G to A polymorphism in exon 45 of the COL1A1 gene

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Source and description of sequence polymorphism

Nucleotide sequencing of type I procollagen (COL1A1) cDNAs from several individuals revealed a nucleotide polymorphism that converted the codon in exon 45 for amino acid 897 of the triple helix from -GCC- (alanine) to -ACC- (threonine) in some alleles. No known endonuclease recognition site was abolished or created. Two alleles were distinguished by allele-specific oligonucleotide hybridization (1) and/or by allele-specific amplification (2).

Allele-specific oligonucleotide hybridization assay

Oligomers used to amplify cDNA

5' - GT GAA TCT GGA CGT GAG GGG - 3'

5' - CCG GTA GTA GCG GCC ACC A - 3'

Oligomers used to amplify genomic DNA

5 - AAG GAT CCC AGG CGG AAG TTC CAT TGG C - 3'

5' - CCG GAA TTC CTG GCC AAG AGC TCA TGC T - 3'

(ASO) hybridization probes

5' - C AGG ACC GGC GGG ACC AGC - 3' (F1: G-allele, antisense)

5' - GCT GGT CCC ACC GGT CCT G - 3' (F2: A-allele, sense)

Allele-specific amplification assay

Oligomer used to amplify G-allele (F1)

5' - GG GCC GAC AGG ACC GGC - 3'

Oligomer used to amplify A-allele, (F2)

5' - GG GCC GAC AGG ACC GGT - 3'

Common oligomer used to amplify both G- and A-alleles

5' - TCC CCT GGA CGA GAC GGT T - 3'

Amplification product was 667 bp.

Frequency: A total of 48 chromosomes were examined. Frequency estimated from 34 chromosomes of unrelated individuals:

F1: G-allele 0.765

F2: A-allele 0.235

	Expected	Observed
G/G	0.585	0.588
G/A	0.360	0.353
A/A	0.055	0.059

Observed Heterozygosity: = 35.3%, PIC = 0.296.

Mendelian Inheritance: Co-dominant inheritance was observed in three two-generation families.

Chromosomal Localization: 17q21.3-q22.

Other Comments: Conditions for amplification were: (a) cDNA for ASO hybridization, 30 cycles consisting of 1 min 30 sec at 94°C, 1 min at 56°C, 3 min at 72°C. (b) Genomic DNA for ASO hybridization, 30 cycles consisting of 1 min 30 sec at 94°C, 1 min 30 sec at 58°C, 3 min at 58°C. (c) Allele-specific amplification, 25 cycles consisting of 1 min 30 sec at 94°C, 1 min 30 sec at 54°C, 2 min at 72°C.

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