Syndrome of the month

Perinatal lethal osteogenesis imperfecta

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Abstract

Perinatal lethal osteogenesis imperfecta is the result of heterozygous mutations of the COL1A1 and COL1A2 genes that encode the $\alpha 1(I)$ and $\alpha 2(I)$ chains of type I collagen, respectively. Point mutations resulting in the substitution of Gly residues in Gly-X-Y amino acid triplets of the triple helical domain of the $\alpha 1(I)$ or $\alpha 2(I)$ chains are the most frequent mutations. They interrupt the repetitive Gly-X-Y structure that is mandatory for the formation of a stable triple helix. Most babies have their own private de novo mutation. However, the recurrence rate is about 7% owing to germline mosaicism in one parent.

The mutations act in a dominant negative manner as the mutant pro α chains are incorporated into type I procollagen molecules that also contain normal pro α chains. The abnormal molecules are poorly secreted, more susceptible to degradation, and impair the formation of the extracellular matrix. The collagen fibres

are abnormally organised and mineralisation is impaired.

The severity of the clinical phenotype appears to be related to the type of mutation, its location in the α chain, the surrounding amino acid sequences, and the level of expression of the mutant allele.

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Osteogenesis imperfecta (OI) is a genetically determined disorder of the connective tissues. The major phenotypic features include bone fragility, dentinogenesis imperfecta, blue sclerae, deafness, and ligamentous laxity. It has long been recognised to be clinically heterogeneous with mild, moderate, severe, and lethal phenotypes. The Sillence classification takes these varying phenotypes into account.¹

Figure 1 OI-IIA: radiograph showing severe osteopenia, continuously beaded ribs, platyspondyly, and broad crumpled long bones.

Figure 2 OI-IIB: radiograph showing severe osteopenia and broad crumpled femora. The ribs which are slender with expanded ends contain few fractures.

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Figure 3 OI-II-C: radiography showing minimal ossification of the skeleton.

Type I is the common mild form, type II is the perinatal lethal form, type III is a severe form, and type IV is a moderately severe form. We will focus on OI type II (OI-II).

Clinical heterogeneity

Babies are classified as having OI-II if they die in the perinatal period. However, the babies

show clinical and radiological heterogeneity which appears to be established early in the pregnancy.² The radiographic appearances are used to subclassify OI-II into groups A to C or into groups I to V.¹³ The Sillence sub-classification will be used here.

OI-IIA

The babies are small for the period of gestation. The face is hypoplastic with micrognathia, deep blue-grey sclerae, and a relatively large calvarium, which is moulded and soft. The chest is small and the abdomen protuberant. The limbs are short and bowed. The hips are flexed and abducted. One or more limbs may be held in a severely deformed position. The radiographs show generalised osteopenia. The diaphyses of the long bones and ribs are as wide as their growth plates owing to the lack of metaphyseal remodelling (fig 1). The thin and crumpled cortices give the long bones a continuously beaded appearance. The pelvis and shoulder girdle have a similar appearance. There is generalised platyspondyly. The skull and face are severely porotic. Numerous wormian bones are present in the skull sutures. The babies are usually stillborn or die at birth.

OI-IIB

The ribs are less affected so that respiratory distress is less and babies may survive for months (fig 2). Otherwise the clinical and radiographic features are similar to those of OI-IIA.

OI-IIC

This is the least common subtype. The babies are very small, osteopenia is severe, and the long bones are slender, poorly modelled, and contain numerous fractures (fig 3). The babies are stillborn or die soon after birth.

Genetic heterogeneity

Numerous studies have shown that the majority

Figure 4 Map of glycine substitutions within the triple helical domains of the $\alpha 1(I)$ and $\alpha 2(I)$ chains of OI-II. Each line represents the triple helical domain of an α chain. The substituting residue is listed on the left. The residue numbers are indicated above each line for the $\alpha 1(I)$ chain and below each line for the $\alpha 2(I)$ chain. The residues are numbered relative to the first glycine residue of the triple helix.

Exon skipping mutations, deletions, and insertions of the triple helical domains of COL1A1 and COL1A2 and the carboxyl-terminal propeptide domain of COL1A1 in OI-II

