**SERPINA1 11478G→A variant, serum α1-antitrypsin, exacerbation frequency and FEV1 decline in COPD**

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**ABSTRACT**

**Background** The α1-antitrypsin 11478G→A polymorphism may be associated with attenuated acute α1-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 α1-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 α1-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 α1-antitrypsin at exacerbation was not demonstrated, even in patients homozygous for the common allele (median exacerbation change 0.07 g/l 11478G, 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 α1-antitrypsin and serum interleukin 6 (IL-6) concentrations.

**Conclusion** The 11478G→A α1-antitrypsin polymorphism is not associated with increased risk of developing COPD, nor accelerated lung function decline. Serum α1-antitrypsin may not be upregulated early at COPD exacerbation. In patients with the 11478G→A polymorphism there was no relationship between the serum α1-antitrypsin and serum IL-6 concentrations.

**INTRODUCTION**

Chronic obstructive pulmonary disease (COPD) is a prevalent condition characterised by airflow limitation and airway inflamma

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cohorts. Secondly, we repeated this analysis in the London COPD cohort in whom exacerbation frequency data, and serum \( \alpha_1 \)-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.\(^6\)\(^-\)\(^7\)

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 \( \leq 80\% \) predicted, FEV\(_1\)/FVC <0.7 and \( \beta_2 \)-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 \( \alpha_1 \)-antitrypsin assay and DNA extraction for \( \alpha_1 \)-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,\(^1\) inflammatory markers\(^9\) and FEV\(_1\) decline.\(^5\) This enabled categorisation of patients into frequent and infrequent exacerbators, defined as \( \geq 3 \) or <5 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 \( \alpha_1 \)-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 \( \alpha_1 \)-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%; \( \alpha_1 \)-antitrypsin intra-assay 4.5–13.1%; \( \alpha_1 \)-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, Minneapolis, 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 TaqI digestion.\(^1\) Individuals were also genotyped for the SERPINA1 S (E264V) and Z (E542K) variants.
**Statistical analysis**

Observed numbers of each genotype were compared with what expected if the subjects were in Hardy–Weinberg equilibrium. Allele frequencies between the different groups were compared using $\chi^2$ 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$\text{}_{1}$ or FEV$\text{}_{1}$ (% 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 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 xtg command in Stata to construct a random effects (patients) linear regression model, with FEV$\text{}_{1}$ as the dependent variable, and independent variables of time, genotype and the interaction between genotype and time.

**RESULTS**

**$\alpha_1$ 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$\text{}_{1}$ 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, $p=0.005$). 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.

**$\alpha_1$ 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$\text{}_{1}$ 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 2** Distribution of 11478G→A genotype frequencies in healthy participants and those with COPD from the ELSA and Whitehall II cohorts

<table>
<thead>
<tr>
<th></th>
<th>ELSA COPD (n=199)</th>
<th>ELSA controls (n=1606)</th>
<th>$\chi^2$ p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG (wild type)</td>
<td>173 (87%)</td>
<td>1383 (86%)</td>
<td></td>
</tr>
<tr>
<td>GA</td>
<td>26 (13%)</td>
<td>214 (13%)</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>0 (0%)</td>
<td>9 (1%)</td>
<td></td>
</tr>
<tr>
<td>HWE p value</td>
<td>0.8170</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Whitehall COPD (n=153)</th>
<th>Whitehall controls (n=2580)</th>
<th>$\chi^2$ p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG (wild type)</td>
<td>124 (81%)</td>
<td>2250 (87%)</td>
<td>0.22</td>
</tr>
<tr>
<td>GA</td>
<td>27 (18%)</td>
<td>321 (12%)</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>2 (1%)</td>
<td>9 (0%)</td>
<td></td>
</tr>
<tr>
<td>HWE p value</td>
<td>0.7028</td>
<td>0.4933</td>
<td></td>
</tr>
</tbody>
</table>

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

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

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

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.
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. The status may change with time, but there were no differences 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 FEV1 decline by genotype (GG 24.2 vs GA/AA 19.8 ml/year; p=0.578).

