RHEUMATOLOGY

Rheumatology 2011;50:v41–v48
doi:10.1093/rheumatology/ker390

Diagnosis of the mucopolysaccharidoses

Thomas J. A. Lehman[1], Nicole Miller[2], Becky Norquist[2], Lisa Underhill[3] and Joan Keutzer[3]

Abstract

The mucopolysaccharidoses (MPSs) often present a diagnostic challenge, particularly for patients who have more slowly progressive disease phenotypes, as early disease manifestations can be subtle or non-specific. However, certain types of bone and joint involvement should always prompt consideration of an MPS diagnosis, such as early joint involvement without classic inflammatory features or erosive bone lesions, claw hand, spinal deformities or dysostosis multiplex. All such patients should be referred to a geneticist or metabolic specialist for diagnostic evaluation. The earlier the diagnosis is made, the better the potential outcome of treatment. Each type of MPS is associated both with deficient activity of a specific lysosomal enzyme that degrades specific glycosaminoglycans (GAGs) and with abnormalities in urinary GAG excretion. MPS patients usually excrete excess GAG in urine and/or have different relative proportions of types of GAG in urine as compared with age-matched normal subjects. Although urinary GAG analyses (both quantitative and qualitative) can suggest the most likely type of MPS, diagnosis must be confirmed by enzyme assay. Multiple assays may be necessary to identify the disease subtype. Correct identification of the MPS type is essential to guide treatment and management decisions.

Key words: Glycosaminoglycan, Laboratory diagnosis, Mucopolysaccharidoses, MPS, Hurler, Hunter, Sanfilippo, Morquio, Maroteaux–Lamy, Sly.

Introduction

The mucopolysaccharidoses (MPSs) are a heterogeneous group of rare inherited disorders caused by deficiency of a lysosomal enzyme that is necessary to break down or catabolize complex carbohydrates known as mucopolysaccharides or glycosaminoglycans (GAGs) [1, 2]. Accumulation of GAGs can cause progressive damage to a broad range of tissues. This damage often manifests as stiff joints, skeletal malformations, retarded growth, pulmonary deficits, and ocular, hepatic, cardiac and sometimes neurological abnormalities [1, 2]. Early and accurate diagnosis of MPS is critical to the provision of appropriate supportive care and, when available, disease-specific treatment—enzyme replacement therapy (ERT) or haematopoietic stem cell transplantation (HSCT) [3–6]. Early recognition and appropriate management profoundly affect patient quality of life and may potentially slow or prevent development of irreversible pathologies [7–12].

