Molecular epidemiology of cystic fibrosis mutations and haplotypes in southern Italy evaluated with an improved semiautomated robotic procedure

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Abstract

We screened for 22 cystic fibrosis (CF) mutations in DNA from a first cohort of 69 CF patients from southern Italy using a semiautomated allele specific oligonucleotide (ASO) dot blot procedure based on two multiplex PCR amplifications. Seven mutations ($\Delta F508$, N1303K, G542X, 1717-1 G→A, W1282X, 1148T, and R553X) identified 77.6% of CF chromosomes. Detection reached 79.8% with the 2183 AA→G mutation analysed with the restriction generating PCR method. Thus, we included the 2183 AA→G mutation in the ASO protocol and set up the conditions to amplify the gene regions that include the eight mutations in a single multiplex PCR reaction. With this method we tested the DNA of the first cohort of 69 CF patients, a second cohort of 63 CF patients, and 300 carrier relatives; we also performed 12 prenatal diagnoses. The results from the 132 CF patients showed differences in the distribution of CF mutations between the south and north of Italy. The XV2c, KM19, and intron 8 VNDR haplotypes suggested the presence, in CF chromosomes bearing undetected mutations, of a limited number of unknown mutations typical of southern Italy. Finally, for six of the eight mutations, we compared the ASO procedure with the methods based on restriction enzymes; the results obtained with the two procedures were identical for all the 57 chromosomes compared.

(*J Med Genet* 1996;33:475-479)

Key words: cystic fibrosis; dot blot analysis; KM19; XV2c.

Cystic fibrosis (CF) is the most frequent hereditary disease in white populations, having an incidence of about 1 in 2500.¹ The disease is caused by molecular alterations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, identified in 1989.²⁻⁴ More than 500 CFTR mutations have been identified so far,⁵ most of which differ greatly in frequency depending on the ethnic group examined. The $\Delta F508$ microdeletion accounts for about 75% of the CF mutations in most of the populations studied from northern Europe and America,¹ but in southern Europe the incidence of the $\Delta F508$ mutation in CF chromosomes is below

or around 50%.¹⁶ Thus, particularly in these populations, the first step in the molecular analysis of CF mutation bearing families is to select a panel of mutations, in addition to $\Delta F508$, with which to maximise the percentage of CF chromosomes that can be characterised.

We studied a series of 264 CF chromosomes from Campania and Basilicata (southern Italy, about 8×10^6 inhabitants), where no mutation other than $\Delta F508$ had been previously studied, using an improved semiautomated procedure based on a single tube polymerase chain reaction (PCR) amplification followed by allele specific oligonucleotide (ASO) dot blotting. We found that (1) a panel of eight mutations (one of which is new for Italy) identified about 80% of CF chromosomes; (2) the method is rapid and efficient and the results are comparable with those obtained using restriction enzyme analysis; (3) the distribution of CF mutations in these regions is somewhat different from that of northern Italy; and (4) the analysis of the most used polymorphisms suggests the presence, in CF patients bearing unknown mutations, of a limited number of mutations typical of southern Italy.

Materials and methods

PATIENTS

Two cohorts of a total of 132 CF patients were enrolled in the study. They were referred to us from the CF Regional Centres of Campania and Basilicata (southern Italy); the families of all patients were native to these regions for at least two previous generations. The diagnosis, made on clinical and anamnestic findings, was confirmed by the sweat test for chloride and sodium (cut off 60 mEq/l for both). The first cohort, which consisted of 69 CF patients, was analysed for the 22 CFTR mutations listed in table 1 using a semiautomatic allele specific oligonucleotide (ASO) procedure^{7,8} and for the 2183 AA→G mutation using the restriction generating (RG) polymerase chain reaction (PCR) method.⁹ The analysis was performed twice on each sample in two different laboratories using a double blind procedure in all cases. The second cohort consisted of 63 CF patients; they were analysed for the panel of eight mutations ($\Delta F508$, N1303K, G542X, R553X, 1717-1 G→A, W1282X, 1148T, and 2183 AA→G) previously identified in the first cohort, with the improved ASO method based

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Received 1 November 1995
Revised version accepted for
publication 30 January 1996

Table 1 Mutations tested on DNA from 138 chromosomes of CF subjects from Campania and Lucania (southern Italy)

