Bone material properties in osteogenesis imperfecta

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Abstract
Osteogenesis imperfecta entrains changes at every level in bone tissue, from the disorganisation of the collagen molecules and mineral platelets within and between collagen fibrils, to the macro-architecture of the whole skeleton. Investigations using an array of sophisticated instruments at multiple scale levels have now determined many aspects of the effect of the disease on the material properties of bone tissue.
The brittle nature of bone in osteogenesis imperfecta reflects both increased bone mineralisation density – the quantity of mineral in relation to the quantity of matrix within a specific bone volume – and altered matrix-matrix and matrix mineral interactions. Contributions to fracture resistance at multiple scale lengths are discussed, comparing normal and brittle bone.
Integrating the available information provides both a better understanding of the effect of current approaches to treatment – largely improved architecture and possibly some macro-scale toughening - and indicates potential opportunities for alternative strategies that can influence fracture resistance at longer length scales.

Introduction and background

Multiple genetic mechanisms give rise to bone fragility (Table 1). Osteogenesis imperfecta (OI) is in danger of becoming a catch-all term for early-onset bone fragility. The key feature that discriminates OI from other early-onset bone fragility conditions is the hyper-mineralisation of the bone material itself – hence the alternative name "brittle bone disease" – although increased mineralisation density is not the only contributor to brittleness. Mutations in the type I collagen synthesis and processing pathways, along with defects in accessory proteins such as PEDF (type VI OI) and those associated with reduced type I collagen production (type V OI) all share the bone material hyper-mineralisation phenotype.
This review of bone material properties in OI describes the multiscale abnormalities from a molecular level up and also assesses their contribution to the brittle phenotype in both animal model systems and, where available, in human tissue.

Normal bone tissue; molecular to fibrillar scale

In the healthy skeleton, mineral accounts for 65-70% of bone mass, water around 10% with the remainder being matrix proteins, principally type 1 collagen, and a small amount of citrate (approximately 2%). (1) Each type 1 collagen molecule comprises two type 1 collagen α1 and one type 1 collagen α2 chains. The internal core of each trimer is hydrophobic.
Each individual heterotrimeric collagen molecule (also called tropocollagen) is approximately 1.5nm wide and 300nm long. (2) The individual collagen molecules self-assemble(3) into a structure that is clearly recognisable by electron microscopy (EM), initially held together by non-covalent interactions. (4) The mature structure is formed of overlapping and cross-linked type I collagen molecules in a quasi-hexagonal array of groups of five collagen molecules, (5) staggered with respect to one another by approximately a quarter of their length (actual overlap on average 67-68 nm), successively longitudinally aligned but separated from the next successive molecule by a "gap space". The arrangement results in the banded appearance under EM that is well

documented, the lighter bands corresponding to the "gap space" of 36 nm. These groups of 5 collagen molecules might reasonably regarded as "microfibrils". Collagen molecules are joined to one another by two types of crosslink, enzymatic and non-enzymatic. Enzymatic crosslinks are the result of the activity of lysyl oxidase and lysyl hydroxylase. (6) Initially such crosslinks are divalent. With increasing maturity some become trivalent; (6) these include the pyridinoline and pyrrole crosslinks that when cleaved during collagen degradation can be measured as biomarkers reflecting bone resorption. The non-enzymatic crosslinks are the result of apparently spontaneous reactions between sugars such as pentosidine and exposed amino acid residues. (7) The number of non-enzymatic crosslinks increases with age. (7) Karim also observed variation in the accumulation of non-enzymatic crosslinks between cancellous and cortical bone. (8) Enzymatic crosslinks are usually regarded as contributing positively to bone strength; non-enzymatic crosslinks, by contrast, are widely held to adversely affect bone material properties. (9)
The collagen molecules within each microfibril group coil gradually around each other, with specific kinks that create pockets and potential binding sites for other matrix proteins (5) as well as interactions with cells through integrin-binding. (10) [INSERT Figure 1 here; use ORGEL doi_10.1073_pnas.0502718103 - figure 3E]
The non-collagenous proteins within bone have been suggested to have a range of functions, from the formation of collagen fibrils and initiation of mineral platelet formation, to mineral maturation and collagen cross-linking. (11) In addition, osteopontin and osteocalcin are proposed to have roles in energy dissipation at the microfibrillar level through the formation of dilatational voids (12) and, additionally for osteopontin, through the forming, loss and reforming of sacrificial bonds with divalent cations such as calcium. (13, 14)
The individual microfibril groups are separated from their near neighbours by so-called "gap channels". (15) Water molecules both within and between the microfibrils contribute to the overall stability of the whole structure through the formation of multiple hydrogen bonds. (16) Individual fibrils are 80-100nm in diameter; fibril length is indeterminate. (17)

