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New Perspectives on Osteogenesis Imperfecta

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Abstract

A new paradigm has emerged for osteogenesis imperfecta (OI) as a collagen-related disorder. The more prevalent autosomal dominant forms of OI are caused by primary defects in type I collagen, while autosomal recessive forms are caused by deficiency of proteins which interact with type I procollagen for post-translational modification and/or folding. Factors contributing to the mechanism of dominant OI include intracellular stress, disruption of interactions between collagen and non-collagenous proteins, compromised matrix structure, abnormal cell-cell and cell-matrix interactions and tissue mineralization. Recessive OI is caused by deficiency of any of the three components of the collagen prolyl 3-hydroxylation complex; absence of 3-hydroxylation is associated with increased modification of the collagen helix, supporting delayed collagen folding. Other causes of recessive OI include deficiency of collagen chaperones, FKBP65 or HSP47. Murine models are crucial to uncovering the common pathways in dominant and recessive OI bone dysplasia. Clinical management of OI is multidiscipinary, encompassing substantial progress in physical rehabilitation and surgical procedures, managment of hearing, dental and pulmonary abnormalities, as well as drugs such as bisphosphonates and rGH. Novel treatments using cell therapy or new drug regimens hold promise for the future.

INTRODUCTION

Osteogenesis Imperfecta (OI), or “brittle bone disease”, is a clinically heterogeneous heritable connective tissue disorder in which the causative defect is directly related to type I collagen, including abnormalities of collagen primary structure, insufficient quantity, abnormal post-translational modification, folding, intracellular transport or matrix incorporation. The clinical features of OI commonly include low bone mass and reduced bone material strength, resulting in bone fragility and easy susceptibility to fracture, bone deformity and growth deficiency. In its various types it occurs in approximately 1/15–20,000 births[1]. Most OI cases have autosomal dominant inheritance. Over 1500 dominant mutations in either *COL1A1* or *COL1A2*, encoding the α-chains (α1(I) and α2(I)) of type I collagen, have been identified[2] (See also the Osteogenesis Imperfecta Variant Database, at https://oi.gene.le.ac.uk). These mutations alter the structure or quantity of type I collagen and cause a skeletal phenotype ranging from subclinical to lethal.

Exciting developments have generated a new paradigm for OI as a collagen-related disorder. Recessive OI with lethal to moderate phenotypes is caused by defects in genes whose products interact with type I collagen. Most recessive cases have null mutations, causing absence of proteins involved in collagen prolyl 3-hydroxylation (*CRTAP, LEPRE1* and *PPIB*)[3–8], or helical folding (*FKBP10* and *SERPINH1*)[9, 10]. Human cases and OI murine

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models are providing insight into common pathways in dominant and recessive OI, leading to re-evaluation of OI definition, classification and therapeutic approaches.

CLASSICAL OI (AUTOSOMAL DOMINANT INHERITANCE)

Defects in *COL1A1* or *COL1A2* cause autosomal dominant OI. OI features include bone fragility and deformity, as well as short stature, dentinogenesis imperfecta (DI) and hearing loss. Blue/grey sclerae and wormian bones are frequent, but not uniform, findings. In addition, there is overlap between the features of individuals with different Sillence types.

The Sillence classification included 4 types based on clinical, radiographic, and genetic criteria[11, 12]. Although proposed before collagen defects were identified in OI, it remains useful in an updated form which accounts for new gene defects or distinctive histomorphometry (Table 1).

The classification shown in this review and elsewhere[13] designates the original 4 Sillence types entirely for mutations in *COL1A1* or *COL1A2*. It separates the novel OI types based on the gene in which the mutation occurs and the general function of that gene (collagen prolyl 3-hydroxylation, collagen chaperone, etc). This classification succinctly communicates the genetic defect and general phenotypic severity, while also generating homogenous groupings for therapeutic approaches and basic investigations of disease mechanism. Several versions of an alternative classification have been proposed[14, 15], in which the recessive types are folded into the original Sillence numeration based on clinical phenotype; these classifications vary in whether the histologically defined types V and VI are retained or also classified clinically. The alternative classification results in children with defects in the same gene (ie *LEPRE1)* being classified as different types because of clinical variability (ie type II for lethal cases, or III for severe survivors), which is likely to confuse genetic counselling.

OI Type I, the mildest form, has a triad of features: fractures, blue sclera, and hearing loss. Fractures often begin with ambulation and decrease after puberty. These individuals have minimal bone deformity, near normal stature and rarely have DI.

OI Type II is perinatal lethal. Affected infants have short, bowed long bones with crumpling from *in utero* fractures, blue/grey sclerae, and a large, soft cranium. Radiographs reveal undertubulated long bones. The most common cause of death is respiratory failure, associated with small thorax, rib fractures, pneumonia, and perhaps with intrinsic collagen-related abnormalities of lung tissue[16, 17].

OI Type III (progressive deforming) is the most severe, non-lethal form. Affected individuals may sustain hundreds of fractures. Most have triangular facies, frontal bossing, blue/grey sclerae, DI, vertebral compressions and scoliosis. Many have platybasia or basilar invagination. They have extremely short stature; about half have “popcorn” formation (sclerotic lines seen on radiographs representing growth plate fragmentation) at femoral growth plates[18].

OI Type IV (moderately severe) has a broad phenotypic range overlapping types I and III. Affected individuals incur dozens of long bone fractures but most achieve ambulation. Scleral hue, DI, basilar impression, hearing loss and final stature are variable.

Genotype-Phenotype relationship

Type I procollagen is a heterotrimer, composed of two pro!1(I) and one pro!2(I) chains, flanked by globular pro-domains at both the amino (N-) and carboxyl (C-) termini (Figure 1). Glycine is obligatory at every third helical residue of collagen because of spatial

constraints inside the triple helix[19]. Procollagen is extensively hydroxylated and glycosylated post-translationally (Box 1)[20].

General principles have emerged for genotype-phenotype correlations in dominant OI. The molecular defect in type I OI is a null *COL1A1* allele due to frameshifts or PTCs, causing reduced synthesis of structurally normal collagen. Also, splice site defects often lead to alternative splicing with subsequent PTCs. The phenotypes of null *COL1A1* alleles and mild helical substitutions may overlap[21, 22]. We propose that type I OI should be limited to cases with type I collagen haploinsufficiency, including those individuals whose haploinsufficiency is associated with a moderate clinical outcome. The overwhelming majority of patients with a type I OI phenotype have a null *COL1A1* allele. The occasional individual with a collagen structural mutation and a very mild phenotype will be designated type IV OI. In this approach, type I OI is a clinically and biochemically homogenous grouping, as well as the only dominant OI form in which structurally abnormal collagen is not present.

Types II – IV OI are caused by defects in type I collagen structure, most commonly glycine substitutions (80%) and splice site mutations (20%)[2]. Glycine substitutions delay helix folding, leading to post-translational overmodification (Box 1). The OI Mutation Consortium examined 832 mutations in Types II-IV OI, representing substitutions at ~ 44% of glycine residues[2]. In both α-chains, substitutions in the N-terminus are non-lethal (Figure 2). Overall, 36% of *COL1A1* glycine substitutions had a lethal outcome, especially those with charged or branched side chains. Substitutions in two α1(I) Major Ligand Binding Regions (MLBR 2 and 3) are exclusively lethal, suggesting collagen-NCP interactions in matrix are essential to bone formation (Figure 2). Most *COL1A2* glycine substitutions are non-lethal (81%). The α2(I) lethal substitutions occur in eight regularly spaced clusters, aligning with proteoglycan binding sites in the collagen fibril (Figure 2)[2, 23, 24]. The different patterns of lethality in α1(I) and α2(I) indicate each chain plays a different role in matrix organization. Also, substitutions at over 40 glycine residues result in both lethal and non-lethal forms of OI[2], supporting the importance of modifying factors[25, 26].

Detailed comparison of collagen quantitative and structural mutations with OI phenotype found higher lumbar spine areal BMD, greater cortical width and lower bone turnover parameters in type I OI[27]. Furthermore, BMD and histomorphometry of patients with non-lethal OI did not correlate with the α-chain containing the mutation, mutation location in the chain, or the substituting residue, suggesting other factors are crucial for outcome severity.

Rare mutations affecting procollagen processing sites or chain register lead to distinctive variants of OI. The procollagen N- and C-propeptides are cleaved by specific propeptidases in the pericellular space (Box 1). Glycine substitutions in the first 90 residues of the α1(I) helical region disrupt a stable N-anchor domain and prevent or delay N-propeptide removal. The pN-collagen is incorporated into matrix, decreases fibril size and causes a phenotype with characteristics of both OI and Ehlers-Danlos Syndrome (EDS)[28, 29]. Disruption of N-propeptide processing by helical defects in α2(I) also leads to OI/EDS[30–34].

For C-propeptide processing, substitutions at the cleavage site Asp-Ala residues result in mild OI with increases in vertebral DXA z-scores and bone mineralization that are counterintuitive for OI, due to accelerated mineralization[35]. Substitutions in the proα1(I) or proα2(I) C-propeptide have broad phenotypic variability, causing types II-IV OI, though the majority are mild or lethal[36–39]. Since α-chains align at the C-terminal end, these mutations delay chain incorporation and helix formation. However, the C-propeptide is not normally incorporated into collagen fibrils, leaving the mechanism of these OI cases unclear.

