association of premature emphysema in ZZ homozygotes who smoke.

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The Z (Glu342Lys) variant of α_1-antitrypsin is common in populations of North European descent. The mutation causes individual α_1-antitrypsin molecules to assemble into polymer chains in the endoplasmic reticulum of hepatocytes. Z homozygotes (PiZZ) have circulating levels of α_1-antitrypsin $\sim$15% of normal and are predisposed to hepatic cirrhosis and severe, early-onset emphysema. The risk of clinically significant disease associated with the heterozygote PiMZ state is minimal. We describe a case phenotyped as PiZZ during family screening, but with surprisingly preserved circulating α_1-antitrypsin levels. Genotyping revealed compound heterozygosity for the Z mutation and a novel, "pseudo-Z" mutation. Biochemical and ion-mobility mass spectrometry characterisation of pseudo-Z α_1-antitrypsin showed that it readily populated a polymerogenic intermediate state under physiological conditions. Cell biological studies of a series of α_1-antitrypsin variants indicated these effects involved disruption of a hydrogen bond stabilising the F-helix-linker region of the protein structure. These data strongly support the hypothesis that stability of this region co-regulates formation of the polymerogenic intermediate. Whilst the intermediate form of pseudo-Z α_1-antitrypsin is more stable than that of the true Z variant, the resultant polymers all share a characteristic neoepitope. Pseudo-Z α_1-antitrypsin is thus a useful model for in vitro screening of potential lead compounds to bind the polymerogenic intermediate state, improving the ability to develop novel therapies to treat α_1-antitrypsin deficiency. Our data predict that the likelihood of severe disease in the PiZ/Pseudo-Z compound heterozygote state will be increased relative to the PiMZ state, but far lower than for PiZZ individuals.

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Pathogenic mutations in the gene for α_1-antitrypsin predispose to aberrant conformational transitions of the translated protein molecules resulting in their self-association to form polymer chains. Polymerisation causes circulating deficiency of α_1-antitrypsin while predisposing to hepatic cirrhosis and severe, early-onset emphysema. Targeting the conformational transitions underlying polymerisation via ligand binding and stabilisation of the physiological native state is therefore a goal of drug design in α_1-antitrypsin deficiency. To complement previous structure-led approaches we have developed NMR spectroscopy and nanospray mass spectrometry as medium-throughput screening tools for such ligands. The coupling of these techniques combines highly sensitive detection of ligand binding with assessment of binding sites, stoichiometry, cooperativity and binding constants. We have used the TTAI peptide, developed within an existing programme of drug design, as a test case. The data demonstrate highly co-operative, slow, tight binding of two copies of the peptide in adjacent parts of the α_1-antitrypsin molecule. TTAI peptide binding is shown to induce widespread conformational change all over the molecule with the exception of β-sheet C. These data prove the utility of NMR spectroscopy and nanospray mass spectrometry in characterising ligand binding whilst providing a highly detailed template for use in specific screening for TTAI peptide-mimetic compounds.

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Chronic Obstructive Pulmonary Disease (COPD), even due to α-1-antitrypsin deficiency (A1AD), is recognised as having distinct radiological, physiological and clinical phenotypes. Little is known about disease progression in physiologically defined phenotypes.[1] We have identified a subgroup of patients with a reduced FEV_1 but normal gas transfer determined by the lower limit of normal (LLN), that is, with standardised residual (SR) value < -1.645. The current abstract reports the progression of these physiological measures with time in this subgroup.

Methods 533 patients with A1AD were studied of whom 43 had isolated FEV_1 abnormality at baseline and also had $\geq$3 years of complete annual follow-up data. These patients were followed for a mean of 5.9 (2.2 SD) years. Of these, 22 remained with isolated FEV_1 abnormality (Group A) whereas 21 had developed evidence of a reduced K,co deficiency (Group B). Group A and B data at baseline and at last follow-up were compared—see Abstract S64 Table 1.

Abstract S64 Table 1

	Group A Mean (SD) n=22	Group B Mean (SD) n=21
Age	53.1 (8.6)	50.4 (9.2)
Males (%)	91	71
Pack year history	20.0 (15.7)	18.1 (18.2)
Total SGRQ	56.5 (16.8)	40.8 (11.3)*
Follow-up, years	5.7 (2.5)	6.0 (1.9)
Baseline FEV_1 (L)	1.53 (0.5)	1.44 (0.5)
Baseline FEV_1 SR	−3.83 (1.1)	−3.87 (1.1)
Last FEV_1 SR	−3.95 (0.9)	−4.04 (1.1)
FEV_1 change (L/year)	−0.038 (0.03)	−0.036 (0.05)
Baseline K,co (mmol/min/kPa/L)	1.38 (0.2)	1.34 (0.2)
Baseline K,co SR	−0.53 (0.5)	−1.01 (0.5)**
Last K,co SR	−0.95 (0.7)	−2.19 (0.6)*
K,co change (mmol/min/kPa/L/year)	−0.034 (0.02)	−0.061 (0.03)**

*$p <= 0.001$, **$p <= 0.005$.

Results There were no differences in FEV_1, smoking, age or sex distribution between the groups. At baseline mean K,co SR was worse in Group B compared to Group A. However, Group A had significantly worse total Saint George's Respiratory questionnaire