Variant of iduronidase deficient mucopolysaccharidoses

Further evidence for genetic heterogeneity¹

B. SHANNON DANES

From the Laboratory for Cell Genetics, Department of Medicine, Cornell University Medical College, New York, New York, USA

SUMMARY An α -L-iduronidase deficiency syndrome has been described in adult male twins, which was phenotypically distinct from that of the Hurler and Scheie syndromes or the chondroitinsulphurias. Multiple dysostosis and stiff joints were present without cloudy corneae, cardiac involvement, and mental or physical retardation. This clinical phenotype appeared to be a newly recognized allelic mutation at the iduronidase locus but does not exclude a non-allelic mutation coding for a subunit of the iduronidase molecule.

With the advent of lysosomal enzyme assays on cultured human dermal fibroblasts, the genetic mucopolysaccharidoses, originally classified on their clinical phenotypes, could be identified by specific lysosomal enzyme deficiencies (McKusick, 1972). One rule substantiated by this approach has been that phenotypic differences within such a group reflect different enzyme deficiencies; α -L-iduronidase appears to be the exception to this rule. The Hurler (I-H) (Matalon and Dorfman, 1971) and Scheie (I-S) syndromes (Bach *et al.*, 1972) as well as four cases of chondroitinsulphuria (Babarik *et al.*, 1974; Spranger *et al.*, 1974; Leisti *et al.*, 1975), each of which are phenotypically different, have a deficiency of α -L-iduronidase. The purpose of this paper is to support this hypothesis for genetic heterogeneity by describing a fourth distinct clinical phenotype associated with a lack of this enzyme.

Clinical history

Identical twin boys were born after a normal gestation of 8 months (birthweight, twin 1: 2097g; twin 2: 2268g. There was no pertinent family history. At birth the boys were considered to be normal except

for club feet which were corrected surgically at age 6 months. During the first year the only clinical abnormality noted was metatarsus adductus (in both twins all 10 fingers locked equally). At 2 years of age clouding of the corneae and mucopolysacchariduria were reported to be absent. At age 5 years bilateral carpal tunnel syndrome required correction and at age 7 years increased restriction of joint movements required hand surgery. Between the ages of 10 and 13 years bilateral hip flexion deformities progressed to give lordotic postures (Figs. 1 and 2). The intelligence of both twins was reported to be normal.

At 13 years of age the twins were referred to our clinic for evaluation of a possible mucopolysaccharidosis using cell culture methodology not available at the time of their initial evaluation 10 years earlier. Their clinical phenotype (Table 1, Figs. 1 and 2) showed distinct skeletal involvement without mental and physical retardation. There was no clinical evidence of storage of mucopolysaccharides in tissues such as cornea, liver, spleen, or heart.

Laboratory studies

Analysis of the urinary mucopolysaccharides (Di Ferrante *et al.*, 1972; Douglas *et al.*, 1973) showed that excretion of large molecular weight mucopolysaccharides over a 24-hour period was three times (55.3, 53.5 mg uronic acid/24 hours) that of normals (range 2.4 to 16.0). The degradation ratio (large

¹This research was made possible through a grant from The National Foundation-March of Dimes and supported in part by The Health Research Council of the State of New York, The Danes Medical Research Fund, Cornell University Medical College, and the Zemurray Foundation.

Received for publication 7 February 1977

Fig. 1 X-ray film of twin 1. (A) Skull, normal; (B) thorax, slight widening of the distal portions of the clavicles near their acromial junction; no rib abnormalities; lungs and heart normal; (C) pelvis, broad femoral heads do not fit into acetabulum with valgus deformity of hips; notice lucent defects along lateral aspects of femoral necks; (D) hands, normal; (E) humerus, short and wide with varus deformity of humeral head and prominent deltoid ridge on the lateral aspect of the shaft; (F) spine, slight posterior scalloping along the posterior aspect of the vertebral bodies and end plates of vertebral bodies somewhat irregular.

molecular weight/degraded fragments) was abnormal (1.5, 1.8; normal 0.03 to 0.8), reflecting a decrease in degradation.

By electrophoresis of CPC-precipitated mucopolysaccharides, there were equal quantities of chondroitin sulphates, dermatan sulphate, and heparan sulphate in the urine in contrast to that found in urine from normals in which chondroitin sulphates are the only major constituents.

Cultured dermal fibroblasts derived from skin biopsies (Danes, 1976) from the twins showed metachromasia and alcianophilia at 0.3 M $MgCl_2$.

Intracellular mucopolysaccharide content was increased more than three times (9.6, 11.8 μg uronic acid/mg cell protein) as compared with that (2.0-3.9 μg uronic acid/mg cell protein) of matched normal cultures (Table 2). Cultures established and assayed at the same time from a Hurler patient and a Hurler/Scheie compound showed five times and from a Scheie patient twice the normal intracellular content (Table 2).

