SERPINA1 11478G→A variant, serum α_1-antitrypsin, exacerbation frequency and FEV_1 decline in COPD

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ABSTRACT

Background The α_1-antitrypsin 11478G→A polymorphism may be associated with attenuated acute α_1-antitrypsin responses. It was hypothesised that patients with chronic obstructive pulmonary disease (COPD) and this mutation have accelerated lung function decline.

Objective To assess whether the 11478G→A polymorphism is associated with attenuated α_1-antitrypsin responses at COPD exacerbation, and therefore accelerated lung function decline.

Methods Lung function decline by genotype was examined in the English Longitudinal Study of Ageing (ELSA; n=1805) and Whitehall II (n=2733) studies. 204 patients with COPD were genotyped in the London cohort and serum α_1-antitrypsin concentration was measured at baseline and (n=92) exacerbation.

Results The 11478G→A genotype frequencies did not vary between COPD cases and controls, or between COPD frequent and infrequent exacerbators. Subjects with the rare A allele experienced more rapid lung function decline in the Whitehall II (A vs non-A: 16 vs 4 ml/year p=0.02) but not ELSA (29 vs 34 ml/year, p=0.46) or London cohorts (26 vs 38 ml/year, p=0.06). Decline was not greater in frequent exacerbator A versus non-A carriers (20 vs 24 ml/year, p=0.58). Upregulation of α_1-antitrypsin at exacerbation was not demonstrated, even in patients homozygous for the common allele (median exacerbation change −0.07 g/l 11478GG, p=0.87 and −0.09 g/l 11478AA/GA, p=0.92; p=0.90 for difference). In patients with the A allele, there was no correlation between serum α_1-antitrypsin and serum interleukin 6 (IL-6) concentrations.

Conclusion The 11478G→A α_1-antitrypsin polymorphism is not associated with increased risk of developing COPD, nor accelerated lung function decline. Serum α_1-antitrypsin may not be upregulated early at COPD exacerbation. In patients with the 11478G→A polymorphism there was no relationship between the serum α_1-antitrypsin and serum IL-6 concentrations.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a prevalent condition characterised by airflow limitation and airway inflammation.[1] Much of the morbidity and mortality relate to episodes of acute deterioration in respiratory health termed exacerbations.[2] Exacerbations are associated with additional airway and systemic inflammation,[3] and drive lung function decline,[4 5] but the mechanism underlying this remains ill defined. Aside from continued cigarette smoke exposure,[6] other factors affecting disease progression in COPD remain incompletely understood.

Approximately 1% of COPD is associated with functional deficiency of α_1-antitrypsin, a glycoprotein produced largely by the liver that inactivates neutrophil elastase.[7] Such patients are generally homozygous for non-M (null, S or Z) variants.[8] The predominant clinical manifestations are emphysema and liver cirrhosis. As in non-α_1-deficient COPD, lung function decline in patients with α_1-antitrypsin deficiency is related to exacerbation frequency.[9]

The gene encoding α_1-antitrypsin (*SERPINA1*) is highly polymorphic,[7] and one such variant in the 3′ untranslated region (UTR), also known as *Taq*I, results in a G→A change at position 11478.[10 11] Baseline serum α_1-antitrypsin levels are not lower in patients with the rare A allele[12] and there should therefore be no increased risk of COPD. Some studies, however, had suggested there may be increased risk,[12] though no signal was detected in more recent genome-wide association analyses.[13]

Importantly, the 11478G→A genotype may result in reduced protein expression in response to acute-phase stimuli,[14] such as may occur during exacerbation of COPD. In addition, the 11478G→A variant has been associated with accelerated progression of atherosclerosis.[15] Atherosclerosis is associated with degradation of elastic fibres in arterial walls. Degradation of elastin in lung results in emphysema and lung function decline.

Consequent on a deficient α_1-antitrypsin protein response during acute insults, we hypothesised that subjects carrying the *SERPINA1* rare 11478A allele would experience more rapid decline in lung function. Further, in patients with COPD, we hypothesised that this association would be most pronounced in those patients susceptible to frequent exacerbations. In the latter group, 11478A carriers would mount an insufficient acute-phase antiprotease response during multiple exacerbations, resulting in unopposed neutrophil elastase activity, greater lung damage and therefore accelerated lung function decline. We designed a study to test this hypothesis, and examine whether 11478A carriers mount an attenuated acute-phase α_1-antitrypsin protein response at exacerbation of COPD.

