A dinucleotide repeat polymorphism at the Kallmann locus (Xp22.3)

P.-M.G.Bouloux, J.-P.Hardelin[2], P.Munroe, J.M.W.Kirk[1], R.Legouis[2], J.Levilliers[2], J.Hazan[2], J.Weissenbach[2] and C.Petit[2]

Academic Department of Medicine, Royal Free Hospital, London, [1]Department of Endocrinology, St Bartholomew's Hospital, London, UK and [2]Unite de Genetique Moleculaire Humaine, Institut Pasteur, Paris, France

Source and Description: Lambda clone 135 derived from a YAC from the CEPH library, mapping to an interval containing at least part of the Kallmann locus (1). DNA sequences flanking a (C-A)17 repeat of the lambda clone were used to design the polymerase chain reaction (PCR) primers. The polymorphism can be typed using PCR as previously described (2).

PCR Primers:
5′ CCCAAAGTAAGGATTTTGCCAC 3′
5′ TAGATCCTATTTGCCAATTTTTG 3′

Polymorphism: Five allelic fragments were resolved on DNA sequencing gels which differed in size from the predicted length (179 bp). These were termed A1, A2, A3, A4, A5 in ascending order of size.

Frequency: Allele frequencies, estimated from 40 CEPH grandmothers:

Frequency	Allele (bp)
A1 0.33	179
A2 0.15	181
A3 0.09	183
A4 0.25	185
A5 0.19	187

Observed heterozygosity 0.61
Calculated PIC 0.72

Chromosomal Localization: The sequence maps to Xp22.3 at a distance of 8700 Kb from the Xp telomere (1 and Hardelin *et al.*, unpublished observations).

Mendelian Inheritance: X-linked inheritance was observed in 4 three generation families.

Other Comments: The PCR reaction was performed in a total volume of 50 µl using 100 ng of genomic DNA and 100 pmol each of each oligonucleotide primer. Conditions of the PCR amplification were as follows: 1) 5 min at 94°C, 2) 2 min at 55°C, 3) 2 min at 72°C, 4) 30 cycles of 1 min at 94°C, 1 min at 55°C and 2 min at 72°C 5), 1 min at 55°C and 6) 5 min at 72°C.

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A polymorphism in intron 20 of the CFTR gene

I.Quere, H.Guillermit, B.Mercier, M.P.Audrezet and C.Ferec*

Centre de Biogénétique, CDTS, BP 454, 29275 Brest, France

Source/Description: We report a sequence variation in the intron 20 of the human CFTR gene.

Chromosome Location: 7q3.1q3.2 (1).

Polymorphism: The nucleotide change is A→G at position 4006–200 of intron 20 (2). To detect that polymorphism, we developed an allele specific amplification assay. The primer corresponding to the most frequent allele was AGGGACTCC-AAATATTGCTG. The second corresponding to the change was AGGGACTCCAAATATTGCTA with the antisense primer CC-AAGTTTTTTCTAAATGTTCCAG.

Protocol: PCR amplification (30 cycles: 30 sec at 94°C, 1 min at 60°C, 1 min at 72°C) was performed in a 100 µl reaction mixture containing 50 mM KCl, 10 mM Tris HCl pH 8.8, 1.5 mM $MgCl_2$, 200 µM dNTPs, 50 pmoles of each primer, 0.3 µg of genomic DNA and Taq polymerase (2 units).

Frequency: (estimated from 60 normal chromosomes):

Normal chromosomes	Allele I (4006–200 = G)	50/60 = 0.83
	Allele II (4006–200 = A)	10/60 = 0.17
CF chromosomes		
chromosomes ΔF508	Allele I (40/40)	Allele II = 0/40
chromosomes non ΔF508	Allele I (69/87) (0.79)	Allele II = 18/87 (0.21)

Other Comments: The ΔF508 accounts for approximately 75% of CF chromosomes worldwide. Many other mutations have been reported. That polymorphism, located inside the CFTR gene, may be useful to increase informativity in family carrier testing or prenatal diagnosis. The allele specific amplification we used works well despite the G-T and A-C mismatches are not a priori specific. Another point is the absence of linkage disequilibrium between non ΔF508 chromosomes and normal chromosomes with respect to that polymorphism and on the other hand the absolute linkage of the ΔF508 chromosomes with the I (4006–200) allele, this strengthens the hypothesis of an identical common ancestral chromosome carrying the ΔF508 mutation.

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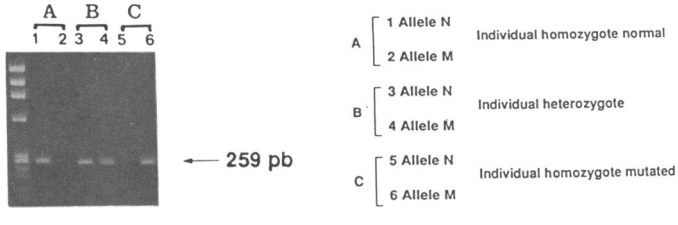
References: 1) Kerem,B.S. *et al.* (1989) *Science* **245**, 1073–1080. 2) Zielenski,J. *et al.* (1991) *Genomics* **10**, 214–228.



* To whom correspondence should be addressed