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ORIGINAL ARTICLE

The natural history and osteodystrophy of mucolipidosis types II and III

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Aim: To assess the natural history and impact of the secondary bone disease observed in patients with mucolipidosis (ML) II and III.

Methods: Affected children and adults were ascertained from clinical genetics units around Australia and New Zealand. Diagnoses were confirmed by the National Referral Laboratory in Adelaide. The study encompassed all patients ascertained between 1975 and 2005. Data focussing on biochemical parameters at diagnosis, and longitudinal radiographic findings were sought for each patient. Where feasible, patients underwent clinical review and examination. Examinations included skeletal survey, bone densitometry, and measurement of serum and urine markers of bone metabolism. In a subset of patients, functional assessment using the Pediatric Evaluation and Disability Inventory (PEDI) and molecular analysis of *GNPTAB* were performed.

Results: Twenty-five patients with mucolipidosis were ascertained over a 30-year period. Morbidity and functional outcomes on living patients were described. Serum calcium and phosphate were normal. All, but one patient, had normal alkaline phosphatase. Serum osteocalcin and urine deoxypyridinoline/creatinine were elevated. Two radiological patterns were observed (i) transient neonatal hyperparathyroidism in infants with ML II and (ii) progressive osteodystrophy in patients with ML intermediate and ML III. Molecular analyses of *GNPTAB* in nine subjects are reported.

Conclusion: ML is characterised by a progressive bone and mineral disorder which we describe as the 'osteodystrophy of mucolipidosis'. The clinical and radiographic features of this osteodystrophy are consistent with a syndrome of 'pseudohyperparathyroidism'. Much of the progressive skeletal and joint pathology is attributable to this bone disorder.

Key words: hyperparathyroidism; mucolipidosis; osteoporosis; 'pseudohyperparathyroidism'.

What is already known on this topic

1. Mucolipidosis (ML) is a rare disorder of lysosomal metabolism characterised by coarse facial features, short stature, hyperplastic gums, organomegaly and retarded psychomotor development due to absent or deficient activity of UDP-GlcNAc 1-phosphotransferase (GlcNAc-PT).
2. Deficient activity of GlcNAc-PT leads to defective modification of numerous degradative enzymes which depend on mannose phosphorylation for uptake and localisation by cells which then leads to intracellular accumulation of partly degraded glycosaminoglycans and sphingolipids.
3. GlcNAc-PT is made up of three subunits, alpha, beta and gamma. Mutations in *GNPTAB*, the gene which encodes for alpha and beta subunits, cause ML II that manifests with more severe phenotype and the milder ML III alpha/beta.

What this paper adds

1. Mucolipidosis (ML) II and ML III alpha/beta can now be recognised as a continuum of disorders resulting from mutations in the alpha/beta subunits of the enzyme GlcNAc-PT.
2. Children with ML have characteristic bone involvement which manifests as neonatal hyperparathyroidism in babies with ML II.
3. Children with a later onset disorder ML III develop a progressive destructive bone disease that has many features of chronic hyperparathyroidism but with normal PTH.

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Mucolipidosis (ML) II and ML III, I-cell disease and pseudo-Hurler polydystrophy, respectively, are rare genetically related inherited disorders of lysosomal metabolism with a combined frequency of 1:422 000.[1] These are characterised by disordered processing of multiple lysosomal degradative enzymes caused

by the deficiency or abnormal function of UDP-*N*-acetylglucosamine : lysosomal enzyme *N*-acetylglucosaminyl-1-phosphotransferase, commonly termed UDP-GlcNAc 1-phosphotransferase (GlcNAc-PT).[2,3] The underlying defects result in deficient post-translational modification of numerous enzymes, which depend on mannose phosphorylation for uptake and localisation by cells where substrate degradation occurs.[4] This in turn results in effective deficiencies of lysosomal degradative enzymes with concomitant intracellular accumulation of both partly degraded glycosaminoglycans and sphingolipids.

