Osteogenesis imperfecta (OI) is a heritable disorder due to mutations in type I collagen. Normal type I collagen forms a heterotrimeric protein comprised of two pro\(\alpha_1(I)\) chains and one pro\(\alpha_2(I)\) chain \([\alpha_1(I)_2\alpha_2(I)]\). The osteogenesis imperfecta murine (oim) model mouse contains a single nucleotide deletion in the pro\(\alpha_2(I)\) gene (COL1A2) resulting in non-functional pro\(\alpha_2(I)\) chains and production of homotrimeric type I collagen containing three pro\(\alpha_1(I)\) collagen chains, \([\alpha_1(I)]_3\), resulting in small body size, increased bone fragility and altered bone mineralization.

The overall goal of this study is to correct the oim defect by introducing normal COL1A2 genes into oim cells. Oim dermal fibroblasts were transfected with a series of COL1A2 gene constructs containing the full-length murine pro\(\alpha_2(I)\) collagen cDNA driven by various lengths of the murine COL1A2 promoter (1.5kb, 3.0kb, and 6.0kb) along with a COL1A2 enhancer. These DNA constructs were cotransfected with pcDNA3 containing a neomycin resistance gene, which allows for selection of stably transfected cell lines. Various assays have been developed to monitor pro\(\alpha_2(I)\) collagen expression at the DNA, RNA and protein levels. A PCR assay was used to confirm genomic incorporation of transgenic COL1A2 gene constructs and an RT-PCR assay used to confirm expression of normal pro\(\alpha_2(I)\) collagen mRNA. Denaturing urea-SDS polyacrylamide gel electrophoresis along with Western blotting analyses using anti-murine \(\alpha_1(I)\) and \(\alpha_2(I)\) collagen antibodies were used to confirm normal pro\(\alpha_2(I)\) collagen expression at the protein level as well as its incorporation into normal heterotrimeric type I collagen.

All the necessary tools have been established for evaluating the efficacy of transfection. Currently, the first series of stably transfected oim cell lines are being expanded for analyses as described above. Although this study is aimed at ‘fixing’ the oim mutation via gene therapy, valuable data will also be collected regarding the effectiveness of the variable length promoter regions in enhancing the expression of the normal COL1A2 gene.