Dinucleotide repeat polymorphism at the human liver-type 6-phosphofructokinase (PFKL) gene

Mihael H. Polymeropoulos, Denise S. Rath, Hong Xiao and Carl R. Merrill

National Institute of Mental Health Neuroscience Center, St Elizabeths Hospital, Room 131, 2700 Martin Luther King Avenue, Washington, DC 20032, USA

Source/Description: The polymorphic (AC)_n repeat begins at base pair 1675 in exon 1 of the human liver-type 6-phosphofructokinase (PFKL) gene (1). The polymorphism can be typed using the polymerase chain reaction (PCR) as described previously (2). The predicted length of the amplified sequence was 135 bp.

Primer Sequences: GTGGTACCTCATTGTGGCTA (AC strand); AGGCATCCTTGTGCTGACAT (TG strand).

Frequency: Estimated from 50 chromosomes of unrelated individuals. Observed heterozygosity = 70%. PIC = 0.66.

Allele (bp)	Frequency	Allele (bp)	Frequency
B1 145	0.02	B5 133	0.24
B2 139	0.16	B6 131	0.04
B3 137	0.06	B7 129	0.02
B4 135	0.46		

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

Chromosomal Localization: PFKL has been assigned to chromosome 21q22.3 (1).

Other Comments: The PCR reaction was performed on 80 ng of genomic DNA using 100 pmoles of each oligonucleotide primer. The samples were processed as described (3) except that the denaturation cycle at 94°C was extended to 1.4 minutes. The dinucleotide repeat was based on a (AC)₁₆ sequence.

References: 1) Elson, A. *et al.* (1990) *Genomics* 7, 47–56. 2) Weber, J.L. and May, P.E. (1989) *Am. J. Hum. Genet.* 44, 388–396. 3) Weber, J.L. *et al.* (1990) *Nucl. Acids Res.* 18, 4637.

A HinfI polymorphism in the cystic fibrosis gene CFTR

Thilo Dörk, Ulrich Wulbrand and Burkhard Tümmler
Abt. Biophysikalische Chemie, OE 4350, Medizinische Hochschule, Konstanty Gutschow Str. 8, D-3000 Hannover 61, FRG

Source and Description: Direct genomic sequencing revealed a C to A polymorphism 65 nucleotides in front of exon 19 (nucleotide 3601–65) of the CFTR gene that is causative defective in cystic fibrosis (CF) (1). This polymorphism generates cleavage sites for HinfI and MboII and is useful as an intragenic marker for the assignment of marker haplotypes of known CF mutations and for carrier screening and prenatal diagnosis in CF families with yet unknown mutations in CFTR.

Primer Sequences:

19i-5 5'-GCCCCACAAATAACCAAGTGA-3' (2);

TUB19 5'-GGTAGGTTTACCTTCTGTTGGCATG-3' (nucleotides 3660–3636 of the CFTR cDNA (1))

Protocol: We carry out PCR in a total volume of 50 µl containing: 0.5 µg genomic DNA, 20 pmoles of each primer, 1.5 mM MgCl₂, 200 µM dNTPs, 50 mM KCl, 10 mM Tris/HCl, pH 8.3, 0.01% gelatin, and 1.5 units of Taq polymerase. Amplification is for 35 cycles with denaturation at 92°C for 60 s, annealing at 57°C for 60 s, and extension at 72°C for 60 s.

Polymorphism: HinfI digestion of the 188 bp PCR product separated on a 3% NuSieve agarose gel identifies a two-allele polymorphism with fragments at either 145 and 43 bp (F1) or 82, 63, and 43 bp (F2).

Frequency: Allele frequencies for 50 non-CF, 75 ΔF508 CF and 71 non-ΔF508 CF German chromosomes:

	non-CF	ΔF508 CF	non-ΔF508 CF
F1	0.76	0.96	0.63
F2	0.24	0.04	0.37

Mendelian Inheritance: Autosomal co-dominant segregation was observed in 25 unrelated two-generation German families.

Chromosomal Localization: The CFTR gene has been assigned to 7q31 (3).

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