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High incidence of the CFTR mutations 3272-26A → G and L927P in Belgian cystic fibrosis patients, and identification of three new CFTR mutations (186-2A → G, E588V, and 1671insTATCA)

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Abstract

We have analyzed 143 unrelated Belgian patients with a positive diagnosis of cystic fibrosis (CF) for mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. An initial screening for 29 CFTR mutations led to mutation identification in 89.9% of the tested chromosomes. Subsequently an extensive analysis of the CFTR gene was performed by denaturating gradient gel electrophoresis (DGGE) in those patients with at least one unknown mutation after preliminary screening. In addition to 10 previously reported mutations we identified 2 new mutations 186-2A → G and E588V. A third new mutation 1671insTATCA was identified during routine screening for ΔF508. Two mutations were detected with a higher frequency than expected: 3272-26A → G, which is the second most common mutation after ΔF508 in our CF population with a frequency of 3.8%, and L927P (2.4%). The clinical data is presented for the mutations 186-2A → G, E588V, 3272-26A→G and L927P. The mutation data are useful for the Belgian population to supplement the initial screening set of mutations.

Keywords: Belgian; Antwerp; Cystic fibrosis; CFTR; DGGE; Genotype–phenotype

1. Introduction

Cystic fibrosis (CF) is one of the most common severe autosomal recessive disorders among Caucasians with an estimated incidence of 1:2500. The clinical expression of the disease is heterogeneous but most patients with CF typically present with chronic obstructive lung disease, elevated electrolyte concentration in the sweat, insufficient pancreatic exocrine function (PI), and male infertility [1,2]. Approximately 10–15% of CF patients has pancreatic sufficiency (PS) [3]. Since the identification of the cystic fibrosis transmembrane conductance regulator (CFTR) gene [4,5,6], more than 1000 CFTR mutations have been reported to the Cystic Fibrosis Genetic Analysis Consortium [7]. The ΔF508 mutation is the most common mutation with an average frequency in Caucasian CF chromosomes of 66% [7] and with a wide variation among different populations [4]. Few mutations have a frequency between 1 and 5%. Most mutations however are rare and occur with a frequency of less than 1% [7].

In the present study, we report the spectrum and frequency of CFTR mutations in Belgian CF patients from the region

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Antwerp. For the frequent and new mutations found in this study the clinical data is presented.

2. Materials and methods

2.1. Samples

A total of 143 unrelated Belgian CF families with at least one affected child with a confirmed diagnosis of CF was studied. The diagnostic criteria used were those proposed by Rosenstein et al. [8]. Genomic DNA was extracted from peripheral blood lymphocytes [9]. If possible DNA analysis of the index cases was followed by analyzing the parents to show that the mutations under study were located on separate chromosomes.

2.2. Detection of known mutations

Twenty-nine frequent CFTR mutations were analyzed by two DNA line probe assays based on the reverse hybridization principle [10]. The Inno Lipa™ CFTR12 assay contains normal and mutant probes for 12 different CFTR mutations (*ΔF508, G542X, N1303K, 1717-1G→A*, *W1282X, G551D, R553X, S1251N, R560T, 3905insT, Q552X, ΔI507*). The Inno Lipa™ CFTR17 assay contains normal and mutant probes for 17 other CFTR mutations (*394delTT, G85E, 621+1G→T, R117H, 1078delT, R347P, R334W, E60X, 711+5G→A, 2789+5G→A, R1162X, 3659delC, 3849+10kbC→T, 2143delT, A455E, 2183AA→G, 2184delA*) (Innogenetics). These assays should allow identification of 87% of the Belgian CFTR mutations.

2.3. Detection of unknown mutations

Denaturating gradient gel electrophoresis (DGGE) analysis was carried out on all coding exons of the CFTR gene including the exon–intron boundaries, except for exon 9 which was sequenced directly. Sequences of primers, PCR conditions, optimal gradient conditions and migration time were kindly provided by Dr. Robert M.W. Hofstra (Department of Medical Genetics, University of Groningen, The Netherlands).

2.4. DNA sequencing

DNA fragments displaying aberrant electrophoretic DGGE patterns were sequenced directly using the Big Dye Terminator Cycle Sequencing Reaction Kit (Perkin Elmer), and analyzed on an ABI 3100 Automated Sequencer.

2.5. Mutation nomenclature

Nucleotide numbers are derived from cDNA CFTR sequences (GenBank accession no. NM_000492). Mutations are named according to the numbering used in the CFTR Mutation Database (http://www.genet.sickkids.on.ca/cftr/).