Molecular changes in Osteogenesis Imperfecta
The vast majority of OI (more than 85% of cases) is caused by mutations in one of the two genes encoding the type I collagen molecules. (18) In broad terms, for "classical OI" as described by Sillence, (19) null alleles (i.e. absent or non-functional gene product) in the COL1A1 gene, resulting in reduced amounts of normal α1 chain give rise to a milder phenotype; missense mutations (point mutation giving rise to altered amino acid sequence) in either COL1A1 or COL1A2 give rise to more severe phenotypes, including lethal disease. No clear skeletal phenotype has been identified arising from a heterozygous COL1A2 null allele. Both collagen genes code for a repetitive, staggered $(Gly\text{-}X\text{-}Y)_n$ amino acid sequence; the small glycine amino acid is key to the ability of the heterotrimer to coil tightly, with the glycine side chain folded into the centre of the triple helix. Most mutations causing more severe OI result from the substitution of glycine by another amino acid that disrupts tight coiling of the triple helix, delaying the process and allowing additional post-translational modification of the collagen molecules to take place, distorting its 3-D structure. The heterotrimeric collagen protein secreted eventually into the matrix is thus structurally significantly different to normal. The tropocollagen molecules nevertheless self-assemble to

create fibrils; the severity of the bone disease that results seems likely to be a consequence both of the alterations in the 3-dimensional fibrillar structure and also the specific siting of the mutation. In particular, two "major ligand binding regions" in tropocollagen are identified as sites of interaction with other matrix proteins that, when disrupted as a result of mutation in the type I collagen genes, often have a severe or lethal OI phenotype. (20)

Fibrillar changes in OI

Much of the exploration of fibrillar mechanics in OI has been undertaken using the *oim* mouse model, which carries a mutation in the cola-2 gene. (21) In *oim-/-* mice, homozygous for the mutation, the altered α2 chain of the type 1 collagen heterotrimer is unable to associate with the α1 chains and the type 1 collagen molecule is thus a homotrimer of α1 chains. The mice have a moderately severe OI phenotype; they are smaller at birth and grow less well than wild type littermates, have low bone mass, fracture with minimal trauma, and develop bone deformities. At a tissue level, cortical thinning and reductions in trabecular number are seen on light microscopy, along with a lack of lamellar architecture and increased osteocyte density. (21)

At an ultrastructural level, collagen content is reduced by 20% (22) and a reduction in the size of the D-space within *oim-/-* collagen fibrils of approximately 1% vs wild type across a range of D-space sizes from 60-70nm has been observed using atomic force microscopy. (23) The individual tropocollagen molecules are more prone to kinking (24) and there is more water associated with the tropocollagen and between the microfibrils (25). The bone matrix compressive elastic modulus of *oim-/-* tibia by nanoindentation was reduced by 20% compared to wild type; in addition, resistance to plastic deformation was 8% higher, implying reduced toughness. (26) The overall picture that emerges from the mouse model reports is of a disorganised bone matrix, more loosely woven, less capable of responding to normal mechanical inputs, and less able to absorb and dissipate energy that might lead to fracture.

