

Adaptation of a UPLC-MS/MS Method for the Analysis of Glycosaminoglycan Fragments for the Diagnosis of Mucopolysaccharidosis in a UK Clinical Laboratory

Kate Neal, Oliver Parkes, Karen Tylee, Teresa Wu

Willink Metabolic Lab, Saint Mary's Hospital



Mucopolysaccharidoses (MPS)

- **Lysosomal Storage Disorder**
- Deficiencies in one of **11 lysosomal acid hydrolases**
- Accumulation of **partially degraded glycosaminoglycans (GAGs)** in various tissues → Progressive multisystemic/neurological symptoms
- **MPS subtypes** = I, II, IIIA, IIIB, IIIC, IIID, IVA, IVB, VI, VII + IX
- Treatments include **enzyme replacement therapy (ERT)**, **hematopoietic stem cell transplant (HSCT)** and **gene therapy** (clinical trials)
- Current first line **diagnosis** in our lab is by **2D electrophoresis** of extracted GAGs from urine
- Rise in **Mass Spectrometry (MS)-based diagnostics**

Adapted Methodology

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BRIEF COMMUNICATION

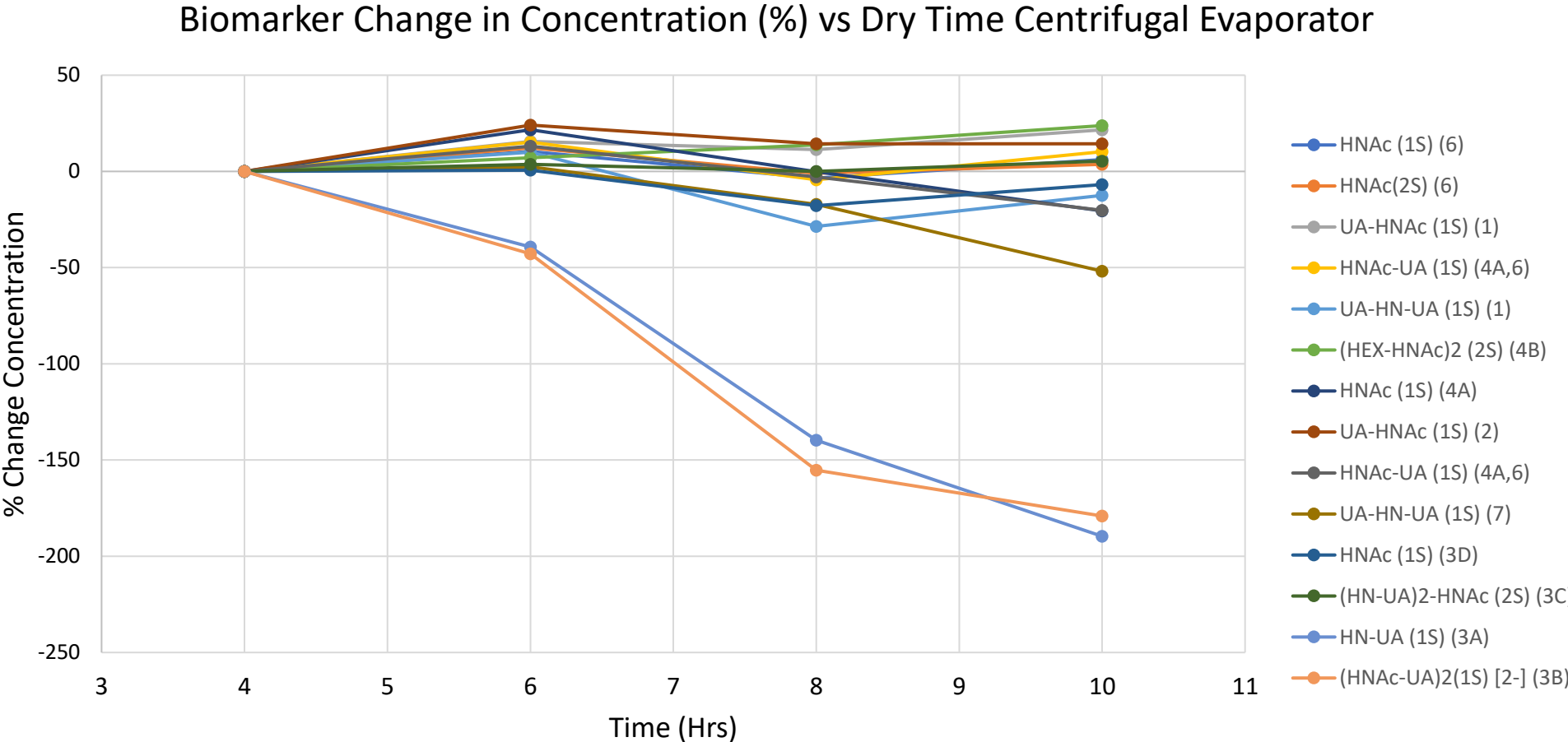
**Genetics
in Medicine**

Disease and subtype specific signatures enable precise diagnosis of the mucopolysaccharidoses

Jennifer T. Saville, PhD¹, Belinda K. McDermott, BSc¹, Janice M. Fletcher, MD^{1,2} and Maria Fuller, PhD^{1,2}

