

Severity of chest disease in cystic fibrosis patients in relation to their genotypes

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Abstract

A detailed comparison of the severity of chest disease with mutational status was carried out by cross sectional study of 127 cystic fibrosis patients, aged 1 to 31 years, living in Wales. Lung disease was classified according to severity, depending on pulmonary function tests (carried out on 76 patients) and chest radiograph status; information was obtained also on age at diagnosis in relation to severity of chest disease and colonisation with *Pseudomonas* species. Genotypes were determined by analysis for the mutations $\Delta F508$, $\Delta I507$, G551D, R553X, G542X, R117H, R560T, 1717-IG>A, and 621+1G>T.

CF patients homozygous positive and heterozygous for the $\Delta F508$ deletion showed a significant decline of lung function with age. Unlike other studies, we did not find patients homozygous positive for the $\Delta F508$ deletion to have poorer lung function compared with heterozygous patients. Patients with the genotype 621+1G>T/ $\Delta F508$ tended to have more severe chest disease than the $\Delta F508$ homozygous patients in the same age group. There was some evidence that four patients heterozygous for R117H have mild chest disease.

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It has long been the impression of clinicians that cystic fibrosis (CF), the most common serious autosomal recessive genetic disorder in Caucasian populations, is heterogeneous, with variable system involvement and disease severity.¹

Since the identification of the most common CF mutation, $\Delta F508$,²⁻⁴ and an increasing number of other mutations, comprehensive studies on phenotype/genotype comparisons have been published.⁵⁻⁸

Johansen *et al*⁶ carried out a cross sectional study of Danish CF patients and found that $\Delta F508$ homozygous positive patients had poorer lung function and an increased incidence of chronic infection with *Pseudomonas aeruginosa* in comparison with heterozygous patients. They also found a significant correlation and decline in FEV₁ and FVC with age in the homozygous but not in the heterozygous patients.

Patients and methods

A cross sectional study was carried out on 127 CF patients living mainly in South Wales, age

range 1 to 31 years. In order to exclude possible complicating factors associated with the presence of more than one affected subject in a family, patients who had sibs with CF (whether alive or dead) were excluded from the group analyses. However, two sets of sibs heterozygous for the R117H mutation were examined separately. The age at diagnosis of CF (determined by clinical features and a positive sweat test) was noted.

Clinical classification of lung disease as 'mild' or 'moderate to severe' was carried out when patients were in a stable clinical state and depended on FEV₁ (% predicted) more or less than 75%. Chrispin-Norman scores were calculated on all patients, whereby a lateral chest radiograph and the four quadrants of the lung fields on a posterior/anterior film were scored with regard to chest configuration and lung markings; a high score indicated a poor chest radiograph (maximum 38 points).⁹

Colonisation with *Pseudomonas* species was noted; patients were scored as positive if the organism was isolated even once, even though the infection was apparently not persistent. All parameters were assessed during the year before the analyses.

GENOTYPE ANALYSES

Data were sought for nine different mutations: $\Delta F508$, $\Delta I507$, 1717-IG>A, G551D, R553X, G542X, R560T, R117H, and 621+1G>T. The $\Delta F508$ deletion was identified by amplification of genomic DNA by polymerase chain reaction (PCR), followed by direct measurement of the size of the PCR product on a denaturing polyacrylamide gel. Multiplex PCR was used in some patients to screen simultaneously for five mutations (J P Cheadle, personal communication).

STATISTICAL ANALYSES

These were carried out using the χ^2 test, Mann-Whitney U test, and Kruskal Wallis test as appropriate. For age dependent data, analyses were done in five year bands.

Results

SEVERITY OF CHEST DISEASE AS MEASURED BY FEV₁% PREDICTED

Data on $\Delta F508$ status, FEV₁ and FVC (% predicted) were available for 77 patients (table 1). A decline of respiratory function with age was shown in all three genotypes.

The median lines drawn for the values of FEV₁% and FVC% with age, shown in figs 1

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Table 1 Decline of FEV₁ and FVC % of predicted with age in CF patients (number of patients in parentheses).

