

Title of the manuscript:

Normalization of glycosaminoglycan-derived disaccharides detected by tandem mass spectrometry assay for the diagnosis of mucopolysaccharidosis.

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Supplemental Table 1. Quantitative analysis of GAG-derived disaccharides by LC-MS/MS assay in confirmed MPS I patients

MPS I Patient No.	Age (years)	Severity	Receiving ERT (Years)	CS-normalized			Creatinine [†] (mg/dL)	Creatinine-normalized		
				DS* (<0.49 µg/mL)	HS* (<0.56 µg/mL)	KS* (<7.81 µg/mL)		DS [†] (<1.56 µg/mg cre.)	HS [†] (<3.1 µg/mg cre.)	KS [†] (<13.13 µg/mg cre.)
1	5.2	I (H/S)	4.1	22.13	2.51	1.78	24.36	11.93	6.69	25.66
2	15.3	I (H/S)	4.6	5.45	0.35	0.23	75.58	1.89	1.32	6.42
3	17.5	I (S)	No ERT	75.72	0.15	0.00	126.55	2.99	0.25	0.00
4	2.0	NBS [#]	No ERT	4.35	9.19	0.00	53.45	2.01	6.59	0.00
5	0.7	NBS [#]	No ERT	4.10	3.38	0.17	21.75	5.24	9.26	16.71
6	1.4	NBS [#]	No ERT	118.46	33.67	0.53	14.76	40.13	11.41	0.18
7	1.4	NBS [#]	No ERT	159.87	28.91	0.33	6.66	120.02	21.70	0.25
8	26.6	I (S)	4.4	18.98	2.14	1.70	47.37	2.00	0.23	0.18
9	38.6	I (S)	3.7	8.48	0.63	0.25	325.61	0.13	0.01	0.00
10	37.2	I (S)	4.7	5.19	0.45	0.04	133.18	0.19	1.12	0.00
11	21.5	I (S)	6.5	13.34	25.09	0.06	127.59	1.03	1.10	1.57
12	2.6	I (H/S)	0.4	68.84	31.26	0.31	98.32	3.43	3.62	4.47
13	16.7	I (S)	No ERT	209.12	40.91	0.39	134.92	2.79	1.22	1.89

*using CS as a basis for GAG-derived disaccharide calculation;

[†]using mg creatinine as a basis for GAG-derived disaccharide calculation;

[#]confirmed MPS I infants from Newborn Screening Centers in Taiwan;

The reference cut-off values were determined in a range of +3SD

The *p* values (Students T-test) of DS and HS were 0.027 and 0.049, respectively (*p*<0.05) when comparing the CS-normalized method to the creatinine-normalized method.

Supplementary Table 2. Quantitative analysis of GAG-derived disaccharides by LC-MS/MS assay in confirmed MPS II patients

MPS II Patient No.	Ages (years)	Severity	Receiving ERT (Years)	CS-normalized			Creatinine [†] (mg/dL)	Creatinine-normalized		
				DS* (<0.49 µg/mL)	HS* (<0.56 µg/mL)	KS* (<7.81 µg/mL)		DS [†] (<1.56 µg/mg cre.)	HS [†] (<3.1 µg/mg cre.)	KS [†] (<13.13 µg/mg cre.)
1	13.6	Mild	8.3	3.56	11.44	6.89	79.81	1.07	3.26	11.72
2	2.7	Severe	No ERT	15.17	346.04	1.43	45.20	2.29	24.34	9.33
3	0.9	NBS [#]	No ERT	21.21	12.06	6.47	15.38	6.90	3.92	2.10
4	22.7	Mild	6.8	21.90	2.09	1.64	255.52	0.43	0.04	0.03
5	24.1	Mild	10.9	2.95	1.94	5.38	238.01	0.20	0.27	3.13
6	4.7	Mild	No ERT	36.66	234.38	2.82	42.64	4.40	22.51	8.42
7	0.4	NBS [#]	No ERT	18.92	103.44	1.27	4.36	35.55	177.75	95.07
8	29.0	IM	6.9	9.71	23.89	2.07	91.08	1.31	4.22	5.27
9	28.0	Mild	10.5	2.58	7.60	0.15	302.58	0.21	0.72	0.74
10	15.0	Mild	10.5	3.00	2.15	0.18	122.32	0.66	1.52	3.63
11	0.3	NBS [#]	No ERT	21.40	30.93	0.09	10.63	16.09	115.71	36.50
12	10.5	Mild	3.7	1.81	2.66	0.22	92.93	0.64	1.30	4.56
13	25.3	IM	7.1	170.76	61.39	0.00	19.80	11.87	33.08	1.96
14	14.0	IM	9.6	8.35	77.72	0.56	95.64	0.90	7.42	2.32
15	2.7	IM	No ERT	41.27	167.46	0.18	14.01	14.06	36.40	26.05
16	6.4	Severe	No ERT	159.47	152.00	0.35	42.20	6.20	24.76	4.24
17	0.2	NBS [#]	No ERT	11.93	175.36	0.08	7.83	15.01	42.35	29.31
18	4.5	Mild	Unknown	3.14	27.44	0.14	46.16	1.97	8.24	13.65

