

Adhalin gene polymorphism

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Source/Description: Adhalin is a 50 kD glycoprotein belonging to the dystrophin-glycoprotein complex (1) missing in severe childhood autosomal recessive muscular dystrophy or SCARMD (2). The corresponding cDNA has been cloned in rabbit (1), and subsequently the cDNA and gene have been cloned in human in which it maps to chromosome 17q12-q21.33 (3). The gene contains a (CA)_n repeat in intron 6 (3). This intragenic marker (D 17S 1319) was used to demonstrate linkage between the adhalin gene and the SCARMD locus in one affected French family (3).

Primer Sequences:

Forward: 5' TATCTCCGCTCTCTGATTGCTCC 3'

Reverse: 5' TTGCGGACTCTGTTGCCCTTGT 3'

Polymorphism: Allele frequencies were estimated from 50 unrelated individuals from the CEPH reference panel.

The observed heterozygosity was 0.76.

Allele	Size (bp)	Frequency	Allele	Size (bp)	Frequency
A1	170	0.1	A5	162	0.36
A2	168	0.2	A6	160	0.24
A3	166	0.02	A7	158	0.04
A4	164	0.04			

Genotypes of CEPH individuals: 1331.01: A2/A2; 1331.02: A3/A5; 1332.01: A2/A5; 1332.02: A1/A2.

Chromosomal Localization: 17q12—q21.33 within the adhalin gene. Multipoint linkage analysis in a number of CEPH families allowed the integration of the (CA)_n repeat to the 17 q regional linkage Généthon/CEPH map (4).

Mendelian Inheritance: Co-dominant segregation was observed in 8 CEPH families.

PCR Conditions: PCR was performed using 100-200 ng of genomic DNA, 100 ng of each primer and 2.5 U *Taq* polymerase (NBL, UK). Amplification reactions were carried out for 35 cycles consisting of 40 s at 92°C and 30 s at 63°C.

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Tri- and tetranucleotide repeat polymorphism in the LIPA gene

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Source/Description: A (TAAA)_n(TAA)_m mixed simple repeat was located at the 3' end of an Alu repeat in the first intron of the human lysosomal acid lipase gene (LIPA) when the exon-intron organisation was analyzed (1). Primers flanking the simple repeat and the Alu repeat were used to amplify a 449 bp fragment in the original λ clone containing the repeat sequence. Upon digestion of the PCR-fragment with *AluI* a 169 bp DNA fragment was generated that is polymorphic in the population.

PCR Primers:

CALIPA1 5'-GGCTGGTCTCAGATGACTTCGTG-3'

CALIPA2 5'-GTCATATATCAACTGGAGGTGGTGG-3'

Allele Frequency: Estimated from 132 chromosomes of unrelated Caucasian individuals. Observed heterozygosity 0.79.

Allele	Size (bp)	Frequency	Allele	Size (bp)	Frequency
A1	193	0.01	A6	176	0.13
A2	190	0.04	A7	173	0.02
A3	189	0.07	A8	169	0.29
A4	186	0.37	A9	164	0.02
A5	182	0.02	A10	157	0.03

Chromosomal Localization: The gene has been mapped previously to 10q23.2—q23.3 using fluorescence *in situ* hybridization on metaphase chromosome spreads (2).

Mendelian Inheritance: Observed in a large (38 member) four generation pedigree (3).

PCR Conditions: DNA (100 ng) was amplified in a volume of 50 μ l containing 50 pmol of each oligonucleotide primer, 0.2 mM of each dNTP, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM MgCl₂; and 1 unit *Taq* polymerase. Following an initial denaturation at 94°C for 2 min a two step amplification was carried out for 35 cycles in a Perkin-Elmer thermal cycler (9600) as follows: 1 min 96°C, 2.5 min 68°C, followed by a final extension at 72°C for 5 min. *AluI* restriction endonuclease (5 units) and 1 X restriction buffer were added to the non-purified PCR products, incubated for 2 h at 37°C, and the resulting DNA fragments were separated by electrophoresis on a 7 M urea, 6% polyacrylamide denaturing gel (equivalents of 1 μ l PCR reaction) and were analyzed by silver staining as described elsewhere (4). Alternatively, 1 μ Ci [³²P]dCTP (3000 Ci/mmol) was added in the PCR reaction and the labelled DNA fragments were visualized on X-ray films. Allele sizes were determined using a pUC19 sequence as molecular weight marker and by cloning and sequencing of different PCR products.

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