CFTR region	Mutations
Multiplex 1	
Exon 4	R117H, Y122X, I148T
Intron 4	621 + 1G→T
Exon 10	Q493X, ΔI507, ΔF508
Intron 10	1717-1 G→A
Exon 11	G542X, G551D, R553X, R560T
Intron 19	3849 + 10 Kb C→T
Exon 20	W1282X
Multiplex 2	
Exon 7	G314E, 1078ΔT, R334W, R347P
Exon 9	A455E
Exon 19	R1162X, 3659ΔC
Exon 21	N1303K

on a single PCR amplification described below. All the chromosomes positive for W1282X, N1303K, G542X, 1717-1 A→G, 2183 AA→G, and R553X were also analysed with the restriction enzyme methodology.

ASO ANALYSIS

DNA was extracted from peripheral blood with the classical phenol/chloroform protocol.¹⁰ The semiautomated allele specific oligonucleotide (ASO) dot blot procedure used to detect the 22 CFTR gene mutations in the first cohort of CF patients has been described elsewhere.⁷⁸ The improved method for the detection of the eight CF mutations in the second cohort of samples, included the following changes. (1) A single multiplex PCR reaction was used, with the following PCR conditions: 95° for five minutes, 10 cycles at 94° for 30 seconds, 65° for 30 seconds, and 72° for 30 seconds (decreasing the annealing temperature 1° per cycle); 30 cycles at 94° for 30 seconds, 60° for 30 seconds, and 72° for 30 seconds, and finally 70° for five minutes. The amplimers described by Bozon *et al*⁹ were used for the 2183 AA→G mutation. (2) The oligonucleotides used for the ASO analysis of the 2183 AA→G mutation were: normal probe: 5'-CTTTGTTTTTTTGT-AGA; mutant probe: 5'-GAAACAAAAA-GCAATCT. (3) The washing temperature for the 2183 AA→G analysis was 42°C.

ANALYSIS OF CF POLYMORPHISMS

The XV2c polymorphism was analysed by PCR amplification and enzyme incubation with *TaqI*¹¹ using the following modified amplification conditions: 95° for four minutes, 30 cycles at 94° for 30 seconds, 52° for 15 seconds and 70° for 45 seconds, and finally 70° for five minutes. The KM19 polymorphism was also detected by PCR amplification and enzymatic digestion with *PstI*.¹² The modified amplification conditions were: 94° for five minutes, then 26 cycles at 94° for 30 seconds, 55° for 15 seconds, and 70° for 45 seconds, and finally 65° for five minutes. The intron 8 VNDR was detected using a modified PCR amplification with the following conditions: 95° for five minutes, 30 cycles at 94° for 15 seconds,

Figure 1 Eight CF mutations in eight CF patients bearing different genotypes, analysed with the improved allele specific oligonucleotide procedure. The genotypes are indicated at the bottom; on the left are indicated the mutations searched for, each with the wild type and mutated oligonucleotide.

50° for 15 seconds, and 65° for 45 seconds, and finally, 70° for five minutes, followed by polyacrylamide gel electrophoresis.¹³ For both XV2c and KM19, the haplotype numbering corresponds to the absence (allele 1) or presence (allele 2) of the restriction sites, and to the number of GT repeats for the intron 8 VNDR.

ANALYSIS OF CF MUTATIONS BASED ON RESTRICTION ENZYMES

The R553X mutation was analysed by digestion of the amplified product with the enzyme *HincII*,¹⁴ W1282X using the restriction enzyme *MnII*,¹⁵ the 1717-1 G→A mutation by PCR mediated site directed mutagenesis followed by digestion with *AvaII*,¹⁶ and the G542X and the N1303K mutations were analysed by the restriction site generating PCR followed by digestion with *ScrFI* and *DdeI*, respectively.¹⁷ The 2183 AA→G mutation was analysed by RG-PCR followed by digestion with *AluII*.⁹

Figure 2 Prenatal diagnosis of CF by direct ASO analysis (panel A), by XV2c polymorphism analysis (panel B), and analysis of intron 8 VNDR (panel C). 1 = Child affected by CF; 2 = father of 1; 3 = mother of 1; 4 = proband (chorionic villi from 3); PC = positive control for TaqI digestion. The ASO analysis excluded, in the index case, the presence of $\Delta F508$ (panel A), XV2c analysis did not exclude or confirm, in the index case, the presence of the unknown mutation of maternal origin (panel B). Intron 8 VNDR analysis in the proband showed the presence of the unknown mutation of maternal origin and thus the carrier state for CF (panel C).

