Natural history of MELAS associated with mitochondrial DNA m.3243A>G genotype

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ABSTRACT

Objective: To describe the natural history of clinical and laboratory features associated with the m.3243A>G mitochondrial DNA point mutation. Natural history data are needed to obtain prognostic information and for clinical trial planning.

Methods: We included 85 matrilineal relatives from 35 families with at least 2 visits in this prospective cohort study. Thirty-one were fully symptomatic with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), and 54 were carrier relatives. Evaluations included standardized questionnaires (medical history and daily living functioning), physical examination, neuropsychological testing, and a battery of imaging and laboratory tests. We evaluated changes in clinical and laboratory features over time and survival. Outcomes are reported over a follow-up period of up to 10.6 years (mean 3.8 ± 2.2 years for patients and 5.5 ± 3.0 for carrier relatives).

Results: Neurologic examination, neuropsychological testing, and daily living scores significantly declined in all patients with MELAS, whereas no significant deterioration occurred in carrier relatives. Cerebral MRI scores declined significantly in patients with MELAS. Magnetic resonance spectroscopy estimates of lactate in the lateral ventricles increased over time, and high lactate was associated with increased mortality. Symptom onset in childhood often was associated with worse outcome. Patients with MELAS had a greater death rate than carrier relatives.

Conclusions: Patients with MELAS carrying the m.3243A>G mutation show a measurable decline in clinical and imaging outcomes. It is hoped that these data will be helpful in anticipating the disease course and in planning clinical trials for MELAS. *Neurology*® 2011;77:1965-1971

GLOSSARY

i.u. = institutional units; **MELAS** = mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; **MRSI** = magnetic resonance spectroscopic imaging; **mtDNA** = mitochondrial DNA.

Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) is an often devastating multisystem syndrome characterized by progressive encephalopathy and stroke-like episodes, leading to disability and early death.¹ MELAS is often associated with the mitochondrial DNA (mtDNA) A-to-G transition at nucleotide 3243.²⁻⁶ Maternal relatives harboring the m.3243A>G mutation often ask about their prognosis, but data that would help predict the clinical course are sparse. The clinical manifestations of the m.3243A>G mutation are probably underrecognized, and the frequency of the mutation seems to be more prevalent than originally thought.⁷ The phenotypic variability is, at least in part, due to heteroplasmy, with varying proportions of mutant and wild-type mtDNA molecules in different tissues.⁸ The clinical features of the m.3243A>G mutation have thus far been described in several case series⁹⁻¹¹ or in retrospective studies based on clinical databases.^{12,13} Only one study to date has described 3-year follow-up data for m.3243A>G mutation carriers and found a significant

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increase in a global disease severity scale, as well as worsening on audiometry, electroencephalography, and echocardiography.¹⁴ We have previously described the phenotype associated with the m.3243A>G mutation, and we have described the decline over time in mutant mtDNA in leukocytes in a subset of our cohort.^{15,16} To provide patients and those caring for them with additional prognostic information, we describe outcomes from a prospective cohort study of 35 families with the m.3243A>G mutation followed for up to 10.6 years using a battery of clinical and laboratory measures.

METHODS **Study population and setting.** We included all m.3243A>G mutation carriers and their matrilineal relatives who participated in an observational study at Columbia University Medical Center in the city of New York. Participants (n = 85) were divided into 2 groups 1): fully symptomatic patients with MELAS (n = 31, termed patients) and 2) asymptomatic or symptomatic relatives who represent obligate carriers by pedigree analysis (n = 54, termed carrier relatives). A fully symptomatic patient with MELAS was defined as having evidence of focal brain involvement in addition to lactic acidosis; i.e., all subjects in this group had a history of stroke-like episodes or focal seizures. We included in our longitudinal analyses all 85 participants with at least 2 in-person visits. In the survival analysis only, we also included 24 additional patients with MELAS who had only 1 in-person visit, but whose survival status was known at some point during follow-up (n = 55).