Small triplet deletion or duplication mutations shift the register of α-chains in the helix. Although the Gly-X-Y sequence is maintained, salt bridges are disrupted by misalignment of X and Y residues between chains. These cases are severe or lethal, and have delayed collagen folding[40, 41]. The register shift can propagate to the end of the helix, and impact N-propeptide cleavage. Interestingly, substitutions for Y-position residues may also propagate a register shift nearly the full length of the collagen helix, interfering with N-propeptide processing and causing variable phenotypes including mild OI, hyperextensibility and Caffey Disease, a transient infantile cortical hyperostosis[42–44]. Several pedigrees with autosomal dominant Caffey Disease have been shown to have the same COL1A1 R836C (p.R1014C) Y-position change[44–46], associated with self-resolving inflammation and subperiosteal new bone formation with reduced penetrance in infancy. The hyperostosis may be the consequence of the mutation disrupting binding of a ligand, such as IL-2, to collagen and causing increased susceptibility to periosteal injury during infancy[44].

Understanding the Disease Mechanism: from gene to tissue

Almost all cases of dominant OI have low bone mass and increased skeletal fragility[47]. Histomorphometry of OI iliac crests revealed decreased trabecular and cancellous bone volume, increased osteoblast and osteoclast surface, and an overall increase in bone formation rate per bone surface. However, deposition of new bone at the single osteoblast level (MAR) is reduced, and is not compensated by the increased cell number[48]. Interestingly, FT-IR and qBEI both revealed elevated bone matrix mineralization. These data support the occurrence of a common defect in OI bone downstream from the collagen quantitative and qualitative mutations, altering bone cell function and the modelling/remodelling mechanisms which normally maintain bone homeostasis[27, 48, 49].

A variety of murine models for OI are now available for investigation of OI mechanism and pilot treatment studies (Table 2). Mov13 mice have a null *Col1a1* allele caused by a proviral insertion and model type I OI[50, 51]. The oim/oim mouse phenotypically resembles type III OI, although its recessive inheritance is atypical for collagen mutations. A spontaneous single nucleotide deletion in the oim *Col1a2* C-propeptide prevents α2(I) incorporation into collagen[52]. However, the resulting α1(I) homotrimer does not account for the severe OI phenotype (see *Gene and Protein Defects*, below). More recent OI models were generated with knock-in technology or ENU mutagenesis. Knock-in Brtl[53] and G610C OI (Amish)[26] mice have classical glycine substitutions in α1(I) or α2(I) respectively, leading to phenotypes representative of type IV OI. Aga2 mice were generated by ENU mutagenesis; they have a proα1(I) C-propeptide mutation causing a type III OI phenotype[54]. Murine OI models provide direct access to intact long bone and tissues such as lung which are not available from patients; they provide large numbers of samples with the same mutation for studies. These models already play an important role in piloting therapy approaches. In Brtl and oim, cell transplantation has led to positive changes in mechanical properties despite low levels of cellular uptake into bone[55–57]. In the same mouse models treated with bisphosphonates, direct access to whole femora revealed both beneficial and potentially detrimental effects[58, 59]; RANKL inhibition has also been piloted in oim[60, 61]. Of equal importance, murine OI models have provided insight into basic mechanism, including elevated osteoclast function (Brtl and oim)[62, 63], varability of expression (Brtl and Amish)[25, 26, 64], ER Stress[54, 65] and apoptosis (Aga2)[54], which provides new targets for therapy.

Factors contributing to the Mechanism of OI

The mechanisms of classical OI encompass the gene mutation, the collagen alteration, and dysfunction at the cellular, matrix (ECM) and tissue levels (Figure 1). The composition and organization of matrix influences the presence of growth factors and cytokines important for

proliferation and differentiation of bone cells[66], as well as matrix mineralization, which confers bone stiffness.

Gene and protein defects—The type I collagen biosynthetic pathway has been extensively reviewed[67] and a brief description is provided in Box 1. The matrix insufficiency of type I OI results from a PTC in the *COL1A1* transcript, which activates NMD, reducing mutant transcripts and leading to the synthesis of half the amount of normal collagen. Absence of α1(I) chains is not compatible with life, as demonstrated by embryonic lethality in the homozygous Mov13 mice[50] (Table 2).

Homozygous null mutations in *COL1A2* lead to a range of phenotypes. Those associated with NMD and loss-of-function lead to assembly of α1(I) homotrimer. Clinically, this causes mild EDS with hypermobility in childhood and cardiac valve disease in adulthood, rather than OI[68]. In contrast, both one patient with OI[69], and the severe oim/oim mouse (Table 2) have a deletion in the α2(I) C-propeptide, which does not result in NMD. They produce normal levels of *COL1A2* transcripts, which are translated into α2(I) chains that cannot incorporate into collagen. Since α1(I) homotrimer alone does not lead to OI, the intracellular accumulation of mutant α2(I) chains (see *Intracellular Stress*, below) may cause the skeletal dysplasia.

Glycine substitutions delay collagen folding and result in overmodified collagen, which may compromise secretion and/or processing[2, 70]. Substituting residues disrupt non-covalent bonds, causing local unwinding. Certain substituting residues have greater lethality, as do substitutions in clusters along α2(I), and in α1(I) MLBRs (Figure 2)[2]. Although the overmodification gradient does not correlate with clinical severity in α1(I), empirical rules correctly assign most lethal or non-lethal outcomes[71]. In addition, the overmodification of structurally normal collagen in recessive OI (**Recessive OI**, below) raises the possibility that excess hydroxylation and glycosylation have a direct detrimental role in matrix.

Intracellular Stress—Misfolded collagen chains in the ER activate the Unfolded Protein Response (UPR), triggering synthesis of chaperones to assist collagen folding or, alternatively, increasing mutant protein degradation[72]. Cellular response varies depending on the type of collagen mutation (Figure 1). Collagen with triple helical mutations is removed by autophagy, as are collagen aggregates in cells lacking HSP47[73]. In Aga2 cells, ER-retention of mutant collagen increases expression of chaperones BiP and HSP47, apoptosis-inducing transcription factor Gadd153/CHOP and activation of caspase-3 dependent apoptosis[54]. In calvaria of Brtl$^{+/-}$ perinatal lethal pups, relative intracellular retention of helices with one mutant chain[65] is associated with increased expression of Gadd153/CHOP, but normal BiP expression, suggesting collagen misfolding activates the UPR through a BiP-independent response[25]. Finally, C-propeptide mutations that impair trimer assembly result in increased BiP expression, retrotranslocation of the misfolded proα chains into the cytosol and degradation via the proteasomal ER-associated degradation (ERAD) pathway[74, 75].

Compromised ECM Structure and Mineralization—In OI types II-IV, the mixture of normal and mutant α-chains results in matrix heterogeneity and may contribute to the generally greater severity of α1(I) defects, since heterozygous α1(I) defects yield helices with two, one or no mutant chains, while α2(I) defects result in two helix compositions. In Brtl mice, homozygosity for the mutant allele leads to matrix homogeneity and, unexpectedly, to a less severe phenotype, suggesting this feature impacts bone properties[76].

The association of lethal OI with MLBRs in α1(I) monomers or proteoglycan binding sites on fibrils for α2(I)[2, 23, 24] most likely reflects compromised interactions of NCPs with fibrils

(Figure 2). The NCP composition of matrix is altered secondarily in OI, which is also likely to impact bone properties. Cultured OI osteoblasts synthesize reduced amounts of osteonectin and proteoglycans, and increased amounts of fibronectin, thrombospondin and hyaluronan[77, 78]. Thrombospondin and decorin bind growth factors, while decorin and fibronectin are important for fibrillogenesis.

The normal D-periodic spacing of fibrils generates gap and overlap regions, which are important for mineral nucleation and collagen cross-links and NCP interactions, respectively[24]. In Brtl$^{+/-}$ bone matrix, the collagen fibril D-period has significantly greater variability in spacing than in wild-type littermates[79]. The abnormal structure of heterotypic fibrils could affect the type and amount of mineral deposited by increasing the density of nucleation sites[80, 81]; OI matrix also contains abnormal levels of NCPs[82] known to regulate crystal deposition and growth[83]. Elevated mineral content has been demonstrated by FT-IR and BMDD in OI bone with collagen quantitative and structural defects, and is also found in murine models[81, 84]. The elevated mineral content and loss of mineralization heterogeneity contribute to the fragility of OI bone[85, 86], possibly through loss of ductility.

Cell-Cell, Cell-Matrix Interactions—Cellular interactions with abnormal matrix and compromised osteoblast development influence signalling between osteoblasts and osteoclasts, increasing bone remodelling and exacerbating the bone weakness caused by the primary collagen change (Figure 1). Osteoblasts sense osteocyte apoptosis via gap junctions, and receive negative feedback from osteocytes through sclerostin[87,88]. Osteoblasts then trigger osteoclast maturation and recruitment[87]. Ultrastructural examination of OI bone revealed increased numbers of osteocytes and multiple osteocytes in some lacunae[89]. In the Brtl mouse, osteoclast numbers are elevated in femora, uncoupled from osteoblast numbers. Brtl osteoclast precursors from marrow are larger, more numerous and more intensely TRAP stained than in wild-type[62]. The RANKL/OPG ratio is normal in Brtl bone, so other soluble factors triggered by the abnormal matrix may increase osteoclast development. In the oim/oim mouse, elevation of the RANKL/OPG ratio and higher expression of TNF-α were detected in sorted immature osteoblasts, supporting cell-cell signalling as a key aspect of elevated bone turnover in OI[63].

