Evaluation of a Biomarker that Differentiates Neuronopathic Forms of MPS I and MPS II

Nidal Boulos, Ph.D.

Director, Clinical Scientist

REGENXBIO Inc., Rockville, MD, USA

Introduction

Mucopolysaccharidoses are a heterogeneous group of disorders characterized by the accumulation of glycosaminoglycans (GAGs) due to deficiencies in lysosomal enzymes.

GAG accumulation leads to widespread cell and tissue damage and subsequent somatic and neurologic manifestations.

Neuronopathic forms of MPS exhibit elevated concentrations of the GAG heparan sulfate (HS) in the brain leading to central nervous system abnormalities and neurocognitive impairment.

There are no predictive or prognostic biomarkers in clinical use capable of differentiating neuronopathic from non-neuronopathic forms of MPS.

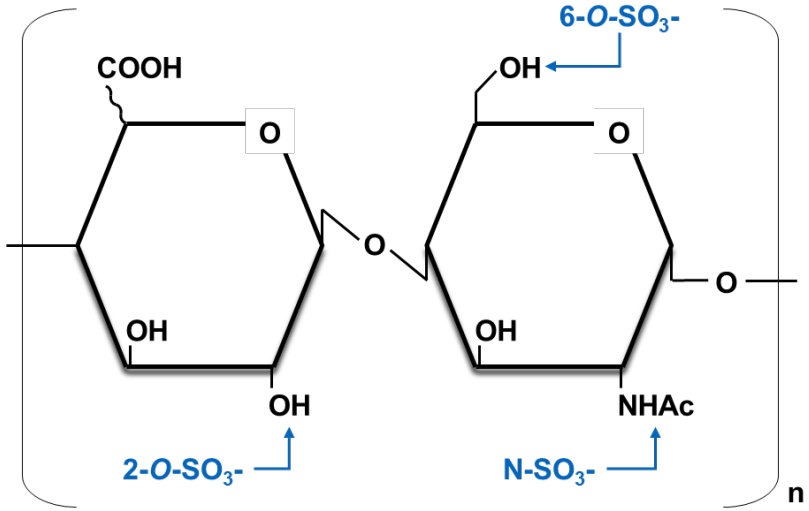
Objectives

Develop and validate a bioanalytical mass spectrometry method to quantify total HS as the sum of four disaccharides in human cerebrospinal fluid (CSF)

Characterize the composition of HS disaccharides (D2S6, D0S0, D0A6 and D0A0) in neuronopathic and non-neuronopathic MPS I and MPS II human CSF samples

Heparan Sulfate (HS) Structure & Biosynthesis

Basic Disaccharide Unit of HS



Uronic acid (glucuronic or iduronic)

Glucosamine

- HS chain is synthesized by the stepwise addition of alternating uronic and glucosamine residues
- Subsequent modifications include sulfation at various positions that generate N-sulfated domains (NS-domains), alternating intermediate sulfation regions (NA/NS-domains) and acetylated regions (NA-domains)
- Increased HS sulfation has been observed in brain tissue from MPS mice and in urine and serum from neuronopathic MPS patients
- Sulfated domains provide numerous docking sites for protein ligands and mediate protein interactions necessary for fundamental processes including brain neuropathology

NS= high sulfation domain

NA/NS= intermediate sulfation domain

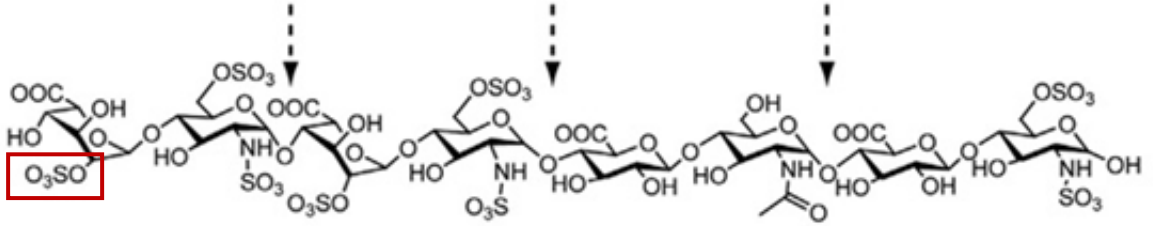
NA= None/Low sulfation domain

(Fuller *et al*., 2004; Holley *et al*., 2011; Wilkinson *et al*., 2012; De Pasquale & Pavone 2019; Maeda *et al*., 2011)