METHODS

We have performed two analyses. The first uses data on lung function decline by 11478G→A genotype in participants from the large Whitehall II and English Longitudinal Study of Ageing (ELSA)

cohorts. Secondly, we repeated this analysis in the London COPD cohort in whom exacerbation frequency data, and serum α_1-antitrypsin concentration at baseline and exacerbation onset were also available.

The Whitehall II and ELSA studies

The Whitehall II and ELSA cohorts have been previously described.[16 17]

In the Whitehall II study, DNA was extracted from blood samples taken at phase 7 (2002–2004). Lung function was assessed by a trained nurse using a portable spirometer (Microplus, Medicalmicro, Basingstoke, UK) at phase 7 and also phase 9 (2007–2009).

The ELSA participants were recruited from respondents of the annual Health Survey for England (HSE) in 1998, 1999 and 2001. Spirometry, measured by a trained nurse (Vitalograph micro, Maids Moreton, Buckingham, UK) was performed in HSE years 2001 and 2004. In ELSA, DNA was extracted from blood samples taken at wave 2 (2004) of the study.

In both studies, height and weight were measured at the same time as lung function to calculate body mass index. Smoking status was ascertained by questionnaire and patients were categorised as never or ever smokers. Participants with COPD were defined at baseline as those who had smoked, who did not report a diagnosis of asthma, and had both forced expiratory volume in 1 s (FEV_1) <80% and FEV_1/forced vital capacity (FVC) <0.7.

DNA was extracted from blood samples using magnetic bead technology (Geneservice, Cambridge, UK). The participants were genotyped as described below. Genotyping error rates were examined from a repeat of 10% of samples in ELSA and 5% of samples in Whitehall II, and were found to be <1% in both studies.

The London COPD cohort patients and controls

Two hundred and four patients with COPD from the London COPD cohort were studied between 1 April 2006 and 31 March 2009. The recruitment and monitoring of these patients have previously been described.[5] In brief, all patients had COPD as defined by a postbronchodilator FEV_1 of ≤80% predicted, FEV_1/FVC <0.7 and β_2-agonist reversibility on FEV_1 of <15% or 200 ml. Patients were excluded if they had other significant respiratory diseases. Patients were recruited when stable, with no exacerbations reported in the preceding month.

Sixty-five smoking and non-smoking control subjects of similar age but without COPD were recruited from a primary care practice. The control subjects had an FEV_1 >80% predicted and an FEV_1/FVC ratio >0.7. Control subjects were excluded if they had a history of significant respiratory disease.

At the initial visit, a medical history was obtained for both patients and controls. Height and weight were measured along with baseline lung function using a volumetric storage spirometer (Vitalograph 2160, Maids Moreton, Buckingham, UK). Blood was collected for α_1-antitrypsin assay and DNA extraction for α_1-antitrypsin genotyping (described below). All patients with COPD were at least 42 days following and >14 days preceding exacerbation at the sampling visit.

This portion of the study was approved by the Royal Free Hospital Research Ethics Committee and patients gave written informed consent.

Exacerbation visits, length and frequency calculation

The London COPD cohort patients complete daily diary cards, as in our previous work,[5] recording any increase in daily respiratory symptoms. They were asked to contact the study team if they experienced an increase in their symptoms and were usually reviewed within 48 h, early in the course of the event and prior to the prescription of any additional treatment. Exacerbations were defined by the presence of two or more new or worsening symptoms for two or more consecutive days, or if in the opinion of the attending clinician the patient was having an exacerbation. To fulfil the exacerbation definition, at least one symptom had to be a major symptom of increased dyspnoea, sputum volume or sputum purulence. Minor symptoms were increased cough, wheeze, sore throat and coryza. Our exacerbation definition has been validated against changes in quality of life,[18] inflammatory markers[19] and FEV_1 decline.[5] This enabled categorisation of patients into frequent and infrequent exacerbators, defined as ≥3 or <3 exacerbations (treated and untreated) in the previous year, respectively.

Exacerbation length was calculated as the number of days from the start of the exacerbation (the first of the two consecutive days) to the last day on which lower airway symptoms (not sore throat or coryza) were still being recorded.

