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Shunji Tomatsu, MD, PhD
Project Title: Non-invasive functional assessment and pathogenesis of Morquio A

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Accomplishments

The long-term goal of this research proposal is to better understand the natural history of MPS IVA in terms of clinical progression and objective outcome measures. The unique aspect of our research is the establishment of innovative non-invasive assessments to evaluate the clinical severity, disease stage, and therapeutic efficacy of skeletal dysplasia. Our central hypothesis is that these assessments, when combined into a disease severity scoring system, can effectively characterize MPS IVA and assess the benefits of therapeutic interventions.

Aim 1. Conduct multidisciplinary and detailed non-invasive assessments in Morquio A patients. Our working hypothesis is that longitudinal non-invasive tests demonstrate accurate prognostic and predictive evaluation of skeletal dysplasia and can be carried out across a broad patient group, including patients who are young, wheelchair-bound, and post-surgical.

Aim 2. Identify potential surrogate biomarkers of skeletal dysplasia. Our working hypothesis is that biochemical biomarkers correlate with disease stage, clinical severity, and non-invasive clinical assessments. These biomarkers should, therefore, serve as prognostic and/or predictive biomarkers of therapeutic response.

1. **Major activities**

Aims 1 and 2.

a. **Team structure:**

As outlined in the grant proposal, we assembled a dedicated and expert study team on May 1, 2021 (Fig. 1), demonstrating our unwavering commitment to the project.

-Monthly-based videoconferences between PI and investigators or board members from 6 Morquio A families have been set up since May 2021 to discuss the recruitment process, research plan, summary, and report on the project.

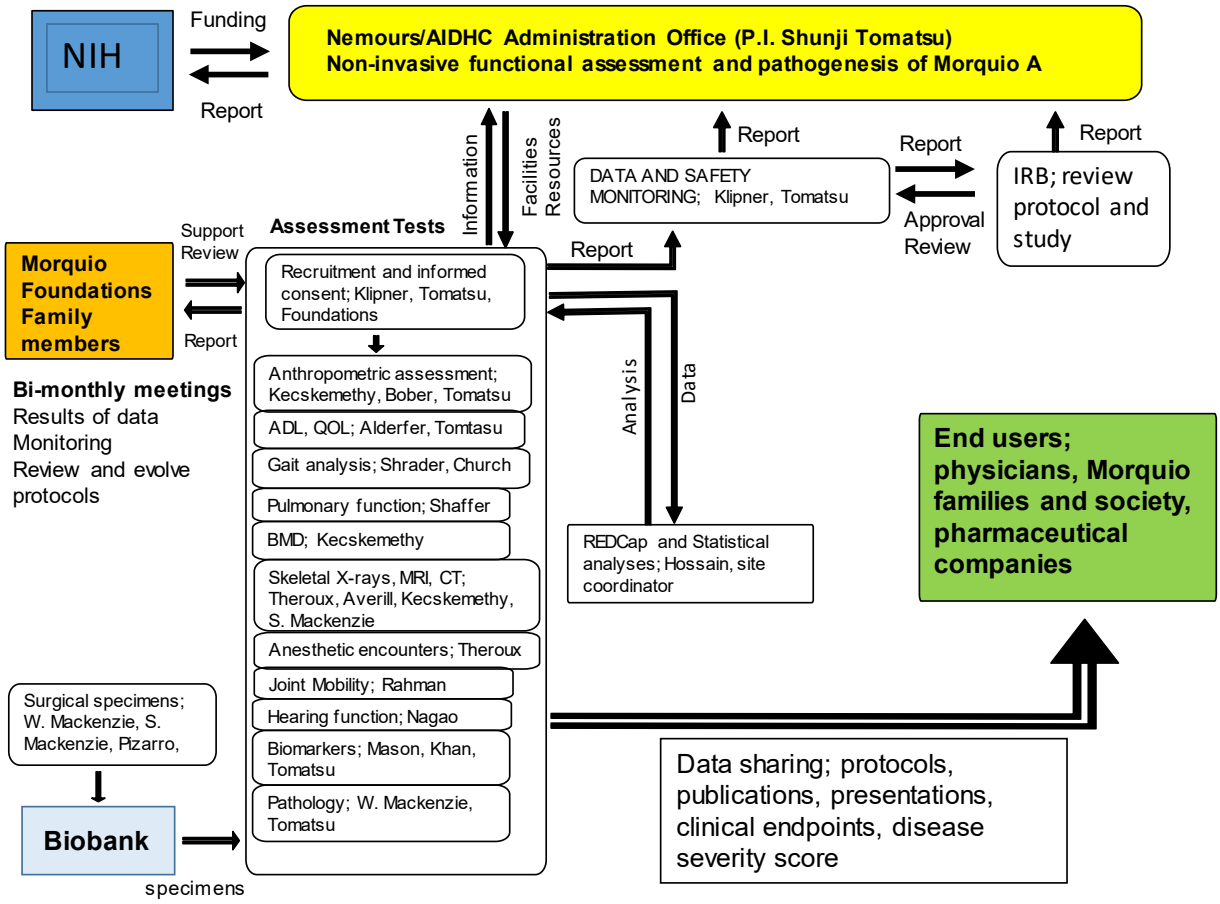


Figure 1. Structure of Study Team of Natural History

b. **Recruitment:** During the COVID-19 pandemic, the recruitment process was significantly impacted, leading to delays due to limited access to the families and the hospital, and the requirement of vaccines to visit the hospital. Despite these challenges, sixty-two patients have consented to the study (Figures 2 and 3). Sixty patients have completed first visits, and thirty-eight have completed second visits. Twenty-one surgical specimens have been collected from participants. See the table below.

c. **Data Collection:** This study includes 15 major assessments: clinical assessment procedures; anthropometric measurements**;** activity of daily living and quality-of-life questionnaires; gait kinematics and kinetics analysis; pulmonary function tests (PFT); skeletal radiographs and dual-energy x-ray absorptiometry (DXA); MRI in cervical spine, temporal bones, and hip; computed tomography angiography (CTA) for tracheal obstruction; CT for temporal bones; anesthetic encounters; joint mobility; hearing function; biochemical analyses; and pathological analyses.

In Aim 1, we have conducted all tests except for biochemical analysis. In Aim 2, we have analyzed some portions of data and will submit it to the articles,

Planned

Ethnic Categories					
	Not Hispanic or Latino		Hispanic or Latino		Total
Racial Categories	Female	Male	Female	Male	
American Indian/Alaska Native	0	0	0	0	0
Asian	3	3	0	0	6
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	4	4	0	0	8
White	20	20	0	0	40
More than One Race	0	0	3	3	6
Total	27	27	3	3	60

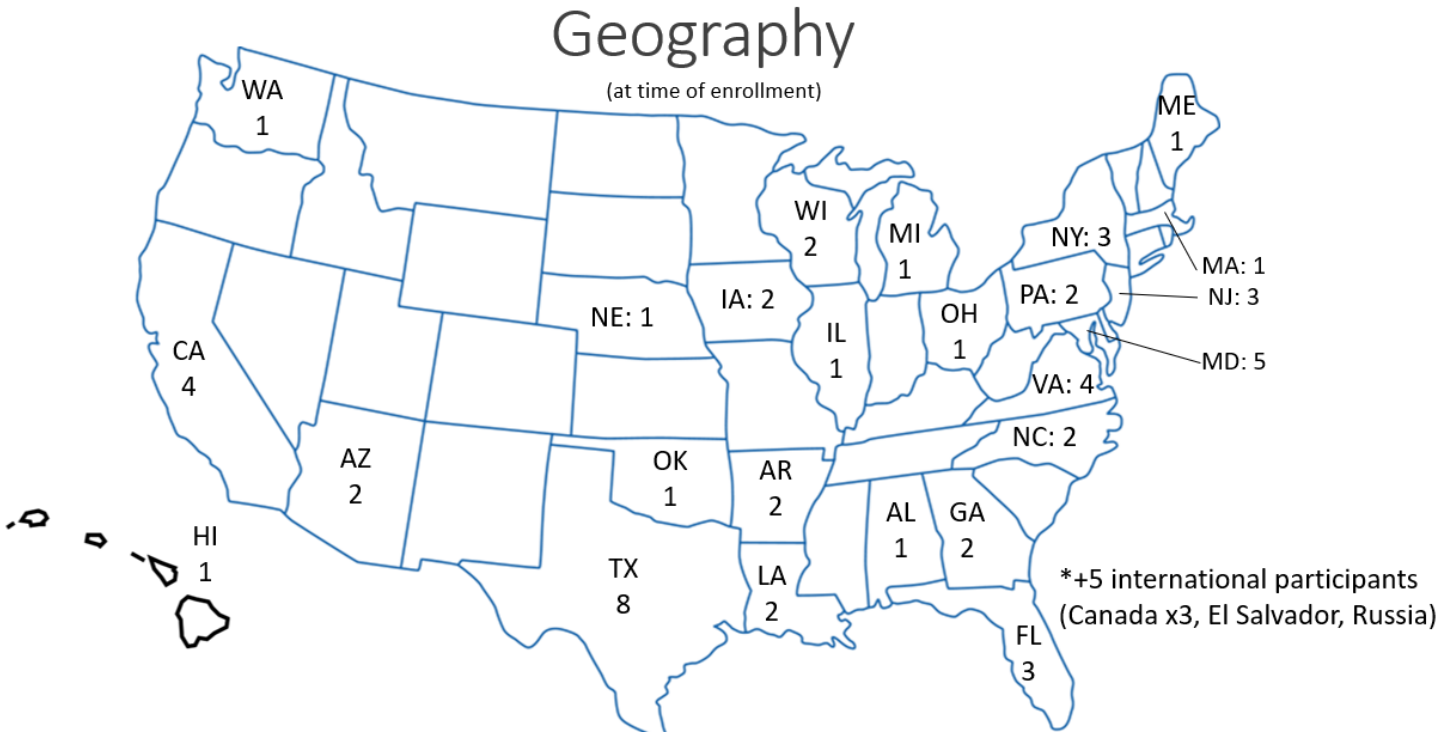
Cumulative (Actual)

Ethnic Categories										
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported			Total
Racial Categories	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/Alaska Native	1	1	0	0		0	0	0	0	2
Asian	3	1	0	0	1	0	0	0	0	5
Native Hawaiian or Other Pacific Islander	0	0	0	0		0	0	0	0	0
Black or African American	1	2	0	0		0	0	0	0	3
White	26	14		4	2					46
More than One Race	1	1	0	1	1	0	0	0	0	4
Unknown or Not Reported	2	1		1	1			1		6
Total	29	18	0	6	4	0	0	1	0	66