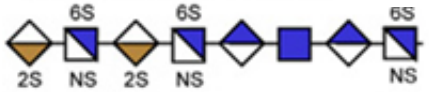
HS Disaccharide Composition Analysis by Heparinase Digestion

- I2S enzyme removes sulfate group at the 2 position from the terminal iduronic acid
- In MPS II, lack of I2S enzyme leads to accumulation of HS with 2-O-sulfated ends

Heparinase Sites for Cleavage

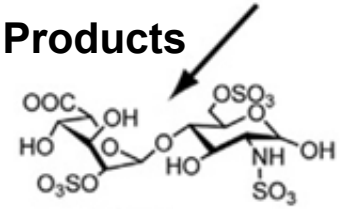


I2S6-I2S6-G0A0-G0S6

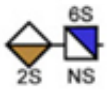


Heparan Sulfate Chain

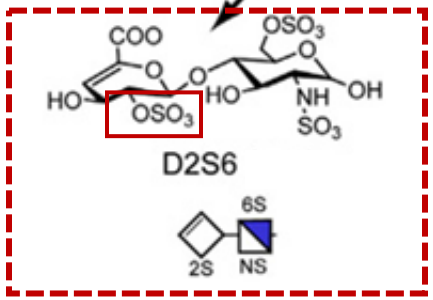
Disaccharide Products



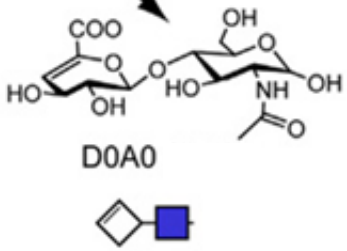
I2S6



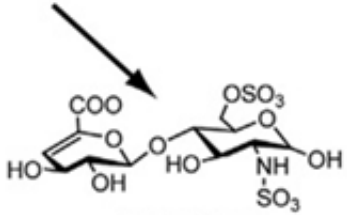
Non-reducing end generated from the terminal end



D2S6

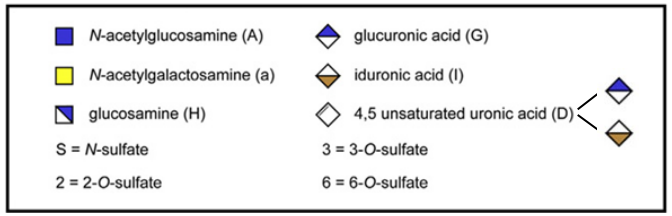


D0A0



D0S6

- D2S6 contains 2-O-sulfated ends which are cleaved by I2S enzyme.
- D2S6 is generated exclusively from internal I2S6 within the NS domains and is more abundant than NRE I2S6
- Quantification of D2S6 provides a method for monitoring I2S enzyme activity and reflects the abundance of NS domain



