Age-Dependent Reference Intervals for Cerebrospinal Fluid (CSF) and Urine Heparan Sulfate (HS) and Dermatan Sulfate (DS) and CSF Gangliosides

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Introduction

- Individuals with mucopolysaccharidoses (MPS) accumulate glycosaminoglycans (GAGs), such as heparan sulfate (HS) and dermatan sulfate (DS), in their cells as a result of insufficient or absent lysosomal enzyme activity.[1,2]
- Cerebrospinal fluid (CSF) and urine biomarkers of GAG accumulation and lysosome function are potential measures of treatment response to disease-modifying therapies for MPS.[1,2]
- Targeting normalization of biomarkers could be an important therapeutic strategy to increase the likelihood of clinical benefit.
- Urine GAGs have been previously reported to be age dependent;[3] however, age dependency of CSF HS, DS, GM2, or GM3 levels has not been evaluated in a pediatric population.
- To enable assessment of biomarker normalization, we sought to establish control reference intervals in a largely pediatric population for key biomarkers of MPS: CSF and urine HS and DS, and CSF gangliosides (GM2 and GM3).
- We also explored whether biomarker levels were dependent or independent of age.

Methods

- CSF samples were collected at the University of California, San Francisco (UCSF) from patients (aged < 1 month to 25 years) as part of diagnosis or periodic evaluation for the recurrence of cancer in the central nervous system, diagnosis of hydrocephalus or suspected meningitis.
- Structured chart abstractions were performed and CSF samples from unique individuals were included based on prespecified criteria. The following CSF samples were excluded:
  - from individuals with a confirmed diagnosis of a lysosomal storage disorder
  - with protein concentrations greater than two times the upper limit of normal (100 mg/dL)
  - with white blood cell count greater than 20 cells/mm^3
  - from individuals with severe and acute illness (e.g. long-term inpatient admission).
- Urine samples were obtained from healthy pediatric and adolescent donors (aged < 1 month to < 18 years) through the Denali biofluid donation program and from samples collected from pediatric donors at UCSF.
  - Urine samples with creatinine levels below the quantification limit (31.2 µg/mL) were excluded.
- CSF and urine HS (sum of D0A0, D0A6, D0S0 and D2S6) and DS (D0a4) levels were quantified using previously published and validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods;[4,5] CSF GM2 and GM3 levels were quantified using a qualified LC-MS/MS method.
  - Biomarker levels below the quantification limit of the assay were imputed as half of the lower limit of quantification.

Statistical analysis[6,7]

- Following evaluation of outliers, the effect of partitioning factors on biomarker levels was evaluated to determine whether separate subgroup reference intervals were required.
  - CSF sample partitioning factors: collection method (ventriculoperitoneal shunt or Ommaya reservoir vs lumbar puncture), low CSF glucose, genetic disorder, autism or cognitive impairment, neurological disorder, sex and age (categorical).
  - Urine sample partitioning factors: collection site, sex and age (categorical).
- The dependence of biomarker levels on age was then assessed.
- Estimate reference intervals were calculated using either a semiparametric model, generalized additive models for location scale and shape (GAMLSS; for age-dependent biomarkers)[8] or a normal distribution-based formula (for age-independent biomarkers with approximately Gaussian distributions).
- For reference intervals estimated from GAMLSS models, the upper limit was set as the 97.5th percentile of the reference biomarker distribution. For reference intervals estimated from Gaussian distribution-based models, the upper limit of the 95% reference interval was reported.
- For all urine sample analyses, reference intervals were calculated for biomarker levels normalized to creatinine.

Scan the QR code for full details of the statistical methods for each biomarker.

