

## ORIGINAL INVESTIGATION

Marc De Braekeleer · André Chaventré  
Giorgio Bertorelle · Claudine Verlingue  
Odile Raguénès · Bernard Mercier · Claude Férec

## Linkage disequilibrium between the four most common cystic fibrosis mutations and microsatellite haplotypes in the Celtic population of Brittany

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**Abstract** Microsatellite haplotypes were determined for 117 chromosomes carrying the four most frequent mutations in the cystic fibrosis (CF) gene identified in the Breton population of Celtic origin, as well as for 83 normal chromosomes (noncarriers of a CF mutation). Each of the three non- $\Delta F508$  mutations was associated with a single haplotype: 1078delT with 16-31-13, G551D with 16-7-17, and W846X with 16-32-13. Although these results suggest identity-by-descent for each mutation, recurrent mutations, although unlikely, could not be completely ruled out. The four most frequent haplotypes on normal chromosomes and the three most frequent haplotypes on  $\Delta F508$  chromosomes are the same as those found in Ireland, Spain, and Italy. This suggests that some haplotypes, associated or not with the  $\Delta F508$  mutation, were present in an ancestral population from which all four populations descended.

### Introduction

Since the identification of the cystic fibrosis transmembrane regulator (CFTR) gene responsible for cystic fibrosis (CF), more than 500 mutations have been reported to the Cystic Fibrosis Genetic Analysis Consortium. Sev-

eral mutations have now been shown to be associated with specific intragenic microsatellite (MS) haplotypes. This association has been used to try to date the origin of  $\Delta F508$ , the most frequent mutation accounting for some 70% of the CF chromosomes worldwide, and some other mutations in different European populations (Russo et al. 1995; Morral et al. 1993, 1994; Cashman et al. 1995 a, b).

Brittany is a region located in northwest France. It comprises four administrative areas called 'départements' – Finistère, Côtes d'Armor, Morbihan, and Ille-et-Vilaine. The population of the first three 'départements' is mostly of Celtic origin whereas the population is much diversified in Ille-et-Vilaine. The region is characterized by a low immigration rate from other French regions and, historically, by high consanguinity and cultural and linguistic isolation (Sutter 1968, 1972; Sutter and Goux 1962; Sutter and Tabah 1955; Fleurio 1980).

Brittany has one of the highest CF incidences, with 1 in 1200 to 1 in 1600 live births and a carrier rate close to 1 in 20 (Bois et al. 1978). During the past years, a major effort was made to identify the CFTR mutations present in the Breton population of Celtic origin (Férec et al. 1991, 1992). It resulted in the identification of 19 different mutations representing over 98% (358/365 CF chromosomes) of the CFTR mutations in this population. Four mutations accounted for 91.3% of the CF chromosomes (Férec et al. 1992); these are  $\Delta F508$  (81.2%), 1078delT (4.9%), G551D (4.1%), and W846X (1.1%).

This study was aimed at determining the microsatellite haplotypes associated with these four mutations, looking for possible linkage disequilibrium and recurrent mutations in this population. Our results were also compared with those reported in a few European populations.

### Material and methods

The CF study in Brittany is based on 902 CF patients from 795 families who were or are still followed at the Centre Hélio-Marin in Roscoff (Brittany) or were subjected to a molecular analysis at the Centre de Biogénétique (ETSBO) in Brest. Diagnosis of CF

M. De Braekeleer (✉)  
Laboratoire de Recherche en Épidémiologie Génétique,  
Département des Sciences Humaines,  
Université du Québec à Chicoutimi,  
555 Boulevard de l'Université Chicoutimi, Québec, Canada  
Tel.: +1-418-545-5011, ext. 2246; Fax: +1-418-545-5012

A. Chaventré  
Laboratoire de Démographie et d'Anthropologie Génétiques,  
UFR Sciences Sociales et Psychologiques,  
Université de Bordeaux II, Bordeaux, France

G. Bertorelle  
Dipartimento di Biologia, Università di Padova, Italy

C. Verlingue · O. Raguénès · B. Mercier · C. Férec  
Établissement de Transfusion Sanguine  
de Bretagne Occidentale (ETSBO), Brest, France

was based both on the typical clinical findings and on at least one positive sweat test.

