

An *Msp*I polymorphism in the human acid sphingomyelinase gene (SMPD1)

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Source/Description: Sequencing of partial and full-length cDNAs encoding human SMPD1 (1, 2) revealed an A (Allele A1) or G (Allele A2) at nucleotide position 1516 which predicted an Arg (Allele A1) or Gly (Allele A2) at codon 506. The deficient activity of SMPD1 results in Types A and B Niemann-Pick disease (NPD).

Polymorphism Frequency: The frequency of the 506-Gly (A2) allele in PCR-amplified genomic DNA from 110 unrelated Caucasian individuals by dot-blot hybridization was 0.84. The 506-Gly allelic frequency among 56 unrelated NPD Type A and B patients was 0.78.

Protocol: To detect the 506 polymorphism, a 567 bp SMPD1 genomic fragment is amplified using sense (5'-AGTAGTCG-ACATGGGCAGGATGTGTGG-3') and antisense (5'-AGTA-GTGTCGACTTGCCTGGTTGAACCACAGC-3') primers. Dot-blot hybridization is performed using allele-specific oligonucleotides for 506-Arg (5'-ACTACTCCAGGAGCTCT-3') and for 506-Gly (5'-ACTACTCCGGGAGCTCT-3'), which are hybridized at 42°C and washed at 51 or 53°C, respectively. This polymorphism also can be detected by restriction enzyme analysis since the 506-Gly polymorphism creates a new *Msp*I restriction site. When the 567 bp PCR-amplified genomic fragment from a 506-Arg allele is digested with *Msp*I, two fragments of 395 and 159 bp are detected on a 1% agarose gel. In contrast, amplification of the 506-Gly allele results in the constant fragment of 395 bp, but the 159 bp fragment is digested into two fragments of 95 and 64 bp.

Chromosomal Location: The human SMPD1 gene has been mapped to the chromosomal region 11p15.1-p15.4 (3).

Other Comments: This polymorphism may serve as a useful marker for the orientation of other polymorphic variants and/or sequence tagged sites in this region of chromosome 11.

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References: 1) Quintern *et al.* (1989) *EMBO J.* **8**, 2469-2473. 2) Schuchman *et al.* (1991) *J. Biol. Chem.* **66**, 8531-8539. 3) Pereira *et al.* (1991) *Genomics* **9**, 229-234.

Dinucleotide repeat polymorphism in CEA gene

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Source/Description: Sequence of cosmid TW7 provided the information necessary for oligonucleotide primer synthesis. The predicted length of the amplified fragment was 104 base pairs.

Primer Sequences: ATGGAGACACCACTGCTGAACCCT (CA strand); GTCCTGAAGCCAAGGTCTCTTCAT (GT strand).

Frequency: Estimated from 94 unrelated Caucasian individuals, 188 chromosomes. PIC = 0.65.

Allele (bp)	Frequency	Allele (bp)	Frequency
A9: 104	0.147	A4: 114	0.032
A8: 106	0.037	A3: 116	0.204
A7: 108	0.497	A2: 118	0.005
A6: 110	0.010	A1: 120	0.010
A5: 112	0.058		

Chromosome Localization: Assigned to chromosome 19q13.1-13.3 by in situ hybridisation and somatic cell hybrids.

Mendelian Inheritance: Co-dominant segregration in 23 families.

Other Comments: Conditions for amplification reactions were 25 cycles consisting of 0.5 min at 94°C, 0.7 min at 70°C, 0.7 min at 71°C. The sizes of the alleles were determined by comparison to pUC9 cut with HpaII and the range of APOC2 'AC' repeat allele sizes. The dinucleotide repeat sequence of CEA is of the imperfect form AC_{13} TCACTCAC, and is located in intron 1 of the CEA gene.

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Reference: 1) Willcocks,T.C. and Craig,I.W. (1990) *Genomics* **8**, 492-500.