Mutation analysis in 600 French cystic fibrosis patients

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Abstract

The cystic fibrosis transmembrane conductance regulator (CFTR) gene of 600 unrelat ed 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 (33%), N1303K (18%), W1282X (1%), 1717-1G→A (1%), 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 Zielen ski et al16 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.18 Haplotype analysis with marker/enzyme pairs XV2C/TaqI and KM19/PuI were performed and are named A, B, C, and D according to Estivill et al19 when the phase in the family is known.

Results

The ΔF508 mutation

The CFTR gene analysis presented here shows that among the 120 CF chromosomes tested, 827 (69%) carry the AF508 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 mutations.

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

<table>
<thead>
<tr>
<th>Mutations tested</th>
<th>No of CF chromosomes with the mutation (% of total CF alleles)</th>
<th>Haplotypes XV2C-KM19</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 3: G55E</td>
<td>4 (0.33)</td>
<td>3C</td>
<td>HinfI/ASO</td>
</tr>
<tr>
<td>394del5TT</td>
<td>2</td>
<td>B</td>
<td>PAGE</td>
</tr>
<tr>
<td>Exon 4: R117H</td>
<td>1</td>
<td>C</td>
<td>ASO</td>
</tr>
<tr>
<td>Y122X</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I148T</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon 5: 711 + 1G→T</td>
<td>8 (0.7)</td>
<td>2C</td>
<td>MnI/sequence</td>
</tr>
<tr>
<td>Exon 7: ΔF507</td>
<td>1</td>
<td>B</td>
<td>MnI/ASO</td>
</tr>
<tr>
<td>1078delT</td>
<td>1</td>
<td></td>
<td>MnI/ASO</td>
</tr>
<tr>
<td>R334W</td>
<td>5 (0.42)</td>
<td></td>
<td>MnI/ASO</td>
</tr>
<tr>
<td>R347P</td>
<td>5 (0.42)</td>
<td></td>
<td>MnI/ASO</td>
</tr>
<tr>
<td>R347H</td>
<td>1</td>
<td></td>
<td>MnI/ASO</td>
</tr>
<tr>
<td>Exon 9: A455E</td>
<td>1</td>
<td></td>
<td>MnI/ASO</td>
</tr>
<tr>
<td>Q493X</td>
<td>1</td>
<td></td>
<td>MnI/ASO</td>
</tr>
<tr>
<td>1609delCA</td>
<td>1</td>
<td></td>
<td>MnI/ASO</td>
</tr>
<tr>
<td>ΔF508</td>
<td>827 (69)</td>
<td></td>
<td>PAGE</td>
</tr>
<tr>
<td>1679delATA</td>
<td>1</td>
<td></td>
<td>PAGE</td>
</tr>
<tr>
<td>Exon 11: 1717-1G→A</td>
<td>16 (1.3)</td>
<td></td>
<td>MnI/sequence</td>
</tr>
<tr>
<td>G542X</td>
<td>40 (3.5)</td>
<td></td>
<td>MnI/sequence</td>
</tr>
<tr>
<td>S549R (T→G)</td>
<td>2</td>
<td></td>
<td>MnI/sequence</td>
</tr>
<tr>
<td>G551D</td>
<td>3 (0.25)</td>
<td></td>
<td>MnI/sequence</td>
</tr>
<tr>
<td>R553X</td>
<td>10 (0.8)</td>
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<td>MnI/sequence</td>
</tr>
<tr>
<td>Exon 12: 1888 +1G→A</td>
<td>1</td>
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<td>MnI/sequence</td>
</tr>
<tr>
<td>1888 +1G→C</td>
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<tr>
<td>Exon 13: 1918delGC</td>
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<td>MnI/sequence</td>
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<td>G944del484</td>
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<td></td>
<td>MnI/sequence</td>
</tr>
<tr>
<td>G6580 (G→A)</td>
<td>2</td>
<td></td>
<td>MnI/sequence</td>
</tr>
<tr>
<td>2188del746</td>
<td>1</td>
<td></td>
<td>MnI/sequence</td>
</tr>
<tr>
<td>2184delA</td>
<td>11 (0.9)</td>
<td></td>
<td>MnI/sequence</td>
</tr>
<tr>
<td>2183A→G</td>
<td>1</td>
<td></td>
<td>MnI/sequence</td>
</tr>
<tr>
<td>K710X</td>
<td>3 (0.25)</td>
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<td>MnI/sequence</td>
</tr>
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<td>Exon 15: S945L</td>
<td>1</td>
<td></td>
<td>MnI/sequence</td>
</tr>
<tr>
<td>17B/L106P</td>
<td>1</td>
<td></td>
<td>MnI/sequence</td>
</tr>
<tr>
<td>L1077P</td>
<td>1</td>
<td></td>
<td>MnI/sequence</td>
</tr>
<tr>
<td>Y1092X</td>
<td>3 (0.25)</td>
<td></td>
<td>MnI/sequence</td>
</tr>
<tr>
<td>Exon 19: R1162X</td>
<td>6 (0.5)</td>
<td></td>
<td>MnI/sequence</td>
</tr>
<tr>
<td>3659del46C</td>
<td>3 (0.25)</td>
<td></td>
<td>MnI/sequence</td>
</tr>
<tr>
<td>Exon 20: G1244E</td>
<td>2</td>
<td></td>
<td>MnI/sequence</td>
</tr>
<tr>
<td>S1251N</td>
<td>2</td>
<td></td>
<td>MnI/sequence</td>
</tr>
<tr>
<td>3008insT</td>
<td>4 (0.33)</td>
<td></td>
<td>MnI/sequence</td>
</tr>
<tr>
<td>W1282X</td>
<td>18 (1.5)</td>
<td></td>
<td>MnI/sequence</td>
</tr>
<tr>
<td>R1303K</td>
<td>22 (1.8)</td>
<td></td>
<td>MnI/sequence</td>
</tr>
<tr>
<td>47 mutations</td>
<td>1031 (85.9)</td>
<td></td>
<td>MnI/sequence</td>
</tr>
</tbody>
</table>