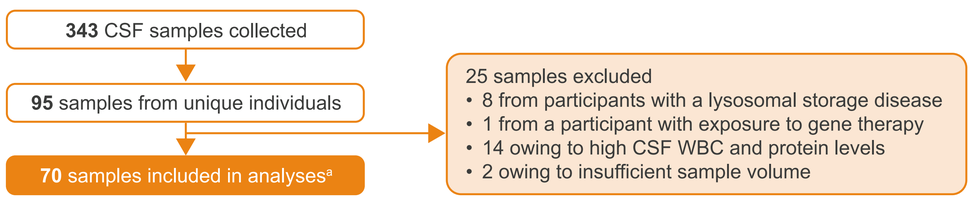
Patients with MPS II

- Baseline CSF and urine biomarker levels were assessed as part of an ongoing, open-label, 24-week, phase 1/2 study and its open-label extension (NCT04251026) in male individuals with MPS II aged ≤ 18 years (n = 47; clinical cutoff date: October 9, 2024).

Results

- Of the 343 CSF samples collected, 70 were included for assessment of GM2 and GM3 levels, and 67 for HS and DS levels (**Figure 1**).
- Overall, 149 urine samples were collected for analysis.
- Participant demographics are summarized in **Table 1** for CSF and urine sample donors included in the calculated reference intervals, and in **Table S1** for patients with MPS II.

Figure 1. Summary of CSF samples included in biomarker analyses



[a]70 samples included in CSF GM2 and GM3 analyses; 67 samples included in CSF HS and DS analyses owing to insufficient sample volume in an additional three samples.
CSF, cerebrospinal fluid; DS, dermatan sulfate; HS, heparan sulfate; WBC, white blood cell.

Table 1. Participant demographics

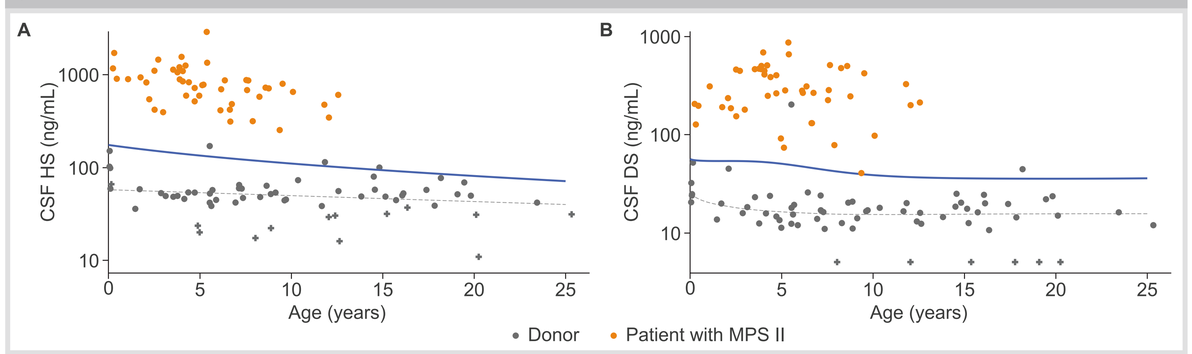
	CSF HS and DS analyses (n = 67)	CSF GM2 and GM3 analyses (n = 70)	Urine analyses (n = 149)
Age, years			
Mean (SD)	9.89 (6.41)	9.68 (6.51)	6.28 (5.03)
Median (min, max)	8.88 (0.06, 25.3)	8.77 (0.06, 25.3)	4.93 (0.05, 17.2)
Sex			
Female, n (%)	29 (43.3)	31 (44.3)	70 (47.0)
Male, n (%)	38 (56.7)	39 (55.7)	79 (53.0)

CSF, cerebrospinal fluid; DS, dermatan sulfate; HS, heparan sulfate; max, maximum; min, minimum; SD, standard deviation.

