

# Comprehensive Cystic Fibrosis Mutation Epidemiology and Haplotype Characterization in a Southern Italian Population

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## Summary

We screened the whole coding region of the cystic fibrosis transmembrane regulator (*CFTR*) gene in 371 unrelated cystic fibrosis (CF) patients from three regions of southern Italy. Forty-three mutations detected 91.5% of CF mutated chromosomes by denaturing gradient gel electrophoresis analysis, and three intragenic *CFTR* polymorphisms predicted a myriad of rare mutations in uncharacterized CF chromosomes. Twelve mutations are peculiar to CF chromosomes from southern Italy: R1158X, 4016insT, L1065P and 711+1G>T are present in 6.3% of CF chromosomes in Campania; G1244E and 852del22 are present in 9.6% of CF chromosomes in Basilicata and 4382delA, 1259insA, I502T, 852del22, 4016insT, D579G, R1158X, L1077P and G1349D are frequent in Puglia (19.6% of CF alleles). Several mutations frequently found in northern Italy (e.g., R1162X, 711+5G>T) and northern Europe (e.g., G551D, I507del and 621+1G>T) are absent from the studied population. The I148T-3195del6 complex allele was present in two CF chromosomes, whereas I148T was present in both alleles (as a single mutation) in another CF patient and in five CF carriers; this could result from crossover events. The haplotype analysis of three intragenic polymorphisms (IVS8CA, IVS17bTA and IVS17bCA) compared with data from other studies revealed that several mutations (3849+10kbC>T, 1717-1G>A, E585X, 3272-26G>A, L558S, 2184insA and R347P) originated from multiple events, whereas others (R1158X and S549R) could be associated with one or more intragenic recombinant events. Given the large population migration from southern Italy, knowledge of the CF molecular epidemiology in this area is an important contribution to diagnosis, counselling and interlaboratory quality control for molecular laboratories worldwide.

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Keywords: CF mutation epidemiology, complex alleles, CF haplotypes, southern Italy CF mutation.

## Introduction

Thus far, about 1300 cystic fibrosis (CF)-causing mutations have been identified in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene (<http://www.genet.sickkids.on.ca/cftr>). Most

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mutations are “private,” but some are frequent in specific regions or ethnic groups (Estivill *et al.* 1997): 2143delT in Germany (Dork *et al.* 1992), W1282X among Ashkenazim (Shoshani *et al.* 1992), Y122X in Reunion Island (Chevalier-Porst *et al.* 1992), 2183AA>G and R1162X in northeast Italy and T338I in Sardinia (Rendine *et al.* 1997). Mutation epidemiology is a crucial component of strategies for CF diagnosis and counselling because it can be used to calculate the residual risk of being a CF carrier in each population. In addition, until the incidence of *CFTR* mutations in individual populations has been defined, it will remain difficult to plan interlaboratory quality control strategies (Dequeker & Cassiman, 1998, 2000), and to produce industrial kits and control samples with known mutations.

Denaturing gradient gel electrophoresis (DGGE) and single-strand conformation polymorphism (SSCP) detection have been widely used to detect mutations in the *CFTR* gene. Whereas the population of northern Italy is genetically and anthropologically closer to central European ethnic groups, the population of southern Italy is more akin to other ethnic groups from the Mediterranean basin. In fact, the incidence of the most frequent *CFTR* mutations differs among Italian regions (Rendine *et al.* 1997). Given the massive migration from southern Italy to other European countries and the Americas, which reached its height at the beginning of the last century, information about the epidemiology of CF mutations in the populations of southern Italy could be particularly telling. Furthermore, the study of *CFTR* haplotypes can help to trace historical events related to the origin and spread of CF mutations, and to identify “recurrent” mutations.

We studied 371 unrelated CF patients from the Campania, Puglia and Basilicata regions of southern Italy. We used DGGE and sequence analysis to screen the whole coding region of the *CFTR* gene, and tested the association of these mutations with three intragenic polymorphisms.

