A Prospective Natural History Study of Mucopolysaccharidosis Type IIIA

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Objectives To characterize the clinical course of mucopolysaccharidosis type IIIA (MPS IIIA), and identify potential endpoints for future treatment trials.

Study design Children with a confirmed diagnosis of MPS IIIA, functioning above a developmental age of 1 year, were followed for up to 2 years. Cognitive status and brain atrophy were assessed by standardized tests and volumetric magnetic resonance imaging, respectively. Liver and spleen volumes and cerebrospinal fluid and urine biomarker levels were measured.

Results Twenty-five children, from 1.1 to 18.4 years old, were enrolled, and 24 followed for at least 12 months. 19 exhibited a rapidly progressing (RP) form of MPS IIIA, and 5, a more slowly progressing form. Children with RP plateaued in development by 30 months, followed by rapid regression after 40-50 months. In patients with RP, cognitive developmental quotients showed consistent steep declines associated with progressive cortical gray matter atrophy. Children with slowly progressing had a similar but more prolonged course. Liver and spleen volumes were approximately double normal size, and cerebrospinal fluid and urine heparin sulfate levels were elevated and relatively constant over time.

Conclusion Developmental quotient and cortical gray matter volume are sensitive markers of disease progression in MPS IIIA, and may have utility as clinical endpoints in treatment trials. For optimal outcomes, treatment may need to be instituted in children before the onset of steep cognitive decline and brain atrophy. *(J Pediatr 2016;170:278-87)*.

Trial registration ClinicalTrials.gov: NCT01047306.

This longitudinal observational study of the rare lysosomal storage disease mucopolysaccharidosis type IIIA (MPS IIIA; also known as Sanfilippo syndrome type A) was conducted to gather standardized data on the natural course of this neurodegenerative disease over 2 years, to assess brain function and structure, and to identify potential endpoints for future treatment trials.

MPS IIIA is a progressive lethal disease caused by deficiency of lysosomal N-sulphoglucosamine sulphohydrolase (SGSH), which degrades heparan sulfate (HS). This autosomal recessive disorder has an incidence of 0.27-1.89 per 100 000 births.[1] MPS IIIA is the least rare of 4 subtypes (types A, B, C, and D), each of which results from an enzyme deficiency in the catabolic pathway for HS. SGSH is localized to 17q25.3, with more than 100 mutations described[2,3] associated with either rapid or slower progression.[1,3] Developmental slowing begins in the second year of life, followed by cognitive decline and behavioral disturbances.[1,3] No therapy is known to modify disease.

A challenge in monitoring new treatments is the lack of knowledge about the rate and variability of clinical disease progression. Although retrospective studies[1,3-5] and studies of the behavioral phenotype[6,7] have been reported,

AEq	Age-equivalent score	MPS IH	Mucopolysaccharidosis type IH
BSID-III	Bayley Scales of Infant and Toddler Development, Third Edition	MPS IIIA	Mucopolysaccharidosis type IIIA
		MRI	Magnetic resonance imaging
		p-tau	phospho-tau
CSF	Cerebrospinal fluid	RP	Rapidly progressing
DQ	Developmental quotient	SGSH	N-sulphoglucosamine sulphohydrolase
FPSS	Four-Point Scoring System		
GAG	Glycosaminoglycan	SP	Slowly progressing
HS	Heparan sulfate	T-tau	Total tau
KABC-II	Kaufman Assessment Battery for Children, Second Edition	VABS-II	Vineland Adaptive Behavior Scales, Second Edition

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prospective studies examining the sequence and timing of cognitive decline and symptom emergence are lacking. We hypothesize that cognitive, imaging, and biomarker changes over a 2-year time period will be associated with disease stage and severity.

Methods

Twenty-five patients with MPS IIIA were recruited to this single-center study. Inclusion criteria were: (1) confirmed diagnosis of MPS IIIA; (2) calendar age ≥1 year; and (3) developmental age ≥1 year as assessed by the Vineland Adaptive Behavior Scales, Second Edition (VABS-II).[8] Exclusion criteria were: (1) history of hematopoietic cell transplantation; (2) presence of significant non–MPS IIIA-related central nervous system impairment; and (3) vision or hearing impairment sufficient to preclude developmental assessment. An Ethics Committee–approved informed consent form was signed by a parent/guardian of each subject.

Assessments were performed at baseline, 6 months, 1 year, and 2 years during a 3-day visit. Developmental testing was conducted on the day before any procedures requiring anesthesia.

Cerebrospinal fluid (CSF) opening pressure was measured via routine manometry at each lumbar puncture. SGSH mutational analysis was performed for each patient.

Adaptive behavior was measured by parent report using the VABS-II. A neurocognitive assessment was performed for each patient. One of 2 standard instruments, the Bayley Scales of Infant and Toddler Development, Third Edition (BSID-III)[9] or the Kaufman Assessment Battery for Children, Second Edition (KABC-II),[10] was used based on anticipated age and ability range. Age-equivalent scores (AEqs) were generated from published normative data,[9,10] and a developmental quotient (DQ) was derived by dividing the AEq by chronological age and then multiplying by 100. This accepted approach avoids the "floor" effects of standardized scores applied to severely cognitively impaired children.[11-13] Either the BSID-III or the KABC-II nonverbal scale was chosen for all of each child's visits according to a specific algorithm as described previously.[14]

The Four-Point Scoring System (FPSS),[4] an MPS III–specific parent-completed disability questionnaire that rates motor, expressive language, and cognitive function on a 3-point scale (normal, 3; beginning regression, 2; severe regression, 1; lost skill, 0), was administered. A total disability score was then calculated as the average of these 3 scores.

The Children's Sleep Habits Questionnaire was completed by the parents.[15] Based on our experience with patients with MPS IIIA, we added 3 additional items to the original questionnaire: disruptive behavior at night, dangerous behavior at night, and sleeping during the day.

Magnetic resonance imaging (MRI) of the head was performed on a 3-T Siemens Trio scanner (Siemens, Erlangen, Germany) with a 12-channel radiofrequency head coil. The protocol included 3-dimensional, T1-weighted, magnetization-prepared rapid acquisition with gradient-echo sequence. Volumetric analysis of gray and white matter and CSF ventricles was performed using FreeSurfer Image Analysis Suite version 5.1 (Martinos Center, Harvard University, Boston, Massachusetts).[16] The resulting segmentations were visually inspected for mislabeling of anatomy and manually adjusted if necessary.