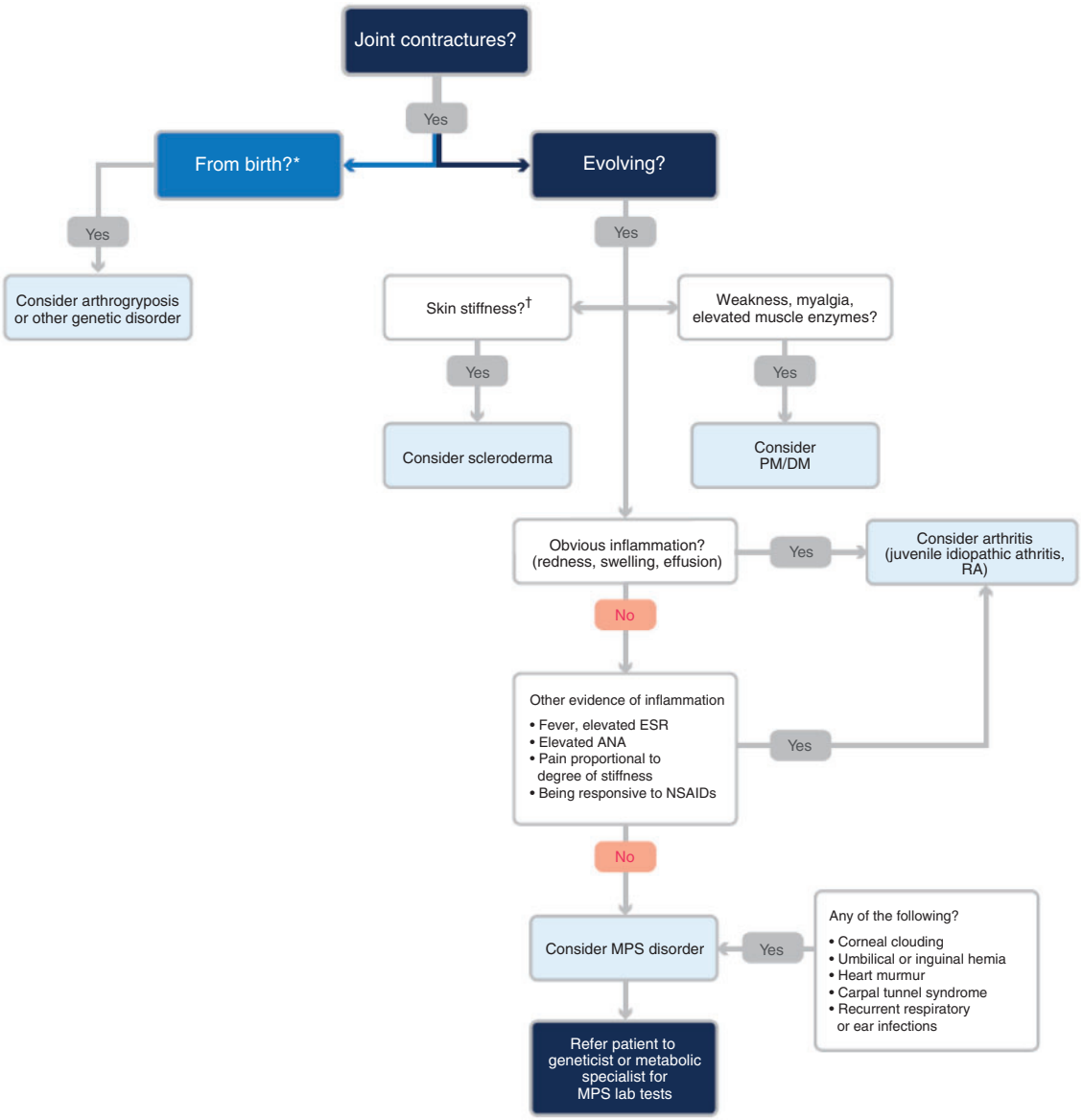
Although recent advances have improved treatment for MPS disorders, lack of disease awareness, the non-specific nature of many symptoms and variable clinical presentations frequently impede rapid and accurate diagnosis. For patients with slowly progressing MPS disease phenotypes, the gap between earliest symptoms and diagnosis is often years, sometimes decades [4, 5, 13–15]. Bone and joint symptoms are common early manifestations of MPS, and undiagnosed patients may initially be referred to a rheumatologist. This is especially likely for patients who have more slowly progressive forms of these disorders. A recent survey of 60 adult and paediatric rheumatologists in North America and Europe found that ~80% were unable to recognize symptoms of MPS I and did not know the appropriate diagnostic tests [16]. This led to the development of a diagnostic algorithm for the evaluation of patients with joint contractures (Fig. 1). The most suggestive rheumatological feature is development of joint pain and joint contractures at an early age without concomitant inflammation. Table 1 lists other key clinical features often apparent in a routine physical exam or medical history that should heighten the suspicion of MPS.

[1]Weill Medical College of Cornell University, New York, NY, [2]Scientific and Medical Affairs, BioMarin Pharmaceutical Inc., Novato, CA and [3]Scientific and Medical Affairs, Genzyme Corporation, Cambridge, MA, USA.

Submitted 29 June 2011; revised version accepted 6 October 2011.

Correspondence to: Thomas J. A. Lehman, Hospital for Special Surgery, 535 E 70 St, New York, NY 10021, USA.
E-mail: lehmant@hss.edu

Fig. 1 Differential diagnosis algorithm for MPS (modified from Cimaz *et al*. [16]). Newborn infants with MPS, although normal appearing, often have radiological evidence of bone and joint abnormalities (as depicted by an asterisk) [58]. Overall skin texture in patients with MPS can be thickened and rough (depicted by dagger). MPS II [2, 14] and rarely MPS I [59] can be associated with a distinctive skin lesion consisting of white pebbly papules 2–10 mm in diameter, sometimes coalescing in ridges.



Enhanced disease awareness among rheumatologists is paramount to ensure that patients whose bone and joint symptoms are suggestive of MPS are referred to a geneticist or metabolic specialist for a diagnostic evaluation.

