Dinucleotide repeat polymorphism in the NEC2 gene

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Source/Description: The isolation and characterization of the intron-exon organization of human *NEC2* has been described (1). The gene was screened for regions of CA-dinucleotide repeats and a sequence of the form $(CA)_{20}$ was identified in intron 2 (GenBank accession no. L21850). Primers were selected from the flanking sequence to amplify this repeat.

Primer Sequences:
PC2S2-CA-05, 5'-AAGTGAAAGTCTGTGATAGC-3' (GT strand)
PC2S2-CA-06, 5'-AGGCCTACCTACTTGTAAGA-3' (CA strand)

Frequency: Seven alleles were observed in 39 unrelated Caucasians. The heterozygosity was 0.76.

Allele	Size (bp)	Frequency	Allele	Size (bp)	Frequency
A1	200	0.04	A5	182	0.27
A2	190	0.01	A6	180	0.33
A3	186	0.04	A7	178	0.12
A4	184	0.19			

The genotypes of CEPH family members 1333-11 and -12 are 3,5 and 5,5, respectively.

Chromosomal Localization: *NEC2* has been localized to chromosome band 20p11.2 (1). Pairwise linkage analysis showed linkage of *NEC2* with other chromosome 20p markers including D20S18 (Z_{max} = 9.3, $\theta_{m=f}$ = 0.03) and D20S58 (Z_{max} = 10.5, $\theta_{m=f}$ = 0.04).

Mendelian Inheritance: Co-dominant segregation was observed in 12 CEPH families.

Other Comments: The PCR was performed using ^{32}P-labelled PC2S2-CA-05 and unlabelled PC2S2-CA-06 for 30 cycles: denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min with a final extension step at 72°C for 10 min. The PCR products were analyzed on a 5% denaturing polyacrylamide gel.

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Reference: 1) Ohagi,S. *et al.* (1992) *Proc. Natl. Acad. Sci. USA* **89**, 4977–4981.

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Single base polymorphism in the DAG1 gene detected by DGGE and mismatch PCR

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Description/Polymorphism: We report a polymorphism in exon 2 of the human dystroglycan gene (accession No. L19711) and two methods for analysis of this polymorphism. A polymorphic synonymous mutation (H752H) which is a C to T substitution in the third nucleotide of the His^{752} codon of the dystroglycan gene was initially identified by DGGE analysis (1, 2).

Primer Sequences for DGGE Analysis:
D2F: 5'AGCCTGACTTTAAGGCCACAAGCA 3'
D2R: 5'CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCGT-CCTGCAGAATGAGTGGCATGCTG 3'

Amplification products were subjected to DGGE on an 8% acrylamide gel containing a 45–75% denaturing gradient (100% denaturant defined as 40% formamide, 7M urea) at 150V fo 7 hours. Sequencing of the polymorphic region revealed alleles A1 (CAC) and A2 (CAT). A mismatch PCR assay was developed which allows rapid and efficient screening for the H752H polymorphism.

Primers for Mismatch PCR:
BspF: 5'ACAGGGACCCTGAGAAGAGCAGTGAGGATGATGTCTA-CGTGCA 3'
BspR: 5'TGAAGGTGGCCTGGTCCTCAAGGGTAAG 3'

The BspF primer was designed to incorporate the polymorphic site into the Bsp1286 I restriction site. This primer has a single base mismatch (underlined) which creates a diagnostic Bsp 1286 1 site for A1 allele (CAC), but not A2 allele (CAT). PCR amplification of the allele A1 followed by Bsp1286 I digestion will produce two bands—119 bp and 42 bp, while only one band of 161 bp will be obtained for allele A2. These products can clearly be detected with a 2% agarose gel.

Allele Frequency: Determined by analysis of 50 unrelated individuals: Allele A1: 0.80; allele A2: 0.20. Observed heterozygosity is 0.32.

Chromosomal Localization: 3p21.

Mendelian Inheritance: Mendelian inheritance was demonstrated in three multigeneration families.

PCR Conditions: 1 min 94°C, 1 min 62°C, 1 min 72°C for 40 cycles with 100 ng genomic DNA for both sets of primers.

Other Comments: This intragenic polymorphism will be useful for linkage studies of neuromuscular diseases.

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References: 1) Myers,R.M. *et al.* (1987) *Methods Enzymol.* **155**, 501–527. 2) Sheffield,V.C. *et al.* (1989) *Proc. Natl. Acad. Sci. USA* **86**, 232–236.

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