**Results** *Pilot study*: There was an exponential relationship between Aα-Val$^{360}$ and the A1AT concentration consistent with theoretical modelling and a negative correlation with FEV1 in the PiZ subjects (r=−0.321, p=0.005). *COPD study (Normal A1AT levels)*: Aα-Val$^{360}$ was greater in subjects with visible emphysema compared to those without (median 21.77 vs 16.98; p=0.013) and correlated well with both physiology and densitometry (Abstract S59 Table 1). Aα-Val$^{360}$ was significantly higher in subjects experiencing a purulent versus non-purulent exacerbation (day 1 median 26.29 vs 21.22; p=0.03), and although values fell, the difference persisted even in the stable state (21.89 vs 17.01; p=0.002). Aα-Val$^{360}$ was also higher on day 1 than in the stable state (23.72 vs 21.28; p=0.005) even when stratified into non-purulent (21.22 vs 20.00; p=0.022) or purulent subgroups (26.29 vs 21.83; p=0.043).

Abstract S59 Table 1 The correlation (r) and its significance (p) between stable state plasma Aα-Val360, physiology and HRCT densitometry

| | r | p |
|---|---|---|
| Body mass index | −0.215 | 0.091 |
| Age | 0.199 | 0.037 |
| FEV1 (% Predicted) | −0.340 | 0.001 |
| KCO (% Predicted) | −0.215 | 0.027 |
| TLCO (% Predicted) | −0.310 | 0.002 |
| Upper Zone Voxel Index | 0.401 | <0.001 |
| Lower Zone Voxel Index | 0.340 | 0.001 |

**Conclusion** Aα-Val$^{360}$ correlates well with physiological and radiological markers of COPD disease severity (in subjects with and without A1AT deficiency) and increases during exacerbations (particularly in those with purulent sputum), both supporting the pathophysiological importance of elastase and demonstrating the potential of Aα-Val$^{360}$ as a valid biomarker in COPD. Further work is required to relate Aα-Val$^{360}$ to longitudinal measures of disease progression.

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## S60 CIGARETTE SMOKE INDUCED OXIDATION OF α-1 ANTITRYPSIN AMPLIFIES THE PULMONARY INFLAMMATORY RESPONSE

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Alpha-1 antitrypsin (AT) is the major elastase inhibitor within the lung. Oxidation of critical methionine residues in AT (Ox-AT) has diminished ability to inhibit neutrophil elastase, which is thought to contribute to the pathogenesis of COPD. Ox-AT may also be pro-inflammatory. We investigated whether cigarette smoke would promote production of Ox-AT and an exaggerated inflammatory response. Adult female transgenic mice for human M-AT and wild type CBA mice (n=9 per group) were exposed to cigarette smoke (CS) from 1R3F research grade cigarettes for 5 days and killed 1 day later. Control mice were exposed to air. Ox-AT and inflammatory chemokines were assessed in BALF and lung homogenates (LH) by ELISA and Western blot. Ox-AT was not detected in control M-AT mice nor CS-CBA mice, but was significantly increased in BALF, 72.3 ng/ml (SEM±11.7), p=0.017 and LH, 1351.3 (±111.6) p=<0.001 of CS-M-AT mice. This was confirmed on western blot of SDS-PAGE using a monoclonal antibody to Ox-AT. There was a significant increase in BAL polymorphonuclear cells (1.53($10^4$) (±0.02) vs 0.16 ($10^4$) (±0.04) p=0.022) and macrophages (16.36 ($10^4$) (±0.69) vs 10.19($10^4$) (±1.94) p=0.008) in CS-M-AT mice compared with CS-CBA mice. There was significantly greater MCP-1 and KC in CS-M-AT vs CS-CBA; BALF, MCP-1 521.35 pg/ml (±46.7) vs 264.63 (±17.65), respectively; p=0.006, and KC 440.5 pg/ml (±53) vs 171.4 (±17), p=0.024. In LH, CS-M-AT MCP-1, 779.6 (±55) vs CS-CBA 368.8 (±30) (pg/ml) p=0.003, and CS-M-AT KC 466.1 (±67) vs 250.9 (±14), p=0.003. Similarly there was significantly increased NF-κB (p=0.015) and AP-1 (p=0.015) activity in CS-M-AT lungs compared with CS-CBA lungs. These findings demonstrate that oxidation of methionines in AT by oxidants released from cigarette smoke not only reduces the anti-elastase lung protection but converts AT into a pro-inflammatory stimulus. Ox-AT generated in the airway interacts directly with epithelial cells to release MCP-1 and IL-8, so enhancing lung inflammation. This mechanism could potentially contribute to the abnormal inflammatory response seen in COPD.