Assay of lysosomal enzyme functions (Neufeld, 1974) showed all (β -galactosidase, β -glucuronidase, β -glucosaminidase, α -glucosaminidase, acid phos-

Fig. 2 X-ray films of twin 2. (A) Skull, normal; (B) thorax, slight widening of the distal portions of the clavicles; (C) Pelvis, femoral heads do not completely fit into the acetabulum; radiolucencies within the femoral necks and in the head of the left femur; (D) Hands, normal; (E) Humerus normal length with widening of the proximal shaft and varus deformity of the head; (F) Spine posterior scalloping of lumbar vertebrae and changes in end plates of vertebral bodies present but not as pronounced as in twin 1.

phatase, aryl sulphatase, α -fucosidase, and α -mannosidase) were within normal range with the exception of α -L-iduronidase. When the activity of α -L-iduronidase was expressed as a ratio to β -galactosidase, the cultured fibroblasts from the twins showed essentially no activity as compared with controls (Table 2, Fig. 3). Samples assayed from the cultures from patients with the Hurler, Scheie and Hurler/Scheie compound syndromes also showed no or minimal α -L-iduronidase activity (Table 2, Fig. 3).

Cell mixing experiments (Danes, 1976) showed that fibroblasts from the twins did not correct fibroblasts

from patients with the Hurler or the Scheie syndromes but did correct fibroblasts from patients with other mucopolysaccharidoses (Sanfilippo A and B, the Hunter and Maroteaux-Lamy syndromes). Their cells were corrected by cells from normals as were those from other mucopolysaccharidoses.

Skin fibroblast cultures from the parents of the twins showed normal culture phenotypes (no metaphase chromasia or alcianophilia, normal intracellular uronic acid content and lysosomal enzyme activities including that of α -L-iduronidase (Table 2, Fig. 3).

Clones developed from single cell platings of

Table 1 Comparison of clinical and biochemical phenotypes of twins with patients with Hurler (I-H) and Scheie syndromes (I-S) and Hurler-Scheie compound (IH/IS)

Clinical criteria*	Hurler (I-H)	Scheie (I-S)	Hurler-Scheie compound (IH/IS)	Twin 1	Twin 2
Age at which clinical signs noted (y)	<1	7-10	1-2	1	1
Retardation					
Physical	+	—	+/-	—	—
Mental	+	—	+/-	—	—
Clinical signs					
Coarsening of facies	+	+	+	+	+
Broad mouth	+	+	+	+	+
Corneal clouding	+	+	+/-	-‡	-‡
Thickened skin	+	+	+	+	+
Hirsutism	+	+	+	+	+
Hand deformities					
Stiff joints (claw hands)	+	+	+/-	+	+
Carpal tunnel syndrome	+	+	+/-	+	+
Cardiac valve involvement	+	+	+	—	—
Metachromatic granulations in polymorphonuclear leucocytes	+	+/-	+	—	—
Hearing defect	+	+/-	+	—	—
Hepatosplenomegaly	+	+/-	+	—	—
Recurrent infections	+	—	+/-	—	—
Urinary mucopolysaccharides†					
Large molecular weight (mg uronic acid/100 ml) (normal=1.4 (range 0.2-4.0))	14.3	9.2	12.6	7.0	7.0
Large molecular weight/fragments (normal=0.03-0.8)	3.4	1.4	2.0	1.5	1.8
Mucopolysaccharides increased	Dermatan sulphate and heparan sulphate				

*Clinical evaluation shown as presence (+), absence (—) or mild expression or variably present (+/-).

†Methodology used in isolation/characterization of urinary mucopolysaccharides—Douglas *et al.* (1973) and Di Ferrante *et al.* (1972). Values from individual patients with each syndrome.

‡Corneal clouding not present but stromal infiltrates which are not uncommon in the general population seen in corneas of both twins and their mother.

cultured fibroblasts according to the dilution technique of Puck *et al.* (1956) had α -L-iduronidase activities which were either in the normal range or slightly lower than normal (Fig. 3).

Enzyme assays did not indicate two groups, one with and without activity, in the clones derived from the mother's line as expected in an X-linked recessive mucopolysaccharidosis (Danes and Bearn, 1967).

Discussion

The clinical disorders of the twins were identified as a mucopolysaccharidosis on the basis of (1) mucopolysacchariduria with increased amount of dermatan sulphate and heparan sulphate, as well as chondroitin sulphates, the major urinary mucopolysaccharide in urine from normals; and (2) increased intracellular mucopolysaccharide content in cultured dermal fibroblasts. Their syndrome was further identified as an iduronidase deficient mucopolysaccharidosis on the basis of (1) deficient activity of α -L-iduronidase in cultured dermal fibroblasts with normal activity of other lysosomal enzymes measured, and (2) no cross-

correction of culture phenotype (increased mucopolysaccharide content measured by increased metachromasia and accumulation of sulphate-³⁵S) when grown with fibroblasts from the Hurler and Scheie syndromes but definite correction with cells from the Hunter, Sanfilippo, and Maroteaux-Lamys syndromes.