Protocol	Step
Dry	Lyophilization
Derivatization	1-phenyl-3-methyl-5-pyrazolone (PMP)
Sample Clean-up	LLE w/ Chloroform
Analysis	UPLC-MS/MS

Sample Dry Down using Centrifugal Evaporator



Adapted Methodology

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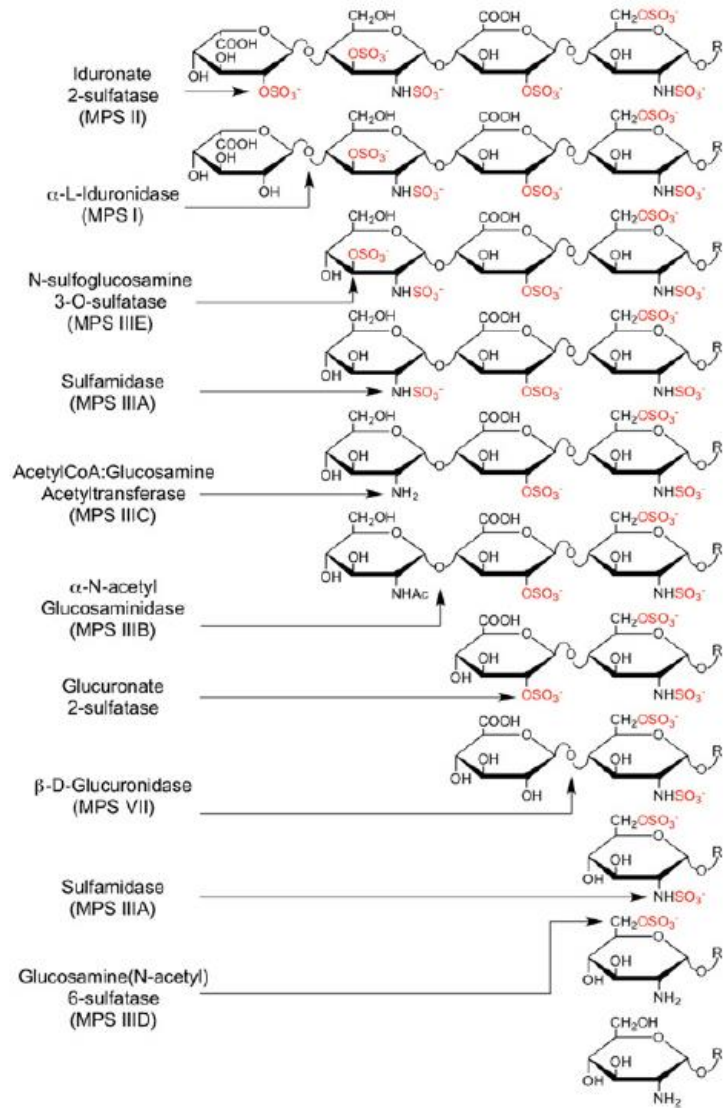
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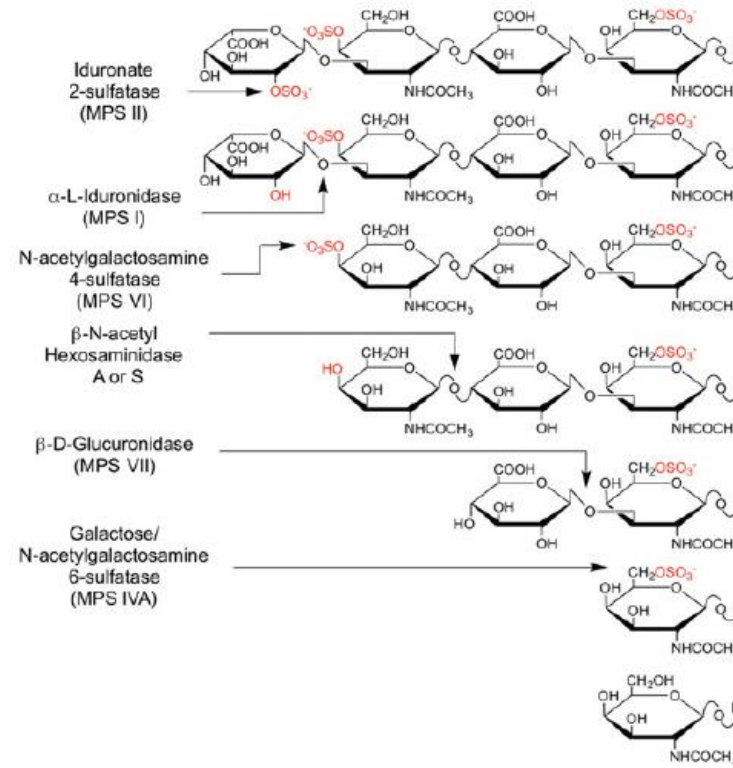
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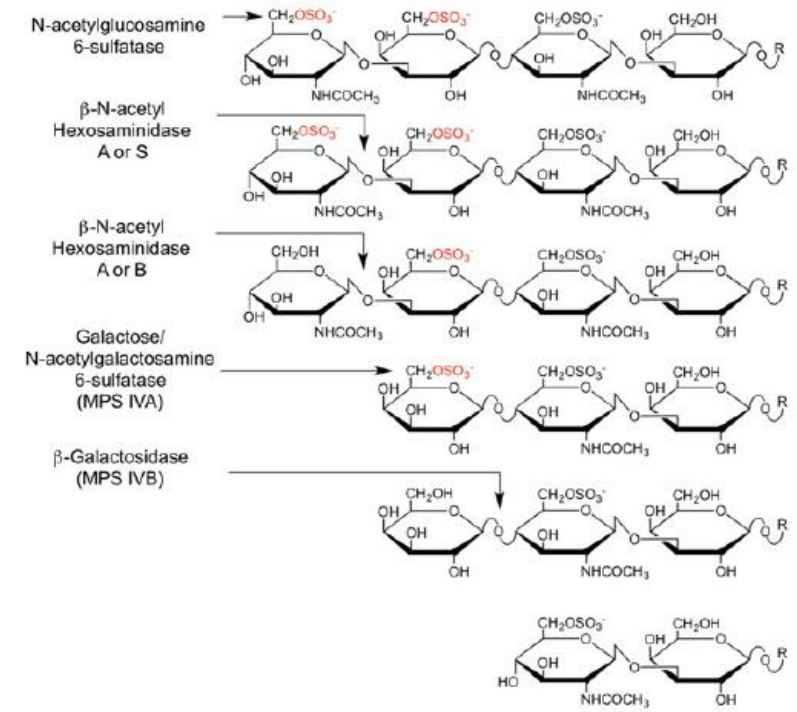
Heparan Sulfate



