α1-Antitrypsin deficiency alleles and the Taq-I G→A allele in cystic fibrosis lung disease


ABSTRACT: Cystic fibrosis (CF) is characterized by progressive and ultimately fatal pulmonary disease although there are notable variations in clinical features. This heterogeneity is thought to lie outside the cystic fibrosis transmembrane regulator (CFTR) gene locus and may stem from deficiencies in the antiproteinase screen that protects the lung from proteolytic attack.

One hundred and fifty seven patients were recruited from two UK CF centres. The serum concentrations of α1-antitrypsin, α1-antichymotrypsin and C-reactive protein (CRP) were determined and patients were screened for the common S and Z deficiency alleles of α1-antitrypsin and the G→A mutation in the 3’ noncoding region of the α1-antitrypsin gene (Taq-I G→A allele).

α1-Antitrypsin deficiency phenotypes were detected in 20 (16 MS, 1 S and 3 MZ) out of 147 unrelated tested CF patients and were, surprisingly, associated with significantly better lung function (adjusted mean forced expiratory volume in one second (FEV1) 62.5% of predicted for deficient group and 51.1% pred for normal alleles; p=0.043). The Taq-I G→A allele was found in 21 out of 150 unrelated patients and had no significant effect on CF lung disease or on levels of α1-antitrypsin during the inflammatory response.

We show here that, contrary to current thinking, common mutations of α1-antitrypsin that are associated with mild to moderate deficiency of the protein predict a subgroup of cystic fibrosis patients with less severe pulmonary disease. Moreover, the Taq-I G→A allele has no effect on serum levels of α1-antitrypsin in the inflammatory response, which suggests that the previously reported association of the Taq-I G→A allele with chronic obstructive pulmonary disease is not mediated by its effect on the serum level of α1-antitrypsin.


Cystic fibrosis (CF) is the commonest autosomal recessive genetic disorder amongst populations of northern European descent. Most patients succumb to progressive pulmonary disease, although there are considerable variations in clinical features [1, 2]. The pulmonary heterogeneity is largely independent of mutations within the cystic fibrosis transmembrane regulator (CFTR) gene on chromosome 7 [2] although recent reports have suggested that milder lung disease may occur in CF patients who are compound heterozygotes for less common mutations [3, 4].

The variability of pulmonary disease in patients with the same genotype has been ascribed to the pancreatic status of individuals, treatment regimes, social class, smoking history and colonization with Pseudomonas aeruginosa or Burkholderia cepacia [5–10]. Studies of twins suggest that genetic factors lying outside the CFTR locus may be linked to pulmonary phenotype [11] and although one such factor has been suggested [12], none have been confirmed to date.

A major cause of the chronic pulmonary disease in CF is a persistent imbalance between neutrophil proteinases, most importantly elastase, and the host defence screen, spearheaded by α1-antitrypsin (α1-AT) [13, 14]. This α1-AT is a 52 kDa acute-phase glycoprotein secreted by the liver and monocytes and is the most important proteinase inhibitor in the lung. Over 70 variants have been described by isoelectric focusing, but the most clinically important are the common S (ValGlu→Val, Z (ValGlu→Lys)) alleles which are found in ap-proximately 12% of the UK population [15] and result in plasma α1-AT levels in the homozygote of 60% and 10%, respectively, when compared to the normal M homozygote. "Relative" α1-AT deficiency may be associated with the Taq-I G→A allele at the polymorphic site in the 3’ noncoding region enhancer binding element of the α1-antitrypsin gene [16]. This mutant allele results in the loss of a Taq-I restriction site and may reduce the rise in the level of α1-antitrypsin in the acute phase response, whilst leaving baseline levels unaffected. The presence of the G→A allele has been found to confer a 13 fold increased risk for the development of chronic airflow obstruction [17, 18].

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In this study we aimed to determine the prevalence of α1-AT deficiency phenotypes and the Taq-I G→A allele in a CF population and to assess their effect on pulmonary disease. In addition, as cystic fibrosis patients have an inflammatory stimulus, we have also tested the hypothesis that the Taq-I G→A allele affects the regulation of α1-AT secretion in vivo by comparing serum levels with other markers of the inflammatory response.

