491. Molecular: pathology of infectious and inflammatory lung disease

P4745
Induced sputum differential gene expression implicates increased p38 signalling activity in severe asthma
Katherine Baines[1,2], Jodie Simpson[1,2], Lisa Wood[1,2], Rodney Scott[3], Peter Gibson[1,2]. *[1]Respiratory Medicine, The University of Newcastle, Newcastle, NSW, Australia; [2]Respiratory and Sleep Medicine, Hunter Medical Research Institute, John Hunter Hospital, Newcastle, NSW, Australia; [3]Information Based Medicine, The University of Newcastle, Newcastle, NSW, Australia*

Rationale: There is an urgent need for further insight into the characteristics and classifications of severe asthma. A thorough understanding of the mechanisms underlying the disease processes is essential to this.
Objective: To investigate the mechanisms of severe asthma through differential gene expression and pathways analysis of sputum gene expression profiles.
Methods: Induced sputum was collected from participants with severe asthma (n=12), defined by the presence of poor asthma control (ACQ>1) or airflow obstruction (FEV_1%predicted<80, FEV_1/FVC%<70), despite treatment with high-dose inhaled corticosteroid (>1000μg) and long-acting β-agonist, and compared to mild and moderate asthma (uncontrolled (n=21) and controlled (n=21)) and healthy controls (n=13). Gene expression profiles were generated (Illumina Humanref-8 V2) from sputum RNA and analysed using GeneSpring GX11.
Results: In severe asthma, 1236 genes were altered compared to controlled asthma, and 1723 genes were altered compared to healthy controls. Only 48 genes were altered between uncontrolled and controlled asthma. There was enrichment of genes in the p38 signalling pathway associated with severe asthma compared to both controlled asthma and healthy controls. The transforming growth factor-β receptor, interleukin-2 and epidermal growth factor receptor 1 pathways were also enriched in severe asthma when compared to healthy controls.
Conclusions: Severe asthma is associated with substantial differences in sputum gene expression that underlie unique cellular mechanisms that are not just associated with loss of asthma control. The p38 signalling pathway may be important in the pathogenesis of severe asthma.

P4746
Sputum gene expression of mast cell tryptase and carboxypeptidase A3 are increased in eosinophilic asthma
Katherine Baines[1,2], Jodie Simpson[1,2], Lisa Wood[1,2], Rodney Scott[3], Peter Gibson[1,2]. *[1]Respiratory Medicine, The University of Newcastle, Newcastle, NSW, Australia; [2]Respiratory and Sleep Medicine, Hunter Medical Research Institute, John Hunter Hospital, Newcastle, NSW, Australia; [3]Information Based Medicine, The University of Newcastle, Newcastle, NSW, Australia*

Rationale: Little is known regarding the presence and activity of mast cells in the

airway lumen across asthma inflammatory phenotypes. Sputum gene expression of the mast cell specific proteases may serve as markers of mast cell activation in asthma.
Objective: To investigate if sputum gene expression of mast cell specific proteases is associated with asthma inflammatory phenotype.
Methods: Induced sputum was collected from participants with asthma (n=59; eosinophilic n=17; neutrophilic n=12; mixed granulocytic n=12; and paucigranulocytic n=18) and healthy controls (n=17). Gene expression profiles were generated (Illumina Humanref-8 V2) from sputum RNA. Mast cell tryptase (TPSAB1), carboxypeptidase A3 (CPA3) and chymase (CMA1) data were extracted and investigated for their relationship to asthma phenotype.
Results: Gene expression of tryptase and carboxypeptidase A3 was significantly different among the 5 groups ($p<0.0001$). There was no difference in chymase expression. Tryptase mRNA was increased ($p<0.01$) in eosinophilic asthma compared to neutrophilic asthma, paucigranulocytic asthma and healthy controls. Carboxypeptidase A3 mRNA was increased ($p<0.01$) in eosinophilic asthma compared to neutrophilic asthma, mixed granulocytic asthma, paucigranulocytic asthma and healthy controls. Tryptase ($r=0.58$ $p<0.0001$) and carboxypeptidase A3 ($r=0.59$ $p<0.0001$) expression was strongly correlated with sputum eosinophils.
Conclusions: Gene expression of mast cell tryptase and carboxypeptidase A3 is increased in eosinophilic asthma. Airway mast cells are important in eosinophilic asthma and have a unique phenotype associated with expression of tryptase and carboxypeptidase A3 but not chymase.

