

GOLD stage II COPD patients had reduced lung function, quadriceps strength and 6MW test despite a similar smoking history. There was increased mitochondrial and intracellular ROS in both skeletal muscle and bronchial biopsies of COPD patients compared to controls. There was a trend for reduced MP in COPD EB mitochondria.

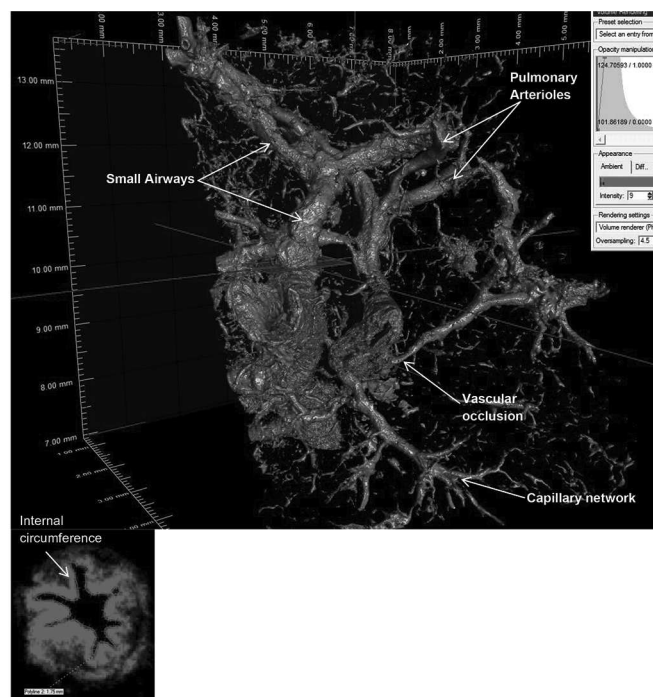
**Conclusion** The presence of excessive ROS in cells from a lung and a non-lung compartment support the existence of a generalised dysfunction of mitochondria in established COPD resulting in increased mitochondrial oxidative stress.

# **P190 CONTRASTING TECHNIQUES FOR THE STUDY OF COPD LUNG MICRO-STRUCTURE WITH X-RAY MICRO-COMPUTED TOMOGRAPHY**

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10.1136/thoraxjnl-2013-204457.342

**Background** Chronic obstructive pulmonary disease (COPD) is a severely debilitating lung condition characterised by airway obstruction within the distal respiratory tree. Micro-CT imaging is a novel radiographic method that can generate a three-dimensional reconstruction of human lung micro-structure at resolutions approaching 1  $\mu$ m. This has revealed an obliteration of terminal bronchioles that may begin before patients have symptoms. A significant challenge when imaging wax-embedded COPD lung tissue is improving the contrast-to-noise for reasonable scan durations. The low contrast within the tissue can result in image analysis taking weeks to perform so addressing this



**Abstract P190 Figure 1.** After segmentation. Small airways and accompanying vessels were almost entirely segmented after 0.1% PTA staining by quickly increasing the threshold applied to the sub-volume (see the higher threshold values displayed on the histogram). The blue marker shows where the airway cross section was taken (bottom left). Measuring internal circumference proved to be highly precise.

issue is critical if micro-structure is to be studied in a more robust and less time-consuming fashion.

**Aims** The study's aim was to compare and quantify the effects of different contrasting techniques on the ability of micro-CT to visualise small airways <2 mm in diameter and micro-vasculature in COPD human lung tissue.

**Methods** Samples were obtained from formalin-fixed sub-pleural lung tissue resected from a patient with moderate COPD and were incubated in 0.1% phosphotungstic acid (PTA), 25% Lugol's iodine, 1% silver nitrate or left unstained to act as a control. Post-incubation, samples were embedded in epoxy resin or paraffin wax and then imaged with a 225kV HMX CT scanner at Southampton University with an average voxel size of 7.6  $\mu$ m. The data was then analysed in Image J and VGI StudioMax.

**Results** Staining with 0.1% PTA and 25% Lugol's iodine significantly improved x-ray contrast ( $p < 0.01$ ) with most intense staining occurring in the small airways and micro-vessels. Staining with 1% silver nitrate failed to improve contrast ( $p = 0.110$ ). PTA staining enabled small airway and vascular occlusions to be three-dimensionally characterised, providing reliable quantification of the micro-structure. Fast and simple image segmentation taking 10 minutes was required to effectively map out most of the branching network of small airways and micro-vessels. Visualising micro-structure in uncontrasted control samples required complex image analysis which took four hours to complete.