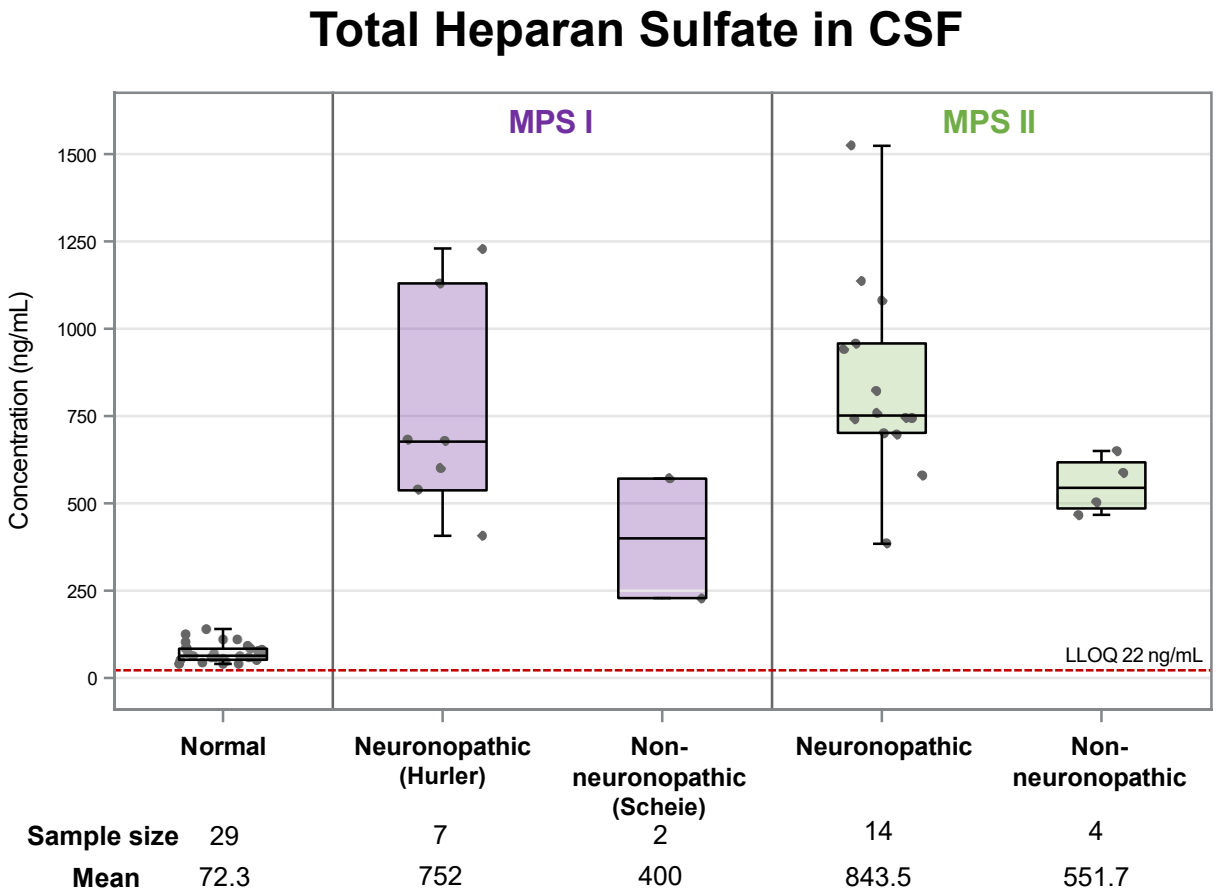
Modified from Lawrence *et al.*, 2014

Bioanalytical Method

- LC-MS/MS assay for the determination of HS disaccharides D0A0, D0S0, D0A6, and D2S6 in human cerebrospinal fluid (CSF) using isotope-labelled disaccharide derivative as internal standard
  - HS polysaccharides are digested by enzymes (Heparinase I, II and III) into disaccharides, which are derivatized before being analyzed with LC-MS
  - Separation of the disaccharides was achieved by reversed-phase HPLC
  - Disaccharides were detected using MS/MS
- Assay showed acceptable intra and inter-assay accuracy and precision

Total HS Concentration in Normal, MPS I and MPS II CSF Samples

- 29 CSF samples from healthy pediatrics and adults (age range 1 month to 89 years) were used to generate normative data using the validated bioanalytical assay
- CSF from MPS I (age range 1 to 28 years) and MPS II patients (5 months to 29 years) were analyzed
- Total HS calculated as the sum of the 4 disaccharides

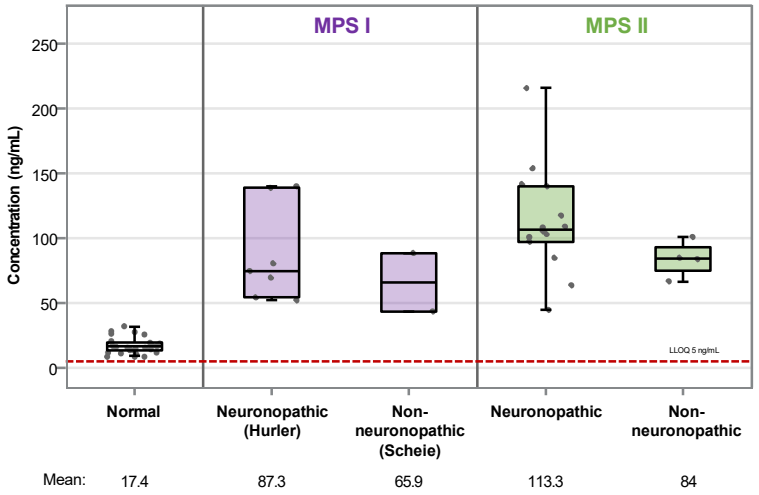


29 Normal CSF samples were purchased from BioIVT (n=20) and Discovery Life Sciences (n=6) or courtesy of Dr. Giugliani (n=3)
3 neuronopathic MPS II, 4 non-neuronopathic MPS II and all MPS I samples are courtesy of Dr. Giugliani
11 neuronopathic MPS II samples are from RGX-121-101

