Phenotypic expression in mucopolysaccharidosis VII

P L J A BERNSEN, R A WEVERS, F J M GABREËLS, K J B LAMERS,
A E H SONNEN,* J H SCHUURMANS STEKHOVEN

From the Institutes of Neurology and Pathology, St Radboud Hospital, University of Nijmegen, and Dr Hans Bergerkliniek, Breda, The Netherlands*

SUMMARY β -glucuronidase deficiency is an extremely rare disorder which is known to have a considerable phenotypic variation. A survey of the clinical findings in 19 previously reported patients with mucopolysaccharidosis VII is presented together with the results of clinical and biochemical studies in two further patients. Because a similar clinical picture is present in a heterozygotic sister it is doubted whether all signs and symptoms can be attributed to the β -glucuronidase deficiency. The probability of a concomitant disorder is discussed. Diagnosis was made both by demonstration of the deficiency in plasma and leucocytes, and by means of hair root analysis. The phenotypic variation and the fact that increased levels of glycosaminoglycans were not found in the urine of the two patients lead to the suggestion that in certain cases a correct diagnosis may be missed if the β -glucuronidase activity in plasma and leucocytes is not determined and only routine urine investigation is performed as a screening for a mucopolysaccharidosis. Hair root analysis may be a useful method to measure the β -glucuronidase activity.

Since the first description of abnormal mucopolysacchariduria in a girl with Hurler syndrome,¹ several types of mucopolysaccharidoses have been recognised.² Mucopolysaccharidosis type VII (MPS VII: β -glucuronidase deficiency) was first clinically observed by Sly *et al*³ in a young child; the enzyme defect in this patient's fibroblasts was established by Hall *et al*.⁴ Since then, 19 patients have been reported (table 1)^{3,5-18} with a considerable phenotypic variation. Sewell *et al*¹⁴ discerned three groups of patients on clinical grounds.

We had the opportunity to study two siblings (24 and 39 years old) with MPS VII. They are the oldest patients to be reported with this condition. We present our clinical and biochemical findings together with a survey of the 19 patients so far reported.

Case reports

Case 1

This man, born in 1945, was the first child of healthy non-consanguineous parents. Pregnancy and delivery were uneventful. Psychomotor development was severely retarded. He was able to walk at the age of 4 years and up to

now speech is limited to 3-word sentences. At the age of 8, he had to be institutionalised because of behavioural disturbances, especially aggressive outbursts. There is no history of recurrent respiratory infections. Since 1979 he has been suffering from tonic seizures, which are difficult to control. The occurrence of the seizures is associated with moments of stress.

Physical examination showed a severely mentally retarded patient with a height of 1.70 m. He had microcephaly (head circumference < 2.5 th percentile) and a coarse facies. The neck was short, the thorax normal. The abdomen showed no abnormalities, and more particularly, there was no evidence of organomegaly or herniae. The vertebral column was normal. The joints were rather stiff. Further general, neurological and ophthalmological examinations revealed no abnormalities.

Case 2

This woman, born in 1960, is a sister of patient 1. Pregnancy and delivery were uneventful. Psychomotor development was mildly retarded. As a child she had been able for a few years to receive school education at a school for learning disabilities. Because of progressive mental deterioration she was institutionalised at the age of 18 years. At the age of 19 she had her first tonic clonic seizure. Later sporadic absences were observed. At the moment the epilepsy is being controlled with medication. Further anamnesis is unremarkable.

Physical examination showed a mentally retarded patient with a height of 1.50 m. Head circumference was between the 2nd and 10th percentiles. Her facial features were not coarse. Thorax and abdomen were, except for a mild

Address for reprint requests: Dr Wevers, Institute of Neurology, St Radboud University Hospital, PO Box 9101, 6500 HB Nijmegen, The Netherlands.

Received 10 June 1986 and in revised form 8 October 1986.

