Prospective cohort study of spinal muscular atrophy types 2 and 3

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Supplemental data at www.neurology.org

ABSTRACT

Objective: To characterize the natural history of spinal muscular atrophy type 2 and type 3 (SMA 2/3) beyond 1 year and to report data on clinical and biological outcomes for use in trial planning.

Methods: We conducted a prospective observational cohort study of 79 children and young adults with SMA 2/3 who participated in evaluations for up to 48 months. Clinically, we evaluated motor and pulmonary function, quality of life, and muscle strength. We also measured SMN2 copy number, hematologic and biochemical profiles, muscle mass by dual x-ray absorptiometry (DXA), and the compound motor action potential (CMAP) in a hand muscle. Data were analyzed for associations between clinical and biological/laboratory characteristics cross-sectionally, and for change over time in outcomes using all available data.

Results: In cross-sectional analyses, certain biological measures (specifically, CMAP, DXA fat-free mass index, and SMN2 copy number) and muscle strength measures were associated with motor function. Motor and pulmonary function declined over time, particularly at time points beyond 12 months of follow-up.

Conclusion: The intermediate and mild phenotypes of SMA show slow functional declines when observation periods exceed 1 year. Whole body muscle mass, hand muscle compound motor action potentials, and muscle strength are associated with clinical measures of motor function. The data from this study will be useful for clinical trial planning and suggest that CMAP and DXA warrant further evaluation as potential biomarkers. *Neurology*® 2012;79:1889-1897

GLOSSARY

AUC = area under the curve; **CMAP** = compound motor action potential; **DXA** = dual x-ray absorptiometry; **FFMI** = fat-free mass index; **GMFM** = Gross Motor Function Measure; **HFMS** = Hammersmith Functional Motor Scale; **HFMSE** = Expanded Hammersmith Functional Motor Scale; **NHANES** = National Health and Nutrition Examination Survey; **FVC** = forced vital capacity; **SMA** = spinal muscular atrophy.

Spinal muscular atrophy (SMA), the second most common recessive lethal pediatric disease,¹ causes proximal muscle weakness, atrophy, and respiratory and orthopedic complications. The severity of the clinical phenotype is heterogeneous with the most severe form (SMA type 1) beginning in infancy. The intermediate (SMA type 2) and mild (SMA type 3) forms cause less severe motor disability.²

Almost all patients have a homozygous deletion of the survival of motor neuron gene (*SMN1*).³ The phenotype is modified by the *SMN2* copy number. *SMN2* partially compensates for the lack of *SMN1* explaining the inverse relationship between disease severity and *SMN2* copy number.⁴

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Several drugs have been explored in clinical trials including hydroxyurea,^{5,6} sodium phenylbutyrate,⁷ acetylcarnitine, and valproate combined with L-carnitine.⁸ Other drugs currently in trials include olexosime⁹ and riluzole.¹⁰ The negative outcome to date may indicate ineffectiveness of the intervention or inadequacy of the trial design characteristics (e.g., sample size or duration of follow-up). Contemporary high-quality clinical data will address this dilemma. Our objective, therefore, is to report longer-term observations from our prospective cohort study, following up on our earlier report that was limited to clinical observations over a 12-month period.¹¹

METHODS Setting. Participants were evaluated at 3 clinical sites of the Pediatric Neuromuscular Clinical Research Network for SMA.

Participants. Eighty-five volunteers with *SMN1*-associated SMA type 2 (SMA 2) or type 3 (SMA 3), diagnosed before age 19 years, were included between May 2005 and February 2009. Exclusion criteria included severe respiratory and other medical conditions precluding safe participation and travel exceeding a reasonable driving distance. Cohort size was based on recruitment feasibility. Data were excluded from 6 subjects: 3 were under age 2 years and 3 did not have postbaseline evaluations. Follow-up data on the remaining 79 participants were included through October 1, 2009.

Standard protocol approvals, registrations, and patient consents. All participants or their guardians provided written informed consent approved by the respective institutional review boards. The study was registered with ClinicalTrials.gov (NCT00443066).