Results

The 69 CF patients in the first cohort were tested twice in double blind conditions by two different operators in two different laboratories (Houston and Naples) and the results obtained were identical. Seven of the 22 tested mutations were identified in the 69 patients tested. The seven mutations identified 77.6% of the CF chromosomes compared to 58.7% when the

Table 2 Frequency of eight cystic fibrosis (CF) mutations in 264 CF chromosomes of patients from Campania and Basilicata (southern Italy)

Mutation	CF chromosomes		Cumulative frequency (%)
	No	%	
$\Delta F508$	149/264	56.4	56.4
N1303K	18/264	6.8	63.2
G542X	15/264	5.7	68.9
W1282X	10/264	3.8	72.7
1717-1 G→A	6/264	2.3	75.0
2183 AA→G	5/264	1.9	76.9
R553X	3/264	1.1	78.0
I148T	2/264	0.7	78.7
Undetected	56/264	21.3	

screening was performed only for $\Delta F508$. The analysis of the 2183 AA→G mutation increased the detection rate to 79.8%.

Fig 1 shows the results of the improved ASO analysis (see Materials and methods) of the eight mutations in eight CF patients bearing different genotypes. Also in this case the results are clear and no cross interference from the different mutations was identified. With this procedure, in addition to the 132 CF patients, we also analysed about 300 CF carriers and 12 samples of chorionic villi for prenatal diagnoses. In all instances the results were clear and unambiguous (fig 2).

The panel of eight mutations detected about 78.7% of CF chromosomes in our sample of 264 CF chromosomes (table 2) compared to 56.4% when the screening was performed only for $\Delta F508$. Table 3 shows the XV2c, KM19, and intron 8 VNDR haplotypes associated with each mutation; the haplotype was not established in a few (about 10%) chromosomes. Most of the chromosomes bearing one of the eight mutations show haplotype 1 and 2 for XV2c and KM19, respectively. These haplotypes are associated with allele 23 (or 17 in a small percentage of cases) for the intron 8 VNDR. However, the majority of CF chromosomes bearing an undetected mutation shows allele 16 on the intron 8 VNDR, which in our population is usually associated with non-CF chromosomes (table 2, last line). Three haplotypes, different from those associated with the eight detected mutations, are expressed by about 70% of CF chromosomes bearing unknown mutations. The 57 chromosomes bearing one of the following mut-

Table 3 Number of haplotypes typed in chromosomes associated with the eight mutations identified in CF patients from southern Italy. Typing is made on the basis of the following three polymorphisms: XV2c (1 or 2), KM19 (1 or 2), and intron 8 VNDR (from 16 to 25)

Haplotype typing	$\Delta F508$	N1303K	G542X	W1282X	1717-1 G→A	I148T	R553X	2183 AA→G	Unknown mutation	Normal subjects
2,2,16	0	0	0	0	0	0	0	0	1	0
1,1,16	0	0	0	0	0	0	2	0	8	7
1,2,16	1	0	0	0	6	0	0	5	7	2
2,1,16	0	0	0	0	0	0	0	0	16	11
1,2,17	27	0	0	9	0	0	0	0	0	1
1,1,17	1	0	0	0	0	0	0	0	2	6
2,2,17	1	0	0	0	0	0	0	0	1	2
1,1,18	0	0	0	0	0	0	1	0	0	0
2,2,21	0	1	0	0	0	0	0	0	0	0
1,2,21	3	0	0	0	0	0	0	0	0	0
1,1,23	0	1	0	0	0	0	0	0	0	0
2,2,23	9	0	0	0	0	0	0	0	3	0
2,1,23	0	0	0	0	0	0	0	0	4	0
1,2,23	84	14	13	0	0	2	0	0	2	0
1,2,24	2	1	0	0	0	0	0	0	0	0
1,2,25	1	0	0	0	0	0	0	0	0	0
Not defined	20	1	2	1	0	0	0	0	12	0

ations, N1303K, G542X, W1282X, R553X, 2183 AA→G, or 1717-1 A→G, were also analysed with restriction enzyme techniques. The results obtained with this technique totally matched those obtained with our improved ASO procedure.