P4747
Coordinate regulation of IL-4 and IL-13 expression in human T cells: 3C analysis for DNA looping
Xin Yao[1], Wei Song[1], Wangjian Zha[1], Peter J. Barnes[2], Ian M. Adcock[2], Andrew L. Durham[2]. *[1]Department of Respiratory Disease, First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China; [2]National Heart and Lung Institute, Imperial College London, London, United Kingdom*

Over 5.4 million adults receive treatment for asthma in the UK. Asthma is a chronic allergic disorder characterised by chronic inflammation. Type 2 T helper (T_H2) are an important component in the development of the asthma and the response to allergens, producing the cytokines interleukin (IL) -4 and IL-13. In T_H2 cells, production of the cytokines IL-4, IL-5, and IL-13 in a co-ordinate manner. Th2 cytokine expression is controlled by cooperation between transcription factors GATA-3 and Nuclear factor of activated T-cells -1 (NFAT1).
The genes encoding these cytokines are located together the same chromosomal region, 5q31.1 in humans. We have investigated the co-ordinated expression of *IL4* and *IL13* in the T_H2 model cell line HUT78. Using chromatin immunoprecipitation (ChIP) and re-ChIP analysis we have shown that in HUT78 cells GATA-3 is associated with the *IL4* and *IL13* promoter regions. Additionally, following stimulation CD3/28 antibodies NFAT1 rapidly translocates to the nucleus and becomes associated with the promoter regions and GATA3. Finally, using Chromatin Conformation Capture (3C) we demonstrate looping of DNA at the *IL4* and *IL13* promoters to bring them into close proximity with each other. This suggests that the formation of a transcriptional hub under the control of GATA-3 and NF-AT drives co-ordinate T_H2 cytokine production.

P4748
microRNA profiling in pulmonary fibroblasts in COPD
Corry-Anke Brandsma[1], Stephanie Christenson[3], Joshua Campbell[3], Marnix Jonker[1], Marc Lenburg[3], Avi Spira[3], James Hogg[4], Dirkje Postma[2], Wim Timens[1]. *[1]Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands; [2]Pulmonary Diseases, University Medical Center Groningen, University of Groningen, Groningen, Netherlands; [3]Bioinformatics Group, Dept of Pulmonary and Critical Care Medicine, Boston University School of Medicine, Boston, United States; [4]Pathology and Laboratory Medicine, University of British Columbia James Hogg Research Centre, Vancouver, Canada*

COPD is characterized by emphysema with loss of extracellular matrix (ECM), and (small) airways disease with increased ECM deposition and airway wall thickening. How both processes can occur in close proximity in one lung is unknown and needs more attention. Fibroblasts are the principal cells involved in ECM production in the lung. MicroRNAs are small RNAs that can cause downregulation of target protein expression. We hypothesize that microRNA-mediated differences between COPD and healthy fibroblasts, and additionally airway and parenchymal fibroblasts, contribute to the airway and parenchymal changes in COPD.
We profiled microRNA expression in pulmonary fibroblasts from severe COPD patients and controls to investigate effects of COPD, smoking (ex-smokers vs current smokers) and fibroblast type (airway vs parenchymal fibroblasts).
Using linear models we found 42 microRNAs differentially expressed in COPD patients, 25 between ex-smokers and current smokers and 45 between airway and parenchymal fibroblasts in COPD ($p<0.01$). Interestingly some of the microRNAs differentially expressed in COPD fibroblasts, i.e. mir-181d and mir-296-5p, are also differentially expressed in lung tissue in relation to emphysema severity *(Christenson, S.A. et al. Am J Resp Crit Care Med 2010;181:A2024)*. COPD and smoking had similar effects on several microRNAs, including mir-181d, mir-296-5p, mir-29b1*, mir-23* and mir-202, suggesting a possible mechanism for the link between smoking and COPD development. Furthermore, mir-155 expression was decreased in COPD and within COPD in airway fibroblasts. Given the role of mir-155 in airway remodeling *(Rodriguez, A. et al. Science 2007; 316:608-11)* this microRNA could be important in the airway changes in COPD.

