The clinical features of three babies with osteogenesis imperfecta resulting from the substitution of glycine by arginine in the pro α1(I) chain of type I procollagen

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Abstract

The features of three babies with lethal perinatal osteogenesis imperfecta resulting from the substitution of glycine by arginine in the pro α1(I) chain of type I procollagen were studied. The babies were heterozygous for this substitution at residue 391 in case 1 (OI24), 667 in case 2 (OI51), and 976 in case 3 (OI30). They were all small, term babies who died soon after birth. The ribs were broad and continuously beaded in OI24, discontinuously beaded in OI51, and slender with few fractures in OI30. The overall radiographical classifications were type IIA in OI24, IIA/IIB in OI51, and IIB in OI30. Histological examination confirmed that the long bones were misshapen and porotic. The calcified cartilage trabeculae were covered with an abnormally thin layer of osteoid and the bone trabeculae were thin and basophilic. There was no evidence of lamellar bone or Haversian systems. The osteoblasts remained relatively large and closely spaced. These babies shared many phenotypic features, but differences in the radiographical appearance of the ribs and long bones suggested that there was a gradient of bone modelling capacity from the slender and overmodelled bones in OI30 to the absence of modelling in OI24.

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Osteogenesis imperfecta (OI) is a genetically determined disorder of the connective tissues in which bone fragility is the main feature. Patients with OI have been classified according to their clinical, radiographical, and inheritance patterns into four main types and several subtypes.[1] This report concerns the type II or perinatal lethal form of OI. It has been subclassified into type IIA, IIB, and IIC.[2] Group A have broad, crumpled bones and beaded ribs with perinatal death. Group B have broad, crumpled long bones but the ribs show minimal or no beading. Some babies live beyond the neonatal period. Group C have thin, cylindrical, and dysplastic long bones with thin, beaded ribs. These babies have very low birth weights and are either stillborn or die soon after birth.

Biochemical heterogeneity has also been noted in the various types and subtypes of OI with the characterisation of a range of mutations of the COL1A1 gene that encodes the pro α1(I) chain and the COL1A2 gene that encodes the pro α2(I) chain of type I collagen.[3 4] As a result, the opportunity now exists to examine the relationships between the genotypes and clinical phenotypes of OI.

We have reported the substitution of glycine by arginine at different sites in the 1014 residue triple helical domain of the pro α1(I) chain of type I collagen in three babies with lethal perinatal (type II) OI.[3 5 6] The babies were heterozygous for this substitution at residue 391 in OI24, 667 in OI51, and 976 in OI30. Our previous studies have also shown abnormal collagen metabolism by cultured fibroblasts and abnormal collagen composition of tissues from these babies.[7 8] The principal findings were slow electrophoretic migration of type I collagen chains owing to overmodification of lysine residues that were amino terminal to the mutation.[9] There were also abnormalities of collagen metabolism including decreased secretion, increased intracellular degradation, and decreased thermal stability. The amount of type I collagen was reduced in the dermis and bone. In this paper we describe the clinical and pathological features of these babies and correlate these findings with the biochemical abnormalities.

Features of the parents, pregnancies, and babies.

Features	OI24 (Gly^{391})	OI51 (Gly^{667})	OI30 (Gly^{976})
Father's age	29	26	29
Mother's age	26	27	24
Parity	1	0	1
Gravida	2	1	2
Gestation (wk)	40	40	40
Presentation	Cephalic	Breech	Cephalic
Delivery	Vaginal	Caesarian section	Vaginal
Sex	Female	Female	Male
Head circumference (cm)		32*	30*
Crown-heel length (cm)		41*	38*
Birth weight (g)	2290*	2620*	1091*
Survival (days)	1	16	7
Type of OI†	IIA	IIA/IIB	IIB
Ribs	Broad, continuously beaded	Discontinuously beaded	Slender, infrequent fractures
Femora	Broad, crumpled	Broad, crumpled	Broad, poorly modelled
Humeri	Broad, crumpled	Broad metaphyses, central overmodelling	Broad metaphyses, central overmodelling
Spine	Generalised platyspondyly	Thoracic platyspondyly	Generalised platyspondyly
Skull	Poor ossification	Poor ossification	Poor ossification
Fibroblasts	Dilated, rough endoplasmic reticulum	Normal	Normal

*Value less than the 10th centile for an Australian hospital population.[10]
†Sillence classification.[2]

Case reports

The main details of the parents, pregnancies, and babies are summarised in the table.

