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 mucopolysaccharidurla 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.

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The mucopolysaccharidoses (MPSs) and mucolipidoses (MLs) consist of approximately 20 genetically distinct inborn errors of metabolism.[1] 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).[2]

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. 1. Two siblings with mucolipidosis III, with normal female.

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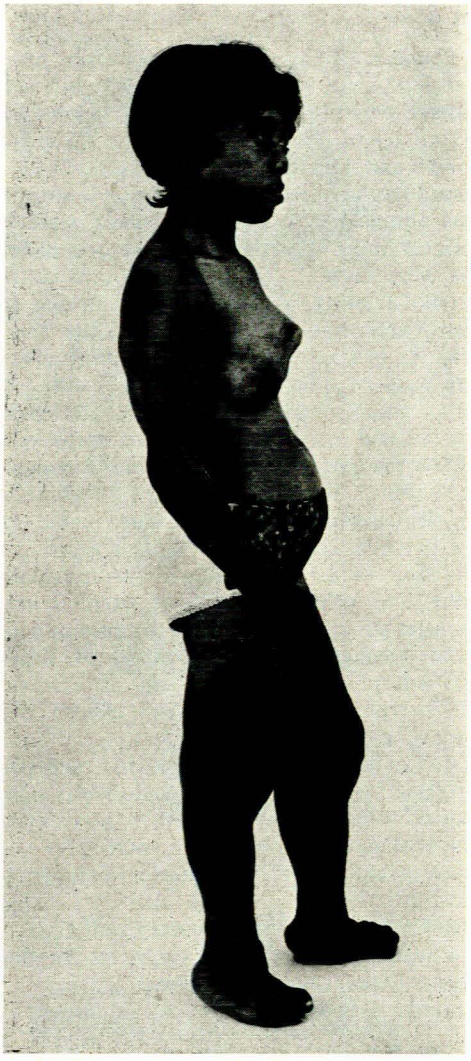

Fig. 2. Patient 1 displaying peculiar facies, sternal protrusion, protuberant abdomen and pigmentary changes on lower limbs.

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).

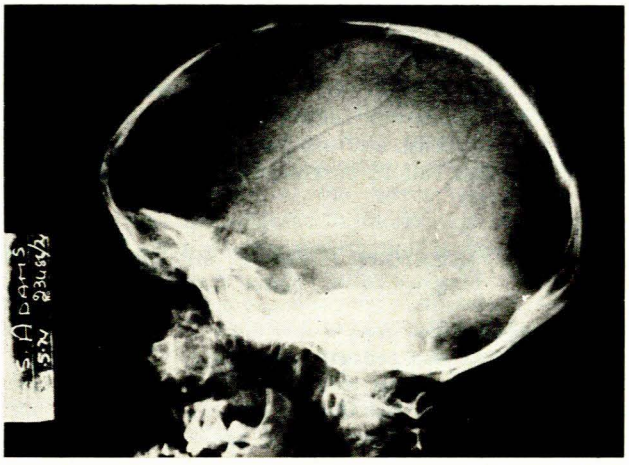

Fig. 4. Skull of patient 1. Note the J-shaped sella turcica and absent mastoid sinuses.

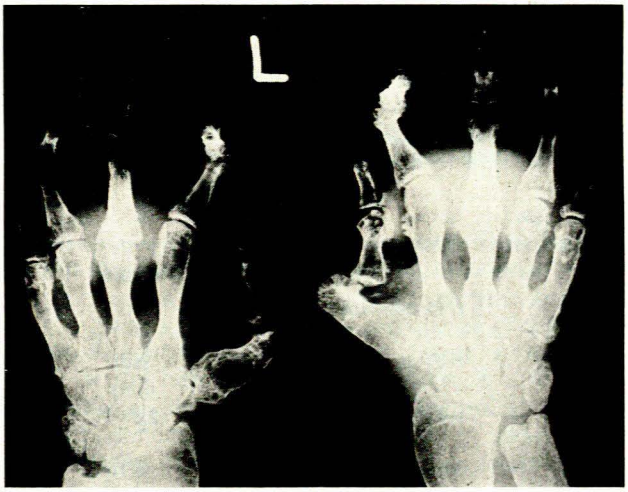

Fig. 5. Radiograph of hands of patient 1 showing camptodactyly, subluxation and exostoses.

multiple small exostoses at the proximal and distal ends of the metacarpals and phalanges in association with camptodactyly.