ML II has symptoms and signs similar to those encountered in patients with mucopolysaccharidoses and to a lesser extent gangliosidoses. It is characterised by coarse facial features, short stature, hyperplastic gums, organomegaly and retarded psychomotor development.[5–7] ML III is a milder disorder with attenuated characteristics and survival to adult life.[8] Intermediate forms of ML II and III have been previously described.[9,10] The presence of clinical heterogeneity has long been recognised and verified in the laboratory by complementation studies.[11–14] Molecular characterisation has further substantiated this heterogeneity and allows for more descriptive disease nomenclature.[3] GlcNAc-PT is a complex enzyme consisting of three subunits that are the products of two genes.[15,16] Mutations in *GNPTAB*, which encodes the alpha/beta subunit, cause ML II and ML III alpha/beta.[17] The gamma subunit is encoded by *GNPTG*, and mutations in this gene lead to the clinically milder condition known as ML III gamma.[16]

Previous radiographic studies of ML II and ML III have focussed mainly on those bone changes collectively called 'dysostosis multiplex', although a few authors have drawn attention to bone changes in infants with ML II that are similar to infantile hyperparathyroidism and rickets.[18–21] In this study, we describe a progressive bone and mineral disorder, its biochemical characteristics and skeletal radiographic findings in patients with ML II and III. In addition, we assess the impact of the secondary bone disease observed in patients on spine and hip morbidity, and assess the frequency and morbidity arising from non-osseous complications of mucolipidosis.

Materials and Methods

Patients were ascertained through clinical genetics units around Australia and New Zealand, the Kazimierz Kozlowski Skeletal Dysplasia Library and the Connective Tissue Dysplasia clinic at the Children's Hospital at Westmead. Patients registered with those units between 1975 and 2005 were included. All diagnoses were enzymatically confirmed by the National Referral Laboratory in Adelaide. Ethics committee approval for the study was obtained from the ethics committee of the Children's Hospital at Westmead. Information was sought from collaborating clinical geneticists on diagnosis, radiographic findings and previous biochemical investigation of mineral metabolism in the patients managed by them. Where feasible, living patients underwent clinical review and investigations. Investigations included skeletal survey, bone densitometry and bone marker measurements (serum calcium, alkaline phosphatase, parathyroid hormone, parathyroid hormone related protein, serum osteocalcin and urine deoxypyridinoline crosslinks). Functional assessment was performed using the Pediatric Evaluation and Disability Inventory (PEDI).[22] This standardised scaling tool measures the domains of life skills, mobility and social function. DNA molecular genetic diagnosis was undertaken in a subset of patients.[23]

Results

Clinical data

A total of 25 affected individuals (ML II = 15; ML III = 5; ML Intermediate = 5) from 16 families were ascertained. The female to male ratio was 1.8 (16F : 9M). In those with ML II, six were diagnosed at birth with median age at diagnosis of 7 months and median survival of 27 months. The five subjects with ML intermediate were siblings with the proband diagnosed at 26 months. Two died at 22 and 23 years of age. The median age of diagnosis in those with ML III was 6 years and all are alive at the time of this report with the oldest being in his mid-forties (Table 1).

Morbidity

Ten patients were available for clinical review. Five patients had ML intermediate, one had ML II and four had ML III. All of the eight subjects in whom height data were available were very short, height Standard Deviation Scores (SDS) = −8.25 ± 3.4. Chronic otitis media was present in 7/10 patients. Cardiovascular complications included aortic and mitral valvular thickening and incompetence present in nine subjects, six of whom had mild dilatation of the left ventricle with or without atrial involvement. Sleep disordered breathing requiring continuous or bi-level positive airway pressures (Continuous Positive Airways Pressure (CPAP) or Biphasic Positive Airways Pressure (BiPAP)) at the level of 8–10 cm water was recorded in 6/10. Four young adults developed upper limb paraesthesia with MRI evidence of thickening of the extradural tissues at the level of C1-C2. One patient required cervical fusion for C1-C2 instability at 8 years of age and a 20-year-old progressed to inoperable