Laboratory testing for an MPS disorder

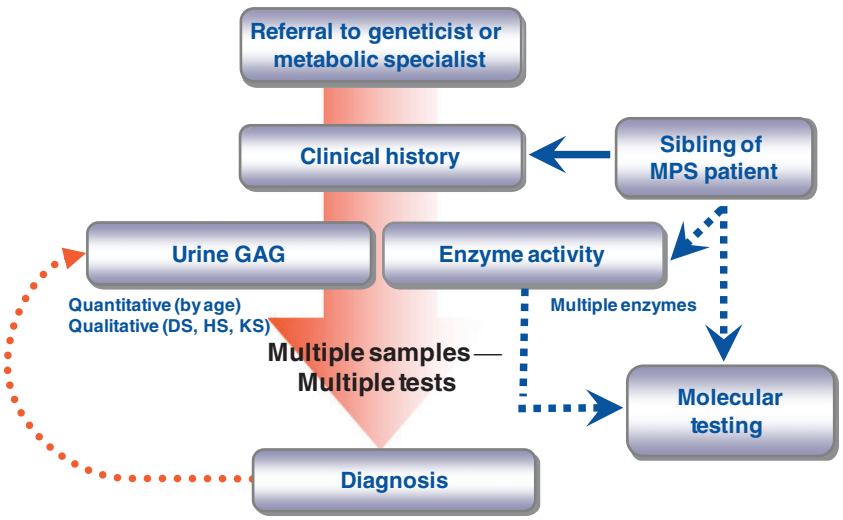
An MPS diagnosis is based on laboratory results from urinary GAG analyses and enzyme activity assays. Enzyme activity assays measure enzyme activity in tissue (blood or fibroblasts). Quantitative GAG assays measure overall elevation of GAG as compared with GAG levels expected in age-matched normal subjects. Qualitative GAG assays detect the type of GAG excreted. Determining the correct MPS type is essential to ensure appropriate therapeutic management. The type of MPS cannot be reliably determined on the basis of clinical presentation or test results alone. High clinical suspicion and thorough testing help ensure an accurate diagnosis of MPS (Fig. 2). Table 2 summarizes the diagnostic assays available for each of the MPS disorders as well as clinical

Table 1 Signs and symptoms suggestive of MPS

Common bone and joint features[a]
Early joint involvement without classic inflammatory features or erosive bone lesions
Claw hand (see Summers and Ashworth in this supplement [49])
Spinal deformity (subtle or overt gibbus, scoliosis, kyphosis, lordosis) (see Muenzer [50], Morishita and Petty [51] and White [52] in this supplement)
Radiological evidence of dysostosis multiplex (see Muenzer [50], Morishita and Petty [51] and White [52] in this supplement)
Other common clinical signs[a]
Coarsening of facial features over time
Corneal clouding (can be mild or severe) (see Summers and Ashworth in this supplement [49])
Short, stiff neck
Frequent respiratory infections, chronic nasal congestion, noisy breathing/snoring
Heart murmur
History of hernia repair surgery (inguinal and/or umbilical) [42, 43]
Short stature
Abnormal gait (especially toe walking)
Abdominal protuberance due to liver and spleen enlargement

[a]Can be subtle or overt. Absence of any particular sign or symptom does not rule out MPS.

Fig. 2 MPSs: the path for laboratory testing. Based on clinical suspicion, a primary care physician or specialist may refer a patient to a geneticist or metabolic specialist who will acquire the patient's clinical history. Using a reliable laboratory, the geneticist or metabolic specialist should order relevant urine GAG analysis (quantitative and qualitative) and enzyme activity assays. If enzyme activity testing identifies a defect in a lysosomal enzyme, molecular testing should also be performed when feasible. The geneticist or metabolic specialist should review the patient's medical history and, in collaboration with the laboratory, interpret the laboratory assay results before completing a diagnosis and providing treatment recommendations. Urine GAG analysis can be used to monitor therapy. When a sibling of an MPS patient is identified, the undiagnosed sibling should undergo the same clinical history and laboratory test process. It may also be possible to proceed directly to enzyme activity testing and molecular testing for the undiagnosed sibling. DS: dermatan sulphate; HS: heparan sulphate; KS: keratan sulphate.



features that can help distinguish one disorder from another. As diagnosis of these disorders can be complex, we recommend that patients suspected of having MPS be referred to a geneticist or metabolic specialist for laboratory testing. Table 3 lists some common diagnostic pitfalls.