To recruit subjects, we disseminated information on relevant web sites and at meetings of patient voluntary organizations.

Study design. Under a prospective cohort design, participants were invited for annual follow-up visits. For subjects who could not return in person, we attempted to obtain basic clinical information over the phone to assess survival status and onset of seizures or stroke. The first patients were enrolled in December 1995, and data were censored on January 31, 2008. At each visit, we conducted a comprehensive medical evaluation that included the following elements.

1. Columbia Neurological Score. A comprehensive physical and neurologic evaluation was conducted as described previously and scored within a range of 0 to 76, with 76 being normal.¹⁵
2. Medical history. We used a questionnaire developed by the investigators based on their clinical observations in patients with mitochondrial disorders to provide a survey of medical and neuropsychiatric problems associated with the m.3243A>G mutation as described previously.¹⁶
3. Neuropsychological assessment. Cognitive function was studied using a battery of tests designed to assess cognitive domains that may be involved in global cognitive function, language, abstract reasoning, visuospatial ability, visual memory, and verbal memory as described previously. Age-adjusted categorical scores were assigned (ranging from 0 to 3, with 0 representing normal performance and 3 representing profound impairment that precluded testing). A global neuropsychological score was derived from the mean score of the separate domain scores, with a range of 0 to 3.^{15,16}

4. Karnofsky score. We evaluated daily living functional abilities using an established scale that ranks, by 10-point intervals, 11 levels of functional ability, with a score of 100 for full functioning and a score of 0 for death.¹⁷
5. Laboratory tests. All laboratory tests were performed in the hospital laboratory and evaluated against established reference ranges. They included a basic metabolic panel, complete blood count, liver function testing, venous lactate and pyruvate, hemoglobin A1c, thyroid panel, and lipid panel.
6. Genetic analyses. The presence of the mtDNA m.3243A>G mutation was confirmed in DNA extracted from leukocytes according to standard procedures as described previously.¹⁸ DNA analyses in urine sediment were available only for a few visits and patients because this was not part of the original protocol.
7. MRI. Magnetic resonance images were acquired using standard methodology. The data were analyzed systematically by regions of interest as described previously. The overall MRI score, resulting from adding regional scores, ranged from 0 to 54, with 54 being normal. Sagittal T1-weighted MRI sequences are used as localizer images for multislice magnetic resonance spectroscopic imaging (MRSI) scans on each patient.¹⁹
8. Proton MRSI. Multislice ¹H-MRSI data were used to estimate lactate. The lactate peak is expressed relative to the mean square root of the background noise in the respective voxel and given in institutional units (i.u.). Lactate values were examined as the average value of all voxels placed over the brain tissue (gray and white matter) and as the value in a voxel placed over the fluid in the lateral ventricle.^{15,19}

Data analysis. The clinical baseline characteristics are presented using descriptive statistics. We calculated the average changes from baseline to years 1 and 4, respectively. Because most visits did not occur exactly at years 1 and 4 because of patients' scheduling preferences, we accepted all measurements obtained within a visit window of ± 2 months. For missed visits, we used the last measurement taken before the missed visit and the first measurement taken after the missed visit to linearly interpolate the missing value. In the case of missing follow-up data, we conservatively chose not to extrapolate. When the missing data were due to MELAS-related death, we imputed the worst value observed in the entire sample for that time point. This was to guard against the error of giving slow progressors with frequent opportunities for follow-up visits too much weight in the analyses and to guard against the error of ignoring death, a negative outcome, by just excluding data from deceased participants.

For the descriptive statistics, the number of participants with available data for a given variable is indicated in each table. Missing follow-up data occurred for several reasons including death, progressive disability, contraindications to MRI (e.g., cochlear implant or pacemaker placement), or difficulty providing a medical history in the absence of a caregiver who could provide this information. In addition to the 31 patients and 54 carrier relatives who had a least 2 in-person visits, we saw an additional 24 patients only once in person but were able to obtain some telephone follow-up information so that we could include these additional 24 patients in the Kaplan-Meier analysis, whereas more detailed longitudinal clinical and laboratory data are available for only 31 patients and 54 carrier relatives. We used a one-sample *t* test to determine whether any change from baseline was statistically significant. We estimated the survival function using a Kaplan-Meier analysis and estimated the median survival time as well as the lower boundary of its 95% confidence interval.