**Conclusion** PTA staining is a simple and effective technique at increasing x-ray contrast and reducing noise in COPD lung tissue. This greatly improves the level of visualisation of micro-structure in COPD tissue, providing more efficient and reliable analysis.

# **P191 BETA-2 ADRENOCEPTOR GENOTYPE AND RESPONSE TO PROPRANOLOL IN PATIENTS WITH PERSISTENT ASTHMA**

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10.1136/thoraxjnl-2013-204457.343

**Background** The arginine-16-glycine (Arg16Gly) beta-2 adrenoceptor (ADR) polymorphism is associated with worse outcomes in patients exposed to regular beta-agonists. We therefore wished to know if Arg16Gly conferred a similar effect in response to beta-antagonists in asthma.

**Methods and observations** We have performed a retrospective composite analysis of two randomised controlled trials looking at effects of Arg16Gly on the chronic response to propranolol in  $n = 25$  mild to moderate corticosteroid treated persistent asthmatics. We evaluated chronic dosing effects of propranolol given for at least 4 weeks (80mg dose at least 2 weeks) on pulmonary

**Abstract P191 Table 1.**

	ArgArg or ArgGly n=15		GlyGly n=10		Genotype Comparison
	Change from Baseline (SEM)	P-value	Change from Baseline (SEM)	P-value	P-value
FEV1 (%)	-3.9 (1.2)	0.006	-2.3 (3.4)	0.51	0.66
FEF25-75 (%)	-5.2 (2.1)	0.025	+2.0 (3.2)	0.55	0.06
R5 (%)	+20.7 (5.5)	0.003	+6.0 (10.3)	0.57	0.27
Recovery FEV1 (%)	-14.3 (3.0)	<0.001	-8.4 (2.8)	0.014	0.18

function (FEV<sub>1</sub>, FEF<sub>25-75</sub>, total airway resistance at 5Hz: R5) and on salbutamol FEV<sub>1</sub> recovery post histamine challenge. Comparisons were made between genotypes comprising one or two copies of Arg (i.e. ArgArg or ArgGly n = 15, FEV<sub>1</sub> = 91.1%, FEF<sub>25-75</sub> = 58.3%) vs. no copies of Arg (i.e. GlyGly n = 10, FEV<sub>1</sub> = 94.1% FEF<sub>25-75</sub> = 60.0%).

**Results** Data are shown in table as change from baseline (i.e. pre vs. post propranolol as means and SEM) within each genotype. Within the Arg genotype there were significant effects of propranolol on FEV<sub>1</sub>, FEF<sub>25-75</sub> and R5 as well as significant blunting of salbutamol response, while in the Gly genotype only salbutamol response was significant. However when comparing the Arg vs. Gly genotypes there were no significant differences for any of the outcomes.

**Conclusion** Propranolol produces significant effects on pulmonary function and salbutamol response in the Arg genotype, although there were no significant differences between Arg and Gly genotypes.

# **P192 A PILOT STUDY TO ASSESS THE INFLUENCE OF 2-ADRENOCEPTOR POLYMORPHISM ON SMALL AIRWAY FUNCTION AND ASTHMA CONTROL**

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10.1136/thoraxjnl-2013-204457.344

**Introduction and Objectives** It is increasingly recognised that small airway dysfunction is associated with suboptimal asthma control. We have previously reported that 2-adrenoreceptor polymorphism at position 16 (i.e. Arg/Gly) is not related to FEV<sub>1</sub> or airway hyper-responsiveness in persistent asthmatics.<sup>1</sup> However effects of 2-adrenoreceptor polymorphism on the small airways are not known. This pilot study in a different cohort of patients evaluated the effects of 2-adrenoreceptor polymorphism on small airway function and asthma control. Impulse oscillometry (IOS) was used to assess small airway function along with FEF<sub>25-75</sub>. IOS is an effort independent test performed during normal quiet tidal breathing and is able to discriminate between changes in central and peripheral airways. Resistance at 5 Hz (R5) and 20 Hz (R20) indicate total and central airway resistance respectively - the difference between R5 and R20 indicates peripheral airway resistance. Asthma control was assessed using the Asthma Control Questionnaire (ACQ-5).