Discussion

We describe an improved ASO method and the results of the molecular analysis of CF patients from Campania and Basilicata (southern Italy), previously analysed in part only for the $\Delta F508$ mutation.⁶ This study confirms the efficiency of the ASO dot blot procedure, and our double blind analysis, performed on the first cohort of cases, shows that it is highly reproducible and robust.

The changes to the original ASO methodology^{7,8} described in this paper, consisting of the use of a single multiplex PCR and the ASO procedure for eight mutations including 2183 AA→G, allow more rapid detection of the eight most frequent mutations in southern Italy. With this method, as many as 96 DNA samples can be tested in a single run and identical results were obtained when we analysed six of these mutations with the restriction enzyme based methodology.

The molecular analysis of the study population of CF patients from southern Italy showed that the eight mutations screened account for 79% of CF chromosomes; this result shows higher genetic homogeneity for mutations in CF patients from southern Italy than in those from northern Italy, where screening for 62 mutations identified about 74% of CF chromosomes bearing 14 known mutations.¹⁸ The incidence of $\Delta F508$ in our population on the basis of 264 CF chromosomes is 56.4%, slightly higher than that (about 50%) previously reported,⁶ but much lower than that in northern Europe.¹ The frequency of the G542X mutation is higher in our regions of southern Italy than in northern Italy (5.7% versus 2.6%).¹⁸ The high frequency of G542X in our regions could result from the spreading of the mutation along Mediterranean coastal areas by the Arabs or from the Spanish domination of southern Italy for several centuries¹⁹; indeed, G542X is the second most frequent mutation in Spanish Mediterranean coastal areas.¹⁹ One of the G542X bearing subjects, who was homozygous for this mutation, was the first case ever among seven showing very severe liver involvement (Castaldo *et al.*, personal communication).

The R1162X mutation, which is the second most frequent mutation (about 10%) in northern Italy, was absent from our population, and the 2183 AA→G, which is present in about 10% of CF patients from northern Italy,²⁰ had a much lower frequency (about 2%) in our CF population. These findings confirm that there are differences in the genetic background between southern and northern Italy. In addition, we found the first two Italian cases of the rare I148T mutation, recently reported in 0.1% of French²¹ and in 9.1% of French Canadian⁵ CF patients, in two patients with compound heterozygosity for $\Delta F508$; the mutation has

never been reported previously in Italy. These patients had very severe pancreatic and lung disease.²²

In agreement with previous results,²³ the analysis of the polymorphisms associated with the CFTR gene showed that in the CF population from southern Italy the most frequent CF mutations are usually associated with homogeneous haplotypes, that is, allele 1 for XV2c, allele 2 for KM19, and allele 23 or, less frequently, allele 17 for the intron 8 VNDR. The $\Delta F508$ mutation is frequently associated with haplotype 1, 2, 23, (XV2c, KM19, and intron 8 VNDR, respectively) and, in a small percentage of cases, with haplotype 1, 2, 17. The G542X mutation was associated with haplotype 1, 2, 23 and this reinforces the notion of the common origin of the mutation and the close ethnic and genetic relationship between the southern Italian and Spanish populations.¹⁹ Of the three chromosomes bearing the R553X mutation, one is associated with haplotype 1, 1, 18 for the three polymorphisms respectively; the other two homozygously show haplotype 1, 1, 16. These findings, together with earlier reports describing the association of this mutation with both alleles 1 and 2 for both XV2c and KM19 polymorphisms,²⁴⁻²⁶ confirm the recurrent origin of the R553X mutation,²⁷ which is located in a hypervariable CpG region.

The haplotype patterns of 1717-1 G→A and W1282X were homogeneous (table 3), which is in agreement with previous data.²⁴ For N1303K all but two chromosomes showed the intron 8 VNDR allele 23, while one single chromosome carried allele 21 and another allele 24; other polymorphisms will be analysed to define whether they could derive from the most frequent variant 23. Finally, all the five CF chromosomes bearing the 2183 AA→G mutation showed haplotype 1, 2, 16 and the two I148T chromosomes were associated with the 1, 2, 23 haplotype. This is the first report of the haplotype pattern for chromosomes bearing these mutations.