Cross-links in collagen fibrils are important for preosteoblast maturation[90]. In OI, collagen located at the surface of fibrils had fewer cross-links than in the fibril interior.[91] Contact with cross-link deficient matrix by OI bone cell populations could contribute to impaired osteoblast maturation and increased osteoclast recruitment. Also, the effects of collagen heterogeneity in dominant OI could be mediated in part by abnormal cross-linking.

RECESSIVE OI

Beginning with the genes encoding the components of the collagen 3-hydroxylation complex, mutations in five genes have now been identified as causing recessive OI (Table 1). Collectively, they account for 2–5% of OI cases detected in North America and Europe[4, 71]. Other genes remain to be identified, including the genes causing types V and VI OI (See Discussion of type VI OI and *FKBP10* mutations). The pattern emerging for OI is of a collagen-related condition, affecting the structure, synthesis, folding, secretion and matrix organization of type I collagen.

Defects in components of the collagen 3-hydroxylation complex

Prolyl 3-hydroxylase 1 (P3H1), cartilage-associated protein (CRTAP) and cyclophilin B (CyPB) assemble into a 1:1:1 complex within the ER that post-translationally modifies specific proline residues in unfolded collagen α-chains[92]. This includes nearly complete 3-hydroxylation of Pro986 residues of α1(I), α1(II) and α2(V) collagen chains, plus several

partially modified sites in α2(I) (80% Pro707), α1(II), α1(V) and α2(V) chains[93]. The complex also has a chaperone function; furthermore, each complex component is a multifunctional protein, with independent extracellular functions[94–96].

The importance of the complex and α1(I) Pro986 hydroxylation to bone development became apparent when the recessive bone defect in the *Crtap*$^{-/-}$ mouse[3] converged with the chromosomal location of type VII OI (3p22–24.1)[97] and with a biochemical approach to recessive OI based on overmodification of structurally normal collagen (Figure 3)[98].

CRTAP, the helper protein of the complex, is highly homologous to the amino end of P3H1, but lacks the C-terminal catalytic domain[95, 98, 99]. *CRTAP* is expressed in the skeletal system by chondrocytes, osteoblasts and osteoclasts. Although it is mainly localized in the ER, it is also secreted and may have a matrix function[3, 95, 100]. The *Crtap*$^{-/-}$ mouse has a moderately severe connective tissue disorder characterized by rhizomelia, kyphosis, growth deficiency, and osteopenia[3, 101] (Table 2). In humans, CRTAP deficiency (type VII OI) presents as a moderate to lethal recessive osteochondrodystrophy, with growth deficiency and rhizomelia as in the mouse, and also with white sclerae, severe osteoporosis with neonatal fractures, and broad undertubulated long bones[3, 4]. Almost all reported *CRTAP* mutations cause frameshifts, resulting in NMD and absence of CRTAP protein, with loss of α1(I) 3-hydroxylation[100]. We had hypothesized that severe OI with collagen overmodification, but without a collagen structural defect, would be caused by defects in one or more proteins that interacted with collagen. However, the OI field had not anticipated that Pro986 3-hydroxylation or defects in members of the 3-hydroxylation complex, such as *CRTAP*, would delay type I collagen folding (Figure 3).

P3H1, the enzymatic component of the complex, is encoded by *LEPRE1*. P3H1 is the only complex component containing a KDEL sequence for ER retrieval; this KDEL-containing isoform is crucial for collagen modification[102]. A P3H1 isoform is also secreted as the chondroitin sulfate proteoglycan leprecan[96]. P3H1 expression is localized to tissues rich in fibrillar collagens, which are abundant during development[103]. Molecular defects in *LEPRE1* (Type VIII OI), most of which led to reduced transcripts, were identified shortly after *CRTAP* mutations[5, 8, 102] and found to outnumber type VII OI cases. Type VIII OI patients present with severe to lethal OI, recessive inheritance, white sclerae, rhizomelia, and undertubulation of long bones. Those who survive into childhood have extremely low BMD, severe growth deficiency and bulbous metaphyses. A *LEPRE1* founder mutation (c. 1080+1G>T), found in West Africans and African Americans, occurs in almost half of type VIII OI cases reported[104]. In Type VIII as in Type VII OI, there is absence of α1(I)Pro986 3-hydroxylation and overmodification of the collagen helical region, supporting delayed folding (Figure 3). The P3H1 null mouse has a milder phenotype than type VIII OI, although they share growth deficiency, rhizomelia, reduced BMD, abnormal hypertrophic chondrocytes, and delayed secretion of overmodified collagen from cultured cells[105] (Table 2).

The third component of the collagen 3-hydroxylation complex, CyPB (encoded by *PPIB*), is a peptidyl-prolyl *cis-trans* isomerase. Isomerization of the naturally-occurring *cis* proline to the *trans* conformation is rate-limiting for collagen folding[106]. CyPB was thought to be the unique collagen isomerase[107]. Several mutations in *PPIB* (Type IX OI)[7, 108] which lead to PTCs or misfolded protein result in severe to lethal OI. Their phenotype and overmodified collagen biochemistry are similar to OI types VII and VIII, except without rhizomelia, consistent with a dysfunctional 3-hydroxylation complex. In contrast, we identified a *PPIB* mutation (c.26T>C; referred to as c2T>G in ref. 6) in siblings with moderate OI and white sclerae, but without rhizomelia[6]. This mutation occurs at position M9 of the protein sequence predicted by GenBank (NM_000942.4). However, it was unproven

(www.uniprot.org/uniprot/P23284), whether M1 or M9 was the initiation codon, and M1 is not fully conserved evolutionarily. Our data supported M9 as the start codon since *PPIB* transcripts were reduced and there was complete absence of CyPB, as expected for an initiator mutation[109]. CyPB was undetectable with multiple antibodies, in guanidinium extracts or after proteasomal inhibition[109]. P3H1 and CRTAP levels are one-third and two-thirds normal in the *PPIB*-null cells, indicating CyPB enhances complex stability. However, activity of the complex in the absence of CyPB is sufficient to normally 3-hydroxylate α1(I) Pro986[6]. Furthermore, normal helical modification in the absence of CyPB (Figure 3), indicating normal folding, argues for redundancy of collagen *cis-trans* isomerases in human cells.

A *Ppib*$^{-/-}$ mouse with an out-of-frame exon 2/4 junction has non-lethal recessive OI with reduced bone volume, kyphosis and growth deficiency[110] (Table 2). *Ppib* transcripts are reduced and CyPB is undetectable, but murine collagen biochemistry differs from both human PTC and start codon mutations. Collagen gel electrophoretic migration had a delayed baseline, rather than a broadened band as in the human PTC cases; helical modification was not quantitated in the mouse. Pro986 3-hydroxylation was absent in murine collagen, although partial persistence of complex components in murine tissue is suggested by detection of reduced levels of P3H1. Functionally, procollagen remained in the ER and was not properly transported into the Golgi. The differences in collagen modification between the KO mouse and human start codon mutation may reflect alternative binding partners supporting P3H1/CRTAP stability in humans, or redundancy for PPIase.

Mechanisms of Recessive OI in Collagen 3-Hydroxylation Defects

Both P3H1 and CRTAP are absent or severely reduced in *LEPRE1*- or *CRTAP*-null cells, although transcript levels of the normal gene are somewhat increased. Transfection of null cells with constructs encoding the absent transcript restored both proteins[111]. These data indicate that CRTAP and P3H1 are mutually protective in the 3-hydroxylation complex, and explain the overlapping phenotypes of types VII and VIII OI. *LEPRE1*- or *CRTAP*-null cells lack the complex chaperone function, as well as collagen 3-hydroxylation. Absence of the P3H1/CRTAP complex also abrogates the collagen chaperone and PPIase functions of CyPB[110], although cellular levels of CyPB are unaffected[111]. Similarly, binding of misfolded CyPB with P3H1/CRTAP appears to interfere with complex function, while P3H1/CRTAP can bind and modify collagen in the total absence of CyPB[6, 7].

Crucial questions remain concerning the enzymatic and chaperone functions of the complex. Bachinger showed that the complex has collagen chaperone as well as PPIase activity[94]. Disregulation of the complex may eliminate 3-hydroxylation, but loss of complex collagen chaperone/PPIase functions may dominate disease mechanism. CyPB loses its ability to bind gelatin without CRTAP/P3H1, and knock-down of P3H1 severely reduces the ability of CyPB to bind nascent collagen chains *in vitro*[110]. The OI type IX case in which the P3H1/CRTAP complex was able to normally 3-hydroxylate collagen in the total absence of CyPB resulted in normal collagen folding and a moderate phenotype[6].

It is unclear how deficiency of the 3-hydroxylation complex leads to collagen overmodification. Pro986 3-hydroxylation minimally affects collagen stability[112]. Although collagen folding has not been measured directly in these cases, overmodification is an established consequence of slow folding[70]. However, the extent of Pro986 hydroxylation does not correlate with overmodification or phenotype; patients with 0–5% Pro986 hydroxylation have the same helical overmodification and lethal outcome as those with 25% Pro986 hydroxylation[100].