Liver and spleen volumetric measurements were obtained by MRI, using the same instrument as used for the head MRI. Liver and spleen volumes were normalized to body surface area and weight, respectively, as described previously.[17,18]

Biomarker analyses included urinary glycosaminoglycan (GAG) concentration, HS in CSF, and total tau (T-tau) and phospho-tau (p-tau) proteins in CSF. The urinary GAG concentration was determined by a 1,9-dimethyl-methylene blue dye binding assay using the Blyscan assay kit (Biocolor Ltd, Carrickfergus, Northern Ireland, United Kingdom), and was reported relative to creatinine concentration (mg GAG/mmol creatinine). The level of total HS in CSF was determined by liquid chromatography–tandem mass spectrometry (LC-MS/MS). HS in the CSF was first extracted using an anion-exchange resin and then digested by a combination of enzymes, including heparinase I, II, and III. The resultant HS disaccharides were labeled with 12C-4-N-butylaniline by reductive amination and then analyzed by LC-MS/MS. The disaccharides were quantified based on a calibration curve generated using 6 commercially available disaccharide standards that are the most abundant in human CSF HS.[19] CSF levels of T-tau and p-tau proteins were measured by immunoassay.

Control patient data were provided by the University of Innsbruck. These patients and parents provided consent, and the samples were anonymized.

Descriptive statistics were summarized overall and by subgroups described below. Data are reported as mean ± SD for continuous covariates and as frequency and percentage for categorical variables. Cortex volumes at later visits could not be obtained for 5 patients owing to extreme atrophy and so were imputed based on each patient's previous trajectory to diminish bias from missing data. Sensitivity analyses for the imputation were conducted as well. The curved mean developmental trajectory was based on local polynomial smoothing.[20] Longitudinal associations were estimated with generalized estimating equations and robust variance estimates for 95% CIs and *P* values to account for the correlated nature of longitudinal measurements. All analyses were conducted using R version 3.0.1.[21]

Results

The cohort comprised 16 males and 9 females, with a median age of 4.8 years (range, 1.1-18.4 years) at enrollment between February 2010 and May 2011. One patient dropped out after the baseline visit for personal reasons; 24 patients were followed for 12 months, and 20 were followed for 24 months. Six sets of siblings were enrolled, including 1 set of dizygotic twins. The youngest 2 children were identified because of a diagnosis in an older sibling.

Table I. Genetic and demographic data for study subjects

Subject	Allele 1	Allele 2	Phenotypic association in literature (references)	Phenotype observed	Sex	Age at diagnosis, mo	Age at baseline, mo	Cognitive age equivalent, baseline/1 y/2 y*	DQ at baseline/ 1 y/2 y	FPSS total score at baseline/ 1 y/2 y
1	R245H	L12P	Severe/new[22,23]	RP	Female	42	79	14/10/NA	18/11/NA	2.3/1.3/1.3
2	N389S	N389S	New/new	RP	Male	34	38	21/16/14	55/31/21	2.3/2.0/2.0
4	R245H	R245H	Severe/severe[3,22,23]	RP	Male	24	28	25/27/30	89/66/53	3.0/2.3/1.7
5†	S66W	Q380R	Severe/severe[3,22,24]	RP	Male	27	29	23/23/23	79/53/40	1.7/2.0/2.0
6†	S66W	Q380R	Severe/severe[3,22,24]	RP	Male	28	29	22/23/18	76/53/31	1.7/2.0/2.0
7	S66W	R245H	Severe/severe[3,22,23]	RP	Male	65	95	8/8/8	8/7/7	1.0/1.0/1.0
9‡	R245H	R433Q	Severe/severe[22,23,25,26]	RP	Female	50	67	27/24/22	40/30/23	1.7/2.0/1.7
10‡	R245H	R433Q	Severe/severe[22,23,25,26]	RP	Male	23	40	27/27/27	68/52/40	2.0/2.0/2.0
11	R245H	R377H	Severe/severe[2,22-24]	RP	Male	52	88	12/12/13	14/12/11	2.0/1.7/1.7
12‡	S66W	M376R	Severe/new[3,22]	RP	Female	56	80	9/8/NA	11/9/NA	2.0/1.3/1.3
13‡	S66W	M376R	Severe/new[3,22]	RP	Male	19	43	25/17/9	58/30/13	2.0/2.0/1.3
15‡	E447K	int7 +1G>C	Severe/new[2,22]	RP	Male	48	51	14/7/14	27/11/18	1.7/1.3/1.3
16‡	E447K	int7 +1G>C	Severe/new[2,22]	RP	Male	9	13	11/16/16	85/62/41	3.0/2.3/2.0
19	1272del 11bp	1272del 11bp	Severe/severe[2,22]	RP	Female	36	37	25/28/27	68/57/44	2.3/2.0/2.0
21	c.197C>G	c.734G>A	Severe/severe[3,22,23]	RP	Female	29	50	25/15/10	50/24/14	1.7/2.0/1.7
23	c.197C>G	c.1080delC	Severe/severe[3,22,27,28]	RP	Male	29	45	35/28/NA	78/47/NA	1.7/2.0/2.3
24‡	R74C	R74C	Severe/severe[2,28,29]	RP	Female	18	22	20/27/31	91/82/67	3.0/2.7/2.3
25‡	R74C	R74C	Severe/severe[2,28,29]	RP	Male	52	57	17/13/13	30/19/16	1.7/1.7/1.7
22§	R74C	P293S	Severe/severe[2,28-30]	RP	Male	99	105	10/11/11	10/9/8	1.3/1.3/1.3
3	R245H	L59F	Severe/new[22,23]	SP	Male	77	81	46/50/55*	57/53/50	2.0/2.3/2.3
14	P293S	S298P	Severe/mild[3,29,30]	SP	Female	128	201	45/51/NA*	22/24/NA	2.3/2.0/2.0
17‡	1079delC	A311D	Severe/new[24,28]	SP	Male	127	132	28/27/27	21/19/17	1.7/2.0/2.0
18‡	1079delC	A311D	Severe/new[24,28]	SP	Female	150	155	61/70/69*	39/42/38	2.0/2.3/2.3
20	P293S	S298P	Severe/mild[3,29,30]	SP	Female	187	220	6/6/5	3/3/2	1.0/1.0/1.0

NA, not administered; patient untestable.
*6-mo cognitive data not shown.
†Dizygotic twins.
‡Sibling pairs.
§Patient diagnosed after age 6 y; diagnosed with autism at age 3 y, classified as severe/RP.

