Detection by Urinary GAG Testing of Mucopolysaccharidosis Type II in an At-Risk Spanish Population

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Abstract Hunter syndrome (Mucopolysaccharidosis type II) is an inherited lysosomal storage disorder with potentially severe degenerative consequences. Clinical diagnosis is not easy, although biochemical confirmation is straightforward, and sometimes patients are diagnosed at a late age. It is widely believed, for inborn errors of metabolism in general, that early diagnosis and management is of paramount importance for improving the prognosis of the disease. The objective of this study was to identify specific populations at risk of suffering from Hunter syndrome. Urine samples were obtained from children between the ages of 0 to 18, belonging to known risk groups of mucopolysaccharidosis (MPS) type II, for the semi-quantitative (GAG test) and quantitative determination of glycosaminoglycans (GAG). One case of Hunter syndrome was found among the 130 samples that were collected and analysed. This study supports the feasibility of early diagnosis and the usefulness of screening tests for MPS II in specific paediatric populations.

Introduction

Hunter syndrome (Mucopolysaccharidosis type II – MPS II; OMIM 309900) is a genetic storage disease in which the deficiency of the iduronate-2-sulphatase lysosomal enzyme produces a progressive deposition of glycosaminoglycans (mucopolysaccharides) in various organs, leading to degenerative symptoms. It follows an X-linked inheritance (Lonegan et al. 2004; Lyon et al. 2006; Martin et al. 2008; Wraith et al. 2008a).

It is a rare disease, with significant geographical and ethnical variations in the incidence. In studies carried out in different populations, its estimated incidence ranges from one case per 49,000 to one case per 526,000 male live births, and in Europe from one case per 72,000 to one case per 77,000 male live births (Lin et al. 2009; Baehner et al. 2005; Nelson et al. 2003).

Although traditionally patients with Hunter syndrome have been classified into “attenuated” or “severe” subtypes, on the basis of life expectancy and presence or absence of central nervous system (CNS) involvement, the disease should be considered as a continuous spectrum of phenotypes between the two extremes (severe form and attenuated form), being more severe with an early onset of presentation of clinical manifestations (Guelbert et al. 2011; Wraith et al. 2008a). Patients usually appear normal at birth. In patients with the severe form, the age at diagnosis is usually between 18 months and 4 years of age, and CNS involvement causes cognitive impairment and profound mental retardation (Holt et al. 2011a).

The phenotype is characterised by coarse facies, infection and obstruction of the upper airways, hypoacusis, inguinal and umbilical hernia, joint contractures, skeletal

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abnormalities (dysostosis multiplex: thoracolumbar kyphosis, bell-shaped thoracic deformity, subluxated hips, valgus knees), hepatosplenomegaly, cardiac valve disease (Kampmann et al. 2011) and slowly progressive mental deterioration with behavioural alterations between the ages of 2 and 6 (del Toro-Riera 2007; Holt et al. 2011b). Diarrhoea, possibly due to infiltration of the autonomic nervous system, can be a chronic management problem. Neuroimaging may reveal increased lateral ventricles, increased perivascular spaces, leukodystrophy and hydrocephalus. Some patients may present with spinal cord compression with quadriplegia (Gutierrez-Solana 2008; Finn et al. 2008; Holt et al. 2011a, b; Manara et al. 2011). The natural course of the disease leads to death by the age of 10–15.

Most of the patients with the attenuated form are diagnosed between the ages of 4 and 8 years. Early facial appearance is normal and facial dysmorphism is usually mild and more slowly progressive. The most apparent symptoms are limited joint mobility, contractures, carpal tunnel syndrome (Kwon et al. 2011), hernias and hepatosplenomegaly. It differs from the severe forms in the absence of neurodegeneration. However, it should be taken into account that some children may present a slight developmental delay due, at least in part, to external factors such as hypoacusia, sleep apnoeas or visual alterations (Gutierrez-Solana 2008; del Toro-Riera 2007; Burton and Giugliani 2012). Typical bone abnormalities are present but are less conspicuous than in the severe forms. In addition to cardiac valve and respiratory disease, the attenuated form may include hypoacusis, retinopathy and, occasionally, spinal cord compression due to cervical spinal stenosis (Matheus et al. 2004). Some patients may survive for more than 60 years, but others die early, even in the second decade of life, due to heart disease, lung infections or obstruction of the airways (Wraith et al. 2008a; Jones et al. 2009).