P4749
microRNA-34a and microRNA 199a-5p in COPD and their control of HIF-1α expression in pulmonary vasucular endothelial cells
Shiro Mizuno[1], Jose Gomez-Arroyo[1], Aysar Alhussaini[1], Donatas Kraskauskas[1], Carlyne Cool[2], Herman Bogaard[3], Norbert Voelkel[1]. *[1]Pulmonary and Critical Care Medicine Division, Virginia Commonwealth University, Richmond, VA, United States; [2]Department of Pathology, University of Colorado Health Science Center, Aurora, CO, United States; [3]Pulmonary Division, VU University Medical Center, Amsterdam, Netherlands*

Rationale: MicroRNAs (miRNA) are small noncording RNAs that silence target gene expression post-transcriptionally, and their impact on gene expression has been reported in various diseases. In the lungs from COPD patients, increased expression of tumor suppressor p53 and decreased expression of HIF-1α have been reported. However, the regulatory roles of these miRNAs in COPD lungs have not been evaluated.
Objective: To assess the expression of miRNAs known to be associated with p53 and HIF-1α expression in lung tissues from patients with COPD/emphysema.
Methods: Lung tissue samples from 55 patients were included in this study. Total RNA and miRNA extracted from lung tissues were used for RT-PCR analysis focused on miR-20b, miR-34a, miR-107, miR-199a-5p and miR-210. HIF-1α and p53 protein expression, AKT phosphorylation was measured by Western blot analysis. Gene silencing of HIF-1α and transfection of miR34a and miR-199a-5p precursor RNAs were performed in cultured human pulmonary microvascular endothelial cells (HPMVEC).
Measurements and main results: miR-34a and miR-199a-5p expression was increased and the phosphorylation of AKT was decreased in COPD lungs. Transfection of the miR34a precursor decreased the phosphorylation of AKT, and transfection of the miR-199a-5p precursor decreased HIF-1α and VEGF protein expression in HPMVEC.
Conclusions: These data indicate that miR-34a and miR-199a-5p contribute to the pathogenesis of emphysema, via decreasing the expression of HIF-1α and increasing the expression of p53 in the lung and thereby affecting the HIF-1a/VEGF-dependent lung structure maintenance program.

P4750

P4751
Cigarette smoke exposure directly induces increased ubiquination and impairs hypoxic-angiogenic signalling in skeletal muscles
Vladimir Basic[1], Hongwei Yao[2], Irfan Rahman[2], Allan Sirsjö[1], Samy M. Abdel-Halim[3,4]. *[1]Department of Clinical Medicine, Örebro University, Örebro, Sweden; [2]Department of Enviromental Medicine, University of Rochester Medical Center, New York, United States; [3]Respiratory Medicine & Allergology, Uppsala University, Uppsala, Sweden; [4]Respiratory Medicine & Allergology, Danderyd Hospital, Stockholm, Sweden*

Objectives: Pulmonary cachexia syndrome (PCS) is a common occurrence in COPD patients and is directly correlated with increased rate of mortality in these