The patients were sorted post hoc into one of 2 groups, rapidly progressing (RP) or slowly progressing (SP), based on diagnosis before (RP) or after (SP) age 6 years, reportedly a reliable indicator of disease severity,[1] and/or known severe genotype. All analyses were conducted separately in these 2 groups. Six novel mutations were discovered (Table I). Thirteen of the 19 patients the RP group were either homozygous or compound heterozygous for known severe mutations; 5 patients were compound heterozygous for a known mutation associated with severe disease and 1 of 3 novel mutations, and 1 patient was homozygous for a novel mutation, N389S. All of the patients in the SP group were compound heterozygous for mutations; 2 patients had a known severe mutation associated with S298P, previously reported to confer a relatively mild phenotype.[29] Three patients in the SP group, including 1 pair of siblings, had a severe mutation in association with 1 of 2 new mutations, L59F or A311D. Thus, these 2 new mutations are associated with SP disease.

Neurocognitive Assessment

Two experienced psychometrists tested 21 patients (19 RP and 2 SP) with the BSID-III and 3 patients (all SP) with the KABC-II (Table I). Based on AEqs, the 3 youngest children, all aged <28 months, continued to acquire skills, as reflected in an upward trajectory (Figure 1, A). A slowing of development between 36 and 40 months and a loss of skills after 48 months was noted in the RP group. After 66 months, the cognitive decline appeared to reach a nadir. The 5 patients in the SP group displayed no consistent trends over time.

Based on the DQ, patients in the RP group showed a dramatic decline over time, estimated at −9.8 points/year (95% CI, −11.8 to −7.7; $P < .001$) (Figure 1, B). This was most notable up to age 6, where the decline was −14.6 points/year (95% CI, −17.5 to −11.8). In comparison, the DQ decline in the SP group was −3.7 points/year (95% CI, −5.0 to −2.4; $P = .012$).

Adaptive Behavior

AEqs on the VABS-II indicated continuing acquisition of skills in the RP group before age 50 months, with subsequent loss of skills (Figure 2). The scores of patients with SP were notably different, with no clear trends evident.

Disability

FPSS total disability scores were closely associated with DQ at the high and low ends, but showed a lack of association of DQ with FPSS scores in the middle of the range (Table I). The FPSS was able to correctly classify some of the least-impaired children (n = 4) with a score of 2.7 or 3.0 (mean DQ, 87; range, 82-91). The FPSS was also sensitive to the most-impaired children (score of 1 or 1.3) (mean DQ, 11; range, 2-31) but misclassified a few; however, the FPSS was insensitive in the mid-range, with many overlapping DQ scores at FPSS scores of 1.7 (mean DQ, 37; range, 11-79), 2 (mean DQ, 44; range, 11-79), and 2.3 (mean DQ, 55; range, 18-82).

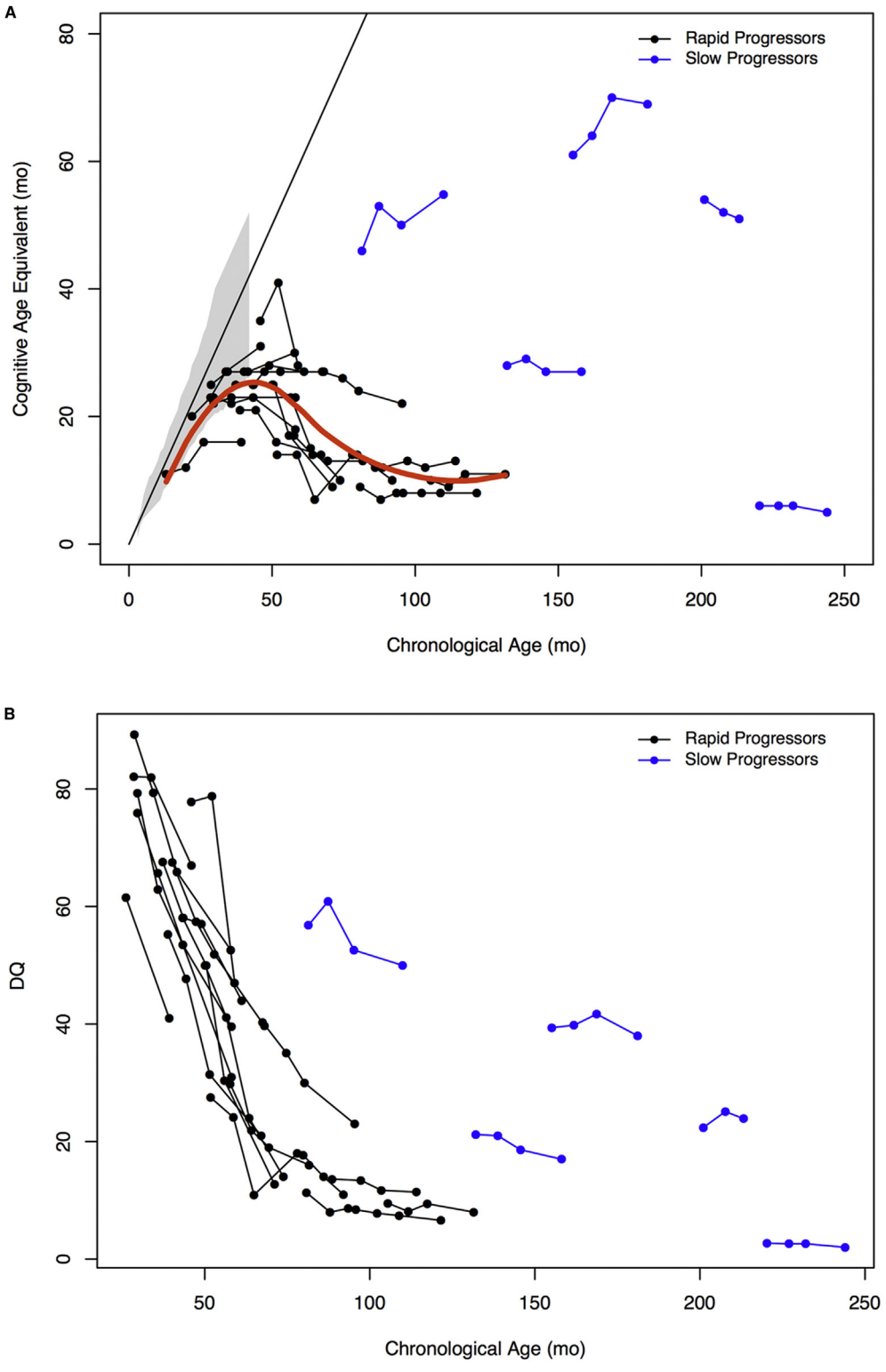

Figure 1. **A,** Trajectory of cognitive growth by age for the RP and SP groups compared with published normative growth data (*gray-shaded area*), with a ceiling at 42 months. The BSID-III was administered to all patients in the RP group and to 2 of 5 patients in the SP group. The KABC-II was administered to the other 3 patients in the SP group, who had a baseline age equivalent of >42 months. The *red line* indicates the growth trajectory for the RP group only. **B,** Change in DQ (100 × age equivalent/chronological age) by age for the RP and SP groups.

