PCR detection of a C/T polymorphism in exon 1 of the porphobilinogen deaminase gene (PBGD)

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Source/Description: Sequencing of exon 1 revealed a C/T polymorphism in exon 1 of human porphobilinogen deaminase gene (PBGD) at position -64 relatively to the initiation translational codon (1). Genetic defects of PBGD are responsible for acute intermittent porphyria. The use of a 5' primer with a mutated sequence to amplify the region containing this polymorphism allows its detection by restriction analysis.

Primer Sequences:

POLY1: 5'CGGTACTCGCCGGCCGGGGCC 3' (exon 1). This primer introduces a mutation in the PCR products, the natural sequence being: 5'CGGTACTCGCCGGCCGGAGCC 3'. The mutation introduces a ApaI site only when the polymorphic base, located 3' of this primer is C. PHI1AS: 5'CTCGTCCAGAA-GCCCAAAGTGTG 3' (intron 1).

Polymorphism: After a ApaI digest of the amplified fragment, two alleles can be identified: F1: 164 bp, F2: 145 bp + 19 bp.

Frequency: Allelic frequency:

F1: 37%

F2: 63%

From 35 unrelated individuals of European Caucasoid origin.

Chromosomal Localization: 11q23 (2).

Mendelian Inheritance: Codominant inheritance was demonstrated in two large families with AIP.

PCR Conditions: The PCR is carried out in a total volume of 50 µl containing 250 ng genomic DNA, 1 unit of Taq polymerase, 25 pmol of each primer, 200 µM dNTPs, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 2 mM MgCl₂, 10% glycerol, 5% formamide. After an initial denaturation step at 95°C for 1 min, the amplification is performed for 30 cycles with denaturing at 94°C 10 sec, annealing at 52°C 30 sec and extension at 72°C for 40 sec, using a Hybaid thermocycler. The amplified fragment is digested with ApaI and the DNA fragments are analyzed on 4% Nusieve-agarose gels.

References: 1) Chretien, S., Dubart, A. *et al.* (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6-10. 2) Wang, A.L., Arredondo-Vega, F.X. *et al.* (1981) *Proc. Natl. Acad. Sci. USA* **78**, 5734-5738.

TaqI polymorphism at the human preproendothelin-1 gene (EDN1)

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Source/Description: The 1.17 kb cDNA insert of plasmid pHET4-3 was used as a hybridization probe (1).

Polymorphism: TaqI digestion identifies a biallelic RFLP with bands of 6.7 (A1) and 5.5 (A2) kb.

Frequency: Estimated from 32 unrelated individuals (Japanese). Observed PIC = 0.34.

A1: 0.67

A2: 0.33

Not Polymorphic For: BglII, HindIII, PstI, AvaI, BamHI. (each enzyme tested on sixteen unrelated individuals).

Chromosomal Localization: The probe pHET4-3 has been previously localized to 6p23-p24 (2).

Mendelian Inheritance: Codominant segregation was observed in a two generation family.

Probe Availability: Contact Dr M.Yanagisawa (1) (2).

References: 1) Ito, Y. *et al.* (1988) *FEBS Letters* **2**, 440-444. 2) Inoue, *et al.* (1989) *J. Biol. Chem.* **264**, 14954-14959. 3) Arinami, T. *et al.* (1991) *Am. J. Hum. Genet.* **48**, 990-996.

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