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Joint Respiratory Research Day
4th May 2016

Held in conjunction with Nottingham Centre for Respiratory Research and Leicester Respiratory Biomedical Research Unit

Nottingham Respiratory Research Unit
· Asthma · COPD · ILD · Lung Infection ·

INSTITUTE FOR LUNG HEALTH
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The event from 9.25 – 16.15 has been financially supported through the purchase of exhibition trade space by the following companies, none of whom have had influence on the agenda or speaker selection:

Astra Zeneca, Novartis, Boehringer Ingelheim, Chiesi

Presentations

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ASM behaviour following heating to a range of different temperatures

Ruth Saunders

R Saunders[1], GE Morris[2], IL Chernyavsky[3], RH Smallwood[4], FRAJ Rose[2], BS Brook[3], CE Brightling[1].

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Bronchial thermoplasty is a newly licensed asthma therapy, which can reduce asthma symptoms in the long term, but can result in worsening of asthma symptoms short term. Cellular changes that occur following thermoplasty have not been studied widely. In addition, computational modelling suggests that during thermoplasty, the airway is exposed to a *range* of temperatures as the radio frequency energy (65°C, 10 seconds) is only applied to the airway wall where there is direct contact with the probes' four electrodes.

We assessed ASM contraction in the collagen gel assay, α-SMA expression, cell number, viability and IL-8/CXCL-10 release by ELISA following heating (37-65°C, 10 seconds). Collagen gels were impregnated with equivalent ASM cell number ($1.8x10^5$).

For results see Table 1.

Table 1:

		37°C	45°C	50°C	55°C	60°C	65°C
Bradykinin stimulated contraction (AUC 45 min, % increase above control)	24 h	27±2	27±3	19±2	15±3*	14±5*	4±2*
	1 week	17±5	18±5	24±5	8±3	14±6	12±4
α-SMA expression (gMFI % 37°C)	24 h	100	105±7	100±6	104±6	107±7	52±6*
	1 week	100	97±12	97±7	118±6*	108±7	69±11*
Cell number (% 37°C)	1 week	100	108±15	90±9	85±8	87±6	37±10*
Viable cells (% 37°C)	1 week	100	98±2	100±4	94±5	90±6	30±6*
IL-8 release post IL-1β (ng/ml/10^5 cells % 37°C)	1 week	100	96±12	88±13	97±10	95±12	192±29*
CXCL-10 release post IFNγ (Δ ng/ml/10^5 cells % 37°C)	1 week	100	110±13	94±10	96±9	97±9	144±21

*p<0.05 vs 37°C, n=3-9, mean±sem.

The transient increase in ASM contractility (55-65°C) and increased ASM inflammatory mediator release (65°C) could have a detrimental effect on asthma symptoms. Heating to 65°C does reduce ASM cell number/viability, however whether the ASM *in vivo* is exposed to sufficient heat during thermoplasty to significantly reduce ASM mass is uncertain.

Functional genetics of lung function associated gene *GPR126*

Robert Hall

Robert Hall, Ian Hall & Ian Sayers

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Rationale: Genome wide association studies (GWAS) have identified several loci associated with lung function and Chronic Obstructive Pulmonary Disease. The chromosome 6q24 locus has reproducibly been associated with lung function in GWAS. The association signal from the recent 1000 genomes SpiroMeta paper which provided greater resolution in this region was from a haplotype encompassing a non-synonymous SNP (rs17280293, Ser123Gly MAF = 2.9%) in *GPR126*.

Objectives: We aim to further define the role of the G-protein coupled receptor GPR126 in the lung and determine the effects of the amino acid change (Ser123Gly) on GPR126 function.

Methods: *In silico* analysis, using SIFT and PolyPhen-2 was carried out to assess the effects of the amino acid change. We also performed a qPCR assay on a range of tissues and lung derived cells to profile GPR126 mRNA expression.

Results: GPR126 mRNA is expressed in whole lung tissue and in airway relevant cells including bronchial epithelial and airway smooth muscle cells. . Ser123Gly lies in the CUB domain of GPR126, a domain which is important for binding collagen type IV. *In silico* predictors suggested that this is a damaging polymorphism.

Conclusion: GPR126 is expressed in the lung and harbours a variant, Ser123Gly, which is associated with lung function and may have damaging effects on protein function. Ongoing studies aim to use an overexpression system to measure downstream s GPR126 signalling activity in Ser123 and Gly123 receptor variants.

The lung fungal microbiome in patients with severe asthma

Eva-Maria Rick

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Rationale
The lung fungal microbiome (mycobiome) in asthma is underestimated due to insensitive detection methods.

Objective
To use amplicon-based high-throughput sequencing (HTS) to determine the lung mycobiome.

Methods
Patients with asthma were classified into three groups: (1) IgE-sensitised to *Aspergillus fumigatus,* (2) IgE-sensitised to non-*A. fumigatus* fungi, and (3) not fungal sensitised. A fourth group comprised healthy controls. All subjects provided sputum and a subset underwent bronchoscopy. DNA was extracted and the internal transcribed spacer region 2 of the fungal nuclear ribosomal operon amplified and subjected to paired-end sequencing on the Illumina MiSeq platform. Bioinformatic analysis was performed using QIIME.