In our sample of patients, most of the CF chromosomes with unidentified mutations showed the three most frequent haplotype patterns, in which alleles 1 and 2 (for either XV2c or KM19) are always associated with allele 16 of the intron 8 VNDR, which is the most frequent allele in non-CF chromosomes. These results suggest that a limited number of unknown mutations might be present with a high frequency in a large percentage of CF chromosomes bearing unknown mutations from our region; we are now screening to try to unravel the nature of these mutations by DGGE and sequencing analysis. This observation is in agreement with studies conducted in other Italian regions on the T338I mutation, which is peculiar to the Sardinian CF population,²⁸ on 2183 AA→G,²⁰ and on R1162X,^{18,20} both of which are very frequent in some areas of northern Italy. However, in other geographical areas such as Spain²⁹ and Germany³⁰ the analysis of the polymorphisms associated with the CFTR gene suggest that there are probably more than 100 different unidentified mutations. Furthermore, in our population the high

frequency of the intron 8 VNDR allele 16 among CF chromosomes bearing unknown mutations and among non-CF chromosomes indicates that no linkage disequilibrium exists between intron 8 VNDR haplotypes and normal or CF chromosomes.

In conclusion, our results confirm the excellent analytical performance of the improved semiautomated robotised ASO dot blot procedure for the analysis of CF mutations. We suggest the semiautomated ASO technique be used if panels of mutations and large series of samples are analysed, whereas the methods based on restriction analysis are more suitable for the study of single cases in families bearing known CF mutations. In addition, the most frequent CF mutations in patients from southern Italy are different from those found in northern Italy, and our analysis may also suggest the presence of a limited number of undetected mutations peculiar to our geographical area. These results have an impact on other populations, given the migration of quite large groups of people from southern Italy in the last 200 years, and the consequent genetic mixing, particularly in northern Italy, Switzerland, Germany, and the United States.

We thank Dr Sue Richards (Baylor College of Medicine, Houston, Texas), in whose laboratory this work was initiated, for her support and advice. Grants from MURST, Regione Campania, AGENSUD, CNR (PF Biotecnologie), and Ministero della Sanità (Rome) are gratefully acknowledged.

