European Journal of Human Genetics (1999) 7, 590–598

http://www.stockton-press.co.uk/ejhg

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Two buffer PAGE system-based SSCP/HD analysis: a general protocol for rapid and sensitive mutation screening in cystic fibrosis and any other human genetic disease

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The large size of many disease genes and the multiplicity of mutations complicate the design of an adequate assay for the identification of disease-causing variants. One of the most successful methods for mutation detection is the single strand conformation polymorphism (SSCP) technique. By varying temperature, gel composition, ionic strength and additives, we optimised the sensitivity of SSCP for all 27 exons of the *CFTR* gene. Using simultaneously SSCP and heteroduplex (HD) analysis, a total of 80 known CF mutations (28 missense, 22 frameshift, 17 nonsense, 13 splicesite) and 20 polymorphisms was analysed resulting in a detection rate of 97.5% including the 24 most common mutations worldwide. The ability of this technique to detect mutations independent of their nature, frequency, and population specificity was confirmed by the identification of five novel mutations (420del9, 1199delG, R560S, A613T, T1299I) in Swiss CF patients, as well as by the detection of 41 different mutations in 198 patients experimentally analysed. We present a three-stage screening strategy allowing analysis of seven exons within 5 hours and analysis of the entire coding region within 1 week, including sequence analysis of the variants. Additionally, our protocol represents a general model for point mutation analysis in other genetic disorders and has already been successfully established for OTC deficiency, collagene deficiency, X-linked myotubular myopathy (XLMTM), Duchenne and Becker muscular dystrophy (DMD, BMD), Wilson disease (WD), Neurofibromatosis I and II, Charcot-Marie-Tooth disease, hereditary neuropathy with liability to pressure palsies, and defects in mitochondrial DNA. No other protocol published so far presents standard SSCP/HD conditions for mutation screening in different disease genes.

Keywords: diagnostics; PCR; SSCP; HD analysis; mutation detection; screening strategy; *CFTR* gene

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Received 3 November 1998; revised 15 February 1999; accepted 22 February 1999

Introduction

The ability to detect mutations or sequence variants in individuals has become increasingly important for the characterisation and screening of disease-causing genes and for linkage analysis of DNA polymorphisms. Different procedures have been designed in recent years to detect small nucleotide changes such as single-base substitutions, deletions or insertions in polymerase chain reaction (PCR) products. The most commonly used techniques include RNaseA analysis of RNA–DNA heteroduplexes,[1] chemical cleavage of DNA–DNA heteroduplexes,[2] denaturing gradient gel electrophoresis (DGGE),[3] and heteroduplex (HD) analysis.[4] One of the most popular methods for the detection of sequence variants in PCR amplified DNA fragments is SSCP analysis.[5] It is based on the assumption that the electrophoretic mobility of single-stranded DNA in nondenaturing polyacrylamide gels depends not only on its size but also on its sequence-dependent folded structure. A single base change can alter this secondary structure and, in consequence, the electrophoretic mobility of the single strands, resulting in band shifts on the gel. A number of studies have tried to assess or modify factors that can be expected to influence the sensitivity of SSCP analysis, but the procedures described so far are either cumbersome and time-consuming, requiring ^{32}P-labelled PCR reactions and gel electrophoresis of 10–24 h followed by autoradiography for 4–12 h, or present different amplification and gel conditions depending on the exon size.[6–9] Thus a general protocol allowing efficient mutation screening of disease genes is still lacking. The main requirement for an adequate screening technology is that it should be able to accommodate the simultaneous analysis of a large number of amplified samples as well as of many different mutations, it should be non-radioactive, rapid and not require too highly specialised skills.

Here we report on appropriate and uniform PCR and SSCP conditions providing sensitive and efficient mutation screening in any disease-causing gene. The protocol we describe augments 'common' SSCP analysis in terms of simplicity and versatility, combines SSCP and HD analysis increasing the detection rate, allows for greater and very fast sample throughput, and extends the boundaries of amplification product analysis into the 500–600 bp range. To prove the utility and power of the method, we present, as an example, the strategy for the screening of the entire coding region (27 exons) of the *CFTR* gene,[10–12] with more than 800 different disease-causing mutations reported so far, and the detection of five new mutations.

