IDENTIFICATION OF NOVEL COLlAI DEFECTS RESPONSIBLE FOR NULL ALLELE PHENOTYPES IN TYPE I OI

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We were interested in identifying mutations in the COLIAI gene which cause "mRNA instability and ultimately lead to a quantitative defect in type I collagen. We therefore selected 12 Ol type I patients and tested the MnlI RFLP in both genomic DNA and cDNA obtained from total RNA. Eight patients showed loss of heterozygosity at the cDNA level, 4 were uninformative. In a few informative cases we purified also nuclear RNA and tested it by RT-PCR/MnlI RFLP as well. Screening for mutations was achieved by amplifying the genomic DNA (a region of 5542 bp, spanning from exon 10 to exon 31) in 15 overlapping fragments, subjected thereafter to SSCP and/or heteroduplex analysis (oligonucleotide primers were kindly provided by MC Willing, Univ. of Iowa). Positive fragments were then cloned and sequenced.
The causal mutation could be identified in 5 out of 12 patients, two unrelated patients (#4 and 5) shared the same mutation.

Pt # mutation transcript In cytoplasmic RNA transcript in nuclear RNA 01 Arg75-Stop absent present 02 G+1IVS17-A absent nd. 03 G+1IV521-A absent absent 04 C-5IVS29-A absent present 05 C-5IVS29-A n.d. n.d.

In two cases (pt. #1 and 4, respectively) we could demonstrate the presence of the transcript from the mutant allele only in the nucleus, thus confirming that mRNAs bearing PTC (premature termination codons) mutations or involved in NAS (nonsense-associated altered splicing) most likely are scanned within the nucleus. Interestingly, the two G+1 substitutions (Pt. #2 and 3, respectively), did not produce exon skipping: in both cases no transcript was detectable from the mutant allele. Downstream sequence analysis showed the presence, within suitable distance, of alternative donor sites, which presumably led to aberrantly spliced, unstable RNAs. We conclude that splicing mutations, so commonly found in collagen genes, can give quite different and unpredictable phenotypic outcomes, depending on the way they affect mRNA metabolism and stability.

A few sporadic intronic sequence variations were found in other patients, whose causal mutations we are still missing; one recurrent intronic sequence variation was found to be polymorphic within the general population, another one is being characterized.

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