

Prediction of neuropathology in mucopolysaccharidosis I patients

Maria Fuller^{a,b,*}, Doug A. Brooks^{a,b}, Marco Evangelista^a, Leanne K. Hein^a,
John J. Hopwood^{a,b}, Peter J. Meikle^{a,b}

^a *Lysosomal Diseases Research Unit, Department of Genetic Medicine, Women's and Children's Hospital, 72 King William Road, North Adelaide, SA 5006, Australia*

^b *Department of Paediatrics, University of Adelaide, Adelaide, SA 5005, Australia*

Received 18 July 2004; received in revised form 7 September 2004; accepted 7 September 2004
Available online 11 November 2004

Abstract

Mucopolysaccharidosis I is a lysosomal storage disorder caused by a deficiency of the lysosomal hydrolase α -L-iduronidase, which is required for the degradation of heparan sulphate and dermatan sulphate. Given the wide spectrum of disease severity in mucopolysaccharidosis I patients, one of the challenges for managing the disorder is to accurately predict clinical phenotype. Enzyme replacement therapy by intravenous infusion is unlikely to make a significant impact on central nervous system pathology and patients displaying this clinical manifestation may respond better to bone marrow transplantation. In order to predict whether mucopolysaccharidosis I patients are going to develop central nervous system pathology, we investigated a number of biochemical parameters in cultured skin fibroblasts from patients of different genotype/phenotype. Residual levels of α -L-iduronidase activity and protein were determined using sensitive immune-quantification assays and fibroblast cell extracts from patients with central nervous system pathology generally had lower levels of α -L-iduronidase than patients with no evidence of central nervous system disease. A total of 15 oligosaccharides, derived from heparan sulphate and dermatan sulphate, was measured in fibroblast extracts using electrospray-ionisation tandem mass spectrometry and all were shown to discriminate mucopolysaccharidosis I from controls. Of these, two trisaccharides were able to group patients based on the presence/absence of central nervous system disease. Moreover, a ratio of α -L-iduronidase activity to these trisaccharides provided clear discrimination between mucopolysaccharidosis I patients with and without central nervous system pathology. We suggest that this type of analysis may be very useful for predicting disease severity in mucopolysaccharidosis I patients.

© 2004 Elsevier Inc. All rights reserved.

Keywords: Mucopolysaccharidosis I; α -L-Iduronidase; Heparan sulphate; Dermatan sulphate; Oligosaccharides; Central nervous system pathology; Electrospray-ionisation tandem mass spectrometry

Introduction

The mucopolysaccharidoses (MPS) are a group of genetic disorders characterised by a deficiency in one of a series of lysosomal enzymes, required to degrade glycosaminoglycans (GAGs). MPS I (Hurler syndrome; Scheie syndrome; McKusick 25280) is the most common of the MPS disorders in Caucasians and has an incidence

of 1:120,000 live births [1]. MPS I is caused by a deficiency of α -L-iduronidase (IDUA; EC 3.2.1.76), which is required for the sequential degradation of the GAGs heparan sulphate (HS) and dermatan sulphate (DS). An IDUA deficiency results in the failure to remove α -L-iduronic acid residues from the non-reducing end of these GAGs, causing the accumulation of these substrates in the lysosomes of affected cells. This lysosomal storage leads to the chronic and progressive deterioration of cells, tissues and organs, and the urinary secretion of partially degraded GAGs [2].

* Corresponding author. Fax: +61 8 8161 7100.

E-mail address: maria.fuller@adelaide.edu.au (M. Fuller).

MPS I patients display a wide spectrum of clinical presentation, ranging in gradations from the archetypal severe Hurler syndrome to the more attenuated Scheie syndrome [2]. Classically, three clinical descriptions have been documented for MPS I patients, with Hurler–Scheie syndrome representing an intermediate clinical presentation between the two extremes listed above. Hurler syndrome patients are usually diagnosed within the first year of life and, if untreated, usually die before 10 years of age. The disease process is progressive with symptoms that include skeletal and joint deformities, coarse facial features, corneal clouding, hernia, cardiac disease, hepatosplenomegaly, fatigue, hydrocephalus, enlarged tongue, and mental retardation. Scheie syndrome patients have a more attenuated clinical phenotype, with variable presentation and delayed onset of the latter clinical symptoms, but these patients can have normal intelligence, stature, and lifespan. Nonetheless, there are many Hurler–Scheie patients whose clinical presentation is broad and lies between the two extremes of Hurler and Scheie.