The majority of children with Hunter syndrome appear healthy at birth, but typical signs and symptoms appear in the early stages of life that could allow for the identification of patient groups at risk of developing the disease. These include patients suffering from thoracolumbar kyphosis (del Toro-Riera 2007; Wraith et al. 2008b), recurrent inguinal and umbilical hernias (Kliegman et al. 1996; Mendelson et al. 2010) and nodular skin lesions (Demitsu et al. 1999; Schwartz et al. 2007).

Objective

The objective of this study was to identify MPS II patients from population groups considered to have a possible higher incidence of children suffering from Hunter syndrome:

- Patient group with thoracolumbar kyphosis of unknown aetiology (in the first 3 years of life)
- Groups of children with bilateral inguinal hernias, recurrent hernias, umbilical hernia that require surgery or those hernias requiring surgery where the surgeon believes there are signs of connective tissue involvement
- Group of patients with typical nodular skin lesions, for example, cobblestone, hypopigmented, ivory or skin-coloured lesions around the shoulder area, upper limbs, and the lateral portion of the thighs, neck and chest

Any of these groups, either alone, in combination with each other or with other MPS signs and symptoms (recurrent respiratory symptoms, facial dysmorphism, hepatosplenomegaly, cardiac valve disorders, etc.) would increase the index of suspicion for the diagnosis of MPS II.

Patients and Methods

Study Population

The study population included children from the ages of 0 to 18 belonging to the above-mentioned “MPS II” risk groups. Patients were selected retrospectively (where data were available) and prospectively, at the Paediatric Trauma, Paediatric Surgery and Paediatric Dermatology Departments of four Spanish reference centres (Hospital de Cruces in Bilbao, Hospital Infantil Universitario Niño Jesús and Hospital Universitario 12 de Octubre in Madrid and Hospital Virgen del Rocío in Seville).

The inclusion criteria were children under the age of 3 suffering from thoracolumbar kyphosis of unknown cause; children with recurrent or bilateral inguinal hernias or with umbilical hernia requiring surgery; children undergoing hernia surgery where the surgeon detected possible signs of connective tissue involvement; and male patients with typical nodular lesions (hypopigmented or skin coloured, cobblestone appearance in the shoulder area, upper limbs, outer part of buttocks, neck or chest). The medical history and examination of children with kyphosis should not be consistent with other known paediatric causes of kyphosis, such as congenital malformation, infection, malignancy, osteogenesis imperfecta, rickets or neurofibromatosis type 1.

This study was conducted in compliance with the recommendations of the Declaration of Helsinki and the Good Clinical Practice (GCP) Guidelines. The study was submitted to the appropriate Independent Ethics Committee for approval, and complied with Spanish Act No. 15/1999 on Personal Data Protection in relation to the confidentiality of patient data. In all cases, signed informed consent was obtained from patients and/or their parents or guardians.

Table 1 Distribution of subjects by hospital department

Urinary quantitative GAG	Negative (<i>n</i> = 103)	Inconclusive (<i>n</i> = 15)	Positive (<i>n</i> = 12)	Total (<i>n</i> = 130)
Trauma	18 (17.48 %)	2 (18.75 %)	1 (8.33 %)	21 (16.15 %)
Surgery	85 (82.52 %)	13 (81.25 %)	11 (91.66 %)	109 (83.85 %)
Dermatology	0	0	0	0

Methods

All subjects identified in the “MPS II” risk groups underwent a screening test using a first morning urine sample, which were sent to Hospital de Cruces (Barakaldo), for the determination of glycosaminoglycans (GAG) (semi-quantitative using the colorimetric method and quantitative using ultraviolet-visible spectrophotometry). In the case of detection of increased excretion of urinary GAG, an enzymatic assay of a blood sample on absorbent paper (dried blood spot) was performed to determine the activity of iduronate-2-sulphatase. In all patients with increased levels of GAG and normal activity of iduronate-2-sulphatase, tests were carried out, looking for another MPS.