Results

Preliminary data from 61 sputa and 14 bronchoscopy samples revealed >350 species from >200 fungal genera. Fourteen species were present in >50% of sputum samples, dominated by *A. fumigatus*, *A. niger, Candida albicans* and *Cladosporium* spp. The number of species detected per individual was highly variable (4 to 93 species) with a trend towards greater diversity from sensitised asthmatics (median diversity group 1 to 4 respectively; 33.5, 34.0, 23.0 and 23.0). *Candida dubliniensis* and *Hyphodontia radula* were among the fungi detected that were more prevalent and at greater relative sequence abundance in asthma compared to healthy controls. The top five prevalent species were consistent between sputum, bronchial wash, bronchial brushings and bronchial lavage.

Conclusions
HTS is a sensitive method to assess the lung mycobiome. The main fungal species detected were comparable between different patient groups and between different sample types, however, fungal diversity appeared to be higher amongst patients sensitised to fungi.

Comparative Validation of Community Assessment Tools (CATs) vs. CRB-65 for Severity Assessment of Acute Lower Respiratory Tract Infections (ALRTI) in Adults: an external validation study

Ruby Armon

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Background: CATs is a recently developed hospital admission triage tool for influenza and pneumonia, and it is made up of 7 criteria (A: severe respiratory distress, B: increased respiratory rate, C: oxygen saturation ≤ 92%, D: respiratory exhaustion, E: severe clinical dehydration or shock, F: altered consciousness level, G: causing clinical concern to the clinician), any one or more of which is expected to trigger hospital admission.

Objectives: Evidence on the predictive performance of prognostic tools across a range of ALRTIs in resource-poor settings is limited. A multicentre study was conducted to validate CATs and compare its performance with that of the British Thoracic Society's CRB-65 in cases of suspected ALRTI in Nigerian adults.

Methods: This was a prospective cohort study; data specific to the CATs and CRB-65 tools were recorded on initial consultation. The performance of both tools with regard to predicting the need for acute care interventions (supplemental oxygen, IV antibiotics and IV fluids), or any deaths on admission were assessed using Area under Receiver Operating Characteristic Curves (AUROC) with 95% confidence intervals, and Hosmer-Lemeshow goodness-of-fit test.

Results: A consecutive sample of 207 (57.5% female) patients with a median age of 42 (interquartile range 31 to 58) years were enrolled between December 2013 to July 2014.CATs predicted all study outcomes better with higher AUROC (95%CI) values ranging between 0.72-0.92 (0.65-0.95), compared to the CRB-65 which had AUROC values ranging between 0.68-0.76(0.62-0.86).

Conclusion: CATs performs better than CRB-65 for severity assessment of adult patients with ALRTIs in a low-resource setting.

Investigating Signal Transduction in Type 2 Innate Lymphoid Cells

Cate Weston

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Type 2 innate lymphoid cells (ILC2) are a non-B non-T cell source of the type 2 cytokines, IL-13, IL-5 and IL-4 and are thought to contribute to the airway inflammation observed in asthma exacerbations [1]. ILC2s respond to epithelial-derived cytokines IL-33 and IL-25. Despite the apparent overlapping functions of IL-33 and IL-25 in regulating ILC2 activity [2], we have found, using microarray analysis, that the two cytokines influence distinct subsets of genes including the transcription factor c-myb. In this study we wished to determine the role of c-myb in ILC2 signal transduction.

ILC2s, identified as lineage (CD2/3/14/16/19/56/123/235a) negative, CRTh2 positive cells, were obtained from human PBMCs using antibody-linked magnetic selection and cultured *in vitro* for up to 6 weeks. Cultured ILC2s were transduced 1 day after isolation with lentiviral particles containing shRNA to reduce myb expression. ILC2s were stimulated with PMA/ionomycin (5 and 500 ng/ml respectively) and cytokine release determined by flow cytometry.

Enriched ILC2s remained a non-T-cell source of type 2 cytokines throughout the culture period. The introduction of c-myb shRNA reduced the level of the protein by >90%. Reduction in c-myb expression inhibited the rate of ILC2 proliferation and significantly affected the production of inflammatory mediators such as IL-4 ($p<0.01$, $n=3$).

These results demonstrate that ILC2s can be isolated and cultured from human blood. The transcription factor c-myb is required by ILC2s for both proliferation in response to IL-33 and the production of inflammatory cytokines. These data provide mechanistic evidence for differential responsiveness by ILC2s to the cytokines IL-25 and IL-33.

1. Jackson *et al*., (2014) *Am J Respir Crit Care Med* **190**: 1373–1382.

2. Neill *et al*., (2010) *Nature* **464**: 1367-1371.

Inhalation of diesel exhaust and allergen alters human bronchial epithelium DNA methylation

Rachel Clifford

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Background: Allergic disease affects 30-40% of the world's population and its development is determined by interplay between environmental and inherited factors. Air pollution, primarily consisting of diesel exhaust emissions, has increased at a similar rate to allergic disease. Exposure to diesel exhaust may play a role in the development and progression of allergic disease, in particular allergic respiratory disease. One potential mechanism underlying the connection between air pollution and increased allergic disease incidence is DNA methylation, an epigenetic process with the capacity to integrate gene/environment interactions.

Objective: To investigate the effect of allergen and diesel exhaust exposure on bronchial epithelial DNA methylation.

Methods: We performed a randomized crossover-controlled exposure study to allergen and diesel exhaust in humans, and measured single site (CpG) resolution global DNA methylation in bronchial epithelial cells.

Results: Exposure to allergen alone, diesel exhaust alone, or allergen and diesel exhaust together (co-exposure) led to significant changes in 7 CpG sites at 48 hours. However, when the same lung was exposed to allergen and diesel exhaust but separated by approximately four weeks, significant changes in over 500 sites were observed. Furthermore, sites of differential methylation differed depending on which exposure was experienced first. Functional analysis of differentially methylated CpG sites found genes involved in transcription factor activity, protein metabolism, cell adhesion and vascular development, among others.