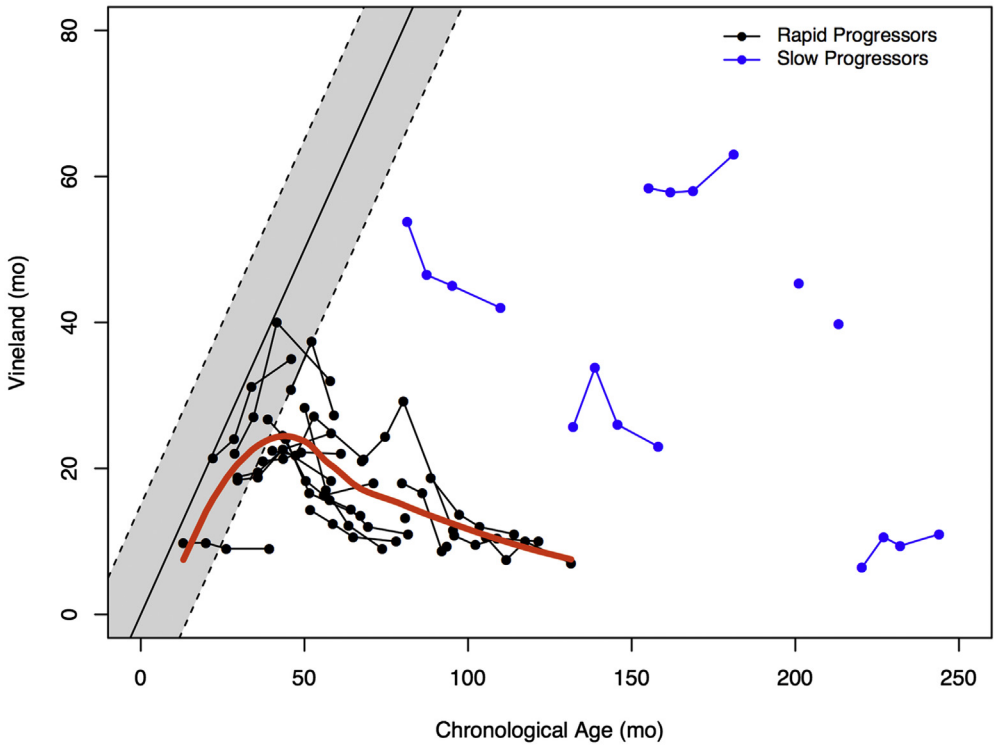

Figure 2. Developmental growth trajectory of mean AEqs in the RP and SP groups for composite score compared with normal published data for the VABS-II. The initial assessment was based on the survey interview form with parents, and subsequent assessments were based on the parent rating form.

Sleep Habits Questionnaire

Although many sleep problems were reported, no pattern or change over time was observed (data not shown). The data were insensitive for use in a clinical trial even as a secondary marker.

Imaging

Volumetric analysis was completed for all but 10 MRI scans. Two children, aged <24 months, exhibited a lack of gray matter and white matter differentiation, thought to reflect immaturity of myelination,[31] which precluded volumetric assessment at baseline and at 6 months for 1 child and at baseline for the other child. In 5 children, progressive atrophic changes resulted in analytic failure, even with manual adjustment, noted at the 24-month visit. For these patients, values were imputed from the slope of previous visits. In 1 child with a ventriculoperitoneal shunt, artifact precluded volumetric analysis at all 4 assessment points.

The trajectories of cortical gray matter volumes over time in the RP and SP groups are shown in **Figure 3**, A. A striking decline was seen in patients in the RP group, consistent at −41.1 mL/year (95% CI, −52.7 to −29.4; $P < .001$), compared with −26.4 mL/year (95% CI, −37.4 to −15.4; $P < .001$) in the SP group. The estimates for the RP group were not significantly different when imputed values were not used.

In the youngest patients, white matter volume exhibited an upward trajectory until age ~40 months, with subsequent declines evident in most children in the RP group. Patients in the SP group showed little or no change (**Figure 4**, A; available at www.jpeds.com).

Ventricular volumes increased in all age groups and in both the RP and SP groups, although with a steeper upward trajectory in the RP group (**Figure 5**; available at www.jpeds.com).

The association between a decline in DQ and gray matter volume over all visits is graphed in **Figure 3**, B. No association between DQ and white matter volume was observed (**Figure 4**, B).

Liver and spleen volumes were increased at baseline in most patients across age groups, with an approximate doubling of organ volume compared with expected values (**Figure 6**; available at www.jpeds.com).[17,18] No trends were apparent over the observation period (data not shown).

Biomarkers

Total urine GAG level remained elevated in all patients relative to control values (**Figure 7**, A). The apparent overall decline with increasing age confounded interpretation of lower levels in the patients in the SP group. Similarly, CSF HS levels were elevated in all patients compared with controls (**Table II**; available at www.jpeds.com), with