CSF biomarkers

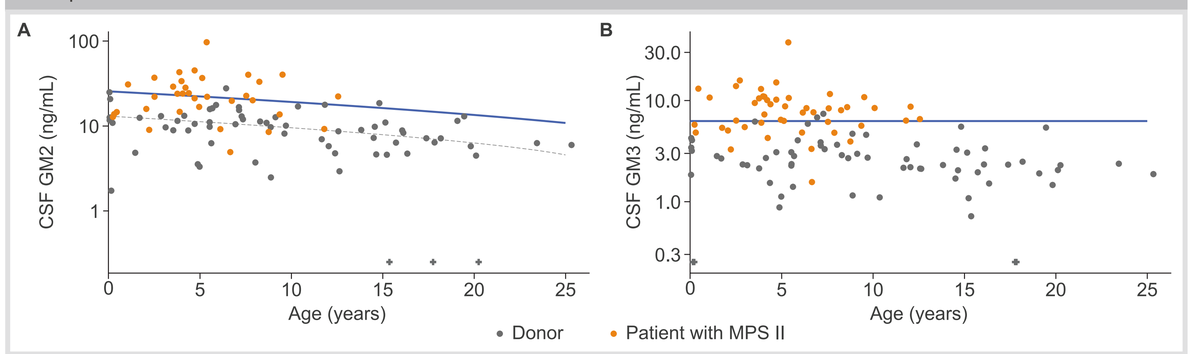
- Estimated reference intervals were calculated for CSF total HS and DS levels (**Figure 2**) and GM2 and GM3 levels (**Figure 3**).
  - Estimated reference intervals were also calculated for CSF HS disaccharides D0S0, D2S6, D0A0 and D0A6 (**Figure S1**; scan the QR code for supplemental files).
  - All CSF biomarker reference intervals, except for GM3, were age dependent.
- Patients with MPS II had elevated baseline CSF HS and DS levels that were 6.1- and 6.6-fold above the upper limit of the age-based reference interval, respectively (**Figure 2**).
- As previously observed, CSF ganglioside levels were elevated at baseline in many patients with MPS II.[1]

Figure 2. Estimated upper limit of the age-based reference interval for CSF HS (A) and DS (B), and baseline levels from patients with MPS II relative to these reference intervals



n = 67 starting reference samples. Gray lines show estimated age-specific 50th percentiles and blue lines show estimated upper limits of the age-based reference intervals (97.5th percentiles); + symbols represent samples with individual disaccharide concentrations that were below the quantification limit and imputed as half the lower limit of quantification.
CSF, cerebrospinal fluid; DS, dermatan sulfate; HS, heparan sulfate; MPS II, mucopolysaccharidoses type II.

Figure 3. Estimated upper limit of the age-based reference interval for CSF GM2 (A) and GM3 (B), and baseline levels from patients with MPS II relative to these reference intervals

