Genetic heterogeneity within the chondroitinsulphaturias

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SUMMARY The approach, identification of clinical phenotype followed by lysosomal enzyme assays in cell culture, used in the classification of the genetic mucopolysaccharidoses I-VI has been applied to the chondroitinsulphaturias. There was evidence of heterogeneity in the first 9 patients reported.

Although the six major genetic mucopolysaccharidoses have now been classified on the basis of specific enzyme deficiencies, one group, the chondroitinsulphaturias, seems to be a heterogeneous group not reflecting a single enzyme deficiency (Sly et al., 1973). Of the seven families reported to have members showing chondroitinsulphaturia one had the clinical signs of mucopolysaccharide storage in association with \( \beta \)-glucuronidase deficiency (Sly et al., 1973, 1974) and was designated Type VII in the McKusick classification (1972). Affected individuals in three (Spranger et al., 1974; Leisti et al., 1975; Babarik et al., 1974) of the other six families showed a deficiency of \( \alpha \)-L-iduronidase, the enzyme lacking in the Hurler and Scheie syndromes (Neufeld, 1974), whereas no enzyme studies have been reported in the other three (Thompson et al., 1971; Onisawa et al., 1971; Schimke et al., 1974).

This report describes the phenotype (clinical and biochemical) of a patient with chondroitinsulphaturia from an eighth family and discusses the evidence for genetic heterogeneity within the chondroitinsulphaturias.

Case report

The patient, a negro boy, was referred when 7 months' old for investigation of 'minimal knee stiffness', noted by his mother, and inability to sit independently. He was the product of a full-term, uncomplicated gestation and was considered to be normal at birth. There was no pertinent family history.

The following abnormalities were present. (1) abnormal facies (flat nose-bridge, frontal bossing, hyper-macroglossia; (2) minimal corneal clouding by slit-lamp examination; (3) hepatomegaly (3-4 cm below RCM); (4) enlarged patellae and limitation of joint extension; (5) limited hand supination; (6) slight thoracolumbar gibbus (Fig. 1), with radiographic features of the skeleton compatible with a mucopolysaccharidosis (Fig. 2); and (8) a two-month delay in gross motor development with adequate fine motor, social, and language scores.

At age 2 years there was slight motor developmental retardation commensurate with the degree of skeletal deformity. Weakness of 4/5 was noted in the gastrocnemius muscles and of 3/5 in the deltoid muscles and dorsiflexor muscles of the hands. Mental development was considered to be close to normal with minimal delay in speech production and normal

Fig. 1 Lateral views of patient at 11 months of age showing (A) flaring of lower ribs and (B) thoracolumbar gibbus.
hearing. The patient has had to be hospitalized several times for recurrent respiratory ailments.

LABORATORY STUDIES
The mucopolysaccharides in the urine and cultured fibroblasts were quantitatively and qualitatively assayed by methods previously described (Danes et al., 1972; Douglas et al., 1973). Activities of lysosomal enzymes studied were assayed as described for fibroblastic homogenates (Neufeld, 1974). Analyses of urinary mucopolysaccharides (Table 1) showed that the excretion of large molecular weight mucopolysaccharides (1.9 mg/24 h) was within normal limits (normal range 2.4-6.0) while excretion of degradation products was low (0.6 mg/100 ml urine), giving a degradation ratio of 2.3 (normal range 0.03-

Fig. 2 Radiographs of patient at age 7 months. (A) Broad and spatulated ribs. (B) Flaring of distal end of femur. (C) Broad phalanges and distal ends of metacarpals, which taper proximally. (D) Thoracolumbar kyphosis with beaking L2-5. (E) Pelvis with angle of femoral neck straightened and other anomalies.
0.80). The CPC-precipitated mucopolysaccharides showed two components which migrated identically with purified chondroitin-4-sulphate and chondroitin-6-sulphate.

Cultured fibroblasts (Table 1) showed metachromasia and alcianophilia at 0.3 M MgCl₂. Intracellular mucopolysaccharides were only slightly increased as compared with that found in cultured fibroblasts from normal persons matched for age and sex. The uronic acid content was 3.1 µg uronic acid/mg cellular protein (normal range 1.4-2.0 µg/mg cellular protein) and sulphate incorporation was 590 cpm ³⁵S/µg uronic acid/mg cellular protein (normal range 210-397).