Age Enrollment Report											
Age Categories	0-1	2--5	6--12	13--17	18--25	26--45	46--64	65-75	76+	Unknown/ Not Reported	Total
Total	2	13	19	5	12	14	1	*	*	*	66

- Currently Consented/Enrolled: 66
- Withdrawn: 11
- First visits: 64 complete, 2 scheduling
- Second Visits: 49 complete, 0 scheduling, 6 missed visits
- Third Visits: 4 complete, 17 scheduling
- Surgical Samples Collected: 25

d. Summary of Demographics



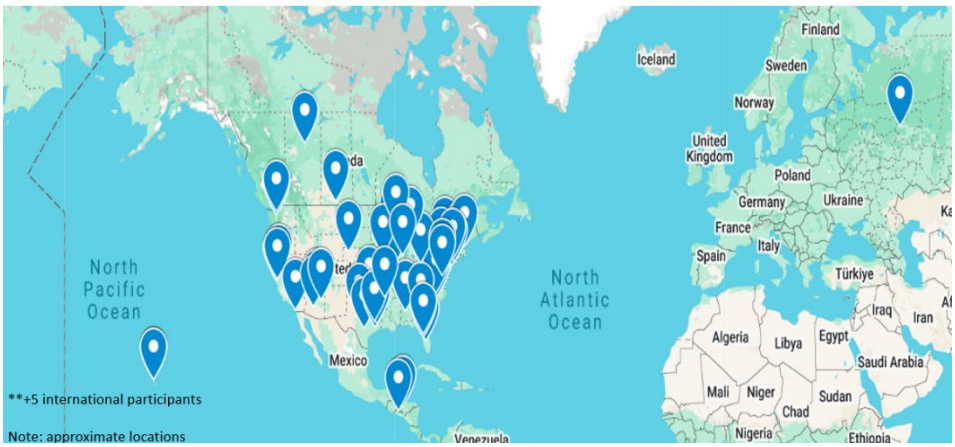


Figure 2. Geographic maps (Global and US only)