<u>Mineralisation of bone matrix</u>

Collagen in sites outside the skeleton and teeth should not mineralise. This implies either the presence of one or more inhibitors of mineralisation in non-skeletal sites, or the presence of factors that promote mineralization in bone, or both. In remodeling sites, mineralisation of osteoid is linked both to local mineral concentrations and the incorporation of osteoblasts into the matrix (27)– clearly, osteoblasts are not present in non-mineralising tissues. During endochondral ossification occurring with growth or fracture repair, matrix vesicles create mineral crystals within themselves that then seed into the local environment as they grow and rupture the vesicle walls. (28) The incorporation of mineral into tissue in endochondral ossification begins with the calcification of the cartilaginous matrix laid down by hypertrophic chondrocytes, with longitudinal septae more heavily calcified than transverse septae. The invasion of endothelial cells precedes the ingress of both osteoclasts, which remove the transverse septae, and osteoblasts, which deposit bone onto the remaining longitudinal calcified cartilage cores to form the bony trabeculae of the primary spongiosa. (29)

Remodelling of the primary spongiosa takes place over a defined space in close proximity to the growth plate; (30, 31) the precise mechanisms controlling remodelling here are unclear. In remodelling sites in the rest of the skeleton, a

variety of inter-linked mechanisms have been identified that regulate the site and degree of mineralisation activity, including the presence of mineralisation inhibitors such as pyrophosphate (32) and osteopontin (33), and the potential mineral nucleation initiator bone sialoprotein which may act in concert with alkaline phosphatase. (34) Multiple other factors may also have a role, recently reviewed in Staines et al. (35) In both endochondral ossification and matrix mineralisation, however, it seems likely that an initial phase of amorphous mineral deposition precedes the formation of more highly organised mineral. (36, 37)

Bone mineralisation in OI

In most cases of OI, mineralisation processes seem to be in place and normally functioning. Two exceptions, however, may be the types V and VI OI initially identified as having distinct bone histological appearances (type V mesh-like under polarised light; type VI osteomalacic) at the level of light microscopy. (38, 39) Type V infants initially display altered metaphyseal modelling suggestive of a delay in endochondral ossification, (40) with a subsequent increase in metaphyseal density, but no alteration in mineralisation rate or extent of remodelling sites in the trabecular bone of transiliac bone biopsies. (38) By contrast, although there is a clear mineralisation defect at remodelling sites in type VI patients, (39) there is no apparent defect in endochondral ossification. Despite these appearances at a light microscopic level, primary osteoblasts from type V OI patients demonstrate increased mineralisation in culture,(41) and bone tissue from type VI patients have been shown to have hyper-mineralised bone tissue by back scattered electron imaging. (42) These findings suggest that the final degree of mineralisation at small length scales is not directly dependent on the rate of mineralisation observed at larger scale lengths. The genetic origins of these forms – an activating mutation in IFITM5/BRIL for type V, (43, 44) and loss of function in SERPINF1 (encoding pigment epithelium derived factor, PEDF) for type VI (45, 46) – suggest no direct connection with collagen synthesis or processing. However, reduced type 1 collagen production has been reported recently in primary cultured osteoblasts from type V patients, (41) and PEDF binds to the secreted collagen heterotrimer at two distinct sites. (47) IFITM5/BRIL may have a role in PEDF regulation; osteoblast PEDF production was reduced and typical type VI histology seen in a patient with a novel inactivating mutation in IFITM5/BRIL. (48)

<u>Mineral platelets</u>

There is general agreement that the mineral platelet's long axis is aligned with the long axis of the collagen fibrils (49) and that some staggering of the platelets occurs. (50) There is, however, a lack of consensus concerning the exact spatial relationships of the collagen molecules with the mineral platelets that contribute to the material properties of bone.