Table 1 Clinical features at diagnosis

Clinical features at diagnosis	ML II *N* = 15	ML intermediate *N* = 5	ML III *N* = 5
Coarse facial features	14	5	4
Corneal clouding	1	3	1
Chest deformity	5	0	1
Umbilical hernia	3	3	0
Inguinal hernia	5	1	0
Hepatomegaly	3	0	0
Splenomegaly	3	0	0
Joint contractures	8	5	2
Congenital hip dislocation	6	2	0
Evidence of hyperparathyroidism	15	5	NK
Progressive osteodystrophy	NA	5	5

NA, not applicable; NK, not known.

occipitocervical dislocation. Five patients had chronic constipation requiring almost daily laxatives and occasional bowel evacuation. All patients had progressive joint stiffness. The patient with ML II had significant joint stiffness at 5 years of age such that she could only sit and stand with support. She moved around with her small wheelchair for less than 90 minutes a day. She cried when handled, a response similar to the bone pain observed in high bone turnover osteogenesis imperfecta. The other nine patients suffered from significant back and joint pains, mainly in the hips by age 3 years. They became confined in their wheelchair by the time they reached 10 years of age. One ML III patient had bilateral hip replacements at 36 years. All subjects had limitation of shoulder movement with difficulty reaching, more noticeable in the ML II subject and the older subjects with ML intermediate. Stiffness of the small joints of the hands was progressive with age and more marked in the ML II and older ML intermediate subjects. All patients had locomotor disabilities to some degree.

Intellectual disability

Intellectual disabilities were present in 7 of the 10 subjects. The one with ML II had few words at the age of 5 but could follow simple commands. The patients with intermediate ML and one subject with ML III had normal speech, were enrolled in special school and learned to read and write. The remaining three patients with ML III had normal intelligence. The intellectual capabilities of the subjects in each sub-classification were similar to the findings in the studies reported by Cathey and colleagues.[23]

Functional assessment (PEDI)

Five subjects had PEDI, one with ML II and 4 with ML intermediate. The ML II subject had scores −2 standard deviations (SDs) below the level of age-normative functional capability and performance in all domains except for the Social Function domain with score slightly higher at SD = −1. In the ML intermediate subjects, scores were below −1 SD with highest scores in the Social Function domain. Almost all of the scaled scores in all subjects were below 70. Inability or difficulty to reach and problems with hand grip markedly impacted functional skills. However, the subjects understood requests and instructions and they were able to provide information about their own activities and needs.

Biochemical findings

Eight patients underwent biochemical evaluation (Table 2). All had normal serum calcium (median 2.35 mmol/L range 2.16–2.64), phosphate (median 1.51 mmol/L range 1.17–1.72) and parathyroid hormone (median 2.9 pmol/L range 1.8–5.0). Parathyroid receptor protein concentrations were available in six patients and all were within normal levels (median < 0.6 range <0.6–1.4). Alkaline phosphatase was elevated in only one out of the eight (median 155 u/L range 102–495). Seven had osteocalcin levels and were elevated in six (median 3.8 nmol/L range 0.8–7.5). Levels of urine deoxypyridinoline/creatinine were elevated in all patients (median 26.7 nM/mM range 14.2–45.5).