Serum α1-antitrypsin concentration by genotype in COPD
There were no differences in serum α1-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 α1-antitrypsin at exacerbation onset in either genotype, and that changes in α1-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 α1-antitrypsin, IL-6 and CRP concentrations
As the major stimulus to α1-antitrypsin (and CRP) release is IL-6, we examined correlations between the baseline serum concentrations of α1-antitrypsin, IL-6 and CRP by genotype, with the results reported in table 8. While IL-6 was correlated with α1-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 α1-antitrypsin. In addition, at exacerbation, while there was a significant relationship between IL-6 and α1-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).

Serum α1-antitrypsin concentration, exacerbation frequency and exacerbation severity in COPD
There were no differences in serum α1-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 (1.52–2.94) g/l (p=0.56) and exacerbation 1.94 (1.38–3.05) versus 2.04 (1.67–2.83) g/l (p=0.56), respectively. The serum α1-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 FEV1 from baseline to exacerbation, or absolute FEV1 levels at exacerbation (all estimates of exacerbation severity) or time to the next exacerbation by α1-antitrypsin genotypes. These data are reported in table 9.

Table 7 Baseline and exacerbation serum α1-antitrypsin, IL-6 and CRP concentrations by 11478G→A genotype in chronic obstructive pulmonary disease

<table>
<thead>
<tr>
<th></th>
<th>GG</th>
<th>GA/AA</th>
<th>p for GG vs GA/AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline α1-antitrypsin</td>
<td>1.91 (1.33–3.21)</td>
<td>2.17 (1.54–4.31)</td>
<td>0.58</td>
</tr>
<tr>
<td>Exacerbation α1-antitrypsin</td>
<td>2.01 (1.54–2.99)</td>
<td>1.98 (1.67–2.12)</td>
<td>0.75</td>
</tr>
<tr>
<td>p (baseline vs exacerbation)</td>
<td>0.87</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>Change in α1-antitrypsin</td>
<td>−0.07 (−1.24 to 1.17)</td>
<td>−0.09 (−0.60 to 1.00)</td>
<td>0.90</td>
</tr>
<tr>
<td>Baseline CRP</td>
<td>4.0 (2.0–7.0)</td>
<td>2.0 (1.0–4.8)</td>
<td>0.09</td>
</tr>
<tr>
<td>Exacerbation CRP</td>
<td>9.0 (4.0–26.5)</td>
<td>5.5 (1.3–74.3)</td>
<td>0.54</td>
</tr>
<tr>
<td>p (baseline vs exacerbation)</td>
<td>&lt;0.001</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Change in CRP</td>
<td>3.0 (0.0–17.5)</td>
<td>2.0 (0.0–79.8)</td>
<td>0.90</td>
</tr>
<tr>
<td>Baseline IL-6</td>
<td>3.14 (1.60–6.42)</td>
<td>3.03 (0.61–8.23)</td>
<td>0.52</td>
</tr>
<tr>
<td>Exacerbation IL-6</td>
<td>5.27 (2.23–13.5)</td>
<td>5.08 (0.28–55.3)</td>
<td>0.67</td>
</tr>
<tr>
<td>p (baseline vs exacerbation)</td>
<td>0.002</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Change in IL-6</td>
<td>2.03 (−1.2 to 6.9)</td>
<td>4.4 (−5.0 to 58.0)</td>
<td>0.49</td>
</tr>
</tbody>
</table>

α1-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.
DISCUSSION

This study was designed to test the hypothesis that patients with COPD and the z1-antitrypsin SERPINA1 11478G→A variant may not sufficiently upregulate serum z1-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 TaqI 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 z1-antitrypsin concentration or function.21 22 It is generally accepted that for an z1-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 z1-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 expression24 and diminished IL-6-induced z1-antitrypsin responses in vitro.14 We provide the first in vivo evidence demonstrating the absence of correlation between serum IL-6 and z1-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 z1-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 ischemic 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.

