REVIEW OF LITERATURE

Bone formation, in embryonic and postnatal life is carried out by mesenchymal stem lineage (MSC) (66,67). MSC are pluripotent cells which differentiate into bone specific cells (68). Bone is a hard connective tissue whose structure and composition depicts an equilibrium of two major functions a) locomotion and protection to all soft organs (69) b) involvement in various metabolic pathways(70).In addition to that, bone has a niche for hematopoiesis (71) and role in immune metabolism (72). Microscopic studies suggested that bone is an inert organ but, bone histomorphometry showed that it is dynamic organ (68, 73).Classically bone consists of organic and inorganic component. About 60% of the bone is made up of inorganic component, 10% is water and the remaining is organic component.(74, 75). The inorganic component consists of $Ca_{10}[PO_4]_6[OH]_2$ (hydroxyapatite), that is made up of calcium phosphate. The organic component primarilycontains (98% by weight) of type I collagen and a range of NCPswhich have role in organization of the extracellular matrix,coordinating cell-matrix and mineral-matrix interaction and regulating the mineralization process and cells make up the remaining 2% of this component (74, 76).

2.1 Cellular Component of the bone

Bone is composed of four types of cells osteoblasts, bone lining cells, osteocytes and osteoclasts. Bone structure is maintained by bone cells through constant bone remodeling in which old bone is replaced by new bone and requiresbiochemical and mechanical signal (77). The Bone remodeling cycle is comprised of four phases: a) activation of osteoclast cells b) initiation of resorption, c) transition, d) bone formation (**Fig. 3)** (78). When bone formation and bone resorption are balanced, bone mass usually maintained. Remodeling process occurs due to close association of osteoblasts, bone lining cells, osteocytes and osteoclasts which together forms bone multicellular unit(11).

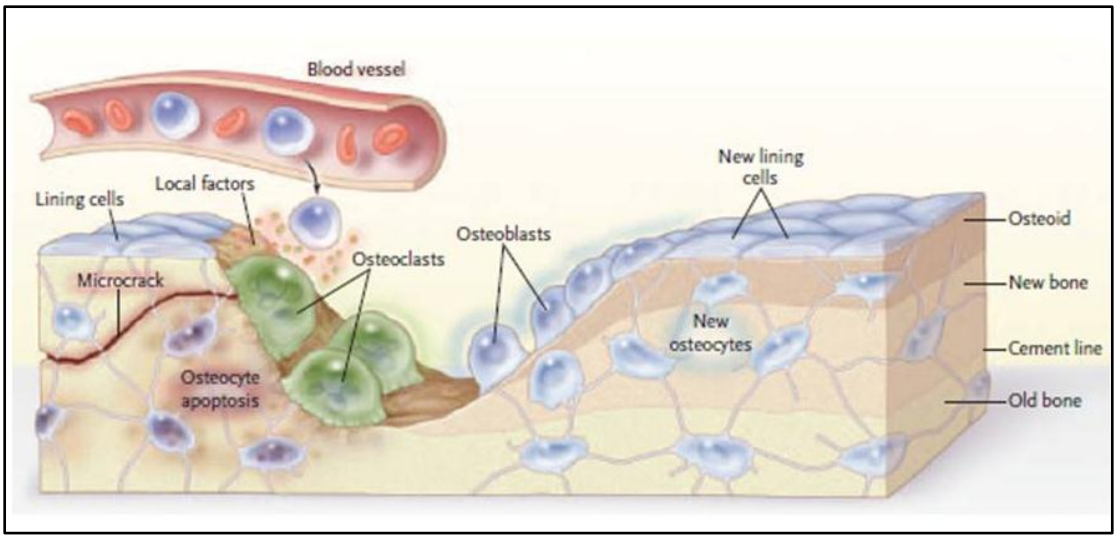

Fig. 3: The Remodeling cycle in the bone.
Source: Seeman et al.N Engl J Med. 2006 ;354(21):2250-61.

2.1.1 Bone cells

Bones cells regulate the 'bone remodeling'through a complex interplay between systemic hormones (Parathyroid hormone and calcitriol), mechanical stimuli, and locally produced cytokines (Transforming growth factor, TGF-β) and bone morphogenic protein growth factors (Insulin like growth factor, IGFs) and other mediators. Locally produced factors communicate with mechanical stimuli to regulate many of these activities and affect bone remodeling (79).

1) Osteoblasts

Pluripotent mesenchymal stem cell can differentiate into multiple types of cells like osteoblasts, adipocytes, chondrocytes, myoblast and fibroblasts(11).Osteoblast–lineage cellscirculate in the peripheral blood in relevant numbers(80). Osteoblasts are cuboidal in shape and located on the bone surface. Osteoblasts produces protein matrix (osteoid) which is formed of type I collagen and various NCPs. This forms an outline for mineralization and creation of mature bone (66).

2) Bone lining cells

These are quiescent flat-shaped osteoblast cells whichare present on the bone surfaces, where neither bone formation or bone resorption occurs (12). These cells have a thin and flat nuclear silhouette and their cytoplasm extend along the bone

surface and show a small number of cytoplasmic organelles such as RER and Golgi body (81).These cells participate in bone remodeling by entering into the lacuna and cleaning its bottom from bone matrix left overs. This allow subsequent deposition of first layer of collagenous proteins at the site of resorption (13).

3) Osteocytes

Initially, osteocytes are long forgotten players in the roster of bone cells, but these cells are the most prevalent bone cell type, constituting approximately 95% of the cellular component bone cells (18). During encapsulation into the bone matrix, osteoblasts change their morphology, losing most of the cellular organelles and cytoplasm and acquiring a stellar shape with thin extensions which create an inter-intra cellular communication with neighbouring osteocytes and bone surface lining osteoblast cells (82).However, osteocytes escaped apoptosis at finishing of bone formation cycle. Osteocytes are long-lived cells with an estimated lifespan of 25 years, while on the other side osteoblast have 3 months lifespan (83). Osteocytes are found throughout the skeleton. , the short-lived osteoblasts and osteoclasts are found only transiently on surface of the bone. Osteocytes dynamically secrete growth factors which stimulate development of bone, as well as sclerostin, which inhibits bone formation (84).