Therapies are available for MPS I patients. Bone marrow transplantation has some value in treating both somatic tissue and central nervous system (CNS) pathology, however this procedure carries significant risk [3]. For effective treatment of Hurler syndrome patients *via* bone marrow transplantation, early diagnosis and treatment, before the onset of irreversible pathology, is essential. Enzyme replacement therapy has recently become available for MPS I patients, but as intravenous infusion of enzyme is not effective in accessing sites of brain pathology, due to the blood–brain barrier, it is currently limited to the treatment of patients without CNS disease [4,5].

When patients are identified early in the disease process, as a result of astute clinicians or potentially following the introduction of newborn screening programs, the decision of therapy options will be arduous in the absence of methods to predict phenotype, in particular CNS pathology. There are currently no accurate methods for assessing the clinical phenotype of the patient or the rate of disease progression following treatment, compared to that expected had the patient remained untreated. A suitable set of biomarkers is required to effectively predict disease severity and progression in presymptomatic MPS I patients to decide on the most effective therapeutic strategy, and additionally to monitor patients receiving therapy.

Approximately 89 IDUA gene mutations have been reported¹ in MPS I patients and mutation analysis has enabled the prediction of clinical severity in some patients, mainly at the severe end of the clinical spectrum [6–8]. For example, two relatively common missense mutations W402X and Q70X have been associated with

a severe Hurler syndrome presentation. Collectively missense alleles account for over three-quarters of the MPS I mutant alleles in Caucasians, but in most cases, clinical criteria are still used to define the patient phenotype [9]. This reflects the overall conclusion that genotype is often not informative for predicting clinical severity and disease progression in MPS I patients.

In this paper, we report on the use of specific substrates, in combination with genotype, level of mutant protein and residual IDUA activity to provide a coordinated biochemical picture in relation to disease severity in MPS I. We have measured DS- and HS-derived oligosaccharides by electrospray ionisation-tandem mass spectrometry (ESI-MS/MS) and used sensitive immunquantification assays to measure IDUA activity and protein in cultured skin fibroblasts from MPS I patients. The ability to evaluate patients using biochemical parameters will be important in deciding on therapeutic alternatives, such as bone marrow transplantation and/or enzyme replacement therapy for MPS I and for monitoring patients receiving therapy.

Experimental

Materials

Cell culture materials were from Gibco BRL (Glen Waverley, Vic) and JRH Biosciences (Lenexa, KS). Recombinant human IDUA was prepared from a CHO-K1 expression system as previously described [10]. Sheep anti-IDUA polyclonal antibody was produced against recombinant human IDUA and affinity purified against the same protein as described for the anti- α -glucosidase polyclonal antibody [11]. The affinity purified polyclonal antibody was labelled with Eu³⁺ as described previously [12]. Microtitre plates and DELFIA wash and assay buffers were obtained from Wallac (Turku, Finland). All solvents used for LC–MS were HPLC grade (Unichrom), and ammonia (28%) and formic acid (90%) were of analytical grade (Univar), all from APS Finechem (Seven Hills, NSW, Australia). Solid phase co-polymeric (C18 and aminopropyl) 50 mg extraction cartridges were from United Chemical Technologies (Bristol, PA) and 1-phenyl-3-methyl-5-pyrazolone was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). The internal standard, 4-deoxy-L-threo-hex-4-enopyranosyluronic (1 → 3) *N*-acetylgalactosamine-4-sulphate (UA-GalNAc4S), was purchased from Sigma Chemical Co. (St. Louis, MO).

Cell culture

Human diploid fibroblasts were established from primary skin biopsies submitted to this Hospital for diagnosis [13]. Human unaffected and MPS I skin fibroblasts were cultured in BME containing 10% (v/v) FCS

¹ <http://archive.uwcm.ac.uk/uwcm/mg/hgmd0.html>

in 75-cm² culture flasks in a humidified atmosphere containing 5% CO₂ at 37 °C. For fibroblasts cultured for 10-weeks post-confluence, the cells remained in the same culture flask and the media were replenished at weekly intervals. At culture times one- and 10-weeks post-confluence, cells were trypsinised and harvested by centrifugation (1000g for 5 min). After two 10 ml washes in phosphate-buffered saline, the pellet was resuspended in 20 mM Tris, 0.5 M NaCl, pH 7, and sonicated for 20 s. Total cell protein was determined by the method of Lowry et al. [14].