19	41.1	Mild	8.1	7.59	28.45	0.28	148.80	0.88	1.48	2.18
20	6.5	Severe	2.7	33.85	9.68	0.05	58.86	3.80	8.47	2.99
21	0.9	NBS [#]	No ERT	1.60	6.45	0.03	96.12	0.64	5.36	1.46
22	3.4	NBS [#]	No ERT	2.87	4.20	0.02	47.16	1.74	9.40	3.54
23	10.2	Severe	No ERT	91.93	384.03	0.16	35.72	8.9	17.08	6.47
24	3.4	Severe	1.1	13.27	47.98	0.20	26.32	6.91	22.42	18.2
25	9.5	Severe	No ERT	65.11	332.58	0.08	17.10	19.33	80.7	15.73

*using CS as a basis for GAG-derived disaccharide calculation;

†using mg creatinine as a basis for GAG-derived disaccharide calculation

[#]confirmed MPS II infants from Newborn Screening Centers in Taiwan;

The reference cut-off values were determined in a range of +3SD.

The *p* values (Students T-test) of DS, HS, and KS were 0.010, 0.017, and 0.011, respectively (*p*<0.05) when comparing the CS-normalized method to the creatinine-normalized method.

Supplemental Table 3. Quantitative analysis of GAG-derived disaccharides by LC-MS/MS assay in confirmed MPS IIIB patients

MPS IIIB Patient No.	Ages (Years)	Severity	Receiving ERT (Years)	CS-normalized			Creatinine† (mg/dL)	Creatinine-normalized		
				DS* (<0.49 µg/mL)	HS* (<0.56 µg/mL)	KS* (<7.81 µg/mL)		DS† (<1.56 µg/mg cre.)	HS† (<3.1 µg/mg cre.)	KS† (<13.13 µg/mg cre.)
1	9.5	N/A	No ERT	0.16	22.16	0.01	99.07	0.13	4.87	0.73
2	4.3	N/A	0.42 (clinical trial)	0.00	92.42	3.59	93.44	0.00	4.95	0.19
3	6.3	N/A	No ERT	0.00	48.59	2.68	92.63	0.00	2.62	0.14
4	5.3	N/A	No ERT	0.73	179.54	0.57	75.67	0.05	11.86	0.04
5	5.7	N/A	No ERT	0.30	74.30	0.36	25.8	0.06	14.40	0.07
6	8.3	N/A	No ERT	0.08	20.88	0.03	49.03	0.18	15.40	3.14
7	5.4	N/A	No ERT	0.11	8.75	0.03	94.01	0.13	4.6	1.55
8	4.3	N/A	No ERT	0.25	38.43	1.03	312.32	0.09	2.23	3.14
9	5.3	N/A	No ERT	0.18	271.56	0.02	26.31	0.47	24.71	3.61
10	13.2	N/A	No ERT	0.44	500.43	0.52	288.07	0.23	1.96	0.79
11	22.7	N/A	No ERT	0.40	94.16	0.69	100.28	0.27	1.27	1.04

*using CS as a basis for GAG-derived disaccharide calculation;

†using mg creatinine as a basis for GAG-derived disaccharide calculation

The reference cut-off values were determined in a range of +3SD.