Materials and Methods

DNA Samples of Known Mutations

DNA samples of 100 known mutations and polymorphisms used in this study were either obtained by previous analysis of our patients or kindly provided by the authors who first reported these mutations. This group consists of 80 disease-causing mutations and 20 polymorphisms distributed among the 27 exons of the *CFTR* gene. The distribution of analysed known mutations is similar to that of the total number of mutations in the entire *CFTR* gene: missense mutations account for 35% (G27E, G85E, R117H, A120T, I148T, H199Y, R334W, T338I, R347P, R347H, A455E, M718K, S5449N, S5449I, G551D, R560T, R560S, S945L, S977P, I1005R, R1066C, R1070Q, M1101K, D1152H, S1235R, R1283M, N1303K, N1303H), followed by 28% of frameshift mutations (175delC, 394delTT, 457TAT->G, 905delG, 1078delT, I507, F508, 1609delCA, 1677delTA, 2143delT, 2176insC, 218delA, 2184insA, 2869insG, 3659delC, 3732delA, 3821delT, 3905insT, 4016insT, 4172delGC, 4382delA), 21% of nonsense mutations (Q30X, Q39X, Q220X, W401X, Q525X, G542X, Q552X, R553X, V569X, E585X, K710X, R792X, Y1092X, R1162X, S1255X, W1282X, E1371X), and 16% of splice site mutations (621+1G->T, 711+1G->T, 711+5G->A, 1717-1G->A, 1898+1G->A, 1898+5G->T, 2789+5G->A, 3271+1G->A, 3272-26A->G, 3601-17T->C, 3849+4A->G, 3849+10kbC->T, 4374+1G->T). Finally, in the ascertained group of sequence polymorphisms, variations in the coding regions of the *CFTR* gene comprise 60% (545T/C, 1540A/G, 1716G/A, 2134C/T, 2694T/G, 3030G/A, 3032T/C, 3212T/C, 3617G/T, 4002A/G, 4050C/T, 4521G/A) and variations in the noncoding regions account for 40% (125G/C, 405+46G/T, 1001+11C/T, 1525-61A/G, 1898+152T/A, 3041-92G/A, 3601-65C/A, 4374+13A/G) corresponding to the pattern observed for all reported polymorphisms in the *CFTR* gene.

Polymerase Chain Reaction Amplification

The 27 exons (including exon/intron boundaries) as well as intron 19 of the *CFTR* gene were amplified by the polymerase chain reaction (PCR)[13] using approximately 200 ng of genomic DNA, 10 mM dNTPs (PCR Nucleotide Mix, Boehringer/Roche Diagnostics Rotkreuz, ZG, Switzerland), 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 2.5 unit Taq polymerase (Boehringer/Roche Diagnostics Rotkreuz, ZG, Switzerland) and 20 pmol of each primer in a total volume of 50 µl. Twenty-eight cycles of PCR with denaturation at 94°C for 15 s, annealing at either 61°C (exons 1, 2, 5–12, 13CD, 15, 16, 18–22, 24) or 53°C (exons 3, 4, 13AB, 14a, 14b, 17a, 17b, 21/N1303K, 23) for 15 s and extension at 72°C for 45 s were carried out in PE 9600 and PE 2400 thermocyclers. PCR products to be analysed by direct sequencing were purified using QIAquick spin columns (PCR Purification Kit, QIAGEN, Basel, BS, Switzerland) to remove primers and dNTPs.

Sequences for the primers 1–10, 11 (forward), 12–16, 17b, 18–20, 21 (forward), 22, 23, 24 (forward) have been reported by Zielenski *et al*;[14] exon 17a was amplified using the primer sequences described by Cheadle *et al*;[15] the primers for the

amplification of intron 19 were designed by Highsmith *et al*;[16] and the primer sequence for the detection of N1303K was reported by Friedman *et al*.[17] The following primer sequences were derived from the present study:

Exon 11 (reverse): 5' - GTGATTCTTAACCCAC-TAGCC - 3'

Exon 21 (reverse): 5' - AAGTGTGTAGAATGATGT-CAGC - 3'

Exon 24 (reverse): 5' - CGAGCTCCAATTCCAT-GAGG - 3'

