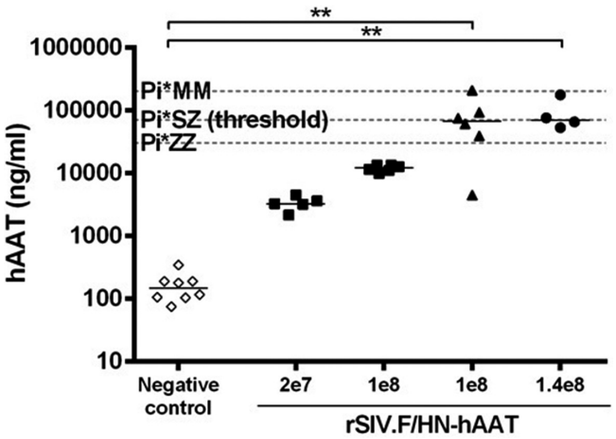
REFERENCE

1 Chapman KR, Burdon JG, Piitulainen E *et al*. Intravenous augmentation treatment and lung density in severe α1 antitrypsin deficiency (RAPID): a randomised, double-blind, placebo-controlled trial. *Lancet*. 2015;**386**:360–8



Abstract S127 Figure 1 Expression of hAAT in epithelial lining fluid following treatment with rSIV. F/HN-hAAT. Mice were given between 2e7 and 1.4e8 TU virus and sacrificed 7–10 days post-treatment

S128 SOLUBLE ADAM33 CAUSES AIRWAY REMODELLING TO PROMOTE ALLERGIC AIRWAY INFLAMMATION

[1]ER Davies, [2]JA Whitsett, [1]DE Davies, [1]HM Haitchi. *[1]University of Southampton, Southampton, UK; [2]Cincinnati Children's Hospital Medical Center, Cincinnati, USA*

10.1136/thoraxjnl-2015-207770.134

Introduction and objectives *ADAM33* is an asthma susceptibility gene associated with bronchial hyperresponsiveness (BHR). It encodes a membrane-anchored protein with metalloprotease (MP) activity whose ectodomain can be shed from the cell surface as a soluble protein (sADAM33-MP). sADAM33-MP levels are increased in asthmatic airways and inversely correlated with FEV1. We have previously generated a pulmonary epithelium-specific, doxycycline (DOX)-inducible double transgenic (dTg) mouse expressing human (h)sADAM33-MP and found that the transgene caused airway remodelling in the absence of inflammation or BHR. Therefore, as asthma involves gene-environment interactions, we postulated that there is a synergistic relationship between ADAM33-remodelled airways and responses to the common aeroallergen, house dust mite (HDM).

Methods DOX was administered to dTg mice to induce hsADAM33 expression and airway remodelling for up to 6 weeks; single transgenic (sTg) littermate controls were similarly treated. Mice were then sensitised to HDM and challenged with HDM or saline. Airway resistance was measured in response to increasing concentrations of methacholine using the forced oscillation technique in anesthetised mice. Inflammatory cell counts were performed on bronchoalveolar lavage fluid (BALF) and indices of inflammation measured by RTqPCR and Luminex ELISA.

Results We first performed a concentration-response experiment with HDM extract with a standard sensitisation protocol to determine the amount of HDM extract (6.25 μg), which elicited minimal BHR and eosinophilia. This low-dose allergen challenge protocol was then applied to dTg *Ccsp*/*ADAM33* and sTg control mice. Allergen challenge of dTg mice resulted in a significant increase in methacholine-induced airway resistance and eosinophilic airway inflammation compared to HDM-challenged sTg controls. The dTg mice also showed a significant increase in airway inflammatory mediators IL-5, IL-13 and eotaxin, in addition to markers of remodelling.

Conclusions This study demonstrates that hsADAM33-MP driven airway remodelling enhances susceptibility to HDM with increases in BHR and inflammation. These functional studies demonstrate, for the first time, a gene-environment interaction involving ADAM33 to cause remodelling and the disproportional inflammatory responses seen in the asthmatic airway. sADAM33 might be a potential target for novel disease-modifying therapies.

S129 A TWO SPECIES PROTEOMICS APPROACH TO DETERMINE MMP-12 SUBSTRATES IN COPD

[1]B Mallia-Milanes, [2]A Dufour, [1]H Bailey, [1]G Meakin, [3]A Leme, [1]C Bolton, [3]S Shapiro, [2]C Overall, [1]S Johnson. *[1]University of Nottingham, Nottingham, UK; [2]University of British Columbia, Vancouver, Canada; [3]University of Pittsburgh, Pittsburgh, USA*

10.1136/thoraxjnl-2015-207770.135

Background Genetic variability in MMP-12 is associated with COPD; the matrix Metalloproteinase (MMP)-12 knockout (KO) mouse is resistant to emphysema despite cigarette smoke exposure, strongly implicating MMP-12 in COPD pathogenesis. However, the complete MMP-12 substrate profile (degradome) in COPD remains unknown. Terminal amine isobaric labelling of substrates (TAILS) is a novel proteomic technique allowing identification of a protease degradome on an organism-wide scale. Identification of the MMP-12 degradome will lead to novel drugs, desperately needed in COPD.

