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Research article

Survey of CF mutations in the clinical laboratory

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Abstract

Background: Since it is impossible to sequence the complete CFTR gene routinely, clinical laboratories must rely on test systems that screen for a panel of the most frequent mutations causing disease in a high percentage of patients. Thus, in a cohort of 257 persons that were referred to our laboratory for analysis of CF gene mutations, reverse line probe assays for the most common CF mutations were performed. These techniques were evaluated as routine first-line analyses of the CFTR gene status.

Methods: DNA from whole blood specimens was extracted and subjected to PCR amplification of 9 exons and 6 introns of the CFTR gene. The resulting amplicons were hybridised to probes for CF mutations and polymorphisms, immobilised on membranes supplied by Roche Molecular Systems, Inc. and Innogenetics, Inc.. Denaturing gradient gel electrophoresis and sequencing of suspicious fragments indicating mutations were done with CF exon and intron specific primers.

Results: Of the 257 persons tested over the last three years (referrals based on 1) clinical symptoms typical for/indicative of CF, 2) indication for in vitro fertilisation, and 3) gene status determination because of anticipated parenthood and partners or relatives affected by CF), the reverse line blots detected heterozygote or homozygote mutations in the CFTR gene in 68 persons (26%). Eighty-three percent of those affected were heterozygous (47 persons) or homozygous (10 persons) for the ΔF508 allele. The only other CF-alleles that we found with these tests were the G542X allele (3 persons), the G551D allele (3 persons), the 3849+10kb C-T allele (2 persons) the R117H allele (2 persons) and the 621+1G-T allele (1 person).

Of the fifteen IVS8-5T-polymorphisms detected in intron 8, seven (47%) were found in males referred to us from IVF clinics. These seven 5T-alleles were all coupled with a heterozygous ΔF508 allele, they make up 35% of the males with fertility problems (20 men) referred to us.

Conclusions: In summary, the frequency of CF chromosomes in the cohort examined with these tests was 26%, with the ΔF508 allele affecting 83% of the CF chromosomes. It is a substantial improvement for routine CF diagnostics to have available a test system for 30 mutations plus the polypyrimidine length variants in intron 8. Our results show that this test system allows a routine first-line analyses of the CFTR gene status.

Table 1: Exons and introns that are amplified with the line probe assay, and the mutations they encompass

Roche assay:	
Amplicon	Mutations
exon 4	R117H,621+1G → T
exon 7	R334W, R347P
exon 9	A455E, 5/7/9T polymorphism
exon 10	Δ1507, ΔF508. F508C, I507V, I506V polymorphism
exon 11	1717-1G → A, G542X, S549N, G551D, R553X, R560T
exon 20	W1282X
exon 21	N1303K
intron 19	3849+10kb C → T
Innogenetics assay:	
exon 3	394delTT, G85E, E60X
exon/intron 4	621+1G-T, R117H
exon 7	1078delT, R347P, R334W
exon 13	2143delT, 2183AA-G, 2184delA
exon 19	R1162X, 3659delC
intron 5	711+5G-A
intron8/exon 9	A455E,, 5T,7T,9T
intron 14b	2789+5G-A
intron 19	3849+10kb C-T

Background

Diagnosis of hereditary human disease to date requires arduous techniques and intense manual handling. When a disorder is caused by mutations in a large gene and at multiple loci, the demands pose considerable challenges to the testing laboratory. In some cases, the underlying mutations can not be found at all, because it is impossible to sequence complete genes routinely. Consequently, clinical laboratories must rely on test systems that screen for a panel of the most frequent mutations causing disease in a high percentage of patients, in order to minimise the need for further elaborate protocols.