The GAG test was performed using a colorimetric method based on the change in colour produced by the complexes formed between methylene blue (DMB) and GAG (Lage et al. 2011). A solution of DMB, ethanol and sodium formate was prepared, whose concentrations were previously optimised and adjusted to an acidic pH that also required optimising. This solution was encapsulated in transparent vials, to which first morning urine was then added using a syringe. If the concentration of GAG in the urine is high, as occurs in patients with MPS II, the blue colour of the DMB turns to purple when mixed with urine. By contrast, when adding urine from a healthy individual to DMB, the colour does not change and remains blue.

The quantitative determination of urinary GAG was performed using ultraviolet-visible spectrophotometry. As such, a dilution curve of chondroitin sulphate 100 mg/ml (by diluting 5, 10, 25, 50 and 100 µl in 500 µl of water), a blank (500 µl of water) and the patient samples for analysis (100 µl of urine diluted in 500 µl of water) were used. Afterwards, 2.5 ml of DMB is added and readings are taken on the differences in absorbance between 520 and 600 nm.

The semi-quantitative and quantitative techniques do not always give matching results. In this case, the determinant is the result of the ultraviolet-visible spectrophotometry. If a positive result in this determination was obtained, the analysis was repeated on a new urine sample to confirm the positive result, prior to continuing on to the enzymatic assay.

For the enzymatic assay, we determined the enzymatic activity of iduronate-2-sulphatase in blood on filter paper (Voznyi et al. 2001). This determination is based on the reaction of a substrate containing 4-methylumbelliferone-iduronate-2-sulphatase to the test sample in a first incubation and then adding the enzyme α -iduronidase in a second incubation, releasing fluorescent 4-methylumbelliferone, thus measuring the fluorescence emitted with the fluorometer (360–450 nm).

Statistical Analysis

This study was designed to provide descriptive information and as such the statistical analysis was descriptive in nature. Categorical variables were described according to the number and percentage of subjects within each category. The mean, standard deviation, median and minimum and maximum values were used for the description of continuous variables.

Results

From May 2007 to May 2011, a total of 130 samples were collected from four reference centres. Most of these patients (112 subjects) were recruited at the surgery departments: 45.7 % were included for umbilical hernia, 51.4 % for bilateral inguinal hernia and 2.8 % for recurrent inguinal hernia.

After analysis of the 130 urine samples, there were 12 positive GAG tests and 15 inconclusive, while the remaining 103 were negative. Table 1 shows the distribution of subjects by department, mainly surgical (109 subjects, 85 %). Table 2 shows the age and gender of the subjects participating in the study based on whether the result was negative, inconclusive or positive. It should be noted that the mean age of positive subjects was significantly lower than the age of the remaining subjects. Similarly, in subjects with positive results, the percentage of male children was higher than that of female children (67 % vs. 33 %, respectively). Moreover, there are high mean concentrations of GAG in positive subjects.

All patients with a negative GAG test showed quantitative GAG values within the normal range. Of those with

Table 2 Demographics of patients

Urinary quantitative GAG	Negative (<i>n</i> = 103)	Inconclusive (<i>n</i> = 15)	Positive (<i>n</i> = 12)	Totals (<i>n</i> = 130)
Mean age in years (range)	4.41 (0.17–16.51)	4.19 (0.33–12)	3.43 (0.03–14)	4.22 (0.03–16.51)
Gender				
Male, <i>n</i> (%)	63 (61.16 %)	9 (60.00 %)	8 (66.67 %)	73 (55.15 %)
Female, <i>n</i> (%)	40 (38.83 %)	6 (40.00 %)	4 (33.33 %)	50 (38.46 %)

inconclusive GAG tests, 14 out of 15 showed quantitative GAG values within the normal range, whereas of those with positive GAG tests, 3 out of 12 had quantitative values within normal limits. Enzymatic determination was carried out on seven out of the ten patients with increased GAG levels. No enzymatic assay was carried out on three patients because they were lost to follow-up. There were three cases with high quantitation (two with positive GAG tests and one with inconclusive GAG test) where values were within the normal range upon repeating the test, after which no further tests were carried out. In all patients with increased levels of GAG and normal activity of iduronate-2-sulphatase, tests were carried out, looking for another MPS, with normal results in all cases. All results of the enzymatic assay were normal except one case that was eventually diagnosed with Hunter syndrome. In this case, the enzymatic deficiency was confirmed in fibroblasts.