Table 1 Baseline characteristics and follow-up data for patients with MELAS and carrier relatives^a

	Gender	Age, y	m.3243A>G mutation, % mutant	Follow-up, y	Age at death, y
Patients with MELAS (n = 32)	48% M/52% F	30 ± 15 (4–61)	26 ± 21 (1–65)	3.8 ± 2.2 (0.25–9)	34.5 ± 19 (10–82) (n = 21)
Carrier relatives (n = 54)	28% M/72% F	38 ± 17 (4–76)	19 ± 16 (0–62)	5.5 ± 3 (0.7–10.5)	NA (deaths not related to MELAS; n = 3)

Abbreviations: MELAS = mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; NA = not applicable.

^a Data are mean ± SD (range).

RESULTS Subjects. The 31 patients with MELAS had a mean ± SD age of 30 ± 15 years at baseline (range 4–61 years), and 48% were male. The 54 carrier relatives had an age of 38 ± 17 years (range 4–76 years), and 28% were male. The proportion of mutant mtDNA in blood at baseline was 26 ± 21% (1%–65%) in patients with MELAS and 19 ± 16% (0%–62%) in carrier relatives (table 1).

Neurologic examination (Columbia Neurological Score). In patients with MELAS there was a significant decline in Columbia Neurological Scores ($p = 0.0014$) from baseline (57.6 ± 10) to 1 year (52.2 ± 4) and to all subsequent time points studied. No significant decline was seen in carrier relatives (table 2).

Karnofsky assessment of daily living functioning. Patients with MELAS as a group showed a decline in Karnofsky scores over time. In 48% (14 of 29) of patients with MELAS, this decline occurred within the first follow-up year with an average annual decline of -11.9 ± 9.4 . However, 10 patients with MELAS remained stable in terms of their Karnofsky scores, whereas another 5 patients showed improve-

ment. Forty-five of 54 carrier relatives showed stable Karnofsky scores after 1 year.

Neuropsychological global score. All patients with MELAS showed a progressive worsening in the neuropsychological global score over time. The decline from baseline (1.5 ± 1) is significant at 1 year ($p = 0.038$) and even more evident after 4 years (2.1 ± 0.8 , $p = 0.0017$) (normal = 0 and worst score = 3). No significant decline was seen in carrier relatives (table 2).

Mutation load. The mutation load in blood decreased slightly over time but did not change significantly over the period of observation (table 3). The mutation load in urine was measured only in a few patients and at a few visits but when available was consistently higher than that in blood and was higher in the patient group than in the carrier relative group. For the few available measurements, there were no significant changes in the proportion of mutant mtDNA over time (table 3).

Neuroimaging. The global MRI score did not significantly change in carriers. However, in patients, there was a significant decline in MRI scores (from 43.7 ± 8 at baseline to 37.7 ± 8 at year 4, $p = 0.021$).

Brain lactate. MRSI ventricular lactate increased in the MELAS patient group by approximately 30% over 4 years to 10.9 ± 3 i.u. (baseline 7.7 ± 3 , $p < 0.001$) compared with a 10% increase in carrier relatives to 5.6 ± 1 (baseline 4.9 ± 2). Whole-brain lactate increased to a lesser degree ($p < 0.001$) (table 3).

Other laboratory findings. Venous lactate did not change significantly in either the patient or carrier group. However, values were higher than normal at all time points in the MELAS patient group and in the upper range of normal limits for the carrier relative group. In addition, there was no significant change over time in the laboratory values measured, including creatinine, aspartate aminotransferase, alanine aminotransferase, triglycerides, triiodothyronine, and thyroxine.

