over time in nonambulatory participants. For the GMFM score, the mean rate of change was 3.96 (95% CI, 1.20 to 6.72) in ambulatory participants and −0.70 (95% CI, −2.86 to 1.46) in nonambulatory participants ($P=.01$). For the HFMSE score, the mean rate of change was 1.62 (95% CI, 0.14 to 3.10) in ambulatory participants and −0.47 (95% CI, −1.66 to 0.72) in nonambulatory participants ($P=.03$). To explore these associations further, we reanalyzed the data and included an interaction term between ambulatory status and age (<5 years vs ≥5 years) in the mixed-effects linear regression model. We found that the difference in GMFM score slope between ambulatory and nonambulatory participants was 6.3 for those younger than 5 years compared with 4.2 for those at least 5 years of age. Similarly, for the HFMSE score slope, the difference between ambulatory and nonambulatory participants was greater in those younger than 5 years (3.8) than in those at least 5 years old (1.5).

All participants were homozygous for a deletion of *SMN1*. The distribution of *SMN2* copy number differed between participants with SMA 2 and SMA 3, with all participants with SMA 2 having 3 *SMN2* copies and participants with SMA 3 having between 3 and 5 *SMN2* copies;

Table 4. Analyses of Rate of Change Over Time for Participants With SMA 2 and SMA 3[a]

Variable	Mean Rate of Change, Slope (95% CI)	*P* Value
HFMS	0.06 (–0.82 to 0.93)	.90
HFMSE	0.25 (–0.65 to 1.15)	.58
GMFM	0.75 (–1.07 to 2.57)	.41
FVC, % of predicted	–1.13 (–4.18 to 1.91)	.46
Elbow flexion, kg	–0.58 (–2.62 to 1.47)	.57
Knee extension, kg	–0.07 (–0.98 to 0.84)	.87
Knee flexion, kg	–0.03 (–1.00 to 0.94)	.95

Abbreviations: CI, confidence interval; FVC, forced vital capacity; GMFM, Gross Motor Function Measure; HFMS, Hammersmith Functional Motor Scale (participants with SMA 2 only); HFMSE, expanded Hammersmith Functional Motor Scale; SMA 2, spinal muscular atrophy type 2; SMA 3, spinal muscular atrophy type 3.

[a] Mean rates of change, CIs, and *P* values were obtained from a mixed-effects linear regression model that included SMA type as a covariate and time (continuous) as the independent variable of interest; see text for details.

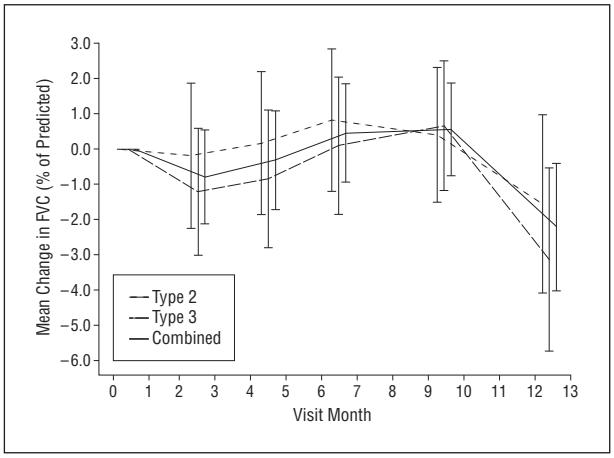

Figure 3. Mean change in forced expiratory vital capacity (FVC) (percentage of predicted) over time estimated using a repeated-measures analysis of covariance model. Error bars indicate 1 SE of the mean. Mean changes are plotted for spinal muscular atrophy (SMA) type 2 and SMA type 3 combined, SMA type 2 only, and SMA type 3 only.

most participants with SMA 3 carried 3 or 4 *SMN2* copies (Table 1). There was no significant association between any measure of the rate of disease progression and *SMN2* copy number.

Sixteen of the 65 participants (25%) used at least 1 medication that might be considered as a potential treatment for SMA at some time during the 12-month follow-up period. These medications included albuterol (6%), carnitine (11%), creatine (8%), hydroxyurea (3%), steroids (2%), and valproic acid (8%). There were no significant differences between those who did and did not take these medications with respect to the mean rate of change over time in motor function or pulmonary function.

Four participants had scoliosis surgery during the 12-month follow-up period. One 14-year-old participant with SMA 3 had surgery between months 6 and 12 and the HFMS score dropped from 21 to 8 after surgery. Only a slight decline in FVC was noted. A 6-year-old participant with SMA 2 had surgery between months 6 and 12 and the HFMS score dropped from 20 to 7 after surgery. This participant also had a decline in FVC from 0.98 L (67% of predicted normal) to 0.68 L (38% of predicted normal). A 5-year-old participant with SMA 2 had surgery shortly after enrollment, but the HFMS score at baseline remained very low throughout follow-up (ranging from 0-2); FVC was not measured in this participant. Finally, a 12-year-old participant with SMA 3 had surgery between months 9 and 12 and a preoperative HFMS score of 17. At the first postoperative study visit, the motor function testing could not be performed because of pain. The FVC changed only slightly in this participant.