Urine GAG analysis

In normal subjects, urinary GAG excretion varies with age, higher values being found during the first years of life, followed by a slow and constant decrease thereafter. Qualitatively, ~90% of the GAG content in normal urine consists of chondroitin-4 and -6 sulphate, with the remaining

Table 2 Diagnosis of MPS disorders

MPS type[a]	Other names	Qualitative GAG assay: type of GAG excreted	Enzyme activity assay: enzyme defective in disorder	Distinguishing clinical features relative to other MPS disorders[a]	Caveats for MPS laboratory tests
MPS I	Hurler, Hurler–Scheie, Scheie	DS, HS	α-L-iduronidase	Children with cognitive impairment rarely have overt behavioural issues [4] Progressive corneal clouding [2]	Total urinary GAG is usually elevated, but false negatives can occur, especially with spot testing [16]
MPS II	Hunter	DS, HS	Iduronate sulphatase	X-linked, so family history can be important and most patients are male [14] No corneal clouding [14] Behavioural/cognitive changes common in severely affected patients [14]	Total urinary GAG is usually elevated, but false negatives can occur, especially with spot testing [16]
MPS III	Sanfilippo, types A–D	HS	A: heparan *N*-sulphatase B: α-*N*-aceytylglucosaminidase C: acetyl-CoA: α-glucosaminide acyltransferase D: *N*-acetylglucosamine-6-sulphatase	Behavioural/cognitive changes precede overt bone and joint involvement [53] Corneal clouding not common and usually mild [2, 54]	
MPS IV	Morquio, types A and B	A: KS, CS B: KS	A: galactose 6-sulphatase, also known as GALNS or *N*-acetylgalactosamine 6-sulphatase B: β-galactosidase	Cognition is usually fully preserved, even in severely affected patients [2] Distinctive skeletal dysplasia with joint hyper-extendibility [2] Corneal clouding develops in some patients [2]	Total GAG level is frequently in the normal range [18], galactose-6-sulphatase activity can be labile when assayed by DBS; additional controls may be necessary to ensure accuracy when diagnosing MPS IVA [39]
MPS VI	Maroteaux–Lamy	DS	Arylsulphatase B (ASB) or *N*-acetylgalactosamine 4-sulphatase	Cognition is usually fully preserved, even in severely affected patients [1] Mild to severe corneal clouding usually develops; may not be an early sign [2, 54, 55]	Measure qualitative (DS), quantitative urine GAGs, multiple sulphatases and multiple enzymes; total urinary GAG may be within the normal range in slowly progressing patients [60]
MPS VII	Sly	DS, HS, CS	β-Glucuronidase	Hydrops fetalis is common at birth [1, 2] Corneal clouding common [54]	
MPS IX	Hyaluronidase deficiency	Unknown; four patients described in the literature [56, 57]	Hyaluronidase	No ocular features Periarticular soft masses seen in some patients Joint symptoms prominent	Elevated plasma hyaluronan levels

[a]Note that clinical features alone should not be used to rule out or rule in a particular type of MPS. DS: dermatan sulphate; HS: heparan sulphate; CS: chondroitin-4 and -6 sulphate; KS: keratan sulphate.

Table 3 Potential diagnostic pitfalls

Incorrect tests ordered
Reliance on a single test or sample (no follow-up testing)
Inappropriate sample storage and handling
Enzyme assays performed without inhibitors of isoenzymes
Interassay variability not reported or considered
Lack of appropriate controls
Heparan sulphate can be present in trace amounts in urine of normal individuals
Lab tests may be routed to non-MPS-specialized lab

being heparan sulphate. Most MPS patients have higher GAG excretion in urine compared with age-matched normal subjects; however, as not all MPS patients have a clear elevation of total GAG excretion, an accurate diagnosis requires a full GAG profile including both quantitative and qualitative analysis done in tandem [17–19]. In fact, patients with different types of MPS differ not only in the total amount of GAG excreted in urine, but also in the relative proportion of various types of GAG [18]. As a consequence, the demonstration of an abnormal pattern is diagnostic for an MPS disorder; moreover, the presence of specific GAGs can suggest the MPS subtype and may direct the appropriate enzyme analyses. For example, high amounts of heparan sulphate or keratan sulphate essentially characterize MPS III and IV, respectively. Within a single subtype of MPS, GAG excretion can also vary depending on the severity of the disease phenotype [20]. Thus an MPS diagnosis should neither be confirmed nor ruled out on the basis of a single GAG test, although GAG testing usually is the first step in the diagnostic pathway.

Enzyme activity

The diagnosis of MPS should be confirmed by enzyme activity testing (Table 2). Geneticists and metabolic specialists should also test for related diseases, such as multiple sulphatase deficiency and mucolipidosis, both of which have some clinical features in common with MPS. Enzyme activity typically is measured in leucocytes or cultured fibroblasts (skin biopsy or punch). For many types of MPS, enzyme activity can be measured from a dried blood spot (DBS). DBS-based assays offer considerable practical advantages with respect to sample collection, storage and transport, and multiple enzyme activity tests can be performed on a single DBS. Recent technological advances have made these assays sensitive and specific if appropriate controls are performed. It is recommended that a positive result from a DBS be confirmed by a tissue-based assay. Due to the complexity of these assays, referral to a geneticist or metabolic specialist is essential.