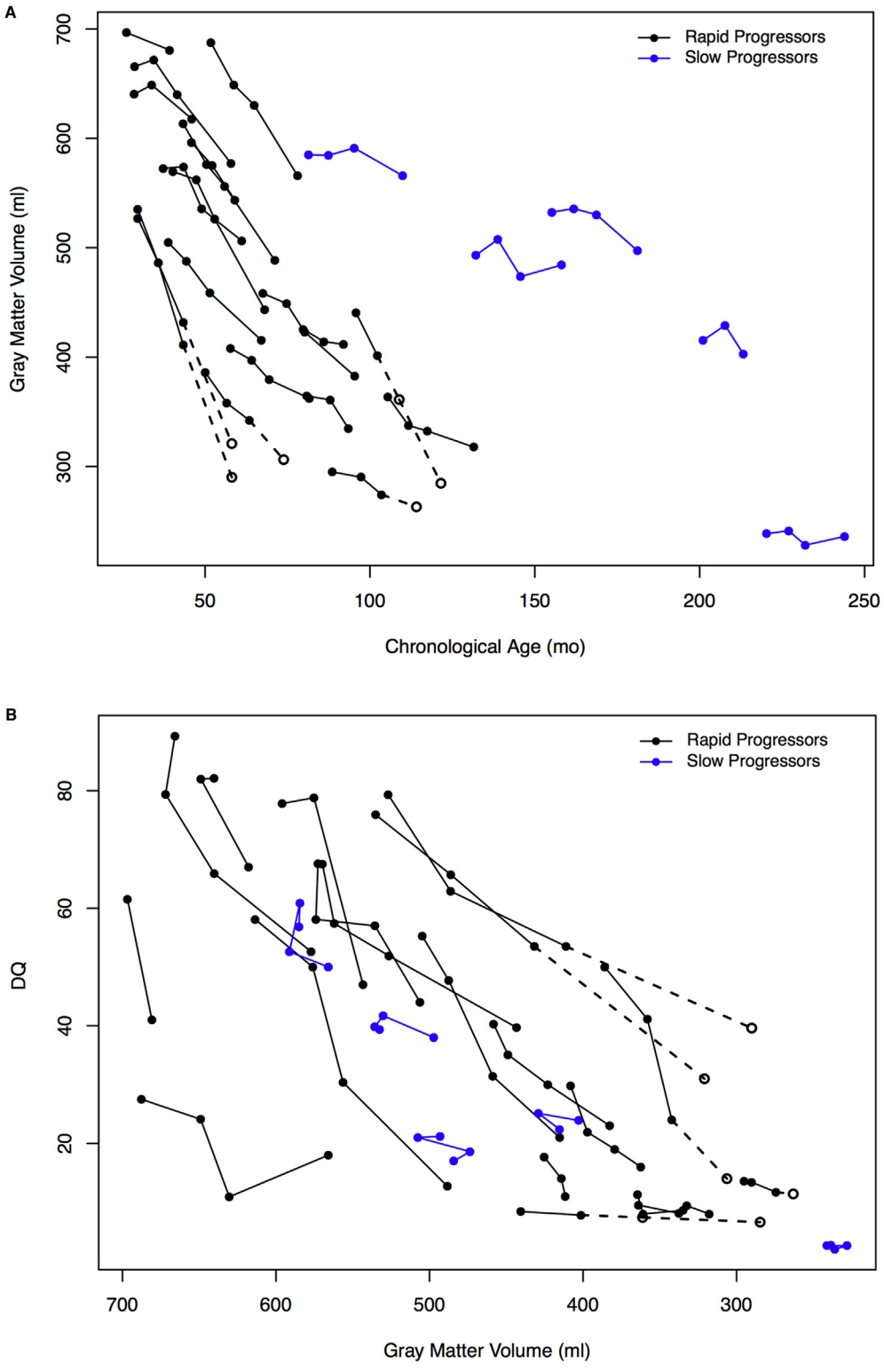

Figure 3. **A,** Change in gray matter volume for the RP and SP groups by age. **B,** Association of decline in DQ with gray matter volume for the RP and SP groups. *Open circles* indicate the imputed values, which are connected with *dotted lines* (values imputed from slope of previous visits) for 4 patients at the 24-month visit only and for 1 patient at both the 12-month and 24-month visits.

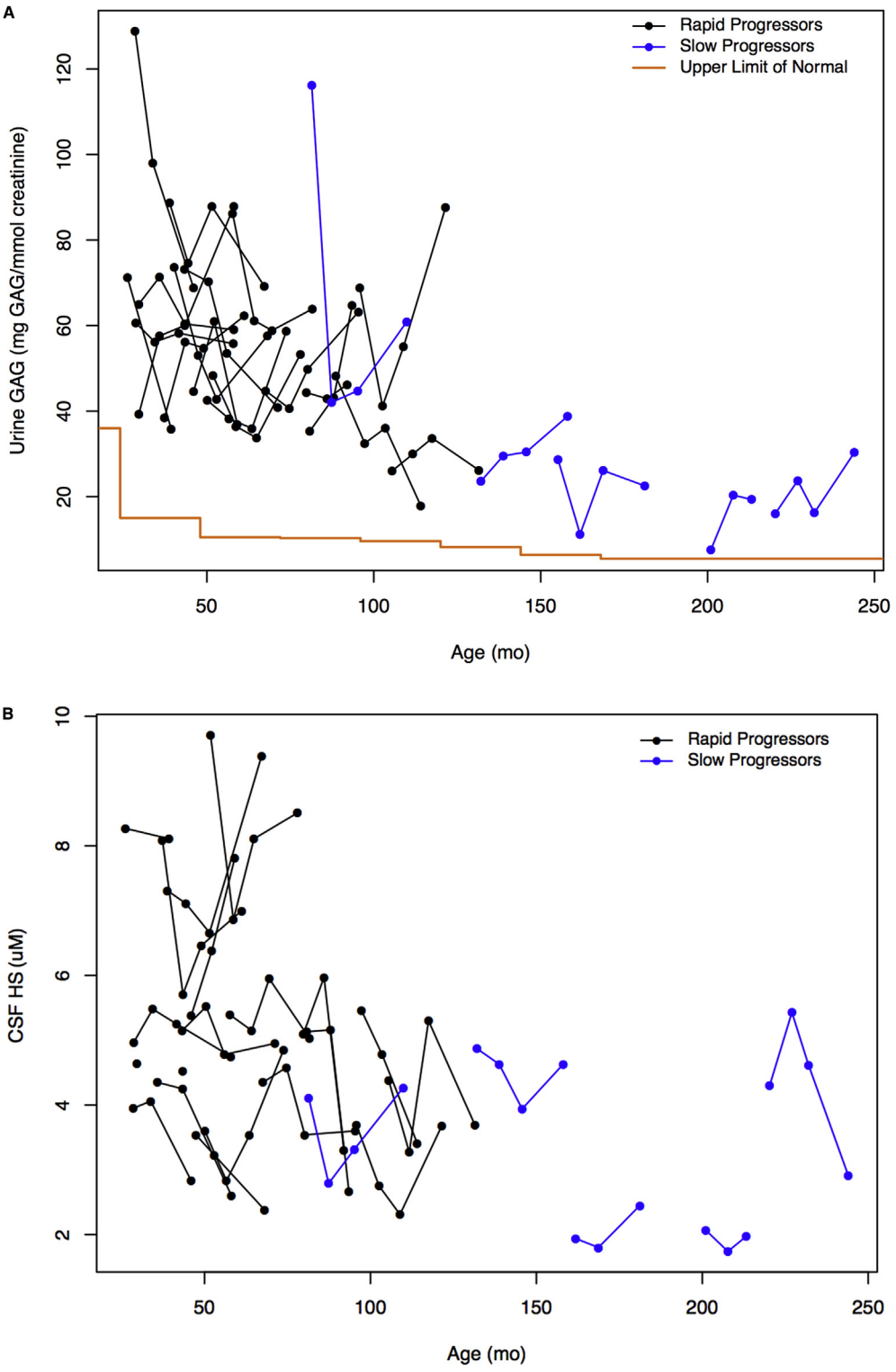

Figure 7. **A,** Change in urine GAGs by age for the RP and SP groups. Upper limit of normal values were taken from Naimy et al,[19] who provided values in 2-year increments from 0 to 14 years and older with the step at the end of each increment. **B,** Changes in CSF HS level by age for the RP and SP groups. Control values are listed in **Table II**.