Chondroitin Sulfate



Keratan Sulfate



Solid Phase Extraction (SPE)

Ion-Exchange Guidelines

First, determine analyte type.

Then, follow corresponding arrows down for recommended particle and mobile phase pH.

Analyte Type	Weak ACID e.g., $pK_a \sim 5$		Strong ACID	Weak BASE e.g., $pK_a \sim 10$		Strong BASE
Charge State vs. pH*	No charge at $pH < 3$	— [anion] at $pH > 7$	— [anion] Always Charged	+	No Charge at $pH > 12$	+

*Note: pH Ranges are approximate. They will depend upon specific analyte and particle characteristics.

Adapted Methodology

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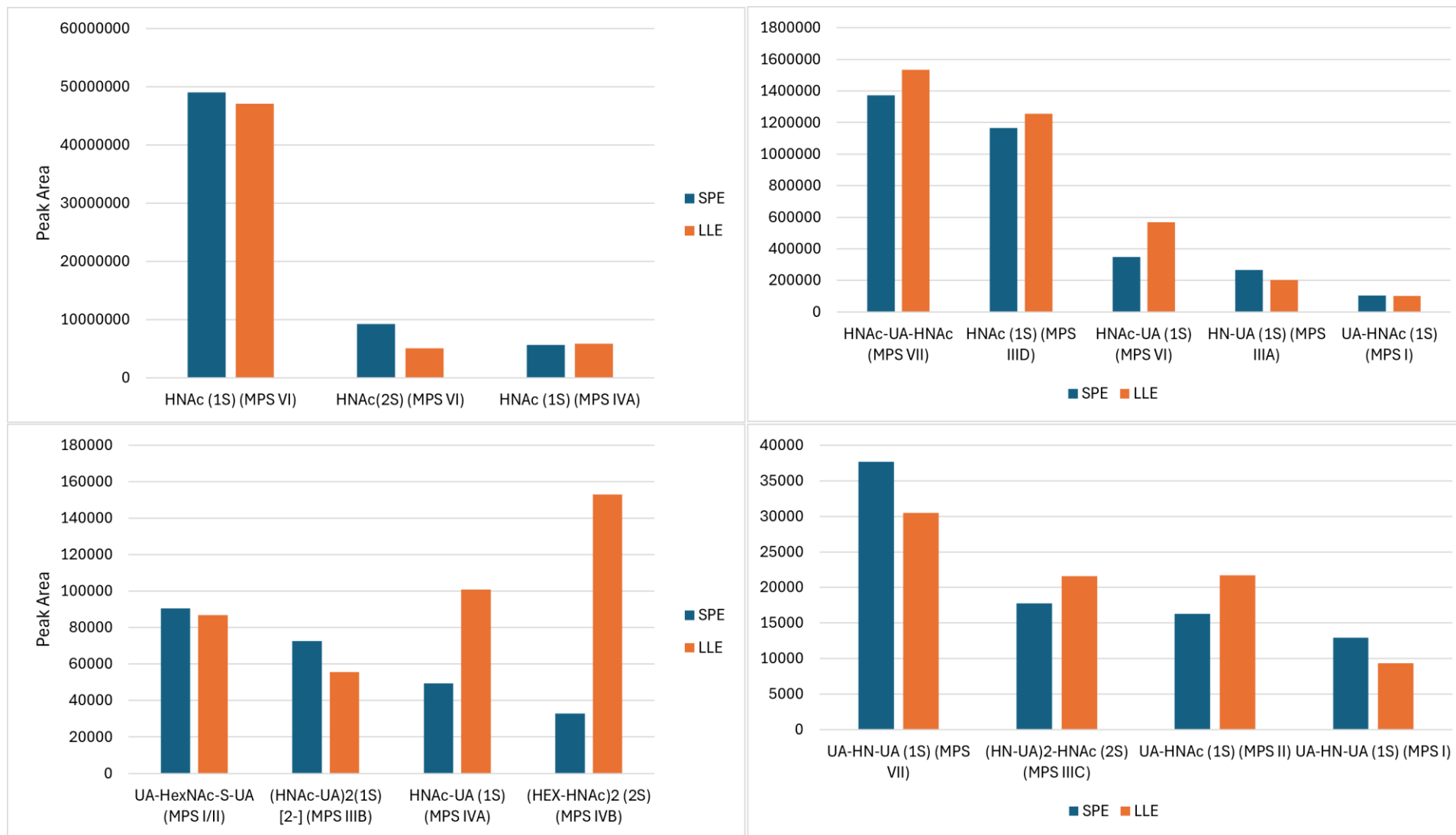
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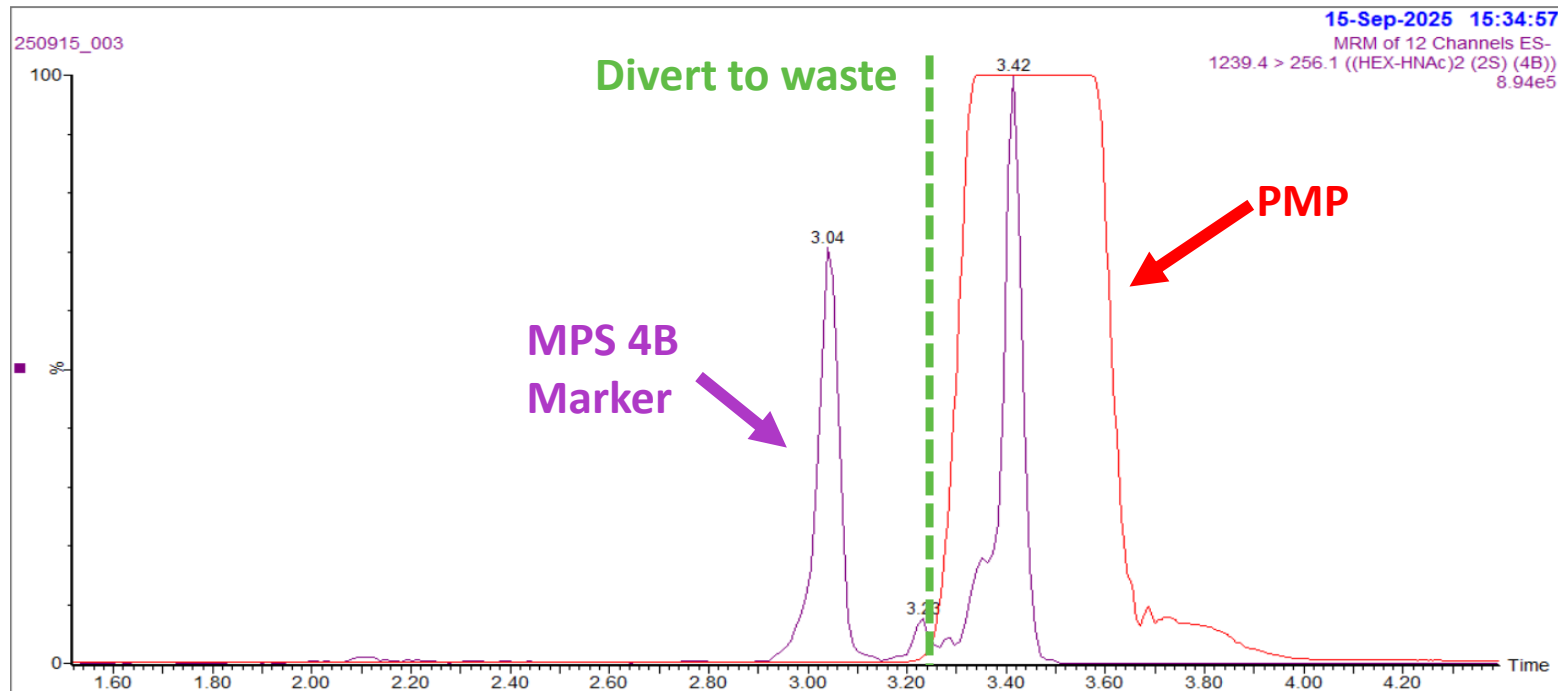
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Protocol Comparison



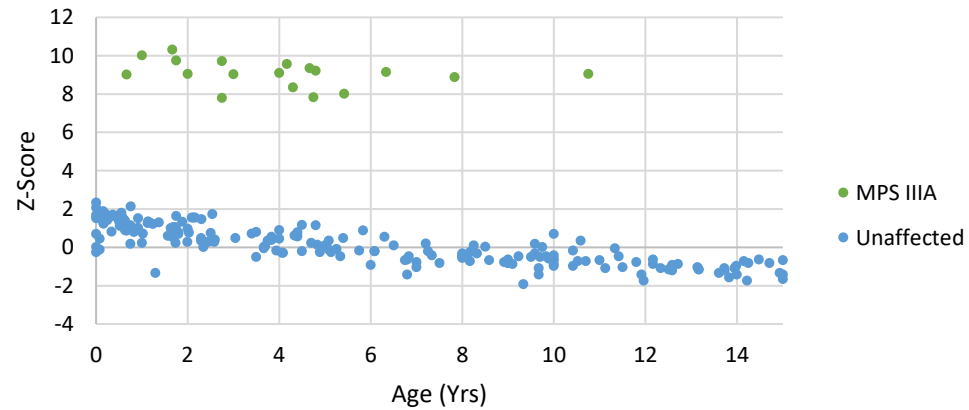
Limitations

- Measured values semi-quantitative
- No commercially available standards – Yet!!
- PMP removal inferior with SPE than LLE

