Circulating polymers in α_1-antitrypsin deficiency

To the Editor:

Most individuals carry two wild-type M alleles of the *SERPINA1* gene which encodes α_1-antitrypsin. 95% of severe deficiency of α_1-antitrypsin is associated with the Z allele (Glu342Lys; denoted PiZZ in the homozygote), and with the retention and polymerisation of α_1-antitrypsin within hepatocytes [1]. These polymers are contained within periodic acid–Schiff-positive, diastase-resistant inclusions that are associated with neonatal hepatitis, cirrhosis and hepatocellular carcinoma. The concomitant lack of circulating α_1-antitrypsin predisposes the Z α_1-antitrypsin homozygote to early-onset emphysema. Polymers of α_1-antitrypsin form within the lung as a result of local inflammation and exposure to cigarette smoke [2]. They have also been identified in the skin of an individual with α_1-antitrypsin deficiency and panniculitis [3] and in a renal biopsy from an individual with α_1-antitrypsin deficiency and vasculitis [4]. It is unknown whether these polymers form locally or are deposited in these tissues from a circulating source, and whether extrahepatic polymers are associated with any disease phenotypes. We have assessed whether polymers of α_1-antitrypsin are present within serum, from where they originate, and whether they are associated with clinical features in individuals with PiZZ α_1-antitrypsin deficiency. In this investigation we used ELISA with the anti-α_1-antitrypsin polymer monoclonal antibody (2C1) [5] to assess the presence of polymers in the plasma of 1) 518 individuals with PiZZ α_1-antitrypsin deficiency; 2) an individual with α_1-antitrypsin deficiency who underwent liver transplantation; and 3) 293 individuals with a mixture of α_1-antitrypsin phenotypes. The specificity of the 2C1 antibody was confirmed by using it to immunoprecipitate polymers from the plasma of individuals with and without a positive signal on ELISA (fig. 1a).

Blood samples from 518 PiZZ individuals from the α_1-Antitrypsin Genetic Modifier Study [6] were assessed for the presence of circulating polymers. Spirometry was undertaken according to standardised American Thoracic Society criteria; chest radiography and methacholine challenge tests were not performed. There was no difference in age ($p=0.13$), forced expiratory volume in 1 s (FEV_1) ($p=0.36$), FEV_1/forced vital capacity (FVC) ratio ($p=0.91$) and pack-years of cigarettes smoked ($p=0.24$) between the subjects enrolled in this study and the 372 subjects reported in 2007 [6]. 517 of the 518 PiZZ individuals had quantifiable polymers, with the one polymer-free individual having previously undergone orthotopic liver transplantation. Polymers were present in the augmentation therapy when assessed by ELISA and Western blot analyses and, therefore, the 248 individuals reporting current or past augmentation therapy and the 26 individuals with incomplete demographic data, including the individual who had undergone liver transplantation, were excluded from further analysis. Circulating polymers were in the range 8.2–230.2 $\mu g \cdot mL^{-1}$ in the remaining 244 individuals with PiZZ α_1-antitrypsin deficiency. The mean$\pm$SD concentration was 36.3 ± 33.3 $\mu g \cdot mL^{-1}$, with higher levels in males (mean 42.8 $\mu g \cdot mL^{-1}$, range 8.2–230.2 $\mu g \cdot mL^{-1}$) compared with females (mean 32.2 $\mu g \cdot mL^{-1}$, range 8.2–183.0 $\mu g \cdot mL^{-1}$; $p=0.02$) and in subjects with COPD (42.6 $\mu g \cdot mL^{-1}$ *versus* 32.5 $\mu g \cdot mL^{-1}$; $p=0.02$). Univariate analysis revealed an association between polymer concentration and FEV_1/FVC ratio (-0.411, SE 0.116; $p<0.0005$) but no association between concentration and a history of ever-smoking, pack-years of smoking or age started smoking. Each unit increase in log-transformed polymer level was associated with higher odds for chronic obstructive pulmonary disease (COPD) (OR 3.6, 95% CI 1.4–9.1). The mean$\pm$SD plasma α_1-antitrypsin in 233 out of the 244 individuals with PiZZ α_1-antitrypsin deficiency from whom measurement was obtained was 0.26 ± 0.08 $mg \cdot mL^{-1}$, with a correlation between the concentration of circulating polymer and total α_1-antitrypsin ($r=0.41$, $p<0.05$). Total α_1-antitrypsin levels in individuals with and without COPD were 0.29 and 0.24 $mg \cdot mL^{-1}$, respectively, with the proportion of polymers being 14.8% and 13.3%, respectively, for the two groups. This cohort was not designed to assess liver disease and so any associations must be considered to be exploratory. Nevertheless, those individuals who self-reported abnormal liver function, liver disease or cirrhosis had higher polymer levels (as well as higher proportions of polymer to total α_1-antitrypsin) than those without a self-report of these conditions.