Accepted 10 October 1986

Table 1 Summary of clinical signs and symptoms of both 19 previously reported cases with mucopolysaccharidosis type VII and the authors' cases

	Review of literature			Case 1	Case 2	Sibling of cases 1 and 2
	Group I*	Group II†	Group III‡			
Coarse facies	6/6	9/9	2/4	+	—	+
Corneal clouding	2/6	4/9	1/4	—	—	—
Short neck	2/6	3/9	0/4	+	—	—
Mental retardation	3/6	7/9	0/4	+	+	+
Sternal protrusion	3/6	6/9	0/4	—	—	—
Recurrent respiratory infections	2/6	7/9	1/4	—	—	—
Splenohepatomegaly	6/6	6/9	1/4	—	+	—
Diastasis recti	1/6	5/9	0/4	—	—	—
Herniae	4/6	5/9	0/4	—	—	—
Metatarsus adductus/talipes equinovarus	4/6	4/9	1/4	—	—	—
Dysostosis multiplex	5/6	9/9	3/4	—	?	?
Granulations	4/6 (2 NR)	3/9 (6 NR)	4/4	+	+	—
Mucopolysacchariduria	4/6 (2 NR)	9/9	4/4	—	—	—
Epilepsy	0/6	0/9	0/4	+	+	+

*Reference numbers 7, 12, 13, 15–17, †3, 6, 8, 10, 11, 14, 18, ‡5, 7, 9
NR = not reported.

splenohepatomegaly, normal. The thoracolumbar spine showed a scoliosis to the left. A slight hirsutism was present. Further general and neurological examination showed no abnormalities.

Family history

A second sister, born in 1952, turned out to be a carrier of MPS VII. She had a non-progressive severely retarded psychomotor development and was institutionalised at the age of 17 years. Since she was 15, she has been suffering from sporadic tonic clonic seizures, which are being controlled with medication. Physical examination showed a severely mentally retarded patient with a height of 1.50 m and a microcephaly (head circumference <2.5th percentile). Her facies was coarse. Further general and neurological examination showed no abnormalities.

All the remaining family members, three siblings included, are in good health and the family history gives no evidence of epilepsy, mental retardation or other relevant particularities.

Once the diagnosis was established in the institutionalised members of the family, the parents refused all further investigations. The healthy family members refused blood analysis for carrier detection.

Radiological studies

Radiographs of skull, chest, ribs, spine, hands and feet of case 1 as well as computed tomography scanning were normal. Radiological studies of case 2 and the sister were not performed.

Electrophysiological studies

The EEG of case 1 showed a low-voltage fast background activity with bilateral but predominantly right-sided temporal sharp theta paroxysms. Later recordings showed diffuse spike to wave complexes. The EEG of case 2 demonstrated a normal pattern in the posterior region but too much diffuse fast activity. Bilateral paroxysmal irritation existed in the frontal regions. The EEG of the sister showed a low voltage fast pattern.

Routine laboratory studies

The following laboratory tests and data of all three patients were normal: complete blood cell count, urinalysis, renal and hepatic function tests, serum electrolyte levels, serum protein content and creatine kinase. Appropriate studies ruled out endocrinological, immunological, chronic infectious or metabolic diseases, deficiencies, and disorders caused by toxic agents.

Chromosomes of case 1 were normal (G-banding).

Specific laboratory studies

Biochemical methods

β -glucuronidase in leucocytes was assayed on a Cobas centrifugal analyser (Hoffmann-La Roche, Basel, Switzerland) at 37°C using 4-MU- β -D-glucuronide (Koch-Light, Colnbrook, England, no 4012-00) as a substrate. The substrate concentration during the incubation amounted to 4.0 mmol/l. 10 μ l sample and 125 μ l 0.1 mol/l NaAc/HAc buffer pH 4.2 containing the substrate were incubated for 7 minutes. The reaction was stopped with 75 μ l of 0.83 mol/l glycine buffer pH 10. Fluorescence was measured using a primary wavelength of 367 nm and a secondary band filter passing light having wavelengths between 445 and 455 nm. Plasma glucuronidase was assayed manually using a similar method. Enzyme analyses were also carried out with the substrate paranitrophenyl- β -D-glucuronide (Koch-Light, Colnbrook, England, no 4281-H).

Urine analysis

Bound uronic acid was determined according to Di Ferrante *et al.*¹⁹ while the method of Abeling *et al.*²⁰ was used for two-dimensional electrophoresis of urinary glycosaminoglycans.