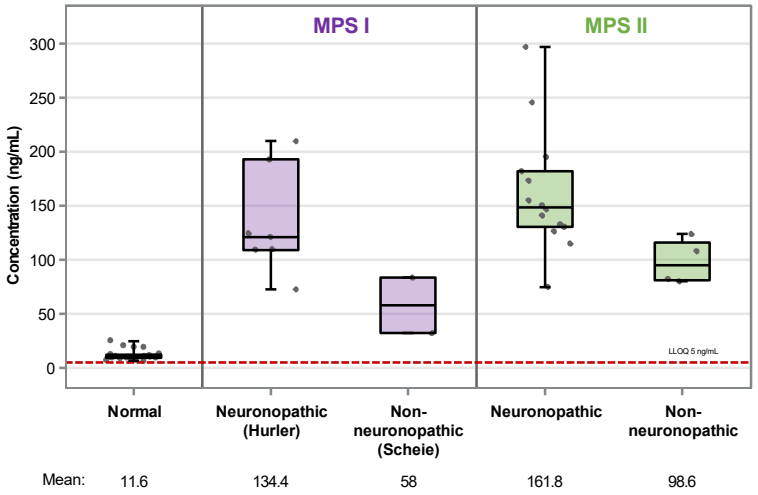
HS Composition in Normal, MPS I and MPS II CSF Samples

HS composition revealed differential disaccharide concentrations in MPS I and MPS II compared to normal controls