Objectives To identify the MMP-12 degradome in COPD by comparing cigarette smoke exposed MMP-12 KO and wildtype (WT) controls by TAILS and validating these targets against the human COPD sputum proteome during exacerbations and stable disease.

Methods C57BL/6J MMP-12 KO and WT mice (n = 4) were exposed to cigarette smoke and airways sampled by bronchoalveolar lavage (BAL). BAL fluid was analysed by TAILS, high performance liquid chromatography (HPLC) and tandem mass spectrometry (MS/MS). Matched COPD exacerbation and stable disease sputum samples (n = 9) were analysed by TAILS, HPLC and MS/MS.

Results The following new MMP-12 targets in the COPD mouse model were identified: alpha-2-HS glycoprotein, anti-thrombin III, clusterin, complement C3, complement C4b, complement factor H-related protein-1, hemopexin, serotransferrin and serum albumin, alpha-2-macroglobulin, beta-1, 4-galactosyltransferase 2, transmembrane protease 7, DEP domain-containing mTOR-interacting protein, kininogen-1, tumour necrosis factor ligand superfamily member 11. Of these, alpha-2-HS-glycoprotein, anti-thrombin III, complement factors C3 and C4B, hemopexin and serum albumin were identified in both exacerbation and stable COPD human sputum. Furthermore, 1,116 peptides were identified in COPD exacerbation and stable disease sputum, grouped into the following categories: cell adhesion/migration, complement system, acute phase response, extracellular matrix structure/function, anti-microbicidal activity, cytoskeletal function/remodelling, carbohydrate metabolism, oxidoreductase activity, cell death regulation/DNA synthesis/repair, immune response, protease activity, protease inhibition and ATP synthesis/function.

Conclusion This study identifies the MMP-12 degradome in COPD and provides the most comprehensive analysis of the

proteome of COPD sputum at exacerbation and stable disease. It suggests a role for MMP-12 in complement regulation and haemostasis in COPD. Thus an important peptide library has been unravelled, providing an ideal tool in developing drugs and understanding COPD pathogenesis.

S130 AXL RECEPTOR TYROSINE KINASE ON AIRWAY MACROPHAGES HAS A KEY ROLE IN LUNG IMMUNE HOMEOSTASIS

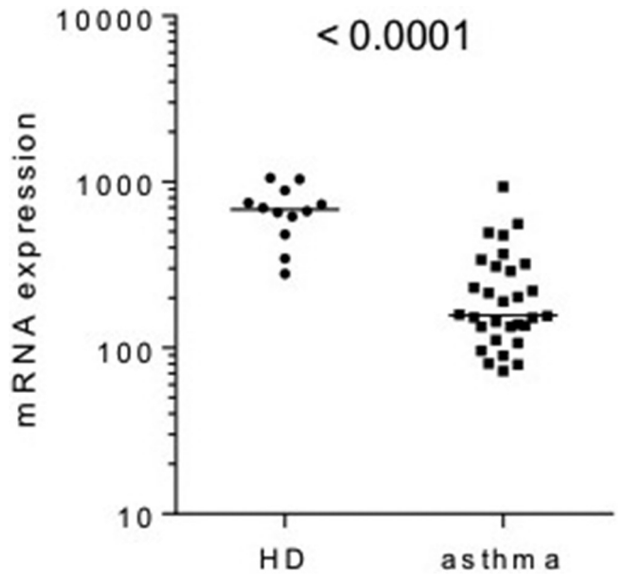
[1]N Denny, [1]AM Grabiec, [2]G Tavernier, [2]S Holden, [2]H Francis, [2]D Ryan, [2]R Niven, [2]SJ Fowler, [2]A Simpson, [1]T Hussell. *[1]Manchester Collaborative Centre for Inflammation Research, The University of Manchester, Manchester, UK; [2]Institute of Inflammation and Repair, Manchester Academic Health Sciences Centre, University of Manchester and National Institute of Health Research Respiratory and Allergy Clinical Research Facility, University Hospital of South Manchester, Manchester, UK*

10.1136/thoraxjnl-2015-207770.136

Rationale Apoptotic cell uptake (efferocytosis) by airway macrophages (AMs) is critical for lung immune homeostasis and is defective in chronic lung diseases, including asthma,[1] although the molecular mechanism behind this remains unknown. The TAM (Tyro3, Axl, MerTK) receptor tyrosine kinases are one of the main receptor classes that mediate efferocytosis but little is known about their regulation and function in inflammatory lung diseases.

