Circulating polymers in α₁-antitrypsin deficiency

To the Editor:

Most individuals carry two wild-type M alleles of the SERPINA1 gene which encodes α₁-antitrypsin. 95% of severe deficiency of α₁-antitrypsin is associated with the Z allele (Glu342Lys; denoted PiZZ in the homozygote), and with the retention and polymerisation of α₁-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 α₁-antitrypsin predisposes the Z α₁-antitrypsin homozygote to early-onset emphysema. Polymers of α₁-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 α₁-antitrypsin deficiency and panniculitis [3] and in a renal biopsy from an individual with α₁-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 extrahaepatic polymers are associated with any disease phenotypes. We have assessed whether polymers of α₁-antitrypsin are present within serum, from where they originate, and whether they are associated with clinical features in individuals with PiZZ α₁-antitrypsin deficiency. In this investigation we used ELISA with the anti-α₁-antitrypsin polymer monoclonal antibody (2C1) [5] to assess the presence of polymers in the plasma of 1) 518 individuals with PiZZ α₁-antitrypsin deficiency; 2) an individual with α₁-antitrypsin deficiency who underwent liver transplantation; and 3) 293 individuals with a mixture of α₁-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 α₁-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 (FEV1) (p=0.36), FEV1/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 were excluded from further analysis. Circulating polymers were in the range 8.2–230.2 μg·mL⁻¹ in the remaining 244 individuals with PiZZ α₁-antitrypsin deficiency. The mean ± SD concentration was 36.3 ± 33.3 μg·mL⁻¹, with higher levels in males (mean 42.8 μg·mL⁻¹, range 8.2–230.2 μg·mL⁻¹) compared with females (mean 32.2 μg·mL⁻¹, range 8.2–183.0 μg·mL⁻¹; p=0.02) and in subjects with COPD (42.6 μg·mL⁻¹ versus 32.5 μg·mL⁻¹; p=0.02). Univariate analysis revealed an association between polymer concentration and FEV1/FVC ratio (r=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 ± SD plasma α₁-antitrypsin in 233 out of the 244 individuals with PiZZ α₁-antitrypsin deficiency from whom measurement was obtained was 0.26 ± 0.08 mg·mL⁻¹, with a correlation between the concentration of circulating polymer and total α₁-antitrypsin (r=0.41, p<0.05). Total α₁-antitrypsin levels in individuals with and without COPD were 0.29 and 0.24 mg·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 α₁-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 α₁-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
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 α1-antitrypsin were initially low but rose from 0.2 mg·mL$^{-1}$ to 2.1 mg·mL$^{-1}$ following hepatic transplantation. Circulating α1-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
have shown that other severe deficiency alleles, including Mmalton (52Phe deleted) produce within the liver.Indeed, although α1-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 α1-antitrypsin produced within the liver.

Finally, a cohort of 293 individuals with mixed α1-antitrypsin phenotypes was used to establish whether the presence of circulating polymers could identify individuals with α1-antitrypsin deficiency. The cohort originated from the Alpha-1 Foundation DNA and Tissue Bank (Coral Gables, FL, USA), and consisted of a mix of α1-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 α1-antitrypsin augmentation therapy. Results showed that the presence of circulating polymers was 100% sensitive and 89% specific in detecting 20 PiZZ α1-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 α1-antitrypsin phenotype (PiMM), but in none of the three SS homozygotes, who have mild α1-antitrypsin deficiency. We have shown that other severe deficiency alleles, including Mmalton (52Phe deleted) α1-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 α1-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 α1-antitrypsin gene [5]. It will therefore detect circulating polymers generated from other polymer-forming alleles. This may represent a useful screening test for severe α1-antitrypsin deficiency.

Taken together, our data show that circulating polymers are present in all individuals with PiZZ α1-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 clinical outcomes in individuals with PiZZ α1-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].

Circulating polymers of α1-AT are present in all individuals with PiZZ α1-AT deficiency and are associated with COPD http://ow.ly/tP8uT

Lu Tan1,18, Jennifer A. Dickens1,18, Dawn L. DeMeco2,3,18, Elena Miranda1, Juan Perez2, S. Tamir Rashid1, James Day1, Adriana Ordoñez1, Stefan J. Marciniak1, Imran Haq1, Alan F. Barker6, Edward J. Campbell7,8, Edward Eden9, Noel G. McElvaney10, Stephen I. Rennard11, Robert A. Sandhaus12, James M. Stocks13, James K. Stoller14, Charlie Strange15, Gerard Turino6, Farshid N. Rouhani16, Mark Brantly16 and David A. Lomas17

1Dept of Medicine, University of Cambridge, Cambridge Institute for Medical Research, Cambridge, and 2Division of Medicine, University College London, London, UK. 3Channing Division of Network Medicine, Dept of Medicine, Brigham and Women’s Hospital, Boston, MA, 4Division of Pulmonary and Critical Care Medicine, Dept of Medicine, Brigham and Women’s Hospital, Boston, MA, 5Dept of Medicine, School of Medicine, Oregon Health and Science University, Portland, OR, 6Intermountain Health Care, Provo, UT, 7HerediLab Inc., Salt Lake City, UT, 8St Luke’s–Roosevelt Hospital, New York, NY, 9Dept of Pulmonary and Critical Care Medicine, University of Nebraska Medical Center, Omaha, NE, 10National Jewish Health, Denver, CO, 11University of Texas Health Science Center at Tyler, Tyler, TX, 12Cleveland Clinic, Cleveland, OH, 13Dept of Medicine, College of Medicine, Medical University of South Carolina, Charleston, SC, and 14Dept of Medicine, College of Medicine, University of Florida, Gainesville, FL, USA. 15Dept of Biology and Biotechnology “Charles Darwin”. Pasteur Institute – Cenci Bolognetti Foundation, University of Rome La Sapienza, Rome, Italy. 16Dept of Cell Biology, Genetics and Physiology, University of Málaga, Málaga Spain. 17Beaumont Hospital, Dublin, Ireland. 18These authors contributed equally.

Correspondence: D.A. Lomas, Division of Medicine, University College London, 1st Floor Maple House, 149 Tottenham Court Road, London, W1T 7NF, UK. E-mail: d.lomas@ucl.ac.uk

Received: June 29 2013 | Accepted after revision: Nov 25 2013
Support statement: L. Tan is supported by the Cambridge National Institute for Health Research (NIHR) Biomedical Research Centre; J.A. Dickens is a Medical Research Council (MRC) clinical training fellow and recipient of a Sackler Studentship; D.L. DeMeo was supported by a Doris Duke Clinical Scientist Career Development Award; S.J. Marciniak is a MRC senior clinical fellow; and I. Haq is supported by GlaxoSmithKline. This work was funded by the MRC (UK) and the NIHR University College London Biomedical Research Centre.

Conflict of interest: Disclosures can be found alongside the online version of this article at www.erj.ersjournals.com

Acknowledgements: The authors would like to thank all the participants in the α1-Antitrypsin Genetic Modifier Study for their enthusiastic support and participation. We would also like to thank E. Silverman (Boston, MA, USA) for his comments on the manuscript and R. Crapo (Salt Lake City, UT, USA) for his review and quality assurance of a subset of the spirometry data.

References