Hair root analysis

Enzyme activity in hair roots was expressed as a ratio between β -glucuronidase and acid phosphatase activities. The latter enzyme was determined as described by Vermorken *et al.*²¹ The enzymes were extracted from the hair roots by five cycles of freezing (–20°C) and thawing in 75 μ l

Table 2 Lysosomal enzymatic activity in leucocytes (nmol/h/mg protein) and in plasma (nmol/h/ml)

	Leucocytes			Plasma	
	Protein (mg/l)	β -glucuronidase		β -glucuronidase	
		4MU-gluc	PNP-gluc	4MU-gluc	PNP-gluc
Case 1	238	32	20	8.5	1
Case 2	520	44	55	9	0
Sister	498	362	197	70	32
Father	ND	ND	ND	ND	ND
Mother	345	396	236	116	51
Controls:					
Number	83	91	75	25	18
Mean	297	943	316	168	70
Range	170–567	400–1450	114–583	55–478	36–226

ND = not determined.

BSA (1 g/l in aq. bidest.). For the determination of β -glucuronidase activity 20 μ l of extract and 20 μ l of 20 mmol/l 4-MU- β -D-glucuronide in 0.2 mol/l NaAc/HAc buffer pH 4.8 were incubated for 4 hours at 37°C. The reaction was stopped with 400 μ l 0.1 mol/l of ethylene-diamine buffer pH 11.4. Fluorescence was determined using primary and secondary wavelengths of 367 and 445 nm respectively.

Results

Biochemical analysis

The deficiency of β -glucuronidase has been established in leucocytes, plasma and hair roots of both patients (table 2, fig 1). Hair root analysis has been carried out in case 1 as well as in four controls. For the latter, essentially similar results were obtained. The enzymatic activity in 14 hair roots of case 1 was in all cases <9% of the mean activity found in the controls. The mean residual activity in his hair roots was 3.5% of the mean activity in controls. There was no overlap in activity between hair roots of case 1 and that of the controls. Table 2 shows the enzymatic activity found in plasma and leucocytes of the family members. Both cases are deficient in enzymatic activity. One sib (the second sister) and her mother seem to have intermediate values indicating a heterozygosity for the defect. β -glucuronidase activity in plasma and leucocytes measured with paranitrophenol- β -D-glucuronide as a substrate, gave essentially similar results (table 2). Other lysosomal enzymes in this family, like N-acetyl- β -glucosaminidase, showed levels within the reference range of leucocytes and plasma (data not shown). Fibroblasts of case 1 have been kept in culture but failed to grow under normal culturing conditions.

Storage products

No elevated uronic acid values could be detected in the urine samples of case 1 and the second sister. A slightly increased value was found in case 2 (8.8 mg uronic acid/mg creatinine; age-matched reference <6.2). The alcian blue spot test was negative in all cases. Two-dimensional electrophoresis of the urine of case 1 showed only small amounts of chondroitin sulphate.

The peripheral granulocytes of cases 1 and 2 showed cytoplasmic vacuoles with the aspect of Alder-Reilly granulation. Those of the sister were

Fig 1 β -glucuronidase activity in hair roots of case 1 and control.

Fig2 Granulocyte of case 1 with vacuoles containing light-floccular material (magnification 9900 $\times$).

normal. Ultrastructural studies of the granulocytes in the peripheral blood of case 1 revealed that the vacuoles contained light-floccular material (fig 2). Lymphocytes were normal.

Discussion

A considerable phenotypic variation is known to exist in MPS VII. It is, however, possible to recognise similar features in the majority of cases. They correspond more or less to the symptoms of the patient described by Sly *et al*,³ symptoms which include a coarse facies, splenohepatomegaly, a gibbus deformity, diastasis recti, umbilical and inguinal herniae, metatarsus adductus, recurrent respiratory infections, and growth and development retardation. Later reports revealed the existence of cases with sometimes only minor clinical symptoms which were usually diagnosed at adolescence.^{5,9} Sewell *et al*¹⁴ proposed, on clinical grounds, a subdivision into three groups of patients with MPS VII: (a) patients in whom the disease had a severe and early fatal course, (b) patients showing the more classical symptoms of MPS VII and a relative stable clinical picture, as described by Sly *et al*,³ Gehler *et al*⁶ and Sewell *et al*,¹⁴ (c) patients who

had exhibited only very mild symptoms, often diagnosed at adolescence.