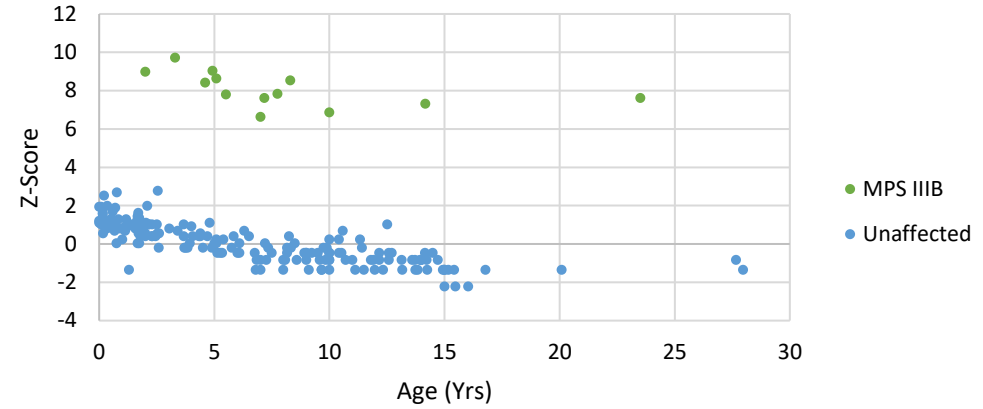


MPS III

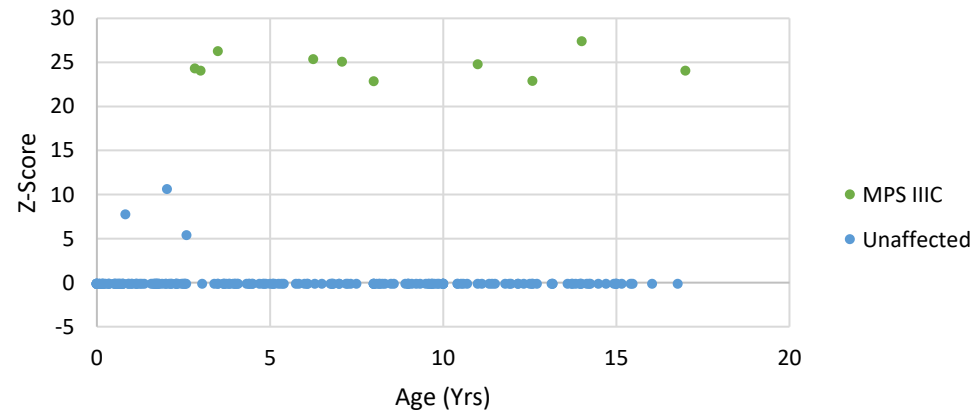
HN-UA (1S) MPS IIIA, n=18



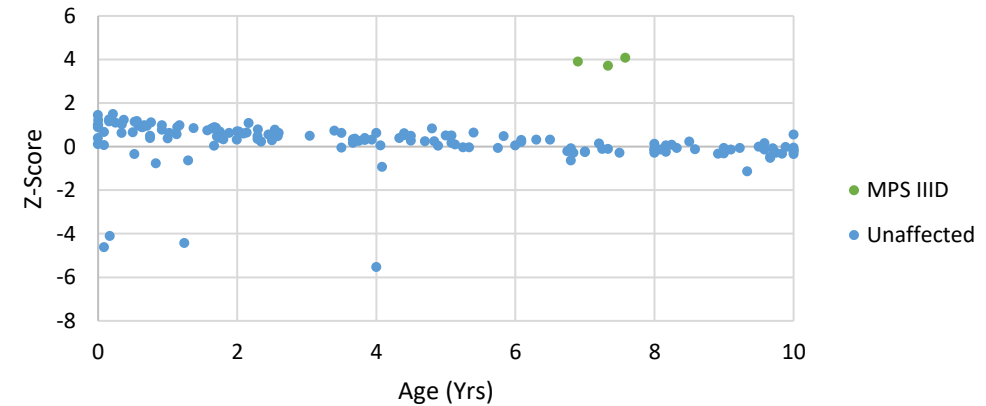
(HNAc-UA)2(1S) [2-] MPS IIIB, n=13



(HN-UA)2-HNAc (2S) MPS IIIC, n=10



HNAc (1S) MPS IIID, n=3

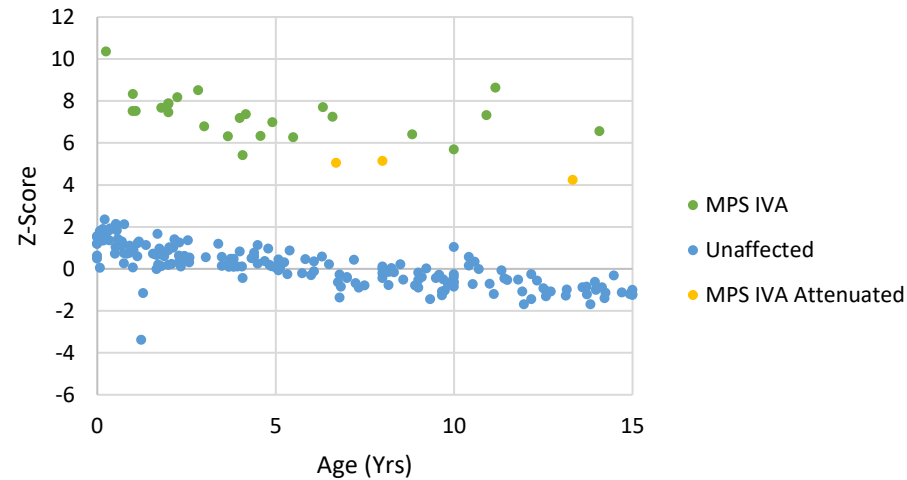


Total Affected MPS, n=142

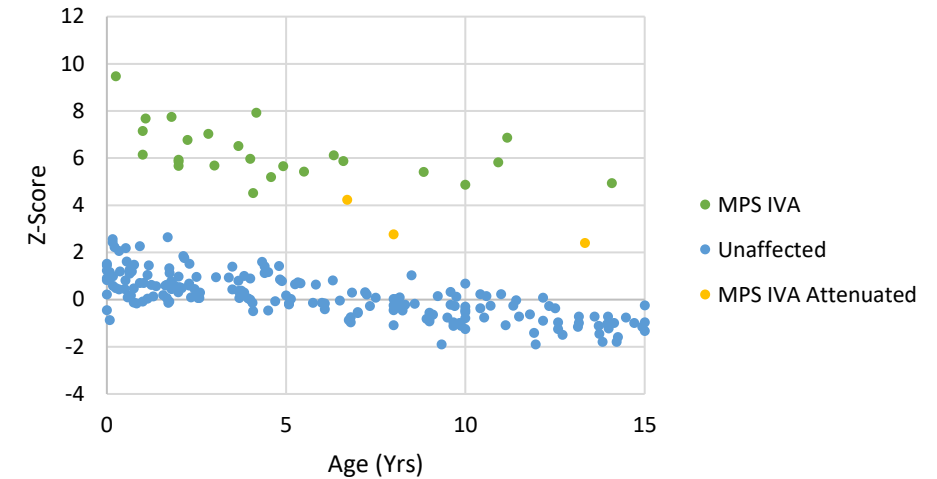
Total Unaffected, n=205 (0-80 yrs)