MPS I patient phenotype

The MPS I fibroblast cell lines used in this study were grouped as to whether or not they were from patients who developed CNS disease. The primary basis for this classification was clinical observations, which reported normal intelligence for five of the 13 patients at four-years of age or older, and symptoms pertaining to CNS disease in the other eight patients. We noted that many clinical observations can be subjective and therefore have only reported on age at diagnosis, genotype and presence of CNS pathology.

Immune-quantification of IDUA protein and activity

Microtitre plates were coated with affinity purified sheep anti-IDUA polyclonal antibody (5 µg/ml in 0.1 M NaHCO₃, pH 8.5, 100 µl/well) overnight at 4 °C. The plates were then washed twice with DELFIA wash buffer and blocked by incubating with 0.02 M Tris–HCl, pH 7.0, containing 0.25 M NaCl, and 1% (w/v) ovalbumin (200 µl/well), for 1 h at 20 °C. The plates were then washed (2×) and 100 microlitres of calibrator or cell extract diluted in DELFIA assay buffer containing 200 µg/L of Eu³⁺ labelled anti-IDUA monoclonal antibody (ID1A) [15], was added to each well. A calibration curve (0–1 ng/well) was included in each assay. The microtitre plates were shaken at room temperature for 10 min and then incubated overnight at 4 °C. The plates were then washed (6×) and 200 µl of DELFIA enhancement solution was added to each well. The plates were shaken at room temperature for 10 min and the fluorescence was read on a 1234 DELFIA Research Fluorometer. Concentrations of IDUA protein were calculated from a 12-point calibration curve using Multicalc Data analysis software (Pharmacia, Wallac Oy).

Immune-quantification of IDUA activity was performed essentially as previously described [16], with the exception that the substrate was added directly to the wells and the fluorescence was read on a Wallac, Victor 2, 1420 Multilabel Counter (Perkin–Elmer Life Sciences), and IDUA activity was calculated from the calibration curve using Multicalc Data analysis software.

Derivatisation of oligosaccharides

The equivalent of 200 µg of total cell protein from cultured skin fibroblast extracts was lyophilised prior to derivatisation, resuspended in 100 microlitres of 250 mM 1-phenyl-3-methyl-5-pyrazolone, 400 mM NH₄OH, pH 9.1, containing 2 nmol of internal standard and derivatised at 70 °C for 90 min. The samples were then acidified with a twofold molar excess of HCO₂H, made up to 500 µl with H₂O, extracted with an equal volume of CHCl₃ to remove excess 1-phenyl-3-methyl-5-pyrazolone, and centrifuged at 13,000g for 5 min. Solid phase extraction cartridges were primed with methanol (1 ml) and water (1 ml), after which the sample was applied and allowed to enter the solid phase. Samples were desalted with three consecutive 1 ml H₂O washes, columns were dried completely on a Supelco Visiprep24 vacuum manifold, and any remaining 1-phenyl-3-methyl-5-pyrazolone was removed with two 1 ml CHCl₃ washes. The columns were again dried thoroughly, and derivatised oligosaccharides were eluted in 600 µl of 50% (v/v) CH₃CN/NH₄OH, pH 11.5, lyophilised and resuspended in 50% (v/v) CH₃CN/0.025% (v/v) HCO₂H in H₂O.

Mass spectrometry

Mass spectrometry was performed on a PE Sciex API 3000 triple–quadrupole mass spectrometer with a turbospray source (200 °C) and Analyst 1.1 data system. Samples (20 µl) were injected into the electrospray source with a Gilson 233 autosampler using a carrying solvent of 50% (v/v) CH₃CN/0.025% (v/v) HCO₂H in H₂O at a flow rate of 80 µl/min. Nitrogen was used as the collision gas at a pressure of 2×10^{-5} Torr. Quantification of PMP-derivatised oligosaccharides were performed using the multiple-reaction monitoring (MRM) in negative ion mode. Each ion pair was monitored for 100 ms with a resolution of 0.7 amu at half-peak height and averaged from continuous scans over the injection period. Relative oligosaccharide levels were calculated by relating the peak heights of the PMP-oligosaccharides to the peak height of the internal standard.