This may reflect the variety of techniques and preparation methods used, the hydration state of the samples (51) and the difficulty in creating model systems that allow accurate recapitulation of the microfibrillar-mineral platelet relationships in vivo. (50) The platelets appear to grow and mature over time; hence older bone has a higher mineralisation density. The platelets extend beyond the individual fibrils into the gap channels; estimates of the distribution of mineral between the intra and extra-fibrillar regions vary, but typically are around one third inside, two thirds outside. (15)

The mineral platelets are composed of hydroxyapatite; estimates of their size vary, (52) in part as a function of their maturity but also again reflecting the methodologies used to provide the estimates and the conditions in which such estimates are made. A recent study using ion-milled cryogenic femoral bone found consistent platelet sizes of 5nm thick, 70nm wide and >200nm long. (53) The platelets may be arranged in stacks, (1) so that several are placed side by side in a particular gap channel, or extending between fibrils across a series of gap channels. (15) Whilst their long axes align with that of the adjacent collagen fibril, the planes of stacks around and between fibrils may not be similar; there is some evidence that they vary significantly across local areas. (54)
In addition to the highly ordered mineral platelet arrays/stacks, there is a disorganised hydrogen-phosphate phase, with both the ordered and disordered mineral elements being strongly associated with both citrate and water. (1) A recent paper suggests that citrate acts to maintain the ordered platelet parallel arrays and also holds non-platelet hydrogen-phosphate in a relatively immobile, highly hydrated phase between the platelets, thus maintaining a degree of disorder and preventing crystals increasing in size or thickness (which would result in increased bone fragility). (1) This arrangement could be conceptualised as a series of stacked multilayer sandwiches (see Figure 2c). Water may also play an important role in the interactions between matrix and mineral. (55) Layered water provides multiple hydrogen bonds at interfaces between mineral platelets, and also allows interactions between platelets and fibrils. (56) Molecular dynamics simulations indicate that water can significantly alter the interactions between platelets and fibrils during loading. (57)

Mineral platelets in OI
Investigations of mineral platelet size and orientation, and the effects of mineral on intrinsic material properties in OI have been undertaken in both human tissue and in mouse models. There is general agreement that mineral platelet size is reduced; (58) that there are more, thinner, platelets; (59) that the composition of the platelets is altered in terms of the ratio of phosphate to carbonate; (23) and that although the alignment of the platelets is generally concordant with the fibrils, there is less overall homogeneity of alignment of platelets within the tissue, (58) likely reflecting matrix disorganisation. Overall, tissue mineralisation density is increased in OI, more so in more severely affected individuals, (60) and even more so specifically in those with c-propeptide cleavage site mutations, (61) BMP-1 mutations (62) and in OI type VI. (42) The roles of water and citrate have not been studied in OI bone; simulation studies of mineralised OI bone have not been undertaken as yet.
The overall increase in tissue mineralisation density is likely to be a major contributor to the brittle nature of OI bone tissue. It is possible that the observed changes listed above reflect increased size of fibrillar gap channels, or possibly the orientation of stacks of platelets across the gap channels, in a manner similar to that suggested by Alexander (15) (see also Figure 2).

<u>Energy dissipation in bone and fracture toughening mechanisms</u>
Fractures occur when the force acting at a particular site or sites in bone exceed the capacity of the bone to dissipate the related energy without suffering damage that results in a substantial loss of structural integrity. Testing bone material properties for factors related to fracture resistance is not straightforward – elegantly reviewed in Wagermaier et al. (63)

Intrinsic material properties that contribute to increased fracture resistance include those that promote plasticity and toughness i.e. ductility, energy absorption and dissipation. (64, 65) This requires cooperative deformation of mineral and matrix and is accomplished through multiple mechanisms including inter and intrafibrillary crosslink breakages, (6) shearing between mineral platelets, (66) sliding of mineral platelets relative to the fibrils, fibril deformation and platelet deformation. (67) Fritsch and colleagues have proposed layered water-induced ductile sliding of minerals followed by rupture of collagen crosslinks based on a continuum micromechanics model, upscaled for elastic properties and then applied to a multi-scale representation of bone materials. (68) A multiscale model summarising toughening mechanisms has been proposed by Ritchie. (65)