Table 3 includes quantitative and enzymatic determination data for subjects with positive or inconclusive results.

Discussion

This is one of the first MPS II screening studies to take place in Spain aimed at the early detection of MPS II. The objective of any screening programme is to reduce morbidity and mortality caused by the disease in the tested population, by providing the opportunity for early treatment of detected cases. Early diagnosis is thought to be of great importance in diseases such as Hunter syndrome, the outcome of which is usually fatal at an early age for the most severe forms (Mendelson et al. 2010). Although Hunter syndrome is a rare condition, its severity makes it very important as a health problem. This is because it could cause premature death, severe neurological disorders, mental retardation and poor overall quality of life, dependency on others, institutionalisation, higher healthcare costs and, therefore, notably higher family, social and economic burdens (Imarzuki et al. 1994).

Inguinal hernia is very common in childhood, occurring in 10–20 of every 1,000 people. It is predominant in males by a 4:1 ratio. Half of these cases manifest in the first year of life, usually within the first 6 months. Some

60 % occur on the right side, 30 % on the left and 10 % are bilateral. In some cases, inguinal hernia may be familial but not associated with familial MPS II. Premature infants have a nearly 30 % incidence of suffering from inguinal hernia, while the incidence is higher in infants with connective tissue disorder such as Ehlers-Danlos syndrome and MPS (Hunter-Hurler) (Mendelson et al. 2010).

We have found 1 enzymatically confirmed case of Hunter syndrome among the 109 subjects examined with hernias, which is significantly higher than that reported among the general population, which is approximately 1 case per 72,000–77,000 male live births in Europe (Baehner et al. 2005). This case was a 3-year-old boy (at the time of analysis) with umbilical hernia who was operated on at the age of 2 months for bilateral inguinal hernia. At the time of diagnosis, he had already suffered moderate bilateral hypoacusis of conduction due to bilateral seromucous otitis, mild bilateral carpal tunnel syndrome, mild hepatomegaly and emerging joint contractures. His psychomotor development was normal, except for a slight language delay. He is currently being treated with enzyme replacement therapy (ERT) with idursulfase (Elaprase®, Shire Human Genetic Therapies, Inc., Lexington, MA) at the standard dose of 0.5 mg/kg, for the past 14 months. Urine GAG values are almost normalised (17.3 mg GAG/mmol Cr for normal levels between 7.9 and 16.2) and hepatomegaly has been significantly reduced (initial liver volume of 652cc, compared with 600cc after 6 months of treatment). He underwent surgery being submitted to adenoamigdalectomy and placement of transtympanic drainage, resulting in improved hearing and normalisation of language. He is now 5 years old and is scholarised in the year of studies in accordance with his age. No behavioural problems have been developed. The results of the neuropsychological assessments carried out were within normal limits (total intellectual coefficient of 111). He seems to have an attenuated form of the disease. The genetic study is currently still pending.

Within the kyphosis group, we found one subject with very high GAG values (364.09 mg GAG/mmol Cr for normal values between 9.5 and 25.7), which may indicate that the patient suffers from MPS. This case was a 4-year-