Results

Cultured human skin fibroblasts

Thirteen human skin fibroblast cell lines were used in this study, representing a selection of different genotypes as shown in Table 1. Not all fibroblast cell lines were available at one- and 10-weeks post-confluence. Fibroblasts were cultured from eight MPS I patients known to develop CNS pathology and five MPS I patients with no evidence of CNS disease at the time of diagnosis. The MPS I patients with CNS pathology were all diagnosed

Table 1
Skin fibroblast cell lines

Age at diagnosis (years)	CNS pathology	Genotype
0.75	Yes	Q70X/Y343X
0.76	Yes	Q70X/W402X
0.77	Yes	W180X/c134-145
0.82	Yes	W402X/474-2a > g
0.90	Yes	Q70X/Q70X
0.90	Yes	W402X/W402X
1.16	Yes	P533R/W402X
2.56	Yes	W402X/W402X
4.00	No	R89Q/1060-2t > c
6.41	No	P533R/R89W
13.00	No	W402X/678-7g > a
22.00	No	Unknown
Unknown	No	R383H/R383H

at a younger age compared to the MPS I patients with no evidence of CNS disease.

Immune-quantification of IDUA

Sensitive immune-quantification assays were developed for the detection of residual levels of IDUA enzyme activity and protein and had limits of detection of 0.2 pmol/min/mg and 0.02 ng/mg of total cell protein, respectively. There were only small differences in the levels of either IDUA protein or activity in the cell extracts at one- or 10-weeks post-confluence. Fig. 1 shows the levels of IDUA activity and protein in the MPS I fibroblasts that were grouped according to the presence/absence of CNS disease. All MPS I patients with CNS pathology, with the exception of one, had no detectable IDUA activity but variable levels of IDUA protein. At one-week post-confluence the logarithmic plots of IDUA activity against IDUA protein, distinguished the unaffected from the MPS I fibroblasts, and with the exception of one cell line, also broadly grouped patients based on CNS pathology. However, at 10-weeks post-confluence there was much better distinction of MPS I patients with and without CNS disease.

Oligosaccharide analysis

A total of 15 oligosaccharides was measured by ESI-MS/MS using MRM for each oligosaccharide. These oligosaccharides, derived from the partial degradation of HS and DS, ranged in size from di- to octasaccharides, with various degrees of sulphation. All 15 oligosaccharides were shown to be elevated in the MPS I fibroblasts compared to the unaffected cell lines and this difference was more pronounced at 10-weeks post-confluence (data not shown). The relative levels of these oligosaccharides were also greater in the MPS I fibroblasts at 10-weeks compared to one-week post-confluence, but the relative amounts in the unaffected fibroblasts remained unchanged over this time (see Fig. 2).

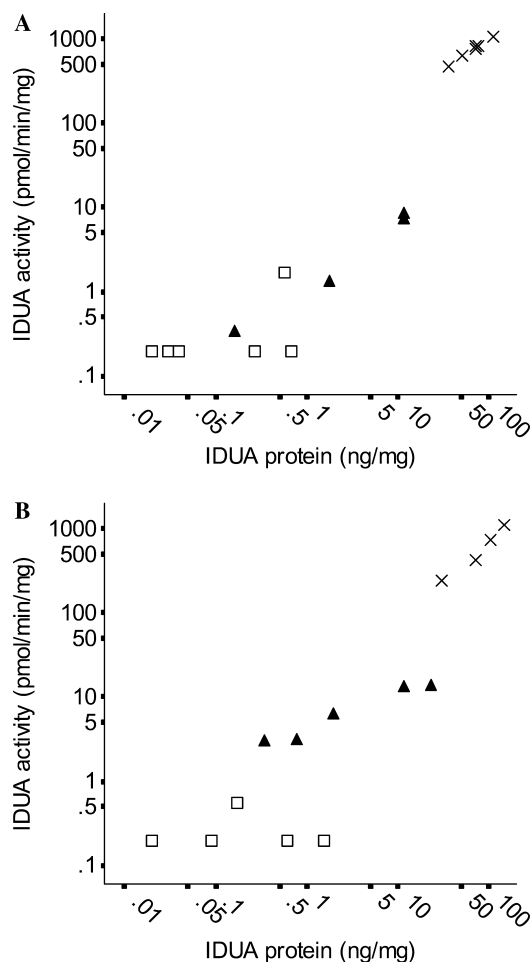


Fig. 1. Correlation between IDUA activity and protein in skin fibroblasts from control and MPS I patients. Fibroblasts from control (x) and MPS I patients with CNS pathology (□) or without CNS pathology (▲), were harvested at one-week (panel A) and 10-weeks (panel B) post-confluence. Cell extracts were assayed for total cell protein, and IDUA activity and protein were determined by immune-quantification.