Toughness is a measure of energy dissipation and cannot be easily estimated in an anisotropic material. Both strength and toughness are influenced by inhomogeneity (30) and interface properties, (64) which can be highly localised. Fracture resistance extends beyond the intrinsic material properties of bone to encompass all levels of scale up to the whole bone. (17) Whilst stiffness can be considered as an averaged property across a material, strength and toughness cannot. Strength is affected by the "weakest link" problem. (63) In addition, scale matters in strength testing – small defects can result in large decreases in strength; as scale reduces, the number and size of defects possible decreases and sample strength increases as a consequence.

At scales beyond those at which material properties are assessed, extrinsic biomechanical factors act to shield an existing crack from forces that would extend that crack – usually on a scale of 10-1000 microns, i.e. osteonal level. (69) Fibre orientation can affect crack propagation, and the successive alteration in fibre orientation within the concentric lamellae of an osteon is thought to reflect such an adaptation in bone. (70, 71) Osteonal borders, defined by cement lines, act as barriers to crack propagation, deflecting cracks into more tortuous paths. (72) Cement lines are also found around remodeled bone packets. Mechanisms have to be able to operate in high strain situations i.e. real life rather than the laboratory where the strain rates are often very low. Experimental evidence suggests that the crack-shielding mechanisms are more effective at preventing crack propagation when they are dealing with the lower "every day" strains, (73) rather than the higher strains more often associated with a fall or moderate degree of trauma likely to result in fracture in real life. With higher strains, cracks are seen to be straighter, crossing osteons rather than deflecting around them, with less energy dissipation. (74)

Accumulated microdamage may contribute to increased fracture risk (75) if appropriate remodelling of damaged areas is not undertaken.

Intrinsic mechanisms of fracture resistance are also affected by strain rate; higher strain rates are associated with increased material stiffness and reduced ductility (post-yield plastic deformation). Thus at abnormally high strain rates, bone behaves as if it is more "brittle". (74)

Many studies have reported the occurrence of microcracks "ahead" of a propagating fracture (76)– the production of these microcracks consumes and dissipates energy that might otherwise be used to further propagate the crack. Bridging across cracks also increases bone toughness, removing some of the load from the propagating crack. (77, 78) Similar results have been shown in both C57B6 and CH3 mice using submicron resolution synchrotron imaging. (79)

Fracture resistance is difficult to measure in vivo. A novel approach using microindentation has been developed that provides information on the ability of bone to resist a localised force – effectively, the resistance of separation of mineralised fibrils - at an intermediate scale. (80) The output for the in vivo, hand-held device (Osteoprobe™) is given as "Bone Material Strength" and is reported to be reduced independently of bone mineral density in patients with fragility fractures. (81) Multiple outputs are provided by the ex vivo benchtop device (BioDent™); "indentation distance" and "indentation distance increase" indicate the extent to which the probe penetrates initially and further after 10 cycles of indentation into a bone sample and are thought to reflect fracture resistance. (82)

Fracture resistance in OI bone

When a long OI bone breaks, the fracture line tends to be transverse, suggesting that some of the mechanisms that normally promote energy dissipation are abrogated. At lower scale lengths in OI, multiple factors likely interact to reduce the ability of the disorganised matrix to effectively absorb or dissipate fracture-causing energy. In the *oim* mouse, these include fewer enzymatic and more non-enzymatic crosslinks with associated increased mineralisation density and consequent reduced material elasticity and toughness, (26, 83) as well as smaller, more densely packed mineral platelets with disordered orientation. (26) These features likely impact on the ability of bone to dissipate energy through sacrificial bond breakage, sliding of platelets relative to fibrils and shearing between platelets.

