Mucolipidosis III: Two Patients Displaying Genetic Pleiotropism

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SUMMARY

Two Cape Coloured siblings with typical features of Hurler's syndrome, but without mucopolysacchariduria or mucopolysaccharide accumulation in tissues, are presented. The clinical features, in conjunction with raised β-D-galactosidase and α-L-fucosidase levels in fibroblast cultures from one of the patients, suggest the diagnosis of a mucolipidosis.

Theories relating to the intracellular deficiency and extracellular excess of lysosomal enzymes in these conditions are reviewed. Phenotypical and cell culture differences between 2 siblings who display the same overall clinical syndrome, illustrate the genetic pleiotropism inherent in this group of diseases.


The mucopolysaccharidoses (MPSs) and mucolipidoses (MLs) consist of approximately 20 genetically distinct inborn errors of metabolism. These conditions display great overlapping of phenotypical findings and are excellent examples of genetic heterogeneity (multiple genetic causes of a similar phenotype) and pleiotropism (a single gene resulting in several different phenotypic manifestations).

In this article, 2 sisters with clinical and biochemical findings representative of ML III are described. They were the third of four generations in the pedigree. The pattern was compatible with autosomal recessive inheritance, there being no affected members in either the second or fourth generations. The elder sister has borne a normal female child who is now 7 years old. The parents of the 2 ML siblings were not blood relations.

The variability of manifestations, even in affected siblings, is illustrated by the clinical and biochemical findings presented here.

CASE REPORTS

Patient 1

The proposita was a 27-year-old woman of normal intelligence. The most prominent external manifestations included short stature (134 cm), coarse facies, sternal protrusion, thoracolumbar kyphosis, abnormal hands and feet and symmetrical pigmentary changes in the skin of the lower limbs. Other facial features were proptosis and a flattened nasal bridge (Fig. 2).

A skeletal examination revealed a limitation of articular movement in hands, elbows, hips, ankles, and feet. There was bilateral genu valgum of 15°. Clinical osseous abnormalities were present in the hands, feet, lumbar spine and sternum. The hands (Fig. 3) showed extreme camptodactyly, rigid deformities in flexion and palmar subluxation of the middle phalanges. The thenar muscles were concave bilaterally and, Tinel's sign being present, there was a suggestion of bilateral carpal tunnel syndrome. There was marked bilateral pes valgoplanus. Metacarpophalangeal joints were dorsally subluxated and rigid deformities of the toes in flexion were present.

There was an aortic regurgitant murmur, but in other
Fig. 2. Patient 1 displaying peculiar facies, sternal protrusion, protuberant abdomen and pigmented changes on lower limbs.

Respects the cardiovascular and respiratory systems were normal. The abdomen protruded, but there was no visceromegaly or pathological condition.

There was bilateral cornea farinata with small areas of increased density in the stroma. Arcus juvenilis was present. Funduscopy revealed widespread stippling, especially of the macula and posterior poles; the pigmentary epithelium was absent in these areas and a yellowish infiltrate was seen, more marked at the posterior pole.

A radiological survey showed that the skull had a J-shaped sella turcica, absent frontal sinuses, and sclerotic mastoid processes (Fig. 4). The cervical vertebral bodies were decreased in height, but there was no odontoid hypoplasia. There was pectus carinatum and increased lumbar lordosis with a wedging of L1 and T2, and there were remnants of horizontal vertebral clefts in the lower thoracic vertebrae. There was osteoporosis of femora and humeri. The pelvis was normal. In the hands (Fig. 5), there were

Fig. 3. Hands showing camptodactyly with flexion and extension deformities (patient 1).

Fig. 4. Skull of patient 1. Note the J-shaped sella turcica and absent mastoid sinuses.

Fig. 5. Radiograph of hands of patient 1 showing camptodactyly, subluxation and exostoses.
multiple small exostoses at the proximal and distal ends of the metacarpals and phalanges in association with campotoadactyly.

The metacarpophalangeal joints of the first and second digits were dislocated and the metacarpals and phalanges were rather thick. There was pes cavus with campotoadactyly.

A biopsy of skin taken from the pigmented area of the left upper leg showed a chronic nonspecific inflammatory cell infiltrate with slight vasculitis. Collagen, elastic tissue and acid mucopolysaccharides were normal. A biopsy of the left cheek showed focal, dense, plasmacellular and lymphocytic accumulations. Occasional mast cells were present in the two specimens, as well as increased dermal iron. Bone marrow investigation revealed a mast cell count of 1%, but it was normal in other respects. No Buhot or Gasser cells were noted, nor were there any vacuolated ‘foam’ cells, Reilly granulations, or other inclusions in bone marrow or peripheral leucocytes. Screening for mucopolysacchariduria by CTAB, acid albumin turbidity, and mucopolysaccharide spot tests, was negative. Tests that yielded normal results in both patients are listed in Table I. Alcian blue and toluidine blue staining for metachromatic granules in cultured fibroblasts was negative. $\text{S}^\text{35}$SO$_4$-kinetic studies on cellular culture were normal. Lysosomal enzymic assay from cultures of fibroblasts showed raised values for $\beta$-D-galactosidase and $\alpha$-L-fucosidase (Table II). These raised fibroblast culture enzymic values are diagnostic for ML II and III.