4) Osteoclasts

Osteoclasts mediate bone resorption. Osteoclast formation, activation and resorption are regulated by the ratio of receptor activator of NF-κB ligand (RANKL) to osteoprotegerin, Interleukin-1, Interleukin-6, colony-stimulating factor, PTH, 1, 25-dihydroxyvitamin D and calcitonin (85). In the process of bone resorption osteoclast release H^+ ion create acidic enviroment that disolve minerals in bone. The exposed organic bone (86)matrix which is primarly composed of type I collagen degarded by osteoclast which secretes various enzyme degarde bone matrix during bone resorption in addition to bone resorbing (20). Osteoclasts communicate with osteoblast, signalling them to initiate bone formation(87).

2.1.2 Mineralization of bone

Bone mineralization is necessary for its hardness and rigidity in which crystals of calcium phosphate are secreted by bone-forming cells and laid down in specific amounts within the bone matrix. If the process is dysregulated, resulting in too little or too much of the mineral whichever can affect bone health. Osteoblasts produce Type I collagen which get deposited in concentric or parallel patterns to generate mature (lamellar) bone. Collagen fibrils/aggregates are organized in different combinations and variable concentrations in tissues to provide diverse tissue properties. In bone, entire collagen triple helices arrange in a parallel, staggered array, 40 nm gaps between the ends of the tropocollagen subunits. Probably serve as nucleation sites for the deposition of long, hard, fine crystals of the mineral component, which is hydroxyapatite(88). Type I collagen gives bone its tensile strength. Osteoblasts also synthesize and secrete the non-collagenous protein, such as proteoglycans, glycosylated proteins, glycosylated proteins with potential cell-attachment activities, and gamma-carboxylated (Gla) proteins. The main glycosylated protein present in bone is alkaline phosphatase, which plays a role in mineralization of bone.

2.2 Type I Collagen protein structure

The triple helix structure of the collagen was proposed in year 1954 (89). Triple helix is formed of supramolecular complex, which is further cross-linked by hydrogen bonding between other charged residues and hydroxyproline, that self-assemble to create a right-handed superhelix (**Fig.4)** (90). The inter-chain hydrogen bonding between N-H and the C=O of the adjacent peptide chains, electrostatic interactions maintain. The structure also has glycine side chain that is short enough to be incorporated in triple helix, and allow tight inter-chain hydrogen bonding. Also, rather than usual α-helices, these α-chains are left-handed polyproline helical proteins. Each molecule of glycine is aligned with extreme precision and in a quarter-staggered array to maintain the stability in the structure and produce a collagen fibril(91). Type I collagen has an active role in intrafibrillar mineralization forming bioapatite structure by regulating nucleation pathways and energy barriers (92).The most common structural defect in type I collagen that causes OI is the glycine substitution in the helical domain (91).

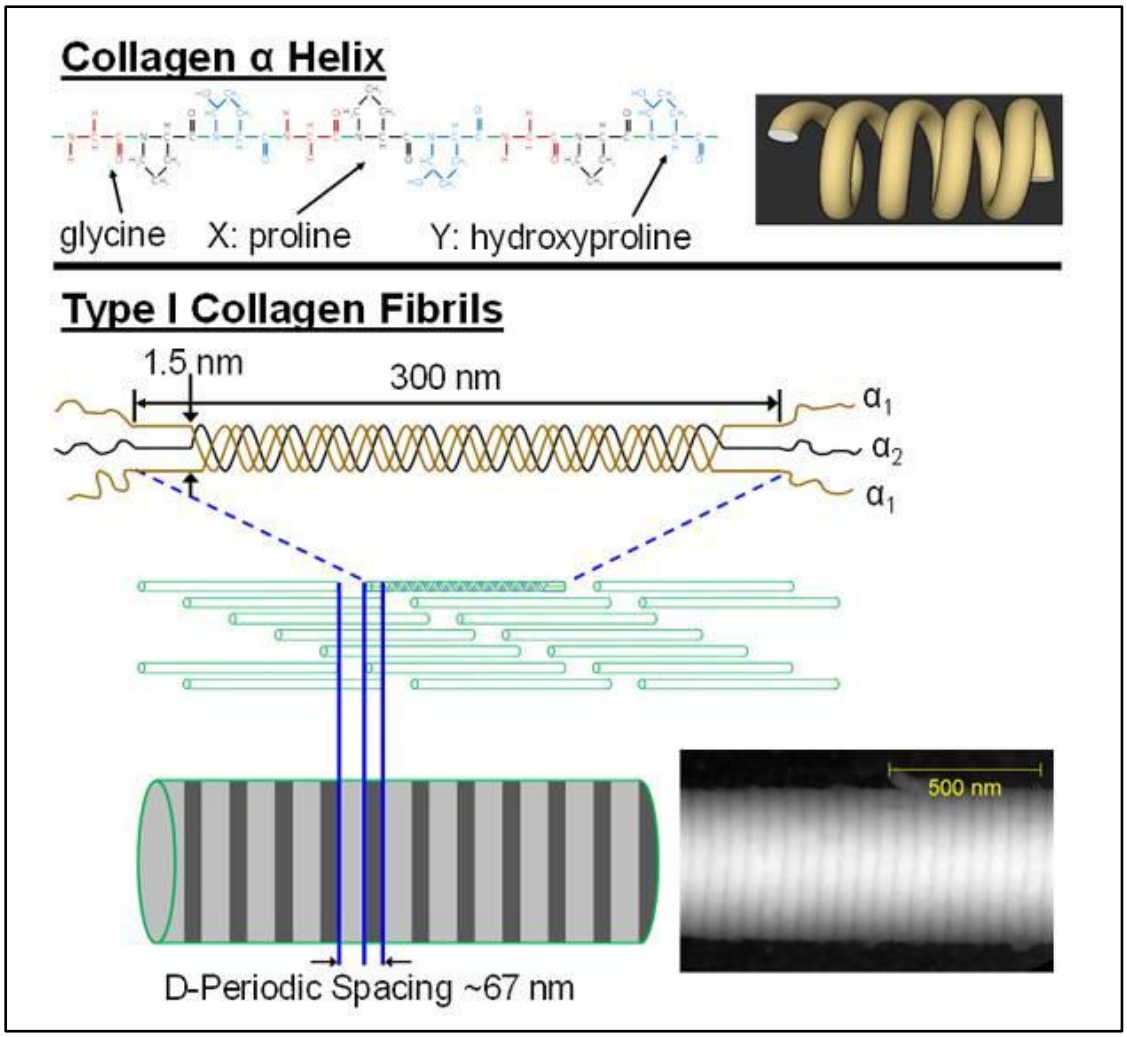

Fig. 4: Ultra structure of type collagen I

Source: Shoulders MD and Raines RT. Collagen structure and stability. Annu. Rev. Biochem. 2009; 78: 929–958.