Methods

Clinical details

One hundred and fifty seven patients were recruited from two regional CF centres in Cambridge and Manchester, UK. Clinical information was obtained by patient interview and scrutiny of the patient case notes by one investigator (table 1). Details of pulmonary function, pancreatic status, smoking history, liver function and age at onset of colonization with P. aeruginosa and B. cepacia were recorded when available. Colonization was defined as two consecutive positive sputum cultures 6 months apart. Patients were classified according to their need for pancreatic enzyme supplements as "pancreatic sufficient" (PS) and "pancreatic insufficient" (PI). The presence of liver disease was confirmed by clinical features, liver imaging and interpretation of liver function tests. The severity of pulmonary disease was documented by recording the patient’s best measurement of forced expiratory volume in one second (FEV1) in the previous 6 months and was compared to age/sex matched normal controls [19], to give a value for FEV1% predicted. A chest radiograph taken when the patient was stable, was assessed using the Northern scoring system [20] by a radiologist and a CF pulmonary physician who were blinded to patient details. This system scores a postero-anterior (PA) chest radiograph in quadrants out of a maximum of twenty, with higher scores representing more marked radiographical abnormalities. CF genotypes were determined by the Dept of Clinical Genetics at Addenbrooke’s Hospital, Cambridge and the Dept of Molecular Genetics, The Royal Manchester Children’s Hospital, Manchester.

Determination of α1-AT phenotype, Taq-I genotype and serum level of α1-AT, α1-antichymotrypsin and C-reactive protein

Ten millilitres of blood (5 mL clotted, 5 mL ethylene diamine tetra-acetic acid (EDTA)) was obtained from each patient at the time of interview. Anticoagulated blood was

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal phenotype</th>
<th>Deficient phenotype</th>
<th>p-value</th>
<th>Non Taq-I group</th>
<th>Taq-I group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age yrs (n)</td>
<td>24.3 (7.1)</td>
<td>25.7 (6.1)</td>
<td>0.432*</td>
<td>24.7 (7.2)</td>
<td>22.7 (5.3)</td>
<td>0.218*</td>
</tr>
<tr>
<td>Sex (n/%)</td>
<td>70 (56.9/14 (70)</td>
<td>71 (56.3/16 (80.0)</td>
<td>0.333*</td>
<td>11 (5.5)</td>
<td>4 (20.0)</td>
<td>0.052*</td>
</tr>
<tr>
<td>CF genotype n(%)</td>
<td>61 (55.5/15 (75)</td>
<td>68 (60.2/11 (55.0)</td>
<td>0.176*</td>
<td>38 (33.6/6 (30.0)</td>
<td>0.364*</td>
<td></td>
</tr>
<tr>
<td>Liver disease n(%)</td>
<td>104 (84.6/19 (95)</td>
<td>107 (84.9/18 (90.0)</td>
<td>0.4308*</td>
<td>19 (15.1/2 (10.0)</td>
<td>0.739*</td>
<td></td>
</tr>
<tr>
<td>Pancreatic status n(%)</td>
<td>114 (92.7/20 (100)</td>
<td>118 (93.7/19 (95.0)</td>
<td>0.360*</td>
<td>19 (15.1/2 (10.0)</td>
<td>1.00*</td>
<td></td>
</tr>
<tr>
<td>P. aeruginosa n(%)</td>
<td>24 (19.5/3 (15)</td>
<td>22 (17.5/6 (30.0)</td>
<td>0.766*</td>
<td>104 (82.5/14 (70.0)</td>
<td>0.221*</td>
<td></td>
</tr>
<tr>
<td>B. cepacia n(%)</td>
<td>98 (79.7/16 (80)</td>
<td>100 (79.4/16 (80.0)</td>
<td>1.000*</td>
<td>26 (20.6/4 (20.0)</td>
<td>1.000*</td>
<td></td>
</tr>
<tr>
<td>Smoking status n(%)</td>
<td>112 (91.8/18 (90)</td>
<td>114 (91.2/18 (90.0)</td>
<td>0.677*</td>
<td>11 (8.8/2 (10.0)</td>
<td>0.803*</td>
<td></td>
</tr>
<tr>
<td>Centre n(%)</td>
<td>82 (66.7/14 (70)</td>
<td>85 (67.5/13 (65.0)</td>
<td>1.000*</td>
<td>41 (32.5/7 (35.0)</td>
<td>0.803*</td>
<td></td>
</tr>
</tbody>
</table>