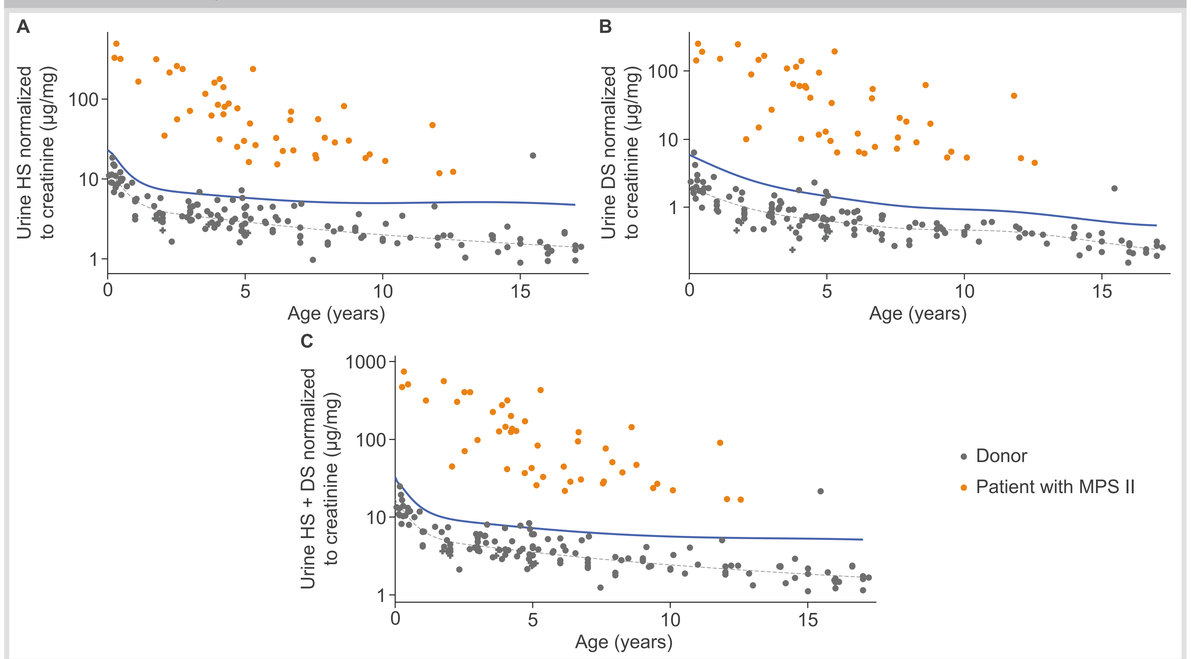


n = 70 starting reference samples. Gray lines show estimated age-specific 50th percentiles and blue lines show estimated upper limits of the age-based reference intervals (97.5th percentiles for CSF GM2 and upper limit of the 95% reference interval for CSF GM3); + symbols represent samples with values that were below the quantification limit and imputed as half the lower limit of quantification.
CSF, cerebrospinal fluid; MPS II, mucopolysaccharidoses type II.

Urine biomarkers

- Estimated reference intervals were calculated for urine HS, DS and the sum of HS and DS (HS + DS) levels (**Figure 4**).
  - Urine HS, DS and HS + DS reference intervals were age dependent.
- Patients with MPS II had elevated baseline urine HS, DS and HS + DS levels that were 13.0-, 30.4- and 17.0-fold above the upper limit of the age-based reference interval, respectively (**Figure 4**).

Figure 4. Estimated upper limit of the age-based reference interval for urine HS (A), DS (B) and HS + DS (C), and baseline levels from patients with MPS II relative to these reference intervals



n = 149 starting reference samples. Gray lines show estimated age-specific 50th percentiles and blue lines show estimated upper limits of the age-based reference intervals (97.5th percentiles); + symbols represent samples with individual disaccharide concentrations that were below the quantification limit and imputed as half the lower limit of quantification.
DS, dermatan sulfate; HS, heparan sulfate; HS + DS, sum of HS and DS; MPS II, mucopolysaccharidoses type II.

Differences between sexes

- There were no significant differences between sexes for any biomarkers analyzed.

Study limitations

- CSF samples were not collected from healthy pediatric populations owing to the invasive nature of the procedure; thus, stored clinical samples were utilized. CSF HS, DS, GM2 and GM3 are not known to be elevated in cancer, hydrocephalus or meningitis/encephalitis.
- Reference intervals were estimated based on small sample sizes using regression modeling.
- The exact reference interval values in this study may not be applicable to other studies that utilize different methodologies; however, our methodology provides a robust framework that can be adopted by others to facilitate the establishment of age-specific reference intervals.

Conclusions

- Reference intervals were established for CSF and urine biomarkers, which are elevated in patients with MPS II.
  - All biomarker reference intervals, except for CSF GM3, were age dependent. Data for CSF biomarkers were previously lacking.
  - All age-dependent biomarkers decreased with age.
- The process of establishing age-based reference intervals should be applied to other studies to assess the degree of biomarker normalization in clinical studies of MPS and other rare diseases, ultimately benefiting clinical research and patients with MPS.

ACKNOWLEDGMENTS

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DISCLOSURES

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Supplemental file

Supplemental methods

Cerebrospinal fluid (CSF) heparan sulfate (HS): n = 67 (n = 62 included for calculation of reference interval; n = 2 excluded as outliers; n = 3 excluded because evaluation of partitioning factors showed that there was a significant effect of collection method and that the ratio of the standard deviations between collection methods and between individuals exceeded the predefined threshold of 0.3; only samples collected by lumbar puncture were included). Given that there was a significant effect of age on CSF HS, generalized additive models for location scale and shape (GAMLSS) models were used to estimate continuous age-specific reference intervals.