Visit schedule. Participants were evaluated at baseline, months 2, 4, 6, 9, and 12, and every 6 months thereafter. All evaluations were performed on a single day, and, to the extent feasible, by the same evaluator in the same order. Because of imperfect follow-up, inability to tolerate the complete testing series, or premature withdrawal, the actual number of evaluations varied by visit (table 1 and table e-1 on the *Neurology*[®] Web site at www.neurology.org).

Outcome variables. Motor function. We used 3 previously described motor function measures¹¹: 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 for use in SMA 2 patients.¹² The GMFM has 88 items organized in 5 domains.¹³ The HFMSE is an expanded version of the HFMS.^{14,15}

Pulmonary function. We recorded the best of 3 consecutive attempts for forced expiratory vital capacity (FVC) using a Koko spirometer.¹¹ We expressed the result as percent predicted for age and height or a surrogate for height (see below).¹⁶

Quality of life. We used the PedsQL[™] 4.0 Generic Core Module, previously described for use in SMA, that encompasses 1) physical functioning (8 items), 2) emotional functioning (5

items), 3) social functioning (5 items), and 4) school functioning (5 items).^{17,18}

Muscle strength. We performed hand-held dynamometry to measure elbow flexion, knee extension, and knee flexion strength. Myometry data were incomplete because they were added to the protocol after study initiation, and some children could not cooperate with the instructions. We did not adjust for developmental growth because normative data are lacking.^{19,20}

Anthropometrics. We measured standing height. Otherwise, we summed head, trunk, and leg segment measurements, or used ulna length as a surrogate.²¹

Electrophysiologic testing. We recorded the compound motor action potential (CMAP) amplitude (mV) and area under the curve (AUC) (mV × ms) from the hypothenar eminence with supramaximal stimulation of the ulnar nerve at the wrist as previously described.¹¹

Muscle imaging. We used dual energy x-ray absorptiometry (DXA) using Lunar model DPX with pediatric software 3.8 and DPX-L with pediatric software 1.5 G (GE Lunar Corporation, General Electric, Madison, WI). Patient positioning and measurement protocols were standardized across sites as previously described.²² The raw data were processed centrally (M.P.). Using DXA-estimated lean mass and measured height, we calculated a fat-free mass index (FFMI = lean mass in kg/squared height in m²).²² The FFMI was referenced to available data from healthy volunteers to generate an age- and gender-based Z score for FFMI. For subjects over age 8 years, we used data from the National Health and Nutrition Examination Survey (NHANES)²³; for those under 8 years, we used the Pediatric Rosetta Body Study data.^{22,24}

Concurrent medications. Participants taking drugs or supplements intended as SMA treatment were included because no effective treatment has been identified to date. Concurrent medication usage was recorded at each visit.

Medical events. Hospitalization data were collected. Because scoliosis surgery causes decreases in postoperative motor function,¹¹ we excluded postoperative observations.

Laboratory testing. The *SMN1* exon 7/8 deletion and determination of *SMN2* copy number were confirmed as described.¹¹ Hematologic and metabolic panels were obtained every 6 months when feasible.

Training and quality control. Evaluators used a common procedure manual and were trained at annual in-person meetings. The flow volume curves were all reviewed centrally by a single pulmonologist (A.C.), and the CMAP waveforms by a single electromyographer (C.L.G.). Technically deficient or biologically implausible measurements were excluded from the analysis. A central, Web-based data management system was used and regular queries were circulated for missing or implausible data. We followed the STROBE criteria for the reporting of observational studies.²⁵

Statistical analysis. Cross-sectional associations between motor function and indirect measures of SMA severity (CMAP amplitude and AUC, DXA FFMI and FFMI Z score, muscle strength, and *SMN2* copy number) were examined at baseline using Spearman correlation coefficients and Wilcoxon rank sum test (to compare participants with 3 *SMN2* copies to those with 4–5 copies). Analyses of the change over time in motor and pulmonary function were performed using a mixed-effects re-

