

Screening for common cystic fibrosis mutations in Romanian patients: analysis of twenty-one cases

Screening pentru mutațiile comune în fibroza chistică la pacienții din România: analiza a 21 de cazuri

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Abstract

Twenty-one patients with a clinical suspicion of cystic fibrosis (CF) were analyzed for common mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. The purpose of the study was the identification of mutations in order to confirm the diagnoses. The methods of analysis were: extraction of deoxyribonucleic acid (DNA) from blood, amplification of DNA by Polymerase Chain Reaction (PCR), analysis of PCR products by electrophoresis or by digestion with restriction enzymes followed by electrophoresis; the latter technique is called Restriction Fragment Length Polymorphism Analysis (RFLPA). Seventeen chromosomes with the F508del mutation, 3 with G542X, 1 with W1282X, and 1 with R347P were detected. These results update the profile of mutations present in CF patients from Romania.

Keywords: Cystic fibrosis, mutations, Romania

Rezumat

Au fost analizați 21 pacienți cu suspiciune clinică de fibroză chistică (FC) pentru mutații comune în gena CFTR (Cystic Fibrosis Transmembrane Conductance Regulator). Scopul studiului a fost identificarea mutațiilor pentru confirmarea diagnosticelor. Metodele de analiză au fost: extracția ADN din sânge, amplificarea ADN prin tehnica Polymerase Chain Reaction (PCR), și analiza produșilor PCR prin electroforeză sau prin digestia cu enzime de restricție urmată de electroforeză; această ultimă metodă este numită Restriction Fragment Length Polymorphism Analysis (RFLPA). S-au detectat 17 cromozomi cu mutația F508del, 3 cu G542X, 1 cu W1282X și 1 cu R347P. Aceste rezultate actualizează profilul mutațiilor prezente la pacienții cu fibroză chistică din România.

Cuvinte cheie: fibroza chistică, mutații, România

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Introduction

Cystic fibrosis (CF) is the most frequent autosomal recessive genetic disease in Caucasian populations and one of the most severe, and therefore better documented, chloride ion channel defects (1). The CFTR gene, situated on the long arm of chromosome 7 (2,3,4), encodes the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein, a cyclic AMP-dependent chloride ion channel, and mutations in the CFTR gene give rise to defective chloride ion transport. The subsequent reduced hydration of secretion from liver, gallbladder, duodenum, pancreas, lung, salivary glands and genital tracts and the viscid mucus-rich secretions are deleterious to normal function in the aforementioned organs (1), and hence responsible for the clinical features of the disorder: characteristic lung disease and pancreas insufficiency. These are criteria for diagnosis, together with the increased concentration of sweat chloride, a positive family history and at least one mutation detected in the CFTR gene (1,5). The most frequent CFTR gene mutation is a deletion of three bases (CTT) resulting in the deletion of the phenylalanine residue at position 508 of the CFTR protein; this mutation, named F508del [formerly known as Δ F508; HGVS (Human Genome Variation Society) nomenclature: p.Phe508del; c.1521_1523delCTT], was described by Kerem et al. (1989) (4), and accounts for 66% of CF chromosomes worldwide (6,7).

We studied a group of twenty-one patients for common mutations in the CFTR gene, in order to establish their genotypes and, consequently, to confirm the diagnoses. These results update our previous data (8).

Material and methods

The patients were recruited in the pediatric hospitals from Bucharest, Cluj-Napoca and Constanța. We selected for this study

twenty-one patients from twenty-one unrelated families, with relevant clinical symptoms and sweat test values. The samples of venous blood were taken in EDTA at the place of consultation and were sent by mail or courier to our laboratory. DNA extraction was performed using the phenol-chloroform method (9).

The specific DNA fragment for each mutation was amplified by PCR in a Hybaid thermal cycler (Hybaid Limited, Teddington, UK) and was analyzed by electrophoresis. We used recognized methods: heteroduplex analysis (HA) (9), multiplex PCR (10,11) and RFLPA (9,11). The following 18 mutations could be identified (*Table 1*): F508del, 1677delTA, G542X, W1282X, N1303K, CFTRdele2,3(21kb), I507del, R117H, 621+1G->T, R334W, R347P, R553X, G551D, 2143delT, 2184insA, 394delTT, 3821delT and 1717-1G->A.