patients. We have recently identified overexpression of von Hippel-Lindau (VHL) mRNA and protein in skeletal muscles of COPD patients. This would exert a negative impact on muscle capillarization by impairing the signal transduction of hypoxia-inducible factor (HIF) to vascular endothelial growth factor (VEGF). It is unclear if VHL overexpression is a primary event induced by cigarette smoke (CS) or if it occurs secondary to development of COPD.
Methods: *129SvJWT* littermates mice were exposed to CS or air during 6 months. The gastrocnemius skeletal muscles were collected and analyzed for expression of the various components of the hypoxic-angiogenic signalling cascade including HIF-1, VHL and VEGF using quantitative RT-PCR and western blots.
Results: Exposure of *129SvJWT* mice to cigarette smoke induced a significant overexpression of HIF-1 mRNA (p*=0.03 vs. controls). Likewise, significant VHL mRNA and protein overexpression were elicited (p***=0.00063 vs. controls). By contrast, VEGFa mRNA expression was not altered (p=0.88).
Conclusion: The current results demonstrate VHL overexpression in skeletal muscles of normal mice exposed to CS. This is in analogy and further confirms our recent findings in skeletal muscles of COPD patients. Thus VHL overexpression appears to be an early primary event related to CS exposure rather than a secondary occurrence to development of the COPD pathology.

P4752
BAMBI – A TGF-β pseudoreceptor with possible functional involvement in COPD and NTHI infection
Sebastian Marwitz[1], Daniel Droemann[2], Jan Rupp[2], Kristina Rohmann[2], Sinia Osbahr[2], Arthur-Jochen Ulmer[3], Kristina Röschmann[3], Mahdi Abdullah[1], Holger Schultz[1], Ekkehard Vollmer[1], Peter Zabel[4], Klaus Dalhoff[2], Torsten Goldmann[1]. *[1]Clinical and Experimental Pathology, Research Center Borstel, Borstel, SH, Germany; [2]Medical Clinic III, University of Lübeck, Lübeck, SH, Germany; [3]Department of Immunology and Cell Biology, Research Center Borstel, Borstel, SH, Germany; [4]Medical Clinic, Research Center Borstel, Borstel, SH, Germany*

TGF-β signaling in non-malignant pulmonary diseases to date is still controversially discussed. Nontypeable Haemophilus influenzae (NTHI) may play a role in the pathogenesis of chronic obstructive pulmonary disease (COPD) as an infectious trigger. Comparably few data are available regarding the influence of acute and persistent infection on tissue remodeling and repair factors such as transforming growth factor (TGF)-β. Here we show that the TGF-β pseudoreceptor BAMBI is expressed in the human lung.
NTHI infection was analyzed in lung tissues obtained from COPD patients and controls utilizing a human ex vivo and in vitro models. Detection of NTHI was achieved by in situ hybridization (ISH). For characterization of TGF-β] signaling molecules, transcriptome array analyses were conducted. Expression of the TGF-pseudoreceptor BMP and Activin Membrane-bound Inhibitor (BAMBI) was analyzed using immunohistochemistry (IHC), ISH and RT-PCR. CXC chemokine ligand (CXCL)-8, tumor necrosis factor (TNF)-alpha and TGF-β expression were evaluated in lung tissue and cell culture using ELISA.
An infection with NTHI was detected in vivo in 38% of the COPD patients in contrast to 0% of controls. Transcriptome arrays showed no significant changes of TGF-β receptors 1 and 2 and Smad-3 expression, whereas a strong expression of BAMBI with upregulation after in vitro infection of COPD lung tissue was demonstrated. BAMBI was expressed ubiquitously on alveolar macrophages (AM) and on alveolar epithelial cells (AEC).
We show for the first time a considerable expression of the TGF pseudoreceptor BAMBI in the human lung. BAMBI is upregulated in response to NTHI infection in COPD lung tissue in vivo and in vitro.

P4753
Elevated osteoprotegerin in COPD is potentially regulated by oxidative stress dependent glycogen synthase kinase-3β and β-catenin signalling
Masako To, Catherine E. Charron, Sergei A. Kharitonov, Peter J. Barnes, Kazuhiro Ito. *Airway Disease Section, National Heart and Lung Institute, Imperial College, London, United Kingdom*