2.2.1 Type I Collagen gene organization and synthesis

The COL1A1 gene, located on chromosome 17q21.33 and consists of 52 Exons and 51 introns that encodes for the alpha chain 1 of the type I collagen. The COL1A2 gene which is located on chromosome 7q22.1and have 52 Exons and 51 introns. It encodes the amino acids for the two alpha chains of the type I collagen. The triple helical domain contains 44 exons; out of which 23 have a size of 54bp, 8 are of 108bp, five are of 45bp, five are of 99bp,and one is of 162bp long **(Fig.5)**. The initial and ending part of the triple-helix are coded for telopeptide and propeptide sequences

respectively. If the mutation occurs in the triple helical domain, the mutation will be dominant in nature, and it can cause abnormal phenotype.

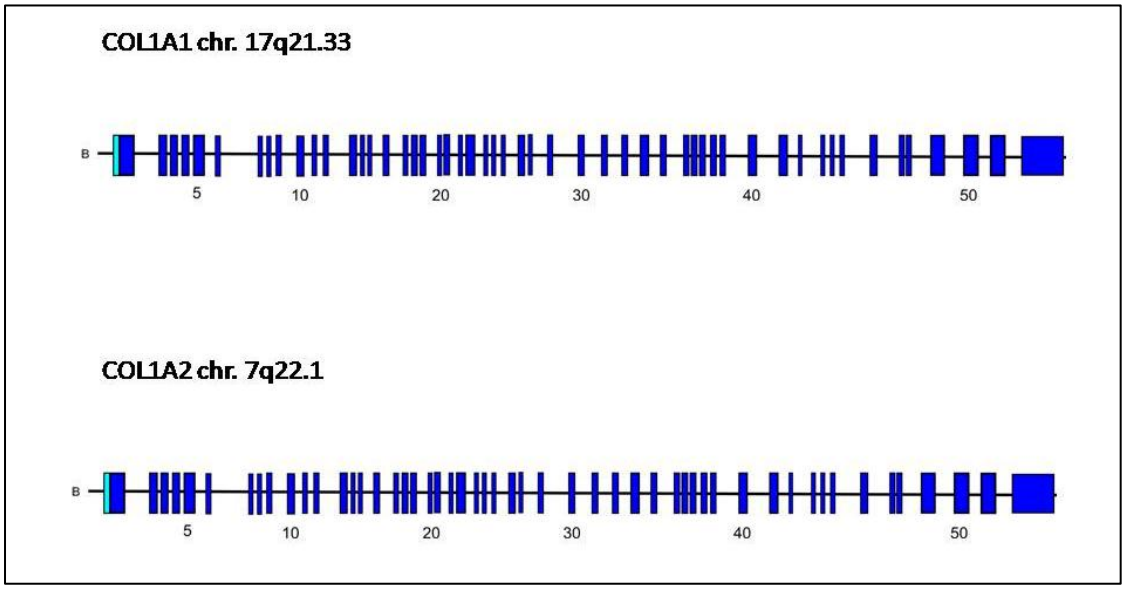

Fig.5: Type I collagen gene organization(COL1A1 and COL1A2)
Source: Kaneto et al. BMC Medical Genetics 2014, 15:45.

In the present study, mutations in COL1A1 and COL1A2 exons are screened to understand pattern of the mutation.

2.2.2 Assembly and processing

For assembly to occur, three steps are involved such as chain selection, registration and nucleation. For chain selection, before the C- propeptide concentration needs to be critical in the lumen of the ER. Previously reported, that anchoring of the procollagen chains to the ER membrane may be important for chain assembly, as this restricts motion of the chains and decrease the concentration required (30). After selection of proαchains registration of three chains through C-terminal, so that nucleation of the triple helix occurs, then propagation of the chain occurs from nucleus to the N terminus of the collagenous domain **Fig.6**.Calcium flux regulates collagen synthesis in ER lumen into the cytoplasm via IP3 (inositol triphosphate receptor) and ryanodine receptors(93).SERPINH1 and FKBP65 interact with triple helix to prevent the premature fibril formation. SERPINH1 stabilize and assist the shuttling of the triple helix into the *cis*-Golgi.

2.2.3 Collagen trafficking and folding

Like other proteins, procollagen is transported from ER to Golgi in coated protein complex II (COPII) pathway. Sec24D the gene encodes part of COPII complex mutation in which leads to OI phenotype (28, 94). Trafficking 300nm long procollagen molecules requirelarger than usual (60-80nm) vesicles to increase vesicle size to accommodate procollagen fibres to controls the size and function of a vesicle coat (95). For shuttling long protein molecules such as procollagen and chylomicrons, largevesicles are required. This is carried out by SEC31protein of COPII ubiquitin ligase CUL3-KLHL12 which act as a regulator of COPII coat formation (96). N-propeptides are cleaved earlier (*cis*-Golgi) in COPII secretory pathway than C-propeptides (97). The cleavage of the C-propeptides in post-Golgi compartments indicates assembly of collagen fibrils at the cell-matrix interface (98). Procollagen molecules are transported from the ER to ER-Golgi intermediate compartment (ERGIC) in large COPII- covered vesicles along with bound HSP47. HSP47 is then transported back to the ER through Golgi after the dissociation from procollagen in ERGIC/Golgi, (97).