Conclusion: These findings suggest that specific exposures can prime the lung for changes in DNA methylation induced by a subsequent insult.

Respiratory cilia and epithelial abnormalities are features of Primary Ciliopathies

Robert Hirst

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Background: A ciliopathy is a genetic disorder of primary non-motile cilia. Although over one hundred ciliopathies are recognised they are rare with multiple systems affected. Respiratory problems in primary ciliopathies have been noted but little is known of the involvement of the ciliated respiratory epithelium.

Aims: To review the structure and function of motile cilia in patients with Bardet Beidl (BB), Alstroms (AS), Oral-facial-digital (OFD1) and Sensenbrenners (SBS) syndromes referred to our national PCD diagnostic service because of chronic respiratory symptoms.

Methods: Nasal brush biopsies were taken from 6 BBS, 9 AS, 2 OFD1 and 1 SBS. The cilia were visualised by a light microscope and recorded using a high speed video. Transmission electron microscopy was used to visualise the epithelial and ciliary ultrastructure. The recordings were analysed in a blinded fashion and were replayed at reduced rate for determination of ciliary beat frequency, cilia beat pattern. Ciliated cell culture was performed and where successful tests were repeated.

Results: The respiratory epithelial cilia of all patients were motile with ciliary beat frequency ranging from 7 to 15Hz. Epithelial abnormalities included extremely dense microvilli, cystic ciliary (Figure 1) tips, swollen cilia membranes along the shaft and microtubular disarrangement. Racket shaped microvilli and very long motile flagella were seen. These were not seen in epithelial samples from patients with a very wide spectrum of respiratory disease.

Conclusion: Patients with primary ciliopathies presenting with chronic respiratory problems may have specific epithelial and ciliary abnormalities. The significance of these findings remains unclear.

Coding region variation in *IL1RL1* regulates receptor response to IL33 and IL33/ST2 blocking approaches

Michael Portelli

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Rationale
Several co-inherited single nucleotide polymorphisms (SNPs) present in the TLR/IL1R (TIR) signalling domain of the IL33 receptor (IL1RL1/ST2) (Glu501Arg/Thr549Ile/Leu551Ser) are associated with asthma and may modify cellular responses to IL33 and the efficacy of targeting IL33 for clinical benefit.

Objectives
To identify whether expression of *IL1RL1* haplotypes in an NFKβ/AP1 reporter cell line (HEK-SEAP) affects IL33 driven signalling and response to IL33 and ST2 blocking approaches.

Methods
HEK-SEAP cells were transiently transfected to express different ST2 coding region variation in the TIR domain, i.e. risk (Glu-Thr-Leu) or common (Arg-Ile-Ser) haplotypes, as well as the empty vector (pCDH-EF1-MCS-T2A-copGFP). Following 24hrs, each cell population was treated with anti-IL33, anti-ST2 or media for 1hr, followed by IL33 stimulation (0.01ng/ml-50ng/ml). After 24hrs cell supernatants were collected and NFKβ/AP1 driven SEAP expression was determined using a colorimetric SEAP assay (n=3).

Results
A dose dependent response to IL33 was observed in both common and risk haplotypes, however response to IL33 increased twofold in the risk haplotype when compared to the common at a concentration range of 10ng/ml-50ng/ml (P<0.001). A statistically significant response to IL33 and ST2 blocking was observed in the risk haplotype, reducing SEAP reporter levels to those observed in the common haplotype (P<0.001). No effect was observed in the common haplotype (P>0.05).

Conclusions
The presence of the ST2 risk haplotype leads to an exaggerated NFKβ/AP1 signalling response to IL33 which is more amenable to blocking strategies. Carriers of the risk haplotype could therefore have a more pronounced IL33 driven inflammatory response and be more receptive to anti-IL33/ST2 therapy.

Funding - Asthma UK

The role of WNT5a in Non-Th2 asthma

Tariq Daud

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Background
Asthma maybe characterised by distinct tissue molecular phenotypes (Choy, Hart et al. 2015). However the process of repair and remodelling remains ambiguous in this context.
WNT5a acting through the non-canonical axis exhibits functional cross-talk with TGF-β1, which may influence repair and remodelling.

Aims and objectives
We sought to evaluate expression of WNT5a and TGF-β1 in the asthmatic epithelium, stratified by pathological phenotype and whether WNT5a potentiates epithelial repair and remodelling via cross talk with TGF-β1.

Methods
Endobronchial biopsies and brushes from a previously described cohort of subjects (9 healthy and 23 asthmatics) in whom the gene expression profiles of TGF-β1, WNT5A and Th2 / Th17 asthma were evaluated. Tissue Sections were immunostained for WNT5a and TGF-β. Cultured BEAS-2B epithelial cells were evaluated for markers of epithelial to mesenchymal transition (EMT) and SMAD2/3 nuclear translocation post stimulation with TGF-β1 [10ng/ml] or WNT5a [1ug/ml].

Results
Quantitative thresholding displayed an increase in epithelial WNT5a in asthma (p=0.0085). Interestingly this was constrained to patients with a Th17 gene expression endotype (p=0.0188). Additionally, we found a significant correlation between TGF-β and WNT5a immunostaining (but not gene expression) in the epithelium (R^2=0.5818, p=<0.0001).
Both WNT5a (R^2=-0.42, p=0.039) and TGF-β1 (R^2=-0.45, p=0.025) gene expression in the epithelium were independent predictors of airway hyper responsiveness (Model R^2=0.364, WNT5a β=-0.407; p=0.026. TGF-β1 β=-0.437; p=0.017).
Stimulation of BEAS-2B cell with TGF-β1 increased the expression of markers of EMT, WNT5a and ROR2 expression. Similarly, both TGF-β1 and WNT5a were shown to induce SMAD2/3 nuclear translocation, which was inhibited by Box-5.