We recently reported that sputum osteoprotegerin (OPG) in COPD is higher than that in controls and is a potential biomarker in COPD (To M *et.al*. *CHEST* 2010 Dec 2 [Epub ahead of print]). However, the molecular mechanism involved in OPG elevation in COPD was not clarified yet. Here we investigated the role of glycogen synthase kinase-3β (GSK-3β) and β-catenin in OPG transcription, especially under oxidative stress.
A549 cells, an alveolar epithelial cell line, were treated with cigarette smoke conditioned media (CSM), and β-catenin protein was determined by westernblot. CSM induced OPG release (measured by ELISA) with a maximum induction of 147% and also increased β-catenin protein expression in a concentration-dependent manner with a maximum induction of 161%. A549 cells were transfected with β-catenin siRNA using lipofectamine for specific knockdown (KD) of β-catenin, and IL-1β stimulated OPG production by the A549 cells was measured by ELISA. β-catenin KD showed significantly lower OPG production at baseline and after IL-1β stimulation compared to non- transfected controls (466±13 pg/ml in β-catenin KD and 1712±35pg/ml in wild type without IL-1β, and 1213±37 pg/ml in β-catenin KD and 2669±16pg/ml in wild type under IL-1β stimulation). The inhibitor of GSK-3β, which controls β-catenin stabilisation, significantly increased OPG production with a maximum induction of 161% and with EC_{50} of 1.2 mM. Thus, defect of GSK-3β causes β-catenin expression, leading upregulation of OPG. This will be a new insight on molecular mechanism of COPD pathogenesis.

P4754
Overexpression of chitinase 3-Like 1/YKL-40 in lung-specific IL-18-transgenic mice, smokers and COPD
Yuki Sakazaki, Tomoaki Hoshino, Satoko Takei, Masanori Sawada, Hanako Oda, Shin-Ichi Takenaka, Haruki Imaoka, Kazuko Matsunaga, Hisamichi Aizawa. *Resipirology, Neurology and Rheumatology, Kurume University, Kurume, Japan*

In this study, we analyzed the lung mRNA expression profiles of murine model of COPD, lung-specific IL-18-transgenic mice using microarray analysis. In the IL-18-transgenic mice, the expression of 608 genes was found to fluctuate more than 2-fold in comparison with control WT mice, and was clustered into 4 groups. The expression of 140 genes was constitutively increased at all ages, that of 215 genes increased gradually with aging, that of 171 genes decreased gradually with aging, and that of 82 genes decreased temporarily at 9 weeks of age. Interestingly, the levels of mRNA for the chitinase-related genes chitinase 3-like 1 (Chi3l1), Chi3l3, and acidic mammalian chitinase (AMCase) were significantly higher in the lungs of transgenic mice than in control mice. The level of Chi3l1 protein increased significantly with aging in the lungs of IL-18 transgenic, but not WT mice. Previous studies have suggested that Chi3l3 and AMCase are IL-13-driven chitinase-like proteins. However, IL-13 gene deletion did not reduce the level of Chi3l1 protein in the lungs of IL-18 transgenic mice. Furthermore, the protein level of YKL-40, the human homolog of Chi3l1, was significantly ($P < 0.01$) higher in serum samples from smokers (331.8±37.0 ng/mL, n = 28) and COPD patients (268.9±32.3 ng/mL, n = 45) than in those from non-smokers (177.8±22.6 ng/mL, n = 30). In COPD patients, there was a significant negative correlation between serum level of YKL-40 and%FEV_1. Chitinase-related genes may play an import role in establishing pulmonary inflammation and emphysematous changes.

P4755
Lung fibroblasts from COPD patients presented a reduced response to TGF-β1 for proliferation and elastin synthesis
Jing Zhang[1], Lian Wu[2], Ming-Xiang Feng[3], Paul Sexton[2], Chun-Xue Bai[1], Mervyn J. Merrilees[4], Peter Black[2], Peter Black[2]. *[1]Department of Pulmonary Medicine, Zhongshan Hospital of Fudan University, Shanghai, China; [2]Department of Pharmacology and Clinical Pharmacology, University of Auckland, Auckland, New Zealand; [3]Department of Thoracic Surgury, Zhongshan Hospital of Fudan University, Shanghai, China; [4]Department of Anatomy, University of Auckland, Auckland, China*