## Materials and Methods

### Patients

All procedures used in this study were in accordance with the Helsinki Declaration of 1975 (revised). We



**Figure 1** A map of Italy showing Campania (1), Basilicata (2) and Puglia (3).

studied 371 unrelated CF patients residing in the Campania ( $6 \times 10^6$  inhabitants), Basilicata ( $0.5 \times 10^6$  inhabitants) and Puglia ( $5 \times 10^6$  inhabitants) regions of southern Italy for at least two generations. About 50 other patients were excluded from the study because they were related to the 371 cases. The studied patients account for about 90% of the known CF patients from these regions. The remaining 10% of known CF patients born in the three regions studied are currently being followed by centres in other Italian regions. Figure 1 shows a map of Italy, and the three regions in which our patients were recruited. The diagnosis of CF was confirmed in all patients by sweat chloride levels (cut-off = 60 mEq/L) and supported by clinical findings.

### Molecular Analysis

The CF patients were first examined for a panel of 13 CF mutations, with the allele specific oligonucleotide (ASO) dot blot procedure (Castaldo *et al.* 1996, 1999). The 13 mutations in this panel are: F508del, N1303K, G542X, W1282X, 2183AA>G, 1717-1G>A, R553X, I148T, R1158X, 711+1G>T, 4016insT, L1065P and

G1244E. We then analyzed CF chromosomes bearing unknown mutations with DGGE of all exons of the *CFTR* gene, using the primers and the conditions described elsewhere (Fanen et al. 1992; Costes et al. 1993) followed by sequencing analysis to characterize the mutations. For each DGGE run, we used a negative control (DNA with no *CFTR* mutations) and two positive controls (DNA with known mutations in the fragment analyzed). The aberrant fragments were analyzed by automated sequencing with the Applied Biosystems 373A apparatus (Perkin Elmer, Foster City, CA).

Intragenic IVS8CA (Morrall et al. 1991), IVS17bTA and IVS17bCA (Zielinski et al. 1991) polymorphisms were analyzed with polymerase chain reaction (PCR) followed by polyacrylamide electrophoresis. In each run, we used at least one control sample whose alleles were known; the allele numbering of the three polymorphisms is based on the number of repeats. Lastly, we calculated the CF chromosome detection rate (i.e., the percentage of CF chromosomes identified) in southern Italian patients obtained with four commercial kits: CF-12 ARMS (12 mutations; Zeneca Diagnostics, UK); CF29 (29 mutations; Elucigene, UK); OLA PCR (32 mutations; PE, Applied Biosystems, CA, USA); and INNO-Lipa reverse dot-blot (29 mutations; Innogenetics, Belgium).

## Results

The 13 mutations tested by ASO were detected in 77.2% of CF chromosomes (85.3% from Campania, 78.7% from Basilicata and 68.8% from Puglia). Thus, 169 CF chromosomes carried an unidentified mutation. These chromosomes were analyzed by DGGE, which detected 30 further mutations in 106 chromosomes. In total, 43 mutations (all previously described) were detected in 91.5% of CF chromosomes from our population (Table 1).

Fourteen mutations were identified in more than 1.0% of CF chromosomes (cumulative frequency: 82.3%). Including 14 other mutations (to give a total of 28 mutations) the detection rate increased to 90.0%. The 15 remaining mutations were identified on a single chromosome, and 63 alleles (8.5%) remained uncharacterized. More than 25 different haplotypes were identified in these latter alleles, indicating that a myriad of

different *CFTR* mutations are present on uncharacterized CF chromosomes.

Several mutations that are rare or absent in other ethnic groups were frequent (1.0 to 6.0%) in CF patients from southern Italy, with several differences among the three geographical regions. In particular, 4016insT, R1158X, 711+1 G>T and L1065P had a cumulative frequency of 6.3% in CF chromosomes from Campania; G1244E and 852del22 a cumulative frequency of 9.6% in CF chromosomes from Basilicata; and 4382delA, 1259insA, I502T, 852del22, 4016insT, D579G, R1158X, L1077P and G1349D a cumulative frequency of 19.6% in CF chromosomes from Puglia. Table 1 shows the epidemiology of *CFTR* mutations in our population and a procedure for the large-scale analysis of several mutations that are frequent in southern Italy.