At longer scale lengths, *oim-/-* mice demonstrate reduced stable crack extension, crack-initiation toughness and crack-growth toughness with increasing severity of OI and amounts of woven bone; in addition, increased cortical vascular porosity in *oim* reduces stable crack growth. (83, 84) Although excessive woven bone is not a clear feature in human OI bone biopsies, increased cortical porosity is, and likely also contributes to increased fracture risk.(85-88) Figure 2 summarises the factors contributing to bone fragility at the different scale lengths.

In the *oim-/-* mouse, use of microindentation showed an increase in initial indentation distance and total indentation distance in one study, (23) but no relationship of microindentation outcomes was found with stress intensity fracture toughness in another. (89) Microindentation has not been applied in vivo in OI as yet.

<u>Effects of current interventions in OI</u>

The therapeutic options currently employed or under investigation in the treatment of OI in humans either reduce bone remodelling (bisphosphonates, (90, 91) denosumab (92, 93)) or increase bone formation (PTH (94), anti-sclerostin antibodies (95)). None of these interventions have been found to affect tissue material properties in OI bone.

However, improved femoral geometry and biomechanical strength in the *brtl* OI mouse was offset by reduced predicted (not measured) elastic modulus following 12 weeks of alendronate treatment. (96)

Tissue mineralisation density is not affected by pamidronate therapy in children with OI and material properties are not worsened. (97) Two groups have found an increase in non-enzymatic cross-linking following 3 years' bisphosphonate treatment in healthy beagles. (98, 99) In the earlier study, no clear changes in

intrinsic material properties were shown; the most recent work found reduced toughness (post-yield plastic deformation) in alendronate-treated bone, in association with an increase also in the phosphate to carbonate ratio of mineral crystals reflecting slower bone remodelling and increased mineral crystal maturity.

Thus for the most commonly used intervention, bisphosphonates, there is evidence for improved extrinsic biomechanical properties consequent on macro-scale architectural change (increased bone width, increased cortical thickness, reduced cortical porosity, retention of new trabeculae in growing bone(86)) which in mild OI may result in reduced fracture risk. (90, 100) There is no evidence, however, that bisphosphonates alter bone material properties in such a way as to further reduce fracture risk. It follows that there is a limit to what current treatment can achieve regarding fracture risk reduction in terms of off-setting increased bone mass and improved macro-architecture against the poor material properties that characterise OI bone.

Anti-TGFβ antibody treatment of two moderate-severe mouse models of OI (*crtap-/-* and *Col1a2*$^{tm1.1Mcbr}$ mice; not *oim-/-)* has been reported to restore bone architecture and reduce hyperosteocytosis, but did not affect bone material properties. (101)

In terms of future therapeutic interventions, new approaches to improving the intrinsic material properties of bone would appear attractive but may not be practicable given the underlying issue of matrix disorganisation. Increasing the proportion of normal collagen within the matrix would require implementation of cellular or genetic approaches. Murine (*oim-/-*) studies suggest that mesenchymal stem cells (MSCs) can engraft and produce normal collagen ameliorating the OI phenotype and reducing bone brittleness, (102-104) and human chorionic cells transplanted into newborn *oim-/-* mice also improved the clinical phenotype. (105) Previous human studies of bone marrow transplantation shon little mesenchymal lineage engraftment and failed to deliver clear benefit (106, 107) but the recent report of fetal stem cell infusion both before and after delivery in a child thought to have type IV OI was encouraging. (108)

Ex vivo manipulation of cells ex vivo and then their reintroduction has been widely discussed following the recent successful treatment of a child with leukaemia. In OI, an approach of this type could substantially impact on tissue phenotype. (109) An alternative approach using siRNAs to knock down mutant alleles in MSCs ex vivo has been shown to reduce mutant collagen production by 42% in fibroblasts from the Brtl mouse model of OI. (110)