Ninety-two of the 204 patients were sampled at exacerbation onset for assay of serum α_1-antitrypsin. At these exacerbation visits the diagnosis was confirmed by examination of the diary cards, spirometry was performed and blood was obtained. All exacerbations were treated with bronchodilators, antibiotics and/or oral corticosteroids, at the discretion of the attending clinician. All the blood samples were taken prior to the initiation of treatment.

Lung function decline in the London COPD cohort

London COPD cohort patients attend quarterly for spirometry in the stable state and this allows accurate estimation of the rate of lung function decline (disease progression) as described further below.

Blood sampling and measurement of inflammatory markers

At baseline and exacerbation visits, 7 ml of venous blood were collected and centrifuged (224 *g* for 10 min at 4°C) within 2 h of collection. The serum was then separated and stored at −80°C for later analysis. Serum α_1-antitrypsin was quantified using commercial ELISA kits (Immunodiagnostik AG, Biosupply, Bolden, UK). The limit of detection was 0.018 g/l. To assess the systemic acute-phase response, we also assayed serum interleukin 6 (IL-6) and C-reactive protein (CRP). IL-6 was measured using commercial ELISA kits (R&D Systems, Abingdon, UK). The limit of detection was 0.7 pg/ml. The manufacturer reported variation in these assays is stated as: IL-6 intra-assay 1.6–4.2%; IL-6 interassay 3.3–6.4%; α_1-antitrypsin intra-assay 4.5–13.1%; α_1-antitrypsin interassay 9.8–14.8%. CRP was measured using a Tina-quant C-reactive protein (Latex) method (Roche/Hitachi) in the Department of Clinical Biochemistry at the Royal Free Hospital, London, UK.

Genotyping

For DNA extraction, 6 ml of venous blood was taken in an EDTA tube and stored at −80°C. DNA extraction was performed using a Gentra Systems Puregene genomic DNA purification kit following the Whole-Blood-Enhanced Productivity protocol supplied by the manufacturer (Gentra Systems, Minneapopils, Minnesota, USA). This method had four stages and yielded between 100 and 300 μg of DNA. The 11478G→A variant was genotyped as described previously, by PCR and *Taq*I digestion.[15] Individuals were also genotyped for the *SERPINA1* S (E264V) and Z (E342K) variants.

Statistical analysis

Observed numbers of each genotype were compared with that expected if the subjects were in Hardy–Weinberg equilibrium. Allele frequencies between the different groups were compared using χ^2 analysis.

In the ELSA and Whitehall II cohorts, change in lung function could be calculated from two time points only. Linear regression was used to examine change in lung function, calculated as the difference between time 1 and time 2, per year of follow-up adjusted for age and smoking status at baseline using FEV_1 or FEV_1 (% predicted). Analyses were performed with SAS version 9.1.

London cohort data were analysed using SPSS version 15 or STATA version 8.2. The Kolmogorov–Smirnov test of normality was applied. Normally distributed data were expressed as mean and SD, skewed data as median and IQR. Pearson correlation was used to assess parametric data, and Spearman rank was used to assess non-parametric correlations. Wilcoxon and Mann–Whitney U tests were used for paired and unpaired non-parametric tests, respectively.

Differences in lung function decline by genotype in the London cohort were examined using the xtreg command in Stata to construct a random effects (patients) linear regression model, with FEV_1 as the dependent variable, and independent variables of time, genotype and the interaction between genotype and time.

RESULTS

α_1 Genotype, COPD prevalence and lung function decline in the ELSA and Whitehall II studies

The baseline characteristics of the ELSA and Whitehall II patients, by genotype, are reported in table 1.

Table 2 reports that, as expected, 11478G→A carriers were not at increased risk of COPD, in either of the cohorts.

In a multivariable model including smoking and genotype, using Whitehall II data, the mean decline in FEV_1 in patients with the rare A allele was indeed greater than in those without this variant (table 3), and greatest in the A homozygotes (AA/GA/GG decline 76 vs 14 vs 4 ml/year, respectively, p=0.003). Lung function decline did not differ significantly between participants who were and were not 11478A carriers in the ELSA study. There was no interaction with COPD status, and this analysis is therefore reported for all study participants.