However, their clinical phenotype was distinctly different from that of the other known α -L-iduronidase deficient syndromes (Table 1). Mental retardation, dwarfism, decreased hearing, and cloudy corneas, present in the Hurler syndrome (McKusick, 1972), were not present in the twins.

The appearance of the twins was similar to that of the Scheie syndrome for (1) mild dysostosis multiplex, (2) normal intelligence, and (3) coarseness of facies and hirsutism, but was dissimilar as (1) corneas were clear and (2) there were no cardiac valvular lesions (in particular aortic valve). The Scheie syndrome was the initial clinical diagnosis based on the mild somatic involvement with normal intelligence. However, as the twins had only one—stiff joints—of the three clinical stigmata (the other two being major clouding of the cornea and aortic regurgitation) of

Table 2 Intracellular mucopolysaccharide content and α -L-iduronidase/ β -galactosidase activity of cultured skin fibroblasts derived from normals, twins and their parents, patients with the Hurler (I-H), Scheie (I-S), Hurler/Scheie compound (I-H/I-S) syndromes, and heterozygotes for Scheie syndrome

Individual studied	Cultured skin fibroblasts*		
	Intracellular mucopolysaccharides†		Lysosomal enzyme activity‡
	μ g uronic acid/mg cell protein	Sulphate- ³⁵ S DPM/mg cell protein	α -L-iduronidase/ β -galactosidase
Normals (12)§	2.0-3.9	261-468	0.39-0.82
Twin 1	9.6	718	0.00
Twin 2	11.8	867	0.09
Mother	2.5	375	0.50, 0.70
Father	3.3	276	0.44, 0.53
Scheie			
Homozygote	7.4	825	0.00, 0.10
Heterozygote (8)§	3.4-5.4	342-620	0.18-0.70
Hurler	16.8	945	0.10
Hurler/Scheie compound	19.0	825	0.09

*Range indicated by a -.
†Chemical methods used in mucopolysaccharide determinations—Danes (1976).
‡Method for lysosomal enzyme assays—Neufeld (1974).
§Number of individuals studied indicated in parentheses otherwise 1 individual for each syndrome.

Fig. 3 α -L-iduronidase activity in frozen-thawed extracts of cultured skin fibroblasts from the twins, their parents, homozygotes and heterozygotes for the Hurler and Scheie syndromes and normals. Activity expressed on the basis (ratio) of activity of another lysosomal enzyme, β -galactosidase (Bach *et al.*, 1973).

this syndrome (McKusick, 1972), their phenotype would have to be considered to be a variant of the Scheie (I-S) syndrome.

Four patients in three families have previously been reported with α -L-iduronidase deficiency associated with chondroitinsulphaturia. One (Benson

et al., 1972; Babarik *et al.*, 1974) had a clinical phenotype similar to that seen in the Hurler syndrome (McKusick, 1972). The other three (Philippart and Sugarman, 1969; Spranger *et al.*, 1971, 1974; Leisti *et al.*, 1975) had mild clinical expressions, as did the twins reported in this paper, but had stigmata (cloudy corneae, abnormal facies, short stature, hepatomegaly, and only chondroitin-4-sulphate in the urine) giving phenotypes distinctly different from that of the twins.

As their clinical phenotype had atypical features in association with α -L-iduronidase deficiency, there was the possibility that the twins represented genetic compounds of the Hurler (I-H) and Scheie (I-S) genes. Their clinical phenotype (Table 1) was dissimilar to that recognized for the Hurler-Scheie (I-H/I-S) compound (mild mental retardation, cloudy corneae, hepatomegaly, and short stature) as was the phenotype of their cultured fibroblasts (Table 2). Another line of evidence is provided by the heterozygote status of the parents. It had previously been shown (Danes 1974, 1976) that the carrier of the Hurler gene could be distinguished from that of the Scheie gene by differences in culture phenotype (staining characteristics and intracellular mucopolysaccharide content) but not on α -L-iduronidase activity as there is an overlap between the values for normals and heterozygotes (Bach *et al.*, 1973) (Fig. 3). The Hurler and Scheie genes could not be assigned to either of the twins' parents as their culture phenotypes (staining, mucopolysaccharide content and α -L-iduronidase activity) were normal (Table 2, Fig. 3). On the basis

J Med Genet: first published as 10.1136/jmg.14.5.346 on 1 October 1977. Downloaded from <http://jmg.bmj.com/> on 06 May 2020 by guest. Protected by copyright.

of the previous cell culture studies in which the Hurler and Scheie genes could be assigned to individual parents of Hurler/Scheie compounds (Danes, 1974, 1976), it seems unlikely that these twins are compound heterozygotes resulting from a Hurler/Scheie mating.

