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.

REFERENCES

1. *Proc Am Thorac Soc* 2009;**6**:524–6.
2. *Am Rev Respir Dis* 1979;**120**:1081–6.
3. *J Clin Invest* 1999;**104**:337–44.

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

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S Alam, Z Li, R Mahadeva. *University of Cambridge, Cambridge, UK*

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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S Alam, Z Li, S Janciauskiene, R Mahadeva. *University of Cambridge, Cambridge, UK*

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