**TABLE I. LABORATORY FINDINGS SHOWING NORMAL RESULTS IN BOTH CASES**

<table>
<thead>
<tr>
<th>pH electrolytes</th>
<th>Urea</th>
<th>Glucose</th>
<th>Full blood count</th>
<th>Sedimentation rate</th>
<th>Liver function screening tests</th>
<th>Effective thyroxine index (ETI)</th>
<th>Calcium and phosphate metabolism</th>
<th>Alkaline phosphatase</th>
<th>Humoral and cellular immunity</th>
<th>Bone marrow investigation</th>
<th>Urinary screening for amino-aciduria</th>
<th>Mucopolysacchariduria</th>
<th>Glucagon stimulation test for glycogenoses</th>
<th>Fluorescent staining and Giemsa chromosome-banding studies</th>
<th>Metachromatic staining of cultured fibroblasts</th>
<th>Uptake of radio-labelled $\text{S}^\text{35}$SO$_4$ in fibroblast culture</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient 1</strong></td>
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<tr>
<td>$\beta$-D-galactosidase</td>
<td>17.17</td>
<td>7.97</td>
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<td>$\beta$-D-glucuronidase</td>
<td>Not</td>
<td>1.59</td>
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<td>$\alpha$-L-fucosidase</td>
<td>2.51</td>
<td>0.85</td>
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<td>$\alpha$-L-mannosidase</td>
<td>0.15</td>
<td>0.25</td>
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**Table II. LYSOSOMAL ENZYMIC STUDIES ON FIBROBLAST CULTURES**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>X</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$-D-galactosidase</td>
<td>17.17†</td>
<td>7.97</td>
<td>9.89</td>
<td>9.66 - 10.34</td>
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<tr>
<td>$\beta$-D-glucuronidase</td>
<td>Not</td>
<td>1.59</td>
<td>2.34</td>
<td>1.62 - 3.48</td>
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<tr>
<td>$\alpha$-L-fucosidase</td>
<td>2.51†</td>
<td>0.85</td>
<td>1.05</td>
<td>0.42 - 1.4</td>
</tr>
<tr>
<td>$\alpha$-L-mannosidase</td>
<td>0.15</td>
<td>0.25</td>
<td>0.34</td>
<td>0.15 - 0.53</td>
</tr>
</tbody>
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* Department of Human Genetics, University of Cape Town.

N = normal.
mast cells were present in the areas of acute inflammation. Dermal acid mucopolysaccharides were normal. Light and electron microscopical biopsy of a needle specimen of the liver showed only very slight focal fatty change. No meta-chromasia of cultured fibroblasts was noted, no mucopolysacchariduria or tissue mucopolysaccharide accumulation was present, and lysosomal enzymic assays on fibroblast cultures gave normal results.

**DISCUSSION**

MPS phenotypes were ascribed to these patients on account of their short stature, sternal protrusion, coarse facies, metaphyseal chondrodysplasia with limited range of articul-
menon' (generally regarded as a unique feature of ML II) by ML III patients as well, indicates that the various hypotheses which have been proposed for this anomaly may be relevant to both conditions. Hickman and Neufeld have shown that earlier ideas of leaking lysosomal membranes were incorrect. These investigators made use of the ability of fibroblasts to take up enzymes from the medium and they showed that ML II cells are just as retentive of ingested enzyme as are normal cells. The fact emerged that the hydrolases released by ML II cells into the medium are not taken up by other fibroblasts as efficiently as are the normal enzymes. Therefore, they have suggested that the defect probably lies in defective enzymes that fail to reach their normal destination.

The accepted view of lysosomal formation states that a primary lysosome filled with hydrolases buds from the Golgi apparatus or endoplasmic reticulum and coalesces with a pinocytotic vacuole that contains only substrate — the GERL concept of Novikoff. The detailed hypothesis of Hickman and Neufeld states that 'packaging of lysosomal enzymes requires intercellular cooperation. Hydrolases synthesized and secreted by a cell are taken up and sequestered into the lysosomes of its neighbours, the uptake requiring specific recognition of the enzyme at the surface of the recipient cells. The recognition site on the hydrolases would have a function analogous to that of $\beta$-galactosyl termini in directing plasma proteins into parenchymal liver cells. In I-cell disease, the mutation would affect this presumed site so that the hydrolases are not recognised, and therefore not taken up by the specific and efficient mechanism. Those defective hydrolases that are stable in the culture medium would accumulate until there is measurable excess over the normal.'

The study of recognition and complementary sites in the mucolipidoses is important in view of the preparation of purified lysosomal enzymes for possible treatment of lysosomal storage disease.

Whereas some of the clinical findings are seen in other conditions, none of the conditions listed in McKusick's authoritative textbook on connective tissue diseases displays all the features present in these cases. A sign which may suggest the diagnosis is the presence of bilateral carpal tunnel syndrome, especially in childhood when it is most rare. This condition was present in the 27-year-old patient. The radiological findings of ML III have been reviewed by Melhem et al. Our findings supported the various bone changes and the more severe affliction of the upper limbs compared with the lower. While Spranger and Wiedemann emphasised the specificity of pelvic changes, only 1 of the 6 patients of Melhem et al. had the definite changes described by these authors and neither of our 2 patients showed any pelvic changes. This does not appear as a highly specific radiological sign. The diagnosis thus rests on nonspecific but characteristic clinical and radiological evidence and enzymic assays.

**CONCLUSION**

The new concepts in the study of these diseases may increase our clinical understanding of the osteochondrodysplasias and our understanding of normal enzymic transport and lysosomal 'packaging' on the cellular level. The ultimate aim is treatment with the purified enzyme encapsulated within a biological envelope (liposome).

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**REFERENCES**