Domain	Locus	Mutation	Reference
Helical	COL1A1	Skipping of exon 14	4
		Duplication of exons 14–17 causing insertion of 60 aa	5
		84 aa deletion, 328–411	6, 7
		Skipping of exon 27	8
		75 bp insertion derived from intron 35	9
		Skipping of exon 43	8
		3 aa deletion (GlyAlaPro) in the region of 868–876	10
		3 aa deletion (GlyAlaPro) 874–876	11
		Skipping of exon 44	8
		Skipping of exon 47	12
	COL1A2	Skipping of exon 28	13
		Skipping of exon 33	14
		Deletion of exons 34–40	15
		Asp 2099His	16
Carboxyl-propeptide	COL1A1	Trp 1134Cys	17
		Val1146Cys plus frameshift and truncation	18
		2 aa deletion (Glu 1159–Tyr 1160)	16
		Leu 1210Arg	16
			16

of babies with OI-II are heterozygous for mutations of the COL1A1 or COL1A2 genes that alter the structure of the triple helical domains or the carboxyl-terminal propeptides of the pro α 1(I) or pro α 2(I) chains of type I procollagen (fig 4, table). Each procollagen molecule contains two pro α 1(I) chains and one pro α 2(I) chain. The propeptides are enzymatically removed outside the cell to produce type I collagen that is laid down in the extracellular matrix of the dermis, bone, dentine, sclera, ligaments, and fascia, the tissues affected in OI.

The first mutation defined in OI-II was a heterozygous deletion within COL1A1 that resulted in the loss of 84 amino acids from the triple helical domain of the α 1(I) chain.⁶⁷ However, few deletions have subsequently been identified (table). Exon skipping mutations are more common. They usually maintain the translational reading frame and the repetitive Gly-X-Y amino acid triplet structure as the exons encode complete triplets (table).

The majority of mutations in OI-II involve the substitution of Gly residues in Gly-X-Y triplets within the triple helical domain of the α chains. The ability of such mutations to produce the OI-II phenotype was confirmed by reproducing it in transgenic mice bearing a COL1A1 mutation that substituted Cys for Gly 859.¹⁹ Numerous point mutations of the GGN codon for Gly have been identified (fig 4). Substitutions by Ser are the most common while substitutions by Ala and Glu are rare and substitutions by Trp have not been identified to date. Approximately two-thirds of the substitutions involve the α 1(I) chain and a similar proportion involve the carboxyl-terminal halves of the triple helical domains of the α 1(I) and α 2(I) chains. The non-uniform nature of the substitutions, α chain involved, and sites within the α chains are unlikely to be the result of ascertainment or technical biases. Similar results were found when all babies born with OI-II in Victoria, Australia were studied.¹⁷ Similar results have also been obtained with modern methods of mutation detection that screen full length α 1(I) and α 2(I) cDNAs.²⁰

Most unrelated babies with OI-II have their own private de novo mutation (fig 4). Exceptions include recurrent substitutions of Gly

352, 415, and 1003 by Ser in the α 1(I) chain.^{20–24} These mutations occurred at CpG dinucleotides, in a manner consistent with deamination of a methylated cytosine residue.²¹ However, there do not appear to be any major mutational hot spots in the COL1A1 or COL1A2 genes in babies with OI-II.

A few mutations in the carboxyl-propeptide of pro α 1(I) chains have been identified in OI-II (table). They alter sequences that are important in chain association, disulphide linking of the chains, and interactions with chaperones.^{16 18}

In many instances, the mutations in OI-II are new dominant mutations, while in the remainder the mutation is inherited from a mosaic parent.²⁵ Isolated germline mosaicism has not been identified to date.^{25 26} The mosaic parent may appear to be clinically normal or to have a mild or moderate form of OI. However, leucocytes, hair follicles, and dermal fibroblasts contain differing proportions of the normal and mutant alleles.^{25 26} The proportion within the osteoblasts, which is likely to be the most important determinant of the phenotype, is usually unknown. The recurrence risk of OI-II is approximately 7% because of the occurrence of germline mosaicism in one parent.²⁷

Pathogenesis

All of the mutations that produce OI-II appear to act in a dominant negative manner. The mutant pro α chains are incorporated into type I procollagen molecules and produce major abnormalities in collagen metabolism.

Deletions, skipping mutations, and insertions in the triple helical domain produce abnormal alignment of the pro α chains. Substitutions of Gly residues interrupt the mandatory repetitive Gly-X-Y structure required for normal formation of the triple helix. They may also produce a bulge or kink as a means of accommodating the bulkier side chains. For example, kinks at the mutant site have been observed in type I collagen molecules containing mutant α 1(I) chains bearing substitutions of Gly 718 or 748 by Cys.^{28 29} The lysine residues, particularly on the amino-terminal side of the substitution, are often over-modified by the prolonged action of lysyl

hydroxylase and glycosyl transferases owing to a delay in the zipper-like folding of the collagen molecules.³⁰ The thermal denaturation temperature of the abnormal collagen is often reduced but it is independent of the level of overmodification of the α chains.³¹ The variable levels of overmodification and thermal stability of the mutant molecules are likely to reflect regional differences in helix stability and in the propensity for renucleation of folding of the helix beyond the mutation.^{32–34}

