

Detection of PstI RFLP in human ADA by the polymerase chain reaction

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Source/Description: Two primers derived from the sequence flanking the polymorphic PstI site in intron 2 of the adenosine deaminase (ADA) gene (1, 2) were selected for polymerase chain reaction (PCR) to amplify a 356 bp fragment that spans the PstI polymorphism. Polymorphism was detected by PstI digestion of the PCR product.

Primer Sequences

Forward primer: 5'-AGAACTGCATGTCCACCTAG-3'

Reverse primer: 5'-AACTACCTTCATGCACGTATG-3'

Polymorphism: K1 allele that lacks the polymorphic PstI site generates a full-length 356 bp fragment on PstI digestion. K2 allele that contains the PstI site generates fragments at 239 and 117 bp on PstI digestion.

Frequency: Estimated from 55 unrelated Japanese individuals.

K1: 0.20

K2: 0.80

Chromosomal Localization: The ADA gene has been assigned to chromosome 20q12-q13.11 (3).

Mendelian Inheritance: Co-dominant inheritance was observed in two families.

Other Comments: The PCR was performed as previously described (4) with the following modification: (a) denaturation at 94°C for 1 min, (b) annealing at 58°C for 2 min, (c) extension at 72°C for 3 min, and (d) 35 cycles were used. The PCR products were digested with PstI and analyzed on a 9% polyacrylamide gel (Fig. 1).

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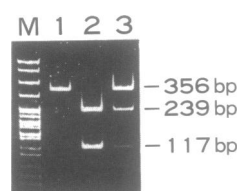


Figure 1. PstI RFLP of the ADA gene detected by PCR. M: molecular weight marker (pBR322 DNA-MspI digest), lane 1: homozygote for A1, lane 2: homozygote for A2, lane 3: heterozygote for A1/A2.

MspI RFLP in the L1 CAM gene in Xq28

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Description: pHK13 is an EcoRI fragment derived from the human neural adhesion molecule L1 CAM (1, 2).

Polymorphism: MspI detects a two-allele polymorphism with fragment lengths of either 2.5 kb (allele A1) or 1.9 kb (allele A2) and constant bands of 1.8 and 1.4 kb.

Frequency: Studied in 154 unrelated Caucasian individuals

A1 = 0.95

A2 = 0.05

Not Polymorphic For: BamHI, BclI, BglI, EcoRI, HincII, HindIII, PstI, PvuII, SstI, TaqI.

Chromosomal Localization: L1 CAM has been localized to Xq28 by in situ hybridization and pulsed-field gel analysis (1).

Mendelian Inheritance: X-linked inheritance was observed in 2 informative families.

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