

## INVESTIGATION AND ANALYSIS OF STORED OLIGOSACCHARIDES IN MUCOPOLYSACCHARIDOSIS TYPE IIIA

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Mucopolysaccharidosis (MPS) type IIIA (Sanfilippo syndrome), one of at least 40 inherited lysosomal storage disorders, is characterised by a deficiency of the exoenzyme sulphamidase needed for the degradation of the polysaccharide heparan sulphate (HS) to inorganic sulphate and monosaccharides. An inability to hydrolyse non-reducing end HS N-sulphate esters leads to the accumulation of partially degraded HS fragments in lysosomes of affected cells and their excretion in the urine. Affected individuals appear clinically normal at birth, but usually develop severe central nervous system degeneration between 2 to 6 years of age. As therapy options become available, the need for early diagnosis, prediction of severity and methods to monitor therapy will become important for the treatment and management of these patients. To address this need, we aimed to identify a range of sulphated oligosaccharides in MPS IIIA urine using electrospray ionisation-tandem mass spectrometry (ESI-MS/MS).

Sulphated oligosaccharides isolated from the urine of a MPS IIIA patient using anion exchange and gel filtration chromatography were derivatised with 1-phenyl-3-methyl-5-pyrazolone, analysed by ESI-MS/MS and identified based on a mass to charge ( $m/z$ ) ratio, and further characterised using product ion scans in negative ion mode. A number of sulphated oligosaccharides, ranging from di- to decasaccharide (10mer), were identified and shown to have non-reducing end glucosamine N-sulphate, susceptible to digestion with recombinant sulphamidase. Nitrous acid (pH 1.5) digestion determined partial structural information for these oligosaccharides. We propose that the urinary sulphated oligosaccharide fragments are products of endoglycosidase activities on HS followed by a series of exoenzyme activities that stop at the first non-reducing end glucosamine N-sulphate. We consider these oligosaccharides as potential biochemical markers for the diagnosis and prediction of clinical severity of MPS IIIA patients and anticipate that these findings will lead to improvements in diagnosis and monitoring of therapy for this disorder.

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