Accordingly, for the diagnosis of mutations that lead to cystic fibrosis, a panel of methods is needed, i.e. PCR-based techniques, reverse blots, DGGE, sequencing a.s.o [1]. The demand for improved tools for CF diagnostics is substantial, because cystic fibrosis is among the most common autosomal recessive diseases in the Caucasian population. It affects 1:2000–1:3000 births each year and results in pulmonary failure and death in most cases at around the third decade of life [2]. The molecular defect in CF was elucidated following the cloning of the cystic fibrosis transmembrane conductance regulator (CFTR) gene [3]. So far, over 800 different mutations are recorded that lie in introns and exons. Recently, association of CFTR mutations with other diseases – such as congenital bilateral absence of vasa deferentia (CBAVD) [4,5] disseminated bronchiectasis [6], or allergic bronchopulmonary aspergillosis [7] – have emerged. This is not surprising considering the cellular heterogeneity of CFTR expression and the multiple mutations in this gene [8–10]. In the case of CBAVD a strong association between the repeat number of a polypyrimidine tract in intron 8 of the CF gene with the absence of the vas deferens was found [11,12].

Our laboratory performs CF diagnostics for the north-eastern part of Austria. In this area – including and surrounding Vienna – reside approximately three million individuals. We have been using reverse line blot assays for CF mutations that encompass up to 29 mutations, 3 polymorphisms, and 3 alleles of the intron 8 polypyrimidine tract for a rapid first screening of patient samples (table 1) complemented by DGGE and sequencing as indicated (table 2). The test panel fared well in the ECCACF (European Community Concerted Action for Cystic Fibrosis) Quality Control Trial of 1997, 1998, 1999, 2000, and 2001.

The aim of this study was the exploration of the screening procedure for CF mutations employed in our laboratory as a first-line diagnostic instrument for Cystic Fibrosis. Here, we report our experiences with various test systems and the resultant CF mutations in persons from north-eastern Austria, referred to our laboratory for CF diagnosis since 1999.

Methods

Patients

In all, 135 men and 122 women were analysed. The male group consisted of 64 children (< 1–18 yrs) and 71 adults (mean age of 32 yrs, range 19–69). The group of females included 56 children (< 1–18 yrs) and 66 adults (mean age of 31 yrs, range 19–65). All probands (or their par-

Table 2: Genotypes of patients with mutations, final results

Group 1) (patients with symptoms typical for/indicative of CF)

No.:	Diagnosis[a]	Sex	Age	Intron 8	16 mut.	29 mut.[b]	seq.[c]	DGGE[d]
1	2 × path. IRT, normal sweat test	f	0	7T/9T	DF508/3849+10kb C-T	x		
2	**CF, substantiation**	f	0	9T/9T	621+1G-T/621+1G-T			
3	**CF, substantiation**	f	1	9T/9T	DF508/DF508	x		
4	**CF, substantiation**	f	5	9T/9T	DF508/DF508	x	x	x
5	**CF, substantiation**	f	7	9T/9T	DF508/G542X	x		x
6	**CF, substantiation,** rec. diarrhoe, pancreas insufficiency, pos. sweat test	f	8	9T/9T	DF508/DF508	x		
7	**CF, substantiation**	f	12	9T/9T	DF508/DF508	x		
8	**CF, substantiation**	f	13	9T/9T	DF508/DF508	x		
9		f	13	7T/9T	DF508/WT			
10	**CF, substantiation**	f	16	9T/9T	DF508/G542X			
11	indicative linkage analysis	f	22	7T/9T	DF508/WT	x		
12		f	24	7T/9T	DF508/WT	x		
13	bronchiectasis, bronchopulmonal infections since infancy	f	28	7T/9T	DF508/3849+10kbC-T	x		
14	pos. sweat test	f	28	9T/9T	DF508/WT	x		
15	typical clinic, pos. sweat test	f	31	7T/9T	DF508/WT		x	x
16		f	32	7T/7T	3849+10kb C-T/WT			
17	pulmonal course typical of CF	f	32	7T/9T	DF508/WT	x	x	x
18		f	34	7T/7T	G551D/WT		x	x
19		f	41	7T/7T	DF508/WT			
20	**CF, substantiation**	f	56	7T/9T	DF508/3849+10kb C-T	x		
21								
22	**CF, substantiation**	m	0	9T/9T	DF508/DF508	x		
23		m	1	7T/9T	DF508/WT	x		
24	impaired lung function, intestinal complications	m	3	7T/9T	DF508/WT		x	x
25	**CF, substantiation**	m	5	9T/9T	DF508/DF508			
26		m	12	7T/7T	G551D/WT		x	x
27	**CF, substantiation**	m	17	9T/9T	DF508/DF508			
28		m	18	7T/7T	R117H/WT&1466delAATT/1466delAATT		1466delAATT	x
29	pos sweat test	m	20	7T/9T	DF508/WT			
30	**CF, substantiation**	m	25	9T/9T	DF508/DF508			
31	.	m	26	5T/9T	DF508/WT			
32		m	30	5T/9T	DF508/WT			
33	**CF, substantiation**	m	31	7T/9T	DF508/1248A-G	x	1248 A/G	x
34	2 × pos. sweat tests, bronchopul. infect., azoospermia, pancreatitis	m	31	9T/9T	DF508/WT			
35	**CF, substantiation**	m	33	9T/9T	DF508/DF508	x		
36		m	33	7T/9T	DF508/WT			
37		m	33	7T/9T	DF508/WT			
38		m	38	7T/9T	R117H/G542X			