- Romeo G, Devoto M. Population analysis of the major mutation in cystic fibrosis. *Hum Genet* 1990;85:391-445.
- Rommens JM, Iannuzzi MC, Kerem BS, et al. Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science* 1989;245:1059-65.
- Riordan JR, Rommens JM, Kerem BS, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 1989;245:1066-73.
- Kerem BS, Rommens JM, Buchanan JA, et al. Identification of the cystic fibrosis gene: genetic analysis. *Science* 1989;245:1073-80.
- The Cystic Fibrosis Genetic Analysis Consortium. Population variation of common cystic fibrosis mutations. *Hum Mutat* 1994;4:167-77.
- Santamaria F, Salvatore D, Castiglione O, Raia V, De Ritis G, Sebastio G. Lung involvement, the $\Delta F508$ mutation and DNA haplotype analysis in cystic fibrosis. *Hum Genet* 1992;88:639-41.
- De Marchi JM, Beaudet AL, Caskey CT, Richards CS. Experience of an academic reference laboratory using automation for analysis of cystic fibrosis mutations. *Arch Pathol Lab Med* 1994;118:26-32.
- De Marchi JM, Richards CS, Fenwick RG, Pace R, Beaudet AL. A robotic-assisted procedure for large scale cystic fibrosis mutation analysis. *Hum Mutat* 1994;4:281-90.
- Bozon D, Zielinski J, Rininsland F, Tsui LC. Identification of four new mutations in the CFTR gene: I148T, L1077P, Y1092X, 2183 AA→G. *Hum Mutat* 1994;3:330-2.
- Sambrook J, Fritsch EF, Maniatis T. *Molecular cloning: a laboratory manual*. 2nd ed. New York: Cold Spring Harbor Laboratory Press, 1989.
- Rosenbloom CL, Kerem BS, Rommens JM, et al. DNA amplification for detection of the XV-2c polymorphism linked to cystic fibrosis. *Nucleic Acids Res* 1989;17:7117.
- Feldman GL, Williamson R, Beaudet AL, O'Brien WE. Prenatal diagnosis of cystic fibrosis by DNA amplification for detection of KM 19 polymorphism. *Lancet* 1988;ii:102.
- Morral N, Nunes V, Casals T, Estivill X. CA/GT microsatellite alleles within the cystic fibrosis transmembrane conductance regulator (CFTR) gene are not generated by unequal crossingover. *Genomics* 1991;10:692-8.
- Cutting GR, Kasch LM, Rosenstein BJ, et al. A cluster of cystic fibrosis mutations in the first nucleotide-binding fold of the cystic fibrosis conductance regulator protein. *Nature* 1990;346:366-9.
- Vidaud M, Fanen P, Martin J, Ghanem N, Nicolas S, Goossens M. Three point mutations in the CFTR gene in French cystic fibrosis patients: identification by denaturing gradient gel electrophoresis. *Hum Genet* 1990;85:446-9.
- Cremonesi L, Seia M, Magnani C, Ferrari M. Rapid detection of 1717-1 G→A mutation in CFTR gene by PCR mediated site directed mutagenesis. *Clin Chem* 1991;37:1967.
- Gasparini P, Bonizzato A, Degnini M, Pignatti PF. Restriction site generating-polymerase chain reaction (RG-PCR) for the probeless detection of hidden genetic variation: application to the study of some common cystic fibrosis mutations. *Mol Cell Probes* 1992;6:1-7.
- Gasparini P, Marigo C, Bisceglia G, et al. Screening of 62 mutations in a cohort of cystic fibrosis patients from north eastern Italy: their incidence and clinical features of defined genotypes. *Hum Mutat* 1993;2:389-94.
- Casals T, Nunes V, Palacio A, et al. Cystic fibrosis in Spain: high frequency of mutation G542X in the Mediterranean coastal area. *Hum Genet* 1993;91:66-70.
- Bonizzato A, Bisceglia L, Marigo C, et al. Analysis of the complete coding region of the CFTR gene in a cohort of CF patients from north-eastern Italy: identification of 90% of the mutations. *Hum Genet* 1995;95:397-402.
- Chevalier-Porst F, Bonardot AM, Gilly R, Chazalotte JP, Mathieu M, Bozon D. Mutation analysis in 600 French cystic fibrosis patients. *J Med Genet* 1994;31:541-4.
- Castaldo G, Rippa E, Raia V, et al. Clinical features of cystic fibrosis patients with rare genotypes. *J Med Genet* 1996;33:73-6.
- Morral N, Bertranpetit J, Estivill X, et al. The origin of the major cystic fibrosis mutation ($\Delta F508$) in European populations. *Nature Genet* 1994;7:169-75.
- Dork T, Neumann T, Wulbrand U, et al. Intra- and extragenic marker haplotypes of CFTR mutations in cystic fibrosis families. *Hum Genet* 1992;88:417-25.
- Bal J, Stuhmann M, Schloesser M, Schmidtke J, Reiss J. A cystic fibrosis patient homozygous for the nonsense mutation R553X. *J Med Genet* 1991;28:715-17.
- Cheadle J, Al-Jader L, Goodchild M, Meredith AL. Mild pulmonary disease in a cystic fibrosis child homozygous for R553X. *J Med Genet* 1992;29:597.
- Reiss J, Cooper DN, Bal J, Slomski R, Cutting GR, Krawczak M. Discrimination between recurrent mutation and identity by descent: application to point mutations in exon 11 of the cystic fibrosis (CFTR) gene. *Hum Genet* 1991;87:457-61.
- Rendine S, Piazza A. Genetic history of CF mutations in Italy. *20th European Cystic Fibrosis Conference, Bruxelles, 1995: Abst P204*.
- Chillon M, Casals T, Gimenez J, et al. Analysis of the CFTR gene confirms the high genetic heterogeneity of the Spanish population: 43 mutations account for only 78% of CF chromosomes. *Hum Genet* 1994;93:447-51.
- Dork T, Mekus F, Schmidt K, et al. Detection of more than 50 different CFTR mutations in a large group of German cystic fibrosis patients. *Hum Genet* 1994;94:533-42.