Dinucleotide repeat polymorphism at the D11S528 locus

X.Y.Hauge, G.A.Evans[1] and M.Litt*
Oregon Health Sciences University, Portland, OR 97201 and [1]Salk Institute, La Jolla, CA 92138, USA

Source and Description of Clone: Cosmid 13,1 was from a human chromosome 11q-specific library (1). p13,1−1 a subclone of this cosmid, was sequenced and the sequences flanking a $(AC)_{17}$ repeat (EMBL accession number X56995) were used to design PCR primers.

PCR Primers:
(#42026)-5′-AATGGTGTCCCCACACATGT-3′
(#42027)-5′-TCCTACCTACCGAGCTTAAA-3′

Polymorphism: Allelic fragments were detected on DNA sequencing gels. Lengths (nt) were: A1 = 91, A2 = 89, A3 = 87, A4 = 83, A5 = 81, A6 = 79, A7 = 77, A8 = 75, A9 = 73.

Frequencies: From 28 unrelated European Caucasians.

A1 = .02	A2 = .02
A3 = .02	A4 = .05
A5 = .02	A6 = .04
A7 = .23	A8 = .05
A9 = .55	

The PIC calculated from these frequencies is 0.60.

Chromosomal Localization: Linkage analysis with THY1 (localized to 11q22.3-q23 (2)) in 5 informative CEPH families gave a maximum lod score of 3.2 at θ = .064.

Mendelian Inheritance: Mendelian inheritance was observed in 5 informative CEPH families with a total 39 children.

PCR Conditions: PCR was performed in a total volume of 25 μl containing: 50 ng genomic DNA, 5 pmole of each primer, 1.5 mM $MgCl_2$, 200 μM dNTPs, 50 mM KCl, 10 mM Tris Cl, pH 8.3, 0.6 units of Taq polymerase (Perkin-Elmer/Cetus) and 0.01% gelatin. Primer #42026 was 5′ end labeled with ^{32}P. Amplification was for 35 cycles with denaturation at 94°C for 1 min, annealing at 52°C for 1 min and extension at 74°C for 1 min. Amplified products were resolved on DNA sequencing gels and detected by autoradiography.

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* To whom correspondence should be addressed

G to A polymorphism in the second exon of the BCL2 gene

Shigeki Tanaka, Jeffrey Kant and John C.Reed*
Department of Pathology and Laboratory Medicine, Laboratory for Molecular Diagnosis, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6082, USA

Source/Description: Direct sequencing of the PCR products (1) of the BCL2 proto-oncogene revealed a G to A polymorphism at nucleotide position 21 (relative to the start codon) (see Figure). This transition results in no amino acid substitution for threonine, #7 of the BCL2 protein. The region −40 to 221 bp of the BCL2 gene was amplified by PCR with the primers shown below from genomic DNA of 28 normal individuals, 9 lymphoma specimens with t(14;18) chromosomes, and 3 cell lines. To detect the G/A polymorphisms, chemical mismatch analysis (2) was performed using as a probe the PCR product of a plasmid DNA containing a BCL2 genomic insert with the G allele (p18−21H). PCR-mismatch analysis resulted in bands at 262 bp and 200 bp corresponding to the full-length and the cleaved ($G\text{-}A_{21}$ polymorphism) probes, respectively (see Figure). DNA from heterozygous individuals (A/G) produces a weaker intensity band at 200 bp and can be distinguished from homozygotes (A/A) by densitometry (not shown). All experiments were repeated at least twice with independently derived PCR products.

Oligomers Used for PCR:
5′ AGA GGT ACC GTT GGC CCC CGT TGC 3′ (−40)
5′ GTC TGC AGC GGC GAG GTC CT 3′ (221)

Frequency: Estimated from 80 chromosomes:
G-allele 0.49
A-allele 0.51

Chromosomal Localization: 18q21.

Other Comments: Conditions for PCR: 40 cycles consisting of 1 minute at 95°C, 2 minutes at 55°C, and 2 minutes at 72°C.

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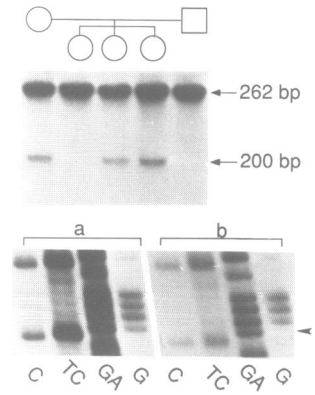

* To whom correspondence should be addressed