**Methods** We collected spirometry, IOS and ACQ data from patients attending a secondary care asthma clinic. A total of 100

**Abstract P192 Table 1: Spirometry, IOS and ACQ-5 according to Arg/Gly-16 polymorphism**

	Arg-Arg / Arg-Gly	Gly-Gly	p-value
FEV <sub>1</sub>	88 (82-94)	85 (78-91)	0.46
(% predicted)			
FEF <sub>25-75</sub>	53 (45-62)	49 (41-57)	0.48
(% predicted)			
R5	149 (132-168)	178 (147-209)	0.58
(% predicted)			
R20	142 (127-156)	146 (130-162)	0.80
(% predicted)			
R5 - R20 (kPa/L/S)	0.07 (0.05-0.09)	0.07 (0.05-0.09)	1.00
ACQ-5	1.38 (0.92-1.84)	1.96 (1.54-2.38)	0.07

Data presented as means (95% CI)

patients all taking inhaled corticosteroids (20% taking long acting beta-agonists) were included with a mean: age 39.2 year FEV<sub>1</sub> 88.4%, FEF<sub>25-75</sub> 55.5%, R5%162%, R5-R20 0.07 kPa/l/s, ACQ-5 1.70

**Results** 48% (n = 48) had 1 or 2 copies of the Arg allele (i.e. Arg/Arg or Arg/Gly genotypes) while 52% (n = 52) had no copies of the Arg allele (i.e. Gly/Gly genotype). There was no significant difference between genotypes in terms of FEV<sub>1</sub>, FEF<sub>25-75</sub>, R5, R5-R20 or ACQ. Furthermore there was no significant effect of LABA according to Arg/Gly polymorphism.

## **REFERENCE**

1. Manoharan A, Anderson WJ, Lipworth BJ. Influence of 2-adrenergic receptor polymorphism on methacholine hyperresponsiveness in asthmatic patients. *Ann Allergy Asthma Immunol* 2013;110: 161-164

# **P193 A ROLE FOR ACTIVE VITAMIN D IN STEROID RESISTANT ASTHMA PATIENTS WHO HAVE ENHANCED PRODUCTION OF IL-17A AND REDUCED IL-10**

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10.1136/thoraxjnl-2013-204457.345

**Background** Steroid refractory (SR) asthma, a distinct disease phenotype, has a high morbidity and mortality and takes up a disproportional burden of healthcare cost. IL-17A is a pro-inflammatory cytokine that is essential for host defence against pathogens but can also lead to damage of the surrounding tissues associated with immune diseases and is linked with severe asthma. IL-10 has crucial immunoregulatory properties, and we have previously shown *in vitro*, that T cells from steroid refractory asthma patients fail to respond to glucocorticosteroids for the induction of IL-10 synthesis.

**Methods** We assessed IL-17A and IL-10 synthesis in steroid sensitive, SS, (mean% change in FEV<sub>1</sub> following 2 weeks of oral prednisolone 16%) versus SR (mean% change FEV<sub>1</sub> 0%) asthma patients and investigated their response to dexamethasone.

**Results** PBMC from SR individuals synthesised 7-fold higher levels of IL-17A than disease-severity matched SS patients (by flow cytometry and CBA). Interestingly IL-17A levels inversely correlated with changes in lung function following oral steroids whereas higher IL-10 levels were associated with an increase in lung function. Dexamethasone failed to inhibit IL-17A, but, surprisingly, increased protein synthesis, an effect that was also seen *in vivo*: inhaled glucocorticosteroid dosages correlated with IL-17A protein levels. This suggests the potentially detrimental effects corticosteroids might have in certain asthma phenotypes. The production of IL-10 by T cells was impaired in cultures from SR asthmatics, but not in healthy controls or SS asthma patients implying an associated impaired IL-10 response with poor asthma control. 1alpha,25-dihydroxyvitamin D3 (1,25(OH)D) not only restored the capacity of T cells to produce IL-10 upon stimulation with dexamethasone in SR asthma patients, but also inhibited IL-17A synthesis in culture independently of steroid.

**Conclusion** High IL-17A levels are associated with poor response to steroids and more severe asthma. Our data supports a steroid-enhancing property of 1,25(OH)D in severe asthma through inhibition of IL-17A and via enhancement of IL-10 synthesis.