Table 1 Baseline participant characteristics and 11478G→A genotype in the ELSA (n=1805) and Whitehall II (n=2733) studies

	GG	GA/AA	p Value
ELSA (wave 0, 2001)			
Men n (%)	724 (46.5%)	112 (45.0%)	0.46
Age (years)	61.6 (61.4 to 62.3)	61.5 (60.4 to 62.6)	0.74
FEV_1 (litres)	2.56 (2.52 to 2.60)	2.56 (2.47 to 2.65)	0.94
FEV_1 (% predicted)	89 (87 to 90)	90 (87 to 92)	0.48
FVC (litres)	3.26 (3.21 to 3.31)	3.26 (3.15 to 3.38)	0.97
Smoking (pack-years)*	19.3 (18.4 to 20.3)	18.6 (16.2 to 21.0)	0.58
BMI (kg m^{-2})	27.7 (27.4 to 27.9)	27.3 (26.6 to 27.9)	0.28
Current smoker %	15.0	15.8	0.58
Whitehall II (phase 7, 2002–2004)			
Men n (%)	1726 (86.6)	648 (87.7)	0.44
Age (years)	61.3 (60.9 to 61.8)	60.9 (60.7 to 61.1)	0.10
FEV_1 (litres)	2.94 (2.86 to 3.01)	2.92 (2.90 to 2.95)	0.59
FEV_1 (% predicted)	77 (76 to 78)	77 (77 to 77)	0.93
FVC (litres)	3.93 (3.85 to 4.02)	3.91 (3.87 to 3.94)	0.57
Smoking (pack-years)	6.72 (6.01 to 7.42)	6.30 (6.02 to 6.58)	0.28
BMI (kg m^{-2})	26.8 (26.4 to 27.1)	26.8 (26.6 to 26.9)	0.96
Current smoker %	13.3	12.2	0.68

Data are expressed as means (95% CI) adjusted for age in linear regression for continuous variables and number (percentage) for categorical variables. Analyses are limited to those participants with lung function data from two time points.
*Calculated in ever smokers.
BMI, body mass index; ELSA, English Longitudinal Study of Ageing; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity.

Table 2 Distribution of 11478G→A genotype frequencies in healthy participants and those with COPD from the ELSA and Whitehall II cohorts

	ELSA COPD (n=199)	ELSA controls (n=1606)	χ^2 p value
GG (wild type)	173 (87%)	1383 (86%)	
GA	26 (13%)	214 (13%)	
AA	0 (0%)	9 (1%)	
HWE p value		0.8170	
	Whitehall COPD (n=153)	**Whitehall controls (n=2580)**	**χ^2 p value**
GG (wild type)	124 (81%)	2250 (87%)	0.22
GA	27 (18%)	321 (12%)	
AA	2 (1%)	9 (0%)	
HWE p value	0.7028	0.4933	

Data are limited to those with complete lung function data at two time points.
ELSA, English Longitudinal Study of Ageing; HWE, Hardy–Weinberg equilibrium.

α_1 Genotype in the London COPD cohort and controls

Two hundred and four patients and 65 control subjects were studied in the London COPD cohort. Their baseline characteristics are reported in table 4. These London patients with physician-confirmed COPD have more severe lung function impairment than the generally healthy subjects enrolled in the ELSA and Whitehall II studies.

Genotype frequencies in the COPD cohort

As in the larger cohorts, there was no difference in the frequency of the rare 11478G→A allele between the patients with COPD and controls. There were also no differences in genotype distribution by GOLD (Global Initiative for Chronic Obstructive Lung Disease) stage, or between COPD frequent and infrequent exacerbators, suggesting that the 11478G→A variant is not associated with increased susceptibility to exacerbation. These data are reported in table 5. Removing carriers of the S and Z variants (n=20 and n=10, respectively) made no difference to the analysis, and therefore all subjects are included in all analyses. None of the patients was homozygous for s and/or z deficiency alleles.

Patient characteristics and 11478G→A genotype in COPD

Baseline FEV_1 and FVC were lower in the GG group compared with those with an A allele; these data are reported in table 6. There were no other differences in any of the baseline characteristics between genotypes in the London cohort.