ΔF508	Mean FEV ₁ , FVC % predicted			Total
	5-9 y	10-14 y	> 15 y	
++ FEV ₁ %	(11) 75.4	(11) 69.5	(15) 56.7	(37)
FVC%	87.6	86.4	75.7	
+ - FEV ₁ %	(7) 85.6*	(7) 67.6*	(16) 50.4*	(30)
FVC%	94.1	85.4	64.5	
-- FEV ₁ %	(3) 86	(1) 72	(5) 43.2	(9)
FVC%	91	78	54.4	

* $p < 0.05$ (Kruskal Wallis test).

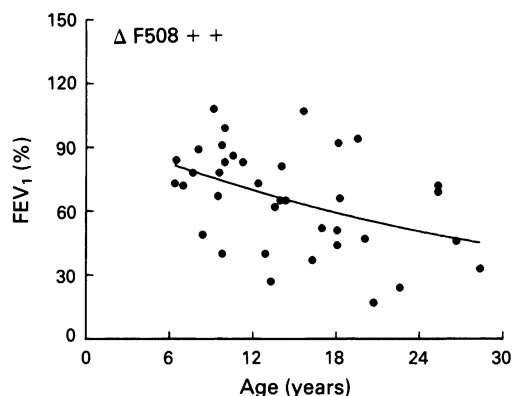


Figure 1 Median curve of FEV₁% predicted, with age, for ΔF508 (++) patients.

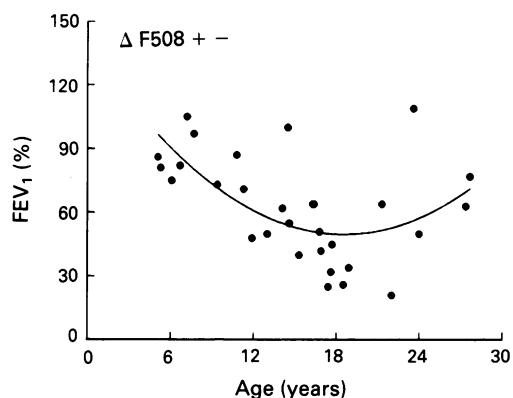


Figure 2 Median curve of FEV₁% predicted, with age, for ΔF508 (+-) patients.

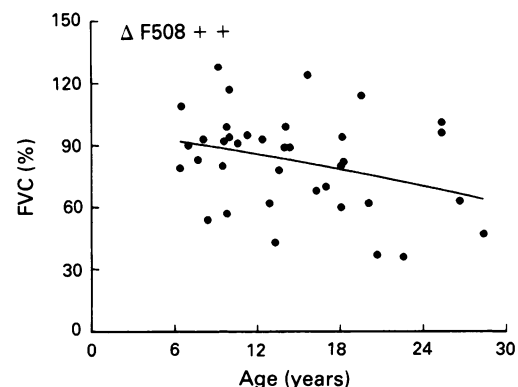


Figure 3 Median curve of FVC% predicted, with age, for ΔF508 (++) patients.

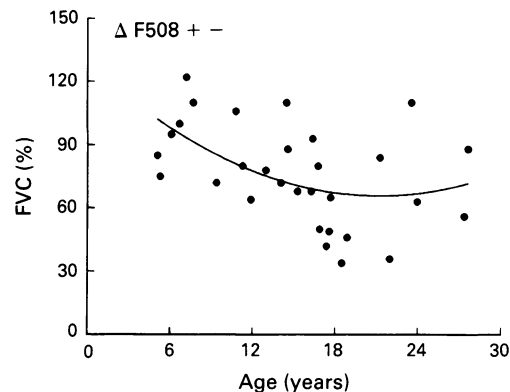


Figure 4 Median curve of FVC% predicted, with age, for ΔF508 (+-) patients.

to 4 for both homozygous positive and heterozygous ΔF508 patients, have been fitted by a quadratic equation. FEV₁% shows a significant linear correlation with age for both ΔF508 (++) ($r = -0.43$, $p < 0.01$) and ΔF508 (+-) patients ($r = -0.39$, $p < 0.01$). Similarly FVC% shows a significant linear correlation with age for both ΔF508 (++) ($r = -0.33$, $p < 0.05$) and for ΔF508 (+-) patients ($r = -0.40$, $p < 0.01$).

The five patients with ΔF508 (+-) who contributed to the upturn in FEV₁% at >20 years of age (fig 2) comprise two patients who carried G551D and three who were screened negative for the nine mutations analysed. These same five patients with ΔF508 (+-) contributed also to the upturn of the curve for FVC% predicted (fig 4).