Conclusions
WNT5a protein is increased in the airway epithelium in patients with asthma displaying a mucosal Th17-dependent gene expression signature. Additionally, we show potential in vitro evidence of TGF-β1-WNT5a cross-talk via the SMAD2/3 axis.

Identification of novel urokinase plasminogen activator receptor function using gene set enrichment analysis

Sangita Bhaker

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Rationale

Expression of the urokinase plasminogen activator receptor (uPAR) is elevated in the airway epithelium in asthma. We hypothesise that the uPAR pathway regulates bronchial epithelial homeostasis. We set out to determine the effect of elevated uPAR variants on human bronchial epithelial cells (HBECs).

Methods

HBECs were engineered to overexpress; i) membrane uPAR (muPAR), ii) a soluble spliced variant (ssuPAR) and iii) a soluble cleaved variant (scuPAR). An empty vector (pcDNA3) control was included. RNA was collected 24 hours post transfection for transcriptomic analyses using RNA-seq. Gene Set Enrichment Analysis was performed using the MSigDB C2 curated gene set collection to assess biologically relevant pathways (FDR<25%).

Results

Overexpression of muPAR and scuPAR led to the enrichment of 150 and 26 pathways, respectively. The Kegg Ribosome pathway (muPAR; enrichment score (ES) 0.77 q <0.01) and Epidermal growth factor targets (scuPAR; ES 0.60 q 0.097) were the most enriched gene sets and both play key roles in cell growth. Pathways upregulated by ssuPAR did not reach 25% FDR in this analysis. Five gene sets were downregulated by muPAR, 29 by scuPAR and 55 pathways by ssuPAR. The most suppressed gene sets regulate cell proliferation and response to viral infections. Pathways consisted of genes downregulated by NRAS signalling (scuPAR; ES -0.58 q 0.08), heparin sulphate biosynthesis (muPAR; ES -0.66 q 0.1) and response to IFNα (ssuPAR; ES -0.75 q<0.01).

Conclusions

In summary these results uncover novel and distinct roles played by uPAR isoforms in HBECs with implications for asthma where uPAR has been shown to be elevated.

Funding: University of Nottingham

Temporal response of muscle mRNA expression to endurance training in chronic obstructive pulmonary disease (COPD)

Lorna Latimer

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Compared to healthy age-matched controls (HC), patients with COPD have impaired muscle metabolism and function, which contributes to exercise intolerance. We aimed to determine whether the temporal transcriptional response of muscle to endurance exercise training in COPD was different to HC, and if so whether any differences could be associated with the magnitude of physiological training adaptation ($\dot{V}O_2^{PEAK}$).

Nineteen COPD patients and 10 HC, none of whom exercised regularly, performed symptom limited incremental cycle ergometry, lean mass assessment (DEXA), physical activity monitoring (tri-axial accelerometery) and underwent resting (fasted state) vastus lateralis muscle biopsies at baseline and after 1, 4 and 8 weeks of supervised cycle exercise training (30min 65% peak power, 3xweek). mRNA was extracted from vastus lateralis tissue and the expression of 94 genes, selected because of their known responsiveness to exercise intervention, was assessed using quantitative RT-PCR. Gene expression data were analysed using Ingenuity Pathway Analysis (IPA; Qiagen) to identify significantly changed biological functions.

Altered biological functions were apparent at weeks 1, 4 and 8 in both groups. A similar response was observed in both groups for networks representing energy production and fuel selection; muscle and connective tissue development and function; and free radical scavenging and inflammation. $\dot{V}O_2^{PEAK}$ increased 15% in the HC group ($p<0.01$) but was unaltered in the COPD group ($p=0.62$) following training.

Both HC and COPD groups experienced similar temporal transcriptional responses to endurance exercise training. Furthermore, functions of gene networks found to be significantly altered were similar. Changes in mRNA expression were not however associated with increases in whole-body $\dot{V}O_2^{PEAK}$ in response to training, with COPD patients showing a clear transcriptional response, but no increase in $\dot{V}O_2^{PEAK}$. These data support the contention that the responsiveness of skeletal muscle to exercise training in COPD is not blunted, at least at the level of mRNA expression.

Expression and Activity of Cathepsin K in Lymphangioleiomyomatosis (LAM)

Arundhati Dongre

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Lymphangioleiomyomatosis is a neoplastic cystic lung disease of women leading to lung destruction, respiratory failure, death. Cyst formation is thought to be a consequence of dysregulated protease expression. The underlying cause of LAM is loss of TSC2 function, resulting in constitutive activation of the mTOR complex. Genotyping analyses suggest that cells without TSC2 loss are common within LAM nodules. We propose that the interaction between wild type and neoplastic cells leads to the production and activation of matrix-degrading proteases. We therefore examined protease expression in LAM tissue and *in vitro* co-culture models.