MPS IVA + IVB/GM1 Gangliosidosis

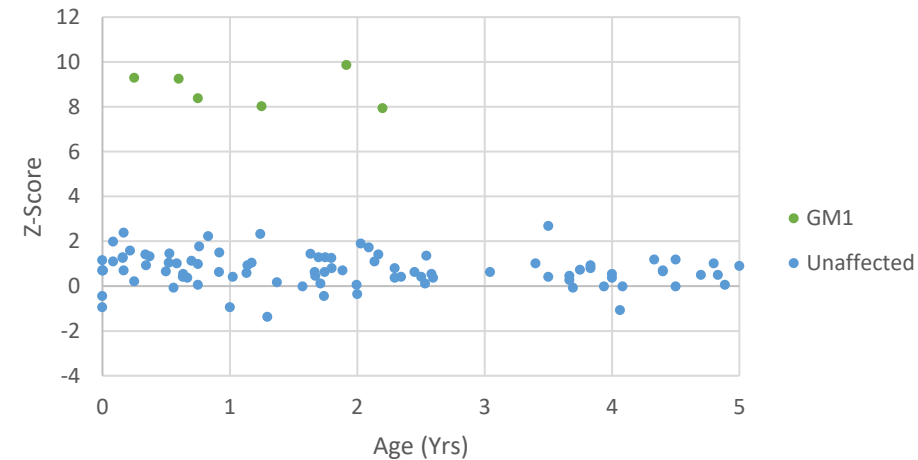
HNAC (1S) MPS IVA, n=29



HNAC-UA (1S) MPS IVA, n=29

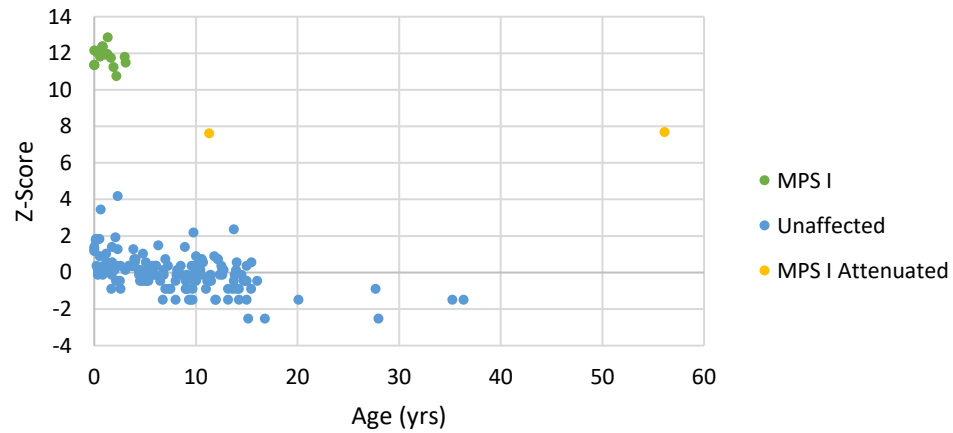


(HEX-HNAC)2 (2S) MPS IVB

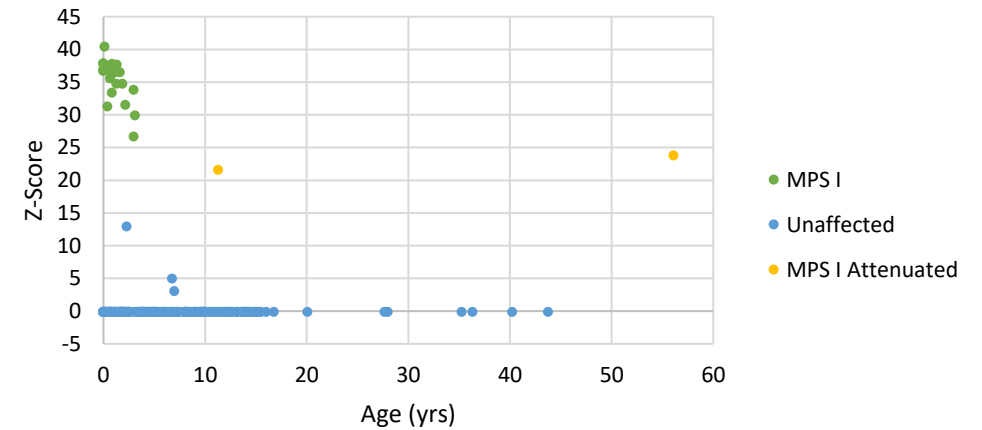


MPS I, II and VII

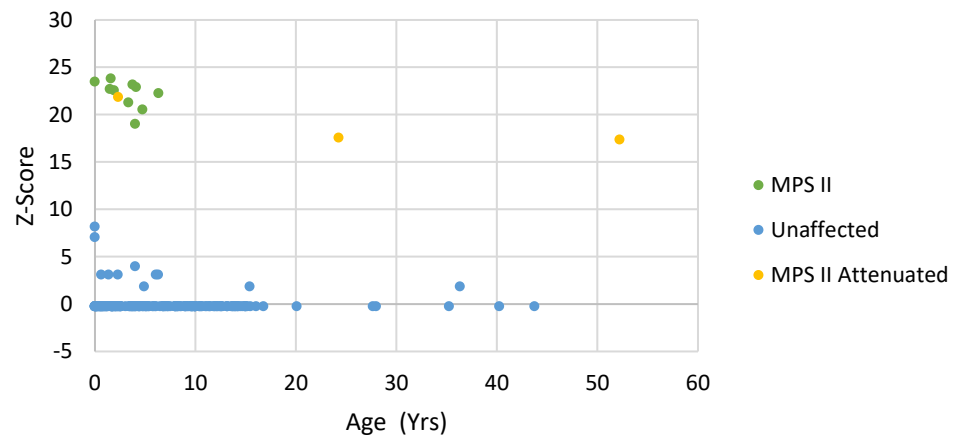
UA-HNAc (1S) MPS I, n=24



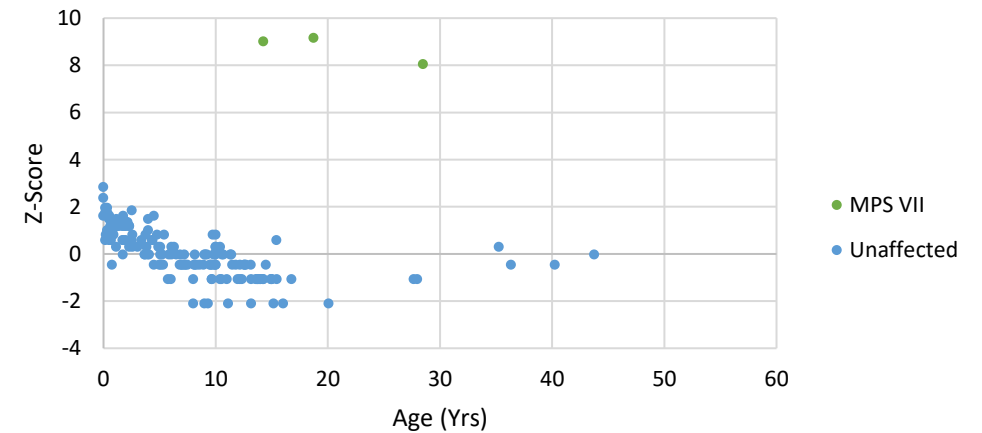
UA-HN-UA (1S) MPS I, n=24



UA-HNAc (1S) MPS II, n=16

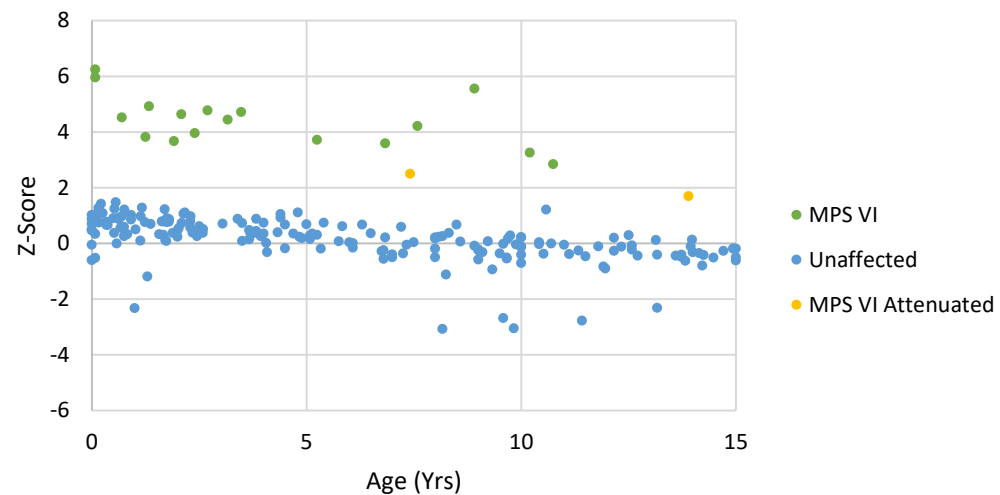


UA-HN-UA (1S) MPS VII, n=3

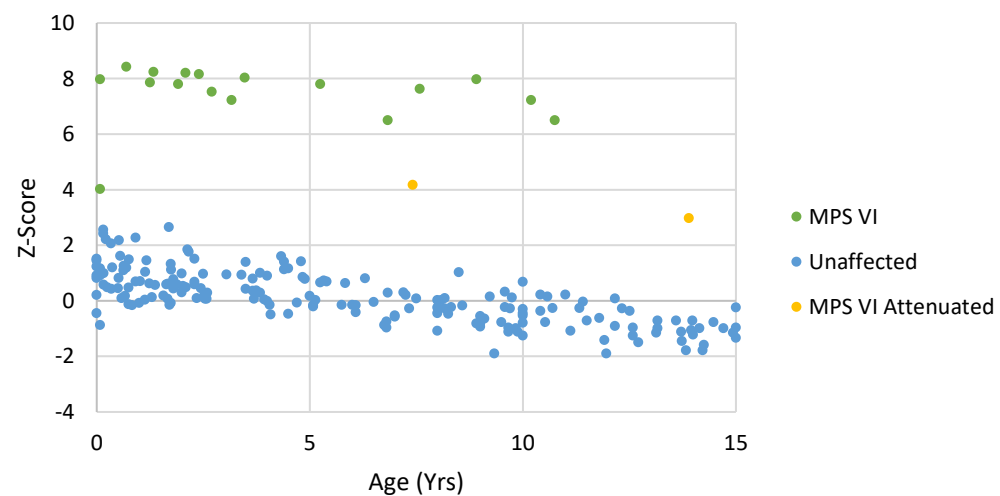