MPS VII is an autosomal recessive hereditary disorder. The variation in clinical picture suggests a genetic heterogeneity. The structural gene of human β -glucuronidase is assigned to chromosome 7.²² The heterozygous carriers of MPS VII apparently do not develop any of the symptoms of the disease. They have intermediate levels of β -glucuronidase activity in leucocytes and serum. Some authors mention the presence of cytoplasmic granulation in leucocytes of heterozygotes.^{8,16}

Our patients differ in several respects from the others reported to date. They are the oldest patients described in the literature. The more typical signs of MPS VII are absent (table 1). Both patients as well as their heterozygous sister are suffering from epilepsy and severe mental retardation. Epilepsy has not been mentioned before in cases of MPS VII, and severe mental retardation is only seen in combination with other symptoms characteristic of this disorder. These facts and the presence of similar features in the heterozygous sister suggest that epilepsy and mental retardation in our patients are not related to MPS VII but to a concomitant disorder. Since the patients show only mild signs of MPS VII, they should in our opinion be classified under group III.

Our patients have a somewhat higher residual β -glucuronidase activity (3–4%) compared with previously described patients, in whom the specific activities of this enzyme for serum and leucocytes are generally reduced to 0.1–2% of the mean control values.^{3,7,9,16} We had the opportunity to perform a hair root analysis in case 1. Such a procedure has not been reported before in cases of MPS VII. It turned out that the deficiency actually can be established in hair roots. A similar level of residual β -glucuronidase activity was found in leucocytes and in hair roots.

The biochemical studies in the patients reported by us are remarkable because no glycosaminoglycans could be detected in their urine by routine procedures; this is in contrast to the findings of all patients with MPS VII described in the literature. The reports mention the excretion of several different glycosaminoglycans. This may be due to variation in the methods of laboratory investigations. The patient reported by Sly *et al*³ excreted chondroitin sulphate, which was also reported by other authors as the principal excreted storage product.^{5,10,14,16} On the other hand, the patients described by Gehler *et al*,⁶ Beaudet *et al*,⁷ Pfeiffer *et al*⁸ and Teyssier *et al*¹² excreted predominantly dermatan and heparan sulphates.

Although no elevated amounts of storage products could be found in the urine of our two patients, cellular morphology clearly provided evidence of a storage disease. Light microscopy showed cytoplasmic granu-

lation in the peripheral granulocytes of both of them. Obvious vacuolation was seen in electron microscopy of the granulocytes, but not in the lymphocytes of case 1. Cytoplasmic granulation in the peripheral granulocytes has been found in almost every patient with MPS VII reported in the literature. They are described as metachromatic granules, prominent granulation, or Alder Reilly anomaly.²³

The clinical findings in cases 1 and 2 emphasise again the phenotypic variation in MPS VII. Because of their nonspecific signs and the absence of glycosaminoglycans in urine they would have escaped diagnosis if determination of lysosomal enzymatic activities had been omitted. Therefore, we consider it advisable to perform enzyme analysis in any patient even if there is a slight suspicion of mucopolysaccharidosis.

We are grateful to Professor S K Wadman and Dr M Duran (Wilhelmina Children's Hospital, Utrecht) for carrying out the two-dimensional electrophoresis of urinary glycosaminoglycans. The skilful technical assistance of Ms C J M G van den Berg and Mrs R I Hekman-Pheiffer is highly appreciated.