COMMENT

We carried out a multicenter, prospective, observational cohort study in patients with SMA under conditions similar to those that would be used in a clinical trial with respect to having a fully developed manual of procedures, evaluator training, and data quality control. Our data suggest that SMA has a relatively stable course over a 12-month period in terms of motor function, pulmonary function, and muscle strength. There was no significant mean change in motor function when measured by 3 different instruments that have been validated for use in SMA: the HFMS, the HFMSE, and the GMFM. Our observational study, however, does not address the issue as to whether 1 scale may be more sensitive than another to the effects of an intervention. Pulmonary function appeared to decline slightly over 12 months, but the change was not statistically significant.

The motor function improvements observed in ambulatory participants may be due to a learning effect that may be more prominent in younger children. It may also in part be associated with the developmental gain of motor milestones in children who walk relatively late because of their underlying condition. The largest gain in motor function occurred in those who were walking and were younger than 5 years, although the number of participants younger than 5 years (n=18, with 5 ambulatory and 13 nonambulatory) was too small to permit definitive conclusions. In contrast, more participants with SMA 2 may have reached their highest level of motor function at the time of enrollment into our study. The slight gain in motor function was surprising, because loss in motor function for patients with SMA 3 had been described in previous studies.[19] A recent open-label study found improvement after drug treatment in younger patients with SMA.[36] While this may be due to biological factors, our results from this observational study suggest the potential for a learning effect or for developmental gains to confound the results of uncontrolled trials that evaluate motor function.

Our data suggest that female sex may be associated with a greater decline in pulmonary function, but not in motor function. This is unexpected because studies of the association between *SMN2* copy number and phenotype had suggested that female sex confers a mitigating effect on disease severity.[9] Additional studies are needed to either confirm or refute this finding.

Participants who had scoliosis surgery during the observation period had postsurgical declines in motor func-

tion. Because of the small number of participants who had surgery during the study period, this finding requires replication. The short-term loss in motor function may be due to reduced axial movements, which are invariably restricted as a result of the stabilizing nature of scoliosis surgery. It is unknown if this is a clinically meaningful functional impairment. From a clinical perspective, surgery is often needed to maintain long-term function and quality of life, and the gain in postural stability and the slowing of progressive worsening of the spine curvature are beneficial to patients in the long-term. From a research perspective, however, it may be important in future clinical trials to select participants who are unlikely to undergo scoliosis surgery during the observation period. For participants who do undergo surgery, exclusion of data collected postsurgery from the statistical analysis is an additional consideration.

A subset of our cohort (25%) used medications intended for the treatment of SMA. Thus, although none of these medications has been shown to have an impact on disease course, our data may not reflect a pure observation of the natural history of SMA. The decision to include participants using supplements and medications intended for the treatment of SMA was based on concerns about recruitment feasibility if the entry criteria had been restricted to entirely untreated patients, as well as the concern that participants may take medications without reporting this to the investigators.

Our results differ from those of previous studies in the United States and Europe that had found a slow decline in motor function in SMA 2 and SMA 3.[18,28,37] A French natural history study that used different measures of motor and pulmonary function found evidence of mild decline in both motor and pulmonary function at 2 and 4 years after baseline, but 1-year data were not reported.[26] The discrepant results may in part be due to differences in methods. Also, it is possible that we would have observed a significant decline in pulmonary function, for example, if we had observed participants for a longer period. The data that we are obtaining through ongoing long-term follow-up of cohort participants may allow a more direct comparison with the French study.

Our results showing relative stability of function over time suggest that future clinical trials will need to be designed to show treatment-associated improvement rather than a slowing or arrest of decline. This design is in contrast to prevailing trial designs for adult neurodegenerative disorders, which often aim to demonstrate treatment-associated slowing of disease progression. There is increasing evidence that key events in the pathogenesis of SMA occur in the distal compartments of the neuron and involve impaired axonal outgrowth and synaptic connections. Clinical trials aimed at detecting improvement would be most appropriate for potential therapies that could promote reinnervation by collateral sprouting or improve function and connectivity in surviving neurons with impaired function.

We have confirmed the feasibility and excellent reliability of the motor and pulmonary function measures used in this study. Importantly, we succeeded in enrolling a relatively large cohort of patients with SMA at only 3 sites in slightly more than 2 years. Given that there was no possibility for direct benefit for participants in this observational study, this success implies that people with SMA and their families are eager to embrace clinical research opportunities and contribute their time and effort.

Continued long-term follow-up of our cohort will determine if the functional stability observed over the initial 12 months continues over a longer observation period. It is also hoped that the planned analyses of additional outcomes including quality of life, electrophysiological outcomes, and muscle mass will enhance our understanding of the clinical longitudinal profile of SMA. The data from this cohort will be valuable for the design of future clinical trials in SMA.

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