Group 2a) (Patients from IVF clinics)

No.:	Diagnosis	Sex	Age	Intron 8	16 mut.	29 mut.[b]	seq.[c]	DGGE[d]
39		m	24	7T/9T	WT			
40		m	25	9T/9T	WT			
41		m	28	5T/9T	DF508/WT	x		
42		m	28	5T/9T	DF508/WT			
43		m	29	5T/9T	DF508/WT	x	x	x
44		m	30	7T/7T	WT	x		
45		m	31	5T/9T	DF508/WT	x	x	x
46		m	31	7T/7T	WT	x		
47		m	31	7T/9T	WT	x		
48		m	33	7T/9T	DF508/WT	x		
49		m	34	7T/7T	WT	x		
50		m	34	9T/9T	DF508/WT	x		
51		m	35	7T/9T	G542X/WT			
52		m	36	5T/9T	DF508/WT			

Table 2: Genotypes of patients with mutations, final results *(Continued)*

53	m	39	5T/9T	DF508/WT	x		x
54	m	40	7T/7T	WT			
55	m	40	5T/9T	DF508/WT	x	x	x
56	m	44	9T/9T	WT			
57	m	45	7T/7T	WT	x		
58	m	47	7T/7T	WT			

Group 3) (Patients with relatives having CF)

No.:	Diagnosis	Sex	Age	Intron 8	16 mut.	29 mut.[b]	seq.[c]	DGGE[d]
59		f	42	7T/7T	3849+10kb C-T/WT	x		
60		f	15	7T/9T	DF508/WT	x		
61		f	20	7T/9T	DF508/WT	x		
62		f	23	7T/9T	DF508/WT	x		
63		f	25	7T/9T	DF508/WT	x	x	x
64		f	26	7T/9T	DF508/WT			
65		f	32	7T/9T	DF508/WT	x		
66		f	40	7T/9T	DF508/WT	x		x
67		f	65	9T/9T	DF508/WT			
68		f	30	7T/9T	DF508/WT	x		
69		m	14	9T/9T	DF508/WT	x		
70		m	16	7T/9T	DF508/WT			
71		m	25	7T/9T	DF508/WT	x	x	x
72		m	28	5T/9T	DF508/WT	x		
73		m	32	7T/9T	DF508/WT	x		x
74		m	45	7T/9T	DF508/WT	x		x
75		m	48	7T/9T	DF508/WT	x		
76		m	69	7T/9T	DF508/WT			
77		m	30	7T/9T	G542X/WT	x		x
78		m	15	7T/7T	G551D/WT	x		

Details for diagnoses, number of mutations analysed, methods used, and other specifics for individuals with found mutations within the three groups are shown. A complete documentation comprising all persons examined is available upon request. Asterisks denote samples that were tested additionally to the Roche assay. In two cases, mutations were found only by sequencing, these mutations are shown in the column "**seq".** Other than that, all mutations were found merely by the Roche assay. [a]The diagnoses are shown for the individuals specified. In the cases, where no diagnosis are given, the referrals were based on clinical symptoms suspicious for CF. No special diagnosis is given for the group of patients from IVF clinics and probands with relatives having CF. [b]The asterisk denotes individuals that were analysed for 29 mutations (see table 1). [c]Fragments (one to five) were sequenced when the DGGE analysis [d] showed bands indicating heterozygosity.

ents, respectively) had given informed consent for genetic analysis as required by the Austrian law. Patients and probands referrals to our laboratory for CF mutation analyses were based on

1) clinical symptoms typical for/indicative of CF,

2) indication for in vitro fertilisation (IVF – group 2a: men, 2b: their spouses), and

3) gene status determination because of anticipated parenthood and partners or relatives affected by CF.