Fig. 2 shows a correlation between a HS-derived sulphated (S) trisaccharide composed of two uronic acid (UA) residues and one hexosamine (HN) (UA-HN-UA (S)) (Fig. 2A and B) and a DS-derived monosulphated trisaccharide containing an *N*-acetylated hexosamine (HNAc) (Fig. 2C and D), with age at diagnosis for each of the MPS I fibroblast cell lines. These oligosaccharides have previously been identified and characterised in MPS I patients [17]. From these plots, it was evident that at 10-weeks post-confluence there was a correlation between age at diagnosis and relative levels of these trisaccharides, resulting in a relatively clear grouping of MPS I patients with and without CNS pathology.

Further discrimination between patients was achieved by plotting the DS-derived trisaccharide against the HS-derived trisaccharide (Fig. 2E and F). At one-week post-confluence, although there was an obvious distinction between MPS I fibroblasts and control cell lines, the

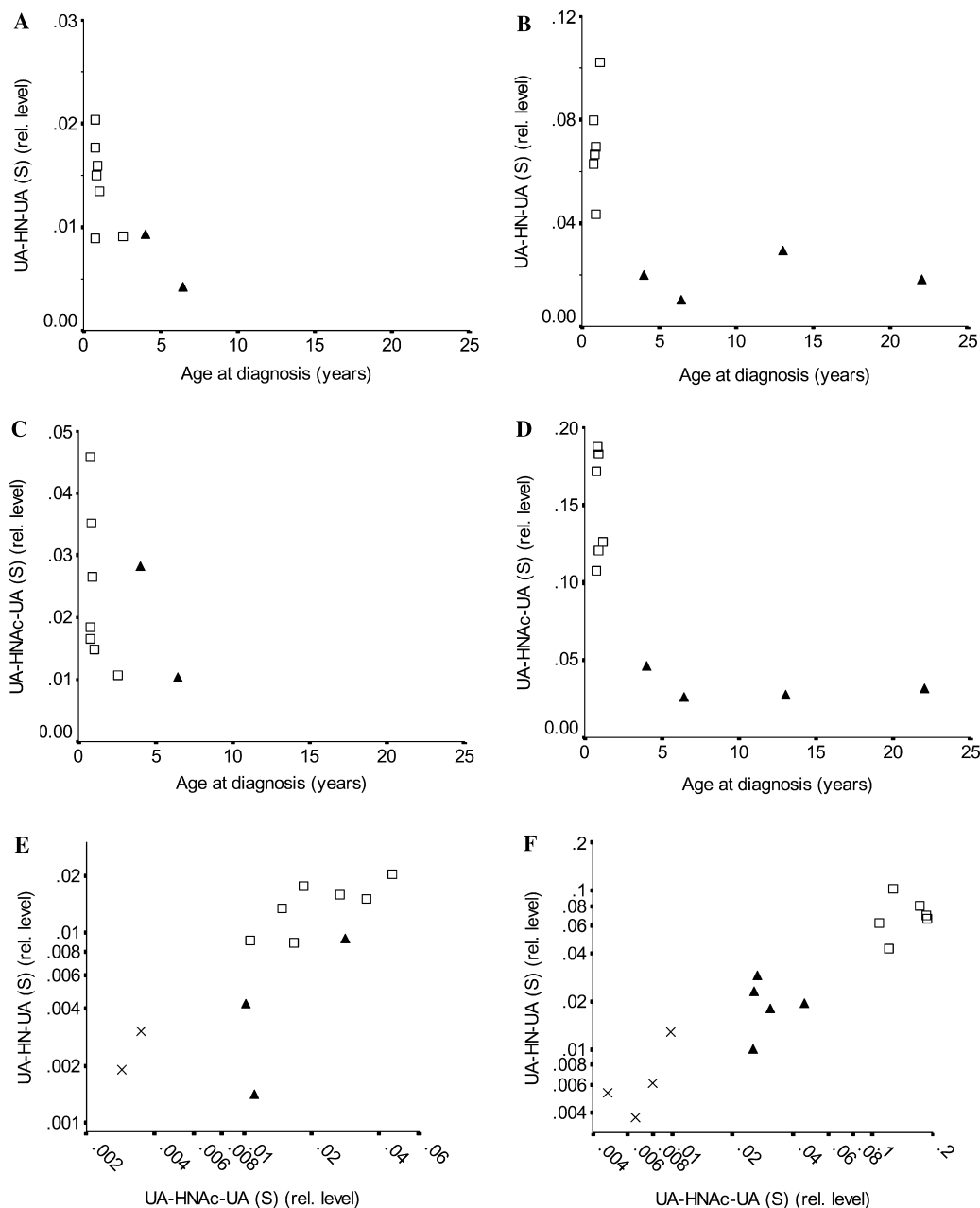


Fig. 2. Relationships between a DS- and HS-derived trisaccharide, and age at diagnosis. MPS I fibroblasts were harvested at one-week (A, C, and E) and 10-weeks (B, D and F) post-confluence. The relative level of a HS derived trisaccharide (UA-HN-UA (S)) was determined with the MRM pair 940/269 (A and B), and a DS derived trisaccharide (UA-HNAc-UA (S)) was determined with the MRM pair 982/269 (C and D), by ESI-MS/MS in the cell extracts. This was plotted as a function of the age