Association of 1078 del T cystic fibrosis mutation with severe disease

P Moullier, M Jéhanne, M P Audrézet, B Mercier, C Verlingue, I Quéré, H Guillermit, O Raguénès, V Storni, G Rault, C Férec

Abstract

Apart from the high frequency of the $\Delta F508$ mutation (81.81%) in Breton cystic fibrosis chromosomes, one mutation, 1078 del T, is also observed frequently (4.96%) in this group, in comparison with the rest of the French where it occurs with a frequency of 0.57%. These two mutations account for more than 86.5% of the total CF mutations identified on Breton chromosomes. We have conducted an unblinded retrospective analysis of 25 patients with the 1078 del T mutation and compared their phenotypes with those of a group of 70 $\Delta F508$ homozygous patients. Both groups of patients had the same ethnic origin and were regularly attending the same CF centre in Brittany, which makes this sample highly homogeneous despite the small size. The 1078 del T mutation appeared to be associated with severe presentation of the disease with, however, a trend to reduced mortality and less *Pseudomonas aeruginosa* colonisation.

(J Med Genet 1994;31:159-161)

Cystic fibrosis (CF) is the most common lethal autosomal recessive disorder affecting Caucasian populations, with an incidence of 1 in 2500 live births.¹ The CF gene encodes for a 170 kDa transmembrane regulator (CFTR) responsible for cAMP dependent chloride channel activity.²⁻⁴ The most frequent CFTR mutation is the deletion of a phenylalanine residue at codon 508 ($\Delta F508$) of exon 10, an exon encoding part of the first nucleotide binding fold of CFTR.⁵

In the Celtic population of Brittany, $\Delta F508$ is present on more than 81% of CF chromosomes. The second most frequent mutation among the Bretons, 1078 del T, occurs on exon 7, which encodes part of the first transmembrane domain, at a frequency of 4.96%.⁶ Through the Cystic Fibrosis Genetic Analysis Consortium, more than 300 different mutations have been reported. This renders genotype/phenotype correlation difficult to perform for rare mutations. In the present study, we document the clinical profile of 25 CF patients carrying the 1078 del T deletion, representing 61% of the total 1078 del T chromosomes worldwide (personal communication). This group was also compared to 70 $\Delta F508$ homozygotes.

Patients and methods

PATIENTS

This study is an unblinded retrospective analysis of 25 1078 del T patients seen on a regular basis at the CF Centre Hélio Marin in Roscoff, Brittany. Data concerning clinical presentation included height, weight, age at diagnosis, age at first infection with *Pseudomonas aeruginosa*, and history of meconium ileus. Information on pulmonary status included percent predicted forced vital capacity (FVC), percent predicted forced expiratory volume in one second (FEV₁), both parameters tested in a stable period. Categorical (yes/no) data regarding pancreatic sufficiency, meconium ileus, chronic presence of *Pseudomonas aeruginosa* colonisation, and liver involvement were studied.

Of the 1078 del T CF patients, 8% (2/25) were homozygous, 64% (16/25) were carrying the $\Delta F508$ mutation on the other chromosome, and the remaining 28% (7/25) were compound heterozygotes (1078 del T/other mutation). In the latter group, we found associated with the 1078 del T mutation one R1066H, one 1221 del CT, one W846X, one 1717-1 G→A, two 4005+1 G→A, and one G551D.

GENETIC ANALYSIS

The strategies that we used include haplotype determination for the $\Delta F508$ CF chromosomes, denaturing gradient gel electrophoresis (DGGE), and DNA sequencing for the remaining non- $\Delta F508$ CF chromosomes, as previously described.^{7,8} We have identified 98% of the molecular defects in our CF chromosomes and three mutations ($\Delta F508$ 81.81%, 1078 del T=4.96%, and G551D=4.13%) account for 90.90% of these molecular abnormalities.^{9,10}

STATISTICS

The risk ratio ($\Delta F508$ /1078 del T) was calculated and represents an estimate of the relative risk of exposure. The Mann-Whitney U test for unpaired differences was used to compare continuous data; a 5% level of significance was chosen (p).