Rationale: Insufficiency in repair capacity of fibroblasts might contribute to this phenomenon. In this study, the stimulative effect of TGF-β1 on proliferation and elastin production was investigated in human primary lung fibroblasts.
Methods: The subjects were classified into COPDand control group according to GOLD criteria. Fibroblasts were cultured from lung tissue. Proliferation was measured with Alamar Blue method. mRNA and protein level for elastin was measured using real-time RT-PCR and Fastin Elastin assay respectively. The percentage of increase of proliferation and elastin production with 1ng/ml TGF-[beta1] incubation was calculated for fibroblasts from each subject.
Results: Totally 18 subjects were included, with 8 in control group and 10 in COPD group. There were no significant differences between the two groups in age, gender or smoking history. FEV1 for COPD group and control group was 1.56±0.38L and 2.25±0.39L respectively (p=0.0012). COPD fibroblasts showed a slower proliferation than control fibroblasts, with a reduced response to TGF-β1 treatment (the percentage of increase being 123±20% vs 174±17%, p=0.0001). The percentage of increase in proliferation was positively correlated with FEV1 (p=0.009). Despite an increase in the mRNA expression and protein secretion for elastin, the promotive effect of TGF-β1 on elastin synthesis which was observed in fibroblasts from control subjects was diminished in fibroblasts from COPD patients.
Conclusions: Our findings suggested that COPD fibroblasts have a decreased response to TGF-β1 in both proliferation and elastin secretion, supporting the impaired repair mechanism in the development of COPD.

P4756
AntagomiR directed against miR-20a restores BMP signaling in hypoxia-induced pulmonary hypertension in vivo
Matthias Brock[1,2], Michelle Trenkmann[1], Colin Schwarzwald[3], Silvia Ulrich[2], Max Gassmann[4], Louise Ostergaard[4], Steffen Gay[1], Rudolf Speich[2], Lars Huber[2]. *[1]Center of Experimental Rheumatology, University Hospital Zurich, Zurich, Switzerland; [2]Working Group of Pulmonary Hypertension, University Hospital Zurich, Zurich, Switzerland; [3]Equine Department, Vetsuisse Faculty, University Zurich, Zurich, Switzerland; [4]Institute of Veterinary Physiology, University Zurich, Zurich, Switzerland*

Introduction: Dysregulated expression of the bone morphogenetic protein (BMP) receptor type II (BMPR2) is a pathogenetic hallmark in the development of pulmonary hypertension (PH). We recently demonstrated that microRNA 20a (miR-20a) represses the expression of BMPR2. Here we investigated the therapeutic potential of antagonizing miR-20a in an in vivo model of PH.

Methods: For in vivo inhibition of miR-20a, antagomiR-20a (A-20a) was injected intraperitoneally into mice with hypoxia-induced PH. After three weeks of hypoxia, morphometry and blood gas analysis were performed. RNA levels of miR-20a, BMPR2, Smad5, and Id2 were assessed by real-time PCR in hearts and lungs.
Results: Intraperitoneal injection of A-20a resulted in significant down regulation of miR-20a in lungs. When exposed to hypoxia, animals treated with A-20a developed less right ventricular hypertrophy as evidenced by relative heart weight. Treatment with A-20a reduced the hemoglobin levels and, moreover, the acid-base status (base deficit) approached the baseline values of normoxic animals. Molecular analysis showed down regulation of BMPR2 mRNA levels in lung tissue by hypoxia (0.79±0.24 fold, p=0.039). A-20a abrogated the hypoxia induced down regulation of BMPR2 and led to significant up regulation of BMPR2 (1.62±0.18 fold, p<0.001). We detected increased expression of Smad5 (1.29±0.19 fold, p=0.003) and the BMP target gene Id2 (1.43±0.40 fold, p=0.02) in lung tissue of A-20a treated mice suggesting improved BMP signaling.
Conclusion: Our data underpin the importance of miR-20a in the BMP signaling and emphasize the need for further studies to address the therapeutic potential of antagomiRs for the human disease.