The HSP47 which is encoded by SERPINH1 gene, binds to procollagen in ER and assist the protein folding (29, 99). Triple helix is stabilized by more than 20 HSP47 molecules per triple helix at 34-35°C body temperature (99, 100). Recent studies have suggested that intracellular Secreted Protein Acidic and Rich in Cysteine (SPARC) may act as collagen chaperone as it interacts with triple-helical domain of procollagens (101).

2.2.4 Type I Collagen and its physical property

Type I collagen is a ubiquitous protein with extremely low solubility but has lower stability. The native procollagen is thermodynamically more favourable than its unfolded chains below 34-35°C. However, they are unfavourable above this temperature (102, 103).

2.3 Osteogenesis imperfecta

OI(OMIM 166200), also called as brittle bone disorder is an inheritable disorder that exhibits typical symptoms of blue sclera, dentinogenesis imperfecta, scoliosis, type-specific multiple pathological fractures, bowing deformities of a long bone and short stature(1).OI caused by the faulty genes which codefor Type I collagen cause OI, which is an autosomal dominant disorder. The recent finding of the recessive causative gene, in 2006, brings new aspects of OI research. The classification of OI was done based on clinical features and radiological findings(104).

2.3.1 Classification of Osteogenesis imperfecta

In 1979, a Sillence classification of OI was proposed based on clinical and genetic findings in OI patients. They distinguished OI as;type I (mild OI, blue sclerae, autosomal dominant inheritance), type II (lethal perinatal OI, autosomal recessive inheritance, later subdivided in II-A, -B, and -C based on radiographic features, type III (progressively deforming, autosomal recessive inheritance), and type IV (dominantly inherited OI with normal sclerae)(105-107).The Sillence classification was used only for the clinical and radiological classification of OI. Thereafter, familial studies and consanguineous cases with OI not caused by pathogenic variants in the COL1A1 or COL1A2 genes(108).

The original Sillence classification was expanded on the bases of unknown genetic etiologyand distinctive clinical manifestations(43). Role of autosomal recessive genes was discovered in 2006, showed alteration in the CRTAP gene causing complete loss of protein function cause OI type VII(109). Currently, other recessive causative genes were identified which are LEPRE1(110), PPIB(111, 112), SERPINH1(113), FKBP10(114),SP7(115)and SERPINF1 (116)concerning genes involve in modification, folding, cross-linking and mineralization(112) presumably X, XI, XII, and XIII, respectively(104).Althoughthe clinical and radiological characteristics of recessive OI don't substantially differ from other types (117-119).**Below Table 1 explain the recent classification of OI (dominant and recessive).**

Table 1. Classification of OI (dominant and recessive).

	OMIM number	Locus	Gene symbol	Sillence type	Clinical variants	Protein	Main location	Bone deformity	Sclerae	Hearing loss	Dentinogenesis imperfecta	Other typical features
Defects in collagen synthesis, structure, or processing (group A)												
Autosomal dominant	166200	17q21.33	*COL1A1*	I	NA	Collagen type I, alpha 1	Extracellular matrix	Rare to very severe	Normal, grey to dark blue	Absent to common	Absent to common	..
Autosomal dominant	166210	17q21.33	*COL1A1*	II	NA	Collagen type I, alpha 1	Extracellular matrix	Rare to very severe	Normal, grey to dark blue	Absent to common	Absent to common	..
Autosomal dominant	259420	17q21.33	*COL1A1*	III	NA	Collagen type I, alpha 1	Extracellular matrix	Rare to very severe	Normal, grey to dark blue	Absent to common	Absent to common	..
Autosomal dominant	166220	17q21.33	*COL1A1*	IV	OI/EDS and HBM/OI	Collagen type I, alpha 1	Extracellular matrix	Rare to very severe	Normal, grey to dark blue	Absent to common	Absent to common	Type IV OI/EDS is due to mutations at the first 85 aminoacids of α1(I); HBM/OI is caused by mutations blocking C-propeptide processing
Autosomal dominant	166200	7q21.3	*COL1A2*	I	NA	Collagen type I, alpha 2	Extracellular matrix	Rare to very severe	Normal, grey to dark blue	Absent to common	Absent to common	..
Autosomal dominant	166210	7q21.3	*COL1A2*	II	NA	Collagen type I, alpha 2	Extracellular matrix	Rare to very severe	Normal, grey to dark blue	Absent to common	Absent to common	..
Autosomal dominant	259420	7q21.3	*COL1A2*	III	NA	Collagen type I, alpha 2	Extracellular matrix	Rare to very severe	Normal, grey to dark blue	Absent to common	Absent to common	..
Autosomal dominant	166220	7q21.3	*COL1A2*	IV	OI/EDS and HBM/OI	Collagen type I, alpha 2	Extracellular matrix	Rare to very severe	Normal, grey to dark blue	Absent to common	Absent to common	Type IV OI/EDS is due to mutations at the first 85 aminoacids of α2(I); HBM/OI is caused by mutations blocking C-propeptide processing
Autosomal recessive	614856	8p21.3	*BMP1*	XIII	NA	Bone morphogenic protein1/ procollagen C proteinase	Pericellular environment	Mild to severe	Normal	Absent	Absent	Umbelical hernia, HBM
Defects in collagen modification (group B)												
Autosomal recessive	610682	3p22.3	*CRTAP*	VII	NA	Cartilage-associated protein	Endoplasmic reticulum	Severe rhizomelia	Normal, grey	Absent	Absent	..
Autosomal recessive	610915	1p34.2	*LEPRE1/ P3H1*	VIII	NA	Leucine proline-enriched proteoglycan1/ prolyl 3-hydroxylase 1	Endoplasmic reticulum	Severe rhizomelia	Normal	Absent	Absent	..
Autosomal recessive	259440	15q22.31	*PPIB*	IX	NA	Peptidylprolyl isomerase B/ cyclophilin B	Endoplasmic reticulum	Severe	Grey	Absent	Absent	..
Autosomal recessive	615066	9q31.2	*TMEM38B*	XIV	NA	Transmembrane protein 38 B	Endoplasmic reticulum membrane	Severe	Normal to blue	Absent	Absent	..
Defects in collagen folding and cross-linking (group C)												
Autosomal recessive	613848	11q13.5	*SERPINH1*	X	NA	Serpin peptidase inhibitor, clade H, member 1/heat shock protein 47	Endoplasmic reticulum	Severe	Blue	Absent	Present	Skin blisters and bullae at birth, inguinal hernia
Autosomal recessive	610968	17q21.2	*FKBP10*	XI	NA	FK506 binding protein 65	Endoplasmic reticulum	Mild to severe	Normal, grey	Absent	Absent	Variable congenital contractures, encompasses Bruck and Kuskokwim syndromes
Autosomal recessive	609220	3q24	*PLOD2*	..	NA	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2	Endoplasmic reticulum	Moderate to severe	..	..	..	Progressive joint contractures