Table 3 Analysis of subjects with positive or inconclusive results

	GAG test	Quantitative determination (mg GAG/ mmol Cr)*	Enzymatic determination (iduronate-2- sulphatase)
Male, 8 months Thoracolumbar kyphosis	Inconclusive	14.18 (normal)	Not carried out
Male, 6 years 6 months Bilateral inguinal hernia	Inconclusive	10.76 (normal)	Not carried out
Male, 3 years Bilateral inguinal hernia	Inconclusive	16.97 (normal)	Not carried out
Male, 2 years Umbilical hernia	Inconclusive	13.27 (normal)	Not carried out
Male, 1 year Thoracolumbar kyphosis	Inconclusive	13.76 (normal)	Not carried out
Male, 3 years Umbilical hernia	Inconclusive	10.84 (normal)	Not carried out
Male, 6 years Umbilical hernia	Inconclusive	12.46 (normal)	Not carried out
Male, 5 years Umbilical hernia	Inconclusive	14.87 (normal)	Not carried out
Male, 2 years Bilateral inguinal hernia	Inconclusive	16.66 (normal)	Not carried out
Female, 12 years Umbilical hernia	Inconclusive	42.93 (increased)	Not carried out
Female, 4 months Bilateral inguinal hernia	Inconclusive	24.09 (normal)	Not carried out
Female, 4 years Umbilical hernia	Inconclusive	14.20 (normal)	Not carried out
Female, 5 years 6 months Umbilical hernia	Inconclusive	13.08 (normal)	Not carried out
Female, 3 years Bilateral inguinal hernia	Inconclusive	9.68 (normal)	Not carried out
Female, 8 years Umbilical hernia	Inconclusive	7.59 (normal)	Not carried out
Male, 3 years Bilateral inguinal hernia	Positive	58.79 (increased)	1.9 μ mol/l h (NR: 9–29) Hunter syndrome
Male, 2 years 5 months Bilateral inguinal hernia	Positive	34.27 (increased)	Normal
Male, 1 year 6 months Umbilical hernia	Positive	89.64 (increased)	Normal
Male, 2 years Umbilical hernia	Positive	126.98 (increased)	Normal
Male, 3 months Bilateral inguinal hernia	Positive	25.40 (normal)	Not carried out
Male, 4 years Umbilical hernia	Positive	11.62 (normal)	Not carried out
Male, 10 days Bilateral inguinal hernia	Positive	70.85 (increased)	Normal
Male, 4 years Umbilical hernia	Positive	19.80 (normal)	Not carried out
Female, 3 years Umbilical hernia	Positive	42.94 (increased)	Not carried out
Female, 3 years Umbilical hernia	Positive	58.29 (increased)	Normal
Female, 4 years Thoracolumbar kyphosis	Positive	364.09 (increased)	Not carried out
Female, 14 years Umbilical hernia	Positive	18.82 (increased)	Normal

*Age (normal range): <1 year (13.3–36.3); 1–2 years (8.1–35.3); 3–4 years (9.5–25.7); 5–6 years (7.9–16.2); 7–10 years (6.7–15.5); 11–16 years (3.3–13.7); 17–18 years (2.4–9.0)

old girl, living in Angola, who was admitted in our hospital due to respiratory decompensation. The physical examination showed macroglossia, severe kyphoscoliosis and serious psychomotor delay. A urine sample was collected for GAG determination. Unfortunately, when the result was obtained, several days later, the family had returned to Angola, where it has not been possible to continue the study.

In this study, we were unable to include any children from dermatology visits with typical nodular lesions, probably because the target population is very limited and very specific to Hunter syndrome. However, this is a group requiring a GAG analysis, as the literature already reports.

Recent publications indicate that GAG test allows for rapid detection, confirms or refutes a suspicion and reduces

the number of individuals who require further analysis (Lage et al. 2011). It is, therefore, a method that could be used as initial screening, but as it can give rise to false positive or false negative, it is also recommended to carry out the quantitative urine GAG test (Martin et al. 2008; Baldellou Vázquez and García Jiménez 2006; Wraith et al. 2008a).

For the definitive diagnosis of MPS II, it is necessary to prove deficient activity of the enzyme iduronate-2-sulphatase in leucocytes, fibroblasts or plasma, in the presence of normal activity of at least one other sulphatase. Genetic analysis to identify the IDS gene mutation associated with MPS II is now also commonly performed, but the actual diagnosis remains an enzymatic one (Scarpa et al. 2011; Martin et al. 2008; Guelbert et al. 2011).

Nowadays, ERT is available for the treatment of patients with MPS II. Idursulfase is a glycoprotein similar to the human iduronate-2-sulphatase produced by genetic engineering in a human cell line. The treatment reduces the size of the spleen and liver and normalises GAG excretion in urine, it improves obstructive sleep apnoea and lung function, it decreases the number of ENT (ear, nose, and throat) infections and it improves quality of life (Muenzer et al. 2006, 2007, 2011a, b). It also has a positive effect on growth, especially when treatment begins before the age of 10 (Schulze-Frenking et al. 2011; Alcalde-Martín et al. 2010). Another recent report suggests that it may be possible for early treatment to slow or prevent the development of irreversible manifestations and therefore modify the natural history of MPS II (Tylki-Szymanska et al. 2012). Another recent study of enzyme replacement therapy with idursulfase showed beneficial effects after only 8 months of treatment in patients under the age of 5 showing neurological abnormalities and other signs and symptoms of being severely affected by the disease (Alcalde-Martín et al. 2010). Thus, enzyme replacement therapy has the potential to benefit many patients with MPS II, especially if started early in the course of the disease (Wraith et al. 2008a).