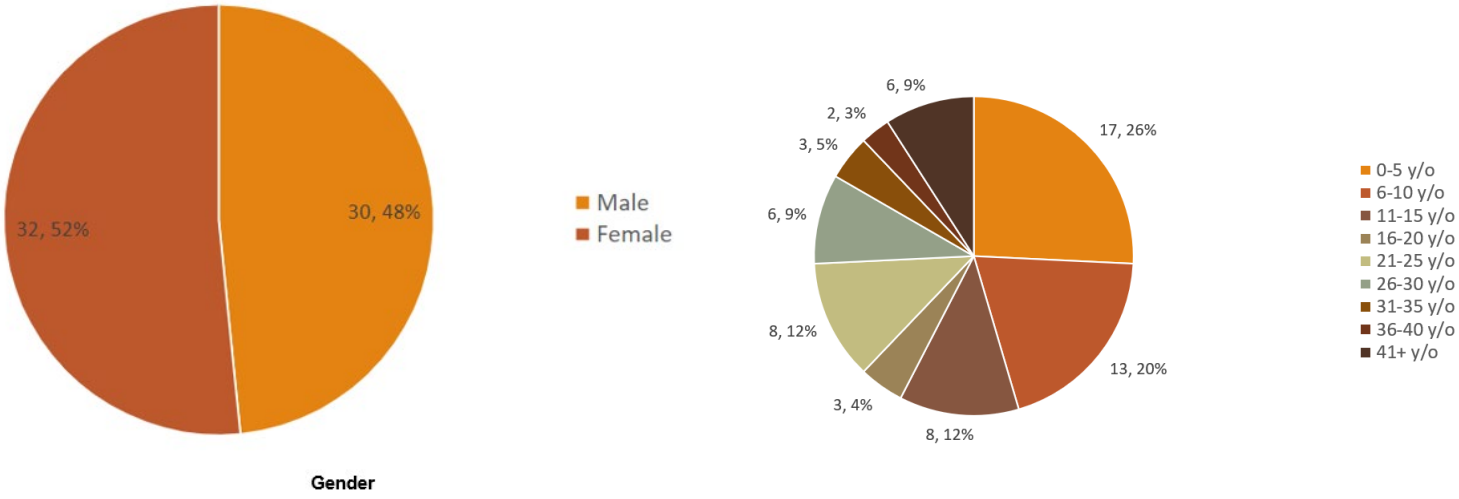


Figure 3. Gender and Age

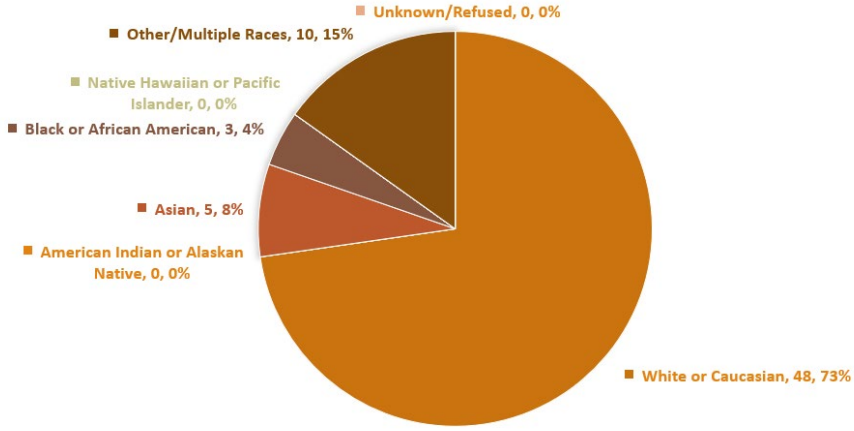


Figure 4. Racial Background

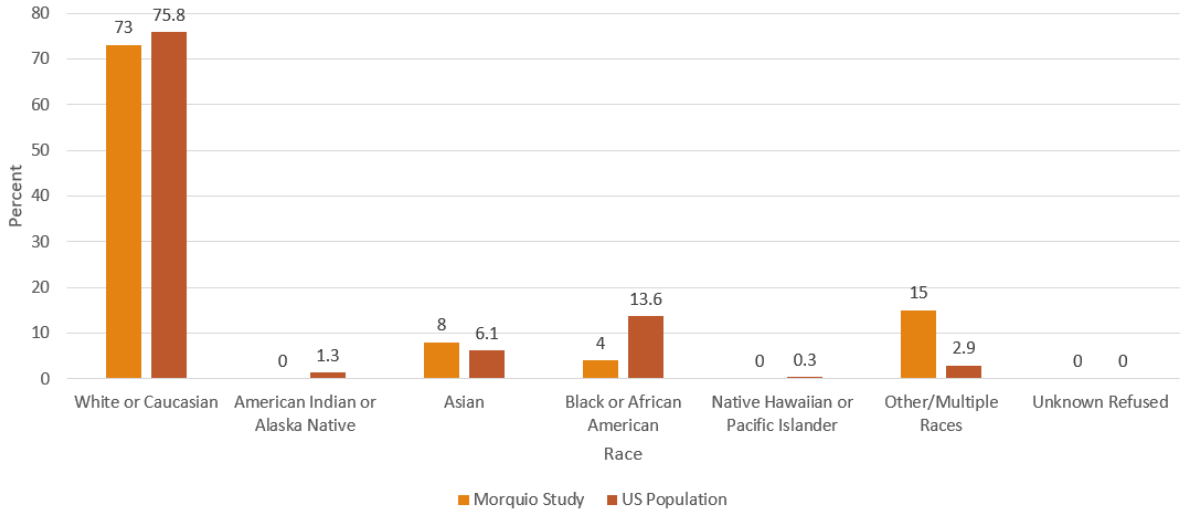


Figure 5. Race: Morquio Study vs. US Population

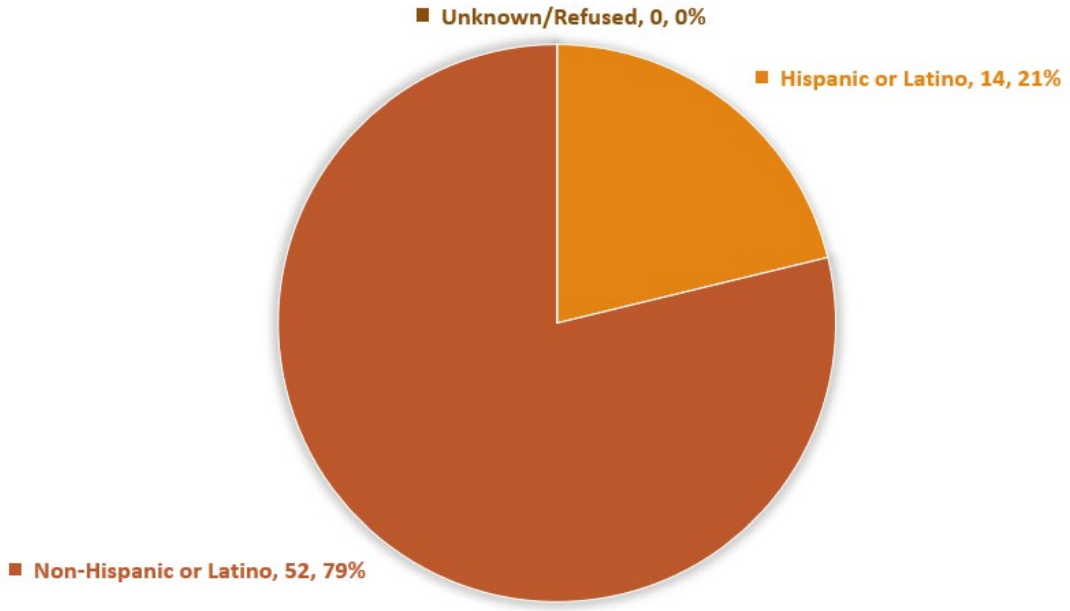


Figure 6. Ethnic Demographics

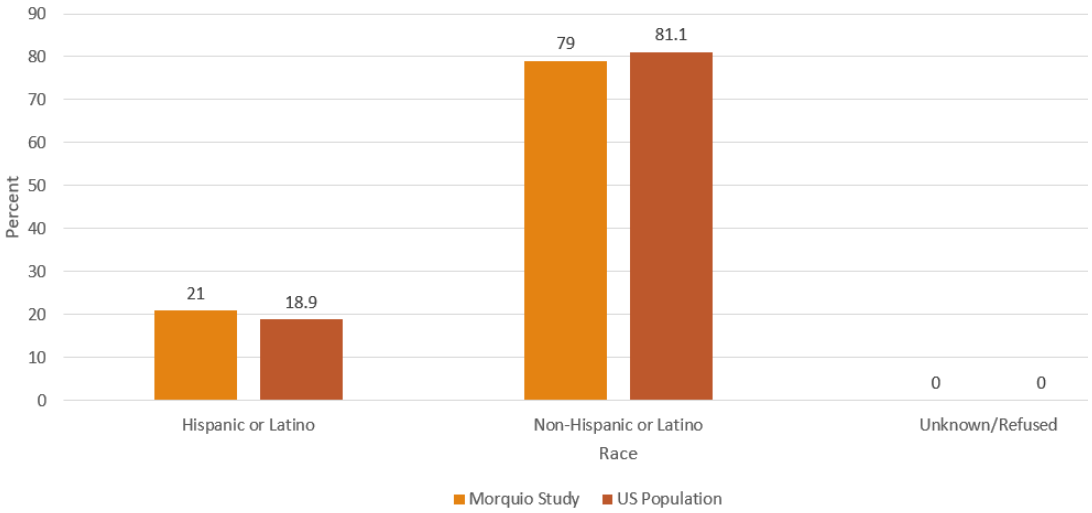


Figure 7. Ethnicity: Morquio A Study vs. US Population

2. Specific objectives

Establishment of innovative non-invasive assessments to evaluate the clinical severity, disease stage, and therapeutic efficacy of skeletal dysplasia.

Aim 1. Conduct multidisciplinary and detailed non-invasive assessments in Morquio A patients. Our working hypothesis is that longitudinal non-invasive tests demonstrate accurate prognostic and predictive assessment of skeletal dysplasia and can be carried out across a broad patient group, including patients who are young, wheelchair-bound, and post-surgical.

Aim 2. Identify potential surrogate biomarkers of skeletal dysplasia. Our working hypothesis is that biochemical biomarkers correlate with disease stage, clinical severity, and non-invasive clinical assessments. These biomarkers should, therefore, serve as prognostic and/or predictive biomarkers of therapeutic response.

3. Significant results (including) major findings, developments, or conclusions (both positive and negative)

Aims 1 and 2.

1. We have completed the first visits for 64 patients, the second for 49 patients, and the third for four patients, 17 scheduling in the problematic situation of COVID-19 after confirming the safety and feasibility of the families. 25 Surgical Samples were Collected.
   We recruited the patients, including 5 international patients. We assume 15 % of the MPS IVA patients in the USA participated in this program if around 400 MPS IVA patients are present. This is very significant in terms of the number of patients. Also, the demographics of the participants are similar to the USA ethnic population pattern, which MPS IVA incidence happens in diverse populations.
2. Until now, no patient has had any discomfort or incapability with the proposed tests, which matches our hypothesis that all non-invasive tests are feasible for patients without age or physical handicap.
   Our non-invasive tests are feasible and acceptable to the majority of patients compared to endurance tests, which include a 6 min walk test and a 3 min climb-up test. This is one of our major goals and a significant result.
3. We found that NTproCNP is currently the most promising biomarker during the study. We plan to measure this biomarker continuously in human blood and urine to identify the correlation between skeletal dysplasia and NTproCNP level. In addition, we are exploring the potential of CNP as a therapeutic agent on the MPS IVA animal models. The discovery of NTproCNP is significant as a potential surrogate biomarker and can be used for the future clinical trial.
4. Based on the natural history program activity and data, our project is selected for the clinical trial the Foundation for the National Institutes of Health (FINH) Accelerating Medicines Partnership® Bespoke Gene Therapy Consortium (AMP® BGTC), which is public-private partnership between the NIH, U.S. Food and Drug Administration (FDA), biopharmaceutical and life sciences companies, and non-profit and other organizations to develop and deliver bespoke (i.e., customized) gene therapies to treat patients with rare diseases. AMP® BGTC aims to accelerate the development and approval of gene therapies for eight rare diseases, including Morquio A Syndrome. This is a very significant result for the entire Morquio Community, Researchers, and
   We have a schedule of the clinical trial for Morquio A in 2027 using AAV gene therapy. The current data will be used for the baseline data of the clinical trial.
5. Because of COVID-19, we have delayed collecting data, which is a serious issue to have longitudinal data (4 visits).

4. Key outcomes or other achievements

Aims 1 and 2.
Identify potential surrogate biomarkers of skeletal dysplasia. Our working hypothesis is that biochemical biomarkers correlate with disease stage, clinical severity, and non-invasive clinical assessments. These biomarkers should, therefore, serve as prognostic and/or predictive biomarkers of therapeutic response.
We collected urine and blood samples from patients who visited the hospital on the first, second, and third visits.

Materials and methods
Subjects
MPS IVA patients
MPS IVA patients commuting to Nemours Children's Hospital in Delaware have participated. Written informed consent was obtained before any study procedures were conducted.

Controls
Residual serum from various patients without skeletal dysplasia at Shimane University Hospital was frozen after biochemical analysis and then sent to our laboratory as control samples for this research. The patients ranged in age from 0 day to 16 years. Blood samples were also collected from 7 healthy adults working in this laboratory, and both plasma and serum were stored. Written informed consent was obtained from them.

a. Collagen type I
Human Collagen Type I (COL1) ELISA Kit (Cat# EKU03297-96T, BIOMATIK, Kitchener, Canada) was used to measure the concentration of collagen type I in patient plasma and control serum and control plasma according to the manufacturer's instructions.
b. Collagen type II

Human Collagen Type Ⅱ, Col Ⅱ ELISA Kit (Cat# EKC40379, BIOMATIK, Kitchener, Canada) was used to measure the concentration of collagen type II in patient plasma and control serum and control plasma according to the manufacturer's instructions.

c. Collagen type X

Human Collagen X ELISA Kit (Colorimetric) (Cat# NBP2-75826, Novus Biologicals, Centennial, Colorado, USA) was used to measure the concentration of collagen type X in patient plasma and control serum and control plasma according to the manufacturer's instructions.

d. NT-proCNP

NT-proCNP ELISA kit (Cat# BI-20812, BIOMEDICA, Vienna, Austria) was used to measure the concentration of amino-terminal pro C type natriuretic peptide (NT-proCNP) in patient plasma and control serum and control plasma according to the manufacturer's instructions.

e. Anti-rhGALNS IgG

Enzyme-Linked Immunosorbent Assay (ELISA) was used to measure the concentration of anti-rhGALNS IgG in each sample.

f. Anti-AAV8 IgG and Anti-AAV9 IgG

The ELISA method [1] was used with modifications to detect these antibodies.

g. Genotype

The genomic DNA of each patient was extracted from frozen WBC, and then all 14 exons in the GALNS gene were PCR-amplified and sequenced by the Sanger method to detect pathogenic mutations.

h. Glycosaminoglycans (GAG) analysis by LC-MS/MS

Control serum and urine: We obtained serum and urine from various patients without skeletal dysplasia at Shimane University Hospital. Control serum ages 5-18 years and urine 5-33 years

Results (outcomes)

Collagen type I and II

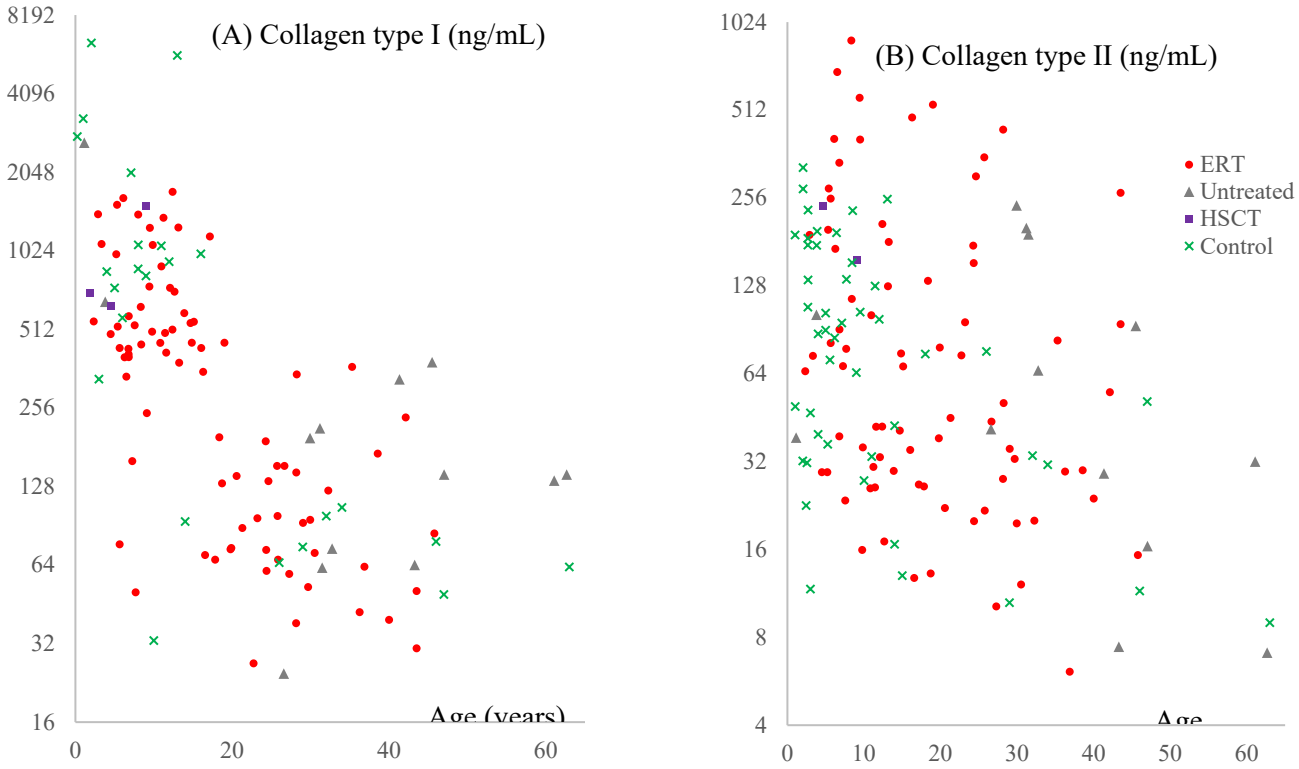


Figure 1. Collagen type I (A) and II (B) levels in patients and controls.
Both vertical axes are presented on a base-2 logarithmic scale.

a. Collagen type I

We found a statistically significant increase in collagen type I in patients older than 20 (**Table 1, Figure 1 (A)**).