Another possible contribution to OI mechanism is a proposed role in matrix for the 3-hydroxylation modification. Eyre and co-workers suggested that 3Hyp sites function in fibril assembly through intermolecular hydrogen bonding[93]. Deficiency of type V collagen 3-hydroxylation could also affect formation of heterotypic fibrils. Alternatively, the 3-hydroxylation modification may not be crucial *per se*, but could serve as a binding epitope for NCPs involved in mineralization[93].

Furthermore, absence of 3-hydroxylation complex components has a metabolic aspect, with intracellular effects beyond collagen hydroxylation. Mutant fibroblasts appear stellate and foamy in culture (unpublished data). The absence of complex chaperone activity would be expected to cause ER stress and ultimately apoptosis. We found a surprising increase in collagen secretion in *LEPRE1*-null fibroblasts that points to a possible role for P3H1 in regulation of proline metabolism or collagen synthesis[5].

Collagen Chaperone Defects

Recently, absence or dysfunction of collagen chaperones HSP47 and FKBP65 have been reported to cause recessive OI[9, 10]. HSP47, encoded by *SERPINH1*, is an ER-resident collagen-specific chaperone that binds to and accompanies the assembled procollagen molecule together with CyPB into the Golgi[113]. *Hsp47*$^{-/-}$ mice are embryonic lethal, demonstrating HSP47 is required for normal development [114]. *Hsp47* defects caused intracellular aggregation and delayed secretion of collagen, with abnormal fibrils; type IV collagen misfolding disrupted basement membranes[73, 115, 116]. Severe bone dysplasia in canine and human cases (type X OI) is associated with *SERPINH1* missense mutations[10, 117]. Dachshunds with a missense mutation (c.977C>T, p.L326P) causing OI with DI survived postnatally, probably due to residual HSP47[117]. The only child reported with HSP47 deficiency was homozygous for the missense mutation c.233T>C, p.Leu78Pro. The proband had a severe OI phenotype, including blue sclerae and DI. Atypical features included transient skin bullae, pyloric stenosis and renal stones requiring nephrectomy[10]. The mutant transcript was stable, but proteasomal degradation led to minimal HSP47 protein. Although collagen secretion was somewhat delayed, total collagen secretion was normal in culture and the secreted collagen had normal post-translational modification, indicating independence of the HSP47 chaperone function and 3-hydroxylation. However, proband types I and III collagen had increased sensitivity to protease digestion *in vitro*, suggesting HSP47 monitors triple helix folding and stabilizes the collagen helix during transit through the secretory pathway.

FKBP65, encoded by *FKBP10*, is ER-localized with chaperone activity for collagen[118]. Turkish and Mexican patients carrying *FKBP10* frameshift mutations were first reported with recessive OI (Type XI OI)[9]. All probands have deforming OI including long bone fractures, ligamentous laxity, platyspondyly and scoliosis, although sclerae and teeth are normal. Biochemically, normal collagen 3-hydroxylation, without evidence of increased helical modification, was reported. *FKBP10* mutations were associated with delayed collagen secretion and dilated ER. Consistent with a defect in the FKBP chaperone activity, intracellular aggregates of collagen were demonstrated. In one case, bone histology with an abnormal lamellar pattern resembling the fish scales of type VI OI was reported, as was elevated alkaline phosphatase in two individuals with the Turkish frameshift mutation[9]. Currently no mutation analysis has associated type VI OI with *FKBP10* mutations; the similarity in bone histology may indicate a common pathway in separate gene defects.

Emerging data does indicate that the phenotypic spectrum of mutations in *FKBP10* exons 5, 6 and 8 overlaps with Bruck syndrome (BRKS), an autosomal recessive condition characterized by osteoporosis, joint contractures at birth, fragile bones and short stature, and often thought of as “OI with congenital joint contractures”[119]. Siblings from Saudi Arabia

have symptoms of Bruck syndrome caused by an *FKBP10* exon 6 frameshift mutation that also alters the third PPIase domain[120]; these authors suggested calling *FKBP10* mutations BRKS3, since *FKBP10* does not map to either of the reported loci for BRKS1 or BRKS2. However, an exon 5 frameshift mutation, (c.831_832insC), predicted to lead to a PTC downstream of the third PPIase domain, has been detected in 5 pedigrees reflecting 4 ethnic groups[121], including the Mexican family in the original report[9], which was not noted to have contractures. Three of these 5 pedigrees had findings of Bruck syndrome, including a sibship with one child diagnosed as type III OI and the second with Bruck syndrome. Kelley *et al* proposed that *FKBP10* mutations were the cause of BRKS1, although the map position of *FKBP10* (17q21.2) does not coincide with the reported chromosomal position of BRKS1 based on a single 2-generation Kurdish family (17p12)[121, 122]. This issue remains unsettled: mapping results can be misleading and the enzyme (bone specific telopeptide TLH, encoded by *PLOD2*) whose deficiency was postulated to be involved in BRKS1 has subsequently been mapped to 3q23–24[123]. Bank and co-workers also reported that the original Kurdish family had no defects in *PLOD2* leaving open the possibility that resequencing of the original Kurdish family might reveal an *FKBP10* mutation[122].

Unclassified OI-like and type I collagen based disorders

There are several OI-like or type I collagen based disorders that do not rise to the level of an OI type because of incomplete information. BRKS2 is a recessive condition caused by mutations in *PLOD2* (3q23–24), which encodes bone-specific collagen telopeptide lysyl hydroxylase (TLH)[124]. Affected individuals with BRKS2 are reported to be clinically indistinguishable from BRKS1, “OI with joint contractures”. TLH deficiency results in underhydroxylation of the lysines of the collagen telopeptide, but not the triple helix, leading to abnormal collagen crosslinking.

Caffey Disease is also a distinctive syndrome, some cases of which are caused by a *COL1A1* R836C (p.R1014C) substitution (see Dominant OI)[44]. The collagen matrix defect causes OI/EDS symptoms, while the partially penetrant cortical hyperostosis is limited to infancy.

Third, is a homozygous genetic defect in osterix (encoded by *SP7*), a member of the SP/KLF family of zinc-finger transcription factors[125, 126]. Osterix mutations might be expected to cause devastating defects of osteoblast differentiation; *Osx*-null mice are perinatal lethal with loss of both endochondral and intramembraneous bone formation, and reduced expression of osteoblast-specific markers Col1a1, BSP, osteonectin and osteopontin[127]. An Egyptian child, with a frameshift mutation in *SP7* that leads to loss of the zinc-finger domain important for DNA binding, has a moderately severe OI-like phenotype with decreased vertebral DXA[125]. Classification of this defect is premature in the absence of biochemical, cellular or bone data, since SP7 (Osterix) does not have a selective direct effect on type I collagen.

CLINICAL ASPECTS OF OI

Secondary features of OI – Hearing, Dental, Neurological, Growth

Hearing loss is a common secondary feature of OI, affecting persons with all Sillence types[128]. It is generally progressive, often with mixed conductive and sensorineural deficiency, mostly bilateral and beginning in the second to fourth decades of life, although about 5% of OI children have been reported to have 20 dB hearing loss[129]. By age 50 years, about half of patients have subjective hearing loss in a Scottish survey[130], while over 60% of Finnish OI adults had hearing loss on audiometry in a population study[131]. All OI types had hearing loss (approximately 60%, 80% and 40% of OI types I, III and IV, respectively). Further analysis of the Finnish OI population showed no correlation of hearing loss with

collagen mutation type (null allele, glycine substitution or splicing defect) or mutated collagen gene, as well as a lack of penetrance in some family members[132]. About half of Finnish OI adults also have vestibular dysfunction, with vertigo generally secondary to inner ear pathology[133].

The hearing loss in OI is clinically otosclerosis-like in that both result in footplate fixation, although the two conditions are distinct. When amplification is not adequate, surgical options may be indicated. Several large series of stapedectomies reported hearing gains of more than 20 dB in over 80% of operated ears, as well as improved bone conduction thresholds[134–136]. More recently, a second Dutch series[137] reported success in type I OI but loss of hearing in a type III OI case, while a Swedish series[138] encompassing multiple surgeons in different hospital settings reported more cautious gains, with worsening of hearing loss in 21% operated ears. For carefully selected OI patients with profound sensorineural hearing loss, cochlear implantation is an option[139]. The procedure is more challenging due to hypervascularity of the middle ear mucosa, but results similar to other sensorineural causes were obtained in most of the 10 cases reported[139].

Dental abnormalities (opalescent teeth, obliterated pulp cavities, and constricted coronal-radicular junctions) were proposed as distinguishing genetic features of OI even before the Sillence classification, with almost complete penetrance in those pedigrees in which it occurred, and subsequently described as Sillence subtypes (A & B, with and without DI, respectively)[140]. Dental examinations with panoramic radiographs revealed 40–80% of children with types III and IV OI had DI in primary dentition[141–143]; microscopy suggests very mild DI may be missed radiographically[144]. Children with yellowish-brown discoloration had more enamel fractures and attrition than those with opalescent grey discoloration, and were more likely to need full-mouth restoration with crowns[141], but discoloration was not related to OI type[143]. DI always improved in permanent teeth. A high incidence of malocclusion, impaction, and both delayed and accelerated tooth eruption were also noted. Interestingly, the majority of patients with quantitative collagen defects did not have DI[145], while DI was not associated with any particular molecular abnormalities in patients with altered collagen structure[145, 146]. Expression of *Col1a1* in homozygous mov13 odontoblasts suggests regulation of collagen expression may differ in teeth[147], which could underlie DI inconsistencies. Histology reveals structurally abnormal dentin, with collagen hyperfibers and vesicles[148]. Reduced number and size variation of dentinal tubules were found on scanning EM of affected teeth, with an abnormally smooth dentin-enamel junction[149]. Recent microscopic and ultrastructural studies found occluded tubules, some with retro-curved processes and occlusion of the pulp chamber, consistent with odontoblast dysfunction[150].