<u>Summary</u>

The characteristic material feature of bone in OI is its brittleness, and this helps differentiates OI from other disorders associated with early onset bone fragility. The brittleness is contributed to both by increased mineralisation density due to smaller more densely packed mineral platelets and increased numbers of non-enzymatic crosslinks. The bone matrix is looser, allowing more space between collagen molecules and fibrils for other matrix proteins as well as the mineral platelets. At longer scale lengths, the contribution of abnormal architecture to fragility is substantial – increased cortical porosity, thinner cortices and narrower bones all contribute to an increase in fracture risk. Current therapies really address only these macro-architectural abnormalities, although there is limited evidence that bisphosphonates may improve toughening. Until, however,

there are therapies that can address the abnormalities at shorter scale lengths, it seems unlikely that there will be substantial improvements in outcome for those with more severe forms of OI. Such therapies are likely to be those that allow the production of larger quantities of normal collagen, and likely require cell or gene-targeting approaches.

Table 1. Early onset bone fragility syndromes

Collagen related

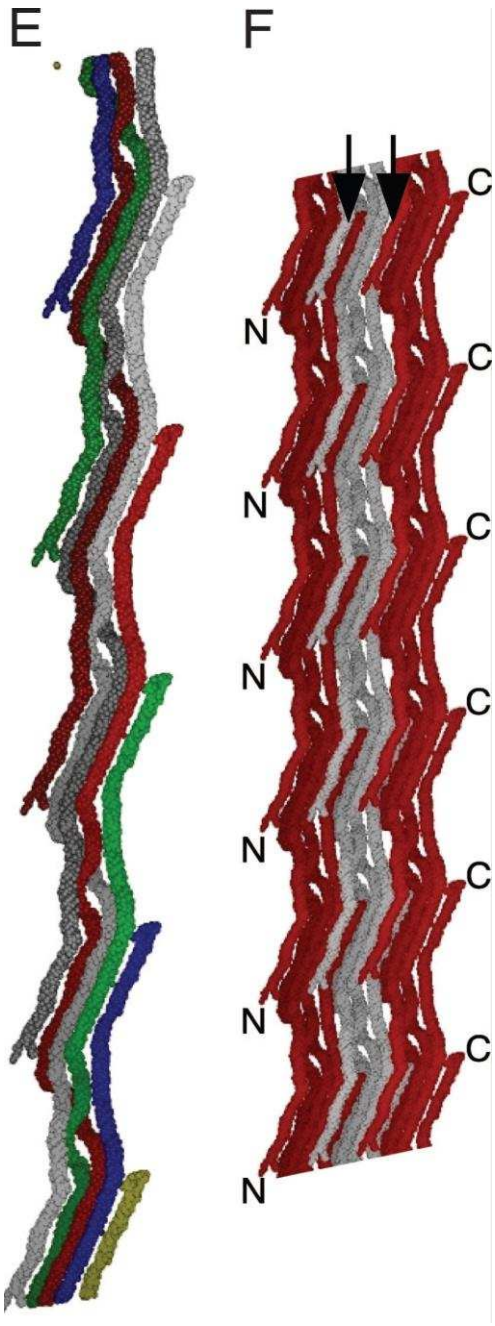
Gene	Protein	Hyper-mineralised?	Phenotype(s)
			Collagen molecule
COL1A1	Type 1 collagen α1 chain	Yes	Mild-lethal OI (18) High bone mass in C-propeptide cleavage site defects (61) Caffey disease with defect at p.Arg1014Cys (111)
COL1A2	Type 1 collagen α2 chain	Yes	Mild-lethal OI (18) High bone mass in C-propeptide cleavage site defects (61)
			Collagen folding
PPIB	Cyclophilin B	Yes	Moderate-lethal OI (112)
LEPRE1	Prolyl-3-hydroxylase	Yes	Severe-lethal OI (113)
CRTAP	Cartilage associated protein	Yes	Severe-lethal OI (114, 115); Cole-Carpenter features (116)
			Collagen stability
FKBP10	FKBP65; 65kD FK506-binding protein	?	Moderate-severe OI; Bruck syndrome (OI with contractures); (117) Kuskokwim syndrome (contractures alone) (118)
PLOD2	Lysyl hydroxylase 2	?	Bruck syndrome (119)
SERPINH1	Heat Shock Protein 47	?	Severe OI, pyloric stenosis, skin bullae, renal stones (120)
SPARC	Secreted protein, acidic, cysteine-rich; osteonectin	Yes	Notable sarcopenia (121)
			Collagen processing/cleavage
BMP1	Bone morphogenetic protein 1; tolloid	Yes	High bone mass, hyperosteoidosis (122)