The eventual goal for the early treatment of MPS II is newborn screening (Marsden and Levy 2010). Assays for lysosomal enzyme activity, including I2S activity, in dried blood spots that may be suitable for newborn screening have been developed (Gelb et al. 2006; Wolfe et al. 2011; Sista et al. 2011). A pilot population-based newborn screening using any of these methods has not yet been reported. In addition, controversy exists on the appropriateness to perform these programmes in MPS as there is not a good genotype-phenotype correlation, with the exception of certain mutations-deletions or rearrangements of the IDS gene that completely abolish I2S transcript production will result in the severe phenotype (Burton and Giugliani 2012). It is currently being discussed if it is ethical and if is justifiable submitting a child, who may have an attenuated form of the disease, to weekly infusions from the first few weeks of life, with the risk for subsequent complications. For the time being, and while addressing all these issues, it remains in the hands of primary care paediatricians and paediatric specialists to recognise and refer patients with suspected MPS II as early as possible.

The patient found in this study, at the time of diagnosis, in addition to hernias presented early stage contractures in hands and elbows, and discrete hepatomegaly which was unnoticed on medical examinations carried out so far. His facial appearance was not striking but characteristic. These data could possibly have been enough to suspect MPS if these have been evaluated by a neuropaediatrician or a paediatrician expert in metabolic diseases, but neither

his primary care paediatrician nor the surgeons being visited had referred him to a specialist until the time of the screening. It is common for children with MPS to have been examined in several hospital departments before reaching the neurologist (surgery, rheumatology, trauma, ear, nose, and throat) (Muenzer et al. 2006). There is often a delay of several years between the onset of signs and symptoms and diagnosis. This is particularly true for patients with the attenuated phenotype, as the disease onset can be insidious. Such delays increase the risk of irreversible organ damage and may decrease the benefit of ERT (Muenzer et al. 2009; Vieira et al. 2008; Wraith et al. 2008a). It is thus necessary that physicians who usually visit these patients know about these diseases and the signs and symptoms for suspecting the existence of MPS. In this sense, the diffusion of groups at risk can be a great help.

The determination of GAG in a single urine sample is a painless, low-cost method that is suitable as a screening test during routine hospital visits. We therefore believe it is worth requesting the test at the slightest suspicion. We recommend the development of a simple referral protocol for suspected MPS II, based on European Guidelines (Working Group for Rare Diseases 2011) and local protocols (González-Meneses et al. 2010), at each centre (especially in surgery departments) in order to aid in the diagnosis, treatment and appropriate follow-up of this patient group. Nevertheless, we believe that further studies, more prolonged and with a higher number of patients, need to be conducted to be able to establish the cost-effectiveness of those programmes.

In summary, our study has demonstrated that MPS screening in these patient groups, with such an easy technique as the determination of GAG in urine, may be a simple but useful approach that allows for the early detection of patients suffering from MPS II and offers an individualised and appropriate follow-up and treatment of these patients.

Synopsis

One case of Hunter syndrome was found among 109 patients with hernias in the first screening study in Europe aimed at the early detection of MPS II.

All authors have made a substantial contribution to the study and all have approved the final draft.