Cell mixing experiments showed that fibroblasts from the patient cross-corrected fibroblasts from patients with β-glucuronidase, β-glucosaminidase, and α-L-iduronidase deficiencies. The activity of β-glucuronidase was within normal limits as well as the activities of other lysosomal enzymes measured (β-galactosidase, α-L-iduronidase, α-glucosidase, β-glucosaminidase, acid phosphatase, aryl sulphatase, α-fucosidase, and α-mannosidase).

Using the same laboratory criteria (Table 1) the urine and cultured fibroblasts from both parents showed normal mucopolysaccaride content and lysosomal enzyme activities. The urinary mucopolysaccharides from a normal half sister were within normal limits.

**Discussion**

The genetic mucopolysaccharidoses were first recognized and classified on the basis of clinical phenotype (McKusick, 1972). The application of cell culture techniques has made it possible to assay for enzymatic defects in connective tissue. The assumption that each represented a different genotype based on the distinct clinical phenotypic differences has been verified by finding a deficiency of a different lysosomal enzyme in cultured fibroblasts from each mucopolysaccharidosis with the exception of the Hurler and the Scheie syndromes which both lack α-L-iduronidase. There seemed to be no heterogeneity within each of the types I-VI (McKusick classification).

It was proposed that the same format could be used for the chondroitinsulphaturias (Table 2). Though some of the published clinical descriptions were incomplete it was possible to compare them according to the major stigmata associated with the mucopolysaccharidoses. All nine patients had physical retardation, short stature, and skeletal dysplasia. The other clinical signs associated with tissue storage of mucopolysaccharides were variably present. The clinical phenotypes of the two affected sibs (patients 3 and 4) (Table 2) were similar.

The clinical criteria used in the McKusick classification (1972) as indicators of different genotypes were compared in the nine patients. (1) Involvement of the cornea—only patient 1 with β-glucuronidase deficiency had clear corneas. (2) Normal intellect—patients 5 and 8 were considered to have normal intelligence. (3) Osseous changes—patients 1, 2, 7, 8, and 9 had a thoracolumbar gibbus, the other 4 did not. All 9 showed dysostosis multiplex indicative of a mucopolysaccharide disorder. (4) Age of onset—all except patient 6 were symptomatic during the first
Table 2  Comparison of clinical and biochemical phenotypes of 9 patients with chondroitinsulphaturia from 8 families

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Patient No.†</th>
<th>1</th>
<th>2</th>
<th>3-4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<th>9</th>
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<tr>
<td>Ages (yrs) at which patient examined</td>
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<td>1/6-2</td>
<td>1/2</td>
<td>1-9</td>
<td>1-11</td>
<td>12</td>
<td>48</td>
<td>4-6</td>
<td>1/2-2</td>
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<td>Retardation</td>
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<td>Physical</td>
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<td>Mental</td>
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<td>Cloudy corneas</td>
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<td>Short stature</td>
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<td>Skeletal dysplasia</td>
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<td>Thoracolumbar gibbus</td>
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<td>Repeated pulmonary infections</td>
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<td>Urinary mucopolysaccharides (X nl)</td>
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<td>X2</td>
<td>X5</td>
<td>X3</td>
<td>X2-4</td>
<td>X7</td>
<td>X6</td>
<td>NL</td>
<td>X100</td>
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<td></td>
<td>Nd</td>
<td>α-1-iduronidase</td>
<td>Chondroitin-4/6-sulphates</td>
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<tr>
<td>Chondroitin-4-sulphate</td>
<td></td>
<td></td>
<td>Nd</td>
<td>Chondroitin-4-sulphate</td>
<td>Chondroitin-6-sulphate</td>
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<td>ND = test not done. +/− = equivocal.</td>
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</table>

*Corneal deposits resembling areas senilis affecting all layers of the cornea at age 41.
†Patient 1—Sly et al., 1973, 1974; 2—Onisawa et al., 1971; 3-4—Spranger et al., 1971, 1974; 5—Philippart and Sugarman, 1969; Leisti et al., 1975; 6—Thompson et al., 1971; 7—Schimke et al., 1974; 8—reported in this paper; 9—Benson et al., 1972; Babarik et al., 1974.