Among our patients we identified 172 homozygotes for F508del, 78 (45.3%) of which had the same haplotype for the three intragenic polymorphisms tested. We also identified three homozygotes for G542X, three for 852del22, two for 2183AA>G, and one each for N1303K, 1717-1G>A, 4016insT, R553X, R1158X and L1065P. In all these cases, the patients were also homozygotes for the haplotypes of the three polymorphisms tested.

Figure 2, panel A shows an example of the multiplex DGGE analysis of *CFTR* exons 8, 5 and 18. The DGGE pattern of exon 5 in the CF patient is suggestive of a heterozygous gene variant, which was identified as the G178R missense mutation. Figure 2, panel B shows an example of the DGGE analysis of the *CFTR* gene exon 6a. The pattern of the CF patient differs from the control pattern and sequence revealed the 852del22 mutation in heterozygosity.

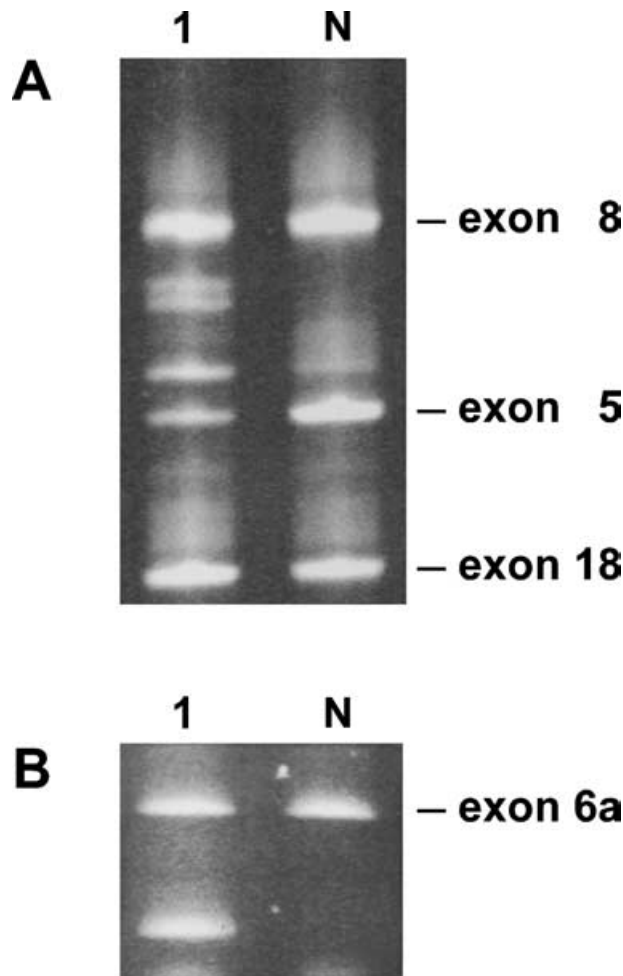
We also evaluated the detection rate of CF chromosomes from southern Italian patients using four commercial kits. The CF-12 ARMS kit detected 71.0% of CF chromosomes; CF29 detected 75% of CF chromosomes; OLA PCR detected 74.8% of CF chromosomes; and the INNO-Lipa reverse dot-blot protocol detected 74.1% of CF chromosomes from our population.