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References

- Alcalde-Martín C, Muro-Tudelilla JM, Cancho-Candela R et al (2010) First experience of enzyme replacement therapy with idursulfase in Spanish patients with Hunter syndrome under the age of 5: case observations from the Hunter Outcome Survey (HOS). *Eur J Med Genet* 53:371–377
- Baehner F, Schmiedeskamp C, Krummenauer F et al (2005) Cumulative incidence rates of the mucopolysaccharidoses in Germany. *J Inherit Metab Dis* 28(6):1011–1017
- Baldellou Vázquez A, García Jiménez MC (2006) Diagnóstico de la mucopolisacaridosis II (síndrome de Hunter) en atención primaria. *Acta Paediatr Esp* 64(10):482–485
- Burton BK, Giugliani R (2012) Diagnosing Hunter syndrome in pediatric practice: practical considerations and common pitfalls. *Eur J Pediatr* 171(4):631–639
- Demitsu T, Kakurai M, Okubo Y et al (1999) Skin eruption as the presenting sign of Hunter syndrome IIB. *Clin Exp Dermatol* 24:179–182
- del Toro-Riera M (2007) Follow-up of patients with Hunter syndrome: the Hunter Outcome Survey (HOS) registry. *Rev Neurol* 19;44 (Suppl 1):S13–S17.
- Finn CT, Vedolin L, Schwartz IV et al (2008) Magnetic resonance imaging findings in Hunter syndrome. *Acta Paediatr Suppl* 97 (457):61–68
- Gelb MH, Turecek F, Scott CR, Chamoles NA (2006) 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* 29:397–404
- González-Meneses López A, Barcia Ramírez A, Díaz Rodríguez JL (2010) Action protocol in mucopolysaccharidosis. *Protoc diagn ter pediatr* 1:24–36
- Guelbert N, Amartino H, Arberas C et al (2011) Guideline for diagnosis, follow-up and treatment of mucopolysaccharidoses type II or Hunter disease. *Arch Argent Pediatr* 109(2):175–181
- Gutierrez-Solana LG (2008) Neurological manifestations of Hunter syndrome. *Rev Neurol* 47(suppl 2):S9–S13
- Holt JB, Poe MD, Escolar ML (2011a) Natural progression of neurological disease in mucopolysaccharidosis type II. *Pediatrics* 127(5):e1258–1265
- Holt J, Poe MD, Escolar ML (2011b) Early clinical markers of central nervous system involvement in mucopolysaccharidosis Type II. *J Pediatrics* 159(2):320–326.e2
- Imarzuki M, Gushi K, Kriwit WI (1994) Long term effects of bone marrow transplant for inborn errors of metabolism, a study of four patients with lysosomal storage diseases. *Acta Paediatr* 36:30–36
- Jones SA, Almassy Z, Beck M et al (2009) Mortality and cause of death in mucopolysaccharidosis type II—a historical review based on data from the Hunter Outcome Survey (HOS). *J Inherit Metab Dis* 32(4):534–543
- Kampmann C, Beck M, Morin I, Loehr JP (2011) Prevalence and characterization of cardiac involvement in hunter syndrome. *J Pediatr* 159(2):327–331.e2
- Kliegman RM, Stanton B, St. Geme J, Schor N, Behrman RE (1996) *Nelson Textbook of Pediatrics*, 19th Edition. W.B. Saunders, Philadelphia
- Kwon JY, Ko K, Sohn YB et al (2011) High prevalence of carpal tunnel syndrome in children with mucopolysaccharidosis type II (Hunter syndrome). *Am J Med Genet A, Part A* 155 (6):1329–1335
- Lage S, Prieto JA, Andrade F, Sojo A, Sanjurjo P, Aldámiz-Echevarría LJ (2011) Reliability of a visual test for the rapid detection of mucopolysaccharidoses: GAG test. *J Clin Lab Anal* 25:179–184
- Lin HY, Lin SP, Chuang CK et al (2009) Incidence of the mucopolysaccharidoses in Taiwan, 1984–2004. *Am J Med Genet A* 149A(5):960–964
- Lonergan C, Payne AR, Wilson WG, Patterson JW, English JC III (2004) What syndrome is this? Hunter syndrome. *Pediatr Dermatol* 21:679–681
- Lyon G, Kolodny EH, Pastores GM (2006) *Neurology of hereditary metabolic diseases of children*, 3rd edn. Mc Graw Hill, New York
- Manara R, Priante E, Grimaldi M et al (2011) Brain and spine MRI features of Hunter disease: frequency, natural evolution and response to therapy. *J Inherit Metab Dis* 34:763–780
- Martin R, Beck M, Eng C et al (2008) Recognition and diagnosis of mucopolysaccharidosis II (Hunter Syndrome). *Pediatrics* 121:377–386
- Matheus MG, Castillo M, Smith JK, Armao D, Towle D, Muenzer J (2004) Brain MRI findings in patients with mucopolysaccharidosis types I and II and mild clinical presentation. *Neuroradiology* 46(8):666–672
- Marsden D, Levy H (2010) Newborn screening of lysosomal storage disorders. *Clin Chem* 56:1071–1079
- Mendelson NJ, Harmatz P, Bodamer O et al (2010) Importance of surgical history in diagnosing mucopolysaccharidosis type II (Hunter syndrome): Data from the Hunter Outcome Survey. *Genet Med* 12:816–822
- Muenzer J, Beck M, Giugliani R et al (2011a) Idursulfase treatment of Hunter syndrome in children before the age of 6: results from the Hunter Outcome Survey. *Genet Med* 13:102–109
- Muenzer J, Beck M, Eng CM et al (2011b) Long-term, open-labeled extension study of idursulfase in the treatment of Hunter syndrome. *Genet Med* 13:95–101
- Muenzer J, Gucavas-Calikoglu M, McCandless SE et al (2007) A phase I/II clinical trial of enzyme replacement therapy in mucopolysaccharidosis II (Hunter syndrome). *Mol Genet Metab* 90:329–337
- Muenzer J, Wraith JE, Beck M et al (2006) A phase II/III clinical study of enzyme replacement therapy with idursulfase in mucopolysaccharidosis II (Hunter syndrome). *Genet Med* 8:465–473
- Muenzer J, Beck M, Eng CM et al (2009) Multidisciplinary management of Hunter syndrome. *Pediatrics* 124:e1228–e1239
- Nelson J, Crowhurst J, Carey B, Greed L (2003) Incidence of the mucopolysaccharidoses in Western Australia. *Am J Med Genet A* 123A(3):310–313
- Sista R, Eckhardt AE, Wang T, Sélos-Moura M, Pamula VK (2011) Rapid, single-step assay for Hunter syndrome in dried blood spots using digital microfluidics. *Clin Chim Acta* 412 (19–20):1895–1897
- Scarpa M, Almassy Z, Beck M et al (2011) Mucopolysaccharidosis type II: European recommendations for the diagnosis and multidisciplinary management of a rare disease. *Orphanet J Rare Dis* 6:72