The CFTR mutations have now been identified in 309 patients born in Brittany. The majority of the families have already been reported (Férec et al. 1991, 1992; Cashman et al. 1995b; Moullier et al. 1994). The microsatellite haplotypes were determined for 21 chromosomes carrying the 1078delT mutation, 16 chromosomes carrying the G551D mutation, 6 chromosomes carrying the W846X mutation, and 74 chromosomes carrying the  $\Delta$ F508 mutation; 83 normal chromosomes, i.e., non-CF chromosomes in the obligate carriers, were also analyzed.

The procedure used for microsatellite markers has been described in detail by Morall et al. (1993) where the conditions for PCR amplification and the relevant primer sequences can be found. A duplex PCR was performed for the microsatellite markers IVS17bTA and IVS17bCA. Primers were end-labeled with  $^{32}$ P- $\gamma$ -ATP using T4 kinase; 25 cycles of PCR were performed with an

annealing temperature of 50°C. A single PCR was performed for IVS8CA with an annealing temperature of 57°C for 35 cycles. A 2- $\mu$ l sample of PCR product was mixed with 2  $\mu$ l of formamide loaded on a polyacrylamide gel and subjected to electrophoresis for 4.5 h at 2000V and exposed overnight to x-ray film. All haplotypes were determined by family studies and were unequivocal.

## Results

For the 200 chromosomes, including 83 normal chromosomes, that were analyzed (Table 1), 41 different MS haplotypes were found. They included 18 different haplotypes among the 117 CF chromosomes and 35 different haplotypes among the 83 normal chromosomes. Twelve

**Table 1** Distribution of the microsatellite (MS) haplotypes among chromosomes carrying the four most common CFTR mutations in Brittany

MS haplotype <sup>a</sup>	$\Delta$ F508 mutation	G551D mutation	1078delT mutation	W846X mutation	Normal chromosomes
16-29-13	1 (1.4%)				1 (1.2%)
16-33-13	1 (1.4%)				
17-30-13	1 (1.4%)				1 (1.2%)
17-33-13	1 (1.4%)				
17-34-13	1 (1.4%)				1 (1.2%)
17-7-17	1 (1.4%)				4 (4.8%)
22-31-13	1 (1.4%)				
23-29-13	1 (1.4%)				
23-32-13	1 (1.4%)				1 (1.2%)
23-33-13	1 (1.4%)				2 (2.4%)
16-31-13	2 (2.7%)		21 (100%)		9 (10.8%)
23-31-14	2 (2.7%)				
23-30-13	4 (5.4%)				
17-31-13	11 (14.9%)				1 (1.2%)
17-32-13	13 (17.6%)				1 (1.2%)
23-31-13	31 (41.9%)				1 (1.2%)
15-7-17					1 (1.2%)
16-22-19					1 (1.2%)
16-28-13					1 (1.2%)
16-30-12					1 (1.2%)
16-30-13					14 (16.9%)
16-31-14					1 (1.2%)
16-32-13				6 (100%)	5 (6.0%)
16-34-13					2 (2.4%)
16-35-13					3 (3.6%)
16-44-13					2 (2.4%)
16-45-13					1 (1.2%)
16-46-13					4 (4.8%)
16-48-13					1 (1.2%)
16-51-11					1 (1.2%)
16-7-17		16 (100%)			11 (13.3%)
17-29-13					1 (1.2%)
17-31-11					1 (1.2%)
17-36-13					1 (1.2%)
17-45-13					1 (1.2%)
17-51-11					1 (1.2%)
17-58-11					2 (2.4%)
18-31-13					1 (1.2%)
18-7-17					1 (1.2%)
21-30-13					1 (1.2%)
23-36-13					2 (2.4%)
Total	73	16	21	6	83

<sup>a</sup> Haplotype is named according to the number of repeats at loci IVS8CA, IVS17bTA, and IVS17bCA, respectively

MS haplotypes were found on normal chromosomes as well as on CF chromosomes (Table 1).