Of all 257 persons analysed for CF mutations, 105 were examined for 16 mutations, 152 for 29 mutations, 31 individuals were tested further by DGGE of all exons and sequencing of suspicious fragments.

DNA studies

DNA was extracted routinely from blood using the Kristal™ DNA extraction kit (Cambridge Molecular Technologies, Cambridge, England). The INNO-LiPa CFTR17+Tn (INNOGENETICS, Ghent, Belgium) assay was performed according to the recommendation of the supplier.

For the evaluation of the Amplicor® Cystic Fibrosis kit (Roche Molecular Systems, Alameda, CA, USA), the extraction protocol included in the kit was tested, as well. For this test, 100 µL blood was incubated 5 to 10 min with 1 ml "Specimen Wash Solution" as supplied in the test kit in Eppendorf vials to lyse red blood cells. The solution was centrifuged for 1 min at maximum speed in a microfuge and the supernatant was discarded. The leucocyte pellet was treated once more with "Specimen Wash Solu-

tion" as above. The final pellet was taken up in "Extraction Reagent" and the subsequent steps were performed analogous to Kristal kit-extracted DNA: Cell pellets or DNA (minimum of 400 ng in a volume of 50 μL or less) were incubated in 200 μL Extraction Reagent for 30 min at 100°C. Twenty-five μL of this solution were PCR-amplified with 25 μL of 16.5 mmol/L Magnesium Chloride solution and 50 μL PCR "Mastermix" according to the manufacturers specification. The resulting amplicons were denatured immediately with "Denaturing Solution" to inhibit the action of uracyl-N-glycosylase (Amperase) present in the Mastermix. The panels with immobilised probes for 16 CFTR mutations and some polymorphisms were prehybridised for 10 min with "Hybridisation Buffer" (3× SSPE + 0.5% SDS). Then, 100 μL denatured amplicons were hybridised to the probes for 20 min at 50°C in hybridisation buffer. After a stringent wash for 12 min at 50°C with "Wash Buffer" (2× SSPE + 0.3% SDS) the panels were incubated with Streptavidin-Horseradish-Peroxidase-Conjugate. After further wash steps, addition of substrate (hydrogen peroxide + tetramethylbenzidine) produces a blue precipitate at the regions of hybridisation. Individual bands can be identified by superimposing a transparent foil with the identification codes imprinted.

DGGE analyses and sequencing

For the individuals specified in table 2), all exons were analysed by DGGE according to Audrezet et al. [20]. Suspicious fragments indicating mutations were sequenced on a Licor 4000L sequencing machine.

Results

Patients

Patients and probands referrals to our laboratory for CF mutation analyses were based on

1) clinical symptoms typical for/indicative of CF, (the clinical symptoms that prompted analysis for CF gene status comprised: prior positive tests for immune reactive trypsinogen (IRT), positive sweat test (> 60 mmol chloride/L), lung disease (bronchiectasies), and others,

2) indication for in vitro fertilisation (IVF – group 2a: men, 2b: their spouses), and

3) gene status determination because of anticipated parenthood and partners or relatives affected by CF.

The first group (symptomatic) was comprised of 83 male (mean age: 29 yrs, range: 19–48 yrs) and 88 female patients (mean age: 31 yrs, range: 19–61 yrs) with 114 children/adolescents (60 male, 54 female) from < 1 to 18 yrs, the mean age of this group was 13.5 yrs. The clinical symptoms for the individuals with mutations (where given) are shown in table 2), a complete list is available upon request.