Various neurological features are associated with OI, including macrocephaly, hydrocephalus, syringomyelia and basilar invagination (BI) (an infolding of the skull base leading to brainstem distortion)[151]. Relative and absolute macrocephaly is common in OI caused by collagen structural defects. BI which progresses to brain stem impingement is relatively rare but its consequences are potentially devastating; progression should be followed with MRI[152]. Early intervention with occipitocervical bracing can delay progression in most cases[153]. Reducible BI (40%) is treated with posterior fossa decompression and occipitocervical fusion, while irreducible BI (60%) is treated with transoral-transpalatopharyngeal decompression. Despite successful decompression, 80% of BI progresses within 6 years post-surgery[152].

Short stature is one of the cardinal features of OI. Endocrine evaluation of the growth axis was normal in most patients with collagen defects, however about half of children had a blunted response to the IGF-I stimulation test[154]. About half of type IV OI children treated

with rGH double their baseline growth rate in the first rGH treatment year[155]. Given the chondro-osseus manifestations of recessive OI, it is reasonable to speculate that the short stature of dominant OI may be related to abnormalities at the transition from cartilage to bone.

Morbidity and Mortality in OI – Pulmonary and cardiovascular features

Extraskeletal manifestations of OI in the respiratory and cardiovascular systems are the most common causes of OI morbidity and mortality[16, 156]. Recurrent pneumonia is well-known in children with severe OI, as is right sided heart failure (*cor pulmonale)* in severe adults[1]. These effects have been considered secondary to skeletal changes[16, 156], such as scoliosis, rib fractures, or thoracic cage deformity[16, 47, 157]. Individuals with OI and scoliosis have striking decline of pulmonary function after 60° curvature[157]. The presence of severe restrictive lung disease with minimal scoliosis raised the possibility that bone-independent pulmonary pathology also contributes substantially to morbidity in Types III-IV OI[157]. Two case studies of OI with lung hypoplasia add to the possible direct role of mutant collagen in lung pathology[17, 158]. *Cor pulmonale* is considered a late effect of pulmonary dysfunction in OI[16]. Pulmonary function data in OI patients with structurally abnormal collagen but without scoliosis, as well as data from both dominant and recessive murine models is needed to delineate the mechanism of pulmonary pathology.

Cardiovascular findings, including valvular insufficiency, aortic root dilatation, atrial septal defects and septal and posterior left ventricular wall thickening have been reported in OI[159]. In OI adults, aortic root dilatation is the most frequent valvular manifestation[159, 160]. When aortic regurgitation occurs, it is more commonly due to abnormal valvular structure than dilatation of the root[160]. Examination of asymptomatic OI adults by 12-lead EKG and 2D echocardiography revealed valvular regurgitation in 95%, with tricuspid and mitral regurgitation with or without aortic regurgitation accounting for 60% of cases, and impaired diastolic function[161]. These changes may reflect greater stiffness of myocardial tissue which may be primary effects of the mutant collagen.

Management of OI – Rehabilitation and physical therapy

The goal of physical rehabilitation in OI is to maximize the patient's gross motor function and daily life competencies. This is especially important in childhood, when the fundamentals of life functioning are established, and for older individuals, who will experience combined OI and aging effects. Upper arm function, mobility, and annual fracture and surgery rates, but not pain, were reported to correlate with DXA z-scores in 20 patients with types I, III, IV[162]. The authors make the reverse inference that improving DXA scores will improve function, but this is not true especially for gross motor skills and is likely to be a simple correlation of baseline DXA and OI severity[163, 164]. Scoliosis and chest wall deformity also correlate with overall measures of physical health[157]. Further, earlier onset of scoliosis correlates with lower mean DXA z-scores and later age of achieving motor milestones[165]. However, children with mild bone disease and hyperlaxity of paraspinal ligaments may also develop early aggressive scoliosis, despite moderate DXA z-scores and earlier gross motor milestones[42].

Physical rehabilitation of children at major OI clinical centers is individualized and actively promotes increased strength and mobility. Functional tests such as the BAMF[166], the GMFM (originally designed for cerebral palsey)[167] and Bleck score[168] have been validated for OI; muscle strength is scored using consistent criteria individual to each center. The results of rehabilitation have been best documented for the Dutch pediatric OI population. A 4- year follow-up of 5–19 year olds showed that joint range of motion decreased significantly over time in type I OI, especially in the lower extremities, whereas types III

and IV had severe motion limitations that did not change with time[169]. Children with all types of OI increased self-care and social function over time, but mobility level plateaued in types III and IV with muscle strength as the best predictor of ambulation. The type I OI children had no pulmonary or cardiac defects at rest[170], while reduced exercise tolerance and muscle strength in types III and IV contributed to fatigue during activities of daily living. Children with types I and IV OI who participated in a low-resistance physical training program had improved peak oxygen consumption, maximal working capacity and muscle strength after 3 months, but the improvements diminished 6 months after program completion[171], suggesting that regular exercise of the correct intensity is important to improving OI fitness. For immobilized children, a pilot study of whole body vibration using a tilt table in 4 type III or IV OI children was reported to achieve upright sitting in 2 children, and walking with minimal support in 2 others, that had not been achieved with several years of bisphosphonate[172].

Management of OI – Orthopaedic Surgery

Orthopaedic surgery remains a mainstay of lifelong OI management, in a complementary function with physical rehabilitation. Osteotomies of long bones with placement of intramedullary rods are undertaken to correct deformity that impedes function and manage fracture recurrence. Corrective surgery is often crucial to ambulation. Currently, surgeons have at their disposal two types of telescoping rods (Fassier-Duval and Baily-Dubow/Sheffield) and non-elongating Rush pins for the immobilization of long bones after osteotomy procedures. Rod migration is a commonly reported complication in OI[173, 174], occurring more frequently in non-telescoping than telescoping rods[175]. The Fassier-Duval rods have the advantage of percutaneous placement, minimizing trauma and allowing multiple bones to be treated in one session, followed by early rehabilitation[176]. Flexible nails unload less weight from the bone and are successfully utilized in single or double nail techniques[177]. Non-union occurs in about 15% of osteotomy in OI[176]; the increase in non-union after pamidronate may be related to thermal damage from use of an electric saw[178].

The course of the scoliosis that is common in OI is minimally affected by bracing[179]. In Europe, successive use of halo traction followed by spine stabilization can be successful to stabilize (and sometimes reduce) the curve, improve respiratory function and pain[180, 181]. In contrast to common progression of scoliosis, basilar invagination uncommonly progresses to clinically significant compression[151]. To prevent hindbrain herniation and CSF obstruction, patients may require shunt placement or decompression with occipito-cervical fusion[152], to be performed by experienced surgeons in specialized centers.

Management of OI – Benefits and limitations of Pharmacological therapy

Bisphosphonates are anti-resorptive compounds widely administered to children with OI, with the rationale that bones with increased volume of OI-quality matrix will be more fracture resistant[182, 183]. Positive effects on bone histology are obtained from treatment of OI children with bisphosphonates, including increased trabecular number and cortical thickness; vertebral DXA z-scores increase[184, 185]. Studies in OI children have shown that these gains are maximized in 2–4 years[163, 186]. While controlled trials concur that bisphosphonates improve vertebral geometry[187, 188], the decrease in long bone fractures is equivocal, even in trials with over 125 children[185, 189, 190]. The claims of improved strength, motor function and decreased pain initially reported in observational trials are unsupported by controlled trials[163]. Human and murine studies have raised concerns about high cumulative doses impairing bone modelling and healing, decreasing bone material quality and mineralization heterogeneity, and impairing bone cells[58, 86,191], but osteonecrosis of the jaw has not been seen in OI[192]. Because bisphosphonates have a decade-plus half-life in bone, it is crucial to determine the lowest effective cumulative dose for improved vertebral

geometry and whether children should be treated until epiphyseal closure to avoid fractures at the junction of treated and untreated bone[193]. Junctional fractures have not been seen on the NIH bisphosphonate regimen; our view is that long-term suppression of bone remodelling is likely to be a greater detriment than junctional fracture.

Two short-acting compounds being investigated in OI murine models may be applicable to OI in the future. Denosumab is a fully humanized antibody to RANKL which has anti-resorptive action[194]. Inhibition of RANKL will shift the RANKL/OPG ratio, and decrease osteoblast signalling that normally stimulates osteoclast development. Animal studies showed OPG treatment inhibits dental eruption[195]; however, it remains to be determined whether denosumab will have a greater clinical impact on tooth eruption than bisphosphonates, which have been associated with a 1.67 year delay in tooth eruption in 6–14 year old children and may be longer in those treated since infancy[196]. Anti-sclerostin antibody works by an anabolic pathway to stimulate osteoblast production of matrix[197] and may provide benefit without anti-resorptive side effects.