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%), 1717-1G→A (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

506M

In exon 10, a C to G substitution at nucleotide position 1650 changes an isoleucine at position 506 into a methionine (506M). This substitution gives, in association with a ΔF508 CF chromosome, a particular pattern when exon 10 PCR products (primed with C16B and C16D\(^2\)) are analysed by heteroduplex formation\(^{17}\) (fig 1). 506M 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,\(^3\) 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 W1282X\(^3\) by MnI 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-
somies with W1282X, apart from one on haplo-
type 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 re-
striction enzyme digestions: abolition of the
MnlI site or creation of an MboII site giving a
pattern similar to GI244E on agarose gel elec-
trophoresis.21 The presence of this new mu-
tation 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)26 and three frame-
shift mutations in exon 13, 1918delGC, 2118del49, and
2372del8.27
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 homozy-
gous 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 Käin et al,25 the two CF
chromosomes (from unrelated patients) positive
for S1251N also carry the polymorphism
F508C,29 but two other CF chromosomes bear-
ing F508C are negative for S1251N and have
unknown mutations.
The splice mutation 711 + 1G→T, first de-
scribed with a high frequency in CF families
living in Quebec,26 was found in three homozy-
gous 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 chromo-
somes carry unidentified mutations.
In our study, W1282X has a higher fre-
quency 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).

The mutation 3905 insT, described with a
frequency of about 9% in Swiss CF chro-
mosomes29 has been found in our study in a large
gypsy population where it is associated with
ΔF508.

Discussion
The present study gives a representative view of
the frequency of 105 CF mutations in an im-
portant 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 chromo-
somes 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, mu-
tation and haplotype analysis made 98.5% of the
family informative.

Besides informativeness, the identification
of the most frequent mutations in a given popula-
tion 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, W1228X, 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 popula-
tion 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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1 Kereem B, Rommens JM, Buchanan JA, et al. Identification
of the cystic fibrosis gene: genetic analysis. Science
2 Rommens JM, Iannuzzi MC, Kereem B, et al. Identification of the
cystic fibrosis gene: chromosome walking and jumping.
3 Rooman JR, Rommens JM, Kereem B, et al. Identification of the
cystic fibrosis gene: cloning and characterization of
4 Cystic Fibrosis Genetic Analysis Consortium. Worldwide
9.
5 Tsui LC. Mutations and sequence variations detected in the
cystic fibrosis transmembrane conductance regulator
(CFTR) gene: a report from the Cystic Fibrosis Genetic
novel mutations in CFTR gene. Hum Mol Genet