The haplotype analysis based on three intragenic *CFTR* polymorphisms revealed that a single haplotype is linked to some *CFTR* mutations (Table 2, group a). Other mutations are associated with haplotypes that

**Table 1** Molecular epidemiology (% frequency) of CFTR mutations in three regions of southern Italy. A procedure for the large-scale analysis of several mutations peculiar to southern Italy is also indicated

Mutation Analytical CF alleles procedure	Campania n = 340	Basilicata n = 52	Puglia n = 350	Total n = 742	
DF508	55.6	55.8	46.8	51.5	
N1303K	7.3	3.8	7.7	7.3	
G542X	5.0	3.8	7.1	5.9	
W1282X	3.5	3.8	0.6	2.2	
2183 AA>G	2.3	5.8	0.8	1.9	
852del22	0	5.8	3.2	1.9	3% agarose
1717-1G>A	2.3	1.9	1.1	1.8	
4382delA	0	0	3.7	1.8	RE (Ear I -)
1259insA	0	0	3.1	1.5	
4016insT	2.1	0	1.1	1.5	ASO
R553X	1.5	0	1.7	1.5	
R1158X	1.5	0	1.3	1.2	ASO or RE (Sfa N 1 -)
L1077P	0.6	0	1.9	1.2	
I502T	0.3	0	2.0	1.1	RE (Mse I -)
3849+10kbC>T	0	1.9	1.6	0.9	
D579G	0	0	1.6	0.8	RE (Avr II +)
G1244E	0.9	3.8	0.3	0.8	ASO or RE (Mbo II +)
G1349D	0	0	1.7	0.8	RE (Sty I -)
2789+5 G>A	0.6	0	0.8	0.7	
711+1 G>T	1.5	0	0	0.7	ASO
L1065P	1.2	0	0	0.5	ASO or RE (Mnl I +)
R347P	0.3	0	0.9	0.5	
2522insC	0.9	0	0	0.4	
E585X	0.6	0	0	0.3	
G85E	0.6	0	0	0.3	
G178R	0.6	0	0	0.3	
D1152H	0.3	0	0.3	0.3	
I148T-3195del6	0.6	0	0	0.3	
I148T (alone)	0	0	0.3	0.1	
R334W	0	0	0.3	0.1	
DI507	0	0	0.3	0.1	
I1005R	0	0	0.3	0.1	
3272-26A>G	0.3	0	0	0.1	
2711delT	0.3	0	0	0.1	
L558S	0	1.9	0	0.1	
W1063X	0	0	0.3	0.1	
D110H	0.3	0	0	0.1	
S549R (A>C)	0	1.9	0	0.1	
2184insA	0.3	0	0	0.1	
3131del22	0.3	0	0	0.1	
R709N	0	0	0.3	0.1	
A349V	0	0	0.3	0.1	
4015insA	0	0	0.3	0.1	
Y849X	0	1.9	0	0.1	
Cumulative	91.6	92.1	91.7	91.5	
Unknown	8.4	7.9	8.3	8.5	
Total	100,0	100,0	100,0	100,0	

RE: restriction enzyme (-/+ : abolition or introduction of a RE site); ASO: allele specific oligonucleotide



**Figure 2** Multiplex denaturing gradient gel electrophoretic analysis of exons 8, 5 and 18 of the cystic fibrosis transmembrane regulator gene in a cystic fibrosis patient (case n. 1) and in a normal control (N). The analysis revealed an altered pattern of exon 5 in the patient; the sequence analysis identified the G178R mutation at the heterozygous state. The electrophoretic pattern typical of point mutations usually shows additional bands (due to the formation of heteroduplexes between normal and mutated alleles). Figure 1 panel B, shows an altered DGGE pattern for exon 6a in a CF patient (case n. 1) as compared to the normal control (N). However, in this case the altered electrophoretic pattern does not show the four bands, but only one additional band. In fact, the 852del22 (a 22-base-pair deletion) does not give rise to heteroduplex formation.

differ at one microsatellite (Table 2, group b), and a third group includes mutations that are associated with at least two haplotypes (Table 2, group c). Finally, two CF patients carried the I148T and 3195del6 mutations in *cis*, giving rise to a “complex allele.” Another CF patient from the study, and five CF carriers (data not

shown), carried I148T without 3195del6 in *cis*. Different haplotypes are linked to I148T alone and to the I148T “complex allele” (see Table 2, group c).