Aim To investigate expression profile of TAM receptors and their ligand Gas6 in human AMs and analyse potential defects in TAM receptor expression in chronic lung inflammation.

Methods AMs from the sputum of patients with asthma (BTS step 3–5) (n = 30) or healthy donors (HD) (n = 12) were enriched by plastic adhesion. Monocytes were isolated from matched whole blood samples by CD14 positive selection and differentiated into monocyte-derived macrophages (MDMs). Total RNA was extracted from all purified cell populations and mRNA expression analysed by qPCR. HD MDMs were stimulated with Th2 cytokines *in vitro* and TAM receptor expression was analysed using qPCR and ELISA.



Abstract S130 Figure 1 mRNA expression of Axl in airway macrophages of healthy donors and patients with asthma

Main results Axl was the dominant TAM receptor expressed in HD AMs whereas monocytes and MDMs predominantly expressed MerTK. Axl expression was significantly reduced in AMs from patients with asthma compared to HD ($p < 0.0001$), while mRNA levels of MerTK and Gas6 was similar in both groups. We found no differences in Axl and MerTK expression in monocytes and MDMs from HD and patients with asthma, indicating that the observed differences were restricted to the site of inflammation. *In vitro*, MDM stimulation with IL-4 or IL-13 downregulated Axl mRNA and protein expression in a time-dependent manner.

Conclusions We have shown for the first time that Axl is the principal TAM receptor expressed in human AMs. Significant reduction of Axl expression in AMs from patients with asthma might be responsible for inefficient clearance of apoptotic cells from the inflamed airways and contribute to persistent airway inflammation. Strategies aimed at restoration of Axl expression or activity may represent a novel therapeutic strategy in asthma and other chronic lung diseases.

REFERENCE

1 Simpson JL, Gibson PG, Yang IA *et al*. Impaired macrophage phagocytosis in non-eosinophilic asthma. *Clin Exp Allergy*. 2013;**43**:29–35

S131 DEFICIENCY MUTATIONS OF α1-ANTITRYPSIN DIFFERENTIALLY AFFECT FOLDING, FUNCTION AND POLYMERISATION

[1]I Haq, [1]JA Irving, [2]AD Saleh, [3]L Dron, [1]GL Regan-Mochrie, [1]N Motamedi-Shad, [1]JR Hurst, [1]DA Lomas, [4]B Gooptu. *[1]University College London, London, UK; [2]Broomfield Hospital NHS, Chelmsford, UK; [3]Royal Free Hospital, London, UK; [4]King's College London, London, UK*

10.1136/thoraxjnl-2015-207770.137

Misfolding, polymerisation and defective secretion of functional α_1-antitrypsin underlie the predispositions to severe liver and lung disease in α_1-antitrypsin deficiency. We have identified a novel (Ala336Pro, Baghdad) deficiency variant and characterised it relative to the wild-type (M) and common severe Z (Glu342Lys) variant. The index case is a homozygous individual of consangineous parentage. Absolute levels of circulating α_1-antitrypsin were in the moderate deficiency range but the biochemical phenotype could not be clearly classified by standard methods. Moreover the majority was polymerised, i.e. functionally inactive, and the purified monomer was only 37% active relative to the wild-type 'M' variant. Together these resulted in 85–95% loss-of-function, a similarly severe functional deficiency to that of ZZ homozygotes. Biochemical, biophysical and computational studies further defined the molecular basis of this functional deficiency. These demonstrated that native Ala336Pro α_1-antitrypsin could adopt the polymerogenic intermediate conformation and polymerised more readily not only than M α_1-antitrypsin but also the severe Z variant. Nevertheless folding was far less impaired in Ala336Pro α_1-antitrypsin than in the Z variant. The data therefore indicate partitions between the contribution of the 'breach' (site of Z mutation) and 'shutter' (Ala336Pro) regions of strand 5A to folding and to polymerisation mechanisms. Moreover the findings demonstrate that in these variants, folding efficiency does not correlate directly with the tendency to polymerise *in vitro* or *in vivo*. They therefore differentiate generalised misfolding from polymerisation tendencies in missense variants of α_1-antitrypsin. Clinically they further support the need to quantify loss-of-function in α_1-antitrypsin deficiency to individualise patient care.