Supplemental Data

Table 1 Baseline characteristics for SMA 2 and SMA 3 participants^a

Variable	SMA 2 (n = 41)	SMA 3 (n = 38)	Overall (n = 79)
Age, y	9.1 (7.4)	13.7 (10.8)	11.3 (9.4)
Age ≤12 y, %	78	55	67
Age at symptom onset, y, median (IQR)	0.8 (0.5, 1.0)	2.0 (1.0, 3.0)	1.0 (0.8, 2.0)
Age at SMA diagnosis, y, median (IQR)	1.1 (0.9, 1.4)	3.5 (2.5, 5.7)	1.5 (1.0, 3.0)
Male, %	39	55	47
Race, %			
White	68	82	75
Black	0	5	3
Mixed	22	8	15
Asian	7	5	6
Unknown	2	0	1
Center, %			
Columbia	51	47	49
Harvard	22	21	22
CHOP	27	32	29
SMN2 copy number, %			
3	100	53	77
4	0	45	22
5	0	3	1
Walking, %	0	71	34
Sitting, %	78	100	89
BiPAP use, %	31	3	18
HFMS (n = 76)	9.0 (8.5)	32.9 (10.3)	20.6 (15.2)
HFMSE (n = 75)	10.7 (10.1)	44.8 (15.4)	27.1 (21.4)
GMFM (n = 72)	17.3 (12.9)	67.4 (27.9)	41.6 (33.1)
FVC (% predicted) (n = 60)	45.6 (21.7)	97.4 (17.5)	72.4 (32.6)
PedsQL (parent) (n = 57)			
Physical health	19.5 (9.9)	44.7 (20.7)	31.9 (20.4)
Psychological health	61.8 (15.4)	75.1 (14.4)	68.4 (16.3)
Total	47.1 (11.9)	64.6 (13.7)	55.7 (15.4)
PedsQL (child) (n = 57)			
Physical health	35.6 (13.9)	51.9 (20.1)	43.6 (18.9)
Psychological health	66.0 (13.7)	70.1 (13.0)	68.0 (13.4)
Total	55.4 (11.3)	63.7 (11.3)	59.5 (12.0)
Muscle strength, kg, median (IQR) (n = 73)			
Elbow flexion	2.0 (1.4, 3.2)	9.5 (6.0, 15.3)	3.9 (2.0, 9.5)
Knee extension	0.7 (0.0, 1.6)	3.0 (2.0, 4.8)	1.7 (0.3, 3.3)
Knee flexion	2.1 (1.4, 3.0)	7.4 (5.1, 10.3)	3.4 (2.0, 7.4)
Fat-free mass index, kg/m ² (n = 40)	8.9 (1.9)	12.2 (1.9)	10.7 (2.5)
Fat-free mass index Z score (n = 40)	−5.1 (2.5)	−2.4 (1.5)	−3.6 (2.4)
Maximal CMAP amplitude (mV), median (IQR) (n = 50)	1.0 (0.7, 1.4)	6.0 (4.6, 8.2)	3.6 (1.0, 6.1)
Maximal CMAP AUC (mV × ms), median (IQR) (n = 47)	1.8 (0.9, 4.7)	16.9 (12.2, 25.4)	11.7 (1.9, 17.9)

Abbreviations: AUC = area under the (negative) curve; BiPAP = bilevel positive airway pressure; CHOP = Children's Hospital of Philadelphia; CMAP = compound motor action potential; FVC = forced vital capacity; GMFM = Gross Motor Function Measure; HFMS = Hammersmith Functional Motor Scale; HFMSE = Expanded Hammersmith Functional Motor Scale; IQR = interquartile range; PedsQL = Pediatric Quality of Life Inventory; SMA = spinal muscular atrophy.

^a Values are mean (SD) unless otherwise indicated.

Figure 1 Association between motor function (Expanded Hammersmith Functional Motor Scale [HFMSE] score) and maximal compound motor action potential (CMAP) area under the curve (AUC) (mV × msec) at baseline

gression model with SMA type as a covariate and linear and quadratic terms for time (continuous), the independent variable of interest. The mean changes at years 2 and 3 estimated from this model were of primary interest. A similar model was used for outcomes that were ascertained less regularly (CMAP amplitude and AUC, DXA fat-free mass, muscle strength, and quality of life), but the quadratic term for time was not necessary and results from that model were summarized as the average rate of change over time (slope). Unstructured covariance patterns were assumed for all models. The parameters of interest were estimated with restricted maximum likelihood using available data from all subjects, with missing data accommodated in an appropriate way under the missing at random assumption.

A description of additional statistical methods is presented online as e-text.