The group 2a) (IVF) referred to us from IVF clinics included 20 men (mean age: 34 yrs, range 24–47 yrs), group 2b (IVF) twelve of their spouses with a mean age of 30 yrs (range: 22–36 yrs) and a presumable risk of being a CF carrier equal to the population risk. Clinical symptoms in men included CBAVD, atrophy of the testes, aplasy of the ductus deferens, aplasy of the epidymes, and azoospermia. The women in group 2b (all without clinical symptoms) were referred to CF analysis for gene status determination in the context of in vitro fertilisation.

Group 3 (mean age: 29 yrs, range: 9–69 yrs) consisted of 26 men and 28 women. These persons wanted their gene status to be determined, because either their children or close relatives were afflicted by CF. The group included 17 couples and 12 individuals that wanted to be tested in the context of planned parenthood.

Amplicor cystic fibrosis test

The Roche Amplicor CF mutation test strip was evaluated by analysing in parallel 100 samples with a panel of techniques established in our laboratory (Inno LiPa reverse dot blot – first generation, DGGE, sequencing). For 27 persons in this cohort this included sequencing of one to five exons. Besides various polymorphisms, only one splice site and one frameshift mutation were found. Thus, 97% of all mutations in this cohort that we could find routinely following our established protocols could be analysed with the Roche Amplicor Cystic Fibrosis test.

The test is based on three major processes: Polymerase chain reaction target amplification, hybridisation of the amplified products to specific oligonucleotide probes, and detection of the probe-bound amplified product by colour formation.

DNA is extracted from minute amounts of patients blood by lysis of the red blood cells and incubation in Extraction Buffer at 100°C. This method is quick (45 min) and results in very good quality of DNA. Subsequently, the DNA is PCR amplified with 8 pairs of biotinylated primers that simultaneously amplify eight different regions of the CFTR gene (Table 1 – amplified regions plus the mutations they encompass). In addition to the analysis of 16 common mutations, the assay yields information about the poly-T tract in intron 8 and polymorphisms at locus F508. Single base exchanges at this locus could lead to a lesser extent of hybridisation to the gene probes that distinguish between F508 and ΔF508. By comparison to the hybridisation pattern at the polymorphic probes (see Fig. 1), misinterpretation can be excluded.

After the PCR, the amplicons are alkali denatured to form single strands which then will hybridise to bound probes. After stringent washes, a horseradish peroxidase-streptavidin complex binds to the biotin-labelled amplicons captured by the membrane bound probes via the streptavidin moiety. This conjugate is reacted after further washing steps with hydrogen-peroxide and TMB to form a colour complex.

DNA studies

A total of 71 patients had CFTR mutations or the 5T allele or both (table 2 – final results for all methods used), 19 patients were found to have two mutations, 49 to have one mutation (not counting the 5T allele).

Among 114 children < 18 yrs in group 1), we found 9 patients to be homozygote for ΔF508, two compound heterozygote for ΔF508/G542X, one compound heterozygote for ΔF508/3849+10kbC-T, five heterozygote for ΔF508, one G551D/WT, one R117H/WT, one homozygote for 621+1G-T, and one girl with 5T/7T alleles in intron 8 (total of 18% with mutations).

Twenty-two percent of the adults in group 1) had CFTR mutations, namely two ΔF508/ΔF508, thirteen ΔF508/WT, one compound for R117H/WT and 1466delAATT (frameshift mutation in exon 9), one R117H/G542X, one G551D/WT, one 3849+10kb C-T/WT, one compound heterozygote for ΔF508/1248+1 A → G (splice mutation in intron 7), and two individuals with ΔF508/3849+10kb C-T. Table 2) gives the details for these individuals. Specifics for persons with no identified mutation albeit clinical indications for CF are not shown because of space limitations. They comprise 133 individuals, 54 of these were analysed for 16 mutations, 79 for 29 mutations of which 13 were examined further with DGGE and sequencing of suspicious fragments (a detailed documentation is available upon request). Virtually none of these developed CF as confirmed by our referring clinicians [22].