SSCP Analysis

Three microliters of the amplification product were added to 2–3 µl of SSCP buffer (95% formamide, 100 mM NaOH, 0.25% bromphenol blue, 0.25% xylencyanol) and denatured at 95°C for 2 min followed by rapid cooling on ice. Three microliters of the mixture were loaded on to a 12% nondenaturing polyacrylamide gel (99% acrylamide, 1% piperazine diacrylamide (PDA)) cast on to GelBond (Bioconcept, Allschwil, BL, Switzerland). The mix for one gel of 220 × 118 × 0.5 mm was: 5.3 ml 40% acrylamide–PDA solution, 8.5 ml Tris-formate buffer (0.75 M, pH 9.0), 3 ml glycerol (41%), 240 µl ammonium persulfate (APS) 10%, and 24 µl TEMED. The trailing ion, contained in soaked blotting-paper strips (Schleicher & Schuell, Feldbach, ZH, Switzerland) was Tris-borate buffer (pH 9.0). Bromphenol blue was added to the electrode buffer to serve as a dye marker for the discontinuous buffer boundary. Electrophoresis was performed using a Pharmacia LKP 2117 Multiphor II Electrophoresis Unit at 350 V for 90 min (12–15°C). Up to 40 samples can be run on one gel. The SSCP and HD banding patterns were detected by the silver staining method described by Budowle *et al*.[18]

DNA Sequence Analysis

Ten microliters of the purified samples of double-stranded PCR products showing aberrant banding patterns following SSCP/HD analysis were subjected to the cycle sequencing method with Taq polymerase for the incorporation of fluorescent-labelled dideoxynucleotides. Sequencing reactions were resolved on an ABI 373A sequencing system (Perkin Elmer, Foster City, CA, USA) and the electrophoretograms were compared using the SEQED software package from the manufacturer. All samples were sequenced in both directions or amplified and sequenced twice for confirmation using the same primers as those for PCR–SSCP analysis with a few exceptions where we used internal primers.

Internal primers used for sequence analysis:

Exon 1 (forward): 5' - GAAGGAGGAGAGGAG-GAAGG - 3'

Exon 9 (reverse): 5' - AAGAAC-TACCTTGCCTGCTCC - 3'

Exon 13A (forward): 5' - TGTGTCTGTAAACT-GATGGC - 3'

Exon 17b (forward): 5' - CTAATTTGATCTTTTT-CAGG - 3'

Results

Mutation Detection Rate

In order to determine the sensitivity and utility of the modified SSCP/HD method for mutation detection in the *CFTR* gene, a total of 100 known variants (80 disease causing mutations and 20 polymorphisms) in the *CFTR* gene was analysed. Intron 19, all 27 exons and their exon–intron boundaries, including the 24 most common mutations worldwide (G85E, R117H, 621 + 1G- > T, 711 + 1G- > T, 1078delT, R334W, R347P, A455E, I507, F508, 1717-1G- > A, G542X, S549N, G551D, R553X, R560T, 1898 + 1G- > A, 2184delA, 2789 + 5G- > A, R1162X, 3659delC, 3849 + 10kbC- > T, W1282X, N1303K) (Cystic Fibrosis Genetic Analysis Consortium 1994), and the 15 most common mutations in our population (I148T, 1078delT, R334W, R347P, F508, 1717-1G- > A, G542X, R553X, 2347delG, D1152H, R1162X, 3849 + 10kbC- > T, 3905insT, W1282X, N1303K), were considered in this study. Figure 1 shows SSCP/HD analyses of 11 DNA samples from one healthy control and 10 CF patients known to contain one or two exon 10 mutations or polymorphisms. All nine genotypes tested can be distinguished from each other on this gel without restriction digest of the 491 bp amplification product, as each variant displays a unique HD pattern. Figure 2 demonstrates a