## S61 CIGARETTE SMOKE PROMOTES POLYMERISATION OF Z α1-ANTITRYPSIN

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Alpha-1 antitrypsin (AT) is an important inhibitor of neutrophil elastase (NE). Z antitrypsin (Glu342Lys) (Z-AT) polymerises within the hepatocyte and the subsequent severe plasma deficiency exposes the lungs to uncontrolled elastolysis and premature emphysema. We have shown that polymeric Z-AT (pZ-AT) are found in emphysematous alveolar walls and are co-localised with neutrophils. pZ-AT does not inhibit NE and are also pro-inflammatory and chemotactic to neutrophils, suggesting a novel role for pZ-AT in Z-AT related emphysema. Cigarette smoking (CS) accelerates decline in lung function in Z-AT homozygotes, but the mechanism involved in this is unknown. We investigated whether CS exposure would induce formation of pZ-AT. Female transgenic mice for human M-AT and Z-AT were exposed to four 1R3F research cigarettes daily for 5 days. BALF and perfused lungs were subsequently collected. Concentrations of pAT and oxidised AT were assessed by ELISA and immunoblot. Neutrophil numbers were assessed by quantifying stained cytospins and neutrophil elastase activity of lung homogenates (LH). pAT was undetectable in non-CS Z or CS-M mice. Polymeric AT was markedly increased in BALF and LH in CS-Z mice; BALF CS-Z 141 (146–114) ng/ml; p=0.001 and LH, 232.5(241.1–218.6) ng/lung, p=0.001. Immunoblot of BALF demonstrated the classical ladders of pATin CS-Z mice.BALF and LH of CS-Z mice had higher neutrophil numbers compared with CS-M mice; NE LH, CS-Z 49(50–45) ng/lung vs CS-M mice 21(25–18); p<0.001. Neutrophil numbers in the lung were tightly correlated with polymer concentrations; correlation coefficient, $r^2$=0.93; p=<0.001. Incubation of plasma purified Z-AT with CS extract (CSE) demonstrated that CSE oxidises Z-AT leading to an accelerated rate of polymerisation; CSE+Z, 114.4 nM/h, Z control 10.3; p<0.001. This was confirmed by the finding that CS-induced polymerisation could be abolished by the antioxidant N-acetyl cysteine CSE+NAC +Z-AT 13.3; p=0.135 vs control. In conclusion, acute CS exposure directly promotes polymerisation of Z-AT via oxidation. The production of pZ-AT further reduces the anti-proteinase protection and attracts neutrophils potentially hastening lung damage. These novel findings provide a molecular explanation for the striking

association of premature emphysema in ZZ homozygotes who smoke.

## S62 CHARACTERISATION OF A NOVEL "PSEUDO-Z" VARIANT OF α1-ANTITRYPSIN

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The Z (Glu342Lys) variant of $\alpha_1$-antitrypsin is common in populations of North European descent. The mutation causes individual $\alpha_1$-antitrypsin molecules to assemble into polymer chains in the endoplasmic reticulum of hepatocytes. Z homozygotes (PiZZ) have circulating levels of $\alpha_1$-antitrypsin ~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 $\alpha_1$-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 $\alpha_1$-antitrypsin showed that it readily populated a polymerogenic intermediate state under physiological conditions. Cell biological studies of a series of $\alpha_1$-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 $\alpha_1$-antitrypsin is more stable than that of the true Z variant, the resultant polymers all share a characteristic neoepitope. Pseudo-Z $\alpha_1$-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 $\alpha_1$-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.

## S63 USE OF NMR SPECTROSCOPY AND NANOSPRAY MASS SPECTROMETRY TO CHARACTERISE BINDING OF LEAD COMPOUNDS FOR DRUG DESIGN IN α1-ANTITRYPSIN DEFICIENCY

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Pathogenic mutations in the gene for $\alpha_1$-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 $\alpha_1$-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 $\alpha_1$-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 $\alpha_1$-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.

## S64 CHANGES IN PHYSIOLOGICAL PHENOTYPES OF α-1-ANTITRYPSIN DEFICIENCY WITH TIME

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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 ≥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