Results

The distribution of genotypes is presented in Table 2. Seventeen chromosomes with the F508del mutation, 3 with G542X, 1 with W1282X, and 1 with R347P were detected. The chromosomes with F508del mutation included 4 homozygotes, 7 heterozygotes with F508del on one chromosome, and two compound heterozygotes with F508del and G542X or R347P on the second chromosome. The chromosomes with the G542X mutation included one homozygote and one heterozygote mentioned above. The chromosome with W1282X was found in a heterozygote with an unknown mutation on the second chromosome. Six cases remained with unknown mutations.

The detection of G542X mutation using RFLPA is presented in Figure 1. G542X (c.1624G>T) destroys a *MvaI* restriction site and is thus distinguishable from the wild-type allele by restriction digestion with *MvaI* followed by agarose gel electrophoresis.

Table 1. Methods employed in the detection of common CFTR mutations

Mutation	HGVS nomenclature		Method	Sequence of primers	Restriction enzyme	Ref.
F508del, I507del, 1677delTA	p.Phe508del p.Ile507del	c.1521_1523delCTT c.1519_1521delATC c.1545_1546delTA	HA	5'-GTT TTC CTG GAT TAT GCC TGG GCA C-3' 5'-GTT GGC ATG CTT TGA TGA CGC TTC -3'		(4, 12, 13, 14, 15)
F508del, I507del, 1677delTA, CFTRdele2,3(21kb), 2143delT, 2184insA, 394delTT, 3821delT	p.Phe508del p.Ile507del	c.1521_1523delCTT c.1519_1521delATC c.1545_1546delTA c.2011delT c.2052dupA c.262delTT c.3689delT	Multiplex PCR and HA with the CF-8 kit			(4, 10, 11)
G542X	p.Gly542X	c.1624G>T	RFLPA	5'-TGC AGA GAA AGA CAA TAT AGT TCC T-3' 5'-GCA CAG ATT CTG AGT AAC CAT AAT-3'	<i>MvaI</i>	(16)
W1282X	p.Trp1282X	c.3846G>A	RFLPA	5'-GTC AGG ATT GAA AGT GTG CA-3' 5'-GTA CAA GTA TCA AAT AGC AGT A-3'	<i>MnlI</i>	(17, 18)
N1303K	p.Asn1303Lys	c.3909C>G	RFLPA	5'-GAA ATA TTT TAC AAT ACA ATA AGG G-3' 5' CTC CAC TGT TCA TAG GGA TCT AA-3'	<i>DdeI</i>	(19)
R117H	p.Arg117His	c.350G>A	RFLPA	5'-CCC GGA TAA CAA GGA GGA GC-3' 5'-GGC CTG TGC AAG GAA GTG TTA-3'	<i>Hin6I</i>	(16)
621+1G>T		c.489+1G>T	RFLPA	5'-CCC GGA TAA CAA GGA GGA GC-3' 5'-GGC CTG TGC AAG GAA GTG TTA-3'	<i>HincII</i>	(16)
R334W	p.Arg334Trp	c.1000C>T	RFLPA	5'-CAG AAC TGA AAC TGA CTC GG-3' 5'-TGC TCC AAG AGA GTC ATA CC-3'	<i>MspI</i>	(20)
R347P	p.Arg347Pro	c.1040G>C	RFLPA	5'-CAG AAC TGA AAC TGA CTC GG-3' 5'-TGC TCC AAG AGA GTC ATA CC-3'	<i>Hin6I</i>	(20)
R553X G551D	p.Arg553X p.Gly551Asp	c.1657C>T c.1652G>A	RFLPA	5'-CAA CTG TGG TTA AAG CAA TAG TGT-3' 5'-GCA CAG ATT CTG AGT AAC CAT AAT-3'	<i>HincII</i> <i>Sau3A</i>	(21)
1717-1 G>A		c.1585-1G>A	RFLPA	5'-GCA CAG ATT CTG AGT AAC CAT AAT-3' 5'-CTC TAA TTT TCT ATT TTT GGT ACT A-3'	<i>DdeI</i>	(19)