Sixteen different MS haplotypes were found on chromosomes carrying the  $\Delta F508$  mutation (Table 1). The three most frequent haplotypes (23-31-13, 17-32-13, and 17-31-13) accounted for 74.3% of the  $\Delta F508$  chromosomes. These three haplotypes were found on only 3 of the 83 normal chromosomes (3.6%). Most of the remaining haplotypes were found on only one  $\Delta F508$  chromosome.

One haplotype (16-32-13) was found on all six chromosomes carrying the W846X mutation. The same haplotype was also found on 5 of the 83 (6.0%) non-CF chromosomes (Table 1). Another haplotype (16-7-17), which was identified on 13.3% (11/83) of the normal chromosomes, was found on all 16 G551D chromosomes (Table 1). All the 21 chromosomes carrying the 1078delT mutation were associated with the 16-31-13 haplotype, which

was also found on 10.8% of the normal chromosomes (Table 1).

## Discussion

Although there are now many reports on the population genetics of CFTR mutations in a large number of populations (Cystic Fibrosis Genetic Analysis Consortium 1994), the distribution of MS haplotypes on CF and normal chromosomes has not been extensively studied yet in populations (Cashman et al. 1995a, b; Russo et al. 1995; Morral et al. 1993).

The Celtic population of Brittany is interesting because some mutations, two of them rarely identified elsewhere (W846X and 1078delT), are rather common, but also because endogamy and consanguinity in this population has always been high with little immigration. The history of

**Table 2** Distribution of the MS haplotypes among normal chromosomes in several European populations

MS haplotype	Brittany		Ireland		Spain		Italy	
	No. of chrom.	%	No. of chrom.	%	No. of chrom.	%	No. of chrom.	%
15-7-17	1	1.2			3	0.6	4	1.1
16-22-19	1	1.2						
16-28-13	1	1.2			7	1.4		
16-29-13	1	1.2	3	2.7	26	5.0	10	2.8
16-30-12	1	1.2						
16-31-14	1	1.2	1	0.9	7	1.4	7	2.0
16-45-13	1	1.2	2	2.7	6	1.6	4	1.1
16-48-13	1	1.2			2	0.4		
16-51-11	1	1.2						
17-29-13	1	1.2			2	0.4		
17-30-13	1	1.2	3	2.7	2	0.4	5	1.4
17-31-11	1	1.2						
17-31-13	1	1.2			6	1.2		
17-32-13	1	1.2						
17-34-13	1	1.2						
17-36-13	1	1.2						
17-45-13	1	1.2						
17-51-11	1	1.2			2	0.4		
18-31-13	1	1.2						
18-7-17	1	1.2						
21-30-13	1	1.2						
23-31-13	1	1.2	1	0.9	6	1.2		
23-32-13	1	1.2			2	0.4		
16-34-13	2	2.4	4	3.7	3	0.6	4	1.1
16-44-13	2	2.4			10	1.9	9	2.5
17-58-11	2	2.4						
23-33-13	2	2.4	1	0.9	12	2.3	4	1.1
23-36-13	2	2.4						
16-35-13	3	3.6	2	1.8	11	2.1		
16-46-13	4	4.8	4	3.7	16	3.1	4	1.1
17-7-17	4	4.8	3	2.7	15	2.9	11	3.1
16-32-13	5	6.0	10	9.2	37	7.1	34	9.5
16-31-13	9	10.8	16	14.7	52	10.0	40	11.2
16-7-17	11	13.3	8	7.3	75	14.5	40	11.2
16-30-13	14	16.9	20	18.3	83	16.0	38	10.6
Other			31	28.4	133	25.7	144	40.2
Total	83		109		518		358	

**Table 3** Distribution of the MS haplotypes on chromosomes carrying the  $\Delta F508$  mutation among several European populations

