A second hypervariable RFLP within the ABR gene

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We previously identified a hypervariable RFLP (VNTR-B) located at 17p13.3 (1). Here we report a second hypervariable RFLP (VNTR-A) located approximately 9 kb 5' of VNTR-B. Like VNTR-B, VNTR-A is within the ABR gene (2).

Source and Description of Clone: pVNTR-A is a 1.1 kb EcoRI/HindIII fragment in puc19. The probe is available from the ATCC.

Polymorphism: TaqI generates bands from 2.4 to 23 kb that are visible upon hybridization with VNTR-A (see figure; each lane represents the TaqI digested DNA isolated from white blood cells of unrelated individuals). Each allele generates one band. The enzymes BamHI, BglII, BstEII, EcoRI, HindIII and SstI also generate polymorphic bands (2).

Frequency: The frequencies of the alleles are based on the TaqI digestion of DNAs from 51 unrelated individuals. 23 kb=0.186, 18=0.098, 10=0.088, 9.4=0.147, 9.2=0.039, 9.0=0.020, 7.0=0.020, 4.8=0.098, and 2.4 =0.304.

Heterozygosity: A heterozygosity of 88% was observed in these individuals for VNTR-A. The heterozygosity of VNTR-B is 78% (1). The combined heterozygosity of VNTR-A and VNTR-B is >99%.

Mendelian Inheritance: Co-dominant segregation was observed in 4 pedigrees.

Other Comments: Post-hybridization washing was to a stringency of $0.15 \times \text{SSC}$ at 65°C.

Acknowledgement: Supported by NIH grant 1 R01-CA 47456.

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PCR detection of a 38 bp length variant in the COL1A2 gene

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We have previously characterised a fragment length variant within the human $\alpha 2(I)$ collagen gene (COL1A2) which results from the presence or absence of a 38 bp DNA insert within an intron (1) — allele system F (HGM10). An *RsaI* site present within the insert gives rise to an RFLP which has been independently reported (2) — allele system G (HGM10). Since the *RsaI* RFLP arises from the presence or absence of the 38 bp sequence, the two allele systems are equivalent. We now report the sequence of PCR primers which allow the rapid detection of the length variant due to the insert.

PCR Primers:

Sense oligo — 5'TCAGTGTATGTTGCTATCAG 3'

Antisense oligo — 5'ATTCCACAGTCAACATCAAC 3'

Polymorphism: The primers amplify two alleles F1 = 641 bp and F2 = 603 bp (see figure; the band lying between the two alleles in the heterozygote results from the formation of heteroduplexes between the alleles).

Frequency: Allele frequencies for 28 unrelated individuals; F1 = 0.64, F2 = 0.36.

Chromosomal Location: COL1A2 has been localised to 7q21.3-q22.1.

Mendelian Inheritance: Co-dominant inheritance and Hardy-Weinberg equilibrium for the alleles have previously been demonstrated (1, 2).

PCR Conditions: PCR amplifications are carried out in a volume of 20 μ l containing: 0.4 μ g human DNA, 1 μ M each primer, 1.5 mM each dNTP, 7.2 mM MgCl_2 , 16.6 mM $(\text{NH}_4)_2\text{SO}_4$, 67 mM Tris, pH 8.8 (at room temperature), 1.5 units Taq polymerase (Amersham) for 30 cycles. Each cycle consisted of: 94°C for 1 min, 50°C for 1 min and 72°C for 1.5 min. The products are analysed directly by electrophoresis through a 2.5% agarose gel in 1 $\times$ TBE at 70 V. The use of TBE and a high voltage gradient results in excellent resolution of the two alleles.

Acknowledgements: We thank John Keyte for synthesising the primers. This work was supported by grants from Action Research for the Crippled Child and the Medical Research Council. C.J.W. was supported by a grant from the European Social Fund.

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