Among the nine patients who were homozygous negative for ΔF508 (table 1) were: three with 621 + IG > T/unknown mutation genotype (ages 6.3, 17.6, and 18 years; FEV₁% predicted 80, 30, and 45 respectively); one with G551D/621 + IG > T genotype (8 years, FEV₁ 81%); one with G551D/unknown mutation genotype (28 years, FEV₁ 77%); one homozygous for R553X (9 years, FEV₁ 97%); and three with unknown mutations (14.2, 16.7, and 19.4 years; FEV₁% predicted 72, 28, and 92 respectively).

CHRISPIN NORMAN SCORE

Values for Chrispin Norman scores were available for all 127 patients. Patients with ΔF508 (++) and (+-) showed a gradual, significant decline of these scores with age (table 2). The pattern of decline appeared to be somewhat different for the 12 patients who were ΔF508 (--), with a sharper deterioration at >15 years, but the small number precludes analysis.

AGE AT DIAGNOSIS AND SEVERITY OF CHEST DISEASE

Correlation coefficients were calculated between ages at diagnosis and FEV₁% predicted at assessment, for both ΔF508 homozygous positive and heterozygous patients, excluding not only sibs, but those who presented with meconium ileus or who were diagnosed after neonatal screening. Although there

Table 2 Mean Chrispin Norman scores of CF patients in various age bands (number of patients in parentheses).

ΔF508	Age bands (y)				Total
	<5	5-9.9	10-14.9	>15	
++	6* (25)	8.7* (12)	11.5* (10)	12.6* (16)	(63)
+ -	6.9* (21)	6.5* (8)	11.2* (7)	12.9* (16)	(52)
--	5.7 (3)	5 (3)	5 (1)	15.6 (5)	(12)

* p < 0.05 (Kruskal Wallis test).

was a tendency for ΔF508 heterozygous patients to be diagnosed later in life, there was a wide overlap and age at diagnosis was not significantly different. There was no significant correlation between age at diagnosis and FEV₁% predicted at assessment for either genotype.

PSEUDOMONAS SPECIES COLONISATION OF THE LUNGS

No association was found between *Pseudomonas* species colonisation and ΔF508 status of CF patients. *Pseudomonas* species were isolated from four ΔF508 homozygous negative patients (ages 6.1, 16.7, 17.6, and 18.9 years) out of 12 patients with a similar genotype (mean age 11.9 (SD 6.4), age range 2.0 to 20.7). The incidence of *Pseudomonas* infection increased with age among all genotypes. *Pseudomonas* colonisation was significantly more common in patients with moderate to severe rather than mild lung disease, both among ΔF508 homozygous positive ($\chi^2=4.30$, p < 0.05) and heterozygous patients ($\chi^2=7.78$, p < 0.01).

621 + IG > T MUTATION

There were 11 patients heterozygous for the 621 + IG > T mutation, of whom seven carried the ΔF508 mutation on the other chromosome. Among the remaining four patients with the 621 + IG > T mutation, one patient carried G551D and three carried mutations so far not identified.

In an attempt to assess the severity of chest disease associated with the 621 + IG > T mutation, comparisons were made between patients homozygous positive for ΔF508 and patients with the 621 + IG > T/ΔF508 genotype, in the same age group, with the use of FEV₁% predicted and Chrispin Norman scores. FEV₁% was expressed as >75%, 75 to 50%, and <50% (table 3). Mean values for FEV₁% predicted for the eight patients who were

ΔF508 homozygous positive and the five patients with 621 + IG > T/ΔF508 genotype, in the 15 to 20 year age group, were 67.9 (SD 24.6) and 45.8 (SD 15.8) respectively. Chrispin Norman scores were a mean value of 11.0 (SD 6.1) for the eight ΔF508 homozygous positive patients and a poorer value of 13.8 (SD 5.0) for the patients with the 621 + IG > T/ΔF508 genotype. Neither of these differences achieved statistical significance.

R117H MUTATION

Four CF patients from two families were heterozygous for the R117H mutation. All were pancreatic sufficient and none had *Pseudomonas* species infection. In one family, the two CF sibs were heterozygous with ΔF508 deletion; ages were 16 and 8 years, values for FEV₁ were 80% and 97% predicted respectively, but Chrispin Norman scores were 12 and 8 respectively. In the other family, the CF sibs were heterozygous with G542X; ages were 4 and 3 years and Chrispin Norman scores 4 and 4.