References

- 1 Dorfman A, Lorincz AE. Occurrence of urinary acid mucopolysaccharides in the Hurler syndrome. *Proc Natl Acad Sci USA* 1957;**43**:443-6.
- 2 McKusick VA. Genetic heterogeneity and allelic variation in the mucopolysaccharidosis. *Johns Hopkins Med J* 1980;**146**:71-9.
- 3 Sly WS, Quinton BA, McAlister WH, Rimoin D. Beta glucuronidase deficiency: report of clinical, radiologic and biochemical features of a new mucopolysaccharidosis. *J Pediatr* 1973;**82**:249-57.
- 4 Hall CW, Cantz M, Neufeld EF. A β -glucuronidase deficiency mucopolysaccharidosis: studies in cultured fibroblasts. *Arch Biochem Biophys* 1973;**155**:32-8.
- 5 Danes BS, Degnan M. Different clinical and biochemical phenotypes associated with β -glucuronidase deficiency. *Birth Defects* 1974;**10**:251-7.
- 6 Gehler J, Cantz M, Tolksdorf M, Spranger J, Gilbert E, Drube H. Mucopolysaccharidosis VII: β -glucuronidase deficiency. *Hum Genet* 1974;**23**:149-58.
- 7 Beaudet AL, DiFerrante NM, Ferry GD, Nichols BL Jr, Mullins CE. Variation in the phenotypic expression of β -glucuronidase deficiency. *J Pediatr* 1975;**86**:388-94.
- 8 Pfeiffer RA, Kresse H, Bäumer N, Sattinger E. Beta-glucuronidase deficiency in a girl with unusual clinical features. *Eur J Pediatr* 1977;**126**:155-61.
- 9 Gitzelmann R, Wiesmann UN, Spycher MA, Herschkowitz N, Giedion A. Unusually mild course of β -glucuronidase deficiency in two brothers (mucopolysaccharidosis VII). *Helv Paediatr Acta* 1978;**33**:413-28.
- 10 Guibaud P, Maire I, Goddon R, Teyssier G, Zabot MT, Mandon G. Mucopolysaccharidose type VII par déficit en β -glucuronidase. Etude d'une famille. *J Genet Hum* 1979;**27**:29-43.
- 11 Hoyne HE, Jones KL, Higginbottom MC, O'Brien JS. Presentation of mucopolysaccharidosis VII (β -glucuronidase deficiency) in infancy. *J Med Genet* 1981;**18**:237-9.
- 12 Teyssier G, Maire I, Damon G, Boyer S, Lauras B, Freycon F. Mucopolysaccharidose de type VII à révélation néonatale. *Arch Fr Pediatr* 1981;**38**:603-4.
- 13 Nelson A, Peterson L, Frampton B, Sly WS. Mucopolysaccharidosis VII (β -glucuronidase deficiency) presenting as nonimmune hydrops fetalis. *J Pediatr* 1982;**101**:574-6.
- 14 Sewell AC, Gehler J, Mittermaier G, Meyer E. Mucopolysaccharidosis type VII (β -glucuronidase deficiency): a report of a new case and a survey of those in the literature. *Clin Genet* 1982;**21**:366-73.
- 15 Wilson D, Melnik E, Sly W, Markesbery WR. Neonatal beta-glucuronidase deficiency mucopolysaccharidosis (MPS VII): autopsy findings. *J Neuropathol Exp Neurol* 1982;**41**:344.
- 16 Capdeville R, Boissinot G, Graveleau D, Maroteaux P. Un nouveau cas de mucopolysaccharidose de type VII avec importantes anomalies squelettiques. *Ann Pediatr* 1983;**30**:689-92.
- 17 Irani D, Kim H-S, El-Hibri H, Dutton RV, Beaudet A, Armstrong D. Postmortem observations on β -glucuronidase deficiency presenting as hydrops fetalis. *Ann Neurol* 1983;**14**:486-90.
- 18 Sheets Lee JES, Falk RE, Ng WG, Donnell GN. β -Glucuronidase deficiency: a heterogeneous mucopolysaccharidosis. *Am J Dis Child* 1985;**139**:57-9.
- 19 Di Ferrante N, Rich C. The mucopolysaccharides of normal human urine. *Clin Chim Acta* 1956;**1**:519-24.
- 20 Abeling NGGM, Wadman SK, Van Gennip AH. Two dimensional electrophoresis of urinary mucopolysaccharides on cellulose acetate after N-cetylpyridiniumchloride (CPC) precipitation: a method suitable for the routine laboratory. *Clin Chim Acta* 1974;**56**:297-303.
- 21 Vermorken ALM, Weterings PJJM, Spierenburg GTh, Van Bennekom CA, Wirtz P, De Bruyn CHMM, Oei TL. Fabry's disease: biochemical and histochemical studies on hair roots for carrier detection. *Br J Dermatol* 1978;**98**:191-6.
- 22 Chern CJ, Croce CM. Assignment of the structural gene for human β -glucuronidase to chromosome 7 and tetrameric associations of subunits in the enzyme molecule. *Am J Hum Genet* 1976;**28**:350-6.
- 23 Peterson L, Parkin J, Nelson A. Mucopolysaccharidosis type VII: a morphologic, cytochemical and ultrastructural study of the blood and bone marrow. *Am J Clin Pathol* 1982;**78**:544-8.