Whist matrix metalloproteinases (MMP) 1, 2, 3, 9, 13 and ADAM 17 were expressed in LAM lung, only cathepsin K gene expression was significantly upregulated when compared with control tissue ($p<0.0001$). Further, cathepsin K protein was observed in LAM lung nodules, but not normal lung using immunohistochemistry. In culture, cathepsin K transcript was strongly expressed in LAF but not 621-101 cells and cathepsin K activity was detected in live LAFs in culture. Extracellular cathepsin K activity is present in LAF culture supernatants which is pH dependent. Cathepsin K activity is increased by almost 40% when LAFs are co-cultured with *TSC2*$^{-/-}$ 621-101 cells ($p\leq0.05$).

Cathepsin K is expressed in LAM nodules by LAM associated fibroblasts and its activity enhanced by the presence of TSC-2$^{-/-}$ cells. This potent matrix degrading protease may play a role in LAM. Further work is required to determine if cathepsin K is a suitable target for therapy in LAM.

Phenotyping Airway Pathology in Adult Asthma Using Topological Data Analysis

Aarti Parmar

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Rationale: Airway remodelling and cellular inflammation are well recognised pathological features of asthma. However the relationship between clinical asthma phenotype, airway pathology and gene expression is poorly understood.

Objectives: We examined common pathological features in adult asthmatic bronchial biopsies to identify whether discrete 'pathological phenotypes/subtypes' map to clinical characteristics and in a subset of patients biopsy gene expression and lung function decline.

Methods: 202 patients (142 asthma/ 60 healthy volunteers) were recruited. Patients underwent bronchoscopy and endobronchial biopsy; bronchial biopsies were evaluated for nine common features of asthma pathology. Topological Data Analysis (TDA), an adaptation of the methods of topology to create compact visual representations of high-dimensional data sets, was applied to the pathological features alone to generate the reported phenotypes.

Results: Three distinct immunopathological clusters were identified, specifically, (i) **Cluster 1**: Late onset eosinophilic, with high tissue and airway smooth muscle (ASM) mast cells, high ASM % area, (ii) **Cluster 2:** Late onset severe eosinophilic asthma, with epithelial and basement membrane remodelling, low ASM % area and the presence of vascular remodelling and (iii) **Cluster 3:** Early onset eosinophilic asthma (tissue and sputum), with high mast cells in the ASM, intermediate ASM% area and vascular remodelling. A number of novel genes were significantly associated with remodelling phenotypes in tissue and the phenotypes reported had differing FEV_1 change trajectories.

Conclusion: We have identified novel pathological phenotypes of asthma with differing features of airway remodelling, cellular inflammation and airway function. Asthma may be characterised by variable pathological phenotype warranting further evaluation

Impact of outpatient neuraminidase inhibitor treatment in patients infected with influenza A(H1N1)pdm09 at high risk of hospitalisation: an IPD analysis

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Rationale: The impact of outpatient/community-based neuraminidase inhibitor (NAI) treatment on hospital admission has not been established.

Objectives: To investigate the association between outpatient/community-based NAI treatment and hospital admission in patients with A(H1N1)pdm09 infection.

Methods: We obtained data collected between January 2009 and December 2010 by nine individual study centres in different countries (n=6,024 patients) on patients with laboratory-confirmed or clinically-diagnosed A(H1N1)pdm09 influenza from the general community and outpatient clinics. We created a standardised, pooled dataset, and performed an Individual Participant Data (IPD) meta-analysis using generalised linear mixed modelling adjusting for NAI treatment propensity and pre-admission antibiotic use, with “study centre” as a random intercept to account for differences in baseline hospitalisation between centres.

Results: Of 6,024 patients, 5,732 (95.15%) had laboratory-confirmed influenza A(H1N1)pdm09, and the remainder were clinically-diagnosed. 1,536 patients (25.50%) received outpatient/community-based NAI treatment. 4,280 (71.05%) hospitalisations occurred, indicating a population at overall high-risk of influenza-related hospitalisation. After adjustment, outpatient/community-based NAI treatment was associated with decreased odds of hospital admission compared to no NAI treatment in the overall study population (OR: 0.36, 95% CI: 0.31 to 0.42), in adults (OR: 0.34, 95% CI: 0.27 to 0.43), in children (OR: 0.43, 95% CI: 0.35 to 0.54) and in high-risk patients (0.17, 95% CI: 0.12 to 0.23). Early NAI treatment (≤2days from symptom onset) was also associated with a decreased odds of hospital admission when compared to later NAI treatment.

Conclusion: In a population with confirmed/suspected A(H1N1)pdm09 at high risk of hospital admission, outpatient/community-based NAI treatment significantly reduced the likelihood of requiring hospital admission.

Posters

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A CEACAM-6-high airway neutrophil phenotype and CEACAM-6-high epithelial cells are features of severe asthma

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Severe treatment-resistant asthma represents a major unmet clinical need and understanding the pathophysiology is essential for the development of new therapies. Gene expression microarrays have the potential to uncover novel molecular targets. Using microarray analysis we previously found three immunological clusters in asthma: T_H2-high, T_H17-high, and $T_H2/17$-low. Although new therapies are emerging for Th2-high disease, identifying molecular pathways in Th2-low disease remains an important goal. Further interrogation of our previously described microarray dataset revealed upregulation of gene expression for carcinoembryonic antigen cell adhesion molecule (CEACAM) family members in the bronchi of patients with asthma. Our aim was therefore to explore the distribution and cellular localisation of CEACAM-6 using immunohistochemistry on bronchial biopsy tissue obtained from patients with mild, moderate and severe asthma, and healthy controls. In addition, soluble CEACAM-6 was measured in sputum and blood by ELISA. Human bronchial epithelial cells were used to investigate cytokine and corticosteroid *in vitro* regulation of CEACAM-6 gene expression. CEACAM-6 gene expression in bronchial biopsies was increased in severe asthma compared to healthy controls. CEACAM-6 protein expression was increased in airway epithelial cells and lamina propria inflammatory cells in severe asthma compared to healthy controls. CEACAM-6 in the lamina propria was localised to neutrophils predominantly, but also eosinophils and macrophages. Neutrophil density in the bronchial mucosa was similar across health and the spectrum of asthma severity, but the percentage of neutrophils expressing CEACAM-6 was significantly increased in severe asthma, suggesting the presence of an altered neutrophil phenotype. CEACAM-6 gene expression in epithelial cells was not modulated by IL-13, IL-17 or dexamethasone. In summary, CEACAM-6 expression is increased in severe asthma and primarily associated with airway epithelial cells and tissue neutrophils. CEACAM-6 may contribute to the pathology of treatment-resistant asthma via neutrophil and airway epithelial cell-dependent pathways.