RESULTS Participants. We analyzed data from 79 SMA participants (41 SMA 2 and 38 SMA 3). The mean (SD) follow-up time was 25 (13) months. Eleven subjects withdrew from follow-up prior to October 1, 2009. The reasons included the time and travel burden associated with visits ($n = 5$), entry into a treatment trial elsewhere ($n = 3$), family illness ($n = 1$), and waning interest in an observational study ($n = 2$).

The mean participant age in years was 11.3 (9.4), 47% were male, and 75% were white. Motor and pulmonary function, quality of life, muscle strength, CMAP amplitude and AUC, DXA fat-free mass, and *SMN2* copy number were better in SMA 3 compared to SMA 2 participants at baseline (table 1).

Cross-sectional associations. Motor function, determined by HFMSE, was significantly ($p < 0.0001$) associated with CMAP amplitude ($r = 0.84$; figure e-1),

CMAP AUC ($r = 0.79$; figure 1), FFMI ($r = 0.64$; figure e-2), FFMI Z score ($r = 0.64$; figure e-3), elbow flexion strength ($r = 0.84$; figure e-4), knee extension strength ($r = 0.70$; figure e-5), and knee flexion strength ($r = 0.69$; figure e-6). The distribution of HFMSE scores was significantly different for those with an *SMN2* copy number of 3 (median = 16, interquartile range 6 to 30) compared to those with an *SMN2* copy number of 4 or 5 (median = 55, interquartile range 47 to 58, $p < 0.0001$). Nearly identical associations were observed when the GMFM was used as the measure of motor function (data not shown).

Longitudinal data. Motor function appeared to decline in a nonlinear fashion when evaluated beyond 12 months, a finding confirmed by each of the 3 motor function measures (table 2; figure 2; figure e-7). The mean change at 36 months was -1.71 for the HFMSE ($p = 0.01$), -4.39 for the GMFM ($p = 0.03$), and -1.26 for the HFMS ($p = 0.07$). In terms of major motor milestones, 2 SMA 2 subjects and 1 SMA 3 subject lost the ability to sit during the study, and 5 SMA 3 subjects lost the ability to walk.

Pulmonary function was measurable in 60 of the 79 participants (76%) at baseline (table 1). As with motor function, the average FVC (% predicted) decreased in a nonlinear fashion over time (figure 3) with mean declines of approximately 3% at both 24 and 36 months (table 2).

Muscle strength. Knee extension appeared to be the weakest of the 3 muscles tested and was the least

Table 2 Estimated mean changes in motor and pulmonary function outcomes at 2 and 3 years obtained from mixed-effects models with linear and quadratic terms for time^a

Variable	2 Years			3 Years		
	Mean	95% CI	p Value	Mean	95% CI	p Value
GMFM	-0.70	(-2.84, 1.44)	0.52	-4.39	(-8.38, -0.40)	0.03
HFMS	-0.34	(-1.26, 0.58)	0.46	-1.26	(-2.65, 0.12)	0.07
HFMSE	-0.54	(-1.45, 0.36)	0.24	-1.71	(-3.02, -0.39)	0.01
FVC (% predicted)	-3.14	(-6.01, -0.27)	0.03	-2.92	(-6.50, 0.66)	0.11

Abbreviations: CI = confidence interval; FVC = forced vital capacity; GMFM = Gross Motor Function Measure; HFMS = Hammersmith Functional Motor Scale (SMA 2 participants only); HFMSE = Expanded Hammersmith Functional Motor Scale.

^a Mean changes, confidence intervals, and p values are obtained from a mixed-effects linear regression model that includes spinal muscular atrophy type as a covariate and linear and quadratic terms for time (continuous); see text for details.

variable measure (table 1). No significant mean changes in knee extension or knee flexion strength were detected over time. There was, however, a significant mean increase in elbow flexion strength over time (0.53 kg/year, $p = 0.02$, table e-2).

Electrophysiologic measures. No significant change was observed in either CMAP amplitude or CMAP AUC, overall or by SMA type (table e-2).

Muscle imaging. Absolute fat-free mass estimated using DXA showed no significant change over time, but the mean annual rate of change in the fat-free mass Z score was significant (-0.18 units/year, $p = 0.01$, table e-2).