Further clarity was sought on the origin of circulating polymers using serial samples from a 54-year-old male with PiZZ α_1-antitrypsin deficiency undergoing orthotopic liver transplantation. He had a 12-month history of peripheral oedema, liver cirrhosis, portal hypertension, gastro-oesophageal varices and normal lung function. After his condition deteriorated and he developed episodes of encephalopathy, he was

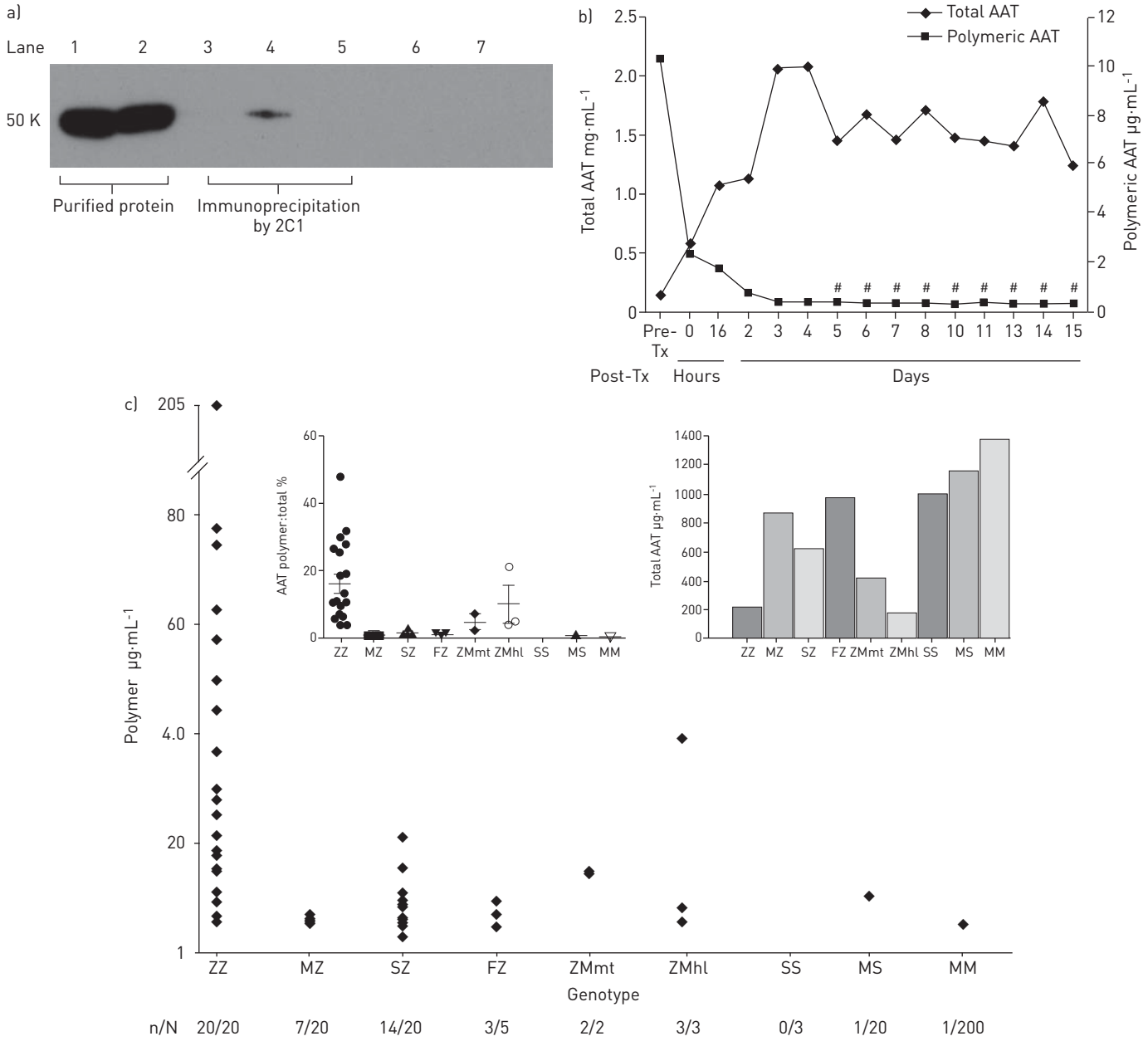

FIGURE 1 Detection of α_1-antitrypsin (AAT) polymers in plasma. a) The specificity of the 2C1 antibody was confirmed by using it to immunoprecipitate polymers from the plasma of individuals with (lane 4) and without (lane 3) a positive signal on ELISA. Proteins were transferred from sodium dodecyl sulfate gel to the membrane and probed using an anti-AAT rabbit polyclonal antibody. Lane 1 and 2 are positive controls of purified M and Z α_1-antitrypsin; three negative controls include immunoprecipitation with no added plasma (lane 5) and blotting of sepharose G beads (lane 6) and 2C1 antibody (lane 7). b) Serum AAT was quantified using sandwich ELISA. Z-AAT polymers are present in the circulation and are cleared following liver transplantation. Mouse monoclonal antibodies produced by our group that detect all conformers of AAT, or only polymeric AAT (2C1 [5]), were used to quantify total AAT and AAT polymers, respectively. c) Concentration of serum AAT polymers in 293 individuals with a mixture of AAT genotypes. The assay is 100% sensitive and 89% specific in PiZZ homozygotes in mixed genotypes. Insets: the total level of AAT and the proportion of circulating polymers for each genotype. n/N represents the number (n) of the total (N) with detectable levels of circulating polymers. ZMmt: ZMmalton; ZMhl: ZMheerlen. #: concentration below the lower limit of quantification (0.4 µg·mL^{-1}).