Table 1. **Comparison of collagen type I levels in 5 age groups.**

Age	No.	Mean	SD	Maximum	Minimum	Mean age	p-value
Control							
≤ 1 y	1	2803.4	0	2803.4	2803.4	0.25	
>1, ≤ 5 y	4	2715.5	2400.5	6397.5	329.6	2.51	
>5, ≤ 10 y	6	1019.0	481.2	2039.0	566.0	7.19	
>10, ≤ 15 y	5	1572.0	2123.6	5734.8	32.9	11.98	
> 15, ≤ 20 y	1	996.1	0	996.1	996.1	15.99	
>20 y	7	76.6	18.6	106.3	49.3	39.57	
MPS IVA							
>1, ≤ 5 y	8	1022.4	681.8	2654.1	490.7	3.05	0.31
>5, ≤ 10 y	24	679.4	469.3	1630.2	50.3	7.38	0.19
>10, ≤ 15 y	14	752.4	398.1	1722.0	381.2	12.52	0.48
> 15, ≤ 20 y	11	323.9	314.8	1161.6	67.0	17.70	N/A
>20 y	41	125.7	92.1	383.0	24.5	33.35	0.0046

b. Collagen type II

We found a statistically significant increase in collagen type II in patients in two age groups, between 5 and 10 years and over 20 years (**Table 2, Figure 1 (B)**). We calculated the partial correlation coefficient between collagen type II and NT-proCNP, controlling for age using the entire age group. There was almost no correlation (r = 0.042, p = 0.69).

Table 2. **Comparison of collagen type II levels in 5 age groups.**

Age	No.	Mean	SD	Maximum	Minimum	Mean age	p-value
Control							
>1, ≤ 5 y	18	129.03	92.41	324.99	11.70	2.68	
>5, ≤ 10 y	12	113.70	53.62	231.40	36.66	6.97	
>10, ≤ 15 y	7	85.64	78.26	253.57	16.66	12.21	
> 15, ≤ 20 y	2	43.85	30.83	74.68	13.02	16.50	
>20 y	7	35.72	22.85	76.23	10.51	39.57	
MPS IVA							
>1, ≤ 5 y	7	105.80	74.15	240.58	29.44	3.23	0.55
>5, ≤ 10 y	21	234.54	233.64	885.55	15.93	7.30	0.037
>10, ≤ 15 y	14	70.07	59.39	208.32	17.03	12.52	0.67
> 15, ≤ 20 y	11	131.71	181.03	534.18	12.77	17.70	0.21
>20 y	40	90.71	105.27	438.58	6.10	33.54	0.0097

c. Collagen type X

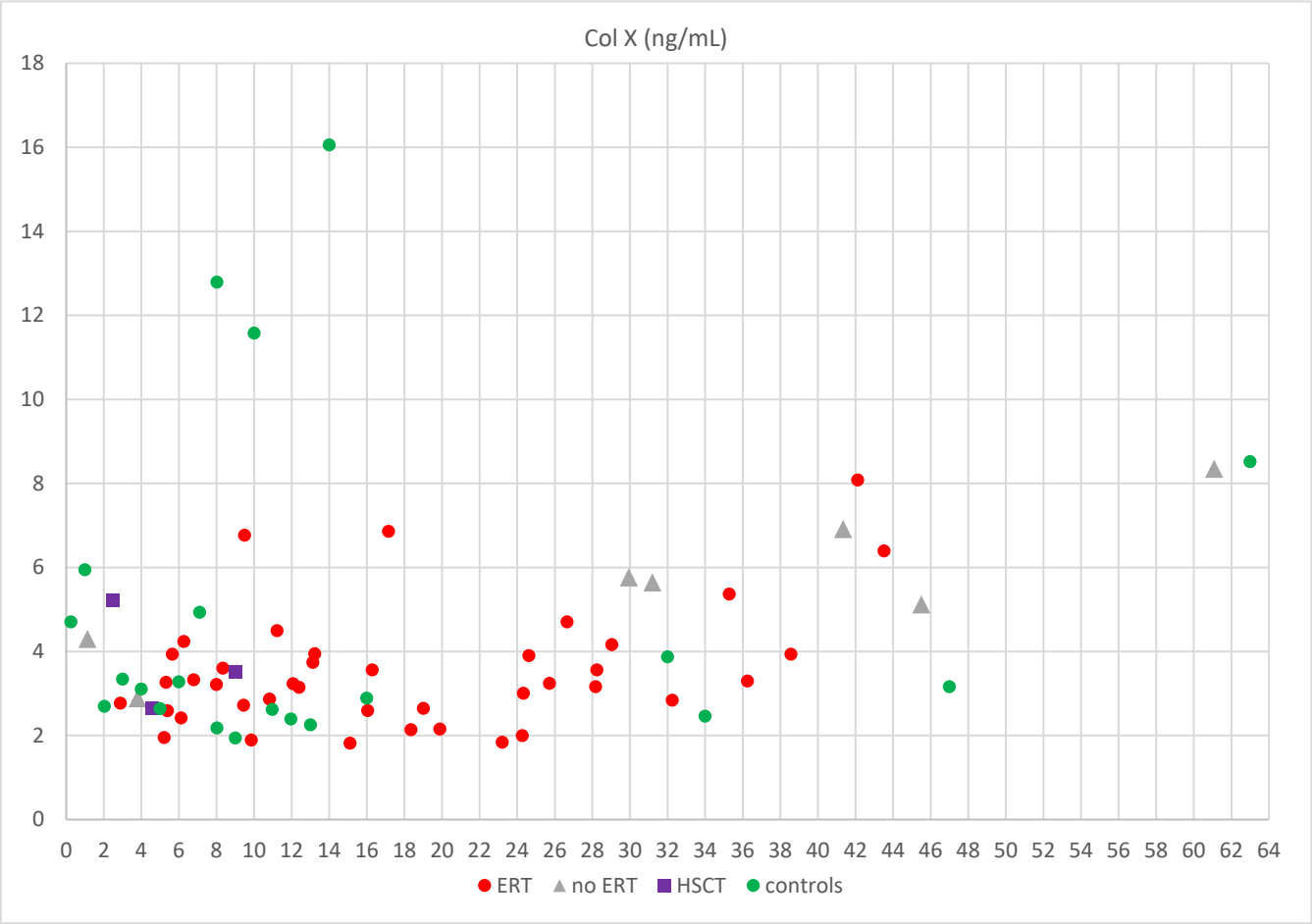


Figure 2. Collagen type X levels in blood
We did not find any significant difference or trend.

d. NT-proCNP

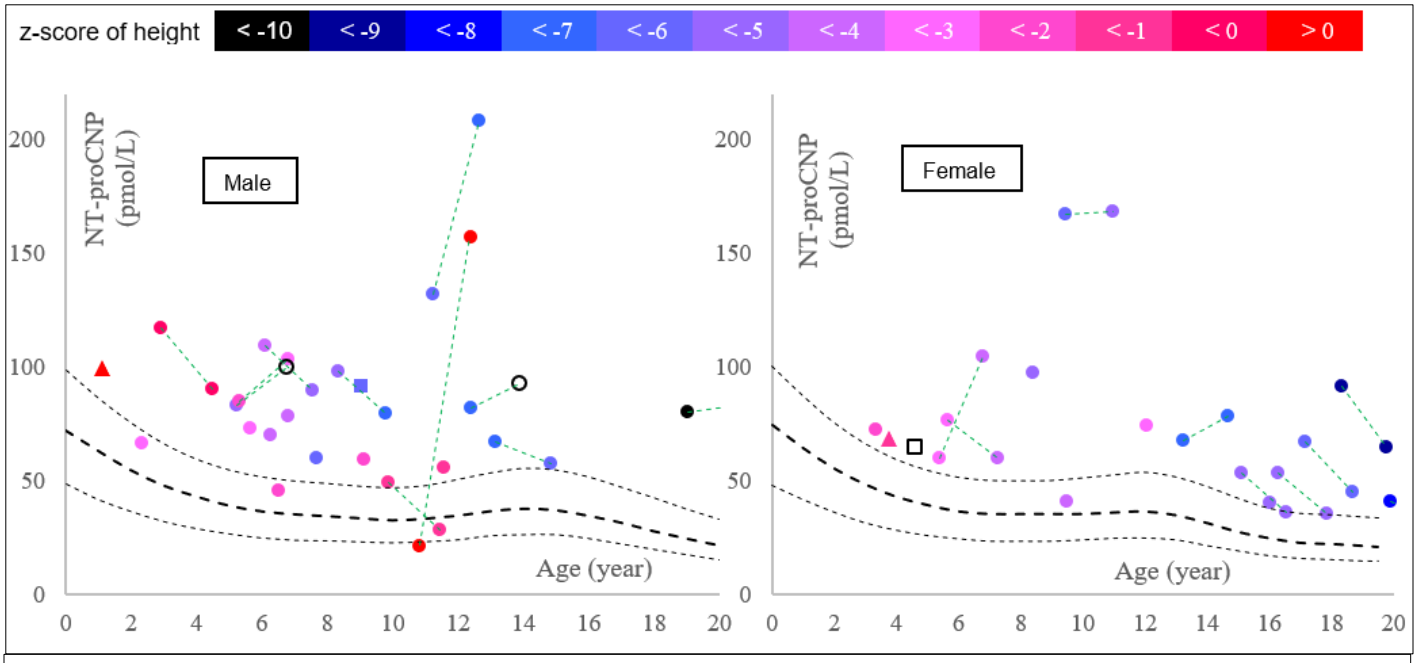


Figure 3: Correlation between age, NT-proCNP level in plasma, and height of patients with MPS IVA. Males and females are shown in the left and right figures, respectively. Round dots represent patients treated with enzyme replacement therapy (ERT). Triangle markers represent patients who were never treated with ERT. Square markers represent patients treated with hematopoietic stem cell transplantation (HSCT). Filled markers represent patients for whom height at blood sample collection was recorded. The color of each marker represents the z-score of each patient's height calculated based on the 2022 CDC growth chart. Black dotted lines show median, 5th and 95th percentiles for healthy controls [30]. When a patient has two data points, each data point is connected by a green dotted line.