Table 3 Decline in lung function per year by 11478G→A genotype in Whitehall II and ELSA cohorts

	Whitehall II (n=2721)	ELSA (n=1805)
GG (wild type)	−4 (−8 to −1)	−36 (−44 to −29)
GA	−14 (−23 to −5)	−23 (−49 to −11)
AA	−76 (−127 to −26)	−60 (−166 to −37)
p Value (for trend)	0.003	0.720

ELSA, English Longitudinal Study of Ageing.
Data are expressed as geometric mean (and 95% CI) change in forced expiratory volume in 1 s (ml/year), adjusted for age, sex and smoking status.

Table 4 Baseline characteristics of the London patients with COPD and control subjects: data are expressed as mean (SD) or number (%)

	COPD (n=204)	Control (n=65)	p Value
Age (years)	70.7 (11.1)	66.9 (6.6)	<0.001
FEV_1 (litres)	1.17 (0.51)	2.50 (0.77)	
FEV_1 % predicted	48.2 (19.9)	99.4 (18.6)	
FVC (litres)	2.50 (0.93)	3.22 (1.04)	
BMI (kg m^{-2})	26.0 (5.5)	26.3 (3.8)	0.34
Smoking (pack-years)	51.6 (38.7)	17.4 (22.2)	<0.001
SpO_2 (breathing air)	95 (2)	96 (1)	<0.001
Male	119 (58%)	21 (32%)	<0.001
Current smoker	52 (26%)	12 (18%)	0.12
Frequent exacerbator*	69 (34%)		
GOLD stage 1/2/3/4 (n)	14/83/73/34		

*≥3 exacerbations/year, calculated from the diary cards.
BMI, body mass index; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; GOLD, Global Initiative for Chronic Obstructive Lung Disease.

Lung function decline did not differ significantly by genotype in the London cohort (GG 38.4 vs GA/AA 26.2 ml/year; p=0.061). The model does not include smoking, as smoking status may change with time, but there were no differences in the number of active smokers between the GG and GA/AA groups (table 6). There was also no difference in pack-years by genotype. In the 69 frequent exacerbators, in whom we hypothesised any effect should be greatest, there was also no difference in the rate of FEV_1 decline by genotype (GG 24.2 vs GA/AA 19.8 ml/year; p=0.578).

Serum α_1-antitrypsin concentration by genotype in COPD

There were no differences in serum α_1-antitrypsin concentration, either in the stable state or at exacerbation onset, in patients by 11478G→A genotype. These data are reported in table 7. Table 7 also reports that there was no detectable upregulation of α_1-antitrypsin at exacerbation onset in either genotype, and that changes in α_1-antitrypsin between baseline and exacerbation did not vary by genotype. This is despite significant upregulation of CRP between baseline and exacerbation in both genotypes, and IL-6 in the wild-type (GG) patients. An increase in IL-6 at exacerbation in patients with the A allele, similar in magnitude to that observed in GG subjects, did not reach statistical significance.

Relationships between serum α_1-antitrypsin, IL-6 and CRP concentrations

As the major stimulus to α_1-antitrypsin (and CRP) release is IL-6, we examined correlations between the baseline serum concentrations of α_1-antitrypsin, IL-6 and CRP by genotype, with the results reported in table 8. While IL-6 was correlated with α_1-antitrypsin concentrations in the GG patients, this relationship was not present (suggesting uncoupling) in those with the A allele. In both groups there was a significant relationship between CRP and α_1-antitrypsin. In addition, at exacerbation, while there was a significant relationship between IL-6 and α_1-antitrypsin in the GG patients (r=0.58, p<0.001), this was not present in those carrying the A allele (r=0.29, p=0.355).

Table 5 11478G→A genotype frequencies in control subjects and patients with COPD, and patients with COPD who were frequent and infrequent exacerbators in the London COPD cohort

	COPD (n=204)	Controls (n=65)	χ^2 p value
GG (wild type)	169 (83%)	56 (86%)	0.35
GA	33 (16%)	8 (12%)	
AA	2 (1%)	1 (2%)	
HWE p value	0.78	0.28	
	COPD frequent exacerbators (n=69)	**COPD infrequent exacerbators (n=135)**	**χ^2 p value**
GG (wild type)	59 (86%)	110 (82%)	0.32
GA	9 (15%)	24 (18%)	
AA	1 (2%)	1 (1%)	
HWE p value	0.36	0.80	

COPD, chronic obstructive pulmonary disease; HWE, Hardy–Weinberg equilibrium.

