MOLECULAR APPROACH FOR THE IDENTIFICATION OF THE UNDERLYING MUTATION IN PATIENTS WITH OSTEOGENESIS IMPERFECTA TYPE I.

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In the majority of patients with Osteogenesis imperfecta (OI) type I, a reduction of type I collagen protein is seen. In these patients the clinical phenotype is the consequence of the translation of only one, normal COL1A1 allele while the mutant COL1A1 allele is not translated into a protein (COL1A1 null-allele). We have developed a COL1A1 null-allele detection assay and applied it to 48 patients with OI type I. Informative results were obtained in 28/48 patients (58%): 18 patients were found to harbour a COL1A1 null-allele and in 10 patients, two distinct COL1A1 transcripts were seen.
In the 18 patients with a proven COL1A1 null-allele mutation analysis of genomic DNA was performed. Three different types of mutations were identified:

frame shift mutations: in 11 patients, single or multiple base pair deletions were detected, resulting in the creation of a premature stop codon upstream of the mutation.

Nonsense mutations: In four patients the substitution of an arginine to a stopcodon was found.

RNA splicing mutations: Splicing defects were found in 6 patients. As no mutant transcript is detectable in the cytoplasmic mRNA pool, the effect of the mutation on the splicing is not known. Probably alternative splice sites are activated resulting in out of frame deletions/insertions with the creation of a premature stop codon as a direct consequence.

As such, the different categories of mutations shown to underly a COL1A1 null-allele, are likely to act via the same mechanism i.e. the creation of a premature stop codon resulting in highly unstable and rapidly degraded mRNA from the mutant allele (nonsense-mediated mRNA decay (NMD).

In the 10 patients in whom the presence of two COL1A1 transcripts was found, mutation analysis of the COL1A1 and COL1A2 cDNA was performed and 7 glycine substitutions were characterised in the alpha1(I) and alpha2(I) collagen chains. These were localised either at the N-terminal end i.e. Alpha1(I)-G79R (in two unrelated patients), alpha1(I)-G160C, alpha2(I)-G103S and alpha2(I)-G112R or at the C-terminal end of the collagen alpha-chain i.e. alpha1(I)-G906S and alpha2(I)-G751S.

In summary, our molecular approach either through the detection of a COL1A1 null-allele or gDNA screening has immediate clinical implications in situations in which an accurate diagnosis of OI type I is requested or prenatal/preimplantation diagnosis is wanted.

Reference: Proceedings of the 7th International Conference on Osteogenesis Imperfecta. Montreal, Canada, 1999.