Table 2 Baseline biochemical findings ($n = 8$)

Biochemistry and markers	Median	Range	Normal values
Calcium	2.35	2.16–2.64	2.10–2.65 mmol/L
Phosphorus	1.51	1.17–1.72	1.00–1.80 mmol/L
ALP	155	102–495	80–355 U/L
Osteocalcin	3.8	0.8–7.5	0.5–2.3 nmol/L
PTH	2.9	1.8–5.0	1.0–7.0 pmol/L
PTHrP	<0.6	<0.6–1.4	<1.3 pmol/L
Deoxypyridinoline/Creatinine	26.7	14.2–45.5	Variable ranges

Table 3 Mutations in the 'Clinical' cohort of mucolipidosis ($n = 9$)

	Mutation 1	Mutation 2
Mucolipidosis II (1)	c.2591insG	c.3503ΔTC
Mucolipidosis intermediate (5)	c.10A > C	c.1399ΔG
Mucolipidosis III (1)	c.1399ΔG	c.3335 + 6T > G
Mucolipidosis III (2)	c.3565C > T	IVS17 + 6T > G

Lysosomal enzyme biochemistry

Diagnosis of 24/25 patients, was confirmed enzymatically by measurement of markedly elevated plasma lysosomal hydrolases and the demonstration of markedly deficient lysosomal hydrolases in cultured fibroblasts. In one patient, diagnosis was accomplished by review of radiographs after her siblings had been confirmed to have mucolipidosis on enzymology testing. This particular subject was stillborn and was also found to have 45,X on chromosome analysis.

Molecular DNA analysis

Mutations in *GNPTAB* were detected in nine of nine tested patients (Table 3). The clinical patient with ML II was doubly heterozygous for a single nucleotide insertion and dinucleotide deletion resulting in a frame shift predicting a severe disorder.[19] The dinucleotide deletion c.3503delTC is the most common mutation observed in patients with ML II worldwide. The three patients with ML III are also doubly heterozygous for either a frame shift or a nonsense and a missense mutation. The family with ML intermediate is double heterozygous for a specific missense mutation c.10A > C which predicts substitution of glutamine for lysine at codon 4 (K4Q) predicting a milder phenotype combined with a frame shift predicting a severe phenotype.

Radiographic findings

Skeletal radiographs showed distinctive patterns in patients at different ages. These were grouped as evidence of:

1 neonatal hyperparathyroidism,
2 osteodystrophy (similar to chronic osteitis fibrosis cystica) and
3 dysostosis multiplex.

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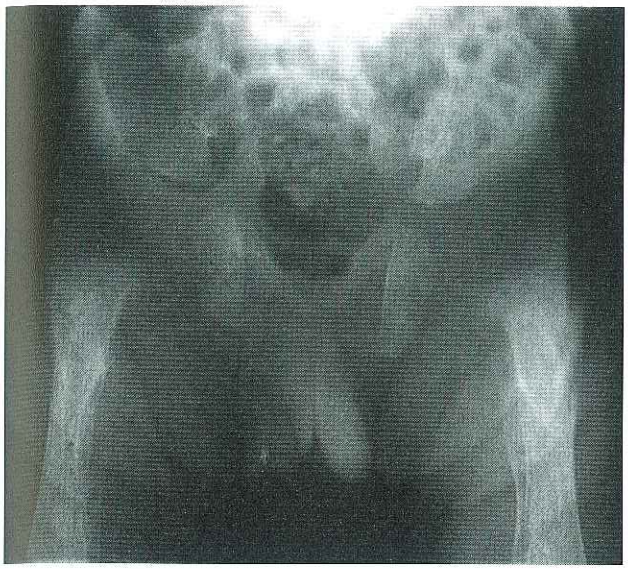

Fig. 1 Radiograph of pelvis and femurs in a neonate with mucolipidosis type II showing features of neonatal hyperparathyroidism including periosteal cloaking and destructive bone lesions.

The radiographic findings of neonatal hyperparathyroidism were seen in all babies with ML II. These changes included generalised bone demineralisation, subperiosteal bone resorption around the shafts of the long bones or periosteal cloaking and fractures of long bones and ribs (Fig. 1). In addition, there were features of rickets with metaphyseal cupping or fraying. Three patients had punctate calcifications in the region of the coccyx, pubis and tarsals at birth.