MPS VI

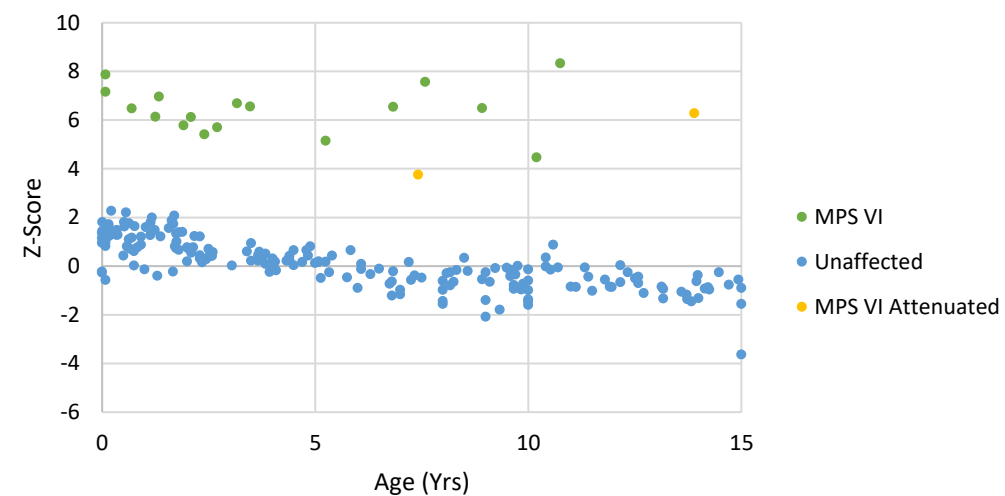
HNAC (1S) MPS VI, n=20



HNAC-UA (1S) MPS VI, n=20



HNAC (2S) MPS VI, n=20



Conclusions

- Clinical validation data shows this adapted method is effective at diagnosing MPS
- Method will be implemented as a first line diagnostic test for MPS in our lab once clinical validation is complete
- Detailed manuscript and protocol currently in submission – other labs can adopt this methodology
- Also working on a method to analyze GAG fragments by MS in DBS for newborn screening

Acknowledgements

- Oliver Parkes, Karen Tylee, Teresa Wu, James Cooper
- GEM Appeal Charity – Equipment, Funding



The background is a solid magenta color. It features several large, light pink triangles pointing upwards, some of which are partially overlapping. There are also three dark purple circles of different sizes scattered across the top half of the image. The text "Thank you for listening!" is centered in the middle of the image in a white, bold, sans-serif font.

Thank you for listening!