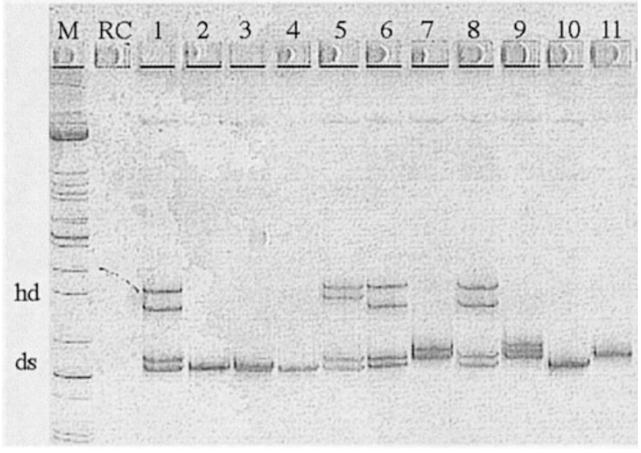

Figure 1 *SSCP mutation screening in exon 10 of the* CFTR *gene. Slot 11 refers to a control person presenting with wild type (wt) sequences, whereas slots 1–10 demonstrate PCR products from individuals with genotype ΔF508/wt (1), ΔF508/ΔF508 (2), F508/ΔI507 (3), ΔI507/ΔI507 (4), I507/wt (5), ΔF508/wt + nt 1525–61 G/A (6), nt 1540 G/A (7), ΔF508/wt (8), nt 1525–61 G/A + nt 1540 G/A (9), ΔF508/ΔF508 (10). RC = reaction negative control; M = size marker; hd = heteroduplex formation; ds = double stranded DNA.*

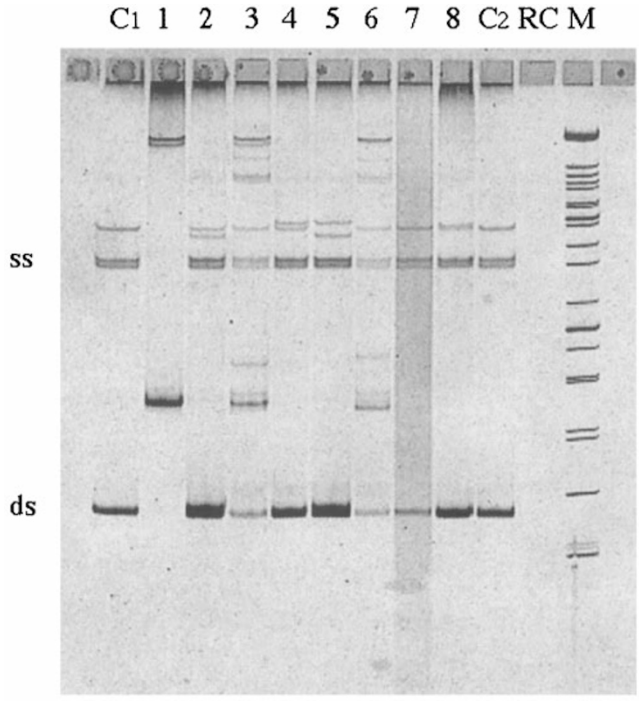

Figure 2 *SSCP analysis of CF mutations in exon 11 of the* CFTR *gene after digestion with HindII. The slots C present wild type (wt) sequences, 1–8 present amplification products from CF patients with the following genotypes: 1 = R553X/R553X; 2 = 1717-1G->A/wt; 3 = R553X/wt; 4 = G542X/wt; 5 = G542X/1717-1G->A; 6 = G551D/wt; 7 = R560T/wt; 8 = S549N/wt.*

mutation screening of exon 11 after restriction enzyme digestion using Hind II. Six different mutations and 8 different genotypes are tested and easily detectable by their specific SSCP pattern. The sensitivity of our SSCP/HD analysis conditions for the detection of CF mutations is presented in Table 1. The R792X nonsense mutation in exon 13 and the 4374 + 1G->T splice site mutation in exon 23 were not detectable with our screening method, whereas 78 out of 80 mutations and all 20 polymorphisms showed band shifts either in the single or in the double strands of the amplified DNA fragments. Three mutations (R1066C, M1101K, E1371X) could only be identified after restriction enzyme digestion of the amplification product, and five mutations (711 + 1G->T, R347H, T338I, Y1092X, S1255X) were discovered in the uncut, but not in the digested, PCR product. All fragments presenting with abnormal band patterns were directly sequenced and all mutations could be identified and confirmed. Based on our findings and on the fact that there is no random distribution of mutations within the *CFTR* gene we developed a mutation screening strategy using our SSCP conditions that allows in three stages and within one week analysis of the entire coding region and, in consequence, the detection of variants and their identification as polymorphisms or as disease-causing mutations (Figure 3).