The *p* value (Students T-test) of HS was 0.027 (*p*<0.05) when comparing the CS-normalized method to the creatinine-normalized method.

Supplementary Table 4. Quantitative analysis of GAG-derived disaccharides by LC-MS/MS assay in confirmed MPS IVA patients

MPS IVA Patient No.	Ages (Years)	Severity	Receiving ERT(Years)	CS-normalized			Creatinine† (mg/dL)	Creatinine-normalized		
				DS* (<0.49 µg/mL)	HS* (<0.56 µg/mL)	KS* (<7.81 µg/mL)		DS† (<1.56 µg/mg cre.)	HS† (<3.1 µg/mg cre.)	KS† (<13.13 µg/mg cre.)
1	16.8	N/A	3.1	0.42	0.03	35.92	94.51	0.02	0.00	1.90
2	7.1	N/A	No ERT	0.04	0.09	11.04	163.20	0.00	0.17	10.11
3	32.1	N/A	No ERT	0.00	0.08	53.24	231.65	0.00	0.00	1.15
4	23.1	N/A	6.3	0.24	0.08	12.09	96.85	0.01	0.00	0.62
5	20.0	N/A	6.1	0.49	0.08	60.15	36.55	0.07	0.01	8.23
6	28.2	N/A	No ERT	0.24	0.01	102.16	27.54	0.04	0.00	18.55
7	1.9	N/A	No ERT	0.04	0.00	103.65	39.70	0.00	0.01	109.57
8	8.6	N/A	5.8	0.14	0.01	37.11	151.02	0.00	0.17	25.36
9	28.2	N/A	2.6	0.82	0.24	23.37	39.10	2.12	0.68	15.60
10	7.3	N/A	2.6	0.57	0.12	42.26	81.89	1.25	0.56	27.00
11	0.7	N/A	0.6	0.69	0.09	14.95	87.02	0.45	0.33	39.93
12	30.6	N/A	0.6	0.43	0.10	32.24	153.04	0.28	0.19	27.39

*using CS as a basis for GAG-derived disaccharide calculation;

†using mg creatinine as a basis for GAG-derived disaccharide calculation

The reference cut-off values were determined in a range of +3SD.

The *p* value (Students T-test) of KS was 0.017 (*p*<0.05) when comparing the CS-normalized method to the creatinine-normalized method.

Supplementary Table 5. Quantitative analysis of GAG-derived disaccharides by LC-MS/MS assay in confirmed MPS VI patients

MPS VI Patient No.	Ages (Years)	Severity	Receiving ERT (Years)	CS-normalized			Creatinine [†] (mg/dL)	Creatinine-normalized		
				DS* (<0.49 µg/mL)	HS* (<0.56 µg/mL)	KS* (<7.81 µg/mL)		DS [†] (<1.56 µg/mg cre.)	HS [†] (<3.1 µg/mg cre.)	KS [†] (<13.13 µg/mg cre.)
1	12.5	N/A	6.6	34.43	0.01	1.78	94.18	1.83	0.00	0.09
2	25.6	N/A	13.9	4.17	0.01	0.86	24.92	2.57	0.12	13.90
3	19.3	N/A	11.8	6.67	0.01	0.12	14.70	7.76	1.00	21.67
4	19.3	N/A	11.8	12.16	0.09	0.74	220.43	0.74	0.16	2.70
5	28.4	N/A	11.6	16.30	0.40	0.25	36.7	2.56	0.88	3.69
6	22.0	N/A	12.7	25.90	0.31	0.12	18.97	5.98	0.15	2.87

*using CS as a basis for GAG-derived disaccharide calculation;

[†]using mg creatinine as a basis for GAG-derived disaccharide calculation.

The reference cut-off values were determined in a range of +3SD.

The *p* value (Students T-test) of DS was 0.049 (*p*<0.05) when comparing the CS-normalized method to the creatinine-normalized method.