Table 6 Baseline patient characteristics and 11478G→A genotype in COPD; data are expressed as mean (95% CI) or %

	GG	GA/AA	p Value
FEV_1 (litres)	1.14 (1.06 to 1.23)	1.33 (1.16 to 1.50)	0.024
FEV_1 (% predicted)	47.0 (43.9 to 50.1)	54.4 (47.0 to 61.8)	0.069
FVC (litres)	2.43 (2.29 to 2.57)	2.82 (2.47 to 3.17)	0.029
FEV_1/FVC	48% (44 to 50)	48% (44 to 52)	0.974
Smoking (pack-years)	52.3 (45.9 to 58.7)	49.6 (33.4 to 65.9)	0.457
BMI (kgm^{-2})	26.1 (25.1 to 27.0)	26.0 (23.9 to 28.0)	0.782
Current smoker	26%	29%	0.735

BMI, body mass index; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity.

Serum α_1-antitrypsin concentration, exacerbation frequency and exacerbation severity in COPD

There were no differences in serum α_1-antitrypsin concentrations in the stable state, or at exacerbation, between frequent and infrequent exacerbators: stable baseline median 2.00 (1.54–3.63) versus 1.81 (1.32–2.94) g/l (p=0.36) and exacerbation 1.94 (1.38–3.03) versus 2.04 (1.67–2.88) g/l (p=0.56), respectively. The serum α_1-antitrypsin concentration did not vary in the baseline state between patients prescribed less versus greater than the mean daily inhaled corticosteroid dose of 846 μg of beclomethasone equivalent (14 patients not prescribed inhaled corticosteroids were excluded from this analysis): 1.97 (1.34–2.96) versus 2.23 (1.51–3.86) g/l; p=0.177.

There was no difference in symptom duration at exacerbation, change in FEV_1 from baseline to exacerbation, or absolute FEV_1 levels at exacerbation (all estimates of exacerbation severity) or time to the next exacerbation by α_1-antitrypsin genotypes. These data are reported in table 9.

Table 7 Baseline and exacerbation serum α_1-antitrypsin, IL-6 and CRP concentrations by 11478G→A genotype in chronic obstructive pulmonary disease

	GG	GA/AA	p for GG vs GA/AA
Baseline α_1-antitrypsin	1.91 (1.33–3.21)	2.17 (1.54–4.31)	0.58
Exacerbation α_1-antitrypsin	2.01 (1.54–2.99)	1.98 (1.67–2.12)	0.75
p (baseline vs exacerbation)	0.87	0.92	
Change in α_1-antitrypsin	−0.07 (−1.24 to 1.17)	−0.09 (−0.60 to 1.00)	0.90
Baseline CRP	4.0 (2.0–7.0)	2.0 (1.0–4.8)	0.09
Exacerbation CRP	9.0 (4.0–26.5)	5.5 (1.3–74.3)	0.54
p (baseline vs exacerbation)	<0.001	0.03	
Change in CRP	3.0 (0.0–17.5)	2.0 (0.0–79.8)	0.90
Baseline IL-6	3.14 (1.60–6.42)	3.03 (0.61–8.23)	0.52
Exacerbation IL-6	5.27 (2.23–13.5)	5.08 (0.28–55.3)	0.67
p (baseline vs exacerbation)	0.002	0.18	
Change in IL-6	2.03 (−1.2 to 6.9)	4.4 (−5.0 to 58.0)	0.49

α_1-Antitrypsin is expressed as median (IQR) g/l, C-reactive protein (CRP) as mg/l and interleukin 6 (IL-6) as pg/ml. Paired analysis, n=92.

Table 8 Correlations between baseline serum α_1-antitrypsin, and interleukin 6 (IL-6) and C-reactive protein (CRP) by 11478G→A genotype in chronic obstructive pulmonary disease

	GG		GA/AA	
	r	p Value	r	p Value
IL-6 vs α_1-antitrypsin	0.227	0.029	0.132	0.528
CRP vs α_1-antitrypsin	0.172	0.050	0.479	0.021

DISCUSSION

This study was designed to test the hypothesis that patients with COPD and the α_1-antitrypsin *SERPINA1* 11478G→A variant may not sufficiently upregulate serum α_1-antitrypsin at exacerbation, and therefore experience more rapid decline in lung function. Our data do not support this hypothesis.