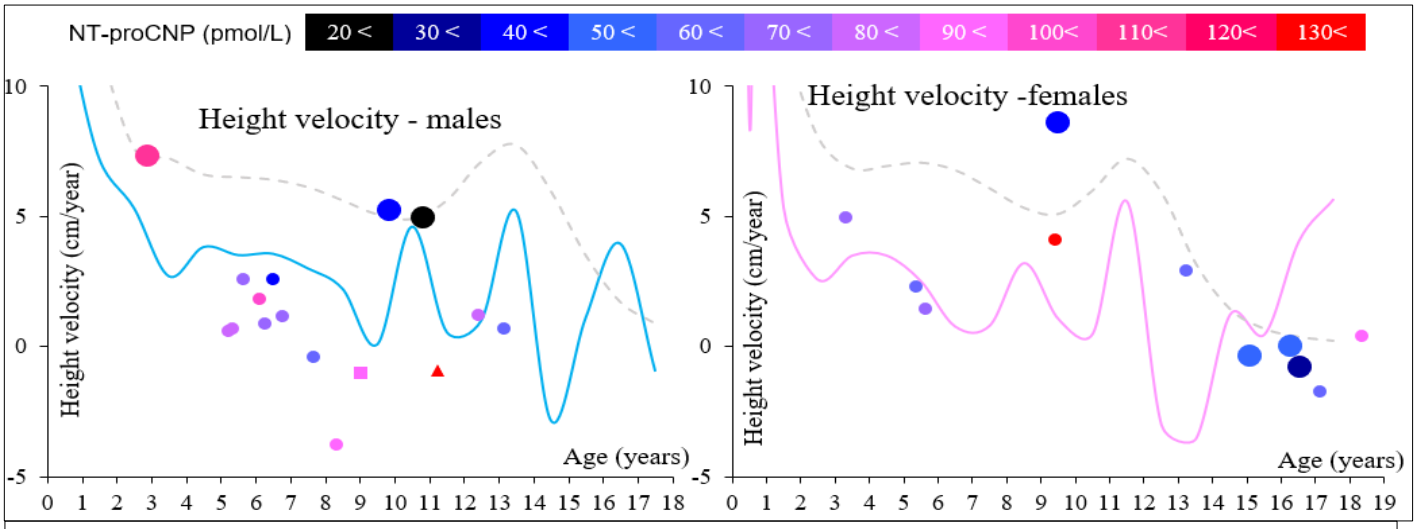


Figure 4: The relationship between each patient's plasma NT-proCNP level, age, and subsequent height growth rate (height velocity): Height velocity was calculated from two sets of height data measured at different times, 7-20 months apart. Each dot represents the age of each patient at which the first height was measured (horizontal axis) and the corresponding later height velocity (vertical axis). Round dots represent patients who received ERT continuously until the second height data was obtained. The square dot represents a patient who had received HSCT. The triangular dot indicates a patient who never received ERT until his second height data was obtained. Larger round dots represent the attenuated phenotype, while smaller round dots represent the severe phenotype. Each dot is filled with a color corresponding to the plasma NT-proCNP level at the first height measurement. The light blue and pink lines show the 50th percentile for height velocity for male and female MPS IVA patients, respectively [29]. The gray dotted line shows the 50th percentile for height velocity for healthy males and females.

We revealed a significant increase of NT-proCNP in patients under 20 years (Welch's t-test, p = 2.67 x 10^{-12}). The increase was particularly evident just before the age of pubertal growth spurt in healthy subjects (**Fig. 3**). If we focus only on patients who have two data points at different ages before the age of pubertal growth spurt (all had received ERT during these two periods), 5 out of 10 boys and 2 out of 3 girls showed the increase in NT-proCNP level. This suggests that plasma NT-proCNP would not be a pharmacodynamic biomarker differing from urinary KS [10] before the age of pubertal growth spurt.

The concentration of NT-proCNP in plasma from patients under 20 years showed a negative correlation with age, and the correlation coefficient (r) was -0.612 (p = 7.44 x 10^{-11}). The Z-score of each patient's height showed a significant negative correlation with age, as current therapy is ineffective to prevent growth failure in MPS IVA (r = -0.703, p < 1.21 x 10^{-6}). We then calculated the partial correlation coefficient between NT-proCNP and z-score of height controlling for age, which is summarized in **Table 3 (below, partial correlation coefficient between NT-proCNP and z-score of height, controlling for age)**. We found a negative correlation after the age of 8 years.

Age group	number of subjects	Partial correlation coefficient	p-value
≤ 8 y	20	0.19	0.4355
>8, ≤ 13 y	17	-0.568	0.0217
>13, ≤ 20 y	15	-0.837	1.865×10^{-4}

In addition, for patients with more than two visits, height velocity was calculated from two sets of height data measured at different times, 7-20 months apart, as summarized in **Fig. 4**. These findings are consistent with the hypothesis in achondroplasia that elevated levels of CNP-related peptides are natural responses to disturbed bone growth [11, 12]. Further accumulation of data from MPS patients is essential and urgent to determine the accurate correlation between NT-proCNP and skeletal symptoms, including height.

e. Anti-rhGALNS IgG

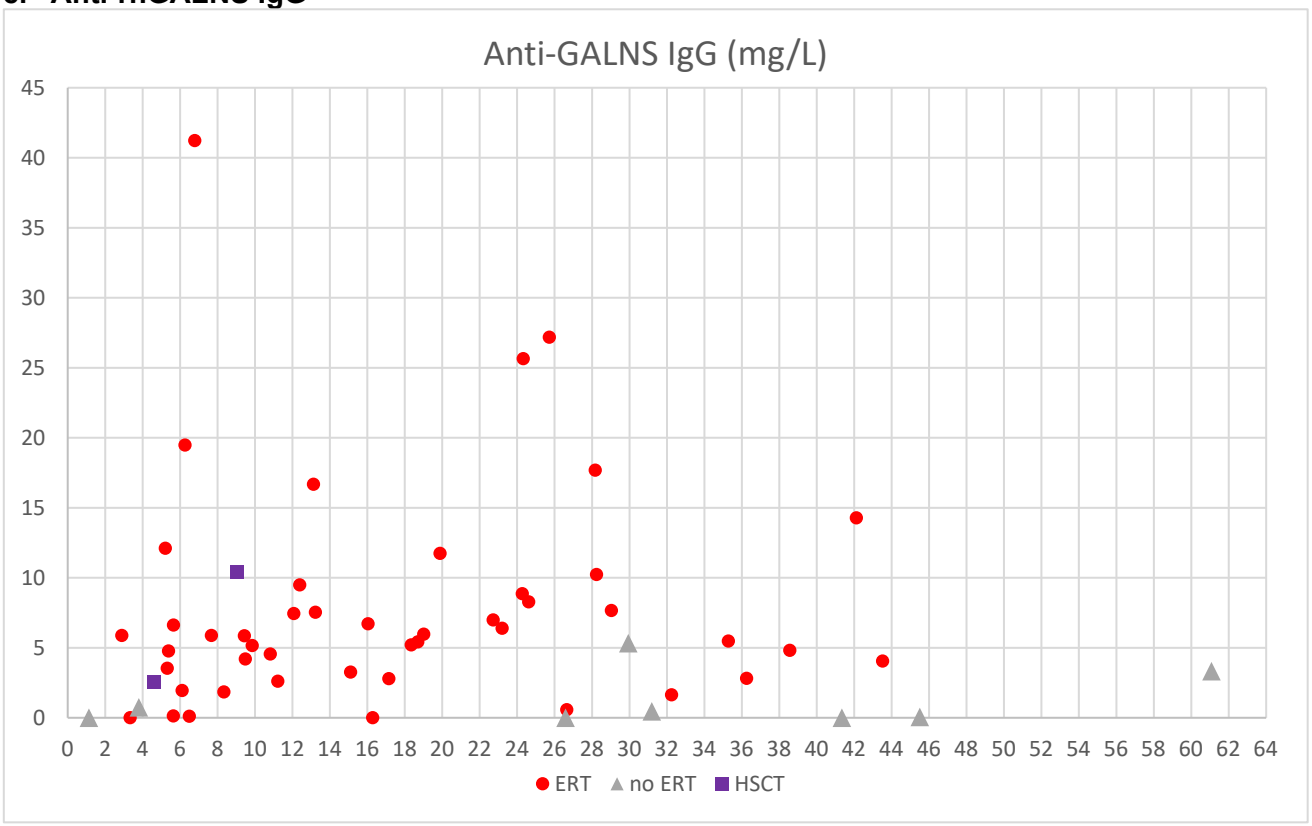


Figure 5. Anti-GALNS antibody levels in blood
ERT is strongly associated with the elevation of anti-rhGALNS antibodies. One patient who received HSCT at 8 years had received ERT until HSCT.

f. Anti-AAV8 IgG and Anti-AAV9 IgG
Under investigation.

g. Genotype: we have identified the following genotypes from participants.