The diagnosis of CF in the first family was made at the ages of 15 and 6 years, the 6 year old being diagnosed first on account of respiratory symptoms and her brother shortly afterwards, following sweat tests on the other sibs in this family. On review, he too had had mild respiratory symptoms. In the second family, the diagnosis followed neonatal screening in the younger sib, when the older, then aged 2 years, was confirmed to have CF also. He was found retrospectively to have had a false negative value for immunoreactive trypsin (IRT) on neonatal screening.

Thus for measurements of FEV₁% predicted, the older sib pair belonged to our 'mild' group of chest disease patients, but even so their Chrispin Norman scores were appreciable. The younger pair was too young for lung function tests, but Chrispin Norman scores at 4 were somewhat lower than the mean score of 6.9 for the ΔF508 heterozygous patients in the same age band (table 2).

Discussion

Chronic lung disease is the main clinical determinant of the course of CF, which despite earlier diagnosis and considerably improved therapy still leads to death in adolescence and early adulthood in the majority of patients.

The identification of the most common CF gene defect, ΔF508, in September 1989 and 150 other gene deletions, mutations, and base

Table 3 FEV₁% predicted in patients homozygous positive for the ΔF508 deletion in comparison with patients carrying the 621 + IG > T/ΔF508 genotype in different age bands.

	Age band (y)												Total
	5-9.9			10-14.9			15-20			>20			
FEV ₁ % predicted	>75	75-50	<50	>75	75-50	<50	>75	75-50	<50	>75	75-50	<50	
Genotype	No of patients												
ΔF508 (+ +)	6	3	2	5	5	2	3	3	2	0	3	4	38
ΔF508/621 + IG > T	2	0	0	No patients			0	3	2	3	No patients		7

substitutions since, has provided the opportunity to study the relationship between CF phenotype and allelic heterogeneity at the CF locus.

This cross sectional study involved 127 CF patients living in various parts of Wales, more than 50% of whom were from south Wales. Respiratory function tests showed a significant decline of lung function with age for both $\Delta F508$ (+ +) and (+ -) patients. Although the shapes of these lung function curves were quite similar to those of Johansen *et al*,⁶ these authors did not find a significant decline in lung function tests among their heterozygous patients. From our data, we disagree with their conclusion that the homozygous patients necessarily have poorer lung function. In our study, the median lines for decline of FEV₁ and FVC (% predicted) correlated significantly with age, for both homozygous and heterozygous $\Delta F508$ patients. In both studies (that of Johansen *et al*⁶ and our study) an examination of FEV₁% predicted for the $\Delta F508$ heterozygous patients shows two populations at least: one population where lung function deteriorates severely with age and finally incapacitates the patients at around 16 to 20 years of age, and another population comprising the fitter, more mildly affected patients, contributing to the upturn in the curve. In the first population of the $\Delta F508$ heterozygous patients in our study, a number of patients fell well below the FEV₁% curve of median fit; among these were two patients heterozygous for 621+IG>T, one patient heterozygous for G551D, and four who screened negative for the nine mutations analysed but who may carry one or more 'minor' mutations causing severe chest disease. The second population contributing to the upturn in the curve included five patients, of whom two carried G551D and three were screened negative for the nine mutations analysed.

A number of explanations could account for the upturn in the curve of median fit among the older heterozygous patients. This could relate to the death of the more severely affected patients and survival of a rather different group with an advantageous genotype; patients of different ages may have received different standards of treatment; or the shape of the curve could have been produced purely by a statistical fluctuation. It is not possible to say which of these factors, singly or in combination, is responsible. It may be relevant that one heterozygous female patient, who screened negative for the eight other mutations, died during the survey at 22 years with an FEV₁ of 21% of predicted.

Graphs were not drawn for the $\Delta F508$ (- -) patients because numbers were small; however, it was noted that five of these nine patients for whom respiratory function data were available showed a severe decline of lung function at more than 15 years of age. Among those five patients were one who carried (G551D/-) genotype with FEV₁ of 77% and two who carried (621+IG>T/-) genotype with FEV₁ of 30% and 40% and two who

screened negative for the nine mutations analysed, with FEV₁ of 28% and 92%.

