G to A polymorphism in exon 31 of the COL3A1 gene

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Source and Description of Sequence Polymorphism: Nucleotide sequencing of type III procollagen (COL3A1) cDNAs from several individuals revealed a nucleotide polymorphism that converted codon for amino acid 531 of the triple-helix from -GC-T- (alanine) (1) to -ACT- (threonine) in some alleles. The PCR was used with the primers below; cDNA products were 301 bp and genomic products were 294 bp in length. Digestion of PCR products from cDNA with AluI yielded fragments of 84, 100 and 117 bp for the alanine allele and fragments of 100 and 201 bp for the threonine allele. Digestion of PCR products from genomic DNA yielded fragments of 86, 95 and 113 bp for the alanine allele and fragments of 86 and 208 bp for the threonine allele. Allele-specific oligomer hybridization was used to study 61 unrelated individuals of the following origins: 32 US American (8 anonymous donors), 4 British, 4 Canadian, 2 Danish, 2 Dutch, 4 Finnish, 1 German, 1 Greek, 2 Japanese and 9 Swedish. Heterozygous individuals were present in all nationalities except for the German and Danish. All experiments were repeated at least three times with different PCR products.

Oligomers Used to Amplify cDNA:

5' CCA CAA GGA TTA CAA GGC TTG 3' (III-5)

5' GACT TCC AAG ACC TCC TCT TT 3' (III-4)

Oligomers Used to Amplify Genomic DNA:

5' AAG TAT ACA AAT TTC TAG ATT G 3' (in IVS 30)

5' ATA AAT GAT CAG AAG GAA ATC A 3' (in IVS 31).

(ASO) Hybridizations Probes:

5' AGA GGT GGA ACT GGT CCC C 3' (Thr-allele, sense)

5' G GGG ACC AGC TCC ACC TCT 3' (Ala-allele, antisense)

Frequency: Estimated from 122 chromosomes:

Ala-allele 0.68

Thr-allele 0.32

	Expected	Observed
Ala/Ala	0.462	0.459
Ala/Thr	0.435	0.443
Thr/Thr	0.102	0.098

Chromosomal Localization: 2q31-q32.3.

Other Comments: Conditions for amplification were:

a) For cDNA amplification, 40 cycles consisting of 1 min 30 sec at 94°C, 1 min at 54°C and 1 min at 74°C.

b) For genomic DNA amplification, 40 cycles consisting of 1 min 30 sec at 94°C, 1 min at 46°C and 1 min at 74°C.

The cDNA sequence data for COL3A1 (1) were submitted to EMBL/GenBank under accession no. X14420.

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Reference: 1) Ala-Kokko *et al.* (1989) *Biochem. J.* **260**, 509–516.

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