Mutation analysis

Mutation analysis or molecular testing includes looking for the known disease-causing mutations in addition to looking for abnormal sequences in the gene coding for a particular enzyme. Molecular testing has limited utility in initial screening because of the extreme genetic heterogeneity that characterizes all types of MPS. Although several MPS disorders are associated with specific commonly occurring mutations, most families have private mutations [3–5]. Thus the primary use of molecular testing is to confirm a diagnosis of a particular type of MPS or to evaluate family members when the type of MPS and the family mutation is known (Fig. 2). Molecular testing can have prognostic value if the mutations that are identified have been well characterized [21–25].

When a patient has been diagnosed with a specific type of MPS and his/her mutation is known, the information can be used for carrier testing and prenatal testing of siblings. With regard to carrier testing, it is important to note that except for MPS II (Hunter), the MPSs are inherited in an autosomal recessive fashion, so carriers have a very small likelihood of having children affected with MPS unless the union is consanguineous.

Biomarkers

Currently there are no established biomarkers for any of the MPS types. Urinary GAG levels usually decrease with treatment [7, 11, 26, 27], but are not an ideal biomarker. Other biomarkers under investigation include heparin cofactor II–thrombin complex measured in serum [28, 29], the ratio of dermatan to chondroitin sulphate in urine [29, 30], and dipeptidyl peptidase IV in plasma [31]. An ideal biomarker would be specific to a particular type or types of MPS, would help to differentiate more severe from less severe disease phenotypes, would respond to treatment and would be easily detected and quantified.

Screening for MPS disorders in newborns and at-risk populations

As early diagnosis and early treatment are helpful to the patient and family, implementation of screening programs can improve opportunities for early detection and intervention. In general, screening falls into two categories: newborn screening (which can be performed as a DBS-based assay that screens for a panel of diseases) [32–36] and screening of populations considered at risk of MPS because of specific signs or symptoms.

Newborn screening offers the hope of disease identification and treatment initiation before development of irreversible disease manifestations. Several of the MPSs are not clinically obvious at birth, but they are progressive, debilitating and often life threatening. MPSs for which treatment is available are excellent candidates for newborn screening because early intervention may have a significant impact on disease outcome [36]. The American Council of Medical Genetics recently published guidelines for newborn screening of nine lysosomal storage disorders, including MPS I, II and VI [37].

The potential impact of early, pre-symptomatic intervention has been demonstrated in case studies of MPS I, II and VI sibling pairs in which the younger sibling was

diagnosed at birth, started on enzyme replacement therapy in infancy and had a much more favourable clinical outcome than the older sibling who began treatment at a later age after symptoms became evident [8, 10, 12]. DBS-based assays, suitable for high-throughput, multiplex newborn screening, are in development for MPS I, II, IV and VI [32, 34, 35, 38–41].

Symptom-based screening programmes among high-risk populations may help identify MPS patients who have more slowly progressive forms of these diseases. Possible symptom targets include corneal clouding, joint manifestations, surgical history [42, 43] and CTS (nearly ubiquitous among children with MPS and very uncommon in the general paediatric population [44–48]). In this regard, routine screening of children presenting at an early age with hand dysfunction without anatomic lesions may be useful.

Standardization of assays

Since MPS diagnostic testing and screening are not currently standardized, open communication between laboratories and physicians is necessary to facilitate the interpretation of specific diagnostic tests and results. Along with clinical signs and symptoms, these diagnostic tests will help ultimately to provide an accurate diagnosis of MPS. We recommend that laboratories implement programs to self-monitor testing proficiency (e.g. for urinary GAG and enzyme activity assays), such as those offered by the European Research Network for Evaluation and Improvement of Screening, Diagnosis and Treatment of Inherited Disorders of Metabolism (ERNDIM; www.erndim.unibas.ch) and the College of American Pathologists (CAP; http://www.cap.org/apps/cap.portal).

Conclusions

An MPS diagnosis should be considered for all patients who present with joint symptoms without signs of inflammation, especially in concert with other suggestive clinical signs, such as corneal clouding, frequent respiratory infections and gastrointestinal complaints, heart murmur, short stature, abnormal gait or history of hernia repair. Such patients should be referred promptly to a geneticist or metabolic specialist, as early and accurate diagnosis can maximize therapeutic outcome. High clinical suspicion informed by specific laboratory diagnostic results from multiple samples across multiple tests ensure an accurate diagnosis of MPS.

