

PvuII polymorphism at the COL1A2 locus

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Source/Description: In the course of sequencing a cDNA clone of the prepro $\alpha 2(I)$ chain of human type 1 procollagen (COL1A2), Kuivaniemi, *et al.* (1) observed a nucleotide variation in codon 392 of the $\alpha 2(I)$ triple helical domain which affected a PvuII cleavage site. Subsequently, we observed the same polymorphism in the amplified products of cDNA from a patient with osteogenesis imperfecta and several patients with osteoporosis. In order to investigate the frequency of this polymorphism, we performed the polymerase chain reaction (PCR) (2) on genomic DNA from 40 individuals using the oligonucleotides 5'-GGGATCCTCGGCCCGCTGGAAAAGAA-3' (CDC60, exon 24) and 5'-CCGAATTCACCTTTATCACCGTTTTG-CCA-3' (DS4, exon 26) and digested the 1040 bp product with PvuII (Bethesda Research Laboratories).

Polymorphism: The sequence change detected is a CpA to CpC transversion which does not affect the encoded proline residue at position 392 of the $\alpha 2(I)$ chain. Digestion with PvuII yields 2 fragments of about 550 and 490 bp if CpA is present. The fragments can be resolved on a 4% NuSieve-GTG agarose gel (FMC, Rockland, Maine) (Figure 1).

Frequency: A total of 68 chromosomes from 40 individuals was examined:

PvuII site present: 41 (60.3%) Allele L1

PvuII site absent: 27 (39.7%) Allele L2

Individuals heterozygous for PvuII site: 18 (45%).

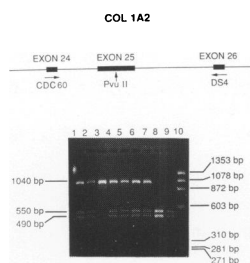
Mendelian Inheritance: Co-dominant inheritance was demonstrated in one three-generation family and in two two-generation families.

Other Comments: The PCR primers have recognition sites for the enzymes BamHI or EcoRI that are useful for cloning the products.

PCR conditions were 1.5 min at 94°C, 1.0 min at 52°C, 1.5 min at 73°C for 5 cycles followed by 30 cycles of 1.5 min at 94°C, 1.0 min at 64°C and 1.5 min at 73°C.

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References: 1) Kuivaniemi, H., Tromp, G., Chu, M.-L. and Prockop, D.J. (1988) *Biochem. J.* **252**, 633–640. 2) Saiki, R.K. *et al.* (1988) *Science* **239**, 487–491.



A HaeIII polymorphism at the D10S101 locus

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Source/Description: KW147 is a recombinant bacteriophage λ clone with a 3.2 kb human insert containing repetitive sequence and was isolated from a human chromosome 10 library constructed by the Lawrence Livermore National Laboratory in the HindIII site of the Charon 21 vector. The library (ID code: LL10N201) was made available through ATCC (American Type Culture Collection). A 0.6 kb HindIII/PstI fragment of the KW147 insert, largely of unique sequence, was subcloned into the phagemid vector (pBluescript II KS+) and designated pKW147a.

Polymorphism: HaeIII detects a simple two-allele polymorphism with bands at either 2.6 kb (A1) or 2.0 kb (A2). A very faint constant band of 1.6 kb was sometimes also seen.

Frequency: Estimated from 40 unrelated Caucasians.

A1: 0.13 ± 0.04

A2: 0.87 ± 0.04

Not Polymorphic For: EcoRI, EcoRV, HincII, HindIII, KpnI, MboI, MspI, PstI and TaqI in ten unrelated Caucasians tested.

Chromosomal Localization: Chromosome 10 origin of this clone has been confirmed by preliminary linkage data showing lod scores greater than 3 with each of the known chromosome 10 markers D10S15, D10S5, and D10S22 (1). Obligate crossovers indicate regional localization of 10p13-q21.1.

Mendelian Inheritance: Co-dominant segregation of the HaeIII RFLP alleles was observed in two of our reference kindreds used for linkage studies (C branch of TSCAN; TSORE) with a total of 125 individuals.

Probe Availability: Contact KKK.

Other Comments: RFLP pattern was observed under normal hybridization and washing conditions for a single-copy probe.

Reference 1) Smith, M. and Simpson, N.E. (1989) HGM10. *Cytogenet. Cell Genet.* **51**, 202–225.