Group 2) consisted of 12 women (all WT/WT and 7T/7T or 7T/9T) and 20 men. Nine of these men (45%) had normal alleles (and a benign thymidine polymorphism in intron 8) at the loci analysed, seven men had a genotype of 5T/9T with ΔF508/WT, one showed 7T/9T with ΔF508/WT, one had 9T/9T with ΔF508/WT, and one had 7T/9T with G542X/WT. As such, of the 20 male patients referred to us because of infertility, 45% had the ΔF508 mutation and 77% of those were additionally affected in intron 8 by the 5T mutation (table 2). The women in this group (all without clinical symptoms and normal genotype) supposedly have the normal population risk of being a CF carrier. Consequently, they are arranged in the separate category 2b.

Of 54 individuals in group 3, tested because their partners or relatives had CFTR mutations, 37% had mutated alleles: seventeen persons had the genotype ΔF508/WT, one had G551D/WT, one had 3849+10kb C-T, and one person had G542X/WT (table 2).

In total, the ΔF508 mutation represented 83% (57 of 68) of all exon mutations in this cohort (table 2). The ΔF508 mutation has a strong association with the 9T allele on the same chromosome. This can be inferred because all ten ΔF508 homozygotes had 9T/9T alleles, and 37 of 38 ΔF508 heterozygotes had at least one 9T allele. On the other hand, only 25 out of 188 WT/WT genotypes had a 7T/9T, four had a 9T/9T polymorphism, but 157 WT/WT genotypes were associated with a 7T/7T polymorphism. This association of ΔF508 and 9T has been observed before [13]. Eight 5T alleles of a total of fifteen were found in individuals other then the men with fertility problems referred from IVF clinics.

Of all 257 persons analysed for CF mutations, 105 were examined only for 16 mutations (Roche assay), 152 for 29 mutations (Roche + Innogenetics assay), 31 individuals were tested further by DGGE of all exons and sequencing of suspicious fragments. Of the 71 CFTR mutations, 69 were found merely by the assay with 16 mutations (n = 257), none was found additionally with the second reverse line probe (n = 152), and 2 mutations were found by DGGE and sequencing (n = 31). Evaluation of our results by contacting the referring clinicians attested good performance of our assays: Virtually all patients with CF were found, whereas probands without mutations (by our tests) did not develop CF later on [22].

In comparison to CF mutation-frequencies in some European countries, the CF alleles we found (>2%) with our tests show the following distribution in our cohort: **ΔF508: 83%** (Romania:27%, Switzerland: 43%, Denmark: 87,2% [19]); **G542X: 4,4%** (France: 3,1%, Italy: 4,8%, Spain: 7,7%); **G551D: 4,4%** (UK: 3,1%, Czechia: 4,0%, Ireland: 6,9%), **3849+10kbC-T: 2,9%** (Germany: 1,2%, Poland: 2,6%, Latvia: 12,5%), **R117H: 2,9%** (Greece: 1,2%, Ireland: 2,0%, Norway: 3,0%)

Because we participate in the European Quality assessment trial for Cystic Fibrosis, we could evaluate the quality of the Roche Amplicor CF test in this regard as well. In the case of the G551D/WT R553X/WT genotype, the Roche test needs careful interpretation (and ought to be improved) because the hybridisation at the one locus destabilised the hybridisation at the other locus which is only 4 bp apart. Accordingly, the line assay showed a G551D/G551D and R553X/R553X (two homozygote mutations) genotype instead of the correct G551D/WT and R553X/WT (compound heterozygote) genotype. The phe-

notypic classification for such patient samples (full CF), however, was accurate, all other samples were typed correctly. This limitation is not unique to the Roche CF assay and is a characteristic of all mutation-specific assays in that sequence variations near the interrogated mutation affect test accuracy.

Discussion

Since the discovery of the cystic fibrosis transmembrane conductance regulator (CFTR) gene [14–16] the number of identified CF mutations has increased enormously. The mutations can be classified according to their influence of CFTR-mediated chloride secretion [17]. Mutations of category I, II, and III lead to complete loss of gene function (associated with pancreatic insufficiency), whereas class IV and V mutations confer altered conductance properties or reduced synthesis of the CFTR protein. Such reduced synthesis caused by varying degrees of exon 9 splicing have been found associated with three length variants within the splice acceptor site in intron 8 [18].