Rheumatology key messages

- Promptly refer all patients with suspected MPS to a geneticist or metabolic specialist.
- Laboratory diagnosis of MPS is based on urinary GAG analyses and enzyme activity tests.
- High clinical suspicion and results from multiple samples and tests inform the diagnosis of MPS.

Acknowledgements

The opinions and conclusions set forth herein are those of the authors and do not necessarily represent the views of Genzyme, BioMarin or Shire.

Supplement: This paper forms part of the supplement entitled 'Rheumatologic Aspects of the Mucopolysaccharidoses'. This supplement was supported by joint educational funding from Genzyme, BioMarin Pharmaceutical and Shire Human Genetic Therapies.

Disclosure statement: N.M. is an employee and shareholder of BioMarin Pharmaceutical Inc. B.N. was a medical writing consultant for BioMarin Pharmaceutical Inc. T.J.A.L. serves as a consultant for Biomarin and Genzyme. L.U. and J.K. are employees of Genzyme Corporation.

References

1 Muenzer J. The mucopolysaccharidoses: a heterogeneous group of disorders with variable pediatric presentations. J Pediatr 2004;144(5 Suppl.):S27–34.

2 Neufeld EF, Muenzer J. The mucopolysaccharidoses. In: Scriver C, Beaudet A, Sly W *et al*., eds. The Metabolic and Molecular Bases of Inherited Disease. New York: McGraw Hill, 2001:3421–52.

3 Muenzer J, Beck M, Eng CM *et al*. Multidisciplinary management of Hunter syndrome. Pediatrics 2009;124:e1228–39.

4 Muenzer J, Wraith JE, Clarke LA. Mucopolysaccharidosis I: management and treatment guidelines. Pediatrics 2009;123:19–29.

5 Valayannopoulos V, Nicely H, Harmatz P *et al*. Mucopolysaccharidosis VI. Orphanet J Rare Dis 2010;5:5.

6 Valstar MJ, Ruijter GJ, van Diggelen OP *et al*. Sanfilippo syndrome: a mini-review. J Inherit Metab Dis 2008.

7 Clarke L, Wraith JE, Beck M *et al*. Long-term efficacy and safety of laronidase in the treatment of mucopolysaccharidosis I. Pediatrics 2009;123:229–40.

8 McGill JJ, Inwood AC, Coman DJ *et al*. Enzyme replacement therapy for mucopolysaccharidosis VI from 8 weeks of age—a sibling control study. Clin Genet 2009;77:492–8.

9 Harmatz P, Yu ZF, Giugliani R *et al*. Enzyme replacement therapy for mucopolysaccharidosis VI: evaluation of long-term pulmonary function in patients treated with recombinant human N-acetylgalactosamine 4-sulfatase. J Inherit Metab Dis 2010;33:51–60.

10 Gabrielli O, Clarke LA, Bruni S *et al*. Enzyme-replacement therapy in a 5-month-old boy with attenuated presymptomatic MPS I: 5-year follow-up. Pediatrics 2010;125:e183–7.

11 Muenzer J, Beck M, Eng CM *et al*. Long-term, open-labeled extension study of idursulfase in the treatment of Hunter syndrome. Genet Med 2011;13:95–101.

12 Tylki-Szymanska A, Jurecka A, Zuber Z *et al*. Enzyme replacement therapy for mucopolysaccharidosis II from 3 months of age: 3-year follow-up. Acta Paediatr 2011, Advance Access published 14 June 2011, doi: 10.1111/j.1651-2227.2011.02385.x.

13 Cimaz R, Vijay S, Haase C *et al*. Attenuated type I mucopolysaccharidosis in the differential diagnosis of juvenile idiopathic arthritis: a series of 13 patients with Scheie syndrome. Clin Exp Rheumatol 2006;24:196–202.

14 Martin R, Beck M, Eng C *et al*. Recognition and diagnosis of mucopolysaccharidosis II (Hunter syndrome). Pediatrics 2008;121:e377–86.

15 Vijay S, Wraith JE. Clinical presentation and follow-up of patients with the attenuated phenotype of mucopolysaccharidosis type I. Acta Paediatr 2005;94:872–7.

16 Cimaz R, Coppa GV, Kone-Paut I *et al*. Joint contractures in the absence of inflammation may indicate mucopolysaccharidosis. Pediatr Rheumatol Online J 2009;7:18.

17 Mahalingam K, Janani S, Priya S *et al*. Diagnosis of mucopolysaccharidoses: how to avoid false positives and false negatives. Indian J Pediatr 2004;71:29–32.