- Schulze-Frenking G, Jones SA, Roberts J et al (2011) Effects of enzyme replacement therapy on growth in patients with mucopolysaccharidosis type II. *J Inher Metab Dis* 34:203–208
- Schwartz IV, Ribero MG, Mota JG et al (2007) A clinical study of 77 patients with mucopolysaccharidosis type II. *Acta Paediatr* 96:63–70
- Tylki-Szymanska A, Jurecka A, Zuber Z, Rozdzyńska A, Marucha J, Czartoryska B (2012) Enzyme replacement therapy for mucopolysaccharidosis II from 3 months of age: a 3-year follow-up. *Acta Paediatr* 101:e42–e47
- Vieira T, Schwartz I, Munoz V et al (2008) Mucopolysaccharidoses in Brazil: what happens from birth to biochemical diagnosis? *Am J Med Genet A* 146A:1741–1747
- Voznyi YK, Keulemans JLM, Bayer EM, van Diggelen OP (2001) A fluorogenic test for the diagnosis of MPS II (Hunter disease). *J Inher Metab Dis* 24:675–680
- Wolfe BJ, Blanchard S, Sadilek M, Scott CR, Turecek F, Gelb MH (2011) Tandem mass spectrometry for the direct assay of lysosomal enzymes in dried blood spots: application to screening newborns for mucopolysaccharidosis II (Hunter Syndrome). *Anal Chem* 83(3):1152–1156
- Working Group for Rare Diseases (2011) Guidelines for the diagnosis, monitoring and treatment of mucopolysaccharidosis type II (MPS II) Hunter's Disease. *Arch Argent Pediatr* 109:175–181
- Wraith JE, Scarpa M, Beck M et al (2008a) Mucopolysaccharidosis type II (Hunter syndrome): a clinical review and recommendations for treatment in the era of enzyme replacement therapy. *Eur J Pediatr* 167:267–277
- Wraith JE, Beck M, Giugliani R, Clarke J, Martin R, Muenzer J (2008b) Initial report from the Hunter Outcome Survey. *Genet Med* 10:508–516