elevated but overlapping values in the RP group compared with the SP group (**Figure 7**, B). Neither CSF HS nor urine GAG levels exhibited within-patient time-dependent increases associated with disease progression. CSF HS levels were strikingly similar in 4 of 6 of the sibling pairs (data not shown).

CSF T-tau and p-tau levels were modestly elevated relative to control values and appeared to decline with increasing age, so no relationship with disease progression was evident (**Figure 8**; available at www.jpeds.com).

CSF opening pressure was normal (<20 cm water) in all but 4 patients (data not shown). In these 4 patients, pressure was normal in 2 or 3 out of 4 lumbar punctures. The highest CSF opening pressure observed was 30 cm water, followed by 3 measurements between 20 and 25 cm.

Discussion

Our patients were sorted into 2 distinctive groups with differing natural histories of cognitive and brain changes and different disease cadences.[3] Classifying patients with MPS IIIA as RP or SP will enhance understanding of each patient's disease stage. In our cohort, the one exception to accurate classification by age of diagnosis[1] was a patient previously diagnosed with autism. This diagnosis, although frequently observed in MPS IIIA,[7] might have contributed to the delayed recognition of MPS IIIA in this patient, who was diagnosed at age 99 months. Although by age >72 months he should have been classified as SP, his known severe mutations classified him as RP. Both age of diagnosis and genetic criteria are necessary to accurately classify patients, but the latter will be limited by the frequent identification of new mutations.

A positive trajectory of cognitive development was evident in patients in the RP group who were aged <28 months at baseline. A ceiling of development at 42-48 months was observed in all but 1 patient in the RP group (**Figure 1**, A). That patient, aged 45 months at baseline, acquired cognitive skills over the first 6 months of the study, but lost significant function by the 12-month follow-up visit. This slowing of development between age 2 and 4 years in patients with MPS IIIA has been described previously,[5,32] and has been reported in other untreated mucopolysaccharidosis disorders as well.[33-35] Given the very rapid development in typically developing children between 2 and 4 years, evidence of slowing at this age should prompt further diagnostic studies.

Disease progression has been described as slowing of speech/language acquisition (phase 1), followed by a halt in cognitive development with emerging behavioral abnormalities (phase 2), and finally a loss of mobility progressing to a vegetative state (phase 3).[36] Median age of death is in the second decade, usually due to neurologic disease.[1,3,4] At baseline, 2 patients of our cohort were in phase 1 (both diagnosed early because of an affected sibling), and the other 23 patients were in phase 2. Although designed to track this progression, the FPSS[4] lacked sensitivity to disease progression during the second stage compared with the DQ.

When cognitive status was expressed as a DQ, all patients in the RP group exhibited marked declines over 1 or 2 years (**Figure 1**, B). Even in the youngest patients who had slower-than-normal development, the BSID-III (used in all patients in the RP group) had the requisite sensitivity for measuring disease progression within a time interval that might correspond to the duration of a therapeutic trial. The mean loss of 15 DQ points over 1 year in children aged <6 years is similar to findings reported in children with mucopolysaccharidosis type IH (MPS IH), a loss of 15-20 points per year at age 1-3 years.[32,33]

Changes in cognition were closely mirrored by quantitative neuroimaging findings. Brain atrophy and characteristic perivascular spaces have been reported clinically,[37,38] but based on quantitative volumetrics, the consistent declines in cortical gray matter volume starting at age ~30 months in patients in the RP group demonstrated a close association with rate of cognitive decline (**Figure 3**, B). This atrophy was accompanied by a compensatory increase in ventricular volumes (**Figure 5**). White matter volumes were less dramatically affected.

Volumetric analysis was not possible in the baseline MRI scans in the 2 youngest patients, owing to a lack of gray matter–white matter contrast resulting from early changes in gray matter water content and lack of myelination.[39] Gray matter growth to age 9 years with a small decline thereafter and continued growth in white matter through adolescence have been shown in typical development.[40,41] Thus, our findings of steep declines in gray matter volume represent gross pathological changes associated with MPS IIIA. The primacy of gray matter volume loss suggests that cognitive decline is associated with loss or damage to cortical neurons. The finding that a quantitative measure of brain structure is associated with neurocognitive assessment points to the validity of the latter as a clinical measure of disease progression in this patient population.

Liver and spleen volumes were approximately double the predicted normal volume for both the RP and SP phenotypes (**Figure 6**).[17,18] Although not usually diagnosed clinically, this finding suggests that volumetric analysis could be a useful treatment response marker.

Not surprisingly, CSF HS, CSF T-tau and p-tau, and urine GAG levels were abnormally elevated. Nonetheless, in contrast to cognitive and neuroimaging measures, there was no relationship between these disease-associated biomarkers and disease progression. Data from animal studies suggest that MPS III is a "tauopathy."[42,43]; however, although there were equivocal elevations in CSF T-tau and p-tau levels, these were not useful markers of disease progression. Our data suggest age-related declines in these markers, as may be observed physiologically in early life.[44]

The present study presents a composite picture of the progression of MPS IIIA over the course of childhood. Although the fact that individual children were followed only for up to 2 years may be considered a limitation of the study, the prospective design, together with the striking conformance of the individual patient trajectories to the overall pattern, suggest that this study represents an accurate picture of the long-term natural history of this disease.