Clinical symptoms and prognostic indicators. Ten of 78 carrier relatives became fully symptomatic with

Table 2 Clinical characteristics of patients with MELAS and carrier relatives

Year	No.	Patients with MELAS	No.	Carrier relatives
Columbia Neurological Score (maximum = 76, minimum = 0)				
Baseline	31	57.6 ± 10	54	69.6 ± 7
Year 1	29	52.2 ± 4	53	69.4 ± 3
Year 4	20	49.2 ± 10	32	68.9 ± 5
Karnofsky assessment of daily living (maximum = 100, minimum = 0)				
Baseline	31	66.1 ± 18	54	93.7 ± 10
Year 1	29	62.9 ± 12	53	92.8 ± 3
Year 4	21	52.3 ± 16	34	88.8 ± 9
Neuropsychological Global Score (0 = normal, 3 = not testable)				
Baseline	30	1.5 ± 1	52	0.22 ± 0.3
Year 1	28	1.6 ± 0.5	52	0.20 ± 0.1
Year 4	19	2.1 ± 0.8	31	0.28 ± 0.5

Abbreviation: MELAS = mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes.

Table 3 Laboratory characteristics of patients with MELAS and carrier relatives^a

Year	No.	Patients with MELAS	No.	Carrier relatives
Mutation (% mutant mtDNA in leukocytes)				
Baseline	30	26 ± 21	49	12 ± 16
Year 1	26	23 ± 9	48	11 ± 4
Year 4	17	25 ± 17	29	10 ± 6
Mutation (% mutant mtDNA in urine)				
Baseline	9	55.4 ± 28	13	44.3 ± 37
Year 1	7	46.9 ± 31	12	39.5 ± 36
Year 4	7	63.1 ± 28	6	38.4 ± 44
MRI (maximum = 54; minimum = 0)				
Baseline	27	43.7 ± 8	45	49.5 ± 4
Year 1	22	43.2 ± 3	26	48.8 ± 0.9
Year 4	8	37.7 ± 8	10	46.7 ± 9
MRSI ventricular lactate, i.u.				
Baseline	28	7.7 ± 3	46	4.9 ± 2
Year 1	25	8.0 ± 1	41	4.9 ± 0.8
Year 4	15	10.9 ± 3	26	5.6 ± 1
MRSI brain lactate, i.u.				
Baseline	27	5.8 ± 2	45	3.9 ± 0.1
Year 1	24	6.0 ± 0.9	40	4.2 ± 0.87
Year 4	14	7.0 ± 2	24	4.6 ± 1
Venous lactate, mmol/L				
Baseline	31	2.9 ± 2	50	1.9 ± 0.9
Year 1	28	3.0 ± 0.6	50	1.8 ± 1
Year 4	20	3.2 ± 1	30	1.9 ± 0.8

Abbreviations: i.u. = institutional units; MELAS = mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes; MRSI = magnetic resonance spectroscopic imaging; mtDNA = mitochondrial DNA.

^a Data are means ± SD.

MELAS during the study. School difficulties, motor or speech delay, small head circumference, or lower Karnofsky score at baseline were significantly associated with development of MELAS. In patients with MELAS, a history of early developmental problems was associated with earlier onset of MELAS, as defined by younger age at first seizure or stroke-like episode. The mean age at onset of MELAS in patients with a history of school difficulties was 13.7 years (SD 9.5) compared with 35.7 (15.9) years for those without. Similarly, for those with a history of growth failure, it was 21.9 (18.4) years vs 30.6 (14.7) years, for those with a history of motor delay it was 13.1 (14.0) years vs 31.6 (15.6) years, and for those with a history of speech delay it was 9.3 (4.5) years vs 29.5 (17.0) years.

Seizures occurred in 35 of 39 patients with MELAS (complex-partial ± generalization), with a mean age at first onset of 24.4 ± 14 years (range 0.5–53 years), and 33 of 39 subjects had strokes,

with a mean onset age of 26.9 ± 16 years (range 6–81 years).