at diagnosis for MPS I patients with CNS pathology (\square) and without CNS pathology (\blacktriangle), and the correlation between the two trisaccharides in the MPS I patients and control individuals (\times) was expressed (E and F).

MPS I patients could not be grouped according to CNS pathology (Fig. 2E). Fibroblasts harvested at 10-weeks post-confluence also showed a discrimination between MPS I and control, but MPS I patients with and without CNS involvement could also be clearly delineated (Fig. 2F). Using a ratio of IDUA activity to the relative levels of the trisaccharides provided a clear resolution of MPS I patients with and without CNS pathology (Fig. 3).

Discussion

The primary disease markers for MPS I include genotype, level of mutant protein, enzyme catalytic capacity, and level of storage product. The different MPS I clinical phenotypes that have been recognised are not easily distinguished using conventional biochemical markers. In theory, the severity of clinical presentation for MPS I

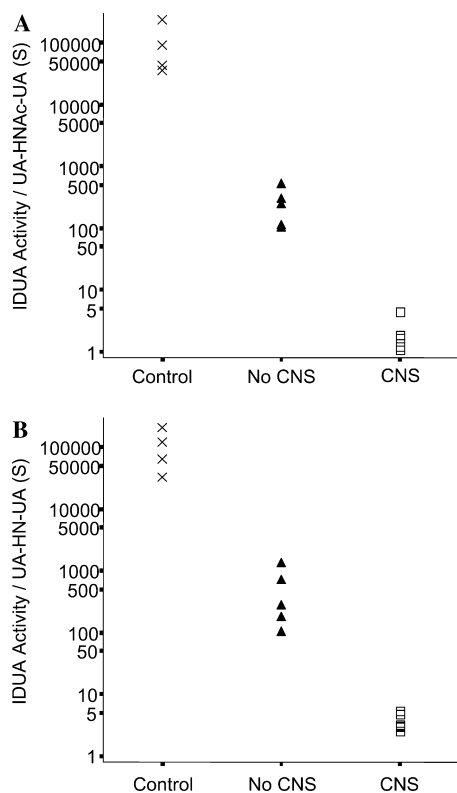


Fig. 3. Discrimination of MPS I patients based on CNS pathology. MPS I and control fibroblasts were harvested 10-weeks post-confluence. The ratio of the level of IDUA activity and the DS-derived trisaccharide (UA-HNAc-UA (S), MRM pair 982/269) (A) and the HS-derived trisaccharide (UA-HN-UA (S), MRM pair 940/269) (B) were determined in control individuals (x) and MPS I patients with CNS pathology (□) and without CNS pathology (▲).