In conclusion, we found that cognitive decline, as measured by DQ and cortical gray matter volume, is a sensitive marker of disease progression in MPS IIIA. The consistent declines in as little as 1 year suggest that these variables

could have utility as endpoints in clinical trials in patients with RP MPS IIIA. The finding that developmental arrest occurs around age 4 years suggests the importance of therapeutic interventions before this stage to confer the greatest benefit. Similar to MPS IH, for which early treatment with hematopoietic cell transplantation benefits cognition,[45] treatment should be instituted in children before the onset of steep cognitive decline and brain atrophy. Unlike in MPS IH, however, in MPS IIIA diagnosis is usually delayed beyond this period, owing to the lack of apparent physical features. Treatment with protein and gene replacement[46] is imminent, and once available, earlier diagnosis will be crucial. This leads inevitably to considerations of newborn screening and increased awareness by community practitioners of the possibility of neurometabolic disease in children with developmental delay. ■

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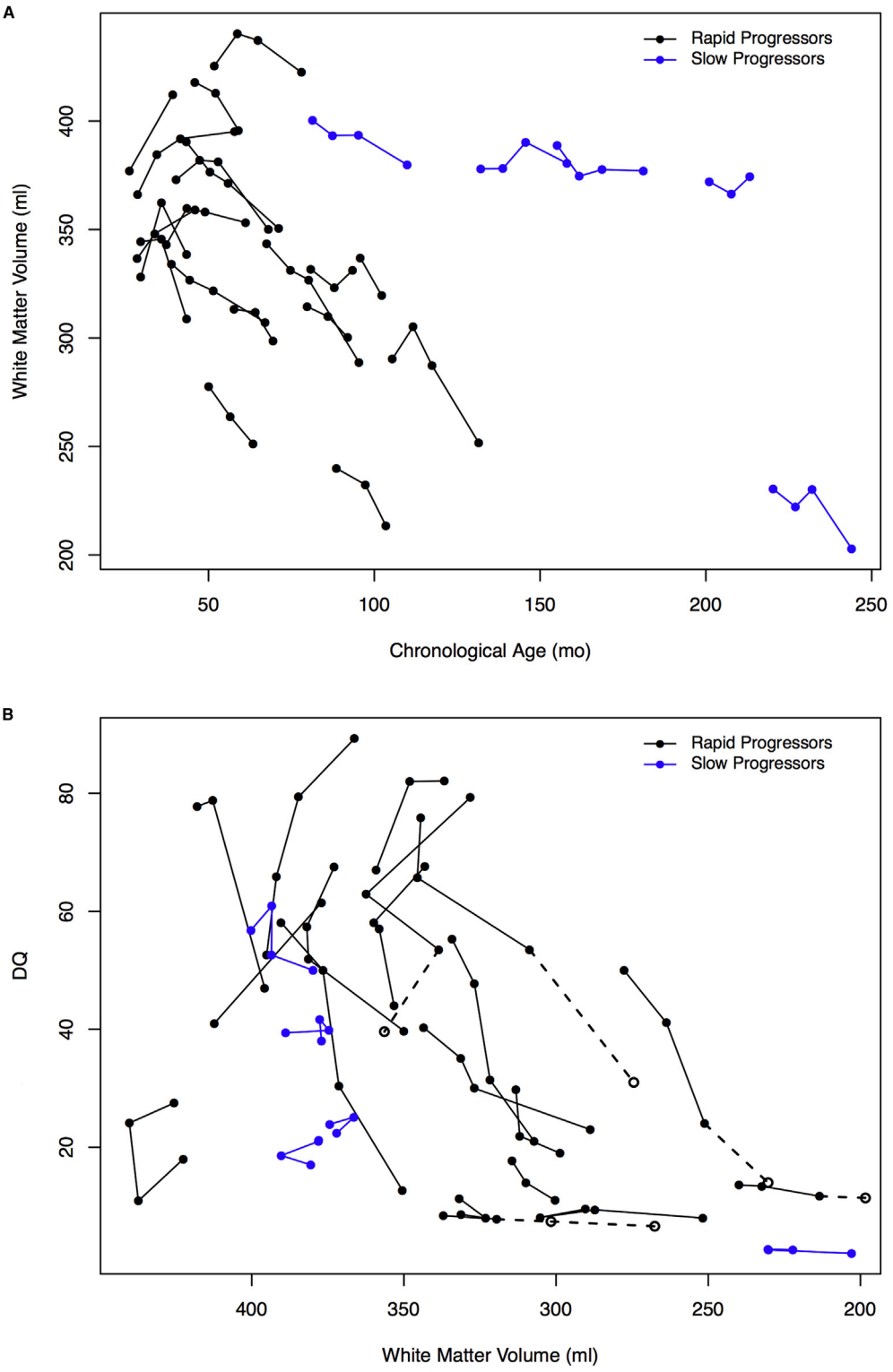

Figure 4. **A,** White matter volumes by age for the RP and SP groups. **B,** White matter volume by DQ for the RP and SP groups. Imputed values are for the same subjects as for gray matter volumes. All volumes were converted from cubic millimeters to milliliters.

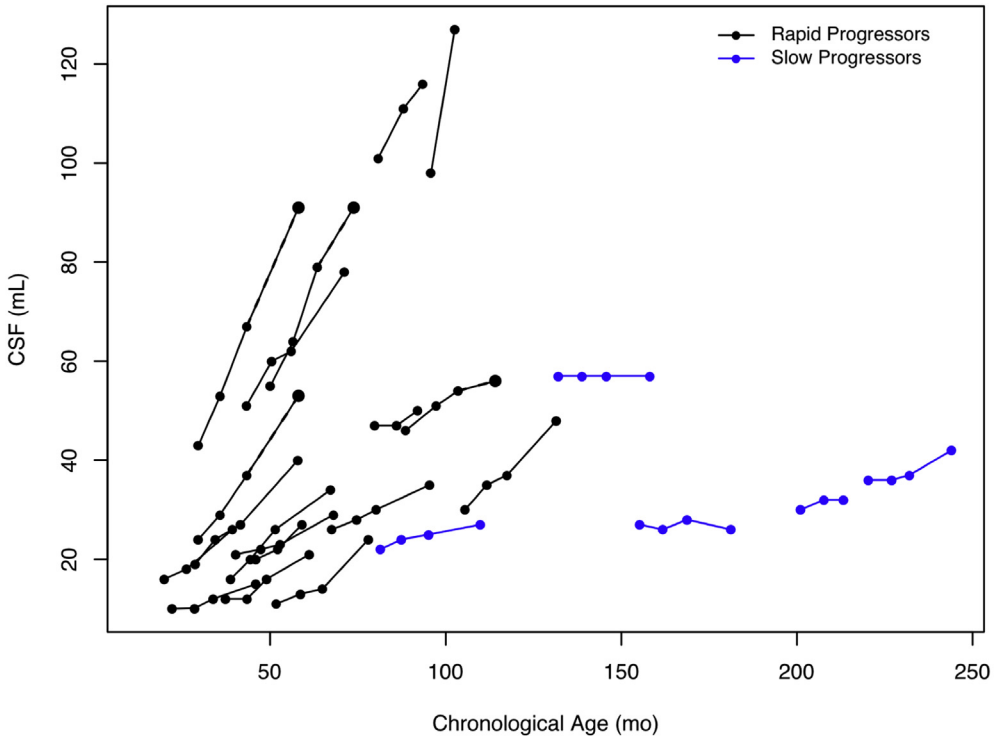

Figure 5. CSF volumes in ventricles by age for the RP and SP groups.