Growth hormone has been administered to both type I OI and type III/IV children in clinical trials[155, 198]. Although the GH axis is generally normal in OI children[154], treatment with standard doses of rGH can produce significant increases in linear growth. In a study of children with types III/IV OI, about half of the type IV children doubled their baseline growth rate and maintained increased linear growth over multiple treatment years; they also experienced significant improvement in bone histology (BV/TV, TbN, BFR/BS) and in vertebral DXA. rGH responders had higher baseline type I collagen carboxyterminal propeptide, a marker for increased matrix production. Similarly, treatment of type I OI children with documented *COL1A1* quantitative mutations resulted in positive changes in bone histology, muscle mass and strength, as well as improved linear growth[198]. The combination of bisphosphonate and rGH is being tested by several groups, and encouraging results are beginning to emerge[199].

Novel molecular approaches to treatment

Potential molecular therapies for OI are modelled on mechanisms of milder disease. Mosaic carrier parents have barely detectable phenotypes, even with high burdens of mutant osteoblasts[200]. Cellular therapy recapitulates the mosaic situation by infusing normal mesenchymal stem cells (MSCs), with osteoblast differentiation potential, into affected individuals. Feasibility studies for MSC therapy were conducted in both humans and mice. Experiments in Brtl and oim showed improved bone properties, despite limited bone engraftment. Transplantation into developing animals[55–57, 201, 202] yielded the highest donor cell engraftment (< 5%), and amelioration of bone structure and integrity. *In utero* transplantation of whole bone marrow into $Brtl^{+/-}$ mice showed that engrafted cells may synthesize a greater amount of normal matrix than the endogenous osteoblasts[56]. Also, MSCs could be used for correction of specific genetic mutations by homologous recombination prior to transplantation[203, 204].

Another approach is allele-specific silencing, which models the null *COL1A1* allele. Agents for targeting mutant transcripts include antisense oligonucleotides, ribozymes and siRNA[205]. Ribozymes, in particular, have specificity for single nucleotide changes, and have shown positive results *in vitro* and in culture[206]. Clinical implementation would require the development of appropriate delivery systems. Further, the importance of ER stress in OI pathology suggests strategies addressing protein folding, perhaps by chemical chaperones[207–209], may be beneficial.

Common features of dominant and recessive OI

The overlapping features of dominant and recessive OI are likely to hold the key to a more complete understanding of mechanism and more targeted therapeutic approaches (Figure 4).

1. **Genetic defects relate to collagen structure, modification, folding or processing**

   Many collagen structural defects and most 3-hydroxylation defects lead to significant overmodification, apparently due to delayed folding. *FKBP10* and *SERPINH1* defects do not cause overmodification, and are not associated with deficient 3-hydroxylation.

2. **Osteochondrodystrophy**

   Because *CRTAP* was first identified in cartilage, it was clear that types VII, VIII, and IX OI were osteochondrodystrophies. Patients with dominant OI have significant growth deficiency, which may represent a defect in transition from cartilage to bone. Patients with *FKBP10* defects have short stature, indicating a cartilaginous component.

3. **ER stress and possible metabolic component**

   Collagen structural, modification and folding defects share swollen ER. Increased stress-related proteins in OI osteoblasts may lead to apoptosis. Dominant and recessive OI may share impaired osteoblast differentiation, altered production of non-collagenous components of matrix and abnormal cell-cell signalling in bone.

4. **Collagen-collagen and collagen-NCP binding**

   For collagen structural defects, alignment of lethal regions on alpha chains with MLBR and proteoglycan binding sites points to important protein-protein interactions in ECM. The Pro986 3-hydroxylation site may be a binding epitope for NCP involved in mineralization. Altered collagen folding from chaperone deficiency could impact protein-protein interactions.

5. **Cell-matrix effects**

   Osteoblasts in contact with abnormal OI matrix may have increased stress and altered cell-cell signalling. Matrix abnormalities including heterogeneity of collagen forms, altered modification and fibril organization may play a role.

6. **Cell-cell signalling and histomorphometry**

   Dominant OI and recessive types VII and VIII OI have high bone turnover, with elevated osteoblast and osteoclast surface. Studies from murine models point to increased osteoblast-osteoclast signalling, perhaps because immature osteoblasts strongly support osteoclast development from marrow precursors.

7. **Hypermineralization**

   OI bone has decreased aBMD, but elevated matrix mineral content. This is a common feature of bone with either quantitative or structural collagen defects causing dominant OI, as well as *Crtap*$^{-/-}$ mice and patients with hypomorphic *CRTAP* defects. The altered matrix content of non-collagenous proteins may disrupt mineralization kinetics.

Abbreviations

BAMF Brief Assessment of Motor Function

qBEI	quantitative Backscattered Electron Imaging
BMDD	Bone Mineral Density Distribution
CSF	Cerebrospinal Fluid
EM	Electron Microscopy
FT-IR	Fourier Transform Infrared Spectroscopy
GMFM	Gross Motor Function Measure
rGH	recombinant Growth Hormone
IGF-1	Insulin-like Growth Factor 1
MAR	Mineral Apposition Rate
NCP	Non-Collagenous Proteins
NMD	Nonsense Mediated Decay
PPIase	Peptidyl Prolyl *cis-trans* Isomerase
PTC	Premature Termination Codon

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Box 1

COL1A1 and *COL1A2* transcripts are translated in the ER into proα1(I) and proα2(I) chains, consisting of a central left handed triple helical domain (1014 aa) flanked by N- and C-terminal globular domains. The terminal C-noncollagenous (C-NC) domain of the proα chains contains interchain disulfide bonds and is important for the assembly and alignment of two α1(I) and one α2(I) chains[210]. Proper chain recognition and heterotrimer assembly through the C-NC alignment region is supported by interactions with ER-resident molecular chaperones such as immunoglobulin-heavy-chain binding protein (BiP), Serpinh1 (HSP47), peptidyl prolyl *cis-trans* isomerases and the recently identified prolyl 3-hydroxylation complex[98, 211, 212]. The assembled trimer then folds the helical domain from C- to N-termini. Proper folding of the triple helical domain requires the presence of a glycine residue at every third amino acid because of steric constrains in the interior aspect of the helix. The helical portions of the collagen chains are subject to a series of post-translational modifications during folding, until the chains become inaccessible in the tight helical configuration. These include prolyl 4-hydroxylation and lysyl hydroxylation of approximately half of proline and one-quarter of lysine residues along the length of the helical region of each chain, catalyzed by prolyl 4-hydroxylase (P4H) and lysyl hydroxylase, respectively, followed by hydroxylysyl glycosylation, catalyzed by glucosyl/galactosyl transferases. Specific proline residues, α1(I)Pro986 and α2(I)Pro707, are fully or partially 3-hydroxylated by the prolyl 3-hydroxylation complex (CRTAP/P3H1/CyPB). After procollagen is secreted into the pericellular space, the terminal propeptides are removed by specific N- and C-proteinases[20]. The mature triple helical collagen molecules participate in a higher order structure in the extracellular matrix, the heterotypic fibril. In the fibril, type I collagen is aligned in a quarter-staggered array, yielding D-period banding with overlap and gap regions. Collagen fibrils are stabilized by formation of covalent cross-links between the telopeptides and adjacent domains of collagen molecules, which are catalyzed by lysyl oxidase. Fibrils interact with non-collageneous proteins, bind soluble factors such as growth factors and cytokines, which regulate cell functions, and constitute the scaffold for mineral deposition[24].