In subjects over 4 months, radiographs show features reminiscent of 'osteitis fibrosa cystica'. There were marked progressive changes in bone texture and density with areas of cystic lucency. Erosive changes especially in the hands and hips were progressive over time (Figs 2,3). The proximal phalanges became broad and under-modelled. The proximal metacarpals showed a mixture of features of osteodystrophy and dysostosis multiplex becoming extremely eroded and narrowed to a point. The carpal bones became extremely osteopenic and hypoplastic, consistent with severe carpal osteolysis. Similarly, there was severe osteolysis of the femoral heads and femoral necks. There was also over-modelling of the long bones and bowing of the proximal end of the humerus and femur leading to coxa valga or 'shepherd's crook deformity' (Fig. 4). The lower third of the ilia became progressively hypoplastic and resorbed (Fig. 3). In addition, there was progressive osteopenia of the spine. The spine showed thoracolumbar kyphosis, beaking of the vertebrae and subluxation typical of lysosomal storage disorders with skeletal involvement.

In all patients surviving the first year of life, skeletal radiographs showed a mixture of osteodystrophic bone changes and atypical changes of dysostosis multiplex. These included proximal pointing of metacarpals in the wrist, dysplastic changes in the lower third of the ilia, marked broadening of the ribs becoming oar-shaped and beaking of the lower thoracic and lumbar vertebrae.

Bone densitometry

In general, patients had evidence of reduced bone density (at all ages or at certain ages). Detailed results are reported in a supplementary paper describing the effect of treatment with cyclic intravenous pamidronate.

Discussion

Many lysosomal storage disorders, particularly the mucopolysaccharidoses are characterised by 'dysostosis multiplex'. The combination of radiographic features includes 'J' shaped sella turcica, oar shaped ribs, anterior inferior beaking of lower thoracic to upper lumbar vertebral bodies, flared iliac wings, constricted iliac bodies, dysplastic femoral heads, 'bullet-shaped' proximal phalanges and central pointing of proximal metacarpals.[24] In this study, dysostosis multiplex developed with age but was not the characteristic feature in newborns.

In addition to dysostosis multiplex, the skeleton in ML II and III is characterised by an osteodystrophy. In ML II, the osteodystrophy has clinical and radiographic features of congenital hyperparathyroidism. In some neonatal subjects, chemical hyperparathyroidism was also demonstrated.

Features of congenital hyperparathyroidism have been reported in ML II as early as 19 weeks of gestation.[25] Osteoporosis, fractures, periosteal new bone formation ('cloaking') and cupped epiphyses have been described in neonates.[18–20,26–31] Histologic examination has confirmed the presence of hyperparathyroidism.[21] Pazzaglia *et al.*[21,32,33] described spontaneous evolution of hyperparathyroidism to dysostosis multiplex in three patients, and they noted resolution of high bone turnover and defective calcification in the older child. In this study, we have observed that in ML intermediate and ML III there are progressive radiographic features that overlap with juvenile hyperparathyroidism or chronic hyperparathyroidism or 'osteitis fibrosa cystica'.

In this study, we have confirmed that ML II is not associated with a disturbance of serum levels of calcium and phosphorus. In our one patient with ML II, serum parathyroid hormone was normal at the time of review but may have been elevated prior to diagnosis at 4 months of age. All subjects had persisting high bone turnover, as measured by deoxypyridinoline/creatinine ratio and progressive osteopenia. Since features of this osteodystrophy are not present in the other lysosomal storage disorders except for Galactosialidosis, we hypothesise that the osteodystrophy is related to the underlying biochemical disorder.

Any postulated pathogenetic mechanism for the osteodystrophy needs to explain both observations of transient hyperparathyroidism of the newborn and the progressive *osteitis fibrosa cystica* which develops from 3–6 months of age. Transient neonatal hyperparathyroidism is also reported where a fetus has inherited a mutation in the calcium-ion sensing receptor mutation from the father, and has an unaffected mother.[34] In this situation there is excessive secretion of parathormone in the fetus with resultant demineralisation, rickets, resorption and cystic changes with periosteal cloaking. Characteristically, the secondary hyperparathyroidism resolves spontaneously by 3–4 months of age.