Mouse airway smooth muscle cells express P2X receptors.

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Rationale: The contribution of extracellular nucleotides to airway smooth muscle (ASM) contraction is unknown.

Objectives: To determine the expression levels of the P2X receptors in mouse tracheal and lung ASM cells.

Methods: The trachea and the lungs from C57BL6/J mice were freshly isolated after the mice were culled by schedule 1 (exposure to isofluorane followed by increasing concentrations of CO_2). P2X receptor transcript expression levels in ASM were measured by qPCR and normalised to 3 reference genes (*Ppia, Nono* and *Tbp* in trachea and *GusB*, *B2m* and *Tbp* in lung). The expression of P2X1, P2X4 and P2X7 receptor proteins was assessed by western blot. The tissue and cellular localisation of these 3 P2X receptor subtype proteins were further investigated using immunohistochemistry (DAB).

Results: Quantitative PCR experiments gave the P2X receptor transcript expression profile P2X4 >> P2X6 ≈ P2X7 >> P2X5 > P2X1 ≈ P2X2 ≈ P2X3 (n=4) in both tissue types. In addition, P2X1, P2X4 and P2X7 receptor proteins were detected in these tissues by western blot (n=3). Immunohistochemistry experiments showed that P2X1, P2X4 and P2X7 receptor proteins co-localise with the ASM in these tissues, whilst P2X4 and P2X7 receptor proteins also co-localise with the epithelium (n=3).

Conclusion: Mouse tracheal ASM cells express both P2X receptor transcripts and protein (in particular P2X1, P2X4 and P2X7).

A role for Interleukin-15 in Human Type 2 Innate Lymphoid Cell Function?

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Rhinoviruses are the most common cause of viral exacerbations of asthma. Infection of human bronchial epithelial cells (HBECs) by rhinovirus induces production of the T-helper 2 (Th2) cytokines Interleukin (IL)-25 and IL-33. Group 2 innate lymphoid cells (ILC2s) produce IL-13 and IL-5 in response to IL-25 or IL-33 in combination with common gamma chain cytokines (e.g. IL-2, IL-7). ILC2s and HBECs express CRTh2, the receptor for Prostaglandin D_2 (PGD_2) as such it may play an important role in asthma exacerbations.

We aim to investigate the relationship between rhinovirus infection, HBECs and ILC2s to identify the molecular and cellular interactions that occur. In particular the nature and source of common gamma chain cytokine and the role of PGD_2/CRTh2.

ILC2s were identified in human peripheral blood as Lineage-, CD123-, CRTh2+ cells. The cells were further phenotyped as $CD45^{Hi}$, CD127+, CD161+, CD25+, $c\text{-}kit^{int}$, $KLRG1^{int}$ and $CD126^{int}$. IL-15 is a common gamma chain cytokine that is increased in humans during rhinovirus infection. Multicolour flow cytometry was used to examine the effect of IL-15 on human peripheral blood ILC2s. Based upon phosphorylation of STAT5, ILC2s were unresponsive to IL-15 treatment directly *ex vivo*; including co-stimulation with IL-15Rα. Similarly, ILC2s did not express IL15Rα (CD215) *ex vivo*. However, stimulation with IL-33 in a purified cell culture of ILC2s leads to an increase of IL15Rα mRNA. Further experiments will investigate combinations of cytokine stimulations to examine IL-15 responsiveness of human ILC2s.

Physical activity heterogeneity in COPD; meaningful analysis of data

Linzy Houchen-Wolloff

Chris Newby, Linzy Houchen-Wolloff, Karam Aboud, Bethan Barker, Leena George, Maria Shelley, Karen Edwards, Chris Brightling, Sally Singh.

INTRODUCTION: Physical Activity (PA) is an important factor to measure in patients with COPD (chronic obstructive pulmonary disease) because low PA is associated with poorer long-term outcomes. However, the optimum way to analyse and report PA data; or if PA is associated with other clinical characteristics, is unknown in COPD.

METHOD: We explored PA within a secondary care COPD cohort (COPDMAP). Sensewear Armband PA monitors (SWM) were used to obtain readings over 7 days for 55 patients. Clinical characteristics including spirometry were also recorded.

PA variables were used in a factor analysis to determine underlying patterns of activity within the cohort and verified in two further separate cohorts.

RESULTS: PA variables were positively correlated with each other ($p<0.05$). Two underlying factors were found, these corresponded to:

1. 'General Daily Activity', 'GDA'associated with the number of 2 min bouts of moderate activity in 24hr, factor loading (FL) =0.94.

2. 'Purposeful Physical Activity', 'PPA' associated with number of 20 min bouts of moderate activity per 24 hours, FL=0.84.