3. Results and discussion

3.1. Molecular analysis

In this study, we first analyzed 29 CFTR mutations in 286 unrelated CF chromosomes from Belgian patients. The ΔF508 mutation was found in 73.4% of the tested chromosomes. Twelve other mutations were found and accounted for another 16.5% of tested chromosomes. Sixteen of the 29 tested mutations were not present in our patient group. In total, we could characterize 89.9% (257/286) of the CF alleles. In those patients with at least one unknown mutation DGGE analysis of the 'negative' CF chromosomes, followed by sequencing analysis of the aberrant fragments, revealed the presence of 12 different CFTR mutations (28/29 CF alleles). In one patient only one mutation (ΔF508) could be identified. This male patient (now 35 years old) presented at the age of 27 a sweat chloride concentration of 100 mEq/l, mild lung disease and pancreatic insufficiency. Two younger sibs also affected with cystic fibrosis died at a much younger age. From the 12 different mutations identified by DGGE 10 mutations are reported before and 2 mutations (186-2A→G and E588V) are new. (Table 1) A third new mutation 1671insTATCA causing a frameshift in exon 10 and creating a premature stop of

Table 1
Spectrum and frequencies of CFTR mutations in unrelated Belgian CF patients (Antwerp region)

	CFTR mutation	Number of CF-chromosomes	Frequency (%)
1	ΔF508 [a]	210	73.4
2	3272-26A→G [b]	11	3.8
3	N1303K [a]	10	3.5
4	S1251N [a]	7	2.4
	G542X [a]	7	2.4
	L927P [b]	7	2.4
5	2789+5G→A [c]	6	2.1
6	1717-1G→A [a]	5	1.7
7	A455E [c]	3	1.0
8	2183AA→G [c]	2	0.7
	E60X [c]	2	0.7
	R334W [c]	2	0.7
9	ΔI507 [a]	1	0.3
	3659delC [c]	1	0.3
	3849+10kb C→T [c]	1	0.3
	186-2A→G	1	0.3
	E588V [b]	1	0.3
	L159S [b]	1	0.3
	G178R [b]	1	0.3
	W401X [b]	1	0.3
	1833delT [b]	1	0.3
	E730X [b]	1	0.3
	P750L [b]	1	0.3
	Y1092X [b]	1	0.3
	M1137R [b]	1	0.3
10	Unknown	1	0.3
Total		286	100.0

[a] Inno Lipa™ CFTR12 mutations.
[b] Mutations identified by DGGE/sequencing analysis.
[c] Inno Lipa™ CFTR17 mutations.

Table 2
Frequency of the mutations 3272-26A→G and L927P for different parts of Belgium and other countries

Country/Region	Frequency 3272-26A→G (%)	Reference
Belgium/Antwerp	3.8	This study
Belgium/Brussels	1.7	W. Lissens, pers. comm.
Belgium/Ghent	0.9	L. Messiaen, pers. comm.
Belgium/Leuven	1.0	[14]
The Netherlands	0.9	H. Scheffer, pers. comm.
France	0.5	[15–17]
Germany	0.9	[18]
Greece	0.8	[19,20]
Spain	0.5	[21]
Canada (Toronto)	0.5	[22]
Portugal	2.0	[11]
South Africa (white population)	4.2	[23]
	Frequency L927P (%)	
Belgium/Antwerp	2.4	This study
Belgium/Brussels	0.7	W. Lissens, pers. comm.
Belgium/Gent	0.3	L. Messiaen, pers. comm.
The Netherlands	0.5	[13]

translation at amino acid 528 was identified in a carrier by routine screening for ΔF508 (heteroduplex analysis). Two of the 10 previously reported mutations were detected with a higher frequency than expected: the mutation 3272-26A→G is the second most common CFTR mutation in the Antwerp patient population (after ΔF508), with a frequency of 3.8%. L927P (together with G542X and S1251N) is the fourth most common CFTR mutation, with a frequency of 2.4%. (Table 2) Remarkably, the incidence of 3272-26A→G differs between different parts of Belgium (from about 1 to 3.8%) and seems to be the highest for Antwerp. More, the incidence of 3272-26A→G in Antwerp is comparable with that of the white population in South Africa. This can probably partially be explained by historical bonds between South Africa and the Antwerp region with migration of inhabitants of Antwerp to South Africa (via the Netherlands) and with direct trade routes between South Africa and the port of Antwerp. Also the incidence of the mutation L927P differs between different parts of Belgium and seems to be the highest for Antwerp (with frequencies from 0.3 up to 2.4%). (Table 2) These numbers are especially useful for carrier screening in the Belgian population, specifically for the population from the Antwerp region.