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

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. $S^{35}SO_4$-kinetic studies on cellular culture were normal. Lysosomal enzymic assay from cultures of fibroblasts showed raised values for β-D-galactosidase and α-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

pH electrolytes urea glucose
Full blood count and sedimentation rate
Liver function screening tests
Effective thyroxine index (ETI)
Calcium and phosphate metabolism and alkaline phosphatase
Serum iron and total iron-binding capacity
Humoral and cellular immunity
Bone marrow investigation
Urinary screening for amino-aciduria and mucopolysaccharidiuria
Glucagon stimulation test for glycogenoses
Fluorescent staining and Giemsa chromosome-banding studies
Metachromatic staining of cultured fibroblasts
Uptake of radio-labelled $S^{35}SO_4$ in fibroblast culture

Patient 2

The younger sibling was 12 years old, and had less severe skeletal changes, but more prominent facial features and massive hepatosplenomegaly. Her height was 1,08 m. She had an IQ of 75, according to the Alexander Performance Test and Fick Scale.

General hirsutism and widespread skin lesions, resembling erythema annulare centrifugum (Darier), were present on the face and limbs. The fingernails were small and dermatoglyphs were normal. There were prominent facial cutis laxa, frontal bossing, a flattened nasal bridge and a long philtrum. There was a prominence of the anterior chest wall (Fig. 6). An aortic regurgitant murmur was present, but there were no other abnormalities of either the cardiovascular or the respiratory system. The abdomen was protuberant without herniation. The liver edge was palpable 7 cm below the costal margin in the midclavicular line and the spleen almost filled the left half of the abdominal cavity (Fig. 1). Sequestration studies indicated a Cr^{51} half-life of 11 days (normal 26 - 30 days), which represented a significantly decreased red cell survival with sequestration in both liver and spleen. A skeletal examination revealed abnormalities of the hands, elbows, knees and feet. There was pectus carinatum and deformities of flexion in the proximal interphalangeal joints and of extension in the terminal interphalangeal joints. The elbows were in 15° of flexion with limited supination and pronation. The knees were in 15° of valgus deformity and 15° of hyperextension in the resting position, but full flexion was possible. The tarsi showed valgus deformity with hyperextension of the metatarsophalangeal joints. There were also cornea farinata, arcus juvenilis, and clear anterior chambers bilaterally. Fundoscopy revealed increased vascular tortuosity and widespread damage to the pigmentary epithelium, especially over the macula and posterior pole, where there was a background of a yellowish infiltrate.

A radiological survey showed less pronounced abnormalities than in patient 1. Irregularity of manual bones was not present and exostoses were absent. The orbits were small and shallow, but there was a large crista galli and no odontoid hypoplasia. The lumbar spine was osteoporotic. A biopsy of the skin from the upper lip showed acute and chronic inflammatory cell infiltration into the dermis and upper subcutis. Many capillaries showed endothelial swelling and 'nuclear dusting'. A pronounced secondary elastolysis and a definite increase in the number of dermal

TABLE II. LYSOSOMAL ENZYMIC STUDIES ON FIBROBLAST CULTURES*

Enzyme	Patient 1	Patient 2	X	Range (μmol/min/mg protein)
β-D-galactosidase	17,17 ↑	7,97	9,89 N=3	9,66 — 10,34
β-D-glucuronidase	Not done	1,59	2,34 N=3	1,62 — 3,48
α-L-fucosidase	2,51 ↑	0,85	1,05 N=5	0,42 — 1,4
α-L-mannosidase	0,15	0,25	0,34 N=3	0,15 — 0,53

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N = normal.

