α_1 -Antitrypsin deficiency alleles and the *Taq-I* G→A allele in cystic fibrosis lung disease

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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 antiprotease 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 α_1 -antitrypsin, α_1 -antichymotrypsin and C-reactive protein (CRP) were determined and patients were screened for the common S and Z deficiency alleles of α_1 -antitrypsin and the G→A mutation in the 3' noncoding region of the α_1 -antitrypsin gene (*Taq-I* G→A allele).

α_1 -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 α_1 -antitrypsin during the inflammatory response.

We show here that, contrary to current thinking, common mutations of α_1 -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 α_1 -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 α_1 -antitrypsin.

Eur Respir J 1998; 11: 873–879.

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Keywords: α_1 -antitrypsin, α_1 -proteinase inhibitor, bronchiectasis, cystic fibrosis, serpins

Received: September 7 1997

Accepted after revision December 15 1997

This work was supported by the East Anglian locally organized research scheme, the Medical Research Council (UK) the European Union (grant no. BMH1-LT13-1592) and Papworth Hospital Research Trust funds. RM is an Anglia and Oxford Research Fellow.

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 α_1 -antitrypsin (α_1 -AT) [13, 14]. α_1 -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 (²⁶⁴glutamic acid (Glu)→valine (Val)) and Z (³⁴²Glu→lysine (Lys)) alleles which are found in approximately 12% of the UK population [15] and result in plasma α_1 -AT levels in the homozygote of 60% and 10%, respectively, when compared to the normal M homozygote. "Relative" α_1 -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 α_1 -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 α_1 -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].

In this study we aimed to determine the prevalence of α_1 -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 α_1 -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 α_1 -AT phenotype, *Taq-I* genotype and serum level of α_1 -AT, α_1 -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 1. – Comparison of characteristics of the two antitrypsin phenotype groups and two *Taq-I* genotype groups used in the analysis

Variable	Normal phenotype	Deficient phenotype	p-value	Non <i>Taq-I</i> group	<i>Taq-I</i> group	p-value
Age yrs (SD)	24.3 (7.1)	25.7 (6.1)	0.432*	24.7 (7.2)	22.7 (5.3)	0.218*
Sex n(%)						
Male	70 (56.9)	14 (70)	0.333#	71 (56.3)	16 (80.0)	0.052#
Female	53 (43.1)	6 (30)		55 (43.7)	4 (20.0)	
CF genotype n(%)						
Δ F508/ Δ F508	61 (55.5)	15 (75)	0.176#	68 (60.2)	11 (55.0)	0.364#
Δ F508/other	39 (35.5)	5 (25)		38 (33.6)	6 (30.0)	
Non Δ F508/non Δ F508	10 (9.1)	0 (0)		7 (6.2)	3 (15.0)	
Liver disease n(%)						
No liver disease	104 (84.6)	19 (95)	0.4308+	107 (84.9)	18 (90.0)	0.739+
Liver disease	19 (15.4)	1 (5)		19 (15.1)	2 (10.0)	
Pancreatic status n(%)						
Insufficient	114 (92.7)	20 (100)	0.360+	118 (93.7)	19 (95.0)	1.000+
Sufficient	9 (7.3)	0 (0)		8 (6.3)	1 (5.0)	
<i>P. aeruginosa</i> n(%)						
Not present	24 (19.5)	3 (15)	0.766+	22 (17.5)	6 (30.0)	0.221+
Present	99 (80.5)	17 (85)		104 (82.5)	14 (70.0)	
<i>B. cepacia</i> n(%)						
Not present	98 (79.7)	16 (80)	1.000+	100 (79.4)	16 (80.0)	1.000+
Present	25 (20.3)	4 (20)		26 (20.6)	4 (20.0)	
Smoking status n(%)						
Nonsmoker	112 (91.8)	18 (90)	0.677+	114 (91.2)	18 (90.0)	1.000+
Current or exsmoker	10 (8.2)	2 (10)		11 (8.8)	2 (10.0)	
Centre n(%)						
Manchester	82 (66.7)	14 (70)	1.000#	85 (67.5)	13 (65.0)	0.803#
East Anglia	41 (33.3)	6 (30)		41 (32.5)	7 (35.0)	

The deficient α_1 -antitrypsin (α_1 -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 α_1 -AT phenotype 508/other group were: six patients G551D, three R117H, three 621+1G→T, two R1162X, two G542X and one each had P67L, 1078delT, 2711delT, 1717-1G→A, V520F, 1898+1G→T, W1310X and N1303K in addition to the Δ F508 mutation. In 15 patients the other CF mutation was unknown. In the 10 non-508/non-508 normal α_1 -AT phenotype group, one patient had each of the following: G551D/G551D, W1282X/2711delT, N1303K/unknown, 977ins/unknown, S549?/unknown, Δ I507/unknown; in four patients both mutations were unknown at the time of the study. In the deficient α_1 -AT phenotype group, the "other" mutations in the five patients in the 508/other group were R560T, 3659delC, 2711delT and two in whom the other mutations were unknown at the time of the study. *: unpaired T-test; #: 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 (26mer) were designed to amplify the region of the α_1 -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 AmpliTaq™ (Perkin Elmer, Applied Biosystems, Warrington, UK), 10 $\times$ PCR buffer, 1.5 mM Mg^{2+} , 200 μM dinucleotide triphosphates (dNTPs) and 100 pmol primers (upper primer 5'-AAT GAC TGA GGC AGA TTC 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 $\rightarrow$ A allele in our control population.