Results

The comparison between clinical parameters of 1078 del T patients and those for $\Delta F508$ homozygotes is shown in tables 1 and 2 and the figure.

Centre de
Biogénétique, Centre
de Transfusion
Sanguine de Brest, 46
rue Félix Le Dantec,
BP 454, 29275 Brest,
France
P Moullier
M P Audrézet
B Mercier
C Verlingue
I Quéré
H Guillermit
O Raguénès
C Férec

Centre Hélio-Marin,
Perharidy, 29684
Roscoff Cedex, France
P Moullier
M Jéhanne
V Storni
G Rault

Correspondence to
Dr Férec

Received 19 April 1993
Revised version accepted for
publication 3 August 1993

Table 1 Comparison of either 1078 del T or G551D mutation with ΔF508/ΔF508 using continuous variables

Variable	ΔF508/ΔF508 Mean (SD)	1078 del T Mean (SD)	p value
Current age (y)	12.5 (6.5)[70]	11.5 (7.0)[25]	ND
Age at diagnosis (y)	2.0 (3.0)[62]	3.7 (6.0)[16]	>0.1
Age at first <i>P aeruginosa</i> (y)	8.0 (6.0)[44]	8.8 (7.0)[9]	>0.1
FEV ₁ (% of predicted)	62.0 (29.0)[55]	67.2 (23.5)[17]	>0.1
FVC (% of predicted)	72.0 (25.0)[55]	75.2 (18.0)[17]	>0.1

ND = not determined.
[] = total number of patients.

Table 2 Comparison of either 1078 del T or G551D mutation with ΔF508/ΔF508 using categorical variables

Condition	ΔF508/ΔF508	1078 del T	Risk ratio
% with onset <6 mth	61 (62)	62 (16)	1.1
% with pancreatic sufficiency	3 (70)	0 (25)	—
% with liver involvement	12 (69)	8 (24)	1.1
% with chronic <i>P aeruginosa</i>	70 (66)	48 (23)	1.5
% Meconium ileus	21 (70)	14 (18)	1.5
% Mortality	19 (70)	4 (25)	4.7

() = total number of patients.

Forced expiratory volume in one second (FEV₁) plotted against age in individual patients according to genotype group. The dashed line denotes the regression line for the patients who had the ΔF508/ΔF508 genotype (open circles). The solid line denotes the regression line for the group who had the 1078 del T mutation (filled circles).

CONTINUOUS VARIABLES (TABLE 1)

The mean age of the two groups was similar, confirming appropriate matching. There were no significant differences between the groups for any of the variables. For instance, FVC and FEV₁ were used to assess pulmonary status (table 1, figure). Because of the strong dependence of lung function on age, we obtained the least square regression line (lung function variable *v* age) from ΔF508 patient data and compared it with that obtained from the 1078 del T group. Although data from both groups showed an equivalent degree of dispersed distribution, this method allowed a rough estimation and comparison of mean values for each group at any given age. The result of this analysis showed no difference (*p*>0.1) between patients from the two different groups who were old enough for lung function testing. When plotted against ideal weight and height

(that is, those observed in the French population), 1078 del T CF patients showed reduced scores, particularly for weight in CF males. However, the curves obtained for these patients were identical to those for ΔF508 homozygotes (data not shown). In addition, a moderate aggravation of disease during puberty was noted in both groups.

CATEGORICAL VARIABLES

Data for each of the categorical outcome variables are shown in table 2. The two groups were no different with regard to liver involvement and pancreatic status. Patients carrying the 1078 del T mutation had a reduced frequency of meconium ileus (14% *v* 21%). Seventy percent of the ΔF508 homozygotes were chronically colonised with *P aeruginosa* and the incidence was only 48% in the 1078 del T group (table 2). Finally, 13 ΔF508 patients out of 70 (19%) have died at ages ranging from 3 to 27 years. In the 1078 del T group, only one patient (4%) died at the age of 10 (mean age = 11.5 years *v* 12.5 for ΔF508/ΔF508).