Patient ID	Sex	Race	Height (Z-score)	Age (year)	Genotype Allele 1	Allele 2	Treatment
M01	F	White or Caucasian	-6.34	17.16	c.740G>A (G247D)	c.901G>T (G301C)	ERT
			-6.73	18.70			
M02	F	Chinese		38.57	c.953T>G (M318R)	c.1567T>G (X523EextX93)	ERT
				40.00			
M03	F	White or Caucasian		24.62	c.448delC (H150Tfs*3)	c.651_652insG (K218Efs*45)	ERT
M04	F	White or Caucasian		42.12	c.675dupC (F226Lfs*37)	unknown	ERT
				43.51			
M05	F	White or Caucasian	-9.93	18.35	c.346G>A (G116S)	c.1156C>T (R386C)	ERT
			-9.76	19.78			
M06	F	White or Caucasian	-8.52	19.89	c.346G>A (G116S)	c.1156C>T (R386C)	ERT
				21.29			
M07	M	White or Caucasian		32.25	c.337A>T (I113F)	c.1171A>G (M391V)	No ERT for 2 years and 8 months
M08	M	White or Caucasian	no record	8.00	c.122T>A (M41K)	c.122T>A (M41K)	ERT
			-6.09	9.03			Received HSCT 1 year ago
M10	M	White or Caucasian	1.22	10.82	c.338T>C (I113T)	c.1219A>C (N407H)	ERT
			1.01	12.37			
M11	F	White or Caucasian	-5.36	15.11	c.421T>A (W141R)	unknown	ERT
			-5.53	16.55			
M12	F	White or Caucasian		26.65	c.697G>A (D233N)	c.1034T>C (L345P)	No ERT for 4 years and 4 months
				28.15			
M13	M	Black or African American	-7.52	13.13	c.251C>A (A84E)	c.319G>A (A107T)	ERT
			-6.75	14.84			
M14	F	White or Caucasian	-5.7	16.29	c.740G>A (G247D)	unknown	ERT
			-5.7	17.83			
M15	M	White or Caucasian	-2.39	5.32	c.498delC (F167Lfs*32)	c.901G>T (G301C)	ERT
			-3.84	6.81			
M16	M	Filipino	-5.09	5.21	c.228C>A (N76K)	c.1480A>G (p.M494V)	ERT
			no record	6.77			
M19	F	Black or African American	-6.49	9.44	c.245C>T (S82L)	unknown	ERT
			-5.64	10.96			
M20	F	White or Caucasian	-4.09	9.49	c.498delC (F167Lfs*32)	c.1474G>A (A492T)	ERT
			-3.53	10.06			
M21	M	White or Caucasian	-4.08	6.12	c.651_652insG (K218Efs*45)	c.1159G>A (G387S)	ERT
			-5.22	7.55			
M22	M	White or Caucasian	-5.45	8.34	c.651_652insG (K218Efs*45)	c.1159G>A (G387S)	ERT
			-7.24	9.78			
M25	M	White or Caucasian		41.34	c.155C>T (P52L)	c.337A>T (I113F)	Never received ERT
				43.28			
M26	M	Filipino	-7.03	12.39	c.93delC (N32Tfs*97)	c.946G>A (G316R)	ERT
			no record	13.89			
M28	F	Mixed		31.20	c.868G>A (G290S)	c.868G>A (G290S)	Never received ERT
		Japanese and Caucasian		32.75			
M30	F	White or Caucasian		43.52	c.121-12T>C	c.121-12T>C	ERT
M31	M	Black or African American	-1.29	9.86	c.935C>G (T312S)	c.1520G>T (C507F)	ERT
			-1.11	11.44			
M32	F	White or Caucasian		45.50	c.121-12T>C	c.121-12T>C	Never received ERT
				47.03			
M33	F	White or Caucasian		29.94	c.1012C>T (Q338X)	c.1171A>G (M391V)	Never received ERT
				31.48			
M34	M	White or Caucasian		28.17	c.121A>T (M41L)	c.121A>T (M41L)	ERT
				29.69			
M35	M	White or Caucasian	-6.86	11.23	c.139G>A (G47R)	c.1156C>T (R386C)	No ERT for 4 years
			-7.69	12.64			
M36	F	Asian Indian		25.72	c.346G>A (G116S)	c.346G>A (G116S)	ERT
				27.27			
M37	M	Asian Indian	-10.12	19.01	c.346G>A (G116S)	c.346G>A (G116S)	ERT
				20.56			
M38	F	White or Caucasian	-5.63	16.04	c.1559G>A (W520X)	unknown	ERT
M39	F	White or Caucasian	-3.54	12.08	c.1559G>A (W520X)	unknown	ERT
M41	M	White or Caucasian		35.29	c.121A>T (M41L)	c.498delC (F167Lfs*32)	ERT
				36.86			
M42	F	White or Caucasian		28.24	c.860C>T (S287L)	c.1055T>C (L352P)	ERT
				29.95			
M43	M	White or Caucasian		29.04	c.121A>T (M41L)	c.901G>T (G301C)	ERT
				30.52			
M44	F	White or Caucasian		24.28	c.1171A>G (p.M391V)	c.502G>A (p.G168R)	ERT
				25.85			
M45	F	White or Caucasian		61.08	c.740G>A (G247D)	c.761A>G (Y254C)	Never received ERT
				62.66			
M46	F	White or Caucasian	no record	6.92	c.167C>A (T56N)	c.502G>A (G168R)	ERT
			-5.54	8.40			
M47	F	Some other race		24.33	c.139G>A (G47R)	c.466T>C (F156L)	ERT
				25.78			
M48	F	White or Caucasian	-7.11	13.23	c.139G>A (G47R)	c.466T>C (F156L)	ERT
			-7.66	14.68			
M49	M	White or Caucasian	-6.44	7.68	c.1156C>T (R386C)	c.1156C>T (R386C)	ERT
M50	F	White or Caucasian	-3.33	5.65	c.451C>A (P151T)	c.477G>A (W159X)	ERT
			-4.81	7.26			
M51	M	White or Caucasian	-0.9	2.89	c.451C>A (P151T)	c.477G>A (W159X)	ERT
			-0.52	4.50			
M52	F	Multiple Races	no record	2.50	c.651_652insG (K218Efs*45)	unknown	Received HSCT 11 months ago
			no record	4.60			
			-2.54	6.21			
M53	F	Chinese		23.20	c.1482+5G>C	c.1498G>T (G500C)	No ERT for 13 months
				24.37			ERT
M54	F	White or Caucasian		36.26	not tested	not tested	No ERT for 5 years and 9 months
M55	F	White or Caucasian	-3.04	5.39	c.1339G>C (D447H)	unknown	ERT
			-4.2	6.80			
M56	F	White or Caucasian	-1.44	3.79	c.740G>A (G247D)	c.1451C>A (P484H)	Never received ERT
M57	M	White or Caucasian	0.41	1.13	c.740G>A(G247D)	c.1451C>A (P484H)	Never received ERT
M58	M	White or Caucasian	-4.38	6.78	c.1012C>T (Q338X)	c.181C>G (R61G)	ERT
M59	M	White or Caucasian	-4.14	6.26	c.337A>T (p.I113F)	c.901G>T (p.G301C)	ERT
M60	F	Some other race		26.58	c.901G>T (G301C)	c.1156C>T (R386C)	Never received ERT
M63	F	White or Caucasian		22.72	c.331C>T (Q111X)	c.1365-2A>G	ERT
M64	M	Black or African American	-3.74	5.64	c.633+1G>C	c.1558T>C (W520R)	ERT
M65	M	Black or African American	-2.9	6.50	c.633+1G>C	c.1558T>C (W520R)	ERT
M66	F	White or Caucasian	-2.1	3.34	c.1156C>T (R386C)	unknown	ERT
M67	M	White or Caucasian	-1.55	11.58	c.1171 A>G (M391V)	c.331C>T (Q111X)	ERT
M68	M	White or Caucasian		45.78	c.1156C>T (R386C)	c.181C>T (R61W)	ERT
M69	M	Asian	-3.03	2.33	c.106_111del (p.L36_L37del)	c.1201C>T (H401Y)	ERT
M71	M	White or Caucasian	-3	9.12	c.1219A>C (N407H)	5.81 kb large deletion	ERT

h. GAGs

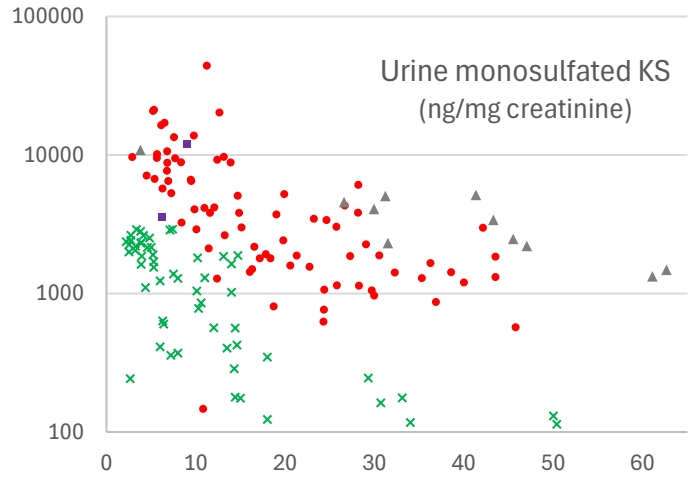
The results of mono-sulfated KS, di-sulfated KS, KS ratio (the rate of di-sulfated KS among total KS), and C6S in urine and blood from patients are summarized in **Table 4** and **Figure 6**. For C6S, the number of available control samples was less than for KS, so the "number of samples" and "mean age" differed from KS in **Table 4**. Partial correlation coefficients controlling for age between z-score of height and each significant GAG (mono-sulfated and di-sulfated KS and urinary C6S) were calculated and summarized in **Table 5**.

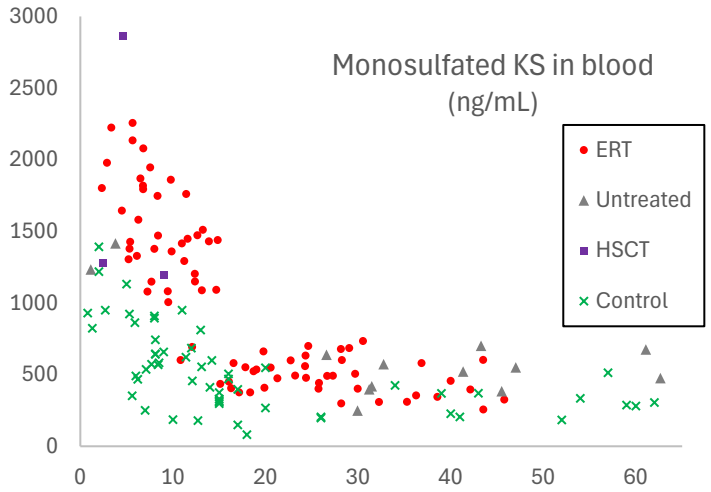
Table 4. Summary of GAG levels in 5 age groups