(and other lysosomal storage disorder) patients can be explained by a balance between the residual IDUA protein/activity and its impact on substrate load. It has been reasonable to assume that there must be residual enzyme activity in milder patients. However, in practice there is not a linear relationship between level of residual enzyme activity and the amount of substrate stored. This translates into a very narrow range of enzyme activity being responsible for a broad range of clinical presentations in MPS I patients. Consequently, mutant protein and enzyme activity measures alone have only been partially informative in predicting the clinical severity of MPS I patients. The relatively crude measures of substrate that have been available have not permitted any additional predictive capacity for MPS I clinical severity. In this report, we have shown that a combination of residual enzyme activity and a particular substrate (trisaccharide), expressed as a ratio (Fig. 3), provides a powerful prediction of CNS pathology.

Recently, we identified a number of low molecular weight GAG fragments in MPS I patients that are likely products of endoglycosidase activities on HS and DS [17, unpublished]. These oligosaccharides were identified by ESI-MS/MS and shown to have α -L-iduronic acid res-

idues on their non-reducing end, characteristic of the deficiency in MPS I. In this report, we have shown that the measurement of these oligosaccharides in cultured skin fibroblasts from MPS I patients correlates with the presence/absence of CNS pathology. It is therefore likely that this reflects the levels of storage product and residual IDUA activity in the CNS. This is a key factor as neurological involvement is likely to be the central issue in deciding treatment between enzyme replacement therapy or bone marrow transplantation. Of the 15 oligosaccharides measured here, most showed a broad correlation with disease severity but two demonstrated a clear correlation with the presence/absence of CNS pathology. The basis of the predictive power of the HS- and DS-trisaccharides compared with the other di- to octasaccharides measured, is unclear at this stage. It may relate to the levels of specific endoglycosidases required for the partial degradation of the stored GAGs or perhaps more likely may be a function of the accuracy of quantification of the different oligosaccharide species. Further work with additional internal standards reflecting the exact structure of the oligosaccharides measured is required to refine the quantification of these compounds.

The correlation between the oligosaccharide markers and the absence/presence of CNS pathology was greater in skin fibroblasts cultured for 10-weeks post-confluence compared with those cultured for only one-week. Previously, we demonstrated that a number of oligosaccharides derived from the partial degradation of HS and DS accumulated in fibroblasts with time in culture [17]. Clearly, this is also the situation in this case, as the relative levels of the oligosaccharide markers are 3- to 6-fold higher at 10-weeks compared to one-week post-confluence (Fig. 2). Progressive accumulation of these oligosaccharide storage substrates may result from a number of factors: the decrease in cell division as the cells reach confluence, the production of GAG in different culture conditions or the level of endoglycosidase activity in the cells. From a practical viewpoint, culturing cells for 10-weeks to obtain a prediction of disease severity would not be optimal, and further studies are required to determine the culture times and conditions to optimise this system.

With the prospects of newborn screening for MPS I looming, it will become increasingly important to yield the best predictive information to enable the most suitable choices for treatment. We have demonstrated that oligosaccharide markers are present in urine from symptomatic patients [17], and therefore believe that they would be present in urine collected from newborns. Given this, extension of the measurement of these HS- and DS-derived trisaccharides reported here into urine, plasma or dried blood spots may provide not only a diagnosis for MPS I, but also a prediction of disease severity and rate of progression. Furthermore, these oligosaccharides may be useful for monitoring the effec-

tiveness of enzyme replacement therapy, bone marrow transplantation or any future therapies for MPS I. This preliminary study has demonstrated that it is feasible to distinguish different subtypes of MPS I clinical presentation. Although the numbers of cell lines used in this study are small they do have representative genotypes for the three broad clinical entities of MPS I. The next challenge is to further subdivide the MPS I clinical spectrum using biochemical markers to generate a severity index for MPS I.

Acknowledgments

We thank Miriam Harkin for assistance with cell culture, Alison Whittle for the purification of the sheep polyclonal antibody and Melanie Lovejoy for the Eu³⁺ labelling of ID1A. This work was supported by the NH&MRC (Australia) and the Wellcome Trust (UK), Grant reference number 060104Z/00/Z.