## Discussion

The data reported herein concern about 90% of the known CF patients from Campania, Basilicata and Puglia, and thus reflect the distribution of CF mutations in southern Italy. Our strategy (ASO dot blot and DGGE screening) detected 91.5% of CF chromosomes. Detection rates obtained with DGGE or SSCP range from 75% in Turkish (Kilinc *et al.* 2002) to 95.4% in Germany (Claustres *et al.* 1993; Hughes *et al.* 1996a; Tzetzis *et al.* 1997; Bonizzato *et al.* 1995; Dork *et al.* 1994). The differences could depend on the variable sensitivity of analytical techniques, but more likely a percentage of mutations in the populations examined lie outside the coding regions and so elude scanning procedures. Furthermore, large deletions of the *CFTR* gene in heterozygosity, and hence undetectable by current scanning procedures (and not tested in the present study), could account for unknown mutations. Finally, *CFTR* sequence variations considered to be likely, as in principle they do not alter the amino acids within the protein sequence, could be associated with impaired mRNA splicing and thus act as disease-causing mutations (Pagani *et al.* 2003).

We confirm that the frequency of F508del was lower in southern Italy than in central and northern Europe (Estivill *et al.* 1997); however, it did not differ between our patients and those of northern Italy (Bonizzato *et al.* 1995). Several mutations frequent in northern and central Europe, i.e., 621+1G>T, I507del, G551D, were not detected in our population and are also rare in other Mediterranean regions (Rendine *et al.* 1997; Casals *et al.* 1997b; Estivill *et al.* 1997). Our study also revealed differences in the epidemiology of CF mutations between southern and northern Italy: R1162X, frequent (9–14%) in Veneto and Trentino (north-east Italy), is absent in CF patients from southern Italy; 2183AA>G has a frequency of about 10% in north-east Italy versus <2.0% in our region and other Mediterranean populations (Rendine *et al.* 1997). Similarly, 1717-1G>A is more frequent in north-western Italy (5–6%) than in southern Italy (about 2.0% in our population), and T338I,

**Table 2** Mutations linked to a single haplotype, characterized at the level of three CFTR intragenic loci (IVS8CA, IVS17bTA, IVS17bCA) by the indication of the repeats number.

Mutation	Present study case (n) references*	Haplotype (n. of repeats)	Other studies case	(n)
W1282X	16	17-7-17	26	1, 2, 3
1259insA	11	16-33-13		
852del22	11	16-33-13		
4016insT	11	16-30-13		
I502T	8	16-30-13		
L1065P	4	16-30-13		
2522insC	4	23-30-13		
2789+5G>A	2	17-7-17	9	1, 2, 3
D1152H	2	16-7-13		
2711delT	1	16-45-13	2	3
D110H	1	16-32-13		
Y849X	1	16-30-13		

\*References:

- 1: Morral *et al.*, 1996
- 2: Claustres *et al.*, 1996
- 3: Hughes *et al.*, 1996b

peculiar to Sardinia, is absent in our population (Rendine *et al.* 1997). However, 12 mutations (4016insT, R1158X, 711+1G>T, L1065P, G1244E, 4382delA, 1259insA, I502T, 852del22, D579G, L1077P and G1349D) have not been found (or have a low incidence) in populations from the British Isles (Cheadle *et al.* 1993; Ferec *et al.* 1992), Spain (Chillon *et al.* 1994; Casals *et al.* 1997a, 1997b), France (Claustres *et al.* 1993, 1996), Ireland (Hughes *et al.* 1996a), Germany (Dork *et al.* 1992) or northern Italy (Bonizzato *et al.* 1995), but have a frequency between 1.0 and 6.0% in southern Italy.

The epidemiological pattern of CF mutations in south and north Italy reflects the pre-Romanic colonisation of Italian regions (Rendine *et al.* 1997): populations of Celtic origin colonized northern Italy and most central European countries, whereas the Greeks colonised southern Italy from the year BC 600. Other mutations appear to have originated in southern Italy and their frequency has probably been amplified through consanguineous marriages; for instance, 852del22, L1065P and others were identified in homozygosity in several CF patients in whom the haplotypes for the three polymorphisms analyzed were also homozygous. However, despite the similar historical background, the frequency of several other mutations differed between the three regions of southern Italy. Some of these

mutations may have been introduced by more recent immigrations.