Ninety percent of patients reported the onset of symptoms associated with mitochondrial disease before age 30 years. In those with disease onset before age 6 years, the most common presenting symptom was developmental delay, in those with onset between 6 and 10 years, it was muscle cramping or pain, and in those within the second and third decades, it was hearing loss.

The most common systemic symptoms were exercise intolerance, gastrointestinal problems (gastric discomfort and constipation), and hearing loss (table 4). The most common early developmental symptoms were school difficulties in 51% of patients and perinatal difficulties in 26% of carrier relatives.

Survival. During the course of follow-up, 24 study participants died. Three were in the carrier relative group, and their deaths were attributed to cancer (n = 2) and complications of surgery (n = 1).

Of the 55 fully symptomatic patients with MELAS, 21 died during follow-up. Death was attributed to MELAS-related medical complications in all patients. Death was considered unexpected and sudden in 3 patients. Two of these underwent autopsy that showed hypertrophic cardiomyopathy. The third patient had status epilepticus and was taking medication for Wolff-Parkinson-White syndrome. In addition to these cases of sudden death, a cardiac cause of death was reported in 1 patient with MELAS. In the remaining patients, neurologic events were prominent in the time leading up to death, including seizures, status epilepticus, and stroke-like episodes. Sepsis complicated the end-of-life period in 3 patients. Gastrointestinal pseudo-obstruction was noted in about one-third of patients during the end-of-life period.

The death rate was more than 17-fold higher in fully symptomatic patients than in carrier relatives. Given the observation period of 120 person-years of observation for patients and 294 person-years for carrier relatives, the death rate was 0.175 deaths in fully symptomatic patients vs 0.01 deaths in carrier relatives per person-year of observation.

The average observed age at death in the MELAS patient group was 34.5 ± 19 years (range 10.2–81.8 years). Of the deaths, 22% occurred in patients younger than 18 years.

The estimated overall median survival time based on 55 fully symptomatic patients was 16.9 years from onset of focal neurologic disease, i.e., seizures or stroke (figure). Among 18 patients who died during follow-up and had ventricular lactate levels recorded at baseline, 17 had ventricular lactate more than 5.41 i.u. (the median ventricular lactate level among

Table 4 Clinical symptoms in patients with MELAS and carrier relatives^a

	Proportion of individuals affected, %			
	Patients with MELAS (n = 31)		Carrier relatives (n = 54)	
	Present at baseline	New onset during study	Present at baseline	New onset during study
Systemic symptoms				
Exercise intolerance	87	6	33	15
Gastrointestinal disturbance	71	19	33	10
Loss of hearing	67	3	29	5
Growth failure	34	6	23	0
Diabetes	26	13	20	10
Hirsutism	25	0	7	0
Night blindness	22	22	11	10
Ptosis	16	25	11	5
Developmental symptoms				
School difficulties	51	NA	10	NA
Childhood motor delay	39	NA	17	NA
Perinatal difficulties	34	NA	26	NA
Special education	34	NA	2	NA
Speech delay	28	NA	8	NA

Abbreviations: MELAS = mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; NA = not applicable.

^a Shown are symptoms that occurred in >5% of individuals and that were present at baseline or that had their onset during the study. (Strokes and seizures are not included because they serve to distinguish the patients with MELAS from the carrier category.)

Figure Kaplan-Meier survival plot

The solid black line represents the 55 fully symptomatic participants with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes. The dotted red lines represent the 95% confidence intervals. Survival is plotted in years from disease onset, defined by first seizure or stroke.

all the patients and carriers at their baseline visits). For this high MRSI lactate group, the median survival from onset to death is 8.65 years, about one-half of the overall survival time. However, 2 carrier relatives had ventricular lactate levels slightly more than 5.4 i.u. but did not develop seizures or strokes.