18 Piraud M, Boyer S, Mathieu M *et al*. Diagnosis of mucopolysaccharidoses in a clinically selected population by urinary glycosaminoglycan analysis: a study of 2,000 urine samples. Clin Chim Acta 1993;221:171–81.

19 Gallegos-Arreola MP, Machorro-Lazo MV, Flores-Martinez SE *et al*. Urinary glycosaminoglycan excretion in healthy subjects and in patients with mucopolysaccharidoses. Arch Med Res 2000;31:505–10.

20 Coppa GV, Galeotti F, Zampini L *et al*. High-throughput determination of urinary hexosamines for diagnosis of mucopolysaccharidoses by capillary electrophoresis and high-performance liquid chromatography. Anal Biochem 2011;411:32–42.

21 Terlato NJ, Cox GF. Can mucopolysaccharidosis type I disease severity be predicted based on a patient's genotype? A comprehensive review of the literature. Genet Med 2003;5:286–94.

22 Karageorgos L, Brooks DA, Pollard A *et al*. Mutational analysis of 105 mucopolysaccharidosis type VI patients. Hum Mutat 2007;28:897–903.

23 Karageorgos L, Brooks DA, Harmatz P *et al*. Mutational analysis of mucopolysaccharidosis type VI patients undergoing a phase II trial of enzyme replacement therapy. Mol Genet Metab 2007;90:164–70.

24 Tomatsu S, Filocamo M, Orii KO *et al*. Mucopolysaccharidosis IVA (Morquio A): identification of novel common mutations in the N-acetylgalactosamine-6-sulfate sulfatase (GALNS) gene in Italian patients. Hum Mutat 2004;24:187–8.

25 Tomatsu S, Montano AM, Nishioka T *et al*. Mutation and polymorphism spectrum of the GALNS gene in mucopolysaccharidosis IVA (Morquio A). Hum Mutat 2005;26:500–12.

26 Aldenhoven M, Boelens JJ, de Koning TJ. The clinical outcome of Hurler syndrome after stem cell transplantation. Biol Blood Marrow Transplant 2008;14:485–98.

27 Harmatz P, Giugliani R, Schwartz IV *et al*. Long-term follow-up of endurance and safety outcomes during enzyme replacement therapy for mucopolysaccharidosis VI: final results of three clinical studies of recombinant human N-acetylgalactosamine 4-sulfatase. Mol Genet Metab 2008;94:469–75.

28 Randall DR, Colobong KE, Hemmelgarn H *et al*. Heparin cofactor II-thrombin complex: a biomarker of MPS disease. Mol Genet Metab 2008;94:456–61.

29 Langford-Smith KJ, Mercer J, Petty J *et al*. Heparin cofactor II-thrombin complex and dermatan sulphate: chondroitin sulphate ratio are biomarkers of short- and long-term treatment effects in mucopolysaccharide diseases. J Inherit Metab Dis 2010;34:499–508.

30 Church H, Tylee K, Cooper A *et al*. Biochemical monitoring after haemopoietic stem cell transplant for Hurler syndrome (MPSIH): implications for functional outcome after transplant in metabolic disease. Bone Marrow Transplant 2007;39:207–10.

31 Beesley CE, Young EP, Finnegan N *et al*. Discovery of a new biomarker for the mucopolysaccharidoses (MPS), dipeptidyl peptidase IV (DPP-IV; CD26), by SELDI-TOF mass spectrometry. Mol Genet Metab 2009;96:218–24.

32 Blanchard S, Sadilek M, Scott CR *et al*. Tandem mass spectrometry for the direct assay of lysosomal enzymes in dried blood spots: application to screening newborns for mucopolysaccharidosis I. Clin Chem 2008;54:2067–70.

33 De Jesus VR, Zhang XK, Keutzer J *et al*. Development and evaluation of quality control dried blood spot materials in newborn screening for lysosomal storage disorders. Clin Chem 2009;55:158–64.

34 Gelb MH, Turecek F, Scott CR *et al*. Direct multiplex assay of enzymes in dried blood spots by tandem mass spectrometry for the newborn screening of lysosomal storage disorders. J Inherit Metab Dis 2006;29:397–404.

35 Zhang XK, Elbin CS, Chuang WL *et al*. Multiplex enzyme assay screening of dried blood spots for lysosomal storage disorders by using tandem mass spectrometry. Clin Chem 2008;54:1725–8.

36 Nakamura K, Hattori K, Endo F. Newborn screening for lysosomal storage disorders. Am J Med Genet C Semin Med Genet 2011;157:63–71.