(Table continues on next page)

	OMIM number	Locus	Gene symbol	Sillence type	Clinical variants	Protein	Main location	Bone deformity	Sclerae	Hearing loss	Dentinogenesis imperfecta	Other typical features
(Continued from previous page)												
Defects in bone mineralisation (group D)												
Autosomal dominant	610967	11p15.5	*IFITM5*	V	NA	Interferon-induced transmembrane protein 5	Plasma membrane	Variable	Normal to blue	Infrequent	Absent	Ossification of the forearm interosseous membrane, radial head dislocation, subephyphyseal metaphyseal radiodense band
Autosomal recessive	613982	17p13.3	*SERPINF1*	VI	NA	Pigment epithelium-derived factor	Extracellular matrix	Moderate to severe	Normal	Absent	Absent	Normal at birth, unmineralised osteoid, fish scale appearance of lamellar bone pattern, raised ALP, loss of serum PEDF
Defects in osteoblast development with collagen insufficiency (group E)												
Autosomal recessive	613849	12q13.13	*SP7*	XII	NA	Transcription factor 7/osterix	Nucleus	Severe	Normal	Absent	Absent	Delayed tooth eruption, midface hypoplasia
Autosomal recessive	615220	12q13.12	*WNT1*	XV	NA	Wingless-type MMTV integration site family, member 1	Extracellular matrix	Severe	White	Absent	Absent	Possible neurological defects
Autosomal recessive	616229	11p11.2	*CREB3L1*	XVI	NA	cAMP responsive element binding protein 3 like 1	Endoplasmic reticulum membrane	Severe	..	..	..	..

OMIM=Online Mendelian Inheritance in Man. NA=not applicable. OI=osteogenesis imperfecta. EDS=Ehlers-Danlos syndrome. HBM=high bone mass. ALP=alkaline phosphatase. PEDF=pigment epithelium-derived factor. MMTV=mouse mammary tumour virus.

Source: Forlino et al. Osteogenesis imperfect. Lancet 2016,387:1657-71

2.3.2 Clinical manifestation of osteogenesis imperfecta

A wide spectrum of clinical variants exists from severe or mild forms, which are evident at birth, are classical and late-onset non-classical forms. The disorder may show significant intrafamilial and interfamilial diversity in frequency of fractures and extent of disability. A spectrum of disease severity exists even within 5-generation family. The patients who are affected most severely exhibit short stature and a mild extent of scoliosis as compared to less severely affected. OI in twin siblings have suggested that disease severity is more or less correlated with the decline in collagen I synthesis (120-122).

2.3.3 Diagnosis of osteogenesis imperfecta and related defects

The diagnosis is based on clinical and genetic criteria (**Table 1**). The diagnosis may be difficult in sporadic cases but secondary osteoporosis and non-accidental injury has to be ruled out. The careful clinical investigations and a detailed family and personal history often reveal OI type I in women with severe postmenopausal osteoporosis. Presently, the molecular characterization in majority of cases is not feasible. As OI is directly linked to collagen defect, the demonstration of reduced synthesis of procollagen I by cultured dermal fibroblasts indicates the disorder (123).On the basis

of linkage analysis, prenatal diagnosis of OI type I is helpful in stopping severely affected offsprings (124). A case study depicted the termination of a normal twin pregnancy after preimplantation, once genetically diagnosed for OI type I (125) which may be due to presence of two blighted ova seen in ultrasound at seven weeks of gestation(106).