Quality of life. Children consistently rated their physical quality of life higher than their parents, particularly those with SMA 2 (table 1). There were no significant changes in quality of life over time (table e-2).

Laboratory results. We found a low mean serum creatinine and CO_2 in patients with SMA. Summary statistics on the baseline laboratory results are shown in table e-5. When looking at all participants who ever had an abnormal value on a given laboratory test, we found that for CO_2 37 participants had results below the range at least once, compared to 35 who did not. For creatinine, 47 participants had re-

Figure 2 Mean change in Expanded Hammersmith Functional Motor Scale (HFMSE) score over time estimated using a repeated measures analysis of covariance model with time treated as a categorical variable

Error bars indicate 1 SEM. Mean changes are plotted for spinal muscular atrophy (SMA) 2 and SMA 3 combined, SMA 2 only, and SMA 3 only.

Figure 3 Mean change in forced expiratory vital capacity (FVC) (percentage of predicted normal) over time estimated using a repeated measures analysis of covariance model with time treated as a categorical variable

Error bars indicate 1 SEM. Mean changes are plotted for spinal muscular atrophy (SMA) 2 and SMA 3 combined, SMA 2 only, and SMA 3 only.

sults below the normal range, compared to 13 who did not. For AST, 12 participants had a result above the normal range at least once, compared to 61 who did not. For ALT, 19 participants had a result above normal, compared to 54 who did not.

Subgroup analyses of change. Subjects with FVC $\geq 70\%$ of predicted normal at baseline had a greater mean decline in pulmonary function at both 24 months ($p = 0.02$) and 36 months ($p = 0.007$) than subjects with FVC $< 70\%$ (table e-3). We did not identify any other significant correlates of changes in motor and pulmonary function among subgroups defined by age, gender, SMA type, ambulatory status, baseline motor function, and baseline pulmonary function (table e-3).

All participants were homozygous for a deletion of *SMN1*. All SMA 2 subjects had 3 *SMN2* copies. SMA 3 participants had 3 to 5 *SMN2* copies (table 1). There were no significant associations between *SMN2* copy number and changes over time in either motor or pulmonary function (table e-3).

Additional analyses. There were no significant differences between those who completed ≤ 18 months of follow-up (17 SMA 2 and 13 SMA 3 subjects) and those who completed > 18 months (24 SMA 2 and 25 SMA 3 subjects) (table e-4). Eleven subjects prematurely withdrew from the study and 11 other subjects had scoliosis surgery during follow-up; the duration of follow-up varied among the other 57

subjects only because of staggered enrollment. There were no important subgroup differences in pulmonary or motor function changes over time when comparing shorter (≤ 18 months) with longer (> 18 months) follow-up.

Nineteen of the 79 participants (24%) used at least 1 medication during the follow-up period. These medications included albuterol ($n = 6$), carnitine ($n = 9$), creatine ($n = 5$), hydroxyurea ($n = 2$), oral steroids ($n = 1$), and valproic acid ($n = 7$). There were no significant differences when comparing medication-exposed to unexposed participants with respect to mean changes over time in motor function and pulmonary functions (table e-3).

DISCUSSION In a prospective, observational study of subjects with SMA 2 and SMA 3, we found slow declines in motor and pulmonary function over time as determined by established motor function measures (GMFM and HFMSE) and spirometry (FVC % predicted). No significant declines were observed in quality of life, CMAP, and lean body mass. The slow declines in motor and pulmonary function may result from greater demands being imposed on the existing motor units due to growth and development, an interpretation we tend to favor. Alternatively, there may be a slow progressive loss of motor neurons. The relatively unchanged CMAP is compatible with either hypothesis because it may reflect either a smaller number of motor neurons supplying

a larger territory or a stable motor neuron pool. However, compared to normal controls, the lean body mass in SMA decreased over time (FFMI Z score). Muscle strength of the elbow flexors showed a slight increase in strength over time, consistent with previous reports and likely reflecting interval growth and development.¹⁹ This observation also recalls the relative vulnerability of different motor neuron pools in SMA; some muscle groups are more affected while others are relatively spared.^{2,26} In contrast to our prior short-term clinical observation,¹¹ neither ambulatory status at baseline nor gender were significantly associated with longer-term pulmonary outcome although female subjects still tended to have a more pronounced decline in FVC at months 24 and 36 (table e-3). We did, however, observe a greater decline in pulmonary function at 24 and 36 months in those with FVC $\geq 70\%$ at baseline. This may be due to regression to the mean. We found an increased variability in motor function outcomes (HFMSE and GMFM) over time possibly due to the smaller number of observations beyond 24 months.