The deficient α1-antitrypsin (α1-AT) phenotype group is a combination of the 16 MS, three MZ and one S patients. The Taq-I group refers to those unrelated patients with the Taq-I G→A allele. One 5 yr old patient with the Taq-I G→A was excluded from analysis as no accurate measure of forced expiratory volume in one second (FEV1) was available. Thirteen patients in each factor group were not assessed for their cystic fibrosis (CF) genotype at the time of the study. The 39 "other" CF mutations in the normal α1-AT phenotype 508/other group were: six patients G551D, three R117H, three 621+1G→A, two R1162X, two G542X and one each had P67L, 1078delT, 271delE, 1717-1G→A, V520F, 1898+1G→T, W1310X and N1303K in addition to the ∆F508 mutation. In 15 patients the other CF mutation was unknown. In the 10 non-508/non-508 normal α1-AT phenotype group, one patient had each of the following: G551D/G551D, W1282X/271delE, N1303K/unknown, 977ins/unknown, S5497/unknown, ∆S507/unknown; in four patients both mutations were unknown at the time of the study. In the deficient α1-AT phenotype group, the "other" mutations in the five patients in the 508/other group were R560T, 3659delC, 271delE and two in whom the other mutations were unknown at the time of the study. #: Chi-square goodness of fit test; *: Fisher’s exact test. P. aeruginosa: Pseudomonas aeruginosa; B. cepacia: Burkholderia cepacia.
stored at -80°C until deoxyribonucleic acid (DNA) extraction was performed. Serum was obtained from clotted blood and stored at -80°C until required. DNA was extracted from peripheral blood leucocytes by phenol/chloroform extraction and ethanol precipitation. Oligonucleotides (20mer) were designed to amplify the region of the α₁-AT gene containing the Taq-I polymorphic site based on the previously published sequence [21]. A product of 832 base pairs was generated by the polymerase chain reaction (PCR) in 100 μL reaction volume with AmpliTaqTM (Perkin Elmer. Applied Biosystems, Warrington, UK), 10×PCR buffer, 1.5 mM Mg²⁺, 200 μM dNTPs and 100 pmol primers (upper primer 5′-AAT GAC TGA GGC AGA TTC CTG AA-3′, lower primer 5′-GTA TTT GTG GAG AGT GAA AGG CTG TC-3′). Reaction conditions consisted of an initial denaturing cycle of 5 min at 100°C followed by 40 cycles of 94°C for 20 s, 55°C for 20 s and 74°C for 40 s. The product was confirmed on a 1% weight/volume (w/v) agarose gel containing ethidium bromide and was digested with Taq-I (Boehringer Mannheim, Sussex, UK) by incubation at 65°C for 4.5 h. Comparison with previously sequenced controls (kindly provided by N. Kalsheker, Dept of Clinical Chemistry, Queens Medical Centre, Nottingham, UK) allowed determination of the Taq-I genotype. All analyses were performed in duplicate and 135 healthy blood donors were screened for the mutation to determine the incidence of the G→A allele in our control population.

α₁-AT phenotypes were determined in comparison with known controls by isoelectric focusing within a narrow pH gradient (pH 4.2–4.9) and immunoblotting as previously described [22]. Serum concentrations of α₁-AT and α₁-antichymotrypsin (α₁-ACT) were measured by immunoturbidimetry in the Dept of Biochemistry, Hinchingbrooke Hospital, Huntingdon, UK, using standards of known concentration. A reference range for α₁-AT concentrations (0.7–1.7 g·L⁻¹; within run coefficient of variation 2.84%) was determined from 106 healthy blood donors. The reference range for α₁-antichymotrypsin (α₁-ACT) has previously been determined (0.2–0.6 g·L⁻¹; within batch coefficient of variation 5.5%). C-reactive protein (CRP) was measured by immunoturbidimetry by the Dept of Biochemistry, Addenbrooke’s Hospital, Cambridge, UK (normal range <6 mg·L⁻¹).