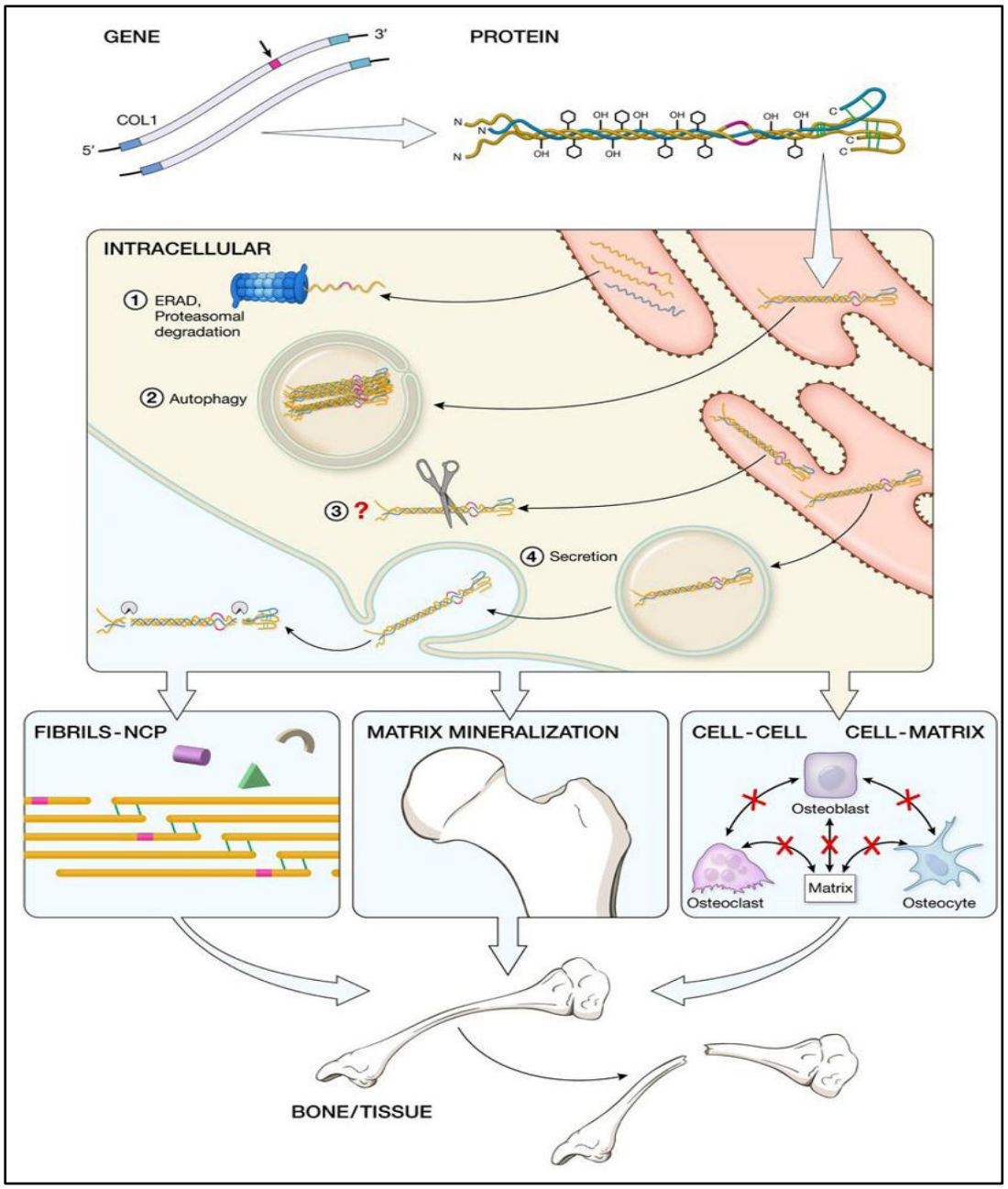

Fig.6: Molecular mechanism contributing autosomal dominant OI.

Source: Forlino et al. Nat Rev Endocrinol. 2012; 7: 540–557.

2.3.4 Mechanism and pathophysiology of type I collagen in OI

Approximately 85-90% of OI cases are caused by structurally abnormal procollagen chains are amplified. Recent studies showed that this disorder is mainly due to impaired maturation of osteoblasts (43,115,126).The mutation in exon 43 of the COL1A1 gene with OI type leads to a premature stop codon in exon 46. All this results in reduction of type I procollagen synthesis.

Defective splicing has also been reported in which fibroblasts established from a child with OI type I was studied which revealed the reduction in production of alpha 1 (I) collagen chains and alpha 1 (I) mRNA (122).

2.3.4.1 Abnormal synthesis procollagen in OI

Collagen biosynthesis occurs in the nucleus and the mutations responsible for synthesis of structurally abnormal proα1 or proα2 or both chains are mostly deleterious (127). There are multiple number of mutations in type I procollagen in OI which cause defect in synthesis of proα chains of type I procollagen that lead to the deleterious effect which is intensified by following mechanisms:

1. Mutated proα chains cause degradation of normal proα chains within the same cell known as procollagen suicide.
2. Normal processing of the N-propeptide of procollagen is prevented by structurally altered pro –α chains and tendency of the N-propeptides interferes with fibril assembly.
3. Mutation in C-propeptide domain of proα chains causing impairing chain association.
4. Substitution of the bulkier or charge residue amino acid for glycine can cause a kink in the procollagen cause abnormal branching of the fibrils (35).
5. Mutation in the 3’ to 5’ splice site that are responsible for the exon skipping effect of this lead to change in Gly-X-Y triplet pattern is not altered ,but chain alignment can be shifted, which affects folding and secretion .

An interesting and distinct group of structural defect is the consequences of duplication or deletion of one or two triplet repeats cause register shift of the chain. It

is evident that OI was a heterogeneous condition. Dominant and dominant lethal mutations are deleterious due to abnormal proα chains are amplified (104, 128).

Previously, OI was considered as an autosomal dominant disease with recurrence due to germline mosaicism since heterozygous collagen type I mutations (*COL1A1* or *COL1A2* mutations) were discovered in all OI types.

2.3.4.2 Defects in ER assembly and processing of procollagen

Structural mutation lead to prolonged retention of the unfolded α (1) chains in the ER lumen expose to excessive post-translational modifications for long time underlying features consequence to bone fragility. Abnormal collagen partially retained in the ER cause ER stress cause to stimulation of autophagy associated with apoptosis activation. Defects in TMEM38B encode TRIC-B, involved in a Ca2+ release from intracellular stores lead to OI phenotype (93). Other protein includes ER-resident Ca^{2+}-binding chaperones including BiP, CyPB (cyclophilin B), PDI (protein disulfide isomerase) and CRT (calreticulin). The study suggests that the homozygous start codon mutation in the peptidyl-prolylisomerase B gene (PPIB) results in a lack of CyPB in OI recessive patients with rhizomelia (shorting of proximal segments of upper and lower limb) (112, 129). However, calcium flux abnormalities likely affect bone cell function, since intracellular calcium signalling is known to control osteoblast proliferation and differentiation, as well as osteoclast survival and resorptive activity (112, 130).

2.3.4.3 Abnormality in procollagen trafficking in OI

HSP 47 plays an important role in the triple helix to prevent premature fibril formation rER. Some studies report that the defect in *SERPINH1* gene leads to a severe recessive form of OI. The mutation leads to degradation of HSP47 via proteasome and to the accumulation of the type I collagen in the ER, resulting in the increase in the marker for showing ER (113).

Mutation in the N terminal of the triple helical domain of the propeptide affects the N propeptide processing and causes generation specific variant of the OI/EDS (131). Cleavage of C- propeptide is more complex than that of N- propeptide. It involves four different c- protease and modulation by enhancer. Mutation in the major enzyme

BMP1/mTLD results in the more severe form of OI than substrate defects. This enzyme has a central role in the initiation of the lysyl oxidase enzyme which helps in crosslinking of collagen fibrils and in the processing of other procollagens (132).Also, some reports suggest that attenuated BMP1 function leads to the fragility of bones in human as wells as Zebrafish (133). We did the expression as well as mutational analysis of the gene SERPINH1 gene. We further validated the role of HSP47.