Osteoblast lineage/function

Gene	Protein	Hyper-mineralised?	phenotype
			Wnt-signalling pathway
LRP5/6	Lipoprotein receptor-related protein 5/6	No	Homozygous – osteoporosis pseudoglioma syndrome; (123) Heterozygous – osteoporosis and/or vitreoretinopathy (124)
WNT1	Wingless-type MMTV integration site family, member 1	No	Homozygous – severe OI; some have brain malformation; autism, learning difficulties in some. (125) Heterozygous – early onset osteoporosis, normal growth (126)
			Osteocyte dysfunction
PLS3	Plastin 3	?	X-linked early onset severe osteoporosis without other OI features (126)
			Mineralisation regulation
SERPINF1	Pigment epithelium derived factor	Yes	Slowly progressively worsening OI; osteoid mineralization defect (no endochondral defect) (45)
IFITM5/ BRIL	Interferon-induced transmembrane protein 5, or, bone-restricted IFITM5-like	Yes	Severe OI; metaphyseal dysplasia and sclerosis, hypertrophic callus, interosseous membrane calcification. (43, 44, 127)
			Osteoblast lineage
SP7/OSX	Specificity Protein 7; Osterix	?	Typical OI features (128)
			ER-related
P4HB	Prolyl 4-hydroxylase; protein disulfide isomerase	?	Cole-Carpenter syndrome; craniosynostosis, ocular proptosis, hydrocephalus (129)
TMEM38B	Trimeric Intracellular Cation Channel Type B; TRIC-B	?	Severe osteopenia and limb fractures without vertebral fractures (130)
CREB3L1	Old Astrocyte Specifically Induced Substrate - OASIS	?	Severe OI; cardiac failure (131)
NBAS	Neuroblastoma Amplified Sequence	?	Early onset osteoporosis, recurrent acute liver failure, developmental delay (132)
SEC24D	Component of COPII complex	?	Cole-Carpenter syndrome; craniosynostosis, ocular

			proptosis, hydrocephalus (133)
			Linker enzyme deficiency
XYLT2	Xylosyltransferase II	?	Vertebral fractures, cataracts, heart defects (134)

Figure 1 – from Orgel - Use only parts E and F



Legend:-
Inter-relationships of tropocollagen molecules within an individual microfibril shown in E; continuous packing of microfibrils with N and C terminal ends labelled in F. Longitudinally compressed by a factor of 5. The arrows in F indicate where neighbouring microfibrils interdigitate.

Figure 2

See previous TIFF file labelled as figure 3

Legend

a. Macro scale –OI bone is narrower, cortices are thinner, there are fewer trabeculae; bone mass is reduced.
b. Sub-macro scale – increased cortical porosity in OI bone; a larger number of vascular channels as well as pores that coalesce and form larger voids.
c. Fibrillar level – fibrils are less consistent in size and shape in OI. The "weave" is looser, allowing more water and mineral between and within fibrils. Mineral platelets are smaller, thinner and more closely packed. Results in hypermineralisation and increased brittleness.
d. Molecular level – increased numbers of non-enzymatic crosslinks (green) reduce collagen molecules ability to absorb energy and increase bone stiffness further.

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