CASE 1 (OI24)

This girl was the second child of healthy, unrelated parents. The pregnancy was complicated by poor weight gain. The membranes ruptured spontaneously at term. After a vaginal delivery, the baby took 15 minutes to establish regular respirations. There was evidence of respiratory distress and supplemental oxygen was required. Ventilation deteriorated and the baby died 29 hours after delivery.

The baby weighed 2290 g and had typical clinical features of type II OI (fig 1). She was small with short, bowed limbs, a very soft head, proptosis, and a flail narrow chest. The sclerae were blue, the pal-
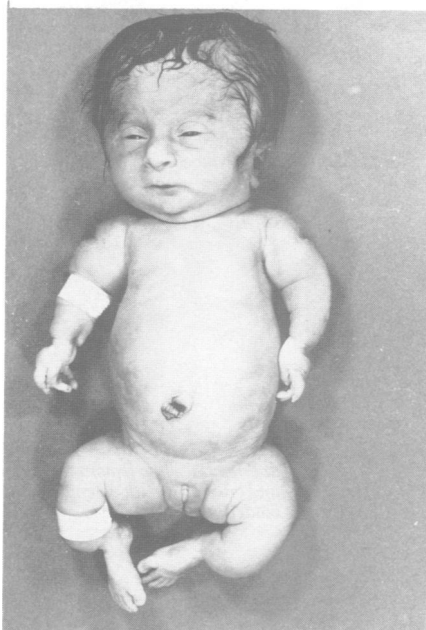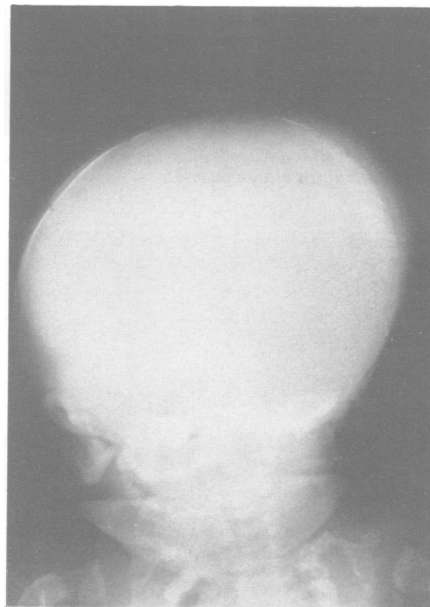

Figure 1   OI24: clinical appearance.

Figure 2   OI24: lateral skull radiograph showing severe osteoporosis.

pebral fissures were short and horizontal, and the nose was beaked. There was widespread crepitus of ribs and limb bones.

The radiographs showed very severe generalised osteopenia and multiple fractures. The calvarium and base of the skull were poorly ossified and there were multiple wormian bones (fig 2). There was generalised severe platyspondyly and the ribs were broad and continuously beaded (fig 3). The long bones were also broad, unmodelled, and crumpled (fig 4). The radiographical features were consistent with those ascribed to type IIA OI.[2] [11]

CASE 2 (OI51)
She was the first child of healthy, unrelated parents

who have subsequently had two normal daughters. Labour started spontaneously at term and she presented by the breech. A caesarian section was undertaken because of lack of progress with labour. Respiration started spontaneously.

She weighed 2620 g and had short limbed dwarfism with widespread crepitus of the limb bones (fig 5). There was marked hypotonia and all long bones were clinically bowed. She also had a narrow chest, soft skull, narrow nose, proptosis, and blue sclerae. Her
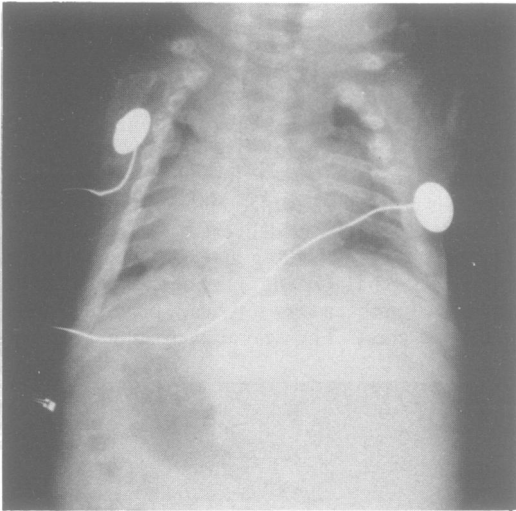