Identification of Previously Unknown Mutations in CF Patients Analysed for Investigation Purposes

Using this newly developed strategy in routine diagnostic analyses, we screened 198 patients (396 chromosomes) at high risk for CF, who had been sent to our CF centre and were of various nationality. Our mutation screening system detected 41 different known mutations in 381 chromosomes (Table 2) including at least 20 common and/or population-specific mutations. Moreover, we identified the following five novel mutations in six chromosomes (Figure 4):

1. *420del9* An inframe deletion of nine bases (GTA CAG CCT) after nucleotide 420 situated in exon 4 and resulting in the loss of the three aminoacids Val97, Gln98, and Pro99.
2. *1199delG* A frameshift deletion of one base (G) after nucleotide 1199 in exon 7 leading to a stop codon seven codons downstream.
3. *R560S* A transversion A->T at nucleotide position 1812 located in exon 12 leading to the exchange of the amino acid Arg by a Ser.
4. *A613T* A transversion G->A at nucleotide position 1969 located in exon 13 leading to the exchange of the amino acid Ala by a Thr.
5. *T1299I* A transversion C->T at nucleotide position 4028 located in exon 21 leading to the exchange of the amino acid Thr by a Ile.

In nine patients we found only one mutation, the second mutation being not detectable by our screening system.

Discussion

Mutation detection is one of the most important areas of molecular biology today playing two fundamental roles in gene discovery. Initially it serves to identify polymorphisms for refining linkage analysis in the chromosomal region of interest and furthermore it makes the characterisation of disease-causing genes possible. Searching for point mutations in a large gene,

Table 1 Sensitivity of SSCP and heteroduplex formation in compound heterozygous CF patients

Exon	*Ampl. product size (bp)*	*Restriction enzyme digest*	*No. of mutations + polymorphisms*	*Detection rate undigested (%)*	*Detection rate digested (%)*
1	424	Ban I	1+1	100	100
2	378	Hae II	3+0	100	100
3	309	Alu I	2+1	100	100
4	438	Hpa II	5+1	100	100
5	395	Xba I	2+0	100	50
6a	384	Sau 3A	2+0	100	100
6b	401	Alu I	1+1	100	100
7	410	Hpa II	5+0	100	60
8	359	Hinf I	1+0	100	100
9	560	Bam HI	1+0	100	100
10	492	Asp 700	6+3	100	100
11	425	Hind II	8+0	100	100
12	426	Ssp I	5+1	100	100
13AB	528	Dde I	5+1	100	100
13CD	497	Hpa I	2+0	50	50
14a	502	Taq I	0+1	100	100
14b	449	Ssp I	1+0	100	100
15	485	Taq I	2+2	100	100
16	570	Sau 3A	1+1	100	100
17a	302	Sau 3A	2+1	100	100
17b	463	Hind II	5+0	60	80
18	451	Alu I	1+0	100	100
19	454	Bgl II	8+2	100	100
20	473	Hae III	4+1	100	80
21	405	Bam HI	3+1	100	100
22	562	Hind II	2+0	50	100
23	400	Xba I	1+1	50	50
24	359	Hind II	1+1	100	100

such as the *CFTR* gene that consists of 27 exons in which a heterogeneous mutation spectrum (> 800) is expected, is an enormous task. Ideally, an optimal technique for mutation detection in such a gene has to be fast, must enable the screening of large stretches of DNA with high sensitivity and specificity, would not require toxic or dangerous compounds, and would provide information about the location of the mutation. SSCP combined with HD analysis holds promise as being such a technique. However, the most fundamental question of any method used for genetic analysis is its sensitivity. Important factors that could play a role in the sensitivity of SSCP analysis include the nature of the mutation and the sequence composition of the DNA fragment as well as the locus of the mutation within the amplification product and the influence of the neighbouring bases. For SSCP there is no theoretical model for predicting the three-dimensional structure and/or mobility shift of single-stranded DNA. On the other hand, it is well known that conformational changes of a single-stranded molecule being induced by a mutation are very sensitive to environmental influences such as temperature, gel composition, ionic strength, and additives.

Thus, varying these parameters, we optimised the sensitivity of SSCP not only for a given fragment but for all 27 exons of the *CFTR* gene. We defined SSCP conditions, such as 12% nondenaturing polyacrylamide gel, 7% glycerol, two-phase buffer system, 90 min electrophoresis at 12–15°C, silver staining, that can be used for mutation screening in any other human disease-causing gene. The use of a discontinuous electrophoresis system allows concentration of the single-stranded species in the samples into a very small volume, thus additionally increasing the resolution of the polyacrylamide gel. Gels from completed SSCP

