# SUMMARY AND CONCLUSION

The prevailing paradigm in bone biology is that differentiation and functions of the specific cell types, osteoblast and osteoclast are most important to maintain the Skelton. In most patients, the disorder is caused by mutations in one of the two genes encoding collagen type 1, but in some individuals no such mutations are detectable. The most important therapeutic advance is the introduction of Bps treatment for moderate to severe forms of osteogenesis imperfecta. However, at present, the best treatment regimen and the long-term outcomes of Bps therapy are unknown. Although this treatment does not constitute a cure; it is an adjunct to physiotherapy, rehabilitation, and orthopaedic care. Gene-based therapy presently remains in the early stages of preclinical research. In present work, we expand the heritable importance of the collagen, which further maintains the proper alignment of the collagen fibrils in the extracellular matrix and also HSP47 molecules are required to triple helix for stability.

**Key highlights of thesis**:

1. Among the fifty OI patients, twenty-six had OI type III, eight type IV, and ten type I and two with types II. Three cases showed limited clinical expression to identify the type of OI. These cases showed extra skeleton manifestation such as blue sclera (n=43), bone deformity (n=42), dentinogenesis imperfecta (n=36), scoliosis (21), hyper-flexibility of the joints (n=12). We observed the onset of the fracture at birth. ALP was elevated in OI patients compared to control group however, other biochemical parameters were within the range. Bone turn over markers were suppressed in OI patients.

2. Total 16 patients showed mutation in type I collagen (Eleven patients showed mutation in COL1A1 and five in COL1A2). The identified mutations are follows

**Table 16 : Identified mutation in type 1 collagen (COL1A1 and COL1A2) and SERPINH**

| Patients ID | Mutant gene | Exon | Mutation pattern | Amino acid change | Existing or novel mutation |
|---|---|---|---|---|---|
| 1 | COL1A2 | Exon33 | c.623 T >A | p.Pro403Pro | Existing |
| 2 | COL1A1 | Exon19 | c.1209 T > A | p.Pro403Pro | Novel |
| 3 | COL1A1 | Exon1 | c.97 G > A | p.Asp33Asn | Novel |
| 4 | COL1A1 | Exon3 | c.87 T > C | p.Thr29Thr | Existing |
| 5 | SERPINH1 | Exon1 | c.233 T>C | p.leu78pro | Existing |
| 6 | COL1A1 | Exon 5 | c.391C>T | p.Arg131X | Existing |
| 7 | COL1A1 | Exon19 | c.1243C>T | p.Arg415X | Novel |
| 8 | COL1A1 | Exon48 | c.3556 C>G | p.Pro1186Ala | Existing |
| 9 | COL1A1 | Exon50 | c.4021C>T | p.Gln1341X | Existing |
| 10 | COL1A1 | Exon34 | c.2321del C | p.gly774leu | Existing |
| 11 | COL1A2 | Exon16 | c.1056 G>A | p.gly352Ser | Existing |
| 13 | COL1A2 | Exon17 | c.435 G>A | p. gly145Ser | Existing |
| 16 | COL1A2 | Exon25 | c.495 C >T | p. gly165Ser | Existing |
| 17 | COL1A1 | Exon48 | c.3702 C > T | p.Thr1234Thr | Novel |
| 23 | COL1A2 | Exon49 | c.3304 C >T | p.gly1102cys | Novel |
| 28 | COL1A1 | Exon 2 | c.141C>A | p.Tyr47X | Existing |
| 32 | COL1A1 | Exon 2 | c.182G>T | p.Cys61Phe | Existing |

3. In COL1A2 gene, the c.3304 C >T mutation substituted ~~a~~ glycine residue by cysteine at codon 1102 (p.gly1102cys). Insilco analysis showed, structural clashes disrupt the inter-chain hydrogen bonding and makes the triple helix highly unstable. (PROVEAN score is equal to or below a predefined threshold (e.g. -2.5), the protein variant is predicted -6.967 to have a "deleterious" effect. Using Polyphen2 tool, we found a prediction of the possible impact of an amino acid substitution on the structure and function of a human protein. This prediction is based on a number of features comprising the sequence,

phylogenetic and structural information characterizing the substitution. This analysis suggested that it is highly conserved region.

4. Functional characterization of HSP47 mutant gene, showed reduced expression of type I collagen and its accumulation in the ER in knockout cells. Insilco analysis showed due to mutation, HSP47 weekly binds to triple helix at binding site.

5. Precursor osteoclast RANK+ cells isolated from OI patients were grouped into two subsets to treat Low (<30 mg per year) Vs high (>30 mg per year) dose of ZOL. We observed that the osteoclast precursor cells received the high dose showed more than 80% caspase-3 activity and lead to apoptosis. BPs at high dose suppressed bone turnover and lead to accumulation of microfractures. X-ray analysis showed long bones with metaphyseal sclerosis and microfracture and delay in fracture healing.

6. As per *In vitro* study, rhPTH (5µg) treatment followed by ZOL (1µM) showed the best anabolic effects.