Figure 3   OI24: chest radiograph showing continuously beaded, wide ribs and generalised platyspondyly.
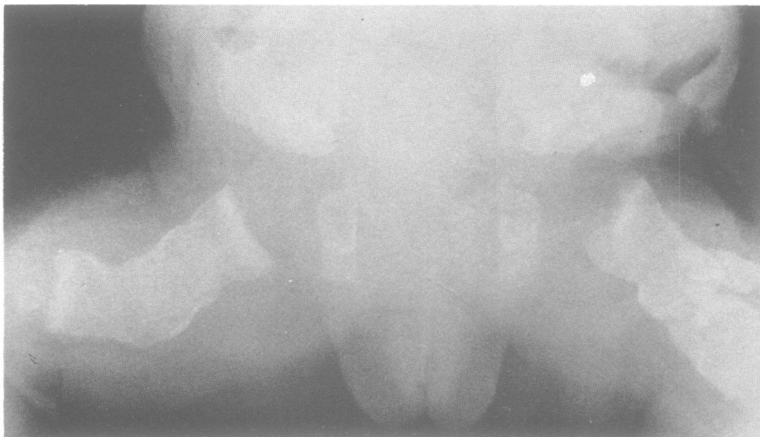

Figure 5   OI51: clinical appearance.

Figure 4   OI24: radiograph of pelvis and femora. The femora are broad and crumpled with no evidence of modelling.

condition gradually deteriorated and she died at 16 days of age.

The radiographs showed severe generalised osteopenia, discontinuously beaded ribs, broad, crumpled femora, thoracic platyspondyly, poor ossification of the skull, and wormian bones (fig 6). The lumbar vertebrae were of normal shape (fig 7). The humeri had broad metaphyses but the diaphyses were over-modelled with mid-shaft fractures. There were features of both type IIA and IIB OI so that OI51 was classified as type IIA/IIB.

CASE 3 (OI30)

He was the second child of healthy, unrelated parents who have had a further normal child. There were few fetal movements noted during the pregnancy. Labour started spontaneously at term and he was born by vaginal delivery.

He weighed 1091 g and was noted to have short limbed dwarfism and widespread crepitus of long bones (fig 8). He showed clinical bowing of all long

Figure 7   OI51: lateral radiograph showing poor ossification of the calvarium. The lumbar vertebral bodies are normally shaped whereas the thoracic vertebral bodies are flat. The humeral diaphyses are overmodelled.

bones, severe softness of the skull, a small face, narrow nose, and blue sclerae. His condition gradually deteriorated and he died at 7 days of age.

Radiographs showed severe generalised osteopenia. The ribs were slender but showed few fractures (fig 9). The femora were broad and poorly modelled, the humeral diaphyses were overmodelled, the skull was poorly ossified with multiple wormian bones, and there was generalised, severe platyspondyly (figs 10 and 11). The features were of type IIB OI.[2 11]

PATHOLOGICAL STUDIES

Necropsy of the three babies showed very similar findings. There were multiple fractures involving the long bones with shortening and malformation of the limbs. The calvarium was soft and poorly calcified. The lungs were mildly hypoplastic, but there were no malformations of the viscera.

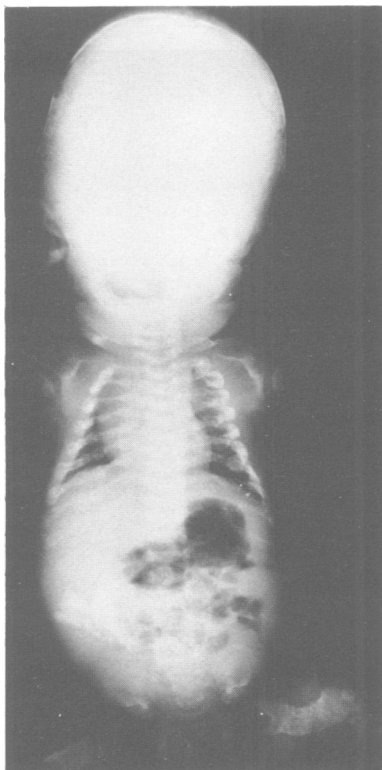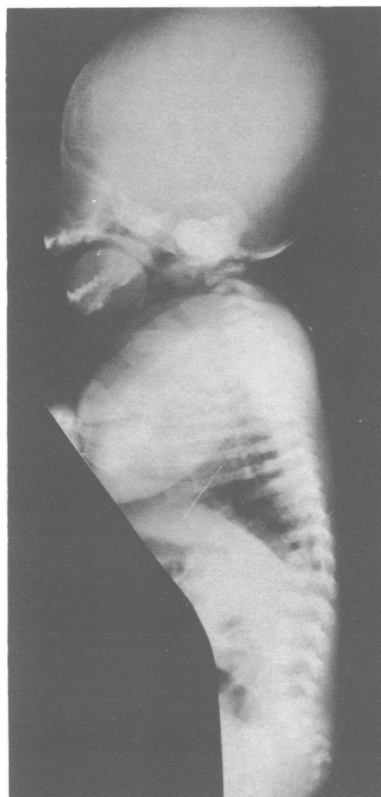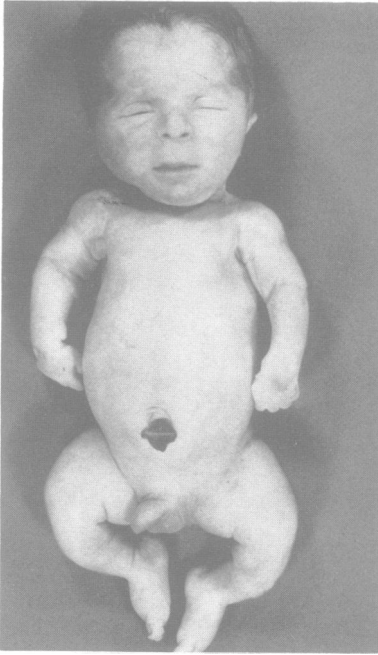Light microscopy showed that the dermis was thin