As expected, there was a significant decline in Chrispin Norman scores with age among $\Delta F508$ (+ +) and (+ -) patients. Although the number of $\Delta F508$ (- -) patients was small, the decline in these scores was more pronounced at 15 years of age and older. It could be argued that the $\Delta F508$ (- -) patients have the genetic advantage earlier in life with well maintained respiratory function and a lesser need for aggressive treatment regimens and hospital admissions. However, they may lose this advantage as they grow older and as environmental factors which are known to influence chest disease have more cumulative effect, so causing the severe deterioration in pulmonary function at an older age. A 17 year old patient with $\Delta F508$ (- -), whose FEV₁% and FVC% were 28% and 60% of predicted, died during the study.

In contrast to other studies, we did not find a significant correlation between age at diagnosis and $\Delta F508$ status,^{5,6} nor did we find an association between $\Delta F508$ status and colonisation with *Pseudomonas* species, which again is different from other surveys.^{6,7} As expected, patients with moderate to severe chest disease had a significantly higher incidence of *Pseudomonas* species infection, whether they carried $\Delta F508$ (+ +) or (+ -) genotypes, compared with those with mild chest disease.

This study has shown that patients heterozygous for the 621+IG>T mutation, which has a high frequency among Welsh patients (Cheadle *et al*, personal communication), may have a predisposition to severe chest disease. Although total numbers were small, three out of five patients with 621+IG>T/ $\Delta F508$ genotype, aged 15 to 20 years, had an FEV₁<50% predicted, compared with two out of eight patients who were $\Delta F508$ homozygous positive in the same age group. Among the total of 77 patients who had pulmonary function tests, 15 patients had an FEV₁% <50% of predicted, of whom five were heterozygous for 621+IG>T mutation. This apparent predisposition has not been reported before.

There is considerable evidence that CF patients carrying the R117H mutation, even in single copy, have milder disease.^{10,11} It may be relevant that one of our sib pairs with this mutation was diagnosed late (at 6 and 15 years) and that the older of the younger pair was recognised at 2 years, when his younger sister was positive on neonatal screening. Owing to the late diagnosis of the older pair (with absence of specific treatment for CF before this) and the youth of the younger pair, we are unable to advance the case that these four patients who are heterozygous for the R117H mutation are definitely 'mild', but they would seem to be so.

A longitudinal study over several years to follow up patients of different genotypes should be more informative than the present cross sectional one.

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- 1 Goodchild MC, Dodge JA. *Cystic fibrosis: manual of diagnosis and management*. 2nd ed. London: Bailliere Tindall, 1982.
- 2 Kerem BS, Rommens JM, Buchanan JA, *et al*. Identification of the cystic fibrosis gene: genetic analysis. *Science* 1989;245:1073-80.
- 3 Rommens JM, Iannuzzi MC, Kerem BS, *et al*. Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science* 1989;245:1059-65.
- 4 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.
- 5 Kerem E, Corey M, Kerem BS, *et al*. The relation between genotype and phenotype in cystic fibrosis-analysis of the most common mutation (delta F508). *N Engl J Med* 1990;323:1517-22.
- 6 Johansen HK, Nir M, Hoiby N, *et al*. Severity of cystic fibrosis in patients homozygous and heterozygous for delta F508 mutation. *Lancet* 1991;337:631-4.
- 7 Santis G, Osborne L, Knight RA, *et al*. Linked marker haplotypes and the delta F508 mutation in adults with mild pulmonary disease and cystic fibrosis. *Lancet* 1990;335:1426-9.
- 8 Santis G, Osborne L, Knight RA, *et al*. Independent genetic determinants of pancreatic and pulmonary status in cystic fibrosis. *Lancet* 1990;336:1081-4.
- 9 Chrispin AR, Norman AP. The systematic evaluation of the chest radiograph in cystic fibrosis. *Pediatr Radiol* 1974;2:101-6.
- 10 Dean M, White MB, Amos J, *et al*. Multiple mutations in highly conserved residues are found in mildly affected cystic fibrosis. *Cell* 1990;61:863-70.
- 11 Keston M, McIntosh I, Brock DJH, Marshall TG, Greening AP. Comparison of clinical phenotype and mutations at cystic fibrosis locus. *Am Rev Respir Dis* 1991;143:605A.