CSF dermatan sulfate (DS): n = 67 (n = 66 included for calculation of reference interval; n = 1 excluded as an outlier; no partitioning factors met the pre-defined criteria to separate reference values). Given that there was a significant effect of age on CSF DS, GAMLSS models were used to estimate continuous age-specific reference intervals.

CSF GM2: n = 70 (n = 68 included for calculation of reference interval; n = 2 excluded as outliers; no partitioning factors had a significant effect except for categorical age). Given that there was a significant effect of age on CSF GM2, GAMLSS models were used to estimate continuous age-specific reference intervals.

CSF GM3: n = 70 (n = 68 included for calculation of reference interval; n = 2 excluded as outliers; no partitioning factors met the pre-defined criteria to separate reference values). Given that there was no significant effect of age on CSF GM3, normal distribution–based formulas were used to calculate the 95% reference intervals.

Urine HS: n = 149 (n = 146 included for calculation of reference interval; n = 3 samples had creatinine levels below the quantification limit. Evaluation of partitioning factors showed a significant effect of collection site and categorical age; however, following consideration of the clinical meaningfulness, partitioning by site was not applied and a single reference interval was established). Given that there was a significant effect of age on urine HS, GAMLSS models were used to estimate continuous age-specific reference intervals.

Urine DS: n = 149 (n = 146 included for calculation of reference interval; n = 3 samples had creatinine levels below the quantification limit. No partitioning factors had a significant effect except for categorical age). Given that there was a significant effect of age on urine DS, GAMLSS models were used to estimate continuous age-specific reference intervals.

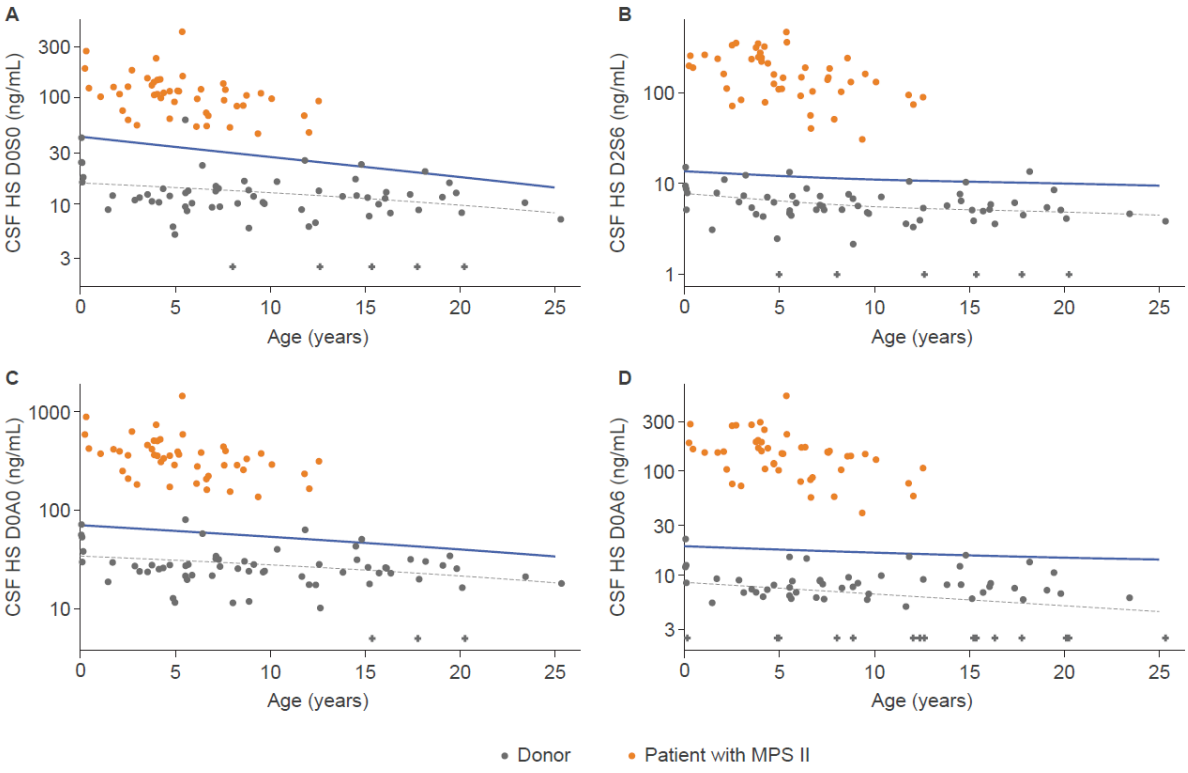
Urine HS + DS: n = 149 (n = 146 included for calculation of reference interval; n = 3 samples had creatinine levels below the quantification limit. Evaluation of partitioning factors showed a significant effect of collection site and categorical age; however, following consideration of the clinical meaningfulness, partitioning by site was not applied and a single reference interval was established). Given that there was a significant effect of age on urine HS + DS, GAMLSS models were used to estimate continuous age-specific reference intervals.