DISCUSSION We have prospectively studied 85 subjects harboring the m.3243A>G mtDNA mutation using a comprehensive battery of medical history questionnaires, physical examination, neuropsychological testing, laboratory tests, and neuroimaging.

In patients with MELAS, there is a progressive decline in neuropsychological and neurologic findings, worsening of MRI abnormalities over time, and progressively increased CNS lactate levels as estimated by MRSI. These findings mirror the clinical experience of patients with MELAS becoming progressively and often rapidly disabled by cognitive and neurologic impairment over time. m.3243A>G mutation carriers frequently develop clinical symptoms associated with mitochondrial disease.

Our study has limitations. Collecting urine samples was not part of the prospective cohort study initially, so we did not collect a sufficient number of samples longitudinally to draw any prognostic conclusions. Future studies should include the longitudinal analysis of tissues other than blood when possible. Another limitation is related to incomplete retention of participants in this long-term observational study because many participants died or became increasingly disabled, making in-person study visits too burdensome. Additional loss to follow-up occurred because of a lack of perceived benefit. Participating in a long-term study without the benefit of an intervention requires an uncommon degree of altruism. We have used several strategies to counteract loss to follow-up including travel support for in-person visits and remote follow-up by telephone for participants who were unable to return.

Statistically, we have taken a conservative approach by including all available data (up to 10.6 years of follow-up) only in the Kaplan-Meier analysis. For all other analyses, we have limited our report to a 4-year follow-up period when a sufficient proportion of the population was still contributing data. We have only interpolated, not extrapolated, based on observed data and provided the number of observations for each time point. The Kaplan-Meier analysis itself has inherent limitations because of the large number of censored observations past follow-up year 10 makes the probability estimates less reliable. However, it is hoped that the survival probabilities together with the descriptive data on timing and

cause of death in our cohort will be useful to those caring for patients with MELAS.

Elevated CNS lactate as estimated by MRSI was associated with shorter survival, confirming our previous observation that elevated lactate is associated with increased disease severity.¹⁵ The presence of developmental delays and growth failure was associated with an earlier onset of MELAS. This finding suggests that a careful developmental history and MRSI studies can guide clinicians who are asked to give prognostic predictions.

Mutation load can vary among tissues (including oocytes) in an individual and within a family.^{20,21} Consistent with our previous report, there was a decline in mutant mtDNA in blood over time.¹⁶ In some cases, no mutation could be found in the blood of obligate carrier relatives. However, it should not be assumed that mutation load is negligible because sufficient mutation must be present to trigger a positive test and because the mutation load may be higher in other tissues. Genetic counselors should advise individuals of this possibility, especially in the context of prenatal and preconception counseling.

The mortality in our cohort was comparable to that reported previously.²² Regarding the presumed causes of death, however, previous studies had found a larger proportion of death attributable to cardiac causes.

To our knowledge, this is the largest prospective study of the natural history associated with the m.3243A>G mutation. Early-phase clinical trials are now under way for MELAS, and the advances in our understanding of the etiology and pathophysiology of MELAS may lead to additional clinical trials. It is hoped that our data will be helpful in the planning of clinical trials as well as in the counseling of mutation carriers.

AUTHOR CONTRIBUTIONS

Dr. Kaufmann: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, acquisition of data, statistical analysis, study supervision. K. Engelstad: analysis or interpretation of data, acquisition of data, study supervision. Dr. Wei: drafting/revising the manuscript, analysis or interpretation of data, statistical analysis. Dr. Kulikova: analysis or interpretation of data, contribution of vital reagents/tools/patients, acquisition of data. Dr. Oskoui: drafting/revising the manuscript, acquisition of data. Dr. Sproule: study concept or design, acquisition of data. V. Battista: drafting/revising the manuscript, acquisition of data, study supervision. D.Y. Koenigsberger: study concept or design, acquisition of data. Dr. Pascual: drafting/revising the manuscript, analysis or interpretation of data, acquisition of data. Dr. Shanske: study concept or design, acquisition of data, study supervision. Dr. Sano: drafting/revising the manuscript, study concept or design, analysis or interpretation of data. X. Mao: analysis or interpretation of data, acquisition of data, statistical analysis, study supervision. Dr. Hirano: drafting/revising the manuscript, analysis or interpretation of data, acquisition of data. Dr. Shungu: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, acquisition of data, statistical analysis, study supervision. Dr. Di Mauro: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, obtaining funding; Dr. De Vivo: draft-