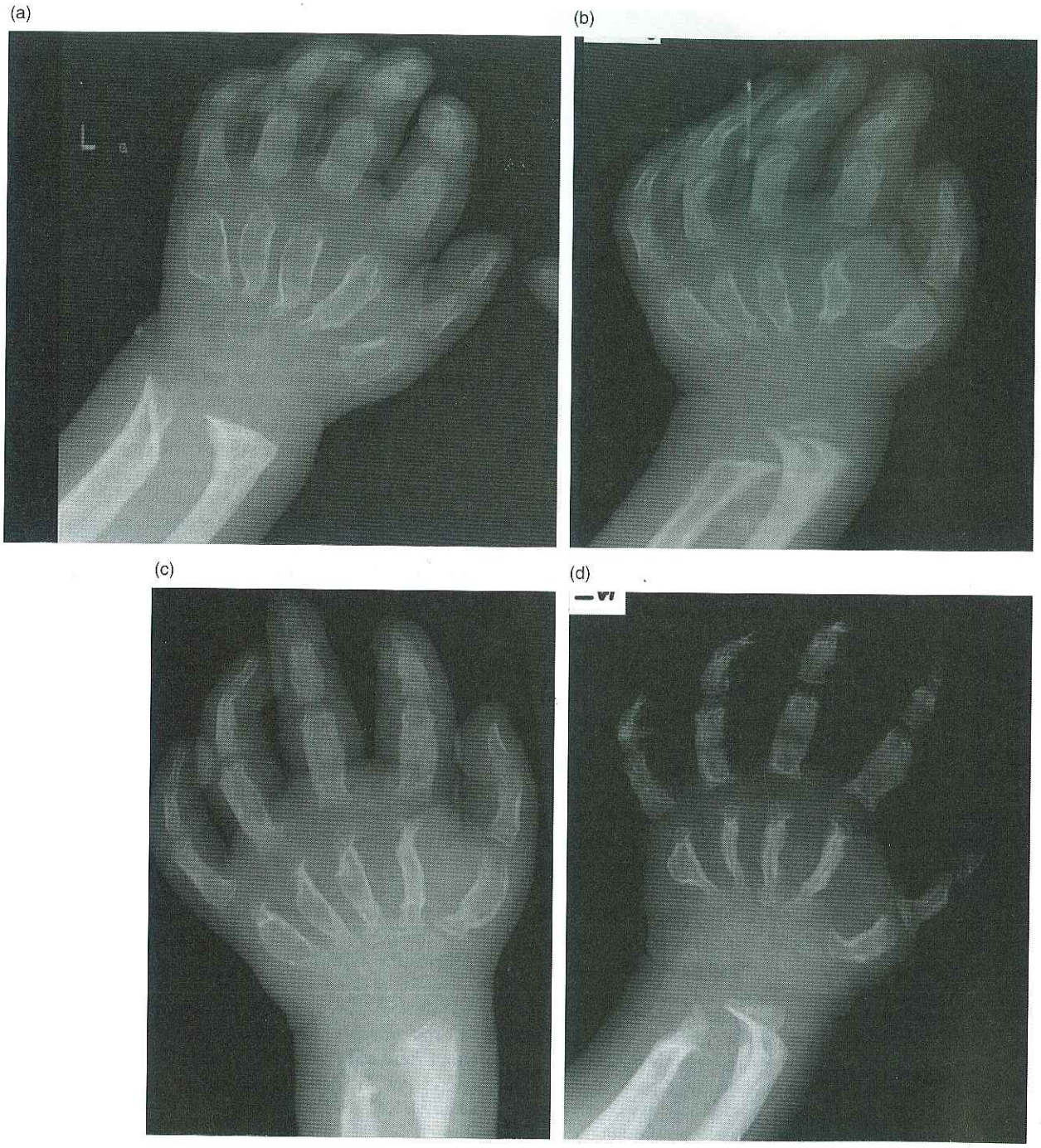

Fig. 2 Radiograph of hands and wrists in four individuals affected with ML intermediate aged (a) 4 years, (b) 16 years, (c) 18 years and (d) 19 years showing progressive osteolysis of carpal centres, proximal metacarpals and distal forearm bones. ML, mucolipidosis.

Following birth, we have confirmed that chemical hyperparathyroidism in ML II resolves but is replaced by a progressive osteodystrophy. We also confirmed that circulating levels of parathyroid-related protein were normal outside the neonatal period. Postnatally, the radiographic features could be consistent with an increased sensitivity of skeletal tissue to normal circulating levels of parathormone.

In a previous report, bone histomorphometry in two teenagers with ML III, demonstrated increased osteoclastic activity.[35] Our biochemical findings support high bone turnover with elevated osteocalcin and deoxypyridinoline. One possible unifying hypothesis is that there is defective targeting of lysosomal enzymes to the osteoclasts with abnormal biofeedback and induction of PTH receptor transduction, with focal areas of