SMA 2 subjects were worse than SMA 3 subjects in terms of motor function, pulmonary function, muscle strength, CMAP, lean body mass, and quality of life. Knee extensors were weaker in SMA 2. These findings are consistent with previous reports.^{11,16,20,26}

CMAP amplitude and AUC, as well as DXA FFMI, all biological measures, were significantly associated with motor function, a clinical measure. On routine laboratory testing, the relatively low creatinine is likely related to decreased muscle mass, while the relatively low CO₂ could be the result of the catabolic state and subtle metabolic acidosis.

Limitations of our study include potential confounding factors such as medication exposure and scoliosis surgery during follow-up. These medications were not found to be effective in clinical trials, and we did not observe any apparent impact of these medications in our study. Therefore, we included data obtained during medication exposure. Our previous observations documented decreased motor function postoperatively. Therefore, we excluded data obtained after scoliosis surgery in 11 subjects. Another limitation is incomplete data from some participants who did not comply with all scheduled visits or tests. A missing at random assumption was made which cannot be formally tested using available data,²⁷ but we did not detect any important differences between those followed for longer vs shorter periods of time in terms of baseline characteristics (table e-4) or patterns of change over time. It remains possible, however, that the missing at random assumption may not hold, producing some degree of bias. Finally, the sample size may have limited our

power to detect potentially meaningful associations between baseline characteristics and changes in motor and pulmonary function over time.

We have shown that pulmonary and motor function outcomes measurably decline over observation periods exceeding 1 year in SMA 2 and SMA 3. This finding extends our previous observation of relative measurement stability over 12 months, and is consistent with a prior report of decreased pulmonary and motor function over a 4-year observation period.²⁸ We did not observe significant progression of muscle weakness as previously described.²⁹ This may reflect different populations (SMA 3 with onset after 3 years of age only), different measurement techniques (manual muscle testing of multiple muscles), or a longer observation period (up to 20 years) in the previous report. Our findings suggest that the observed functional declines are the net result of a failure to gain sufficient strength and muscle mass to meet the increasing demands associated with normal growth and development.

We hope our report will be helpful to clinicians who want to provide more substantive prognostic information to patients with SMA, and researchers as they plan clinical SMA trials, including sample size determination. Until there is a truly effective therapeutic intervention, we will not be able to determine the window of opportunity that exists for phenotypic rescue. We remain optimistic that intervention will be possible at any point in the life cycle. The indolent progression of the condition suggests that relatively long follow-up times with large patient numbers will be needed for SMA type 2 and 3 trials designed to measure a slowing in the disease progression. However, if the agent is capable of rescuing the clinical phenotype, a smaller sample size and relatively short follow-up may be sufficient. While our estimates of variability for several outcome measures are helpful for sample size determination, this determination also depends critically on the minimal clinically important group difference of interest to detect. More research is needed on the specification of this value in order to inform rational sample size calculations.

AUTHOR CONTRIBUTIONS

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P. Kaufmann reports no disclosures. M.P. McDermott serves as a scientific advisory board member for ISIS Pharmaceuticals, Biogen Idec, Inc, The ALS Association/FDA, and the Muscular Dystrophy Association; and is a consultant for the New York State Department of Health, Teva Pharmaceutical Industries, Ltd, Synosia, Inc, Smith and Nephew, Inc, and Impax Pharmaceuticals. He receives research support from the Michael J. Fox Foundation, Spinal Muscular Atrophy Foundation, Muscular Dystrophy Association, American Dental Association, and the NIH. Basil T. Darras is a consultant for ISIS Pharmaceuticals and Quest Diagnostics, received funding for travel from Athena Diagnostics, Honorarium for Virtual Grand Rounds, received research support from PTC Therapeutics, the Spinal Muscular Atrophy Foundation, Muscular Dystrophy As-

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