The features (clinical and cell culture) of the twins described suggested that they represented a newly recognized mutant allele at the Hurler-Scheie locus but did not exclude a non-allelic mutation coding at a separate locus for a subunit of the iduronidase molecule.

I am indebted to Dr Richard H. Nachtigall for referring the family, Dr Patricia Winchester for interpreting the x-ray films, and Dr Peter L. Laino for his ophthalmological evaluations. I wish to acknowledge the assistance of Mrs Beth K. Rottell and Mrs E. Senna for their invaluable assistance. The phenyl α -L-iduronide was a gift from Drs B. Weissmann and E. F. Neufeld under Contract NIH-NIAMDD 73-2205.

References

- Babarik, A., Benson, P. F., Dean, M. F., and Muir, H. (1974). Chondroitinsulphaturia with α -L-iduronidase deficiency. *Lancet*, **2**, 464-465.
- Bach, G., Cantz, M., Hall, C. W., and Neufeld, E. F. (1973). Genetic errors of mucopolysaccharide degradation. *Biochemical Society Transactions*, **1**, 231-234.
- Bach, G., Friedman, R., Weissmann, B., and Neufeld, E. F. (1972). The defect in the Hurler and Scheie syndromes: deficiency of α -L-iduronidase. *Proceedings of the National Academy of Sciences of the United States of America*, **69**, 2048-2051.
- Benson, P. F., Dean, M. F., and Muir, H. (1972). A form of mucopolysaccharidosis with visceral storage and excessive urinary excretion of chondroitin sulphate. *Developmental Medicine and Child Neurology*, **14**, 69-74.
- Danes, B. S. (1974). In vitro confirmation of genetic compound of the Hurler and Scheie syndromes. *Lancet*, **1**, 680.
- Danes, B. S. (1976). Advances in the diagnosis of human genetic disorders. In *Birth Defects: Risks and Consequences*, pp. 297-317. Ed. by S. Kelly, E. B. Hook, D. T. Janerich, and I. H. Porter. Academic Press, New York.
- Danes, B. S., and Bearn, A. G. (1967). Hurler's syndrome: a genetic study of clones in cell culture with particular reference to the Lyon hypothesis. *Journal of Experimental Medicine*, **126**, 509-522.
- Di Ferrante, N., Neri, G., Neri, M. E., and Hogsett, W. E., III. (1972). Measurement of urinary glycosaminoglycans with quaternary ammonium salts: an extension of the method. *Connective Tissue Research*, **1**, 93-101.
- Douglas, C., Nowak, J., and Danes, B. S. (1973). Mucopolysaccharides in urine during normal human development. *Pediatric Research*, **7**, 724-727.
- Leisti, J., Rimoin, D. L., Kabak, M. M., Hollister, D. W., Den Tandt, W., Neufeld, E., Matalon, R., and Philippart, M. (1975). Phenotypic variation in alpha-L-iduronidase deficiency. *Lancet*, **1**, 1344.
- McKusick, V. A. (1972). *Heritable Disorders of Connective Tissue*, 4th ed. C. V. Mosby, St. Louis.
- Matalon, R., and Dorfman, A. (1971). Hurler's syndrome, an α -L-iduronidase deficiency. *Biochemical and Biophysical Research Communications*, **47**, 959-964.
- Neufeld, E. F. (1974). The biochemical basis for mucopolysaccharidoses and mucopolipidoses. In *Progress in Medical Genetics*, vol. 10, pp. 81-101. Ed. by A. G. Steinberg and A. G. Bearn. Grune and Stratton, London and New York.
- Philippart, M., and Sugarman, G. I. (1969). Chondroitin-4-sulphate mucopolysaccharidosis—a new variation of Hurler's syndrome. *Lancet*, **2**, 854.
- Puck, T. T., Marcus, P. I., and Cieciura, S. J. (1956). Clonal growth of mammalian cells in vitro, growth characteristics of colonies from single HeLa cells with and without a 'feeder' layer. *Journal of Experimental Medicine*, **103**, 273-284.
- Spranger, J., Gehler, J., O'Brien, J. F., and Cantz, M. (1974). Chondroitinsulphaturia with α -L-iduronidase deficiency. *Lancet*, **2**, 1082.
- Spranger, J. W., Schuster, W., and Freitag, F. (1971). Chondroitin-4-sulphate mucopolysaccharidosis. *Helvetica paediatrica Acta*, **26**, 387-396.
- Requests for reprints to Dr B. Shannon Danes, Laboratory for Cell Genetics, Department of Medicine, The New York Hospital—Cornell Medical Center, 525 East 68th Street, New York, N.Y. 10021, U.S.A.