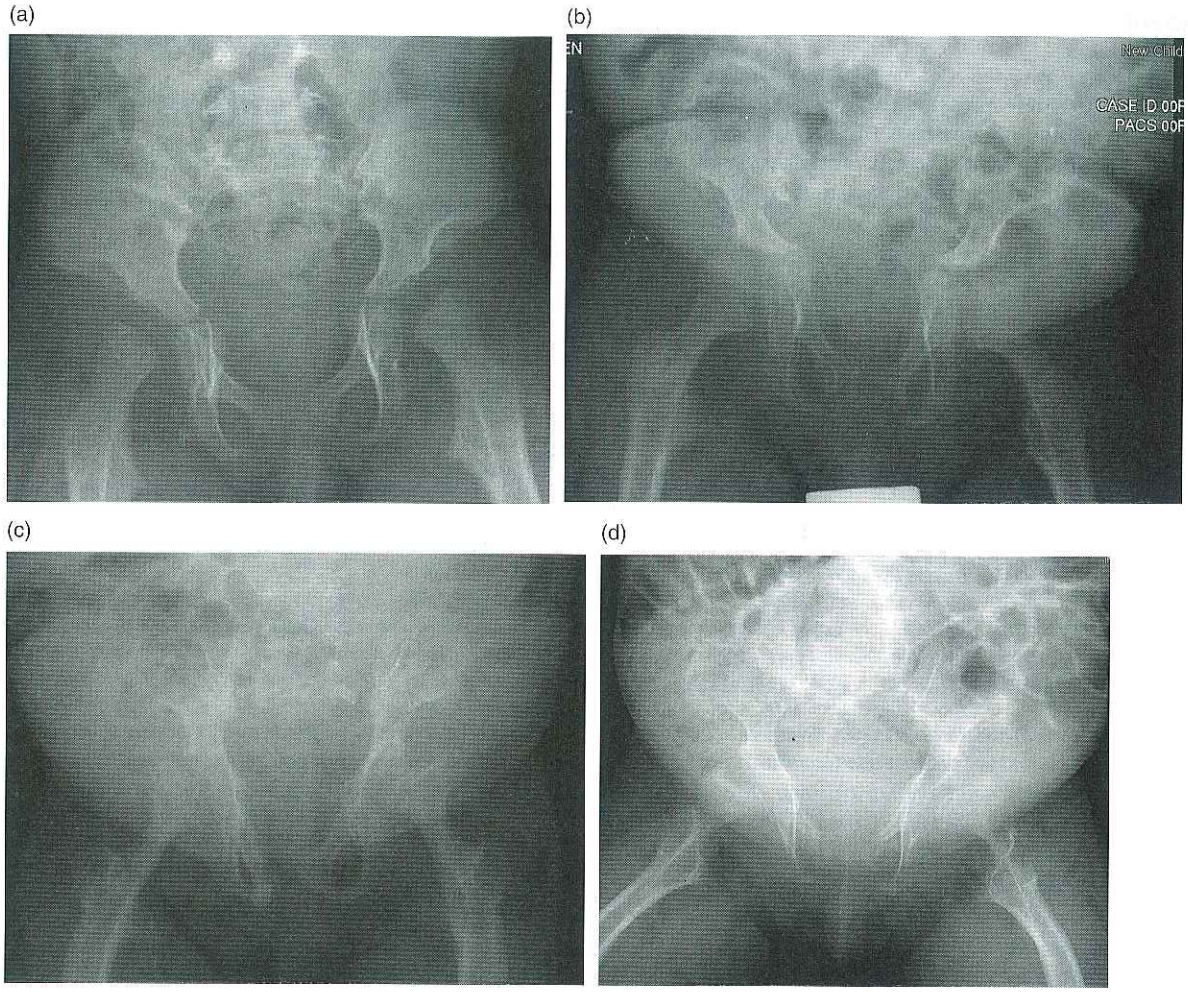

Fig. 3 Radiograph of pelvis and proximal femurs in four affected with intermediate aged (a) 4 years, (b) 16 years, (c) 18 years and (d) 19 years. showing progressive dysplasia/resorption of the low third of the ilia femoral heads and femoral necks.

increased resorption. Another possibility is that mannose-6-phosphate targeting is important for other proteins involved in signal transduction of PTH effects on bone formation and remodelling. The radiographic findings show a remarkable similarity to *osteitis fibrosa cystica*. We hypothesise that the pathology is best explained as 'pseudohyperparathyroidism', that is, tissue hypersensitivity to circulating PTH.

There is a clear need for further systematic studies regarding the pathophysiology of the osteodystrophy in ML II and ML III beginning at birth or time of diagnosis. Additional answers to this perplexing problem may come from study of the cat model of mucolipidosis.[36,37]