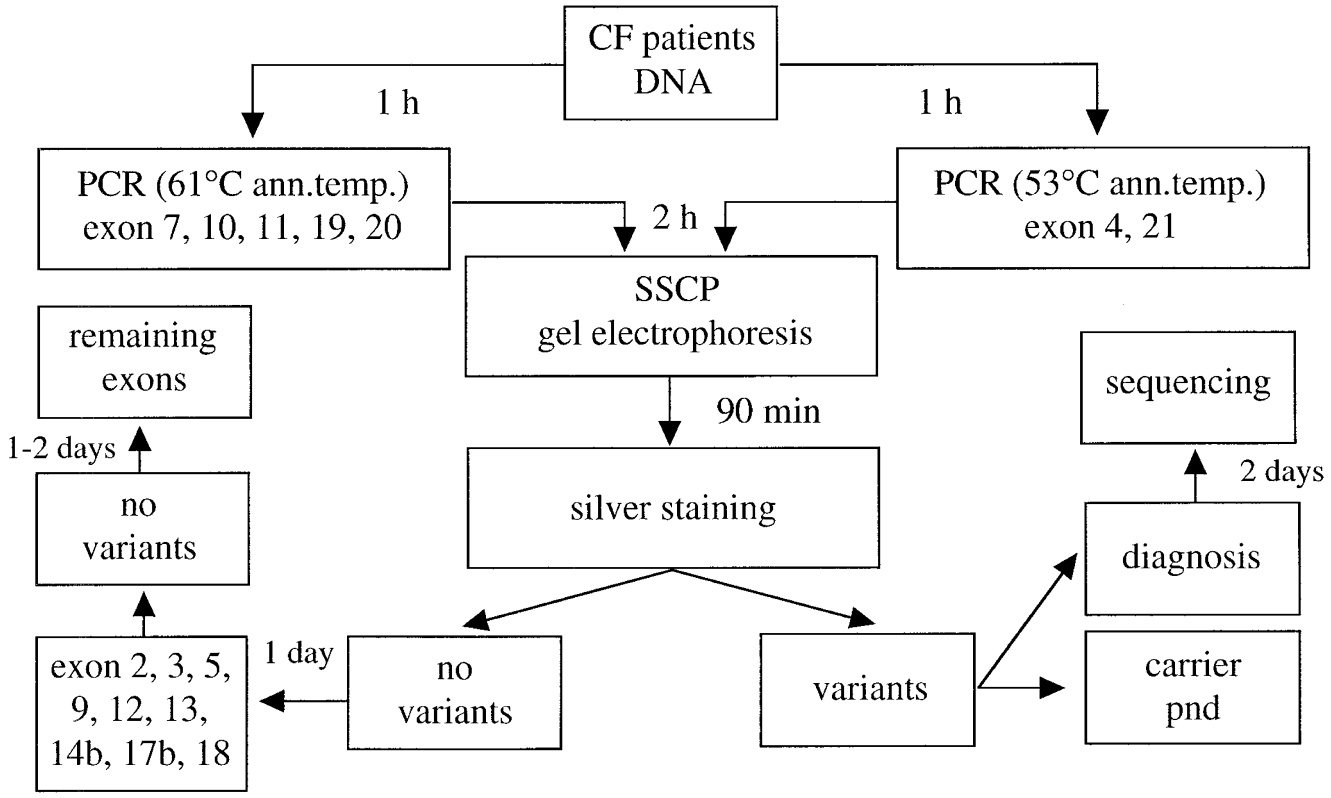

Figure 3 *Screening strategy for mutation detection in the coding region of the* CFTR *gene.*

analysis can be conveniently stored in plastic wrap at room temperature for several months without detectable diffusion of the band pattern. We collected DNA samples containing 100 different mutations and polymorphisms which are distributed along the gene. In total, 27 exons together with their exon/intron boundaries plus intron 19 have been considered. The number of variants analysed within each region ranges from 1 to ten because of the uneven distribution of mutational events. With the conditions described here 100% of the polymorphisms and 97.5% of the mutations were detectable including the 24 most common mutations worldwide. The use of fragments longer than 300 bp (optimal size for SSCP analysis[19,20]) in our SSCP analysis does not seem to be a disadvantage in identifying point mutations. Even the largest fragment used in this screening (exon 16, 570 bp) gave rise to the identification of a base substitution (T->C, S977P) without digestion of the amplification product before SSCP analysis. The reason why particular mutations can only be identified with, or without, digestion of the PCR product may be based on the fact that digestion alters the position of the mutation within the amplification product and in consequence also the conformation of the single-strand resulting or not in a band shift. In addition, during the 90 min SSCP electrophoresis, denatured and partly reannealed DNA fragments remain on the gel allowing the detection of heteroduplex formations (Figures 1, 2, 4). Thus SSCP and heteroduplex analysis can be simultaneously performed on the same gel increasing the sensitivity of both methods.