P4757
Adrenergic receptor blocker reverses cardiac metabolic remodeling and improves right ventricular function in experimental pulmonary hypertension
Jose Gomez-Arroyo[1], Shiro Mizuno[1], Laszlo Farkas[1], Donatas Kraskauskas[1], Daniela Farkas[1], Ayser Husseini[1], Antonio Abbate[2], Herman Bogaard[3], Norbert Voelkel[1]. *[1]Pulmonary and Critical Care, Virginia Commonwealth University, Richmond, VA, United States; [2]Cardiology, Virginia Commonwealth University, Richmond, United States; [3]Pulmonary Division, VU University Medical Center, Amsterdam, Netherlands*

Albeit right ventricular failure (RVF) is a major complication in patients with pulmonary arterial hypertension, the knowledge on the pathobiology of RVF is still incomplete and RV-targeted therapies are practically non-existent. Since a metabolic switch has been reported for left heart failure, we sought to describe any potential changes in cardiac fuel metabolism and mitochondrial function in RVF.
PAH was induced in S-D rats by a combination of a VEGF-receptor blocker followed by 4 weeks of 10% hypoxia (SuHx).Gene expression was evaluated by RT-PCR and confirmed by western blot. Rats were treated with vehicle/carvedilol for 4 weeks after hypoxia exposure.RV function was measured by echocardiogram and reported as TAPSE.
As an initial step, we evaluated the expression of PGC1α, a master regulator of cardiac energetics and mitochondrial biogenesis.SuHx RV tissue had a 60% downregulation of PGC1α when compared to controls (p<0.001).PGC1α target genes codifying enzymes for fatty acid metabolism were decreased, whereas major glycolysis genes were upregulated.These changes strongly correlated with decreased TAPSE (R^2=0.77,p<0.001).Furthermore, SuHx RV tissue showed a downregulation of TFAm (a critical regulator for mtDNA maintenance) which was associated with abnormal mitochondrial morphology shown by electron microscopy.Carvedilol treatment, a drug known to improve RVF, completely restored PGC1α expression and reversed the gene expression profile.
Our data suggests a switch in cardiac substrate utilization and mitochondrial dysfunction during RVF. We propose carvedilol as a potential treatment to reverse this metabolic switch.

P4758
Plasma protein profiling in alpha-1-antitrypsin deficiency
Helen Stone[1], Douglas Ward[2], Robert Stockley[3]. *[1]ADAPT Project/Department of Lung Function and Sleep, Queen Elizabeth Hospital Birmingham, Birmingham, United Kingdom; [2]Department of Cancer Science, University of Birmingham, Birmingham, United Kingdom; [3]ADAPT Project/Department of Lung Function and Sleep, Queen Elizabeth Hospital Birmingham, Birmingham, United Kingdom*

Introduction: Alpha-1-antitrypsin deficiency (ATD) results in an imbalance of proteases and antiproteases with proteolytic destruction of lung parenchyma, and subsequently early onset emphysema. We hypothesised changes in plasma peptides/proteins would be detectable and could provide potential biomarkers to predict a response to treatment in patients with ATD receiving augmentation therapy.
Methods: Mass spectrometry was used to identify proteolytic fragments in plasma following neutrophil activation by ionophore or the addition of neutrophil elastase (NE). 72 paired plasma samples from the EXACTLE trial, taken at baseline and 6 months, were subsequently analysed using weak cation exchange beads and MALDI-TOF-MS, to determine whether any of the protein fragments generated *in vitro* were present in clinical samples and changed significantly in response to augmentation.
Results: Following neutrophil activation or the addition of NE to plasma 325 peptides from 32 proteins were identified. 207 peptide peaks were detected in the spectra of the EXACTLE patients. T Tests were performed to determine whether or not the peak intensities of the peptides changed with treatment. Of these, 12 were significantly different in the treatment group, yet unchanged in the placebo group. The most significant of these had a p value of 2.08E-05.
Conclusion: Changes in the plasma peptidome can be found in patient plasma after the addition of calcium ionophore and NE. Some of these changes can be detected in plasma pre/post augmentation. We aim to use more in depth proteomic approaches to further characterise changes in the plasma peptidome, and determine clinical utility as a guide to monitoring and treatment in ATD.