Numerous methods for analysing CFTR mutations are available, the number of mutations that should be tested for routine diagnostic purposes still being a question of debate. It may be best to have a two-tiered approach for mutation detection, with an economical first step and further more elaborate techniques for identifying rare mutations [19]. Because we perform the "economical first step" in our laboratory routinely, up to 29 of the most common CFTR mutations were analysed with line probe assays.

Persons to be tested for CF mutations are referred to our laboratory because of 1) symptoms typical for/indicative of CF, 2) fertility problems, and 3) CF in relatives/partners. This classification was kept for ease of presentation. Because of financial and personnel restrictions, it is not possible in our laboratory to analyse all specimen by DGGE [20] and sequencing. Thus, reverse line probe assays are used as a first step analysis tool. From DNA extraction to the results the turnaround time is approximately 6 hrs. Both assays used are not automated and thus require various manual steps. But the ease with which 30 mutations and, very importantly, the thymidine polymorphism/mutation in intron 8 can be tested, make these tests adept as a diagnosis tool for CF in the clinical laboratory. Certainly, these tests were developed for the US and Europe and cannot be adapted for other ethnic groups. In summary, the frequency of CF chromosomes in the cohort examined with our tests was 26%, with the ΔF508 allele affecting 83% of the CF chromosomes. This is considerably higher than the proportion of ΔF508 alleles of 55% found in a previous study in southern Austria [21]. Five other mutations in CF chromosomes were found with these tests, four of which with a frequency above 2%. The only other mutations detected by sequencing, were a splice site mutation in intron 7 (1248+1 A-G) and a frame shift mutation in exon 9 (1466delAATT).

Austrian law requires genetic counselling for persons seeking genetic diagnosis. In this context, careful explanation of positive and negative test results must be given. Whereas the meaning of a positive test result would yield clear conclusions, a negative result does not exclude possible mutations at loci not examined by the test used. As more than 800 putative mutations in the CFTR gene are known, this has to be explained thoroughly to the patients seeking advice.

The phenotype/genotype correlation in our cohort was rather moderate: In the group of individuals referred to us because of symptoms indicative for CF, mutations were found in only 22% of the samples. However, our test system is not inappropriate, as we have found practically all patients with CF, whereas probands without mutations did not develop clinical signs of CF later on [22]. Thus, the increasingly pressing need for fast clinical reports was met by our tests. Even slight indications for putative disease must be analysed for their possible significance, nowadays, explaining the high number of samples with no mutations found.

Somewhat higher results (37%) were found for individuals referred to us for genotype determination because their partners/relatives had known CF mutations. Thirty samples with a WT/WT genotype by the reverse line blot assays were analysed subsequently by DGGE and sequencing that resulted in only two further determinations of the mutant genotype. In both groups, the assays did not necessarily depict unknown mutations, but the panel of the commonest CF mutations was determined with certainty.

Very informative, though, were the results for the patients referred from in vitro fertility clinics. For more than half of the afflicted males, a conclusive analysis could be offered by the tests, and the exclusion of the most common mutations in their female partners was of utmost importance for their family planning. In this context, the determination of the 5T mutation in intron 8 was very significant. With the wealth of information now available, the association of the 5T mutation with fertility problems is evident. The 5T polymorphism is considered now to be a mutation with incomplete penetrance [12]. The diminished amount of CFTR gene product generated by the splicing defect in intron 8 no longer sustains the physiological functions required.

Conclusions

Our results show that current techniques allow a routine first-line analyses of the CFTR gene status. It is a substantial improvement for routine diagnostics to have available

a test system for 30 mutations plus the polypyrimidine length variants in intron 8. Certainly. further developments will advance the diagnostic possibilities for CF considerably. In this regard, it will be interesting to review our samples with the new generation line probe assays that are being developed, allowing a survey of up to 60 mutations [23].

Competing interests

None declared

Authors' contributions

H.K.R. conceived and coordinated the work, performed DGGE and sequencing, and prepared the manuscript. M.B. carried out the PCR and hybridisations, N.R., M.A., and S.R developed a reverse line probe assay and were helpful in preparing the manuscript, B.K. participated in the design and coordination of the study and was helpful in preparing the manuscript.

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