2.3.4.4 Abnormality in procollagen folding and crosslinking in OI

Improper folding of procollagen molecule leads to the retention of type I collagen in the cell of OI patients in comparison to the normal individuals. In the normal culture fibroblast, the addition of ascorbate enhances the clearance of type I collagen. But it is not effective in the case of abnormal procollagen which affects the collagen-specific chaperone function(134).

2.3.5 Abnormality in triple helices post-translational modifications

It has been extensively studied in fibril-forming collagens that are synthesized as procollagen molecules. They are comprised of an amino-terminal propeptide followed by a short, nonhelical, N-telopeptide, a central triple helix, a C-telopeptide and a carboxy-terminal propeptide. Individual polypeptide chains which are undergone numerous posttranslational modifications in the endoplasmic reticulum (hydroxylation of proline and lysine residues, glycosylation of lysine and hydroxylysine residues, and sulfation of tyrosine residues (135)are regulated by the formation of the triple helix.

2.3.6 Abnormality in bone formation mineralization in OI

2.3.6.1 Impaired bone mineralization

Correctly folded triple helix is capable of unassisted self-assembly (fibrillogenesis) and is tightly regulated by PEDF and IFITM5 protein molecules. Fibrillogenesis occur in outer surface although N'- and C'-propeptide cleavage begin inside the cell. PEDF encoded by SERPINF1 is a secreted collagen binding protein. The binding sites for PEDF overlap the heparin and heparan sulphate proteoglycan binding sites with the C-terminal majorligand-region having another binding site in N terminal, overlaps

integrin collagen binding site (136). It acts as a potent inhibitor of fibrillogenesis also it significantly slows down the fibre formation and collagen-collagen interactions in the triple helix.

However, PEDF has been reported to upregulate and increase osteoclast number and bone resorption by favouring RANKL binding to osteoclast RANK receptor (137). IFITM 5 is also known as Bone-restricted IFITM-like (BRIL),it has a single transmembrane domain with an extracellular C-terminus. On the cytoplasmic side, BRIL is attached to the membrane through palmitoylation of cysteines amino acid at 52 and 53 positions. It is predominantly present in the osteoblast plasma membrane and expressed throughout the life (138). In addition to that IFITM 5 expresses in the embryo skeleton after 14.5 days and leads to the undifferentiated osteoblast to differentiation and causes mineralization (139). Both the genes have opposite effect; their pathways intersect with each other and suggest the functional relationship. Aberrant regulation of collagen fibrillogenesis by extracellular matrix molecules lead to OI V or VI phenotype.

Mutation in IFITM5 leads to OI type V (140) and SERPINF to OI type VI. The knockout model of SERPINF -/- showed a reduced bone volume and an unmineralised osteoid (141).

2.3.7 Abnormality in osteoblast function and development

Impaired osteoblasts secrete type I collagen formed from pro-collagen. Type I collagen which is a triple helix composed of two α1(I) and one α2 (I) chain which is encoded by COL1A1 and COL1A2 genes respectively. A study by Morike *et al* and Fedarko *et al* have reportedthat*in vitro* bone culture of patients have significantly low level of type I collagen and extracellular matrix proteins (142). Further, they have shown that osteoblast cells of type III and type IV OI patients, the production, processing and accumulation of procollagen is low compared to the normal (142).

The osteoblasts differentiation is a crucial event in bone formation. Runx2 is key transcription factor which regulates the process of osteoblast differentiation. In addition, Runx2 regulates the zinc finger-containing transcription factor osterix, promoter of *Osx (Sp7)* gene (which encode Osterix). It has anRNA binding sequence which suggests that *Osx* is a direct Runx2 target. Three genes such as *WNT1,*

CREB3LI and*SP7*, are reportedly associated with OI. Out of these three genes, more supportive data is available for *WNT1* gene. Homozygous nonsense, missense, frame shift and splicing mutations are observed in *WNT1* gene. Several patients have developed brain malformations, while some mutations impaired the canonical pathway and mineralization (143). CREB3L gene has a role in activation of the COL1A1 promoter (144). Molecular mechanism related to osteoblast differentiation is still unknown. Sp7 gene is a direct target of the *WNT1* pathway. The functional characterization of the mutation in this gene is not yet studied (115).

2.4 Pharmacological and biological available treatments in OI

OI is characterized by low bone mass, increased bone fragility, multiple pathological fractures and considerable alterations in quantity, quality, mineralization and homeostasis of the extracellular matrix. The symptoms are mainly caused by the quantitative defects associated with a mutation in the type 1 collagen gene. Other severe forms of OI like II and III are caused by qualitative defects often resulted by glycine substitutions leading to the collagen over modification. There is no cure found out for OI until now.

The drugs which are currently available in the market, target the activity of bone-resorbing osteoclasts and bone-forming osteoblasts to increase the bone mass. They do not address the defective type I collagen which leads not only to reduced bone quantity but also lead to the abnormal bone matrix and bone quality. Hence, there is a need to look for the new therapeutic targets.