A decade of life, which was considered a typical clinical course for a mucopolysaccharidosis (patient 6 was not studied until the fourth decade of life and it was noted (Thompson et al., 1971) that this was atypical for such disorders). (5) Mild or severe somatic involvement—patients 1, 2, 7, and 9 had severe somatic involvement; patients 3 to 6 and 8 had mild involvement with the major clinical problem in patients 3 to 5 being stiffness of the joints.

Based on these major criteria (McKusick classification, 1972) and enzyme assays done, an attempt was made to subgroup these 9 patients with chondroitinsulphaturia. The first group (patients 1 and 2) had the phenotype of severe somatic involvement (mental and physical), an increase in both chondroitin-4-sulphate and chondroitin-6-sulphate, in addition to recurrent respiratory infections. Patient 1 had previously been designated the prototype (McKusick classification) for β-glucuronidase deficiency. The deficiency of β-glucuronidase has been associated not only with chondroitinsulphaturia as in patient 1 but also in patients with mucopolysaccharidurias showing an increase in dermatan sulphate (Beaudet et al., 1974) or heparan sulphate and chondroitin sulphate (Danes and Degnan, 1974). Patient 2 had a clinical phenotype similar to patient 1. Unfortunately, enzyme studies were not done before death so the genotype could not be established.

The second group (patients 3, 4, and 5) had the phenotype of mild somatic involvement in both mental and physical development, an increase in chondroitin-4-sulphate in the urine, and deficient α-L-iduronidase enzyme activity (Spranger et al., 1974; Leisti et al., 1975). Both their clinical stigmata and types of urinary mucopolysaccharides were dissimilar to those found in the Hurler or Scheie syndromes (McKusick, 1972), the other α-L-iduronidase deficient disorders (Neufeld, 1974). Patient 6 had a clinical phenotype dissimilar to this group but also only chondroitin-4-sulphate in her urine.

On the clinical features associated with mucopolysaccharide storage patient 7 would have been placed in group I as having severe physical and mental involvement. However, only chondroitin-6-sulphate was increased in the urine. The patient also had lymphopenia, defective cellular immunity, and the nephrotic syndrome (Schimke et al., 1974), which are not associated with this group of disorders and were not seen in the other patients in group 1. As enzyme studies were not done before death the genotype could not be determined.

The clinical phenotype of the patient 8 reported in this paper had features in common with group I—
kyphosis, minimal joint restriction, repeated pulmonary infections, and chondroitin-4/6 sulphates in the urine. His other clinical abnormalities were similar to those observed in group 2. The total excretion of mucopolysaccharides in the urine was within the normal range though the increased degradation ratio indicated a defect in the mucopolysaccharide degradation. As deficiencies of β-glucuronidase and α-L-iduronidase were not present in patient 8 (Table 1) it could be concluded that he represented a different genotype than that of group 1 or 2.

Although patient 9 had a deficiency of α-L-iduronidase (Babarik et al., 1974) the severe somatic involvement and presence of both chondroitin-4/6 sulphates in the urine precluded his inclusion in Group 2. Patients 3, 4, 5, and 9 appeared to represent clinical variants of the iduronidase-deficient mucopolysaccharidoses (Stevenson et al., 1976).

The mode of inheritance in all nine patients was considered from the family history to be autosomal recessive. Sly et al. (1973, 1974) reported that the parents and the other family members were identified as carriers by intermediate level of β-glucuronidase activity in their white blood cells. The parents of patient 8 could not be identified as heterozygotes as in all studies on cultured fibroblasts their cells could not be distinguished from those from normals.

Future research on such connective tissue disorders should combine clinical descriptions with cell culture studies as a means of identifying and classifying mutant genotypes first recognized on the basis of variant phenotypes.

References


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