Despite the improved sensitivity of our PCR–SSCP analysis, false negatives cannot be excluded by this technique. As the nature and location of a base substitution as well as the base sequence around a point mutation have also an effect on the mobility shift of single strands, some mutations may not be detected even not under ideal conditions of electrophoresis. Moreover, intronic mutations, creating cryptic or new splice sites as well as large deletions or insertions spanning one or more exons are missed using this technique. Therefore, absence of mutation cannot be proven for certain. False positives can also appear as a result of the annealing of free oligonucleotides to PCR product strands leading to mobility shifts. This problem

Table 2 Known mutations identified in 198 CF patients analysed investigatively

CFTR mutations	*Exon (E) intron (I)*	*Number of chromosomes*	*Patient's nationality*	*Highest prevalence*
F508	E10	212	miscellaneous	
3905insT	E20	025	Swiss	Swiss, Amish, Arcadian
R553X	E11	020	Swiss, German	German
1717–1G–>A	I10	017	Swiss, Italian	Italian
N1303K	E21	011	Swiss, French, Italian	Italian
W1282X	E20	014	Swiss, Italian, Israelit	Jewish-Askhenazi
G542X	E11	009	Swiss, Spanish, Italian	Spanish
2347delG	E13	008	Swiss	
R1162X	E19	006	Swiss, Italian, Russian	Italian
3849+10kbC–>T	I19	005	German, French	
R347P	E07	004	Swiss	
T5	I08	004	Swiss	
R334W	E07	003	Swiss	
Q525X	E10	003	Swiss	
3732delA	E19	003	Swiss	
S1235R	E19	003	Italian, Turkish	
G85E	E03	002	Italian, Greek	
I148T	E04	002	Austrian, Turkish	French-Canadian
621+1G–>T	I04	002	French	French-Canadian
1078delT	E07	002	Swiss	
E585X	E12	002	Italian	
2176insC	E13	002	Swiss, Italian	
2789+5G–>A	I14b	002	Italian	Spanish
D1152H	E18	002	Swiss, French	
4016insT	E21	002	Turkish	
Q39X	E02	001	Swiss	
394delTT	E03	001	Swiss	Nordic, Finnish
R117H	E04	001	Swiss	
A120T	E04	001	Swiss	
G126D	E04	001	Swiss	
711+5G–>A	I05	001	Russian	
M348K	E07	001	Italian	
L568F	E12	001	Italian	
2183AA–>G	E13	001	Italian	Italian
K710X	E13	001	Swiss	
S945L	E15	001	French	
3272–26A.–>G	I17a	001	Swiss	
M1101K	E17b	001	Swiss	Huttite
3601–17C–>T	I18	001	Swiss	
R1158X	E19	001	Swiss	
4005+1G–A	I20	001	Italian	

can be avoided by minimising the primer concentration to less than 25 pmol/50 µl reaction volume or by removing oligonucleotides prior to SSCP analysis.

Within those limitations discussed above, the assay that we have described offers several advantages over current techniques.[21,22,23] Reaction conditions are identical for all DNA fragments recognising all classes of point mutations (base substitutions, deletions, insertions). The assay provides rapid (at least 4 gels- = 160 samples per day) and efficient screening and characterisation of mutations using a modified SSCP analysis followed by direct fluorescent automated sequencing of the variable amplification product. The technique developed demonstrates excellent single-strand separation and non-radioactive visualisation on polyacrylamide gels, and is time-saving and directly