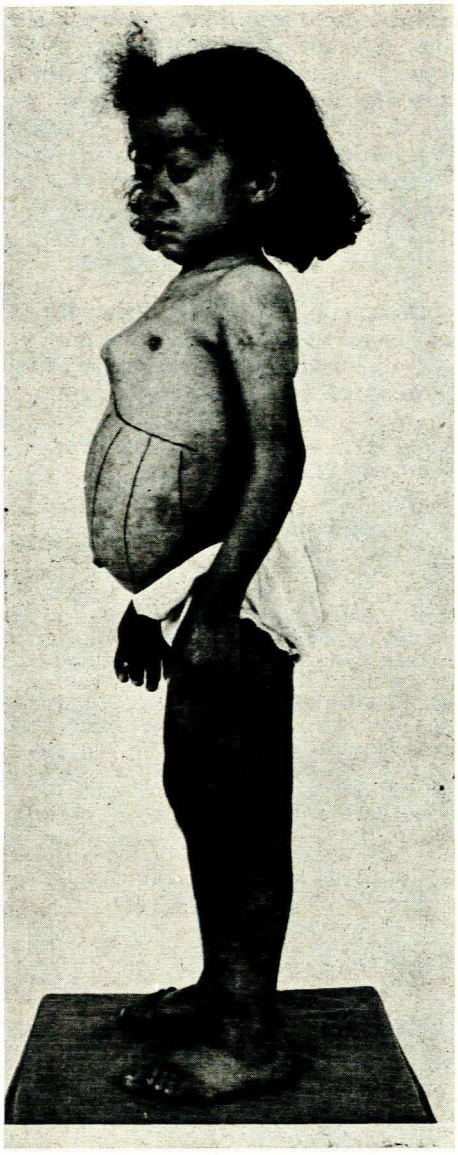

Fig. 6. Patient 2, showing facial cutis laxa, sternal protrusion, large abdomen owing to hepatosplenomegaly and claw hands and feet.

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 metachromasia of cultured fibroblasts was noted, no mucopolysaccharidiuria 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 articular movement, boot-shaped sella turcica (patient 1), hepatosplenomegaly (patient 2) and thoracolumbar kyphosis (patient 1). A normal intellect does not rule out the MPSs and may be found in patients with MPS IS (Scheie's syndrome), MPS IVa and IVb (Morquio's disease and a non-keratin sulphate-excreting allelic variant) and MPS VIa and VIb (usual and allelic types of Maroteaux-Lamy syndrome).[3] The allelic types were taken into consideration in connection with these patients. In such circumstances no excessive mucopolysaccharidiuria is found, but the ratio of urinary glycosaminoglycans is abnormal. This situation was not applicable to the 2 cases reported here.

In 1966 Maroteaux and Lamy first described *'la pseudopolydystrophie de Hurler'* in 4 patients in whom the typical features of Hurler's syndrome were unassociated with mucopolysaccharidiuria.[4] They are now believed to have had ML III. The MLs were separated as a group in 1970 when sufficient patients with MPS phenotypes, but who showed different enzymic defects, had been found.[5] Accumulation of excessive acid mucopolysaccharides, sphingolipids or glycolipids, or both lipids, may be found in visceral and mesenchymal cells of ML patients. They may show manifestations of both MPSs and sphingolipidoses. Four ML types have been recognised. Reviews have been published by Spranger and Wiedemann,[5] McKusick,[10] and Legum *et al.*[1] In a consideration of the whole range of related biochemical abnormalities, various other rare diseases such as Gm_1 gangliosidosis I and II, the Austin type of juvenile sulphatidosis and the oligosaccharidoses such as mannosidosis, fucosidosis and acetylglucosaminuria, have also been included under the MLs. Knowledge of the function of these enzymes as a group is still very rudimentary, although new findings will be acquired as more afflicted individuals are detected by means of recently developed screening programmes.[6]

Recently it has been shown for ML II and III that certain lysosomal enzymes may be in excess in fibroblast culture media in approximately the same proportion as they are deficient within the cells.[7] Although these two conditions are thus biochemically related, they are differentiated by dissimilar clinical features. In Table II the results of assays of some lysosomal enzymes from fibroblast cultures of both patients are shown. The raised values for patient 1 were considered to be diagnostic for the mucolipidoses and in the absence of psychomotor retardation, were ascribed to ML III. The normal culture findings of the affected siblings were ascribed to the pleiotropic effect of the genes responsible for the MLS.

Thomas *et al.*[7] have analysed 5 lysosomal enzymes from 4 ML III patients and compared them with earlier findings in a ML II patient. Two points which are relevant to the cases described in this article emerged from their analysis:

1. The changes in type, quantity and quality of lysosomal enzymes involved in ML patients reflect a range of severity at the cellular level which makes it difficult to decide whether an entirely different biochemical disorder strongly resembling ML III is represented in some cases.

2. The excess of lysosomal enzymes in fibroblast cultures and body fluids in relation to the deficiency within cells in ML III, is similar to that found earlier in mucolipidosis-II (I-cell) patients. The manifestation of the 'I-cell pheno-

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[8] 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.[9] 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 β-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'.[8]

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[10] displays all the features present in these cases. A sign which may suggest the diagnosis is the presence of bilateral carpal tunnel syndrome,[11] 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.*[12] Our findings supported the various bone changes and the more severe affliction of the upper limbs compared with the lower. While Spranger and Wiedemann[5] emphasised the specificity of pelvic changes, only 1 of the 6 patients of Melhem *et al.*[12] 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).[13]

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