Figure 6   OI51: anteroposterior radiograph. The ribs are discontinuously beaded, the thoracic vertebral bodies are flat, and the femora are broad and crumpled.
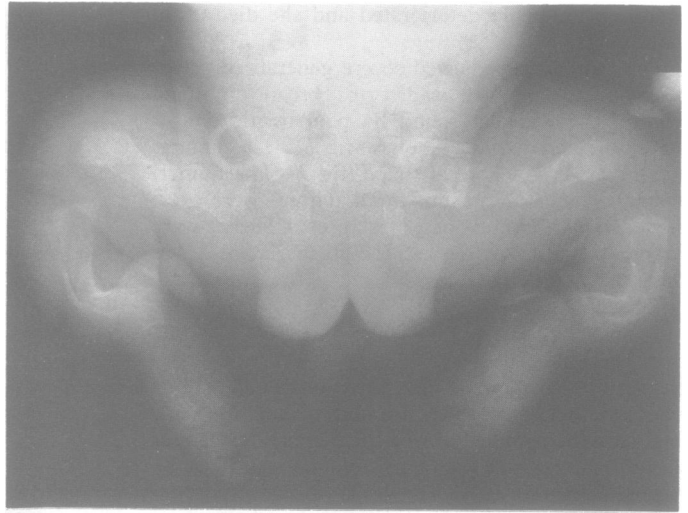
Figure 8   *OI30: clinical appearance.*

Figure 10   *OI30: radiograph of pelvis and legs. The tibiae are bowed with mid-diaphyseal overmodelling. The femora contain multiple fractures and show some modelling of the diaphysis.*

Figure 9   *OI30: chest radiograph showing thin ribs with few fractures, thoracic platyspondyly, and overmodelled humeri.*
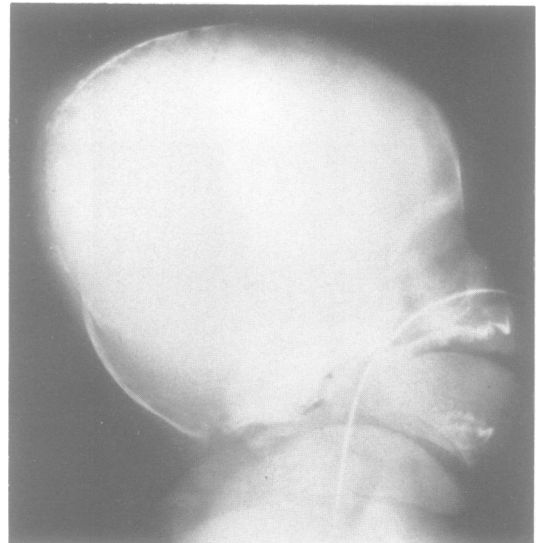