P4759
TRPA1 expression and characterisation in patients with chronic cough
Shoaib Faruqi[1], Ann Campbell[2], Alyn Morice[1]. *[1]Division of Cardiovascular and Respiratory Medicine, Castle Hill Hospital, University of Hull, Cottingham, United Kingdom; [2]Department of Histopathology, Hull Royal Infirmary, Hull, United Kingdom*

Aim: The TRPA1 ion channel is thought to have an important role in the cough reflex. It has been shown that inhalation of cinnamaldehyde, a specific agonist of TRPA1, induces cough in normal human volunteers. We wanted to determine the expression and characterisation of the TRPA1 receptor in human lung tissue.
Methods: Bronchial biopsies were obtained from patients with the Cough Hypersensitivity Syndrome on fibre-optic bronchoscopy. Lung resection samples were obtained from patients undergoing lung resection for lung cancer. Dorsal root ganglia were used as a positive control. These tissue samples were analysed by immunohistochemistry with a specific TRPA1 antibody.
Results: Samples from 14 patients (11 males, mean age 56 years) with the Cough Hypersensitivity Syndrome and 10 lung resection samples (6 males, mean age 68 years) were obtained. All the tissue samples stained for TRPA1. The TRPA1 stain was avidly taken up by the bronchial epithelium, smooth muscle bundles and nerve tissue.
Conclusion: We have described the distribution of TRPA1 ion channel in lung tissue. TRPA1 is agonised by several environmental irritants and endogenous mediators of inflammation. The presence of TRPA1 ion channel in bronchial mucosal nerves and epithelium suggests an important role in the cough reflex. The localisation of these ion channels on smooth muscle could suggest a role in asthmatic inflammation as well.

P4760
Expression of the Bcl-2 binding protein beclin 1 in human idiopathic pulmonary fibrosis
Davide Scozzi[1], Salvatore Mariotta[1], Emanuela Cherubini[2], Vittorio Pietrangeli[1], Manuela Del Forno[1], Luca Tabbì[1], Lara Pisani[1], Pierdonato Bruno[1], Alberto Ricci[1]. *[1]Dipartimento di Medicina Clinica e Molecolare, Università la Sapienza, Roma, Italy; [2]Centro Ricerche, Ospedale San Pietro, Roma, Italy*

Autophagy is the main cellular route for degradation of long-lived proteins and cytoplasmic organelles and its dysregulation contributes to the pathogenesis of different disease. In some settings it seems to be interconnected with apoptosis. In this regard the binding of the anti-apoptotic protein Bcl-2 with a key regulator of autophagy as Beclin 1 seems to down-regulate both apoptosis and autophagy. It is known that fibroblasts in idiopathic pulmonary fibrosis (IPF) acquire resistance to apoptosis but no data are available about the regulation of autophagy in IPF. Here we examined the expression of Beclin 1 and Bcl-2 in human IPF fibroblasts before and after cisplatin exposure.
We performed immunohistochemistry for Beclin 1 on sections of lung biopsies obtained from patients affected with IPF (n=25) histologically normal (n=5) or with emphysema (n=5). In 4 IPF, 2 normal and 2 emphysema the tissue was used to isolate lung fibroblasts. We performed Western Blot analysis and co-immunoprecipitation for Bcl2 and Beclin 1 on cultured fibroblasts before and after cisplatin exposure.
Expression of Beclin 1 in fibroblasts from IPF was down-regulated in comparison with normal and emphysema while the anti-apoptotic protein Bcl-2 was over-expressed. Treatment of fibroblast cell cultures with cisplatin induced a significant increase in Beclin 1 but a reduction in Bcl-2 expression in IPF fibroblasts. The immunoprecipitation of Bcl-2 and the following immunodetection of Beclin 1 indicate that Bcl-2 is mainly bound to Beclin 1.
The modified expression of Beclin 1 and Bcl-2 in human IPF fibroblasts in comparison with normal ones seems to suggest the possibility of an autophagic/apoptosis system dysfunction.