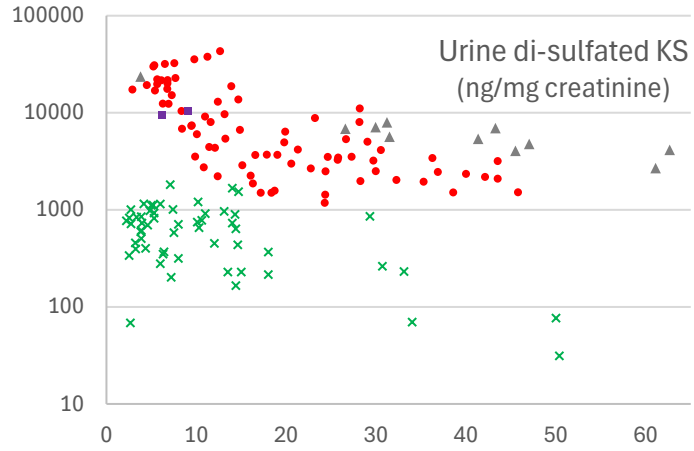
Age group	Number of samples	Mean age	Mono-sulfated KS	Di-sulfated KS	KS ratio (%)	C6S	Number of samples	Mean age
Control	(urine)							
>1, ≤ 5 y	18	3.47	2128 ± 618	663 ± 263	23.4 ± 4.6	N/A	0	N/A
>5, ≤ 10 y	14	6.48	1380 ± 833	768 ± 439	36.8 ± 5.6	164.7 ± 129.7	14	6.48
>10, ≤ 15 y	15	12.75	971 ± 574	802 ± 413	46.5 ± 9.4	178.1 ± 104.1	15	12.75
> 15, ≤ 20 y	3	17	215 ± 96	271 ± 69	57.2 ± 4.9	83.3 ± 17.2	3	17
>20 y	6	37.92	157 ± 45	255 ± 283	48.7 ± 18.6	88.0 ± 19.8	3	31.03
MPS IVA	(urine)							
>1, ≤ 5 y	3	3.73	9202 ± 1563*	20081 ± 2603**	68.6 ± 3.6***	727 ± 282		
>5, ≤ 10 y	23	7.24	9904 ± 5024***	18225 ± 9116***	63.9 ± 8.3***	633 ± 398***		
>10, ≤ 15 y	15	12.36	8143 ± 10725*	12343 ± 11876**	65.1 ± 11.1***	465 ± 247***		
> 15, ≤ 20 y	11	17.7	2340 ± 1178***	3091 ± 1509***	56.7 ± 8.0	289 ± 189**		
>20 y	39	33.56	2267 ± 1391***	4033 ± 2286***	64.4 ± 7.7	245 ± 131***		

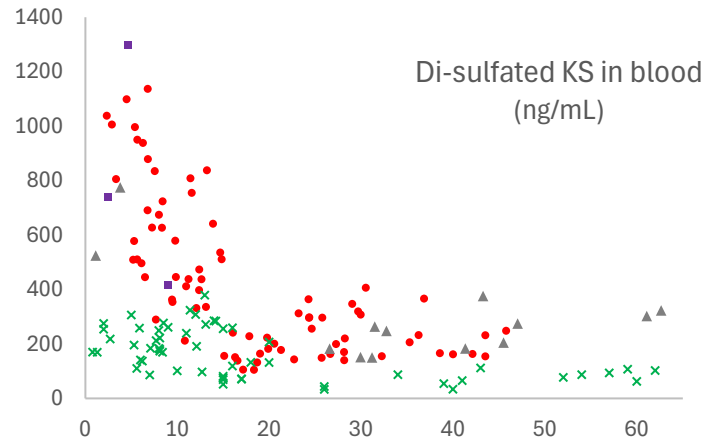
Age group	Number of samples	Mean age	Mono-sulfated KS	Di-sulfated KS	KS ratio (%)	C6S	Number of samples	Mean age
Control	(serum)							
>1, ≤ 5 y	4	2	1095 ± 223	229 ± 40	17.4 ± 1.3	5.15 ± 2.67	4	2
>5, ≤ 10 y	17	7.18	660 ± 220	197 ± 59	23.3 ± 3.6	2.34 ± 2.01	17	7.18
>10, ≤ 15 y	10	12.35	545 ± 235	248 ± 88	32.3 ± 5.0	2.81 ± 2.29	10	12.35
> 15, ≤ 20 y	10	15.9	322 ± 123	118 ± 73	27.8 ± 14.7	0.49 ± 0.06	2	17
>20 y	15	42.2	314 ± 109	86 ± 43	21.4 ± 7.8	N/A	0	N/A
MPS IVA	(plasma)							
>1, ≤ 5 y	8	3.13	1805 ± 514*	910 ± 229***	33.7 ± 4.1***	1.97 ± 2.53		
>5, ≤ 10 y	22	7.33	1556 ± 363***	639 ± 227***	28.7 ± 6.0**	1.97 ± 3.88		
>10, ≤ 15 y	14	12.52	1257 ± 306***	509 ± 181***	28.4 ± 4.3	1.12 ± 0.41		
> 15, ≤ 20 y	11	17.7	482 ± 90**	166 ± 45	25.5 ± 4.6	1.65 ± 1.04		
>20 y	40	33.54	493 ± 132***	238 ± 76***	32.6 ± 6.3	0.85 ± 0.43		

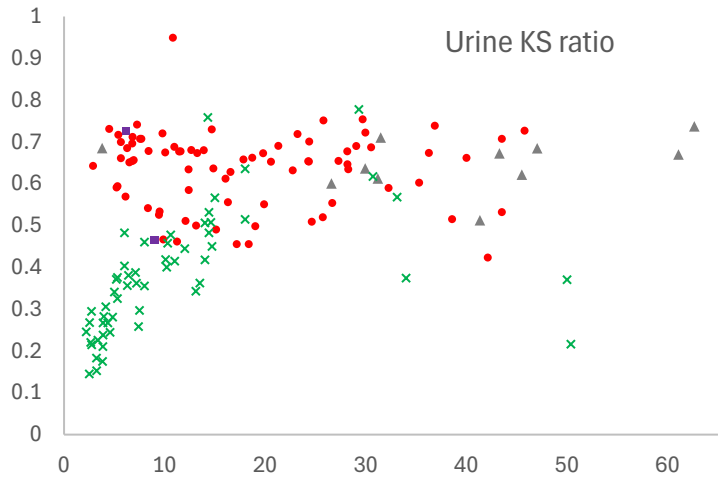
*p < 0.05, **p < 0.01, ***p < 0.001

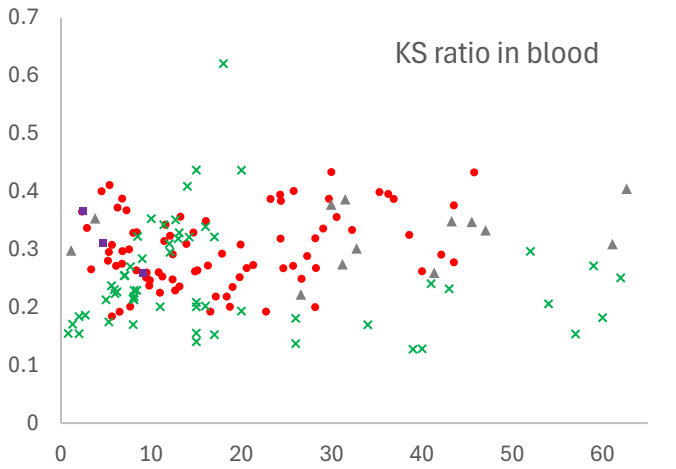

Urine monosulfated KS
(ng/mg creatinine)
100000
10000
1000
100
0
10
20
30
40
50
60

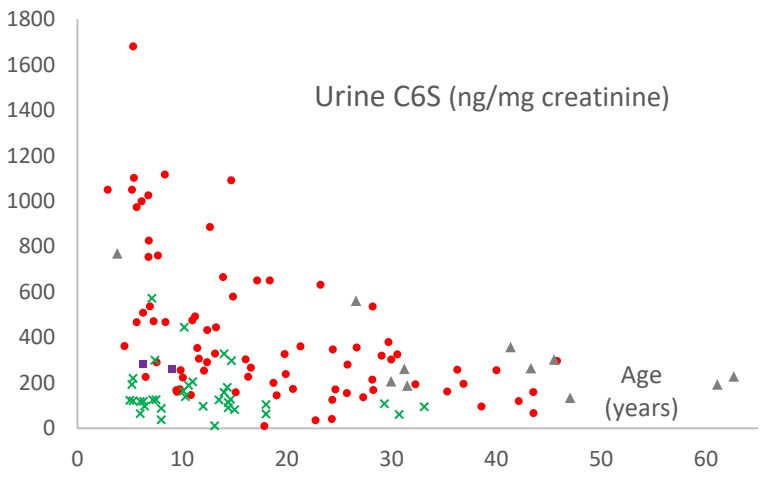

Monosulfated KS in blood
(ng/mL)
ERT
Untreated
HSCT
Control
3000
2500
2000
1500
1000
500
0
0
10
20
30
40
50
60


Urine di-sulfated KS
(ng/mg creatinine)
100000
10000
1000
100
10
0
10
20
30
40
50
60


Di-sulfated KS in blood
(ng/mL)
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
60


Urine KS ratio
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
60


KS ratio in blood
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
60


Urine C6S (ng/mg creatinine)
1800
1600
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
60
Age
(years)

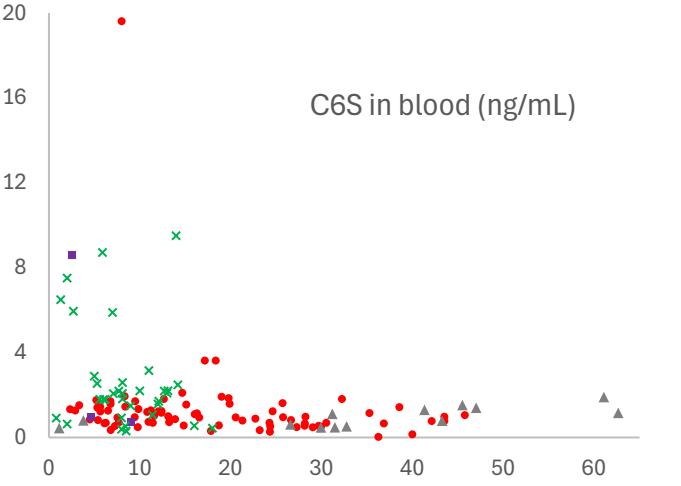

C6S in blood (ng/mL)
20
16
12
8
4
0
0
10
20
30
40
50
60

Figure 6: Glycosaminoglycan levels in urine and blood. Mono-sulfated KS, di-sulfated KS, KS ratio (di-sulfated KS to total KS), and C6S levels in urine and blood are summarized in scatter plots. The horizontal axes indicate the age (years) of each patient or control. For urinary mono-sulfated and di-sulfated KS levels, the vertical axes are shown on a base-10 logarithmic scale. Round red dots indicate patients who ever received ERT. Purple squares are indicated for patients who received HSCT. Gray triangles represent patients who never received ERT or HSCT. Green x marks represent controls. For blood samples, plasma samples were used for patients and serum for controls.