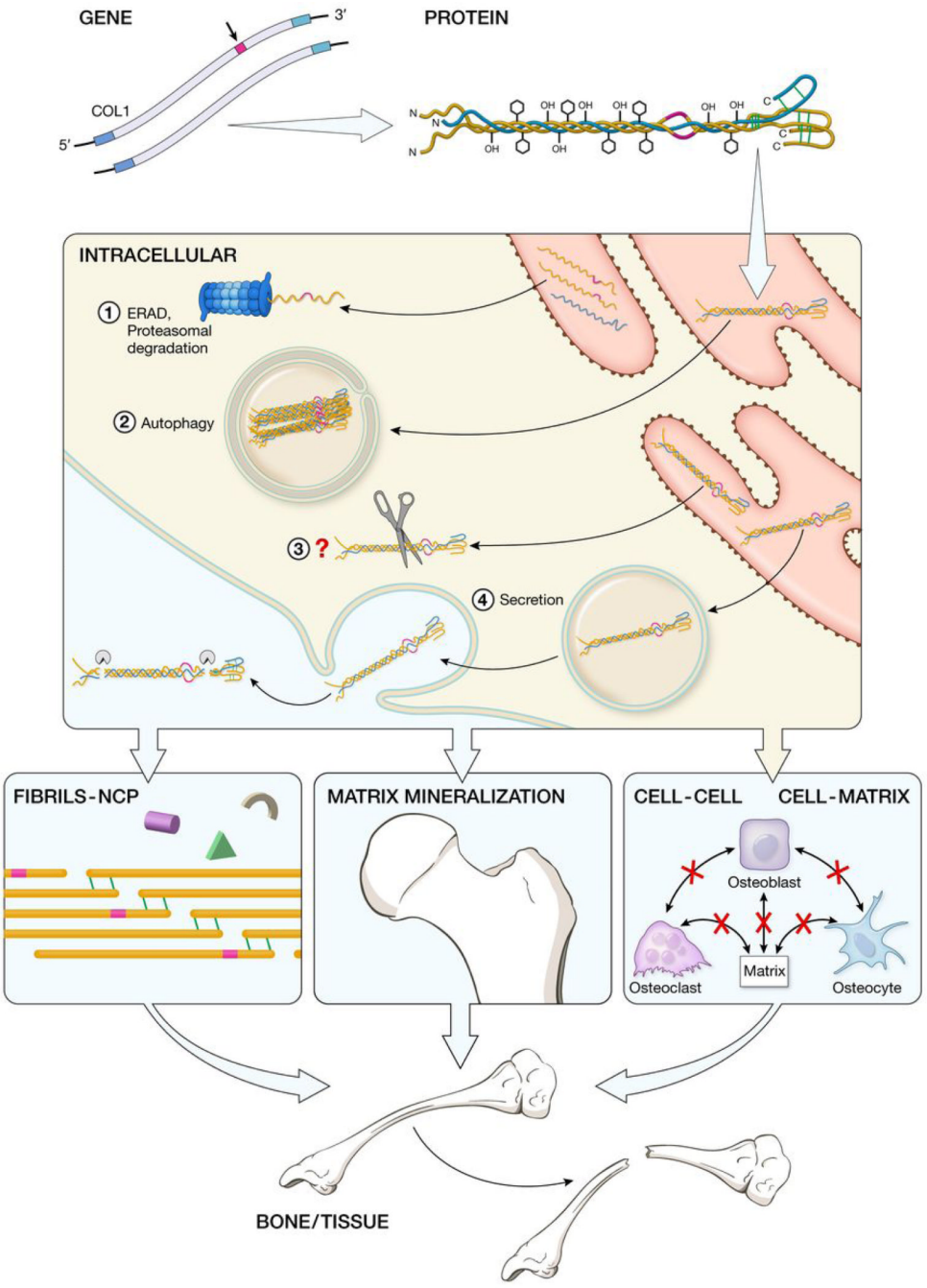

Figure 1. Mechanisms contributing to autosomal dominant OI bone dysplasia: from mutant type I collagen gene to bone defect

Mutations in either *COL1A1* or *COL1A2* are translated into collagen α chains with abnormal structure, which delays folding of the heterotrimer and results in excess post-translational modification of the collagen helical region. Mutant procollagen chains unable to incorporate into heterotrimer are retrotranslocated into the cytosol and degraded by the ER-Associated Proteasomal (ERAD) pathway (1); fully misfolded heterotrimers with structural defects generate supramolecular aggregates that are eliminated by autophagy (2); mutant molecules with triple helical mutations are degraded through an unidentified pathway (3). Finally abnormal procollagen can be secreted, processed and incorporated in

the extracellular matrix (4). The secreted mutant collagen affects fibril structure and interactions of NCPs with matrix, as well as matrix mineralization and osteoblast development and cell-cell and cell-matrix cross-talk. The overall result is bone deformity and fragility, although the relative importance of various contributions is under investigation.

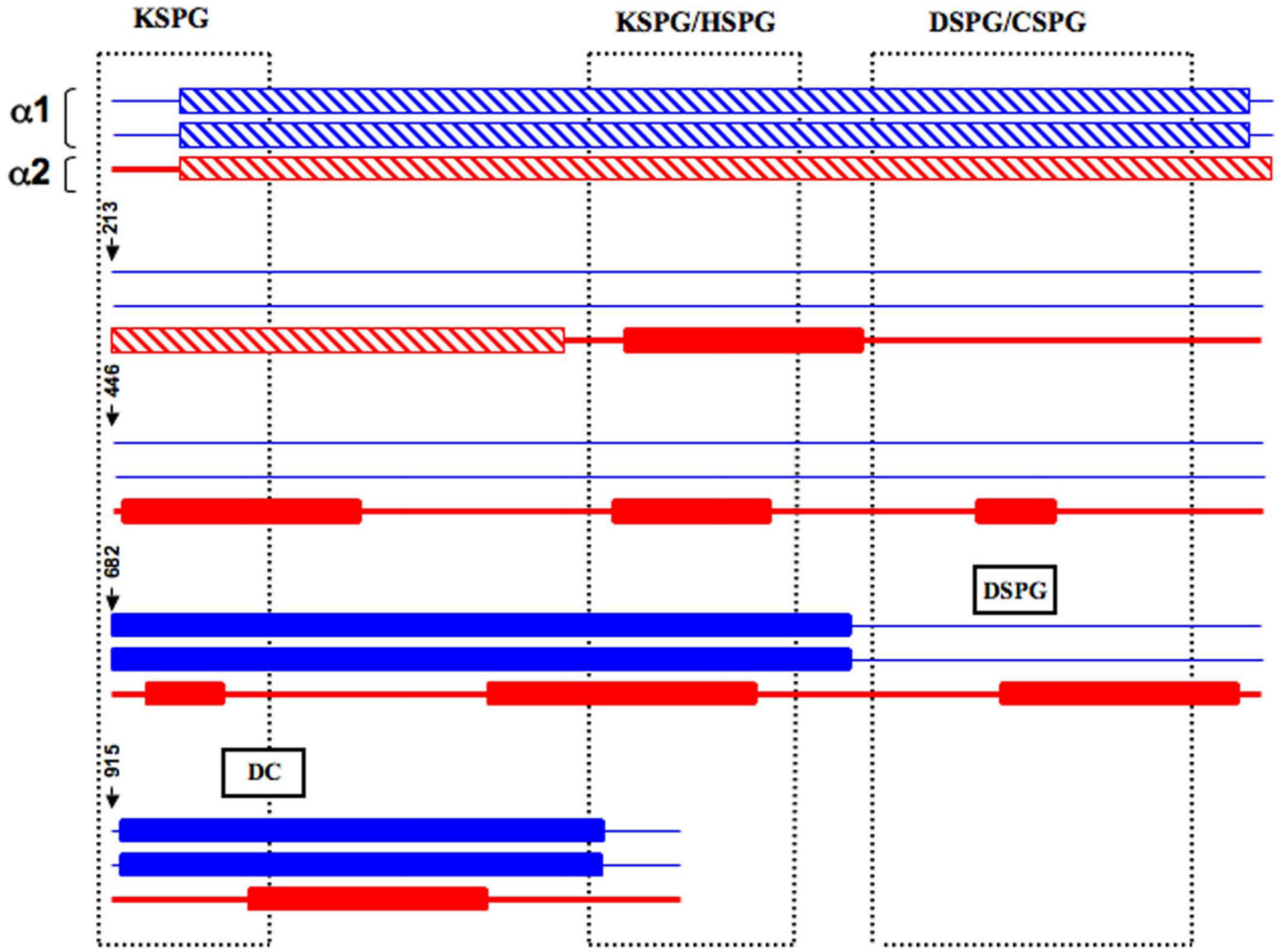

Figure 2. Distribution of lethal and non-lethal glycine substitutions causing OI along the type I collagen monomer and fibril

The α1(I) chains are represented by blue coloration and the α2(I) chain by red coloration. Rectangles with hatched lines indicate the regions with predominantly non-lethal mutations located in the first quarter of both α chains. Filled rectangles symbolize the lethal regions in each chain. In the α1(I) monomer, stretches of exclusively lethal mutations were identified in the Major Ligand Binding Regions (MLBR2 and 3). In α2(I), lethal mutations were clustered in eight regions along the chain. The vertical boxes defined by dots represent the binding regions in the type I collagen fibril for keratin (KSPG), heparan (HSPG), dermatan (DSPG) and chondroitin sulphate (CSPG) proteoglycans. The monomer-binding sites for DSPG and decorin core protein overlap α2(I) lethal clusters. There is substantial alignment of the α2(I) lethal clusters and the proteoglycan binding sites on the fibril.

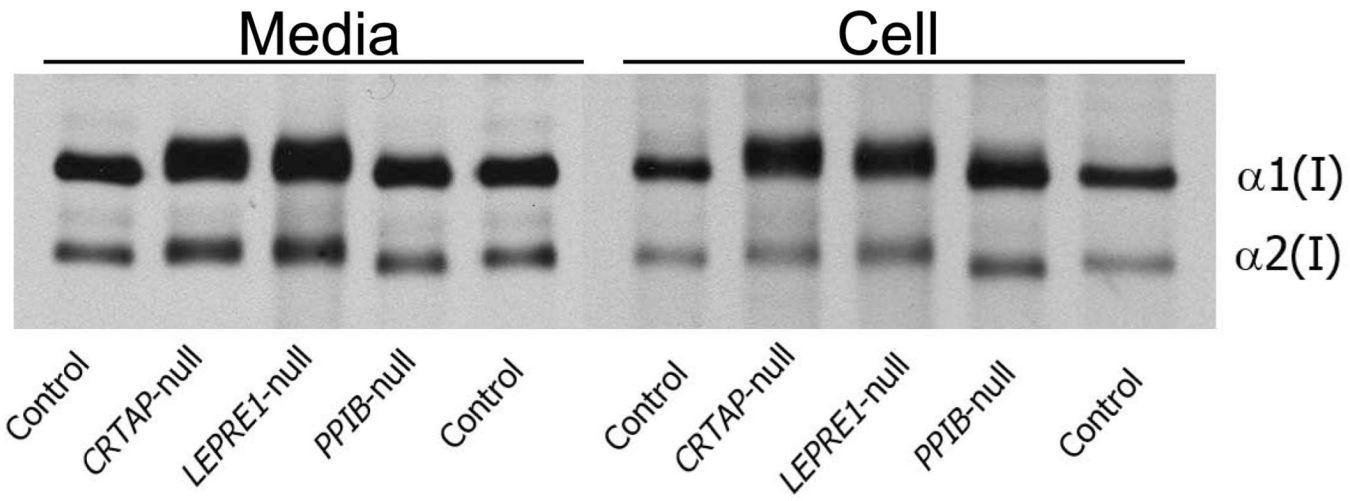

Figure 3. Electrophoretic analysis of type I collagen synthesized by dermal fibroblasts with mutations in genes coding for collagen 3-hydroxylation complex components

Most mutations in *CRTAP, LEPRE1* or *PPIB* cause decreased or absent protein due to nonsense mediated decay. Both [^{3}H]-proline-labelled collagen alpha chains are fully overmodified in media and cell layer from primary fibroblast cultures of OI patients with *CRTAP* or *LEPRE1* null mutations, indicating delayed folding of the collagen helix. However, in the fibroblasts of siblings with a mutated *PPIB* start codon, the secreted α chains have normal electrophoretic migration and collagen in the cell layer fraction has minimal backstreaking of α1(I).