As type I collagen molecules contain two $\alpha 1(I)$ chains it is likely with heterozygous mutations of COL1A1 that 25% of the molecules are normal while 75% of the molecules contain one or two mutant $\alpha 1(I)$ chains. Conversely, as type I collagen molecules contain one $\alpha 2(I)$ chain, it is likely with heterozygous mutations of COL1A2 that 50% of the molecules are normal and 50% contain a mutant $\alpha 2(I)$ chain.²⁷ Procollagen containing one or more mutant pro α chains is poorly secreted and is excessively degraded by cultured fibroblasts.²² Once secreted, cleavage of the amino propeptides by N-proteinase may be reduced even with mutations distant from the cleavage site.²⁸

Histological and biochemical anomalies have been observed in the dermis from babies with OI-II. The fibroblasts often contain dilated, rough endoplasmic reticulum because of impaired secretion of the mutant type I procollagen molecules.³⁵ The collagen fibrils are smaller than normal. There is a reduced amount of type I collagen which consists of a mixture of normal and mutant type I collagen molecules. The mutant collagen chains are more readily extracted which suggests that the type I collagen molecules containing one or more mutant chains may be degraded before they are incorporated into the more insoluble cross linked extracellular matrix.³⁶

Severe anomalies have also been observed in bone from babies with OI-II. The bone is severely porotic. The normal cortical and trabecular bone pattern is replaced by woven bone.^{35,37} There is an abundance of plump osteoblasts surrounded by little extracellular matrix. The osteoblasts may also contain dilated rough endoplasmic reticulum.² The growth plate is normal but cartilage cores persist in the trabeculae.³⁸ The bone matrix contains a reduced amount of type I collagen and increased amounts of type III and V collagens.^{36,39} The type I collagen includes both normal and mutant molecules.^{36,40} The levels of some non-collagenous proteins are also abnormal.⁴¹ Osteocalcin, $\alpha 2$ -HS glucoprotein, and bone sialoprotein levels are increased, osteonectin is reduced, and decorin levels are normal. There are dramatic changes in the collagen fibrils and mineralisation. The collagen fibrils are thin and do not show the normal spatial arrangement of parallel bundles.^{2,42} At the mineralising front, apatite crystallites are not oriented along the collagen fibrils as observed in normal bone. The crystallites are also small and appear to grow by aggregation of new crystallites onto their surfaces.^{2,38,43}

Many of these metabolic findings were also observed in transgenic mouse models of

OI-II.^{19,44} Expression of COL1A1 constructs encoding a Gly 859 to Cys substitution or an in frame deletion in the triple helical domain of $\alpha 1(I)$ chains resulted in the incorporation of mutant type I collagen molecules into the tissues.

Mutations of the carboxyl-terminus of the pro $\alpha 1(I)$ chain can also produce dramatic changes in type I collagen metabolism.^{16,45} In one heterozygous baby with OI-II, a codon frameshift mutation produced a truncated pro $\alpha 1(I)$ chain with other pro α chains.¹⁸ The abnormal type I procollagen molecules were not secreted and were degraded within the cell. The type I collagen content of the dermis and bone was reduced to about 20% of normal. This is the amount of normal collagen that would be expected if all of the molecules containing one or two mutant pro $\alpha 1(I)$ chains were degraded. In contrast, mutations of the carboxyl-terminus of the pro $\alpha 1(I)$ chains that prevent chain association and incorporation of mutant chains into type I procollagen molecules produce one form of mild OI-I.⁴⁶

Genotype-phenotype relationships

There are two broad categories of genotype-phenotype relationships in osteogenesis imperfecta. The first category includes phenotypes associated with haploinsufficiency of COL1A1 and COL1A2. The second category includes phenotypes associated with dominant negative mutations of COL1A1 and COL1A2. There are rare exceptions, such as some forms of autosomal recessive OI that do not appear to be the result of type I collagen mutations.⁴⁷

Haploinsufficiency of COL1A1 results in an approximately 50% reduction of type I collagen synthesis and the mild osteogenesis imperfecta type I phenotype with normal teeth, OI-IA.²⁷ Heterozygous Mov-13 mice, in which one COL1A1 allele is inactivated by insertion of a retrovirus, also show the OI-IA phenotype.⁴⁸ In contrast, homozygous Mov-13 mice die around the twelfth day of gestation as a result of the absence of type I collagen from many tissues.⁴⁹ A human equivalent has not been identified as yet but would probably produce early death in utero and spontaneous abortion.