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DISCLOSURE

Dr. Kaufmann conducted the work reported here while she was a full-time employee of Columbia University. This report is not related to her current work at NINDS. She has received consulting honoraria from the SMA Foundation; has received travel reimbursement to investigator meetings from the NIH, SMA Foundation, the MDA, Santhera Pharmaceuticals, and PTC Therapeutics, Inc.; serves on the editorial board of *Neuromuscular Disorders*; has received research support from Santhera Pharmaceuticals, Penwest Pharmaceuticals Co., PTC Therapeutics, Inc., the NIH/NINDS, the US Department of Defense, and the SMA Foundation; and her spouse serves on the editorial board of *Pediatric Pulmonology*. K. Engelstad, Dr. Wei, and Dr. Kulikova report no disclosures. Dr. Oskoui has received fellowship support from the Canadian Institutes of Health Research. Dr. Sproule has received funding for travel from UCB; receives/has received research support from PTC Therapeutics, Inc., the NIH/NINDS Neurological Science Academic Development Award, the Spinal Muscular Atrophy Foundation, and the American Academy of Neurology Foundation; holds stock and stock options in Pfizer Inc; and his spouse is an employee of Pfizer Inc. V. Battista and D.Y. Koenigsberger report no disclosures. Dr. Pascual serves as an Associate Editor for *Neuroscience Letters*. Dr. Shanske reports no disclosures. Dr. Sano serves on a scientific advisory board for Medivation, Inc.; serves as a consultant for Bayer Schering Pharma, Bristol-Meyers Squibb, Elan Corporation, Genentech, Inc., Medivation, Inc., Medpace Inc., Pfizer Inc, Takeda Pharmaceutical Company Limited, and United Biosource Corporation; and receives research support from the NIH (NIA/NCRR). X. Mao reports no disclosures. Dr. Hirano serves on the scientific advisory board for Telethon Italy; has received funding for travel from the American College of Medical Genetics; serves as Nerve and Muscle editor of *Current Neurology and Neuroscience Reports* and served on the editorial board of the *Journal of Neuromuscular Disorders*; has served on the speakers' bureau for Athena Diagnostics, Inc.; and has received research support from Santhera Pharmaceuticals, Edison Pharmaceuticals, Inc., the NIH, the Muscular Dystrophy Association USA, and the Marriott Mitochondrial Disorder Clinical Research Fund (MMDCRF). Dr. Shungu reports no disclosures. Dr. Di Mauro serves as Chairman, Scientific Advisory Committee, Telethon Italy; serves on the editorial boards of *Muscle and Nerve*, *Neuromuscular Disorders*, and *Acta Myologica*; serves as a consultant for Athena Diagnostics, Inc.; and receives research support from the NIH/NICHD and the Marriott Mitochondrial Disorder Clinical Research Fund (MMDCRF). Dr. De Vivo serves on scientific advisory boards for the SMA Foundation, the Colleen Giblin Foundation, the Pediatric Neurotransmitter Disease Association, the International Reye Syndrome Foundation, and the Will Foundation; serves as a consultant for Isis Pharmaceuticals, Inc.; receives royalties from publishing *The Molecular Basis and Genetic Basis of Neurologic and Psychiatric Disease*, 4th edition (Lippincott Williams & Wilkins, 2008); receives research support from the NIH (NINDS, NICHD), the US Department of Defense, the SMA Foundation, the Colleen Giblin Foundation, the Will Foundation, and the Pediatric Neurotransmitter Disease Association; and has served as an expert witness in a medico-legal case.

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