Variable	2 minute bouts		20 minute bouts	
	r	p-value	r	p-value
Post Bronchodilator Forced Expiratory Volume (FEV1)	0.34	0.001	0.47	0.001
Inspiratory Capacity (IC)	0.35	0.017	0.41	0.004
Post Bronchodilator Forced Volume Capacity (FVC)	0.26	0.082	0.47	0.001
6 Minute Walk Test (6MWT)	0.60	<0.001	0.46	<0.001
Hb levels in blood	0.01	0.928	0.31	0.036

Clinical correlates of 2 min and 20 bouts of moderate activity per 24hours can be seen in the table.

CONCLUSIONS: We have found two distinct types of activity within a secondary care COPD cohort; that of 'General Daily Activity' 'GDA' and 'Purposeful Physical Activity', 'PPA'. These have distinct clinical associates.

Relationship between small airways obstruction and exhaled particles in asthma

Marcia Soares

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Background: Asthma is characterised by dysfunction of the small airways compartment, however its mechanism is not fully understood. Impulse oscillometry (IOS) may be utilized to evaluate small airways dysfunction and has been carefully validated by physical and mathematical models within our group.

Aims: Using a novel approach -Particles in Exhaled Air-PEx (http://pexa.se/en/), we sought to a) sample and analyze exhaled particles and relevant small airway proteins in asthma and b) relate protein concentrations with small airway dysfunction.

Methods: 102 adult patients with asthma (GINA 1-5) were recruited from Glenfield Hospital in Leicester. Markers of small airway dysfunction were evaluated from IOS (R5-R20) after administration of 400mcg of Salbutamol. PEx was collected using a reported technique and analysed with ELISA, for surfactant protein A (SpA) and Albumin. Protein concentration in ng/ml was corrected for the ng of PEx extracted, to give a normalized % of SpA and albumin.

Results: PEx maneuvers were easy to perform, taking 11 minutes in average. 97% and 94% of samples were above the lower limits of quantification for SpA and albumin, respectively. Significant inverse correlations where observed between absolute value of R5-R20 and %SpA and %albumin ($p<0.001$). The accuracy of %albumin to distinguish between patients with normal and abnormal R5-R20 was good, measured by Youden Index (YI=0.51) using the receiver operating curve; similar results were found with %SpA.

Conclusion: We report that PEx collection and analysis is feasible in the presence of airway obstruction. Furthermore, SpA and albumin concentrations can be a predictor biomarker of small airway dysfunction.

Face-masks: A Sampling Route of Lower Respiratory Tract

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Rationale:

We aim to determines most effective breathing patterns in collecting air particles of distal airways using a locally-developed face mask system that, once optimized, will be applied for patients with pulmonary tuberculosis and other lung sepsis as a rapid, non-invasive and point of care testing in the diagnosis of lower respiratory tract infections.

Methodology:

A set of 110 clinical face-mask samples were collected from 15 healthy volunteers during four main activities over 15-, 30- and 60-minute intervals:

A. Normal breathing pattern

B. Instructed breathing pattern

C. Intermittent coughing

D. Reading-out loud

Results:

The samples were quantifiable for universal 16S signals in the range of 10^6-10^8 copies and their phylogenetic identity was characterizable into six main groups: Actinobacteria, Firmicutes, Bacteroidetes, Betaproteobacteria, Gammaproteobacteria and Fusobacteria. We were able to differentiate samples of upper and lower respiratory tract through a panel of lung protein biomarkers including albumin and pulmonary surfactant protein-A (SPA) and to correlate that with the expired microbiome component. The effect of signals-degrading factors such as respiratory lysozyme, DNase and protease was detected. Reading-out loud activities are associated with largest quantities of albumin, SPA and Bacteroidetes.

Conclusion:

Bacterial communities habituating a healthy respiratory tract are not aerosolized into equal doses. Their quantities differ according to the breathing pattern, accumulation time and phylogenecity factor. Increasing the sampling interval does not result in a higher yield of bacterial signals whereas it improves the yield of exhaled lung proteins. Predominance of Bacteroidetes in a face-mask sample can potentially be used as a marker of distal-airways origin.

Bronchial Epithelial Expression of ST2: Stratification based on asthma risk genotypes

Michael Portelli

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Rationale
Polymorphisms spanning the IL33 receptor (*IL1RL1*/ST2) have been associated with asthma; however the mechanisms underlying this association remain unresolved.

Objectives
To i) identify whether human bronchial epithelial cells (HBECs) isolated from asthma patients and cultured *in vitro* have increased expression of total, membrane or soluble ST2 and ii) determine the effect of polymorphisms previously associated with asthma or related traits on ST2 expression in asthma patient HBECs.

Methods
HBEC bronchial brushings obtained from 39 asthma and 17 control subjects were cultured and RNA and supernatants collected. mRNA expression was measured using TaqMan® qPCR and soluble protein levels using Luminex assays. All cells were genotyped using TaqMan® genotype assays.

Results
ST2 mRNA and protein levels were not different between asthma and control HBECs (P>0.05). Cells were genotyped for rs10192157 (41% C:C; 43% C:T; 16% T:T), rs1420101 (41% A:A; 41% A:G; 18% G:G) and rs1420091 (48% C:C; 25% C:T; 27% T:T). Stratification identified that rs1420101 determined total (P=0.0034), membrane (P=0.0101) and soluble (P=0.0034) ST2 mRNA and secreted protein levels (P=0.064) (A:A>A:T>T:T, A= asthma risk allele). While not associated with total ST2 mRNA levels (P=0.203), rs1420091 genotype determined both membrane (P=0.038) and soluble (P=0.0034) mRNA and soluble protein (P=0.042) levels (C:C<C:T<T:T, C= improved FEV_1/VC). We identified no association for rs10192157.