37 Wang RY, Bodamer OA, Watson MS *et al*. Lysosomal storage diseases: diagnostic confirmation and management of presymptomatic individuals. Genet Med 2011;13:457–84.

38 Hwu WL, Chien YH, Lee NC. Newborn screening for neuropathic lysosomal storage disorders. J Inherit Metab Dis 2010;33:381–6.

39 Khaliq T, Sadilek M, Scott CR *et al*. Tandem mass spectrometry for the direct assay of lysosomal enzymes in dried blood spots: application to screening newborns for mucopolysaccharidosis IVA. Clin Chem 2011;57:128–31.

40 Duffey TA, Sadilek M, Scott CR *et al*. Tandem mass spectrometry for the direct assay of lysosomal enzymes in

dried blood spots: application to screening newborns for mucopolysaccharidosis VI (Maroteaux-Lamy syndrome). Anal Chem 2010;82:9587–91.

41 Duffey TA, Khaliq T, Scott CR *et al*. Design and synthesis of substrates for newborn screening of Maroteaux-Lamy and Morquio A syndromes. Bioorg Med Chem Lett 2010; 20:5994–6.

42 Arn P, Wraith J, Underhill L. Characterization of surgical procedures in patients with mucopolysaccharidosis type I: findings from the MPS I Registry. J Pediatr 2009;154: 859–64 e3.

43 Mendelsohn NJ, Harmatz P, Bodamer O *et al*. Importance of surgical history in diagnosing mucopolysaccharidosis type II (Hunter syndrome): data from the Hunter Outcome Survey. Genet Med 2010;12:816–22.

44 Lamberti PM, Light TR. Carpal tunnel syndrome in children. Hand Clin 2002;18:331–7.

45 Van Heest AE, House J, Krivit W *et al*. Surgical treatment of carpal tunnel syndrome and trigger digits in children with mucopolysaccharide storage disorders. J Hand Surg Am 1998;23:236–43.

46 Van Meir N, De Smet L. Carpal tunnel syndrome in children. Acta Orthop Belg 2003;69:387–95.

47 Van Meir N, De Smet L. Carpal tunnel syndrome in children. J Pediatr Orthop B 2005;14:42–5.

48 Wraith JE, Alani SM. Carpal tunnel syndrome in the mucopolysaccharidoses and related disorders. Arch Dis Child 1990;65:962–3.

49 Summers CG, Ashworth JL. Ocular manifestations as key features for diagnosing mucopolysaccharidoses. Rheumatology 2011;50(Suppl 5):v34–v40.

50 Muenzer J. Overview of the mucopolysaccharidoses. Rheumatology 2011;50(Suppl 5):v4–v12.

51 Morishita K, Petty RE. Musculoskeletal manifestations of mucopolysaccharidoses. Rheumatology 2011; 50(Suppl 5):v19–v25.

52 White KK. Orthopaedic aspects of mucopolysaccharidoses. Rheumatology 2011;50(Suppl 5):v26–v33.

53 Valstar MJ, Ruijter GJ, van Diggelen OP *et al*. Sanfilippo syndrome: a mini-review. J Inherit Metab Dis 2008;31:240–52.

54 Ashworth JL, Biswas S, Wraith E *et al*. Mucopolysaccharidoses and the eye. Surv Ophthalmol 2006;51:1–17.

55 Ashworth JL, Biswas S, Wraith E *et al*. The ocular features of the mucopolysaccharidoses. Eye 2006;20:553–63.

56 Natowicz MR, Short MP, Wang Y *et al*. Clinical and biochemical manifestations of hyaluronidase deficiency. N Engl J Med 1996;335:1029–33.

57 Imundo L, Leduc CA, Guha S *et al*. A complete deficiency of hyaluronoglucosaminidase 1 (HYAL1) presenting as familial juvenile idiopathic arthritis. J Inherit Metab Dis 2011;34:1013–22.

58 Mundada V, D'Souza N. Lumbar gibbus: early presentation of dysostosis multiplex. Arch Dis Child 2009;94:930–1.

59 Schiro JA, Mallory SB, Demmer L *et al*. Grouped papules in Hurler-Scheie syndrome. J Am Acad Dermatol 1996; 35(5 Pt 2):868–70.

60 Gottwald I, Hughes J, Stewart F *et al*. Attenuated mucopolysaccharidosis type VI (Maroteaux-Lamy syndrome) due to homozygosity for the p.Y210C mutation in the ARSB gene. Mol Genet Metab 2011;103:300–2.