Figure 11   *OI30: lateral skull radiograph. The calvarium is better ossified than in OI24 and OI51.*

when compared to normal age matched control dermis. The junction between the loose papillary and the dense reticular layers of the dermis was poorly defined. The collagen bundles were well formed and normally birefringent. Silver staining showed a decreased number of small, dark, argyrophilic fibres in the superficial dermis. Elastic fibres were decreased in size and in number. Electron microscopy showed marked dilatation of rough endoplasmic reticulum of fibroblasts in OI24 but not in OI51 or OI30.
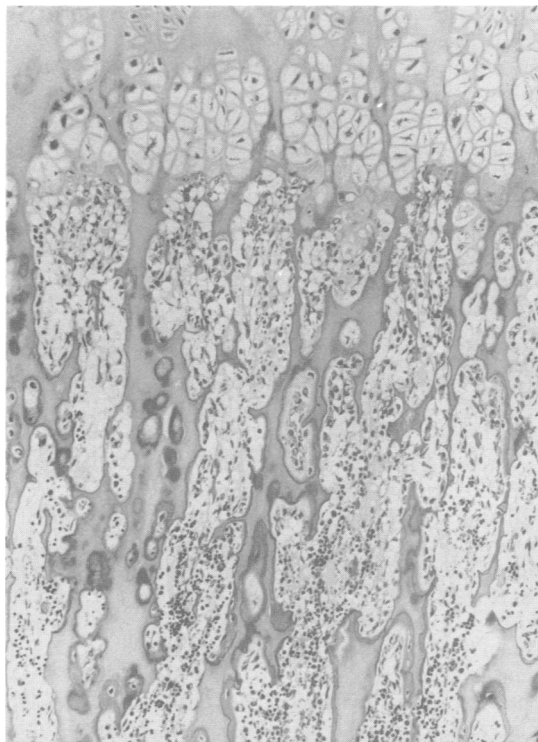
Figure 12   Normal costochondral junction stained with haematoxylin and eosin.
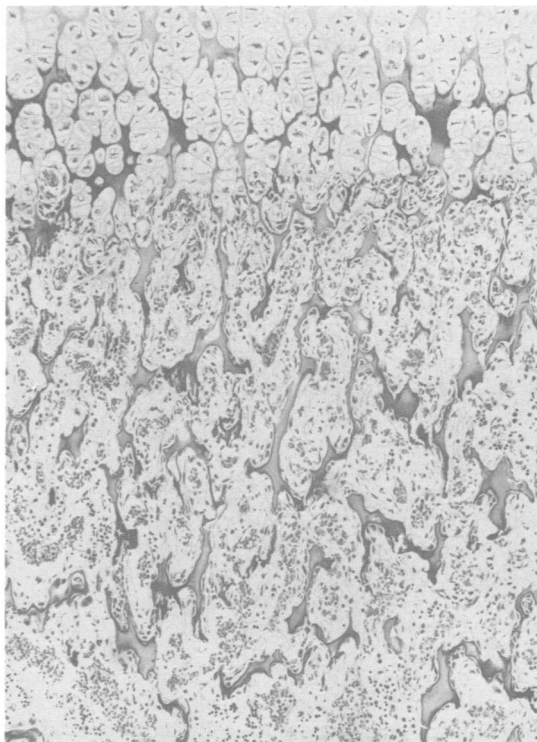

Figure 13   Costochondral junction of rib of OI30. The primary cartilaginous trabeculae are thin and sparse and the layer of osteoid on the trabeculae is thin.

The architecture of the costochondral growth plates was normal in each patient although on ultrastructure some of the chondrocytes in OI24 contained prominent cytoplasmic droplets of fat. In the metaphysis, the calcified cartilage trabeculae were abnormally thin in most sites (figs 12 and 13). However, this feature was variable and in some sites the trabeculae were considerably broader. Both types of trabeculae were only covered by an abnormally thin layer of osteoid and bone. The bone trabeculae in the diaphysis remained thin, woven, and basophilic. Examination under polarised light did not show maturation into lamellar bone. Cortical bone with Haversian systems was not seen. The osteoblasts within the bone trabeculae remained relatively large and closely spaced, without evidence of maturation into small osteocytes. Multiple fractures with callus were also observed.

Discussion
These babies were all small, term babies who died shortly after birth. OI30 was much smaller than the other babies but in other respects their clinical appearances were very similar. However, there were some radiographical differences between them. OI24, with an amino acid substitution near the junction of the amino terminal and middle thirds of the helix, had the typical radiographical features of type IIA OI. In contrast, OI51, with a substitution near the junction of the middle and carboxyterminal thirds of the helix, had features of both type IIA and IIB OI, while OI30, with a substitution near the carboxy terminus of the helix, had features of type IIB OI. The appearances of the ribs highlighted these differences. They were broad and continuously beaded in OI24, discontinuously beaded with fractures in OI51, and slender with infrequent fractures in OI30. These radiographical findings suggest a gradient of bone modelling capacity from OI30 to OI24. A gradient of this type is also in keeping with the appearances of the long bones, as they were broad and unmodelled in OI24, unmodelled or overmodelled in OI51, and thin or overmodelled in OI30.

