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 deoxyriboflavin/creatinine were elevated. Two radiological patterns were observed: (i) transient neonatal hyperparathyroidism in infants with ML II and III, 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 ‘pseudo hyperparathyroidism’. Much of the progressive skeletal and joint pathology is attributable to this bone disorder.

Key words: hyperparathyroidism; mucolipidosis; osteoporosis; ‘pseudo hyperparathyroidism’.

What this paper adds

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 (Gnpt1). 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.

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

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by the deficiency or abnormal function of UDP-N-acetylglucosamine:lysosomal enzyme N-acetylglucosaminyl-
1-phosphotransferase, commonly termed UDP-GlcNAc
1-phosphotransferase (GlcNAc-PT). The underlying defects
result in deficient post-translational modification of numerous
enzymes, which depend on mannose phosphorylation for
uptake and localization by cells where substrate degradation
occurs. This in turn results in effective deficiencies of lysosomal
degradative enzymes with concomitant intracellular accu-
mulation 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 gas-
glucosidosis. It is characterised by coarse facial features, short
stature, hyperplastic gums, organomegaly and retardation psychomotor development. ML III is a milder disorder with attenuated
characteristics and survival to adult life. Intermediate
forms of ML II and III have been previously described. The
presence of clinical heterogeneity has been recognised and
verified in the laboratory by complementation studies. Molecular
characterisation has further substantiated this het-
erogeneity and allows for more descriptive disease nomenclature.
GlcNAc-PT is a complex enzyme consisting of three subunits that are the products of two genes. Mutations in
GNPTAB, which encodes the alpha/beta subunit, cause ML II
and ML III alpha/beta. The gamma subunit is encoded by
GNP1G, and mutations in this gene lead to the clinically milder
condition known as ML III gamma.

Previous radiographic studies of ML II and ML III have focussed mainly on those bone changes collectively called ‘dys-
ostosis multiplex’, although a few authors have drawn attention
to bone changes in infants with ML II that are similar to infantile
hyperparathyroidism and rickets. In this study, we describe a progressive bone and mineral disorder, its biochemical char-
nacteristics 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-
ossseous complications of mucolipidosis.

Materials and Methods

Patients were ascertained through clinical genetics units around
Australia and New Zealand, the Kozlowski Koziolowski 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 Labora-
tory 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 bio-
chemical 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 deoxyribose online croslinks). Functional assessment was
performed using the Pediatric Evaluation and Disability Invent-
ory (PEDI). This standardised tool measures the
domains of life skills, mobility and social function. DNA molec-
ular genetic diagnosis was undertaken in a subset of patients.

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. Cardiovas-
cular 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 Airway Pressure (CPAP) or Biphasic Positive Airway Pressure (BIPAP)) at the level of 8-10 cm water was recorded in 6/10.
Four young adults developed upper limb paresis 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

<table>
<thead>
<tr>
<th>Clinical features at diagnosis</th>
<th>ML II</th>
<th>ML Intermediate</th>
<th>ML III</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 15</td>
<td>N = 5</td>
<td>N = 5</td>
<td></td>
</tr>
<tr>
<td>Coarse facial features</td>
<td>14</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Corneal clouding</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Chest deformity</td>
<td>5</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Umbilical hernia</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Inginal hernia</td>
<td>5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Joint contractures</td>
<td>8</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Congenital hip dislocation</td>
<td>6</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Evidence of hyperparathyroidism</td>
<td>15</td>
<td>5</td>
<td>NK</td>
</tr>
<tr>
<td>Progressive osteoastody</td>
<td>NA</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

NA, not applicable; NK, not known.
occupitocervical 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 pain, 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 I 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.  

### Functional assessment (PEDI)

Five subjects had PEIDI, 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 pmol/L, range 0.8–7.5). Levels of urine deoxyphosphoridolene/creatinine were elevated in all patients (median 26.7 µM/L, range 14.2–45.5).

### 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 enzymeology testing. This particular subject was stillborn and was also found to have 45,X on chromosome analysis.

### Molecular DNA analysis

Mutations in GNPTA8 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. 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.
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, fused iliac wings, constricted iliac bodies, dysplastic femoral heads, ‘bullet-shaped’ proximal phalanges and central pointings of proximal metacarpals. 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 I, 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. Osteoporosis, fractures, periosteal new bone formation (‘cloaking’) and cupped epiphyses have been described in neonates. Histologic examination has confirmed the presence of hyperparathyroidism. Patzakia et al. 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 II and ML III there are progressive radiographic features that overlap with juvenile hyperparathyroidism or chronic hyperparathyroidism or ‘osteo- sis 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 Gaucher disease, 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. 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.
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. Prenatally, 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. 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 osteoclast with abnormal biofeedback and induction of PTH receptor transduction with focal areas of...
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 simi-
laritv 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 signifi-
cantly to the skeletal and joint symptoms. Progressive and
destructive bone disease places an additional burden of weak-
ness, pain and disability over that encountered in mucopoly-
saccharide 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. 36

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.

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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