Polymorphism in the APNH gene, detected by denaturing gradient gel electrophoresis

Yale University School of Medicine, Departments of Internal Medicine and Human Genetics, 333 Cedar Street, New Haven, CT 06510, USA

Description: A 376 bp genomic DNA fragment corresponding to nucleotides -25 — 351 of the published cDNA coding sequence for the Na+/H+ antiport gene, APNH (1), was amplified by PCR using 5'-CGAGGCTGGCTCTGGAAGCAGCACCATG-3' and 5'-TATCTTCATGAGGCAGCAAGGAT-3' as the 5' and 3' primers respectively. PCR was performed with a programmable thermal cycler (MJ Research Inc.) using the following conditions: 94°C 1 min., 72°C 1 min. for 5 cycles, and 94°C 1 min., 65°C 30 sec., 72°C 30 sec for 30 cycles. Each 100 μl sample contained 100 ng of genomic DNA, reaction buffer (50 mM KCl, 10 mM Tris-HCl pH8.3, 1.5 mM MgCl₂, 0.001% gelatin) 1 μM of each primer, 800 μM mixed dNTPs, 2.5U Taq DNA polymerase and distilled deionized water. This product was then subjected to DGGE on a 35 - 65 % gradient (100% = 7M urea, 40% formamide) at 75V for 18 hours. By allowing denatured PCR products to reanneal before DGGE, heteroduplex DNA is created in regions where sequence polymorphism exists.

Polymorphism: As figure 1 demonstrates, a two allele polymorphism was identified.

Frequency: In 86 unrelated chromosomes allele frequency is:

A₁ 0.59
A₂ 0.41

Chromosomal Localization: APNH has been localized to 1p35—p36.1 (2). Using linkage analysis, we have mapped it telomeric to D1S57 and close to RH and ALPL (3).

Mendelian Inheritance: Co-dominant segregation was demonstrated in six families of 202 individuals.

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XmnI polymorphism in the human TPA gene

K.-D. Wohm, M. Ludwig, G. Langer, K. Olek and W.-D. Schleuning
Research Laboratories of Schering AG, Berlin/Bergkamen, Müllerstraße 170—178, PO Box 65 03 11, 1000 Berlin 65 and Institute of Experimental Haematology and Blood Transfusion, Sigmund-Freud-Straße 25, 5300 Bonn 1, FRG

Source/Description: A 971-bp PCR-amplified genomic DNA fragment of the human TPA gene. The sequences of the primers were as follows with the corresponding nucleotides of the TPA gene (1) indicated in parentheses: 5'-TTACAGCCATGCTGTCAGCAGTGGC-3' (27187—27205), 5'-ACTGGCGTCAGTGCCTGATCAGG-3' (28141—28158).

Polymorphism: XmnI (Asp 700) identifies a two allele polymorphism with bands at either 5.9 kb or 5.6 kb.

Frequency: Studied in 23 unrelated Caucasians

5.9 kb allele (A1) 0.59
5.6 kb allele (A2) 0.41

Not Polymorphic For: BamHI, SphI.

Chromosomal Location: Chromosome 8, bands 8p12—q11.2 (2).

Mendelian Inheritance: Co-dominant segregation shown in three families of German origin.

PCR Conditions: PCR was performed using a Perkin Elmer Cetus Kit employing 1 μg of genomic DNA under the following conditions: 35 cycles of 60-s denaturation at 94°C, 60-s annealing at 58°C, and 180-s elongation at 72°C. The elongation time was prolonged for 10 s in each cycle. The amplification product was purified in a Centricon-30 centrifugation device (Amicon). 100 ng were used for nick translation.

Other Comments: The TPA cDNA identifies the same polymorphism together with three invariable bands.

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Figure 1. Lanes 1, 2, 5 and 7 are individuals homozygous for A₁. Lane 3 is homozygous for A₂. Heteroduplexes are seen in individuals heterozygous for each allele (lanes 4 and 6).