The study was approved by the local hospital Ethics Committee.

Statistical analysis

The Chi-squared goodness-of-fit test, Fisher’s exact test and the unpaired t-test were used to test for differences in patient characteristics between the factor groups studied. In addition, the Chi-squared goodness-of-fit test was used to compare the proportions of patients with the Taq-I G→A allele in the study group with a group of healthy controls.

The three outcomes of interest were FEV₁ % pred, chest radiograph score and age at onset of colonization with P. aeruginosa. The effect of the two factors, α₁-AT phenotype and Taq-I genotype formed the primary investigation for this study. Analysis of covariance was used to assess the effect of the factors upon the outcome measures, while adjusting for covariates that could possibly confound the relationship between the factors and the outcome measures. For each factor, both the observed mean (unadjusted for covariates) and the adjusted mean are shown in table 2 with the relevant tests performed on the latter. The covariates considered were age, sex, pancreatic status, P. aeruginosa colonization status, B. cepacia colonization status, CF genotype, smoking status, liver cirrhosis and recruitment centre. Age and P. aeruginosa status were excluded from the covariates when assessing the age at onset of colonization with P. aeruginosa. One of a sibling/twin pair was randomly excluded from these analyses in order to eliminate the effect of unknown genetic factors. One patient who had a liver transplant was only included in the analysis of α₁-AT phenotype on serum α₁-AT levels described below. The correlation between duration of colonization with P. aeruginosa and FEV₁ % pred accounting for age was assessed by analysis of covariance.

CF provides an ideal condition in which to test the effect of the Taq-I G→A allele on serum α₁-AT concentration as many patients have an inflammatory response which may be reduced by the Taq-I G→A allele. There is no way of knowing whether the rise in serum α₁-AT is appropriate for the degree of inflammation within each

The Taq-I group refers to those with a Taq-I G→A allele. FEV₁: forced expiratory volume in one second; CXR: chest radiograph; P. aeruginosa: Pseudomonas aeruginosa.

Table 2. – Outcome measures and the pooled within-treatment group standard deviation following adjustment for covariates for the two α₁-antitrypsin phenotype groups and the two Taq-I genotype groups used in the analysis of unrelated patients

<table>
<thead>
<tr>
<th>Outcome measures</th>
<th>Normal phenotype</th>
<th>Deficient phenotype</th>
<th>p-value</th>
<th>Non Taq-I group</th>
<th>Taq-I group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV₁ % pred</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observed mean</td>
<td>52.6</td>
<td>61.1</td>
<td>0.043</td>
<td>52.8</td>
<td>60.7</td>
<td>0.368</td>
</tr>
<tr>
<td>Adjusted mean</td>
<td>51.1</td>
<td>62.5</td>
<td></td>
<td>54.2</td>
<td>59.3</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>22.86</td>
<td></td>
<td>22.91</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CXR score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observed mean</td>
<td>9.3</td>
<td>8.9</td>
<td>0.127</td>
<td>9.3</td>
<td>8.9</td>
<td>0.813</td>
</tr>
<tr>
<td>Adjusted mean</td>
<td>9.7</td>
<td>8.5</td>
<td></td>
<td>9.2</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>3.12</td>
<td></td>
<td>3.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at onset of P. aeruginosa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observed mean</td>
<td>15.1</td>
<td>14.3</td>
<td>0.899</td>
<td>15.3</td>
<td>12.6</td>
<td>0.146</td>
</tr>
<tr>
<td>Adjusted mean</td>
<td>14.8</td>
<td>14.5</td>
<td></td>
<td>15.7</td>
<td>12.2</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>7.64</td>
<td></td>
<td>7.48</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