A homozygous deficiency of $\alpha 2(I)$ chains has been reported in a child with moderately severe OI-III.⁵⁰ The structure of the carboxyl-propeptide of the pro $\alpha 2(I)$ chains was altered so that the chains would not associate. The metabolic consequences of the mutation were equivalent to homozygous haploinsufficiency of COL1A2. The heterozygous parents were minimally affected.

The majority of deletions, insertions, and splicing anomalies that alter the triple helical domain of the $\alpha 1(I)$ or $\alpha 2(I)$ chains produce lethal phenotypes. However, the more common glycine substitutions in the repetitive Gly-X-Y triplets of the triple helix produce mild to lethal phenotypes. The site and type of substitution have been proposed as important factors in determining the severity of the phenotype. For Gly substitutions by

Cys in the $\alpha 1(I)$ chain, carboxyl-terminal sites produce lethal phenotypes, mid-helical sites produce moderately severe phenotypes, and amino-terminal sites produce mild phenotypes.³⁴ However, exceptions such as the substitution at Gly 244 by Cys in a baby with OI-II have been observed.⁵¹ Gradients of phenotypic severity are less obvious with other Gly substitutions (fig 4). Lethal phenotypes are frequently observed with mid-helical Gly substitutions by Ser and Arg.

There are a few rare instances of unrelated subjects harbouring the same substitutions of glycines in either the $\alpha 1(I)$ or $\alpha 2(I)$ chain. Somewhat unexpectedly, such people do not always exhibit the same phenotype suggesting the possible involvement of epistatic effects. To illustrate this, an $\alpha 1(I)$ -Gly352Ser substitution produces OI-II in one person but OI-IV in two others.^{20,22,23} A similar, though less dramatic, effect has also been noted in two subjects with OI-III resulting from an $\alpha 2(I)$ -Gly859Ser substitution.⁵² In contrast, three subjects harbouring an $\alpha 2(I)$ -Gly502Ser substitution appear to exhibit exactly the same OI-II phenotype.⁵³ Clearly evidence from more instances is required to resolve the question of epistasis.

Gly substitutions were proposed to be less deleterious in the $\alpha 2(I)$ chain as type I collagen molecules only contain one $\alpha 2(I)$ chain. However, a similar proportion of Gly substitutions in the $\alpha 1(I)$ and $\alpha 2(I)$ chains produce lethal phenotypes. The sequences around the Gly substitution may be more important in determining the consequences of the substitution than the α chain involved.^{23,32} For example, within the $\alpha 2(I)$ chain there are non-lethal domains interspersed with lethal domains.²³

The level of expression of the mutant allele has been shown in transgenic mice also to be an important determinant of the severity of the phenotype. A direct relationship was found between the severity of the phenotype and steady state mutant mRNA levels in mice bearing a Gly 859 substitution by Cys in COL1A1.¹⁹ These findings indicated that moderate levels of expression of the mutant COL1A1 were sufficient to lead to dramatic dominant negative effects on the incorporation of type I collagen into the extracellular matrix. Similar findings were also observed in transgenic mice that were high expressors of a COL1A1 gene construct containing an in frame deletion.⁴⁴ However, inbred mice expressing moderate levels of this transgene showed marked phenotypic variability.⁵⁴ The explanation for this variability was not determined.

Little is known about the levels of expression of the mutant alleles and the levels of incorporation of mutant molecules into the extracellular matrix in human OI-II. However, the collagen content of the dermis and bone is reduced and mutant collagen is detectable in babies with Gly substitutions in the triple helical domain of the α chains.³⁶ In one baby with a substitution of Gly 580 by Asp in the $\alpha 2(I)$ chain, the bone matrix was shown to contain a ratio of mutant to normal $\alpha 2(I)$ chains of 0.7:1.⁴⁰

The genotypes associated with subtypes of

OI-II have only been studied in a few babies. The substitution of Gly by Arg at Gly 391 produced OI-IIA, at Gly 667 produced OI-IIA/IIB, and at Gly 976 produced OI-IIB.³⁵ The substitution of Gly by Val at Gly 1006 produced OI-IIC, Gly 973 produced OI-IIA, and Gly 256 produced IIA.⁵⁵ A carboxyl-propeptide mutation of the pro $\alpha 1(I)$ chain produced OI-IIB.⁴⁵

Prenatal diagnosis

OI-II is often detected on routine ultrasound scans of pregnant women with a history of osteogenesis imperfecta.^{56,57} The skull is poorly echogenic and the limb bones are characteristically broad, shortened, and fractured. The chest is also abnormal. OI-IIA is the most common subtype.^{56,58} These scans are often undertaken at about 16 weeks but can be done earlier in families with a history of previous babies with OI-II. In the latter families, intrauterine detection of the COL1A1 or COL1A2 mutations can be used if the mutation has been determined before the pregnancy.

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