The major histological features of OI bone included the paucity of matrix around the osteoblasts, the failure of maturation of osteoblasts, and the lack of lamellar bone and Haversian systems. These findings

are likely to result from the effects of the mutations on the amount and quality of type I collagen, although the exact pathogenesis of these architectural and maturational abnormalities remains unclear.

van der Harten et al[12] reported that the histological appearances of the metaphysis correlated with the radiographical subtypes of type II OI. Thin calcified trabeculae were reported in cases of type IIA and IIB OI while broader and irregularly arranged cartilaginous trabeculae were reported to be characteristic of type IIC OI. In contrast, we observed both patterns at different sites in the same patient. As a result, this difference in pattern is likely to be non-specific.

Many of the findings in the present study are in accordance with our previous biochemical results. The thinness of the dermis is consistent with its reduced concentration of type I collagen.[8] Similarly, the reduced amount of bone is consistent with our previous findings of reduced amounts of type I collagen in this tissue. We also found an increased proportion of type III and V collagens that were localised to the non-calcified fraction of OI bone.[8] This fraction is likely to correspond to the marrow tissue which is abundant between the sparse trabeculae of woven bone.

Our previous studies also showed decreased thermal stability of the mutant collagen and increased intracellular degradation of collagen produced by cultured dermal fibroblasts.[7] [9] Although much of the mutant collagen may have been degraded some of it was secreted into the medium. Collagen secretion was normal in OI51 and OI30 but was halved in OI24. These observations are consistent with the finding of grossly dilated, rough endoplasmic reticulum in fibroblasts from OI24 dermis. They are also consistent with the presence of mutant and normal type I collagen in dermis and bone from these babies.[8]

Although our previous biochemical findings accounted for some of the abnormalities observed in the current study, the mechanisms involved in producing the clinical phenotype are still largely unknown. In addition, many factors may affect the expression of the mutation including whether the $\alpha1(I)$ or $\alpha2(I)$ chain is involved, its location and surrounding sequence in the α chain, the genetic background of the baby, obstetric factors, and postnatal care. For example, the substitution of glycine by arginine at residue 1012 of the triple helix of the $\alpha2(I)$ chain of type I collagen was reported to give rise to a mild to moderately severe autosomal dominant form of OI classified as type IV OI.[4] The phenotypic expression of substitutions of glycine residues in the helix of the $\alpha1(I)$ chain by cysteine have also been studied. Substitutions at residues 988,[13] 904,[14] 748,[15] and 718[16] produce the lethal type II phenotype, substitutions at 526[16] produce type III OI, substitutions at 175[17] produce type IV OI, and substitutions at position 94[16] produce type I OI. At

least for glycine substitutions by cysteine in the $\alpha1(I)$ chain, there appears to be a gradient of severity of phenotype from carboxy terminal to amino terminal substitutions.[16] [18]

In the present study of glycine substitutions by arginine in the $\alpha1(I)$ chain, there is evidence of a gradient of bone modelling. While this proposal might indicate a gradient of severity of phenotype that is the reverse of that observed with glycine substitutions by cysteine, it should be noted that all of the substitutions of glycine by arginine were lethal. We are unable to determine whether OI24, with a birth weight of 2290 g and unmodelled bones owing to a substitution at residue 391, was more or less severely affected than OI30 with a birth weight of 1091 g, slender bones, and overmodelled bones owing to a substitution at residue 976.

These relationships have also been studied using site directed mutagenesis of a mouse COL1A1 gene. Mutations, resulting in the substitution of glycine-859 by arginine or cysteine, were expressed in fibroblasts where they produced metabolic and electrophoretic changes that were identical to those seen in patients with the same substitutions at nearby positions.[19] When the cysteine mutant gene was expressed in transgenic mice it resulted in a lethal perinatal OI phenotype that was almost identical to type IIA OI. The severity of the phenotype was related to the expression of the mutant gene.[19]

Study of further OI babies with defined mutations and the study of additional animal models of OI produced by site directed mutagenesis of collagen genes should provide further insights into the factors involved in the phenotypic expression of type I collagen mutations.

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