The Taq-I group refers to those with a Taq-I G→A allele. FEV₁: forced expiratory volume in one second; CXR: chest radiograph; P. aeruginosa: Pseudomonas aeruginosa.
patient without a control acute phase protein with similar kinetics to α1-AT. As α1-ACT and CRP are unaffected by the Taq-I genotype and the α1-AT phenotype, these proteins can be used to measure the level of inflammatory stress within a given patient. The effect of α1-AT phenotype and Taq-I genotype upon serum α1-AT levels was assessed by analysis of covariance, adjusting for the covariates α1-ACT and CRP. For both analyses, the effect of liver disease was assessed but was excluded from the final model since there were very few patients with liver disease and the Taq-I G→A allele or a deficient phenotype (2 and 1 respectively). In a further assessment of the effect of the Taq-I genotype, the possible confounding effect of the MS α1-AT phenotype was also investigated. The three MZ and one S phenotype patients were excluded from both analyses due to insufficient numbers.

In all analyses a p-value of less than 0.05 was taken to be statistically significant.

Results

A total of 157 patients with CF were recruited (58.6% male, 41.4% female) with a mean age of 24 yrs (SD 7.4 yrs; range 3–50 yrs). There were five sibling pairs and one pair of twins. Twenty out of 147 (13%) unrelated CF patients (excluding six related patients, three patients not tested and one patient with a liver transplant) were found to have α1-AT deficiency phenotypes: 16 MS, one S, three MZ. Two CF patients were found to have the rare FM phenotype. No Z α1-AT homozygotes were detected. Twenty one out of 150 unrelated patients (14%) assessed (excluding six related patients and one with liver transplant) were found to carry the G→A allele at the 3’ Taq-I site (20 heterozygous and one homozygous for the mutation (fig. 1); 13.7% of Manchester patients and 14.5% of Cambridge patients). Nineteen out of 135 healthy blood donor controls (14.1%) were similarly found to be heterozygous for this allele with no statistical difference between the two proportions (p>0.2).

α1-AT phenotypes, Taq-I genotype and the severity of CF lung disease

One of a sibling pair (i.e., six patients) were randomly excluded from the initial analysis of α1-AT phenotypes and the Taq-I genotype on the severity of CF lung disease in order to negate the effect of other possible genetic influences. In addition eight and five patients were omitted from the α1-AT phenotype groups and the Taq-I genotype groups, respectively, due to incomplete data sets, the young children who were unable to perform lung function testing and the patient who had a liver transplant.

α1-AT phenotypes. The characteristics of the patient groups are shown in table 1 and the effect on lung disease severity in table 2. In combination, the S and Z α1-AT deficiency alleles were associated with significantly less severe lung disease as measured by FEV1 % pred (11.4% difference; p=0.043). This effect was independent of the covariates CF genotype (p>0.2) and colonization with P. aeruginosa (p=0.184). The deficiency alleles were also associated with better chest radiograph scores (table 2) although the difference was not significant (p=0.127). The MS α1-AT phenotype group alone had higher FEV1 % pred values (adjusted mean 63.5% versus 51.9% for M homozygotes), but the effect narrowly missed statistical significance (p=0.061). One sibling in a twin or sibling pair had been randomly excluded from this analysis so as to avoid confounding genetic factors. If all siblings were included, the beneficial effect of the MS α1-AT phenotype on FEV1 % pred was highly significant (13.8% difference; p=0.027). The presence of only one S homozygote and three MZ heterozygotes make it impossible to assess the effect of these specific phenotypes on lung disease severity in this group of patients.

The MS α1-AT phenotype group were colonized with P. aeruginosa almost 2 yrs earlier than M homozygote controls (13.1 yrs versus 14.8 yrs), but this effect was not statistically significant (p=0.461) in an analysis that lacked power due to insufficient numbers (n=12). Those colonized with P. aeruginosa in the combined α1-AT deficient group had a longer duration of colonization with the organism than the normal phenotype group: median 97 months (interquartile range (IQR) 33–124 months) and 63 months (IQR 28.5–103.5 months) respectively, although this was not significant, p=0.46. Interestingly, there was no significant correlation between duration of colonization with P. aeruginosa and FEV1 % pred when age was taken into account (r=0.002, p=0.99).