It has been suggested8 that z1-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 z1-antitrypsin response at exacerbation of COPD. Our hypothesis was that wild-type patients would upregulate z1-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 z1-antitrypsin in wild-type patients. There are a number of possible explanations for this. We considered a problem with our z1-antitrypsin assay, but the assay standards performed as expected and our median values were within the expected physiological range for z1-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 z1-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 z1-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 z1-antitrypsin concentration at COPD exacerbation onset. The sputum:serum z1-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 z1-antitrypsin samples. There are data showing that serum z1-antitrypsin concentrations may be higher at exacerbation than baseline in two small studies; however, one of these included patients with PiZZ z1-antitrypsin deficiency,29 and in the other the samples were not paired.30 There is therefore minimal existing evidence that the systemic z1-antitrypsin concentration is generally upregulated at exacerbation, and our current results challenge the suggestion that this is true. Indeed, original data suggesting that z1-antitrypsin is an acute-phase reactant derive from postoperative patients31 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 z1-antitrypsin. Although all exacerbations were judged to require treatment with antibiotics and/or

Table 8 Correlations between baseline serum z1-antitrypsin, and interleukin 6 (IL-6) and C-reactive protein (CRP) by 11478G→A genotype in chronic obstructive pulmonary disease

<table>
<thead>
<tr>
<th></th>
<th>GG</th>
<th>GA/AA</th>
<th>r</th>
<th>p Value</th>
<th>r</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 vs z1-antitrypsin</td>
<td>0.227</td>
<td>0.029</td>
<td>0.132</td>
<td>0.528</td>
<td>0.172</td>
<td>0.050</td>
</tr>
<tr>
<td>CRP vs z1-antitrypsin</td>
<td>0.172</td>
<td>0.029</td>
<td>0.132</td>
<td>0.528</td>
<td>0.172</td>
<td>0.050</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>GG</th>
<th>GA/AA</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exacerbation onset FEV1 (litres)</td>
<td>1.09 (0.51)</td>
<td>1.19 (0.56)</td>
<td>0.37</td>
</tr>
<tr>
<td>Fall in FEV1 at exacerbation (litres)</td>
<td>0.11 (0.33)</td>
<td>0.09 (0.59)</td>
<td>0.74</td>
</tr>
<tr>
<td>Exacerbation length (days)</td>
<td>12.00 (7.00–17.00)</td>
<td>13.50 (9.25–24.00)</td>
<td>0.21</td>
</tr>
<tr>
<td>TTNE (days)</td>
<td>95.50 (41.75–221.00)</td>
<td>117.00 (60.50–322.00)</td>
<td>0.31</td>
</tr>
</tbody>
</table>

COPD, chronic obstructive pulmonary disease; FEV1, forced expiratory volume in 1 s; TTNE, time to the next exacerbation.
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 α1-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 to delay screening for α1-antitrypsin deficiency using protein concentration until after exacerbation, and our data would not support a hypothesis that α1-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 α1-antitrypsin responses in patients with the 11478C→A variant. Previous data have related serum α1-antitrypsin concentration to other inflammatory markers (including CRP) in patients on haemodialysis.\(^\text{23}\) Interestingly, in patients with the variant allele, serum CRP remained correlated with α1-antitrypsin, suggesting there may be additional mechanisms associated with α1-antitrypsin production in these subjects that require further study.

A further important negative finding in our study was that α1-antitrypsin genotype did not vary by exacerbation frequency. There is increasing evidence that the ‘frequent exacerbator’ may represent a distinct phenotype,\(^\text{52}\) and whilst there has been much interest in COPD susceptibility genes,\(^\text{24}\) work examining genetic determinants of exacerbation frequency remains limited despite evidence of familial aggregation.\(^\text{53}\) 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 11478C→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 11478C→A α1-antitrypsin promoter variant results in accelerated lung function decline in COPD. Indeed, we found no evidence to support general upregulation of α1-antitrypsin during exacerbations. We have provided data reporting that the 11478C→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 α1-antitrypsin responses in subjects with the 11478C→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. JHR 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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