Clinical data of the 1078 del T/non-ΔF508 heterozygotes were indistinguishable from the remaining 1078 del T CF patients (1078 del T/ΔF508) as well as from the ΔF508 homozygote group.

Discussion

The distribution of non-ΔF508 mutations with striking regional variations is probably related to founder effect or genetic drift or both. This has permitted some investigators to collect a significant number of CF patients carrying rare genotypes, as recently illustrated by Shoshani *et al*¹¹ in the Ashkenazi Jewish population, where the W1282X mutation represents over 60% of the CF alleles, and also by Gasparini *et al*¹² who reported a high incidence of the R1162X mutation (12%) in Veneto in north east Italy. In the Celtic part of Brittany we have also observed a founder effect for the 1078 del T mutation. Evidence for this comes from analysis of the genealogical register as well as the study of intra and extragenic polymorphisms linked to these chromosomes (unpublished data). Thus, these founder effects have led to a frequency of 4.96% for the 1078 del T mutation in this population.

These patients are particularly homogeneous since they share an ethnic origin and regularly attend the Centre Hlio Marin of Roscoff in Brittany where they are subjected to identical clinical protocols. In this study, we have collected clinical data on these 25 1078 del T patients and have compared them with another homogeneous group of 70 patients carrying the ΔF508 mutation on both alleles, also followed at the Roscoff Centre. The results obtained show that there is (1) no significant difference between the two groups regarding their pancreatic status as all are pancreatic insufficient; (2) no significant difference with respect to pulmonary function tests (FEV₁, FVC) although with a trend towards improved FEV₁ among the 1078 del T CF

patients; (3) mortality in the 1078 del T patients group is only 4% *v* 19% in the $\Delta F508$ homozygotes. It is tempting to correlate this finding with a reduced predisposition to chronic *Pseudomonas aeruginosa* colonisation (48%) detected in the 1078 del T patients compared with 70% of $\Delta F508$ patients, since prognosis is linked to the pulmonary status which in return is highly dependent on the extent of *Pseudomonas* colonisation and resistance to conventional treatment, as recently outlined by Kubesch *et al.*¹³ Since the demonstration of a frequent $\Delta F508$ deletion as well as many other mutations in the CFTR gene, investigators such as Kristidis *et al.*¹⁴ and others¹⁵ have tried to identify genetically determined clinical manifestations of cystic fibrosis. Despite the lack of precise genotype/phenotype correlations, pancreatic function is one symptom which appears to be genetically determined. Indeed, pancreatic sufficiency occurs in patients who have mild CFTR mutations whereas pancreatic insufficiency occurs in patients with two severe alleles such as $\Delta F508$. Here, we show that the 1078 del T mutation is associated with a severe phenotype with respect to pancreatic function. According to Kristidis *et al.*¹⁴ the 1078 del T mutation should be added to the list of severe mutations resulting from a single nucleotide deletion. The 1078 del T/ $\Delta F508$ heterozygotes have identical clinical presentation when compared with the $\Delta F508$ homozygotes. Similar results have been observed for CF mutations other than $\Delta F508$, such as G551D, R1162X,¹⁶ W1282X, N1303K,¹⁷ and G542X. This, in addition to the 1078 del T phenotype described in this study, is consistent with the approximate 85% frequency of CF alleles associated with a severe phenotype. However, our cohort of 1078 del T CF patients have not been studied for a long enough period of time to permit meaningful assessment of survival. Thus, it would be interesting to continue recording the progress of these patients of which the average age is 11.5 years, in order to confirm the reduced prevalence of chronic *Pseudomonas aeruginosa* colonisation observed

in this group, along with a possible lower morbidity.