The strengths of our study include the assessment of both genotype and protein concentration in >200 patients with well characterised COPD, and the analysis of lung function decline by genotype in two separate, large cohorts composed of >4500 participants. We have therefore examined associations across the spectrum of COPD severity.

The history of the 11478G→A variant is complex. First described as a *Taq*I variant in 1985,[10] a subsequent study of 24 patients suggested a higher prevalence in patients with emphysema compared with controls.[20] A higher prevalence in emphysema was also found in further studies,[12 21 22] some of which additionally (and paradoxically) reported that the polymorphism was not associated with differential systemic α_1-antitrypsin concentration or function.[21 22] It is generally accepted that for an α_1-antitrypsin allele to result in clinical disease, serum levels must be <35% of normal values.[8] We report the largest study to date of the 11478G→A variant in COPD. We found no increased risk of COPD in patients with the 11478G→A variant, in three separate cohorts, in keeping with a variant that does not affect serum α_1-antitrypsin protein concentration,[12] a finding also confirmed in this study. That there is no increased risk of COPD in 11478A allele carriers is in agreement with genome-wide and subsequent meta-analysis of studies examining COPD susceptibility genes.[13 23]

In 1992, the polymorphism was sequenced as a G→A change, and found to occur in the 3′UTR of the gene, acting cooperatively with regulatory sequences in the promoter region.[11] The 11478A allele has been associated with decreased gene expression[24] and diminished IL-6-induced α_1-antitrypsin responses in vitro.[14] We provide the first in vivo evidence demonstrating the absence of correlation between serum IL-6 and α_1-antitrypsin acute-phase responses in patients with the 11478G→A variant.

Our primary hypothesis arose from the observation that the 11478G→A polymorphism was associated with accelerated atherosclerosis.[15] Atherosclerosis is associated with stiffening of arteries through degradation of elastin fibres in the arterial wall, a process analogous to the degradation of elastin in airways that results in emphysema and progressive airflow obstruction. Arterial stiffness is known to occur in COPD, independent of any effect on endothelial or fibrinolytic dysfunction.[25] The relationship between α_1-antitrypsin deficiency, blood pressure and cardiovascular risk is complex, as homozygotes for deficiency alleles have lower blood pressure, and even heterozygotes may be protected from ischaemic events.[26] While 11478G→A carriers experienced a more rapid lung function decline in the Whitehall II study, this was not observed in the ELSA subjects or the London cohort, and no effect was observed in COPD 'frequent exacerbators' in whom any effect should be greatest. Genetic studies using more than one cohort often document stochastic variation between populations. All three populations vary in characteristics—one is a clinical cohort, the ELSA subjects are representative of people aged 50 years and older, while the Whitehall II study was originally an occupational cohort. The Whitehall II study is not representative of older age groups as there is evidence of a healthy worker effect. We conclude that the 11478A allele is not associated with accelerated lung function decline in COPD or, if it is, that any effect is small.

Table 9 Clinical indices at exacerbation by 11478G→A genotype in COPD; data expressed as mean (SD) or median (IQR)

	GG	GA/AA	p Value
Exacerbation onset FEV_1 (litres)	1.09 (0.51)	1.19 (0.56)	0.37
Fall in FEV_1 at exacerbation (litres)	0.11 (0.33)	0.09 (0.59)	0.74
Exacerbation length (days)	12.00 (7.00—17.00)	13.50 (9.25—24.00)	0.21
TTNE (days)	95.50 (41.75—221.00)	117.00 (60.50—322.00)	0.31

COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 s; TNE, time to the next exacerbation.

It has been suggested[8] that α_1-antitrypsin concentrations in serum are upregulated during acute-phase responses (such as exacerbations of COPD). It was not previously known whether the 11478G→A variant was associated with an attenuated α_1-antitrypsin response at exacerbation of COPD. Our hypothesis was that wild-type patients would upregulate α_1-antitrypsin at exacerbation, but that this response would be reduced in patients with the 11478G→A allele. Our data do not support this hypothesis either and, indeed, we were not able to detect upregulation of α_1-antitrypsin in wild-type patients. There are a number of possible explanations for this. We considered a problem with our α_1-antitrypsin assay, but the assay standards performed as expected and our median values were within the expected physiological range for α_1-antitrypsin of 1.5—3.0 g/l.[8] We considered a problem with the samples or storage, but we were able to demonstrate upregulation of IL-6 and CRP at exacerbation. A significant relationship between serum CRP and α_1-antitrypsin concentrations has been reported previously,[27] and the presence of this relationship in our data argues against a problem with our samples or assay.