The median durations of colonization with B. cepacia for the normal and α1-AT deficient groups were 48 months (range 0–132 months) and 24 months (range 24–92 months), respectively, but further statistical analysis was not possible as the exact duration of colonization with this organism was known in only three patients.

α1-AT Taq-I genotype. The characteristics of the patient groups are shown in table 1. The Taq-I genotype had no significant effect on FEV1 % pred chest radiograph score or age of onset of P. aeruginosa in patients with CF (table 2). Analysis of covariance also showed that neither CF genotype nor colonization with P. aeruginosa significantly altered the effect of the Taq-I genotype on lung disease as measured by FEV1 % pred or chest radiograph scores.

Serum α1-AT levels

The serum levels of α1-ACT and CRP were used to assess the effect of the inflammatory response in an individual patient as they are unlikely to be affected by α1-AT...
deficiency phenotypes and the Taq-I genotype. Analysis of covariance showed that serum α₁-ACT (p<0.0001; fig. 2), but not CRP, was a good predictor of serum α₁-ACT concentration. The linear relationship between α₁-ACT and α₁-ACT was used to test the effect of the Taq-I genotype and the MS α₁-ACT phenotype on the serum level of α₁-ACT, taking into account an individual patient’s inflammatory response. As expected the MS α₁-ACT phenotype reduced the serum level of α₁-ACT in patients with CF (mean MS serum α₁-ACT 2.0 versus 2.6 g·L⁻¹ for M homozygotes, fig. 2). The low numbers of patients (17) in the MS group resulted in a lack of statistical power and may account for the finding that the marked difference observed in serum levels between the two groups did not reach statistical significance (p=0.225). The mean α₁-ACT level from 23 patients with the Taq-I G→A allele was 2.57 (21 out of 23 had evidence of an acute phase response as defined by α₁-ACT levels >1.7 g·L⁻¹) compared with 2.50 g·L⁻¹ for the non-Taq-I group (p=0.968; fig. 3). Therefore, there was no evidence to suggest that the Taq-I genotype affected the serum level of α₁-ACT during the inflammatory response.

In a further model the effect of α₁-ACT phenotype was also taken into account as a covariate. Again the Taq-I genotype had no significant effect on α₁-ACT levels during the inflammatory response (means 2.55 for non-Taq-I and 2.57 g·L⁻¹ for the Taq-I groups; p=0.578).

Discussion

The mechanism by which a defect in the CFTR gene predisposes to the progressive obstructive pulmonary disease in CF remains to be clarified. Previous studies have shown that there is significant variation in lung damage between patients with the same CF genotype [2] indicating that other factors play an important role in the progression of the pulmonary disease. The lung damage in CF is thought to result from the marked protease-antiproteinase imbalance in favour of neutrophil elastase [13, 14]. The physiological role of this enzyme remains unclear, although it is likely to be important in the digestion of bacterial cell walls and the initiation and regulation of inflammation [23, 24]. Free neutrophil elastase has many deleterious effects, including the destruction of lung elastin [23], cleavage of immunoglobulins [25] and fibronectin [26], a reduction in ciliary beat frequency [27] and the stimulation of mucus gland secretion [28].

Although α₁-ACT phenotypes that result in mild to moderate plasma deficiency of the protein do not have any clinical sequelae in the normal individual, it is logical that any deficiency of this protein is likely to intensify lung damage in CF where the elastase load is considerably greater. Surprisingly, however, our study suggests that the common MS, MZ and S α₁-ACT phenotypes, which result in mild to moderate deficiency of the protein, are not associated with worse lung disease. Indeed, this group of patients have a significantly better FEV₁ % pred when compared to patients with CF who have a nondeficient α₁-ACT phenotype. Similarly, the MS phenotype alone was associated with a 11.6% better FEV₁ % pred which, although this failed to reach statistical significance, but this difference was significant when all siblings were included in the analysis (13.8% difference; p=0.027). The protective effect of mild α₁-ACT deficiency alleles was independent of other factors that can mitigate against progression of CF lung disease such as pancreatic sufficiency, abstinence from smoking and delayed onset of colonization with P. aeruginosa (tables 1 and 2). DÖRING et al. [29] also found that mild to moderate deficiency of α₁-ACT does not confer a disadvantage to the severity of CF lung disease but was associated with a 3% higher FEV₁ % pred, although this failed to reach statistical significance in a univariate analysis. They also reported a significantly earlier onset of colonization with P. aeruginosa in CF patients with α₁-ACT deficiency alleles and their study concluded that the unopposed action of neutrophil elastase results in tissue damage that encourages colonization with P. aeruginosa.