MS haplotype	Brittany		Ireland		Spain		Italy	
	No. of chrom	%	No. of chrom	%	No. of chrom	%	No. of chrom.	%
16-29-13	1	1.4						
16-33-13	1	1.4						
17-30-13	1	1.4					2	1.0
17-33-13	1	1.4			2	1.1	1	0.5
17-34-13	1	1.4	1	0.6			1	0.5
17-7-17	1	1.4	1	0.6				
22-31-13	1	1.4	5	3.0	2	1.1		
23-29-13	1	1.4					2	1.0
23-32-13	1	1.4	12	8.0	2	1.1	7	3.4
23-33-13	1	1.4						
16-31-13	2	2.7	3	2.0	1	0.5		
23-31-14	2	2.7			1	0.5		
23-30-13	4	5.4	3	2.0	2	1.1	4	1.9
17-31-13	11	14.9	12	8.0	29	15.3	25	12.0
17-32-13	13	17.6	34	23.0	34	17.9	42	20.2
23-31-13	31	41.9	72	48.0	106	55.8	102	49.0
Other			7	4.7	11	5.8	22	10.6
Total	73		150		190		208	

this population is marked by the settlement in the fourth century of individuals of Celtic origin who sailed from Ireland. One would expect our results differ from those obtained in the populations of Ireland, Spain, and Italy, but still to be more comparable to those of Ireland (Cashman et al. 1995 a, b) than of Spain (Morral et al. 1993) or Italy (Russo et al. 1995).

Indeed, as shown in Table 2, differences in the distribution of the MS haplotypes among normal chromosomes were observed within the four populations. Only those MS haplotypes found on at least one Breton chromosome were included in Table 2 and in Table 3. However, the four most frequent MS haplotypes in the Breton population are also the four most frequent haplotypes in Spain, Italy, and Ireland ( $P = 0.21$ ). One of these, the 16-31-13 haplotype, is considered to be the most likely ancestral MS haplotype for normal chromosomes (Morral et al. 1993).

The three most common non- $\Delta F58$  mutations in Brittany are each associated with a single MS haplotype. However, since the chromosomes carrying these MS haplotypes are quite frequent in the population of Brittany, recurrent mutations arising on different chromosomes cannot be completely ruled out, although such a possibility is unlikely.

The G551D mutation is associated with haplotype 16-7-17 in all the populations thus far studied, including Ireland, Scotland, England, France, the Czech Republic and Spain (Cashman et al. 1995 b; Morral et al. 1993). Based on the molecular results and the geographical distribution, it has been postulated that G551D is a Celtic or even a pre-Celtic mutation (Cashman et al. 1995 b; Macek et al. 1991). Although this explanation is presumably correct,

recurrent mutations are still a possibility because the 16-7-17 haplotype is found on 7–14% of normal chromosomes (Table 2).

The mutation 1078delT has rarely been described outside the Breton population and the MS haplotypes were only determined on two chromosomes outside Brittany, one in Spain (Morral et al. 1993), the other in Italy (Russo et al. 1995). The 16-31-13 haplotype found on all the Breton chromosomes is also found on the 1078delT chromosome from Spain, whereas in Italy the 1078delT chromosome carries a 16-32-13 haplotype. This latter haplotype was associated with the W846X mutation in Brittany. The fact that each of the three mutations occurs on a single haplotype, in contrast to the  $\Delta F508$  mutation, which shows greater haplotype diversity, suggests that G551D, W846X, and 1078delT are more recent than  $\Delta F508$ .

The  $\Delta F508$  mutation was associated with 16 different MS haplotypes. The distribution of the MS haplotypes on chromosomes carrying the  $\Delta F508$  mutation in Brittany showed some differences with those observed in Ireland, Spain, and Italy (Table 3). However, the three most frequent haplotypes (17-31-13, 17-32-13, 23-31-13) in Brittany are the same as in the other populations. The difference in their frequencies is borderline significant ( $P = 0.064$ ). It suggests that some MS haplotypes, associated with the  $\Delta F508$  mutation or not, were present in an ancestral population from which all four populations descended. Successive differentiation among these European populations affected the frequencies of the MS haplotypes. Most of the variability in the MS haplotypes associated with the  $\Delta F508$  mutation found in Brittany apparently did not evolve in loco in recent times but was introduced into the Breton population by migrations.

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