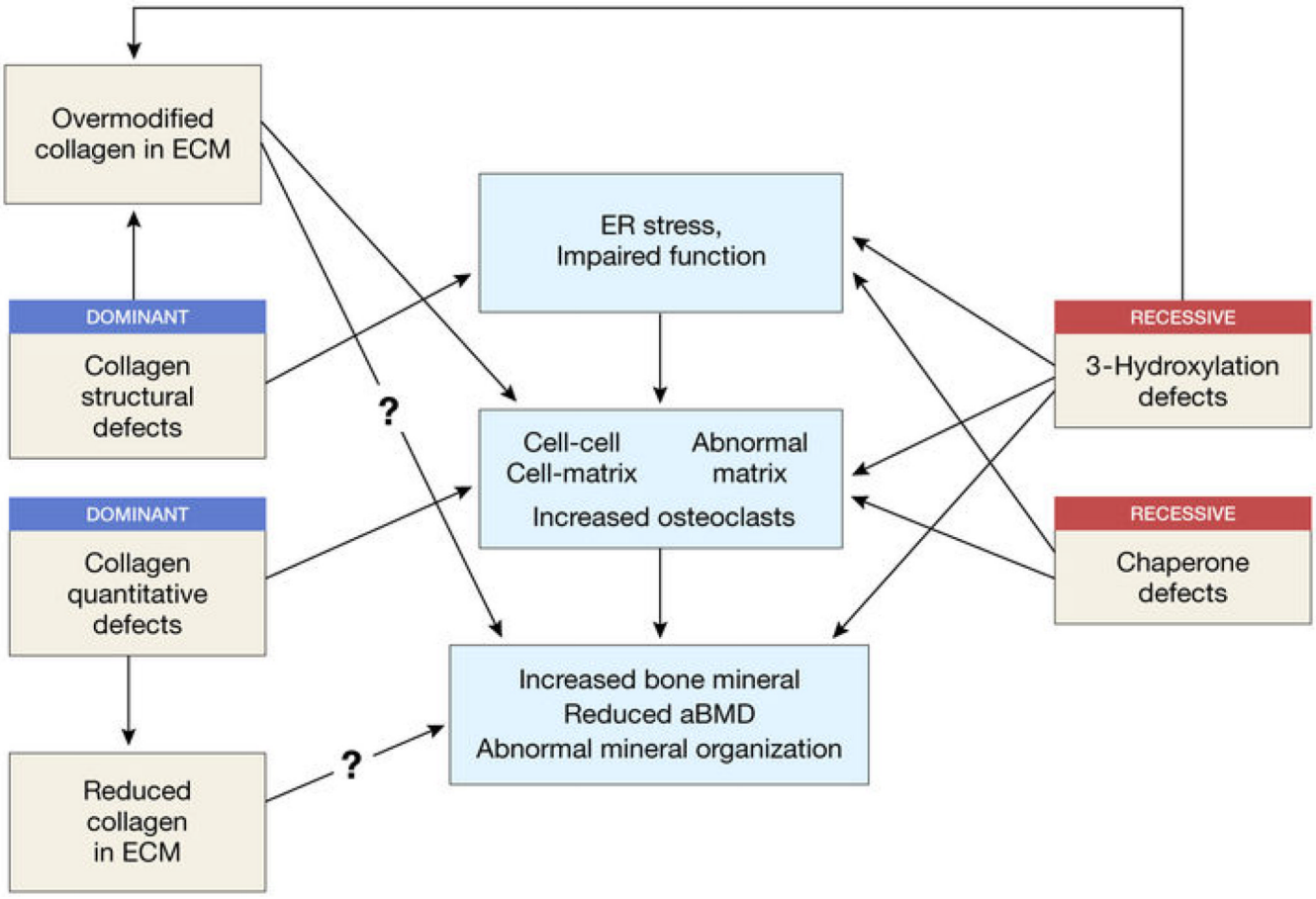

Figure 4. Relationship between dominant and recessive forms of OI

Boxes in the left and right columns identify features of dominant and recessive OI, respectively. The central column of boxes list mechanisms which may be shared by both sets of mutations.

Table 1

OI Nosology

	OI Type	Inheritance	Phenotype	Gene Defect
Classical Sillence Types	I	AD	Mild	Null *COL1A1* allele
	II	AD	Lethal	*COL71A1/COL1A2*
	III	AD	Progressive Deforming	*COL1A1/COL1A2*
	IV	AD	Moderate	*COL1A1/COL1A2*
Unknown Etiology	V	AD	Distinctive Histology	Unknown
	VI	AR?	Mineralization Defect	Unknown
3-Hydroxylation Defects	VII	AR	Severe (Hypomorphic) Lethal (Null)	*CRTAP*
	VIII	AR	Severe to Lethal	*LEPRE1* (P3H1)
	IX	AR	Moderate to Severe	*PPIB* (CyPB)
Chaperone Defects	X	AR	Severe to Lethal	*SERPINH1* (HSP47)
	XI	AR	Progressive Deforming Bruck Syndrome?	*FKBP10* (FKBP65)
Unclassified OI-like or Collagen-based Disorders				
Bruck Syndrome type 2		AR	Joint Contractures	*PLOD2*
Caffey Disease		AD	Cortical Hyperostosis	*COL1A1*
Osteoblast Maturation Defects		AR	Moderate	*SP7* (Osterix)

Table 2

OI Murine Models

Mouse	Gene defect	Protein defect	Transmission	Bone phenotype	OI Type	Ref
Mov 13 $^{+/-}$	M-MuLV insertion in endogenous *Col1a1* intron 1	50% synthesis of normal proα1(I)	AD	Reduced ductility and bone strength, increased tissue porosity and altered collagen organization	Type I	213
Mov 13 $^{-/-}$		Lack of proα1(I)	AR	Lethal in mid-stage gestation due to rupture of major blood vessels	Type II	51
Transgenic	Exogenous mutant murine *Col1a1* cDNA	Gly859Cys	AD	Deformed bones, poor mineralization and underdevelopment of the skeleton	Type II	214
Transgenic	Human *COL1A1* minigene	Human proα1(I) lacking 41 internal exons	AD	Moderate to severe bone phenotype depending on transgene expression level. Multiple fractures, short femurs, reduced mineral and collagen content	Type II–IV	215
Oim $^{-/-}$	Naturally occurring *Col1a2* c.3983delG	Lack of proα2(I) in collagen I and synthesis of $[\alpha 1(I)]_3$ homotrimers	AR	Skeletal fractures, limb deformities, generalized osteopenia, small body size and reduced bone mineral density. Increased osteoclast activity.	Type III-like	52
Brtl $^{+/-}$	Knock-in *Col1a1* c.1546G>T	Gly349Cys	AD	30% perinatal lethality, reduced body size, flared thorax, rib fractures, long bone deformity, bone fragility, and reduced bone mineral density. Increased bone turnover due to increased osteoclast precursors and reduced osteoblast activity.	Type IV	53
Brtl $^{-/-}$			AR	Absence of perinatal lethality, body size intermediate between Brtl$^{+/-}$ and WT, no rib fractures, no flared thorax, normal bone mineral density.	Mild Type IV (near normal)	76
Aga2 $^{+/-}$	ENU induced *Col1a1* IVS50-2T>A c.4216-2T>A	Frameshift of last 48 amino acids and addition of 90 amino acids beyond stop codon	AD	Severe bone phenotype: early lethality, multiple fractures, and reduced bone mass. Disturbed osteoblast function.	Type III	54
G610C OI (Amish)	Knock-in *Col1a2* c.2098G>T	Gly610Cys	AD	Moderately severe phenotype affected by genetic background. Reduced body size, reduced bone mineral density and bone strength.	Type IV	26
Crtap$^{-/-}$	Knock-out of *Crtap* gene	Absence of CRTAP protein	AR	Moderate phenotype: growth delay, skeletal deformity, kyphosis, and reduced bone mineral density.	Type VII	3
P3H1 null	Knock-out of *Lepre1* gene	Absence of P3H1 protein	AR	Moderate phenotype: reduced body size, and reduced bone mineral density.	Type VIII	105
Ppib$^{-/-}$	Knock-out of *Ppib* gene	Absence of CyPB protein	AR	Severe phenotype: premature death, reduced body size, bone deformity, and reduced bone mineral density.	Type IX	110
Osx $^{-/-}$	Knock-out of *Sp7* gene	Absence of Osterix protein	AR	Lethal phenotype: severe bone deformity, and absence of bone mineralization.	OI-like	127, 216

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