References

- [1] P.J. Meikle, J.J. Hopwood, A.E. Clague, W.F. Carey, Prevalence of lysosomal storage disorders, *JAMA* 281 (1999) 249–254.
- [2] E.F. Neufeld, J. Muenzer, The mucopolysaccharidoses, in: C.R. Scriver, A.L. Beaudet, W.S. Sly, D. Valle (Eds.), *The Metabolic and Molecular Basis of Inherited Diseases*, eighth ed., McGraw-Hill, New York, 2001, pp. 3421–3452.
- [3] E.A. Braunlin, N.R. Stauffer, C.H. Peters, J.L. Bass, J.M. Berry, J.J. Hopwood, W. Krivit, Usefulness of bone marrow transplantation in the Hurler syndrome, *Am. J. Cardiol.* 92 (2003) 882–886.
- [4] E.D. Kakkis, J. Muenzer, G.E. Tiller, L. Waber, J. Belmont, M. Passage, B. Izykowski, J. Phillips, R. Doroshov, I. Walot, R. Hoft, E.F. Neufeld, Enzyme-replacement therapy in mucopolysaccharidosis I, *N. Engl. J. Med.* 18 (2001) 182–188.
- [5] Laronidase, *BioDrugs* 16 (2002) 316–318 (Review).
- [6] S. Bunge, W.J. Kleijer, C. Steglich, M. Beck, E. Schwinger, A. Gal, Mucopolysaccharidosis type I: identification of 13 novel mutations of the α -L-iduronidase gene, *Hum. Mutat.* 6 (1995) 91–94.
- [7] H.S. Scott, S. Bunge, A. Gal, L.A. Clarke, C.P. Morris, J.J. Hopwood, Molecular genetics of mucopolysaccharidosis type I: diagnostic, clinical, and biological implications, *Hum. Mutat.* 6 (1995) 288–302.
- [8] C.E. Beesley, C.A. Meaney, G. Greenland, V. Adams, A. Vellodi, E.P. Young, B.G. Winchester, Mutational analysis of 85 mucopolysaccharidosis type I families: frequency of known mutations, identification of 17 novel mutations and in vitro expression of missense mutations, *Hum. Genet.* 109 (2001) 503–511.
- [9] N.J. Terlato, G.F. Cox, Can mucopolysaccharidosis type I disease severity be predicted based on a patient's genotype? A comprehensive review of the literature, *Genet. Med.* 5 (2003) 286–294.
- [10] E.G. Unger, J. Durrant, D.S. Anson, J.J. Hopwood, Recombinant α -L-iduronidase: characterization of the purified enzyme and correction of mucopolysaccharidosis type I fibroblasts, *Biochem. J.* 304 (1994) 43–49.
- [11] K. Umaphathysivam, A.M. Whittle, E. Ranieri, C. Bindloss, E.M. Ravenscroft, O.P. van Diggelen, J.J. Hopwood, P.J. Meikle, Determination of acid α -glucosidase protein: evaluation as a screening marker for Pompe disease and other lysosomal storage disorders, *Clin. Chem.* 46 (2000) 1318–1325.
- [12] P.J. Meikle, D.A. Brooks, E.M. Ravenscroft, M. Yan, R.E. Williams, A.E. Jaunzems, T.K. Chataway, L.E. Karageorgos, R.C. Davey, C.D. Boulter, S.R. Carlsson, J.J. Hopwood, Diagnosis of lysosomal storage disorders: evaluation of lysosome-associated membrane protein LAMP-1 as a diagnostic marker, *Clin. Chem.* 43 (1997) 1325–1335.
- [13] J.J. Hopwood, V. Muller, J.R. Harrison, W.F. Carey, H. Elliot, E.F. Robertson, A.C. Pollard, Enzymatic diagnosis of the mucopolysaccharidoses: experience of 96 cases diagnosed in a five-year period, *Med. J. Aust.* 20 (1982) 257–260.
- [14] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, *J. Biol. Chem.* 193 (1951) 265–275.
- [15] P.R. Clements, D.A. Brooks, G.T. Saccone, J.J. Hopwood, Human α -L-iduronidase. I. Purification, monoclonal antibody production, native and subunit molecular mass, *Eur. J. Biochem.* 152 (1985) 21–28.
- [16] D.A. Brooks, S. Fabrega, L.K. Hein, E.J. Parkinson, P. Durand, G. Yogalingam, U. Matte, R. Giugliani, A. Dasvarma, J. Eshlahpazire, B. Henrissat, J.P. Mornon, J.J. Hopwood, P. Lehn, Glycosidase active site mutations in human α -L-iduronidase, *Glycobiology* 11 (2001) 741–750.
- [17] M. Fuller, P.J. Meikle, J.J. Hopwood, Glycosaminoglycan degradation fragments in mucopolysaccharidosis I, *Glycobiology* 14 (2004) 443–450.