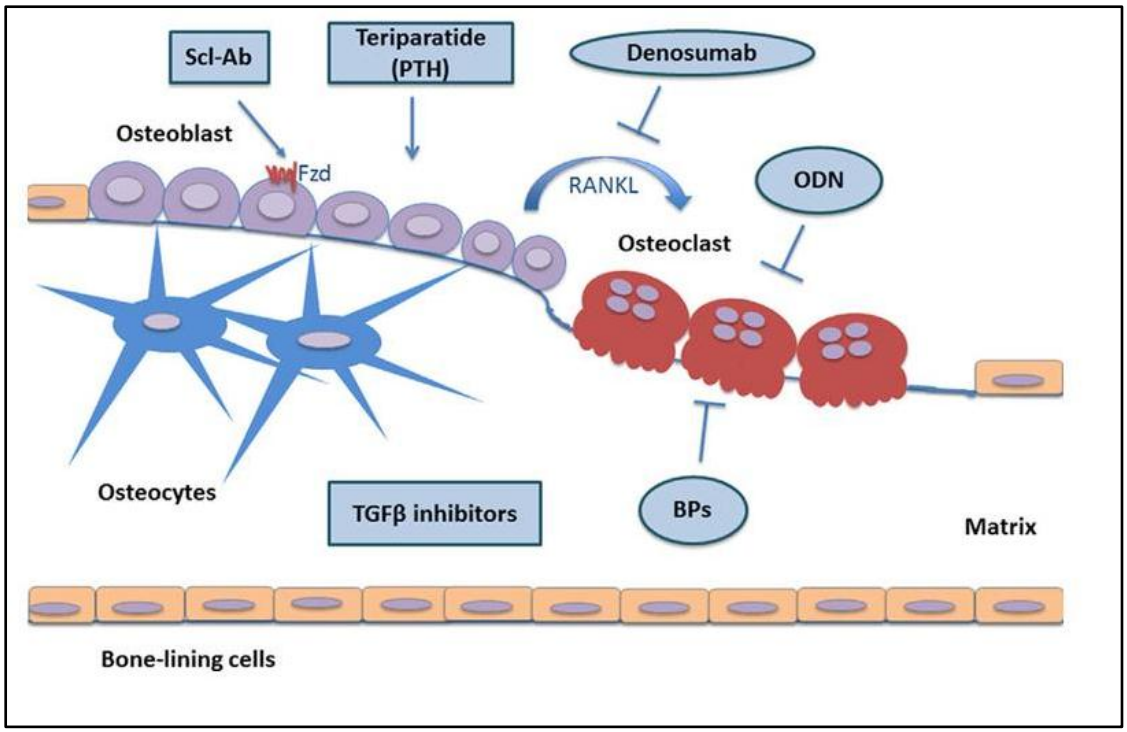

Fig. 7: Mechanism of various drug target of therapeutic interventions

Source: Marom R et al. Pharmacological and biological therapeutic strategies for osteogenesis imperfecta. Am J Med Genet Part C Sem in Med Genet 2016; 9999C:1–17.

2.4.1 Bone antiresorptive agents

The Bisphosphonates (BPs) such as Pamidronate, Alendronate, Risedronate and Zoledronic acid, are most potent antiresorptive agents. Zoledronic acid (ZOL) is FDA approved BPs and is commonly used in clinical practice (145). BPs is the mainstay of therapy for pediatric OI patients with moderate to severe symptoms. BPs areanalogs of pyrophosphate, which inhibit the bone resorption by blocking the key enzyme farnesyl-pyrophosphate(53).In response to the inhibition of prenylation of intracellular proteins by BPs, there is ultimately an increase in osteoclasts apoptosis(8).

BPs can also reduce the number of osteoclasts by inducing apoptosis in macrophages and mature osteoclasts (146). In addition, BPs can directly inhibit the bone-resorbing activity of osteoclasts (54). Fortunately, the half-life of BPs 10 years in human, since systemically available BPs are largely sequestered in the bone also renal excretion is the only route of elimination (147). The mechanism by which are BPs act directly on

osteoclasts and osteoclasts precursors have been reported to be partly due to inhibition of the mevalonate pathway(53). There is also a likely indirect effect via osteoblasts, bone-forming cells which have a cellular link with osteoclast cells due to modulation of osteoblasts secretion of soluble paracrine factors that influence osteoclasts activity(55, 56).

BPs, administered to children with OI, have been shown to increase bone volume by counteracting the high turnover cellular status of bone in classic OI (148-150). The new bone still contains defective collagen. The hypothesis behind the treatment is that an increased bone mass (even of impaired quality) leads to a moderate reduction in the risk of fracture (52). Highly potent BP- Zoledronic acid (ZOL) enhances osteoblastic activity and differentiation(58), which further increases the bone mass in OI patients(151). It is likely that BPs decrease the fracture rate but they increase bone brittleness. They directly do not affect the defective collagen however they inhibit the bone resorption by osteoclasts (59, 60).

The present study was planned to compare the frequency of caspase 3 expressing cells (suggestive of apoptosis) in precursor osteoclast cells of OI children who received ZOL (high dose vs low dose) with age-matched controls.

2.4.2 Bone anabolic agents

A number of other pharmacologic agents are currently being studied in OI such as human parathyroid hormone (rhPTH), sclerostin inhibition and inhibition of transforming growth factor beta (TGFβ) signaling and recombinant growth hormone. Drugs with anabolic action on bone formation, recombinant growth hormone, are undergoing testing in the animal models of OI (65, 152, 153).

Several studies required to justify their role of drugs in osteoblast. Thus, we have employed a novel strategy to exploit the pathway of osteoblastic genes. In the presentstudy, we did the *in-vitro* studies related to BPs and rhPTH in an osteoblastic cell line such as U2OS.

2.4.3 Cell therapy, gene targeting and gene editing

Previous studies suggested that MSCs isolated from the OI patients were successful in genetical correction (154). Earlier, Chamberlain et al had used adeno-associated virus (AAV) vectors to inactivate mutant COL1A1 genes in MSCs from OI patients. The above study demonstrated that both the type I collagen genes liable for OI can be successfully targeted and can be improved by incorporating vector to minimize the limitations of random integration, avoiding unnecessary exon skipping. MSCs are targeted at mutant COL1A2 alleles which produced normal type I procollagen and formed bone, thereby demonstrating their therapeutic potential (155).

The best treatment for OI would be a therapy to administer at the earliest during growth (preferably *in utero*, for healthy bone development and avoid progressive damage to the skeleton) and to enable direct repairing of underlying gene defect to yield a normal bone matrix(42). One approach has been the allogeneic transplantation of bone marrow, bone marrow stromal cells, or marrow-derived mesenchymal stem cells (MSCs) to test whether osteoprogenitor cells that normally reside in the marrow would successfully graft into the OI patient marrow and contribute to the healthy bone formation.