A polymorphic CACA repeat in the 3' untranslated region of dystrophin

Alan H.Beggs and Louis M.Kunkel

Genetics Division, Children's Hospital, Howard Hughes Medical Institute, Boston, MA 02115, USA

Introduction: We describe a rapid assay for non-isotopic detection of polymorphism at a CACA repeat (1) located in the 3' untranslated region of the human dystrophin gene Xp21. Oudet *et al.* (2) have independently described this polymorphism using autoradiography of end labeled PCR products on 50% urea sequencing gels.

Source/Description: A CACA repeat consisting of $(CA)_8TA(CA)_7$ was identified at nucleotides 11727–11758 of the human dystrophin cDNA (3). Oligonucleotide primers for PCR were constructed from sequences 70 bp upstream and 31 bp downstream:

3'CA-F: 5'-GAAAGATTGTAACTAAAGTGTGC-3' (nucleotides 11657–11680)
3'CA-R: 5'-GGATGCAAAACAATGCGCTGCCTC-3' (nucleotides 11789–11766)

Polymorphism: PCR with these primers produces four different sized products designated as A1, A2, A3 and A4 in order of decreasing size. The 133 bp product $[(CA)_8TA(CA)_7]$ sequenced in (3) corresponds to A2.

Frequency: From 9 males and 32 females (unrelated). A1 = 0.22, A2 = 0.70, A3 = 0.07, A4 = 0.01. 50% of females are heterozygous. PIC = 0.46.

Mendelian Inheritance: X-linked co-dominant segregation was observed in two families (3 generation and 2 generation) with 21 total individuals (see figure below).

Other Comments: PCR reactions (50 μ l) using reagents from Perkin Elmer Cetus (Norwalk, CT) GeneAmp kits contained each primer at 0.5 μ M, 5 units Taq polymerase and 250 ng genomic DNA. Amplification conditions were: 94°C, 7 min. followed by 25 cycles of 94°C, 30 sec.; 65°C, 4 min (step cycle) with the last annealing/elongation cycle for 10 min. Reaction products (20 μ l) were separated on 8% polyacrylamide (1X TBE) gels and stained with EtBr. Figure illustrates segregation of A1 with DMD mutation in a family as well as A3 and A4 in four unrelated females (lanes 1–4).

Acknowledgements: Thanks to Ulrich Muller for helpful discussions and Umadevi Tantravahi for DNA samples.

References: 1) Weber, J.L. and May, P.E. (1989) *Am. J. Hum. Genet.* **44**, 388–396. 2) Oudet, C., Heilig, R. and Mandel, J.L. (1990) *Hum. Genet.* In press. 3) Koenig, M., Monaco, A.P. and Kunkel, L.M. (1988) *Cell* **53**, 219–228.

D21S194, a jump clone from D21S16

A.P.Walker, F.S.Collins¹, T.Siddique, L.H.Yamaoka, M.H.Herbstreith, M.A.Pericak-Vance, S.L.Secore, W.-Y.Hung, A.M.Goate², J.A.Hardy², D.Patterson³, A.D.Roses and R.J.Bartlett

Duke University Medical Center, Durham, NC 27710, USA, ¹University of Michigan, Ann Arbor, MI 48109, ²St. Mary's Hospital Medical School, London W2 1PG, UK and ³Eleanor Roosevelt Institute for Cancer Research, Denver, CO 80262, USA

Source and Description of Clone: AW8-1J (D21S194) was isolated from a 100kb jumping library (1) in lambda Ch3AΔlacXba by plaque purification of a clone which showed positive hybridization with pGSE9 (D21S16). The 'landing side' of the jump clone insert is a 3kb EcoRI–MboI fragment; it is defined by a pair of primers derived from flanking vector sequences, permitting direct amplification by PCR (2).

Polymorphism: BamHI (G↓GATCC) identifies two alleles with bands at 4.6kb and 12.3kb, and a faint constant band at 6.9kb.

Frequency: Studied in 61 unrelated individuals (122 chromosomes): 12.3kb allele 0.51; 4.6kb allele 0.49

Not Polymorphic For: BanI, BglII, EcoRI, EcoRV, HincII, HindIII, MspI, NcoI, PstI, PvuII, RsaI, SacI, StuI, TaqI and XbaI with panels of 6 unrelated individuals.

Chromosome Location: 21q11.2 using a panel of human-rodent cell lines segregating portions of CH 21. Two-point linkage data confirm localization to the centromeric region of CH 21.

Mendelian Inheritance: Co-dominant segregation demonstrated in two 7-generation families (54 and 25 individuals tested) and two 6-generation families (17 and 16 individuals tested).

Probe Availability: Available for collaborative studies.

References: 1. Collins, F.S., *et al.* (1987) *Science* **235**, 1046–1049. 2. Walker, A.P. *et al.* manuscript in preparation.

Acknowledgements: Supported by Clinical Research Grants (ADR, RJB) and a Postdoctoral Fellowship (APW) from the M.D.A.; NINDS grant NS19999 (MAPV, ADR); Grants NS21442 (TS), AG00029, HD17449 (DP) and GM34960 (FSC) from the NIH; A.D.A./Allied Signal Faculty Scholar Award (RJB); and a L.E.A.D. award AG07922 from the NIA (ADR). F.S.C. is an Associate Investigator of the Howard Hughes Medical Institute. We thank Dr Gordon D.Stewart for probe pGSE9.