admitted for orthotopic liver transplantation. The procedure was prolonged by a large portal vein thrombus and the patient developed reperfusion injury with hyperkalaemia that necessitated intraoperative haemofiltration. His post-operative care was uneventful. Analysis of blood samples showed that plasma levels of α_1-antitrypsin were initially low but rose from 0.2 mg·mL^{-1} to 2.1 mg·mL^{-1} following hepatic transplantation. Circulating α_1-antitrypsin polymers were detected prior to transplantation, but fell rapidly following the procedure (fig. 1b). This may, in part, relate to the requirement for intraoperative haemofiltration, but the levels continued to fall in the post-operative phase. Fitting the post-operative

decline to an exponential decay function allowed an estimate of the half-life of circulating polymers to be 30 h. This figure should be treated with some caution due to the requirement for intraoperative haemofiltration and blood products (which contain α_1-antitrypsin). However, it is significantly shorter than the half-life of monomeric α_1-antitrypsin (4–5 days), suggesting that polymers are cleared from the circulation by a different mechanism. The level of circulating polymers became undetectable after 4 days (lower limit of quantification of polymers 0.4 $\mu g \cdot mL^{-1}$), demonstrating that they arise from Z α_1-antitrypsin synthesised within the liver. Indeed, although α_1-antitrypsin is also secreted from lung epithelial cells [7], the 11 PiZZ individuals who had undergone lung transplantation still had circulating polymers (data not shown), thereby reinforcing the hypothesis that serum polymers arise from α_1-antitrypsin produced within the liver.