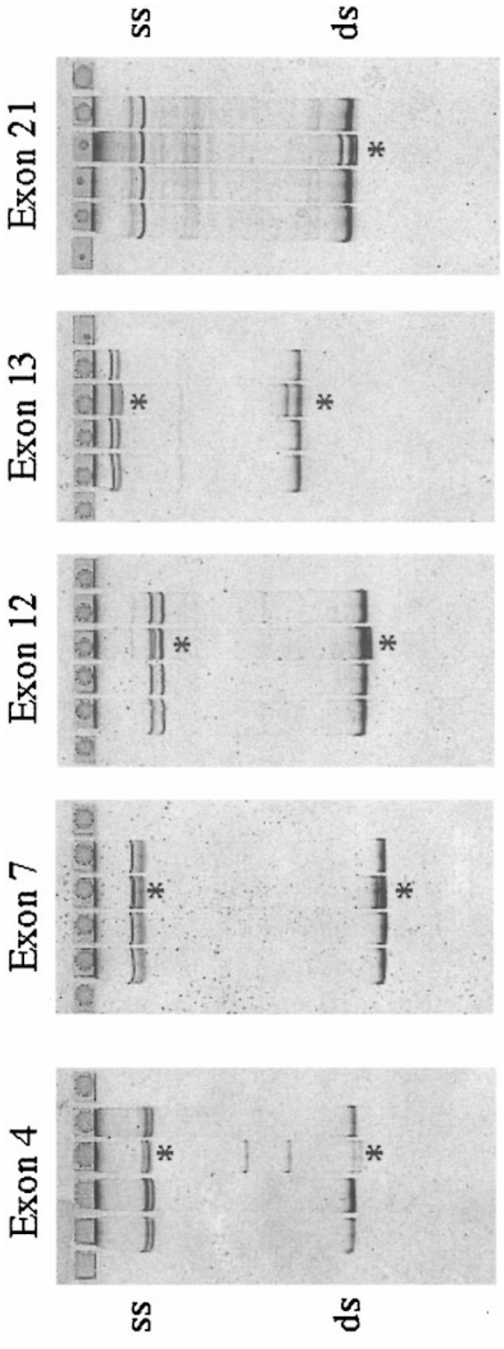

Figure 4 *SSCP gel demonstrating new mutations (*) in exon 4 (420del9), exon 7 (1199delG), exon 12 (R560S), exon 13 (A613T), and exon 21 (T1299I) of the* CFTR *gene compared with three control patterns. The mutation in exon 21 is only detected by heteroduplex formation, whereas the other four mutations show band shifts in the single strands as well as in the double strands.*

applicable to early diagnostic testing, carrier detection and prenatal diagnosis. The screening strategy presented allows analysis of seven exons within 5 hours and analysis of the entire coding region of the *CFTR* gene within 1 week, including sequence analysis of the variants. Moreover, we did not experience any false-positive findings, and the detection of known mutations was not affected by either the nature or context of the mutation.

The ability of this technique to detect mutations independent of their nature, frequency, and population specificity was confirmed by the identification of five previously unknown mutant alleles in Swiss CF patients as well as by the detection of 41 different mutations in 198 patients analysed investigatively (Table 2). In 1997 and 1998 we participated successfully in the ECCACF quality control trials using our screening technique.

Additionally, our protocol represents a general model for point mutation analysis in other genetic disorders and has already been successfully established for OTC deficiency,[24] collagen deficiency,[25] X-linked myotubular myopathy (XLMTM),[26] Duchenne and Becker muscular dystrophy (DMD, BMD), Wilson disease (WD), neurofibromatosis I and II, Charcot-Marie-Tooth disease, hereditary neuropathy with liability to pressure palsies, and defects in mitochondrial DNA.[27] As soon as the *MTM1* gene was identified we screened 47 unrelated XLMTM patients using the SSCP conditions described in Materials and Methods and found 34 novel and six known mutations.[28] Analysing 23 WD patients we detected 10 known and eight novel mutations, and in 38 patients at risk for mitochondrial diseases we identified three novel and seven known mutations as well as many polymorphisms. No other protocol published so far presents standard SSCP/HD conditions for mutation screening in different disease genes.

In conclusion, our study provides firm evidence that this modified SSCP/HD technique is very fast and has the ability to detect at least 97% of all point mutations in the coding region of a disease gene. Thus, we consider it to be a high yield scanning method that can be recommended to laboratories involved in the mutation screening of genetically heterogeneous populations.

Acknowledgements

We thank T Weber for technical assistance, and T Bienvenu, J Cheadle, F Chevalier-Porst, M Claustres, H Cuppens, G Cutting, M Dean, P Fanen, M Ferrari, K Friedman, PF Pignatti, GB Leoni, F Mekus, B Mercier, V Nunes, S Shackleton, and J Zielenski for providing control DNA samples of known mutations.

This work was supported by a grant from the Swiss National Foundation (32-040681.94) and by grants from Solvey Phama AG, Sandoz AG and the Silva Casa Foundation.

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