Mutation analysis in 600 French cystic fibrosis patients

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Abstract
The cystic fibrosis transmembrane conductance regulator (CFTR) gene of 600 unrelated cystic fibrosis (CF) patients living in France (excluding Brittany) was screened for 105 different mutations. This analysis resulted in the identification of 86% of the CF alleles and complete genotyping of 76% of the patients. The most frequent mutations in this population after ΔF508 (69% of the CF chromosomes) are G542X (3-3%), N1303K (1-8%), W1282X (1-5%), 1717-1G→A (1-3%), 2184delA→2183 A→G (0-9%), and R553X (0-8%).

METHODS
Fourteen exons of the CFTR gene and their flanking intron-exon junctions were amplified according to Zielenowski et al9 from genomic DNA. Methods used to detect mutations are: (1) heteroduplex formation followed by polyacrylamide gel electrophoresis (PAGE);17 (2) allele specific oligonucleotide hybridisation (ASO); (3) restriction enzyme digestion showing created or abolished sites. Mutations detected by the abolition of a restriction enzyme site were confirmed by ASO. (4) PCR with modified primers followed by restriction enzyme digestion.19

Haplotype analysis with marker/enzyme pairs XV2C/Taq1 and KM19/PstI were performed and are named A, B, C, and D according to Estivill et al8 when the phase in the family is known.

MATERIAL AND METHODS
Sample composition
Mutation analysis was performed on 600 unrelated CF patients and their families. The diagnosis of CF was made on the basis of at least two positive sweat tests and on clinical findings. This CF population is composed mostly of patients of French origin (about 87%) but also of mixed European origin and North African origin (Algeria, Tunisia, Morocco).

RESULTS
The ΔF508 mutation
The CFTR gene analysis presented here shows that among the 1200 CF chromosomes tested, 827 (69%) carry the ΔF508 mutation and 373 (31%) carry another mutation: 310 patients (51-5%) are ΔF508 homozygotes, 207 patients (34-5%) are compound heterozygotes for ΔF508 and another mutation, and 83 patients (14%) have two other mutations.

Screening for other mutations
The 104 other mutations tested are located in 14 different PCR products corresponding to 14 exons and their splice junctions. At the beginning of this work, the choice of regions to study was influenced by the number of mutations described and their frequency. Then with the rapid increase in the number of mutations identified, the choice was influenced by the origin of the population.


Forty-six other mutations were found on at
least one CF chromosome (table): 21 of them are very rare as they were found on only one CF chromosome in our population. Screening of the 47 positive mutations allowed the characterisation of 1031 (85.9%) CF alleles and complete genotyping of 456 (76%) CF patients.

The most frequent mutations after ΔF508 are G542X (3.3%), N1303K (1.8%), W1282X (1.5%), I717+1G→T (1.3%), 1717+1G→T (1.3%), 2184delA+2183A→G (0.9%) and R553X (0.8%). The search for ΔF508 and these six mutations allowed the identification of 944 (79%) CF alleles and the determination of the complete genotype of 386 (64.5%) CF patients in our study.

### IDENTIFICATION OF NEW SEQUENCE MODIFICATIONS

#### 1506M

In exon 10, a C to G substitution at nucleotide position 1650 changes an isoleucine at position 506 into a methionine (1506M). This substitution gives, in association with a ΔF508 CF chromosome, a particular pattern when exon 10 PCR products (primed with C16b and C16d) are analysed by heteroduplex formation17 (fig 1). 1506M is probably a polymorphism as there is a methionine at this position in two other related proteins: the maltose and the ribose transporters of *Ecoli.*

#### 2184delA

When screening for the frameshift mutation 2184delA+2183A→G,+1321 one CF chromosome was found to have only the deletion of an A (2184delA) without the A→G substitution at position 2183 of the deleted sequence. The same mutation has also been identified in a German CF patient (Dörk et al, personal communication). The 11 other positive chromosomes had both changes.

#### R123K

When screening for W1282X by MnlI digestion, a patient showed a pattern similar to this mutation, except that it was on a CF chromosome bearing haplotype C (all the CF chromo-

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### Table: Distribution of CFTR mutations found in our sample of population (1200 CF chromosomes)

| Exon 3: G555E | 4 (0.33) |
| Exon 4: R117H | 2 |
| Exon 5: 1148T | 1 |
| Exon 6: 621+1G→T | 1 |
| Exon 7: ΔF511 | 1 |
| Exon 8: 711+1G→T | 8 (0.7) |
| Exon 9: A455E | 1 |
| Exon 10: S492F | 1 |
| Exon 11: 1696+1A | 1 |
| Exon 12: G1244E | 1 |
| Exon 13: 1717+1G→A | 16 (1.3) |
| Exon 14: X193delG | 1 |
| Exon 15: X194delB | 1 |
| Exon 16: G1358G→A | 2 |
| Exon 17: 2184delA | 11 (0.9) |
| Exon 18: K148T | 3 (0.25) |
| Exon 19: R1162X | 6 (0.5) |
| Exon 20: G1244E | 3 (0.25) |
| Exon 21: N1303K | 22 (1.8) |

| 47 mutations | 1031 (85.9) |

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### Notes:

- **G555E**: exon 3
- **R117H**: exon 4
- **1148T**: exon 5
- **621+1G→T**: exon 6
- **ΔF511**: exon 7
- **711+1G→T**: exon 8
- **A455E**: exon 9
- **S492F**: exon 10
- **1696+1A**: exon 11
- **G1244E**: exon 12
- **X193delG**: exon 13
- **X194delB**: exon 14
- **G1358G→A**: exon 15
- **2184delA**: exon 16
- **K148T**: exon 17
- **R1162X**: exon 18
- **G1244E**: exon 19
- **N1303K**: exon 20
- **R1162X**: exon 21

### References:

1. [Figure 1: Heteroduplex pattern of a part of exon 10/C16b-C16d)](http://jmg.bmj.com/)

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17 [Heteroduplex formation](http://jmg.bmj.com/) in CF patients with different mutations.
The mutation 3905 insT, described with a frequency of about 9% in Swiss CF chromosomes has been found in our study in a large gypsy population where it is associated with AF508.

Discussion

The present study gives a representative view of the frequency of 105 CF mutations in an important sample of the CF population (600 patients).

From our data, the molecular pathology of the CFTR gene is exceedingly heterogeneous in our population: only 86% of the CF alleles identified with 47 different mutations (out of 105 tested). Most of the unidentified mutations are probably very rare and almost “private”, so that systematic sequencing of these chromosomes would be necessary, but this procedure represents an unrewarding amount of work.

Most of the identified CF alleles belong to haplotype B (96%), while only 62% of groups A and D and 46% of groups C were identified. Consequently, for the patients with non-B chromosomes, polymorphism study (RFLP and microsatellites) will be necessary to identify the heterozygotes and allow prenatal diagnosis for the family. At the end of this study, mutation and haplotype analysis made 98.5% of the families informative.

Besides informativeness, the identification of the most frequent mutations in a given population is necessary for genetic counselling for at risk couples when one of the partners is a proven heterozygote. For the other partner, who has an initial risk of being a carrier of 1 in 25, the screening of the seven mutations AF508, G542X, N1303K, W1282X, 1717–1G→A, 2184delA+2183A→G, and R553X allows a better estimation of this risk; it drops to 1 in 120 if this screening is negative.

Our data also indicate that the goal of population screening for CF mutations (identification of 90 to 95% of the CF alleles) will be very difficult in this population of mixed ancestry because many different mutations will have to be tested. It seems very unlikely that a frequent but still unknown mutation will be identified, as many European CF chromosomes have already been sequenced.

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