α_1 -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 α_1 -AT and α_1 -antichymotrypsin (α_1 -ACT) were measured by immunoturbidimetry in the Dept of Biochemistry, Hinchingsbrooke Hospital, Huntingdon, UK, using standards of known concentration. A reference range for α_1 -AT concentrations (0.7–1.7 $\text{g}\cdot\text{L}^{-1}$; within run coefficient of variation 2.84%) was determined from 106 healthy blood donors. The reference range for α_1 -ACT had previously been determined (0.2–0.6 $\text{g}\cdot\text{L}^{-1}$; within batch co-efficient of variation 5.5%). C-reactive protein (CRP) was measured by immunoturbidimetry by the Dept of Biochemistry, Addenbrooke's Hospital, Cambridge, UK (normal range $<6\text{ mg}\cdot\text{L}^{-1}$).

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 $\rightarrow$ 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, α_1 -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 α_1 -AT phenotype on serum α_1 -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 $\rightarrow$ A allele on serum α_1 -AT concentration as many patients have an inflammatory response which may be reduced by the *Taq-I* G $\rightarrow$ A allele. There is no way of knowing whether the rise in serum α_1 -AT is appropriate for the degree of inflammation within each

Table 2. — Outcome measures and the pooled within-treatment group standard deviation following adjustment for covariates for the two α_1 -antitrypsin phenotype groups and the two *Taq-I* genotype groups used in the analysis of unrelated patients

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

The *Taq-I* group refers to those with a *Taq-I* G $\rightarrow$ 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 α_1 -AT. As α_1 -ACT and CRP are unaffected by the *Taq-I* genotype and the α_1 -AT phenotype, these proteins can be used to measure the level of inflammatory stress within a given patient. The effect of α_1 -AT phenotype and *Taq-I* genotype upon serum α_1 -AT levels was assessed by analysis of covariance, adjusting for the covariates α_1 -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 α_1 -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 α_1 -AT deficiency phenotypes: 16 MS, one S, three MZ. Two CF patients were found to have the rare FM phenotype. No Z α_1 -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 ($p>0.2$).

α_1 -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 α_1 -AT phenotypes and the *Taq-I* genotype on the severity of CF lung disease in

Fig. 1. — Two per cent weight/volume (w/v) agarose ethidium bromide gel showing the 832 base pair product generated from the polymerase chain reaction (undigested fragment). Digestion with *Taq-I* revealed none (+/+), one (+/-) or two (-/-) *Taq-I* G→A alleles. The deoxyribonucleic acid (DNA) size markers are shown on the left of the gel.

order to negate the effect of other possible genetic influences. In addition eight and five patients were omitted from the α_1 -AT phenotypes 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.

α_1 -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 α_1 -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 α_1 -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 α_1 -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 α_1 -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 α_1 -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 α_1 -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.

α_1 -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 α_1 -AT levels

The serum levels of α_1 -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 α_1 -AT

Fig. 2. — Graphical representation of the relationship between serum α_1 -antitrypsin (α_1 -AT) and α_1 -antichymotrypsin (α_1 -ACT) concentrations for cystic fibrosis patients with the nondeficient and MS phenotypes. The lines represent the least squares linear regression line derived from the analysis of covariance for the group with the MS α_1 -AT phenotype (●; ---) and those with a normal phenotype (○; —).

deficiency phenotypes and the *Taq-I* genotype. Analysis of covariance showed that serum α_1 -ACT ($p < 0.0001$; fig. 2), but not CRP, was a good predictor of serum α_1 -AT concentration. The linear relationship between α_1 -ACT and α_1 -AT was used to test the effect of the *Taq-I* genotype and the MS α_1 -AT phenotype on the serum level of α_1 -AT, taking into account an individual patient's inflammatory response. As expected the MS α_1 -AT phenotype reduced the serum level of α_1 -AT in patients with CF (mean MS serum α_1 -AT 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 α_1 -AT 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 α_1 -AT 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 α_1 -AT during the inflammatory response.

Fig. 3. — Graphical representation of the relationship between serum α_1 -antitrypsin (α_1 -AT) and α_1 -antichymotrypsin (α_1 -ACT) concentrations for cystic fibrosis patients with the *Taq-I* G→A allele (●; —) and those without (○; ---). The lines represent the least squares linear regression line derived from the analysis of covariance for the group.

In a further model the effect of α_1 -AT phenotype was also taken into account as a covariate. Again the *Taq-I* genotype had no significant effect on α_1 -AT 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 proteinase-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 α_1 -AT 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 α_1 -AT 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 α_1 -AT phenotype. Similarly, the MS phenotype alone was associated with a 11.6% better FEV₁ % pred which narrowly missed 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 α_1 -AT 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 α_1 -AT 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 α_1 -AT 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 α_1 -AT 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 α_1 -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 α_1 -AT deficiency allele. Interestingly, a heterozygote advantage has been suggested to account for the high incidence of the S and Z α_1 -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 α_1 -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 α_1 -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 α_1 -AT. There was no evidence to suggest that the *Taq-I* genotype had any effect on serum α_1 -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 α_1 -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 α_1 -antitrypsin alleles. This is of current

clinical significance in view of the prevailing interest in nebulized α_1 -antitrypsin therapy for cystic fibrosis.

Acknowledgements: The authors would like to thank L. Sharples, MRC Biostatistics Unit, University of Cambridge for her help with the statistical analysis.

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