Table 5. The partial correlation coefficient (*r*) between each GAG and the z-score of height of patients under 20 years old, controlling for age

	Number of subjects to calculate the *r* value	*r*	p
Urine di-sulfated KS	49	-0.484	0.00049
Urine mono-sulfated KS	49	-0.42	0.00293
Plasma di-sulfated KS	50	-0.041	0.777
Plasma mono-sulfated KS	50	-0.181	0.213
Urine C6S	49	-0.282	0.0517

Conclusions and plan

Collagen type I: This is not a significant biomarker for pediatric patients. Several papers reported that the expression level of mRNA encoding collagen type I increased in chondrocytes from human MPS IVA patients [13, 14]. Comprehensive proteomic analysis of 6-week-old mouse femurs also showed that collagen alpha-1 and 2 (Uniprot IDs: P11087 and Q01149) increased in MPS IVA [15]. Therefore, we predicted that patients would present with higher concentration levels than the control group. However, only adult patients showed such differences. On the contrary, other age groups showed lower concentration in patients, even though it was not statistically significant. Because human collagen type I has a molecular weight of approximately 300 kDa, much larger than NT-proCNP, and is surrounded by hydroxyapatite in bone [16–18]It could be pretty difficult for collagen type I to escape from bone and cartilage and enter the bloodstream compared to NT-proCNP, thereby preventing higher concentrations in the blood of patients. We should measure smaller peptides related to collagen type I.

Collagen type II: This is not a significant biomarker for pediatric patients. Blood procollagen II C-terminal propeptide (CPII) and/or urinary C-terminal telopeptide of type II collagen (CTX-II) are currently the two primary markers of type II collagen metabolism in osteoarthritis [19–21], joint injuries [22], and Kashin-Beck disease (KBD) [23]. CPII reflects the synthesis of type II collagen during cartilage repair and remodeling [24–26], while CTX-II is a marker of type II collagen breakdown, reflecting cartilage catabolism [21, 27]. We will consider focusing on these peptides related to collagen type II.

Collagen type X: No significant findings were obtained. Also, the kit I used (https://www.novusbio.com/products/collagen-x-elisa-kit_nbp2-75826) is no longer manufactured. We will have to purchase other kits if we continue to measure this biomarker.

NTproCNP in humans
This is currently the most promising biomarker. We will measure this biomarker in human urine and a novel MPS IVA rat model. In addition, to increase specificity, we are collaborating with a mass spectrometer manufacturer (SCIEX, Framingham, MA) to establish a method to measure this biomarker by mass spectrometry.

GAGs
Urinary di-sulfated KS may not be a pharmacodynamic biomarker. Complete data analysis is ongoing.

Other biomarkers:

As I showed in the previous review [28], IL-1β, IL-6, IL-18, TNF-α, MMPs may be among the other candidates to measure. We are measuring these biomarkers with the Luminex™ xMAP™ INTELLIFLEX system by technicians at our institute. The results will be available shortly.

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Discussion
Unmet goals
Aim 1.
We have reached our goal of over 60 actively enrolled participants and will continue to have follow-up visits as they are interested.

1. We will not have enough to achieve 4 visits; therefore, we will resubmit the grant based on the achievements.
2. We will summarize the data and will publish each test data.

Aim 2.

1. We will continue to assay the biomarkers, including glycosaminoglycans (C6S and KS), Collagen, and Inflammatory factors.
2. We will submit the manuscripts with data of biological biomarkers.

PUBLICATIONS (other achievements)

2022

Poletto E, Colella P, Pimentel Vera LN, Khan S, **Tomatsu S**, Baldo G, Gomez-Ospina N. Improved engraftment and therapeutic efficacy by human genome-edited hematopoietic stem cells with Busulfan-based myeloablation. Mol Ther Methods Clin Dev. 2022 Apr 19;25:392-409. doi: 10.1016/j.omtm.2022.04.009. PMID: 35573043; PMCID: PMC9065050.

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Supplementary Table 1 (3/11/2025)

Study ID	Sex	Age (Years) at Enrollment	Race	Ethnicity
M01	F	17	White or Caucasian	Non-Hispanic or Latino
M02	F	38	Asian	Non-Hispanic or Latino
M03	F	24	White or Caucasian	Non-Hispanic or Latino
M04	F	42	White or Caucasian	Non-Hispanic or Latino
M05	F	18	White or Caucasian	Non-Hispanic or Latino
M06	F	19	White or Caucasian	Non-Hispanic or Latino
M07	M	32	White or Caucasian	Non-Hispanic or Latino
M08	M	8	White or Caucasian	Hispanic or Latino
M10	M	10	White or Caucasian	Non-Hispanic or Latino
M11	F	14	White or Caucasian	Non-Hispanic or Latino
M12	F	26	White or Caucasian	Non-Hispanic or Latino
M13	M	12	Black or African American	Non-Hispanic or Latino
M14	F	16	White or Caucasian	Hispanic or Latino
M15	M	5	White or Caucasian	Non-Hispanic or Latino
M16	M	5	Multiple Races	Non-Hispanic or Latino
M19	F	9	Black or African American	Non-Hispanic or Latino
M20	F	9	White or Caucasian	Non-Hispanic or Latino
M21	M	5	White or Caucasian	Non-Hispanic or Latino
M22	M	8	White or Caucasian	Non-Hispanic or Latino
M25	M	41	White or Caucasian	Non-Hispanic or Latino
M26	M	11	Multiple Races	Hispanic or Latino
M27	M	9	White or Caucasian	Hispanic or Latino
M28	F	30	Multiple Races	Non-Hispanic or Latino
M30	F	43	White or Caucasian	Non-Hispanic or Latino
M31	M	9	Black or African American	Non-Hispanic or Latino
M32	F	45	White or Caucasian	Non-Hispanic or Latino
M33	F	29	White or Caucasian	Non-Hispanic or Latino
M34	M	27	White or Caucasian	Non-Hispanic or Latino
M35	M	11	Other	Hispanic or Latino
M36	F	25	Other	Non-Hispanic or Latino
M37	M	18	Other	Non-Hispanic or Latino
M41	M	35	White or Caucasian	Non-Hispanic or Latino
M42	F	27	White or Caucasian	Non-Hispanic or Latino
M43	M	28	White or Caucasian	Non-Hispanic or Latino
M44	F	23	White or Caucasian	Non-Hispanic or Latino
M45	F	60	White or Caucasian	Non-Hispanic or Latino
M46	F	6	White or Caucasian	Hispanic or Latino
M47	F	23	White or Caucasian	Hispanic or Latino
M48	F	12	White or Caucasian	Hispanic or Latino
M49	M	6	White or Caucasian	Hispanic or Latino
M50	F	4	White or Caucasian	Non-Hispanic or Latino
M51	M	2	White or Caucasian	Non-Hispanic or Latino
M52	F	3	Multiple Races	Hispanic or Latino
M53	F	22	Asian	Non-Hispanic or Latino
M54	F	35	White or Caucasian	Non-Hispanic or Latino
M55	F	4	White or Caucasian	Non-Hispanic or Latino
M56	F	3	White or Caucasian	Non-Hispanic or Latino
M57	M	10 months	White or Caucasian	Non-Hispanic or Latino
M58	M	6	White or Caucasian	Non-Hispanic or Latino
M59	M	5	White or Caucasian	Non-Hispanic or Latino

M60	F	25	Other	Hispanic or Latino
M63	F	22	White or Caucasian	Non-Hispanic or Latino
M64	M	5	Multiple Races	Non-Hispanic or Latino
M65	M	6	Multiple Races	Non-Hispanic or Latino
M66	F	3	White or Caucasian	Hispanic or Latino
M67	M	11	White or Caucasian	Non-Hispanic or Latino
M68	M	45	White or Caucasian	Hispanic or Latino
M69	M	2	Asian	Non-Hispanic or Latino
M70	M	13	White or Caucasian	Non-Hispanic or Latino
M71	M	8	White or Caucasian	Non-Hispanic or Latino
M72	M	2	Asian	Hispanic or Latino
M73	F	3	White or Caucasian	Non-Hispanic or Latino
M74	F	37	White or Caucasian	Non-Hispanic or Latino
M76	F	10 months	White or Caucasian	Non-Hispanic or Latino
M77	F	2	White or Caucasian	Non-Hispanic or Latino
M78	M	11	White or Caucasian	Non-Hispanic or Latino

Supplementary Table 2 (3/11/2025)

Estimated Number of Actively Enrolled Participants to Complete Each Study Visit Before May 2026	
Baseline	66
18 months	62
36 months	50
54 months	0

ClinicalTrials.gov Protocol Registration and Results System (PRS)

750932-25	NCT05284006	Non-invasive Functional Assessment and Pathogenesis of Morquio A (NIFAMA)

NIH: Study Record | Beta ClinicalTrials.gov
Clinicaltrials.gov: Non-invasive Functional Assessment and Pathogenesis of Morquio A - Full Text View - ClinicalTrials.gov

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We have reached our goal of over 60 actively enrolled participants and will continue to have follow-up visits as they are interested.

1. We will not have enough to achieve 4 visits because of the initial delay due to COVID19 pandemic. We will accommodate the patients as much as possible to increase the number of visits in total. We will also resubmit the grant to accomplish 4 visits.
2. We will summarize the data and will publish each test data in the 5th year period.

3. We will continue to assay the biomarkers, including glycosaminoglycans (C6S and KS), Nt-pro-CNP, Collagen, and Inflammatory factor and will correlate these biological biomarkers with the clinical phenotype, especially skeletal symptoms. We will submit the manuscripts with data of biological biomarkers.

Discuss efforts to ensure that the approach is scientifically rigorous and results are robust and unbiased. Remember that significant changes in objectives and scope require prior approval of the agency (e.g., NIH Grants Policy Statement, 8.1.2).

Include any important modifications to the original plans. Provide a scientific justification for any changes involving research with human subjects or vertebrate animals. A detailed description of such changes must be provided under Changes.