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

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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'-TATCTTCATGAGGCAGGCCAGAGGAT-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 DIS57 and close to RH and ALPL (3).

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

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References: (1) Sardet *et al.* (1989) *Cell* **56**, 271–280. (2) Mattei *et al.* (1988) *Cytogen. Cell Genet.* **48**, 6–8. (3) Dudley *et al.* (1990) *Hum. Genet.* (in press).

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).

Xmnl polymorphism in the human TPA gene

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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'-TTACAGCCATGCTGATGGC-3' (27187–27205), 5'-ACTGGCGTCAGTGCCTGA-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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