The differing epidemiology of CF mutations, even between regions with a similar historical background, underlines the need for thorough investigation of the regional origin of each patient with suspected CF in order to select the appropriate panel of mutations for analysis and hence, to make accurate risk calculations. In fact, an increase in the detection rate of CF mutations greatly reduces the residual risk that a couple negative for the test will have an affected child (Castaldo *et al.* 1999). In this context, the molecular epidemiology of CF mutations in regions of southern Italy could be useful for laboratories in other countries, given the large migratory flow from these regions to other European areas, and to north and south American and Australia in the last two centuries. Furthermore, international quality control strategies should take into account the different epidemiology of CF mutations. For example, the study promoted by the European Concerted Action for Quality Control on CF that involved 136 European laboratories (Dequeker & Cassiman, 1998, 2000) included samples bearing mutations G551D and 621+1G>T, which are virtually absent in southern Europe and Italy (Rendine *et al.* 1997).

Due to the presence of 'local' mutations, the detection rate with commercial kits for CF chromosomes in

**Table 3** Mutations linked to different haplotypes possibly due to slippage events, characterized at the level of three CFTR intragenic loci (IVS8CA, IVS17bTA, IVS17bCA) by the indication of the repeats number

	Present study		Other studies		
	Cases (n) references*	Haplotype (n. of repeats)	cases (n)	Haplotype (n. of repeats)	
R347P	4/4	16-32-13	3	16-32-13	1,2,3
			1	16-31-13	3
			2	17-28-13	1
			1	16-45-13	
			1		
L1077P	3/3	17-7-17	1	17-7-17	1
			1	17-7-16	1
G85E	2/2	16-24-13	9	16-24-13	2,3
			1	16-25-13	2
2183AA>G	14/14	16-31-13	1	16-31-13	3
			4	16-30-13	1
R553X	6/11	17-55-13	3	17-58-13	3
	3/11	18-55-13	1	17-57-11	1
	1/11	16-55-13	2	17-55-13	1,3
	1/11	16-55-11	6	17-55-11	1
			1	17-52-11	1
			1	17-54-11	1
			1	17-56-13	3
G1244E	5/6	16-32-13	1	17-34-13	1
	1/6	16-34-13			
711 +1 G>T	5/5	16-25-13	7	16-25-13	1,2,3
			1	16-26-13	1
G1349D	5/6	16-30-13			
	1/6	16-32-13			
G178R	1/2	16-32-13	1	16-30-13	3
	1/2	16-32-13	2	16-32-13	1

## \*References

- 1: Morral *et al.* 1996.
- 2: Claustres *et al.* 1996.
- 3: Hughes *et al.* 1996b.

southern Italy is low, and the same is true for other populations (Tomaiuolo *et al.* 2003). Thus, customized procedures must be used to detect mutations peculiar to specific ethnic groups, or commercial kits should be produced for different ethnic groups. Alternatively, procedures such as DGGE and D-HPLC could be used in second level laboratories to screen CF chromosomes that are negative for the first panel of mutations.

Two CF patients from the present study carried the I148T mutation *in cis* with the 3195del6 mutation; another CF patient, five patients with CBAVD, and five unrelated CF carriers from our population (data not shown), carried I148T not associated *in cis* with 3195del6. The chromosomes bearing I148T alone are linked to a different haplotype (of two or three loci)

than those linked to the I148T-3195del6 complex allele; a crossover event may be responsible for this. It must be emphasised that all of these cases originated in the same region (Campania). It is not clear whether I148T alone is a disease-causing mutation, even though it was described in a CF patient in whom no other *CFTR* mutation was detected in the whole coding region (Bozon *et al.* 1994). Furthermore, it has been demonstrated that I148T alone alters the transport of  $\text{HCO}_3^-$  across the apical membrane of epithelial cells (Ahn *et al.* 2001).