Table S1. Demographics of patients with MPS II

		Patients with MPS II n = 47
Age, years	Mean (SD)	5.5 (3.1)
	Median (min, max)	5.0 (0.3, 12.6)
Age group, n (%)	< 4 years	14 (29.8)
	≥ 4 years	33 (70.2)
Sex, n (%)	Male	47 (100)
	Female	0
Race, n (%)	Asian	4 (8.5)
	Black/African American	4 (8.5)
	White	27 (57.4)
	Other	1 (2.1)
	More than one race	3 (6.4)
	Not reported/unknown	8 (17.0)
Ethnicity, n (%)	Hispanic/Latino	7 (14.9)
	Not Hispanic/Latino	38 (80.9)
	Not reported	2 (4.3)
MPS II phenotype, n (%)	Neuronopathic	44 (93.6)
	Non-neuronopathic	3 (6.4)
DQ	Mean (SD)	55.1 (28.7)
Variant type, n (%)	Missense/synonymous	22 (46.8)
	Large deletion/rearrangement/ stop/ frameshift shift/splice	25 (53.2)
Prior therapy group, n (%)	ERT (idursulfase IV)[a]	29 (61.7)
	ERT naive[b]	14 (29.8
	HSCT/gene therapy[c]	4 (8.5)

Percentages were calculated based on the number of non-missing values. [a]ERT prior therapy group includes participants who were on a stable idursulfase IV treatment for at least 4 months prior and had not received HSCT or gene therapy. [b]One participant with idursulfase treatment < 4 months was considered under the ERT naive group. [c]Participants who received HSCT or gene therapy were included regardless of their prior ERT status. DQ, developmental quotient; ERT, enzyme replacement therapy; HSCT, hematopoietic stem cell transplantation; IV, intravenous; max, maximum; min, minimum; MPS II, mucopolysaccharidosis type II; SD, standard deviation.

Figure S1. Estimated upper limit of the age-based reference interval for CSF HS D0S0 (A), HS D2S6 (B), HS D0A0 (C) and HS D0A6 (D)



Gray lines show the estimated age-specific 50th percentiles and blue lines show the estimated upper limits of the age-based reference intervals (97.5th percentiles). + symbols represent samples with individual disaccharide concentrations that were below the quantification limit and imputed as half the lower limit of quantification. A, C and D: CSF HS D0S0, D0A0 and D0A6, respectively, n = 67 starting reference samples (n = 65 included for calculation of reference interval; n = 2 excluded as outliers; no partitioning factors met the pre-define criteria to separate reference values). B: CSF HS D2S6, n = 67 starting reference samples (no outliers were excluded; no partitioning factors had a significant effect). A–D: Given that there was a significant effect of age on CSF HS D0S0, D2S6, D0A0 and D0A6, GAMLSS models were used to estimate continuous age-specific reference intervals. CSF, cerebrospinal fluid; GAMLSS, generalized additive model for location, scale and shape; HS, heparan sulfate.