The basis of the apparent protective effect of the MS phenotype on lung function is not obvious and would seem paradoxical in view of the known effects of neutrophil elastase. It is possible that our study has detected a group of CF patients with mild lung disease who would have not presented had they not had an α₁-ACT deficiency phenotype. This would seem unlikely, as the incidence of deficiency alleles in our CF patients is similar to that found in the general population [15]. Moreover, this also
makes it unlikely that patients with mild to moderate α1-AT deficiency have such severe lung disease that it leads to early death and hence exclusion from our study population. A second possible explanation is that this group of patients have inherited an as yet unknown protective factor that is linked with the S α1-AT deficiency allele. Interestingly, a heterozygote advantage has been suggested to account for the high incidence of the S and Z α1-AT alleles in the European population. These alleles have been implicated in protection against pulmonary tuberculosis [30], although this remains unproven. A mechanistic explanation may lie in the regulatory role of elastases in chronic inflammation [24]. The persistence of P. aeruginosa within the lungs of CF patients leads to a continuing neutrophil influx and release of neutrophil elastase which causes much of the lung damage. However, patients colonized with P. aeruginosa can survive many years, implying that a balance between bacterial invasion and the host inflammatory response is reached, with a limit on the release of neutrophil elastase. Elastase and other proteinases can down-regulate inflammation by inhibiting neutrophil activation by cleavage of immunoglobulins, immune complexes, neutrophil receptors and complement [31] and by inducing apoptosis [32, 33]. One might speculate that in the dynamic processes involved in chronic inflammation, deficiency of α1-AT would allow more free neutrophil elastase to perform this regulatory role at an earlier time point in the inflammatory process, thereby limiting its detrimental effect. Regular antibiotic therapy would negate the adverse effect that this would have on bacterial proliferation. The Taq-I G→A allele has been linked to an increased risk of chronic airflow obstruction [17, 18]. In vitro data have suggested that the presence of a G→A allele results in a reduction in the rise of serum α1-AT during the inflammatory response [16]. If correct, this allele may affect the pathogenesis of CF lung disease in a predictable manner. Our data show that the Taq-I G→A allele was present in 14% of both the CF and the healthy blood donor population and was not associated with more severe lung disease in those patients with CF. The frequent finding of an acute phase response in this group of patients has allowed an examination of the effect of the Taq-I genotype on serum levels of α1-AT. There was no evidence to suggest that the Taq-I genotype had any effect on serum α1-AT levels in our cohort of CF patients, most of whom had evidence of an acute inflammatory response. It would seem likely that the association of the allele with chronic obstructive bronchitis [17, 18] is mediated by a mechanism independent of the serum α1-AT concentration.

The complexity of cystic fibrosis lung disease and our lack of understanding of the exact pathological mechanisms involved implies that other, as yet undefined factors are likely to affect the pulmonary phenotype. This presents some problems in the analysis of the effect of any one factor on lung disease. Nevertheless, our study provides the first evidence of an effect from a genetic factor outside the cystic fibrosis transmembrane regulator gene locus to account for the heterogeneity of cystic fibrosis lung disease. Further clinical studies are required to confirm our findings, along with an examination of the proteinase-antiproteinase balance in the lungs of cystic fibrosis patients with variant α1-antitrypsin alleles. This is of current clinical significance in view of the prevailing interest in nebulized α1-antitrypsin therapy for cystic fibrosis.

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**References**