We wish to thank Chantal Baclet for assistance in preparation of the manuscript. This work was supported by the Centre Départemental de Transfusion Sanguine of Brest, the Association Française de Lutte contre la Mucoviscidose (AFLM), the Association Bretonne d'Etude et de Recherche sur la Mucoviscidose (ABER-M), and the CRITT Bretagne.

- 1 Boat TF, Welsh MJ, Beaudet AL. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The metabolic basis of inherited disease*. New York: McGraw-Hill, 1989:2649–860.
- 2 Dalemans W, Barbry P, Champigny G, *et al.* Altered chloride ion channel kinetics associated with the $\Delta F508$ cystic fibrosis mutation. *Nature* 1991;354:526–8.
- 3 Rommens JM, Dho S, Bear CE, *et al.* cAMP-inducible chloride conductance in mouse fibroblast lines stably expressing the human cystic fibrosis transmembrane conductance regulator. *Proc Natl Acad Sci USA* 1991;88:7500–4.
- 4 Bear EC, Li C, Karner N, *et al.* Purification and functional reconstitution of the cystic fibrosis transmembrane conductance regulator (CFTR). *Cell* 1992;68:809–18.
- 5 Worldwide survey of the delta F508 mutation: report from the Cystic Fibrosis Genetic Analysis Consortium. *Am J Hum Genet* 1990;47:354–9.
- 6 Audrézet MP, Mercier B, Guillermit H, Férec C. A mutation in exon 7 of the CFTR gene is common in the western part of France. *J Med Genet* 1992;29:679–80.
- 7 Lerman LS, Silverstein K. Computational simulation of DNA melting and its application to denaturing gradient gel electrophoresis. *Methods Enzymol* 1987;155:482–501.
- 8 Myers RM, Maniatis T, Lerman LS. Detection and localization of single base changes by denaturing gradient gel electrophoresis. *Methods Enzymol* 1987;155:501–27.
- 9 Férec C, Audrézet MP, Mercier B, *et al.* Detection of over 98% cystic fibrosis mutations in a Celtic population. *Nature Genet* 1992;1:188–91.
- 10 Audrézet MP, Mercier B, Guillermit H, *et al.* Identification of 12 novel mutations in the CFTR gene. *Hum Mol Genet* 1993;2:51–4.
- 11 Shoshani T, Augarten A, Gazit E, *et al.* Association of a nonsense mutation (W1282X), the most common mutation in the Ashkenazi Jewish cystic fibrosis patients in Israel, with presentation of severe disease. *Am J Hum Genet* 1992;50:222–8.
- 12 Gasparini P, Nunes V, Savoia A, *et al.* The search for South European cystic fibrosis mutations: identification of two new mutations, four variants, and intronic sequences. *Genomics* 1991;10:193–200.
- 13 Kubesch P, Dörk T, Wulbrand U, *et al.* Genetic determinants of airways' colonisation with *Pseudomonas aeruginosa* in cystic fibrosis. *Lancet* 1993;341:189–93.
- 14 Kristidis P, Bozon D, Corey M, *et al.* Genetic determination of exocrine pancreatic function in cystic fibrosis. *Am J Hum Genet* 1992;50:1178–84.
- 15 Hamosh A, King TM, Rosenstein BJ, *et al.* Cystic fibrosis patients bearing both the common missense mutation Gly→Asp at codon 551 and the $\Delta F508$ mutation are clinically indistinguishable from $\Delta F508$ homozygotes, except for decreased risk of meconium ileus. *Am J Hum Genet* 1992;51:245–50.
- 16 Gasparini P, Borgo G, Mastella G, Bonizzato A, Dognini M, Pignatti PF. Nine cystic fibrosis patients homozygous for the CFTR nonsense mutation R1162X have mild or moderate lung disease. *J Med Genet* 1992;29:558–62.
- 17 Osborne L, Santis G, Schwarz M, *et al.* Incidence and expression of the N1303K mutation of the cystic fibrosis (CFTR) gene. *Hum Genet* 1992;89:653–8.