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→G→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 Zielenki et al.8 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/TaqI and KM19/PstI were performed and are named A, B, C, and D according to Estivill et al.5 when the phase in the family is known.

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 were 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 AF508 are G542X (3.3%), N1303K (1.8%), W1282X (1.5%), 1717-1G→A (1.3%), 2184delA+2183A→G (0.9%), and R553X (0.8%). The search for AF508 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 formation3 (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 E coli.

2184delA

When screening for the frameshift mutation 2184delA+2183A→G,1 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.

R1283K

When screening for W1282X3 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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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 ΔF508.

Figure 2 Direct genomic sequencing of exon 20 PCR product from a heterozygous patient showing a G→A substitution at position 3980 (R1283K).

somess with W1282X, apart from one on haplotype D, are from haplotype B. Direct sequencing of the PCR product of exon 20 from this patient showed a G to A substitution at nucleotide 3980 changing an arginine at position 1283 into a lysine (R1283K) (fig 2). It has not been established if R1283K is a disease causing mutation as the arginine at this position is not conserved in the related proteins.3 R1283K can be detected by two different restriction enzyme digestions: abolition of the MnlI site or creation of an MboII site giving a pattern similar to G1244E on agarose gel electrophoresis.21 The presence of this new mutation highlights the necessity of verification by ASO hybridisation for mutations detected by abolition of a restriction enzyme site.

We also identified four other mutations which have already been reported, a nonsense mutation in exon 4 (Y122X)23 and three frameshift mutations in exon 13, 1918delGC, 2118del4, and 2372del8.22

This study underlines some other factors. The identification of R334W in two affected sibs and the study of the segregation of this mutation through the family showed that the mother, without a history of CF, was homozygous for R334W. Further clinical investigations showed that she had a positive sweat test (80 mmol/l) and was pancreatic sufficient. This mutation has previously been described as a "mild" mutation.24

As reported by Kälin et al.,25 the two CF chromosomes (from unrelated patients) positive for S1251N also carry the polymorphism F508C,26 but two other CF chromosomes bearing F508C are negative for S1251N and have unknown mutations.

The splice mutation 711 +1G→T, first described with a high frequency in CF families living in Quebec,27 was found in three homozygous and two compound heterozygous patients originating from North Africa. This mutation, also associated with haplotype A, accounts for 5% of CF chromosomes in this population and most of the other North African CF chromosomes carry unidentified mutations.

In our study, W1282X has a higher frequency than in the rest of France (CFGAC). This mutation has been reported with a high frequency (60% of CF chromosomes) in the Ashkenazi Jewish population.28 The cause of this frequency in our sample is unknown as the origin of these 18 chromosomes is variable (from the north east to south east of France).

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 ΔF508, 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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