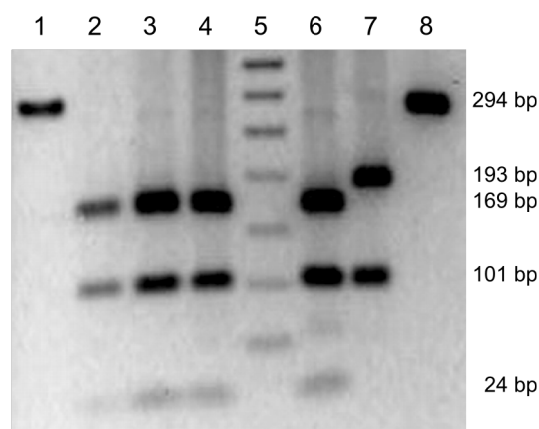
Table 2. The distribution of genotypes in 21 CF patients from Romania

Genotype	Number
F508del/ F508del	4
F508del/x	7
F508del/G542X	1
F508del/R347P	1
G542X/G542X	1
W1282X/x	1
x/x	6

The F508del mutation is the most frequent in these 21 patients, but the relatively small patient cohort prevents accurate calculation of mutation frequencies. Previous data indicate a frequency of 56.3% for F508del, 3.9% for G542X, and 2.3% for W1282X (8). The finding of a homozygote for G542X suggests the importance of this mutation in Romania; this is the second most frequent mutation in our country and after addition of these new data, its frequency will increase. This mutation is of Phoenician origin and is characteristic for the Mediterranean area (6,7). The R347P mutation, which was not found in the previous cases (8), is common in the south of Bulgaria (3.8%) and has a total frequency of 2.2% in CF patients from this country (6,7). This is the second most common CFTR mutation in Slavic populations [after CFTRdele2,3(21kb)] that is present in Romania. The presence of mutations that are frequent in the Mediterranean region, as well as mutations of Slavic origin in Romanian patients, is in agreement with our previous suggestion correlating CF mutations with the origin of Romanian people (8).

Conclusions

We studied the genotypes of 21 CF patients and found mutations in 15 patients, 7 of whom had two mutations. The remaining 8 had one mutation identified. This confirms the clinical diagnosis in about a third of cases and yields a strong suspicion of CF in a further third.

**Figure 1. RFLP analysis for the G542X mutation.**

2% agarose gel, stained with ethidium bromide. Lanes 1, 8: unrestricted PCR product of 294 bp; Lane 5: DNA molecular weight markers; Lanes 2, 3, 4 and 6: restricted PCR products (with *MvaI* enzyme) with size of 169, 101 and 24 bp, corresponding to the normal genotype (x/x); Lane 7: restricted PCR products (with *MvaI* enzyme) with size of 193 and 101 bp, corresponding to the mutant homozygous genotype (G542X/G542X).

The results reported in this work update the knowledge regarding the distribution of CFTR mutations in Romanian patients. On one hand the frequencies of common CF mutations were essentially confirmed; on the other hand new aspects were found, such as an increased frequency of G542X and the identification of a compound heterozygote of F508del and R347P.

Acknowledgements

The authors are grateful to Prof. Dr. Gheorghe Benga, Prof. Dr. Dhc. Mircea Cucuianu (University of Medicine and Pharmacy Cluj-Napoca) and Dr. Martin Schwarz (Department of Medical Genetics of St Mary's Hospital, Manchester, UK) for helpful discussions regarding the preparation of this manuscript. We thank Prof. Dr. Ioan Gherghina, Drs. Paula Avram and Mihai Iordăchescu (Institute for Protection of Mother and Child Bucharest), Drs. Sur Genel, Văleanu Cornel, Filip Mirela, Camelia Mărgescu, Mircea Mărgescu, Ioana Șoșa, Carmen Culcițchi, Daniela Iacob (Cluj-Napoca Childrens' Emergency Hospital), and Dr Elena Leu (Constanța County Pedi-

atrics Hospital) for providing the blood samples and the clinical data.

This work was supported by the 'Iuliu Hațieganu' University of Medicine and Pharmacy Cluj-Napoca and The Cluj County Emergency Hospital.

The authors declare no competing financial interests.

Abbreviations

CF: Cystic Fibrosis

CFTR: Cystic Fibrosis Transmembrane
Conductance Regulator

DNA: deoxyribonucleic acid

HGVS: Human Genome Variation Society

PCR: Polymerase Chain Reaction

RFLPA: Restriction Fragment Length Poly-
morphism Analysis

bp: base pairs

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