It has been reported that complex alleles are sporadic in CF (Dork *et al.* 1991; Duarte *et al.* 1996) but their frequency is probably underestimated (Savoy *et al.* 1995; www.genet.sickkids.on.ca/cftr). Genetists

**Table 4** Mutations linked to different haplotypes due to recombinant or recurrent events, characterized at the level of three CFTR intragenic loci (IVS8CA, IVS17bTA, IVS17bCA)

	Present study		Other studies		
	Cases (n) references*	Haplotype (n. of repeats)	Cases (n)	Haplotype (n. of repeats)	
I148T and 3195del6 (in cis)	2/2	23-7-17	3	23-7-17	2,3
I148T	1/1	23-32-13			
S549R (A>C)	1/1	23-33-13	1	16-33-13	2
1717-1G>A	13/13	16-7-17	23	16-7-17	1,2,3
			2	16-30-13	1
			1	16-32-13	1
R1158X	6/6	16-7-17	1/2	16-7-17	2
			1/2	6-45-13	2
			1/1	16-31-13	3
			1/1	16-45-13	3
3849 +10kbC>T	5/5	23-31-13	2	23-31-13	1
			1	16-14-31	4
			1	16-7-17	1
			3	16-46-13	2
			1	16-17-19	2
			1	17-31-13	2
E585X	2/2	16-7-17	1	16-32-13	2
			1	17-31-13	2
			1	16-7-16	see 2
3272-26G>A	1/1	15-7-17	1	16-32-13	1
			4	16-7-17	5
L558S	1/1	16-32-13	1	16-32-13	1
			1	15-7-17	1
2184 ins A	1/1	16-29-13	1	16-45-13	1
			1	16-7-17	1
			1	16-24-13	3

## \*References

- 1: Morral *et al.* 1996b.
- 2: Claustres *et al.* 1996.
- 3: Hughes *et al.* 1996b.
- 4: Hughes *et al.* 1996a.
- 5: Tzetzis *et al.* 1997.

and researchers should be alert to the existence of complex alleles in genotype-phenotype correlation studies.

The analysis of haplotypes linked to CF mutations indicates that several mutations (Table 2, group a) may have originated from a single event, given the concordance of haplotypes in our population and in other populations, even if not all of them have been studied in other ethnic groups. Other mutations (see Table 2, group b) also seem to derive from a founding event, and haplotypes differing by a single microsatellite could depend on slippage phenomena, as already proposed for mutations G85E (Claustres *et al.* 1996) and L1077P (Morral *et al.* 1996). In particular, the R553X mutation is as-

sociated with a myriad of sequential alleles which are frequently observed on less stable alleles that have more than 50 IVS17b(TA) repeats (Claustres *et al.* 1996); these could derive from a common ancestor via the slippage phenomena, rather than from recurrent phenomena as suggested by others (Dork *et al.* 1994). A recurrent origin has also been postulated for R347P (Morral *et al.* 1994; Claustres *et al.* 1996) since it lies on a triplet with a high mutation rate, and different microsatellite alleles have been associated with this mutation. Similarly, a number of different haplotypes have been associated with the 3849+10kbC>T mutation; thus multiple recurrent events have also been postulated for this high



mutation rate nucleotide (Morral *et al.* 1994). Other mutations (Table 2, group c) have a recurrent origin, or the different linked haplotypes may result from recombination events. Taken together, our data indicate that a notable percentage of CF mutations are linked to recurrent events.

To conclude, the epidemiology of CF mutations differs in southern Italy compared with northern Italy and Europe. Given the significant migration from southern Italy over the last two centuries, these data may have an impact on the results obtained by laboratories worldwide. In this context, scanning techniques can make a valuable contribution as a second level approach to the screening of CF alleles bearing unknown mutations. Similarly, haplotype analysis provides important data about the origin and spread of CF mutations.

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