The osteodystrophy of the mucolipidoses contributes significantly to the skeletal and joint symptoms. Progressive and destructive bone disease places an additional burden of weakness, pain and disability over that encountered in mucopolysaccharide storage disorders. A better understanding of the pathogenesis is important to improve the quality of life of those affected. Treatment with cyclic intravenous pamidronate is a promising adjunctive therapy that is presently being evaluated for those affected with the mucolipidoses.[35]

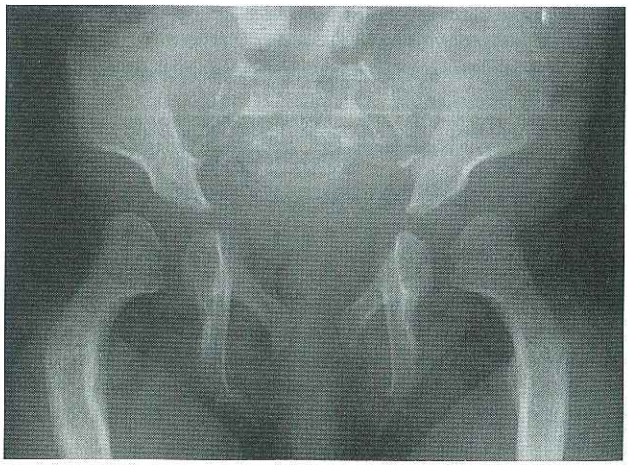

Fig. 4 Radiograph of pelvis and femurs in a 3-year-old with mucolipidosis type II showing over-modelling of the proximal end of the femur leading to coxa valga or 'shepherd's crook deformity'. The lower third of the ilia have become progressively hypoplastic and resorbed.

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