TaqI and HaeIII RFLP polymorphisms in human proteoglycan link gene (CRTL1)

J.T.Hecht, Y.Wang, C.Rhodes[1] and Y.Yamada[1]
University of Texas Medical School, Department of Pediatrics, PO Box 20708, Houston, TX 77225 and [1]National Institute of Dental Research, NIH, Bethesda, MD 20892, USA

Source/Description: Southern blotting was used to detect two new polymorphic sites in the human proteoglycan link gene (CRTL1). Psa002 is a 1.76 kb clone of the human link gene cDNA subcloned into the EcoRI site of pUC18 (Sasaki, unpublished data). It contains the complete coding sequence.

Polymorphisms: TaqI identifies a polymorphic site detected as the presence or absence of a 6.0 kb fragment with constant bands of 10, 5.0, 2.3, 1.9 and 1.8 kb. HaeIII identifies a polymorphic site detected as the presence or absence of a 1.7 kb fragment with constant bands of 2.1, 1.0 and 0.95 kb.

Frequency: TaqI studied in 19 Caucasian individuals and HaeIII in 12.

TaqI	B1+ 0.11	HaeIII	C1+ 0.24
	B2− 0.89		C2− 0.76

Not Polymorphic For: RsaI, MspI, PvuII, StuI, BglI, HindIII, EcoRI, BamHI, BglII and PstI.

Chromosomal Localization: 5q13−14.1 (1).

Mendelian Inheritance: Dominant segregation was observed in 9 and 2 families, respectively, using TaqI and HaeIII. Phase could not be determined and therefore, it is unknown whether these sites are in linkage equilibrium.

Probe Availability: Requests should be sent to Dr Y. Yamada.

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Reference: 1) Osborne-Lawrence *et al.* (1990) *Genomics* **8**, 562−567.

GT repeat polymorphism in the human proteoglycan link gene (CRTL1) promoter region

J.T.Hecht, Y.Wang, C.Rhodes[1] and Y.Yamada[1]
University of Texas Medical School, Department of Pediatrics, PO Box 20708, Houston, TX 77225 and [1]National Institute of Dental Research, NIH, Bethesda, MD 20892, USA

Source/Description: Human proteoglycan link gene (CRTL1) stabilizes the interaction of the proteoglycan core protein to hyaluronic acid in cartilage (1). A GT repeat was identified in the 5′ promoter region of this gene (1, 2). The following sequences were used for PCR amplification: forward primer 5′-CCGCGTGTCCCAGCATCTTC-3′ and reverse primer 5′-TCCTTGGATGACAGAGCTCA-3′. These primers amplified a 219 bp fragment.

Protocol: The reaction was performed using Perkins Elmer Cetus PCR kit, 0.25 μg of DNA and 1 μM of each primer. Genomic DNA was amplified for 30 cycles using a Perkin Elmer Cetus DNA Thermal Cycler with denaturation, for 1 min at 94°C, annealing, for 1 min at 65°C, and extension, for 1 min at 72°C. 10 μl of PCR reaction mixture was analyzed on a polyacrylamide sequencing gel and autoradiographed overnight at room temperature. The amplified fragments ranged in size from 222−240 bp and yielded 10 alleles (Fig. 1).
Observed (%) heterozygosity: 85% in 34 unrelated Caucasians.

Frequencies:

Allele	bp	Frequency	Allele	bp	Frequency
A1	240	1%	A6	228	1%
A2	238	10%	A7	226	9%
A3	236	10%	A8	225	24%
A4	234	12%	A9	224	4%
A5	230	9%	A10	222	19%

Chromosomal Localization: 5q13−14.1 (3).

Mendelian Inheritance: Co-dominant segregation was observed in 9 families in which 44 individuals were typed.

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References: 1) Rhodes *et al.* (1988) *J. Biol. Chem.* **263**, 6063−6067. 2) Rhodes (1991) in press. 3) Osborne-Lawrence *et al.* (1990) *Genomics* **8**, 562−567.

Figure 1. PCR amplification of human link gene GT repeat. Nine of 10 fragments are seen in 11 members of 3 families.