Finally, a cohort of 293 individuals with mixed α_1-antitrypsin phenotypes was used to establish whether the presence of circulating polymers could identify individuals with α_1-antitrypsin deficiency. The cohort originated from the Alpha-1 Foundation DNA and Tissue Bank (Coral Gables, FL, USA), and consisted of a mix of α_1-antitrypsin phenotypes (N): MM (200), MZ (20), ZZ (20), SZ (20), FZ (5), SS (3), MS (20), ZMheerlen (3) and ZMmalton (2). No individual was receiving α_1-antitrypsin augmentation therapy. Results showed that the presence of circulating polymers was 100% sensitive and 89% specific in detecting 20 PiZZ α_1-antitrypsin homozygotes in a mix of 293 genotypes (fig. 1c). Polymers were also detected in individuals who were heterozygous for Z and another allele (M, S, Mmalton and Mheerlen). The presence of circulating polymers was 70% sensitive and 99% specific in identifying the Z allele (PiXZ where X is any allele other than Z). A low signal was detected in one out of 200 individuals with a normal α_1-antitrypsin phenotype (PiMM), but in none of the three SS homozygotes, who have mild α_1-antitrypsin deficiency. We have shown that other severe deficiency alleles, including Mmalton (52Phe deleted) α_1-antitrypsin, also form polymers [8]. Similarly, the mild S deficiency allele (Glu264Val) forms polymers [9], but at a slower rate, in keeping with less retention in the liver and milder plasma deficiency. This is consistent with the absence of circulating polymers in the three PiSS α_1-antitrypsin individuals in this study. The epitope recognised by the 2C1 antipolymer antibody is unknown, but *in vitro* studies have shown it to be polymer specific, irrespective of the underlying mutation in the α_1-antitrypsin gene [5]. It will therefore detect circulating polymers generated from other polymer-forming alleles. This may represent a useful screening test for severe α_1-antitrypsin deficiency.

Taken together, our data show that circulating polymers are present in all individuals with PiZZ α_1-antitrypsin deficiency and that they originate from the liver. Exploratory data suggest a possible association between circulating polymer concentration and lung and liver disease. Further studies are now required to establish the temporal stability of circulating polymers and whether this biomarker is useful in predicting clinically important outcomes in individuals with PiZZ α_1-antitrypsin deficiency. However, circulating polymers will be useful to monitor the efficacy of small molecules designed to block polymerisation, should the current lead molecules progress to clinical trials [10].

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Circulating polymers of α_1-AT are present in all individuals with PiZZ α_1-AT deficiency and are associated with COPD http://ow.ly/tP8uT

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Impact of theophylline/corticosteroid combination therapy on sputum hydrogen sulfide levels in patients with COPD

To the Editor:

Hydrogen sulfide (H_2S) has emerged as a new and important endogenous regulator of inflammation in recent years [1] and may also protect from emphysema induced by cigarette smoke exposure [2]. We have also recently shown that H_2S can inhibit airway smooth muscle cell proliferation and inflammatory mediator release *in vitro* [3]. Serum levels of H_2S positively correlate with the decline in lung function in chronic obstructive pulmonary disease (COPD) and were significantly lower in Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage III patients compared with those in GOLD I [4]. Existing therapies for COPD, such as corticosteroids or long-acting anticholinergic agents, may reduce the exacerbation rate but do not significantly slow disease progression. A previous study has shown that theophylline alone had no impact on serum H_2S levels and is of limited value in the management of stable COPD [5]. Interestingly, sputum H_2S measured in patients with asthma correlated with sputum neutrophil counts and the degree of airflow obstruction measured by forced expiratory volume in 1 s (FEV1) % predicted [6]. Moreover, combination therapy of an inhaled glucocorticoid with low-dose theophylline has been shown to attenuate airway inflammation in patients with COPD and reverse glucocorticoid resistance [7]. We therefore investigated whether the combination of inhaled corticosteroid and low-dose theophylline, as opposed to low-dose theophylline alone, would modulate H_2S levels in the lungs of COPD patients. We now report the levels of H_2S assayed in sputum samples collected during this study (www.clinicaltrials.gov identifier NCT00241631), details of which have already been published [7].