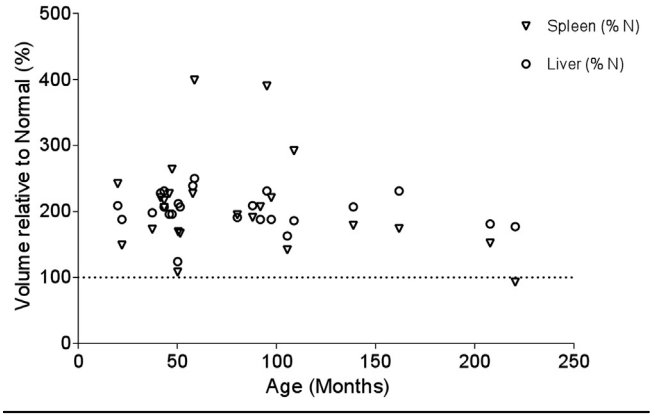

Figure 6. Liver and spleen size relative to normal controls by age based on percentage of normal and based solely on the first reading for each patient.

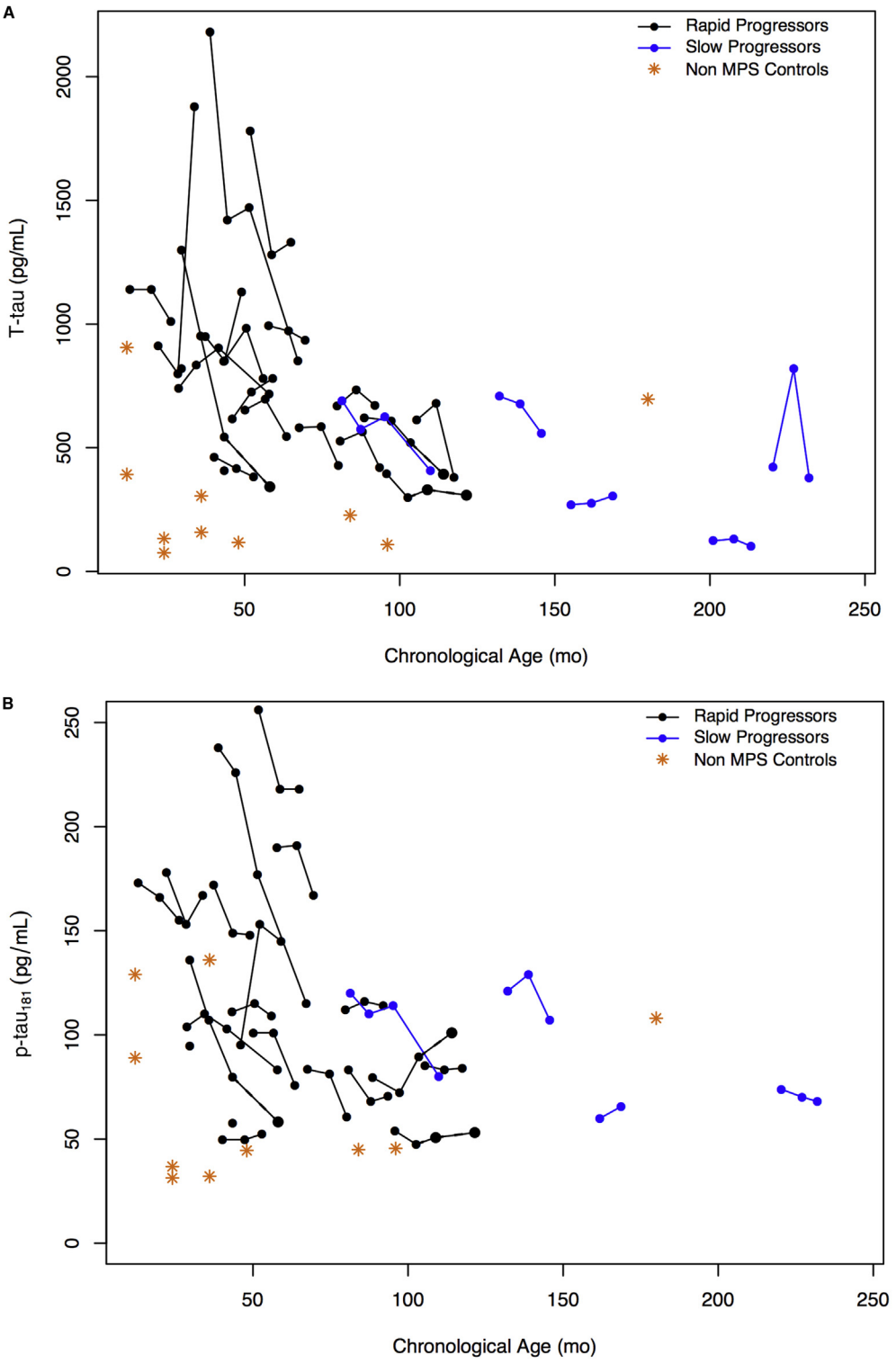

Figure 8. **A,** CSF T-tau protein levels and **B,** CSF T-tau and p-tau protein levels over time for the RP and SP groups compared with controls.

Table II. Control CSF HS levels

	Age group			
	0-27 d	1-23 mo	2-11 y	12-18 y
Number, total	24	52	41	31
Number below LLoQ (%)	0	0	13 (44%)	27 (87%)
Minimum, μM	0.229	0.248	<0.251	<0.300
Maximum, μM	0.463	0.648	0.443	0.426

LLoQ, lower limit of quantitation.

HS levels were measured by tandem mass spectrometry in deidentified CSF samples collected from children without MPS IIIA, obtained from the National Children's Medical Center bio-repository. Samples were assayed from the following age groups: birth to 27 days (24 samples), 1-23 months (52 samples), 2-11 years (41 samples, of which 44% were below the LLoQ), and 12-18 years (31 samples, of which 87% were below the LLoQ). The increasing frequency of samples with HS levels below the LLoQ with increasing age suggests age-related declines in CSF HS levels. The maximum level observed was 0.648 μM in a child in the 1-23 month age group.