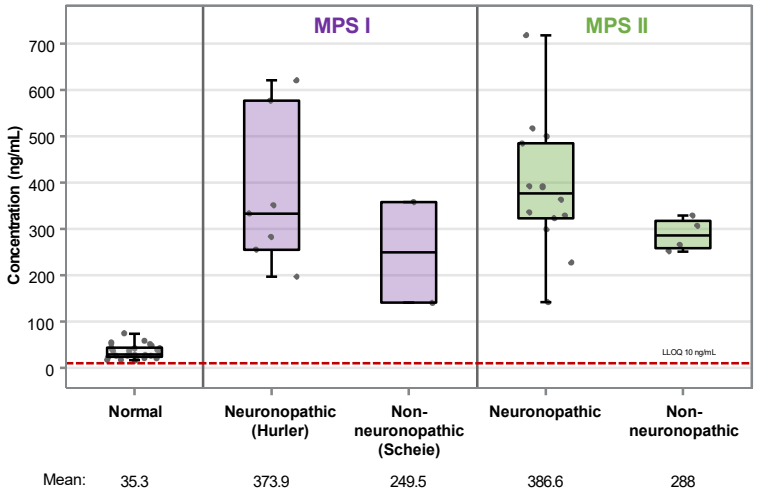
D0S0 Disaccharide



D0A6 Disaccharide



D0A0 Disaccharide



Sample size: Normal (n=29), Hurler (n=7), Scheie (n=2), Neuronopathic MPSII (n=14), non-neuronopathic MPSII (n=4)
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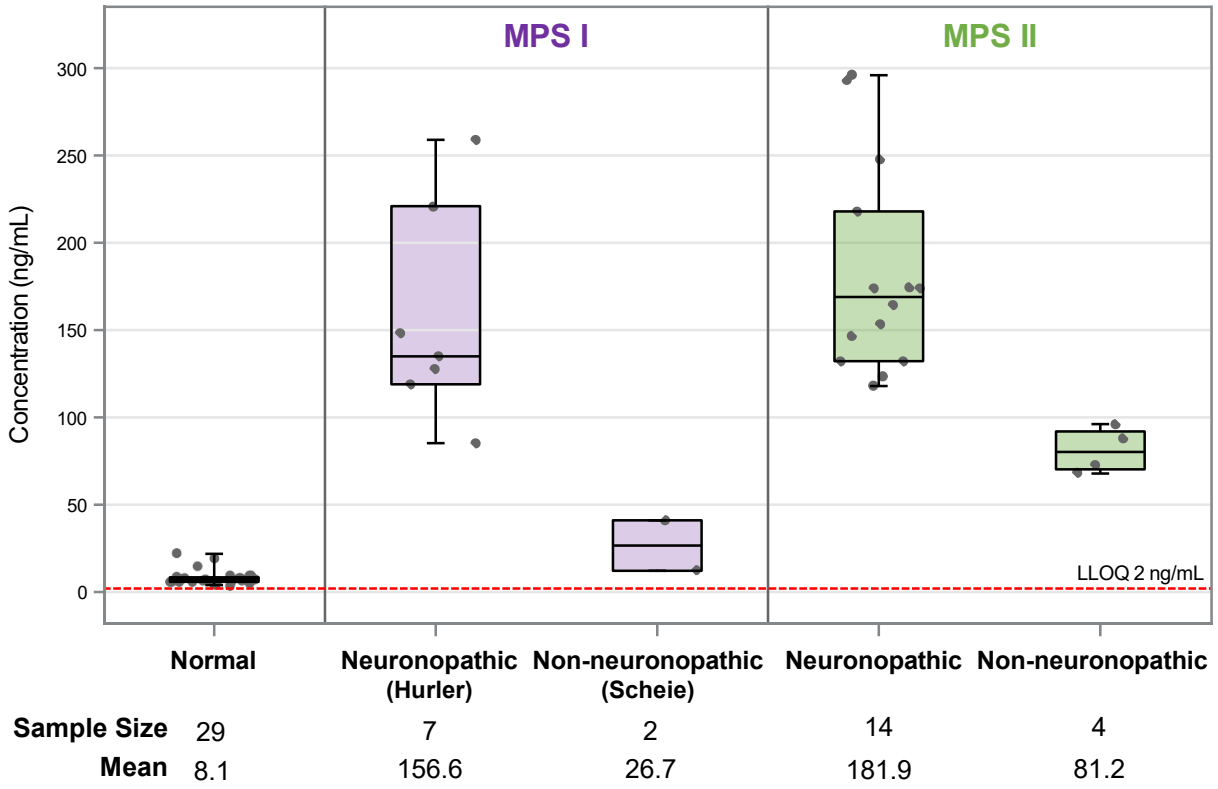
HS Composition in Normal, MPS I and MPS II CSF Samples

D2S6 Disaccharide



	Normal	Neuronopathic (Hurler)	Non-neuronopathic (Scheie)	Neuronopathic	Non-neuronopathic
Sample Size	29	7	2	14	4
Mean	8.1	156.6	26.7	181.9	81.2

D2S6 concentrations were significantly elevated in neuronopathic compared to non-neuronopathic MPS I and MPS II and to normal controls