3.2. Genotype–phenotype studies

3.2.1. Frequent mutations

3.2.1.1. 3272-26A→G. It was previously shown that the 3272-26A→G mutation leads to the creation of an alternative acceptor splice site competing with the normal one during RNA processing and resulting in the occurrence of an alternatively spliced mRNA with 25 extra nucleotides from intron 17a and a premature stop codon soon thereafter [11]. Amaral et al. [12] reported the clinical phenotypes of 60 CF patients with the mutation 3272-26A→G and found that these were significantly milder than those of ΔF508 homozygotes. In this study, the mutation is found in 11/143 CF patients. In 10 patients ΔF508 is the second mutation. One patient is compound heterozygous for 3272-26A→G and 1717-1G→A. The clinical phenotypes of our patients are milder than those of ΔF508 homozygotes, and are in concordance with the phenotypes described by Amaral et al. [12].

3.2.1.2. L927P. The mutation L927P is caused by the transition of a T to C at nucleotide position 2912 in exon 15, and changes a leucine to a proline at position 927 of the protein. The L927P is described before by Hermans et al. [13] in 3 families (5 patients). They concluded that most likely L927P can be classified as a severe CF mutation comparable with ΔF508. We found L927P in 7 CF patients: four patients have ΔF508 as the second mutation, one patient 1717-1G→A, another patient S1251N, and a last patient 2789+5G→A. The clinical phenotypes of these patients are given in Table 3. For the patient with 2789+5G→A extended clinical information was missing. The patients described in Table 3 presented more gastro-intestinal problems and

Table 3
Clinical phenotypes of patients compound heterozygous for L927P and a second mutation

Genotype (L927P/any)	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Second mutation	ΔF508	ΔF508	ΔF508	ΔF508	1717-1G>A	S1251N
Sex	Male	Female	Male	Female	Female	Female
Age at diagnosis	1 month (meconium ileus)	?	<1 year	<1 year	Neonatal (meconium ileus)	?
Current age	1.5 year	9 year	20 year	5 year	10 year	35 year
Pancreatic insufficiency	Yes	Yes	Yes	Yes	Yes	Yes
FEV_1 (% predicted)	/	88.1	86.7	72.6	80.4	70
Pseudomonas aeruginosa colonization	No	No	No	Yes	No	Yes
Sweat Na (mEq/l)	?	142	?	88	89	?
Sweat Cl (mEq/l)	118	160	?	105	106	?

suffered more often with meconium ileus, chronic diarrhea and stagnation of weight. 80% of them were also pancreatic insufficient. Lung problems and spirometry values were similar to the classic ΔF508. Children with L927P were mostly diagnosed in the neonatal period. From this, we conclude that patients with L927P show clinical features comparable to patients homozygous for ΔF508.

3.2.2. New mutations

3.2.2.1. 186-2A→G. The mutation 186-2A→G constitutes a splicing defect in intron 1. The nucleotide substitution A>G at position 186-2 most probably creates an alternative cryptic acceptor splice site that competes with the normal acceptor splice site during RNA processing reducing splicing from the correct site. mRNA sudies should be performed to confirm this. The mutation is found in a male patient displaying a phenotype with a high sweat chloride concentration (102 mEq/l), pancreatic insufficiency and development of nasal polyps. The patient is compound heterozygous for ΔF508 and 186-2A→G.

3.2.2.2. E588V. The missense mutation E588V is caused by a transversion of A to T at nucleotide 1895 in exon 12 of the CFTR gene leading to a change of glutamic acid to valine at amino acid position 588. It is found in a male patient with obstructive lung disease, pancreatic insufficiency, and pseudomonas colonization. The patient is compound heterozygous for ΔF508 and E588V. Arguments for a pathogenic character of E588V are (1) the mutation was not detected in 96 control chromosomes, (2) a relative large charged polar amino acid is substituted by a small apolar amino acid. (3) the glutamic acid at position 588 is conserved in several species.

In conclusion, extensive mutation analysis of the 27 exons and flanking intron sequences of the CFTR gene by DGGE analysis in 143 Belgian CF patients from the Antwerp region led to the identification of almost 100% of the disease-causing mutations. In this study, the detection efficiency of DGGE seems to be high only because CF patients with already one identified mutation after preliminary screening were included. In one patient no second CF mutation could be identified, most probably due to incomplete sensitivity of DGGE. Twenty-five different mutations were identified including two new CFTR mutations 186-2A→G and E588V. Two known mutations 3272-26A→G and L927P were shown to occur with a higher frequency in Antwerp compared to other Belgian regions. We conclude that 3272-26A>G is a milder mutation than ΔF508, as reported before by Amaral et al. [12], and that L927P can be classified as a severe mutation comparable with ΔF508.

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