Our finding that there was no upregulation of α_1-antitrypsin at exacerbation of COPD therefore seems robust, and we have reviewed previous reports in this area. There is very little published information on serum α_1-antitrypsin concentration at COPD exacerbation onset. The sputum:serum α_1-antitrypsin ratio has been shown to fall with exacerbation treatment,[28] but we have been unable to locate any reports of paired pre-exacerbation and exacerbation serum α_1-antitrypsin samples. There are data showing that serum α_1-antitrypsin concentrations may be higher at exacerbation than baseline in two small studies; however, one of these included patients with PiZZ α_1-antitrypsin deficiency,[29] and in the other the samples were not paired.[30] There is therefore minimal existing evidence that the systemic α_1-antitrypsin concentration is generally upregulated at exacerbation, and our current results challenge the suggestion that this is true. Indeed, original data suggesting that α_1-antitrypsin is an acute-phase reactant derive from postoperative patients[31] in whom the inflammatory stimulus may have been much greater than at exacerbation of COPD. It is therefore possible that our exacerbations were too mild to result in upregulation of serum α_1-antitrypsin. Although all exacerbations were judged to require treatment with antibiotics and/or

corticosteroids by the attending physician, the median increase in CRP was only between 2 and 3 mg/l. Alternatively, by sampling exacerbations early in the course of the event, we may not have detected the peak of α_1-antitrypsin release, and further work would be required to ascertain the time-course of such responses. The median (IQR) time between symptom onset and sampling in our patients was 3 (0—5) days. These data have relevance to clinical practice in that it may not be necessary[8] to delay screening for α_1-antitrypsin deficiency using protein concentration until after exacerbation, and our data would not support a hypothesis that α_1-antitrypsin augmentation at exacerbation would be necessary for carriers of the 11478A allele.

As described above, our study provides the first in vivo evidence of uncoupling between serum IL-6 and α_1-antitrypsin responses in patients with the 11478G→A variant. Previous data have related serum α_1-antitrypsin concentration to other inflammatory markers (including CRP) in patients on haemodialysis.[27] Interestingly, in patients with the variant allele, serum CRP remained correlated with α_1-antitrypsin, suggesting there may be additional mechanisms associated with α_1-antitrypsin production in these subjects that require further study.

A further important negative finding in our study was that α_1-antitrypsin genotype did not vary by exacerbation frequency. There is increasing evidence that the 'frequent exacerbator' may represent a distinct phenotype,[32] and whilst there has been much interest in COPD susceptibility genes,[23] work examining genetic determinants of exacerbation frequency remains limited despite evidence of familial aggregation.[33] It is plausible that deficiencies in anti-inflammatory and innate host responses may underlie a susceptibility to exacerbation such that otherwise trivial infections result in clinically significant events. We have excluded the 11478G→A variant as such a susceptibility gene. There was also no evidence that exacerbations were more severe in patients with this variant.

In conclusion, our data refute the hypothesis that the 11478G→A α_1-antitrypsin promoter variant results in accelerated lung function decline in COPD. Indeed, we found no evidence to support general upregulation of α_1-antitrypsin during exacerbations. We have provided data reporting that the 11478G→A variant does not increase susceptibility to COPD, or to exacerbation in COPD, and the first in vivo data demonstrating uncoupling of IL-6 and α_1-antitrypsin responses in subjects with the 11478G→A allele.

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Competing interests None.

Ethics approval The London COPD cohort work was conducted with approval of the Royal Free Hospital Ethics Committee.

Contributors All authors contributed to the design and interpretation of data, and have approved the final version of the manuscript. JRH devised the hypothesis for the study. JKQ coordinated the London cohort studies and led the analysis of this data with GCD. The genotyping was performed in the laboratories of PJT. MK led the analysis of the Whitehall and ELSA data.

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