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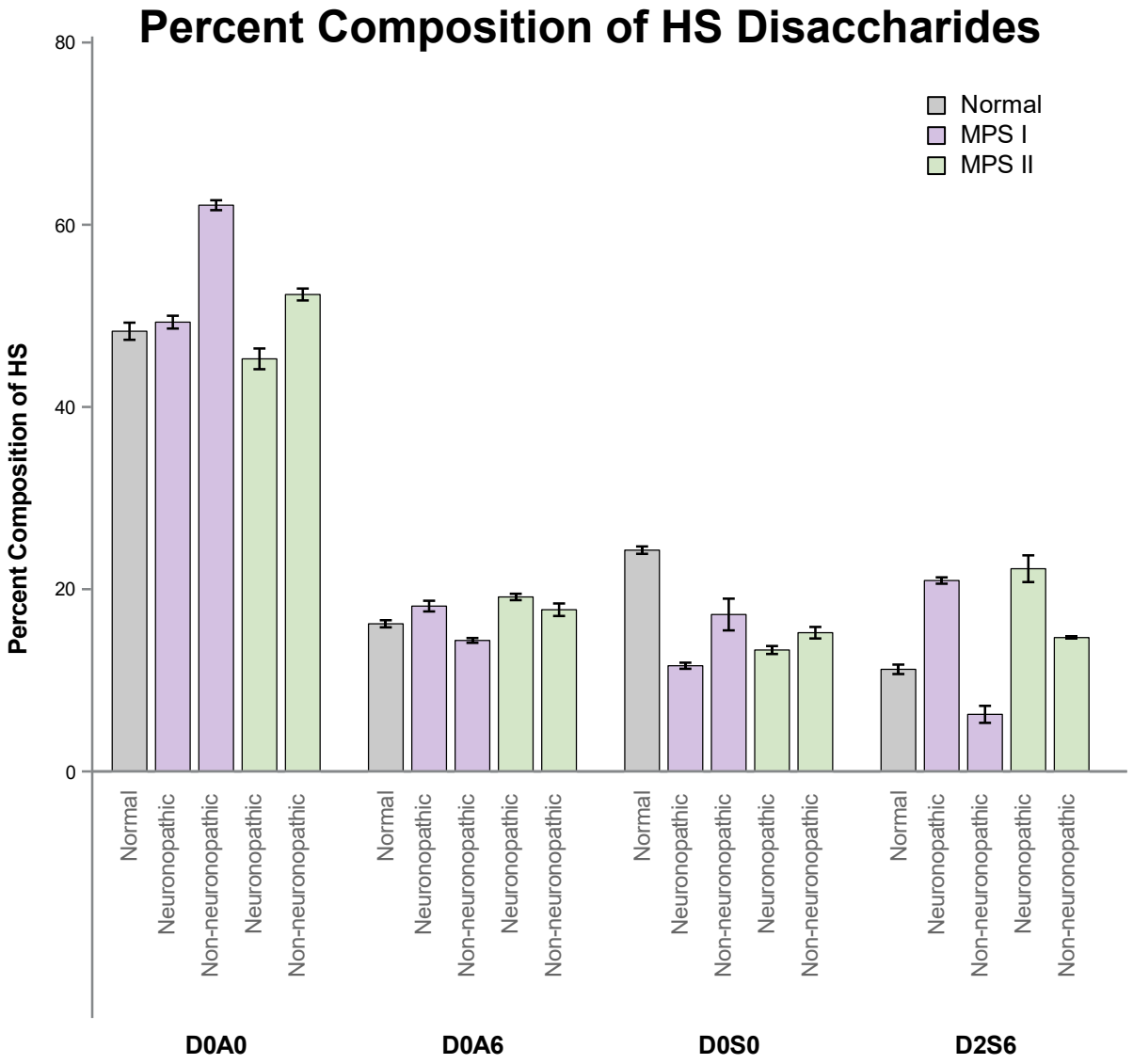
Percent Composition in Normal, MPS I and MPS II CSF Samples



- Composition of HS may play a role in disease pathogenesis
- HS composition analysis showed:
  - D0A0 as the most abundant disaccharide in CSF
  - D2S6 demonstrated the highest increase in CSF of neuronopathic MPS compared to healthy donors
- D2S6 has been shown as the HS analyte most responsive to gene therapy in preclinical models of neuronopathic MPSs, and associated with reductions in neuroinflammation and correction of behavioral abnormalities (Gleitz *et al*., 2018, Sergijenko *et al*., 2013)

Conclusions

The bioanalytical assay developed and validated for the quantitation of HS in CSF showed acceptable intra and inter-assay accuracy and precision.

Increase in the four disaccharides measured was observed in MPS I and MPS II CSF compared to normal control.

D2S6, a fully sulfated disaccharide, was significantly increased in neuronopathic compared to non-neuronopathic MPS I and MPS II and to normal controls.

To our knowledge, this is the first study to identify a potential diagnostic biomarker in CSF that may differentiate neuronopathic from non-neuronopathic MPS and may be useful to monitor response to therapies.

Acknowledgements

Co-authors

- Zhuchun Wu, Regenxbio
- Yoonjin Cho, Regenxbio
- Caroline Mulatya, Regenxbio
- Maria Fuller, University of Adelaide
- Jerome Ausseil, Université Toulouse III
- Luigi Pavone, University of Naples
- Roberto Giugliani, Hospital de Clinicas de Porto Alegre
- Michele Fiscella, Regenxbio
- Marie-Laure Névoret, Regenxbio

RGX-121 Clinical Study

- Clinical Investigators
- Clinical Project Managers
- Study Coordinators and Research Teams at Clinical Sites

The MPS I and MPSII patients and their families