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

L N Al-Jader, A L Meredith, H C Ryley, J P Cheadle, S Maguire, G Owen, M C Goodchild, P S Harper

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 ΔF508, ΔI507, G551D, R553X, G542X, R117H, R560T, 1717−1G>A, and 621+1G>T.

CF patients homozygous positive and heterozygous for the ΔF508 deletion showed a significant decline of lung function with age. Unlike other studies, we did not find patients homozygous positive for the ΔF508 deletion to have poorer lung function compared with heterozygous patients. Patients with the genotype 621+1G>T/ΔF508 tended to have more severe chest disease than the other ΔF508 homozygous patients in the same age group. There was some evidence that four patients heterozygous for R117H have mild chest disease.

(J Med Genet 1992;29:883-7)

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, ΔF508,²⁴ and an increasing number of other mutations, comprehensive studies on phenotype/genotype comparisons have been published.²⁴

Johansen et al.⁴ carried out a cross sectional study of Danish CF patients and found that ΔF508 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: ΔF508, ΔI507, 1717−1G>A, G551D, R553X, G542X, R117H, R560T, and 621+1G>T. The AF508 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 χ² 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 ΔF508 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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Received 18 March 1992.
Revised version accepted 4 June 1992.
Table 1  Decline of FEV, and FVC % of predicted with age in CF patients (number of patients in parentheses).

<table>
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<th>ΔF508</th>
<th>Mean FEV, FVC % predicted</th>
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<tr>
<td></td>
<td>5-9 y</td>
<td>10-14-9 y</td>
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<tr>
<td>++ FEV %</td>
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<td>(11) 69-5</td>
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<td>FVC %</td>
<td>87.6</td>
<td>84.4</td>
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<tr>
<td>+ - FEV %</td>
<td>(7) 85-6*</td>
<td>(7) 67-6*</td>
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<tr>
<td>FVC %</td>
<td>94.1</td>
<td>85-4</td>
</tr>
<tr>
<td>-- FEV %</td>
<td>(3) 86</td>
<td>(1) 72</td>
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<tr>
<td>FVC %</td>
<td>91</td>
<td>78</td>
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</table>

* p<0.05 (Kruskal Wallis test).

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

Figure 2  Median curve of FEV,% 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.

Values for Chrisin 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 FEV,% 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...
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 (χ² = 4-30, p < 0-05) and heterozygous patients (χ² = 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 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>
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<td>8-7*</td>
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substitutions since, has provided the opportu-

nity 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
ΔF508 (++) and (+−) patients. Although the
shapes of these lung function curves were
quite similar to those of Johansen et al.,2 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 significa-

tantly with age, for both homozygous and
heterozygous ΔF508 patients. In both studies
(that of Johansen et al. and our study) an
examination of FEV1 % predicted for the
ΔF508 heterozygous patients shows two popu-
lations 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 ΔF508
heterozygous patients in our study, a num-
ber of patients fell well below the FEV1 % curve
of median fit; among these were two patients
heterozygous for 621+1G>T, one patient
heterozygous for G551D, and four who
screened negative for the nine mutations ana-
lysed 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
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 com-

bination, is responsible. It may be relevant
that one heterozygous female patient, who
screened negative for the eight other muta-
tions, died during the survey at 22 years with
an FEV1 of 21% of predicted.

Graphs were not drawn for the ΔF508
(−−) 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+1G>T−) genotype
with FEV1 of 30% and 40% and two who
screened negative for the nine mutations ana-
lysed, with FEV1 of 28% and 92%.

As expected, there was a significant decline
in Chrispin Norman scores with age among
ΔF508 (++) and (+−) patients. Although the
number of ΔF508 (−−) patients was small, the
decline in these scores was more pronounced
at 15 years of age and older. It
could be argued that the ΔF508 (−−) 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 ΔF508 (−−), 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 ΔF508 status,44 nor did we find an associa-
tion between ΔF508 status and colonisation
with Pseudomonas species, which again is dif-
ferent from other surveys.47 As expected,
patients with moderate to severe chest disease
had a significantly higher incidence of Pseudo-
monas species infection, whether they carried
ΔF508 (+ +) or (+−) genotypes, compared with
those with mild chest disease.

This study has shown that patients hetero-
zygous for the 621+1G>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+1G>T/ΔF508 genotype,
aged 15 to 20 years, had an FEV1,<50% predicted, compared with two out of
eight patients who were ΔF508 homozygous
positive in the same age group. Among the
total of 77 patients who had pulmonary func-
tion tests, 15 patients had an FEV1,<50% of
predicted, of whom five were heterozygous for
621+1G>T mutation. This apparent predis-
position has not been reported before.

There is considerable evidence that CF
patients carrying the R117H mutation, even in
single copy, have milder disease.101 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 ab-
sence 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.

We should like to thank Dr M Alfaham for his
clinical contribution to this research; Drs I
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Campbell, R Prosser, and other clinicians for allowing us to study their patients; and Mrs J Myring for her laboratory assistance. This work was supported for one year by the Joseph Levi Charitable Foundation.