Conclusions
ST2 was not differentially expressed in asthma patient HBECs *in vitro*. Stratification on known asthma/lung function risk polymorphisms identified that rs1420101 (coding region, synonymous) and rs1420091 (5'distal) effect ST2 mRNA and soluble protein expression in asthma cells. Ongoing studies aim to determine these finding's functional significance.

Funding - Asthma UK

The relationship between body mass index (BMI) and respiratory muscle strength in healthy children of primary school age

Nidhal Talib

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Rationale:
Previous work in adults has demonstrated that BMI is negatively associated with respiratory muscle weakness in patients with COPD (chronic obstruction pulmonary disease and CABG (coronary artery bypass grafting) (Nishimura, Tsutsumi et al. 1995) and (Iida, Yamada et al. 2010).The present study aimed to investigate the relationship between BMI and maximal inspiratory and expiratory pressure (MIP &MEP) in healthy children.

Objectives:
We sought to determine whether there was any relationship between BMI and respiratory muscle strength in healthy children.

Methods:
We measured spirometry, height and weight, BMI and respiratory muscle strength in children aged 5-11 in their primary schools.

Results:
Ninety-eight children (43 boys and 55 girls) were studied. All subjects had a normal BMI with the exception of two girls with BMI of 31.0 and 11.6. We obtained MIP on 80 and MEP on 87children. No significant association was detected between MIP or MEP and BMI in boys (regressions analysis $p<0.89$, $p<0.08$ respectively). Similarly, in girls no significant association was found between MIP or MEP and BMI (regression analysis $p<0.14$, $p<0.15$ respectively).

Conclusion:
We have not detected any significant associations between BMI and MIP or MEP in healthy children. We cannot rule out an association if our study population were to include a large number of overweight or underweight children, with abnormal BMIs.

STUDIES ON THE ROLE OF Rv2660c DURING STRESS RESPONSES IN MYCOBACTERIUM TUBERCULOSIS

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Rationale: *rv2660c* encoding a conserved hypothetical protein was shown to be a highly up-regulated gene during *in vitro* starvation model and mice infection. However, the biological role of Rv2660c in *Mycobacterium tuberculosis* during growth and persistence has never been fully explored. Outcome of the study will provide some insights into the pathology of tuberculosis and contribute towards understanding the mechanism where Rv2660c enhances the efficacy of a potential vaccine candidate for prevention of TB reactivation

Objectives: To determine *in vitro and in vivo* phenotypic characterisation of deletion and overexpressing strains of both *rv2660c* and *rv2661c* and analyse mRNA expression during non replicating persistence (NRP) and stress responses

Methods: Unmarked single deletion mutants (Δ*rv2660c* and Δ*rv2661c)* and a double deletion mutant (Δ*rv2660c:2661c*) in *M. tuberculosis* were generated and survival in different stress conditions were examined. In addition, qRT-PCR was performed to analyse expression level of *rv2660c* and *rv2661c* in those environments.

Results: Our study confirmed that Rv2660c and Rv2661c are dispensable for growth *in vitro*. Initial findings demonstrated Δ*rv2660c:2661c* strain showed a survival defect during oxidative stress.

Conclusion: Rv2660c and Rv2661c may have an important contribution *M.tuberculosis's* response to stress conditions.

Genome-wide association study into susceptibility to idiopathic pulmonary fibrosis identifies novel loci and replicates *MUC5B* and *DSP* signals

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Rationale: Idiopathic Pulmonary Fibrosis (IPF) is a lung disease with poor prognosis (median survival time of 3 years) characterised by scarring of lung tissue. IPF has been linked with a number of environmental and genetic factors; the strongest genetic association being in the *MUC5B* gene. Despite this the pathogenesis of IPF is still unclear and more needs to be done to understand the genetic basis of IPF.

Objectives: To identify variants associated with susceptibility to IPF.

Methods: A Genome-Wide Association Study (GWAS) was performed to investigate variants across the whole genome. This is the first IPF GWAS in a UK cohort and the first to use a combined reference panel of the latest versions of 1000 Genomes and UK10K reference panels, allowing for dense imputation and analysis of 12,716,843 variants. Analyses were performed on 612 IPF cases and 3,366 controls from UK Biobank passing stringent quality control measures.

Results: 32 regions showed possible associations with susceptibility to IPF including two previously reported loci, namely *MUC5B* (OR=4.11 (95% CI [3.31,5.11]); $p=1.86\times10^{-37}$) and *DSP* (OR=1.67 (95% CI [1.44,1.92]); $p=4.14\times10^{-12}$).

Conclusion: This analysis has identified 32 regions of interest which will be investigated further in independent populations. *MUC5B* produces mucin in the airways and *DSP* encodes desmoplakin (protein in the desmosome, which is responsible for cell adhesion and is important in areas under mechanical stress - such as the lung during fibrogenesis). Results from this analysis will contribute to an international collaboration bringing together unprecedented sample sizes of IPF cases.

Hyperpolarised Xenon-129 MRI of Lungs in Healthy Adult Volunteers

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Introduction
Hyperpolarised xenon-129 MRI (129Xe-MRI) is a novel technique developed to enhance the applicability of MRI in lung imaging.

Aim
We aimed to assess the feasibility and tolerability of 129Xe-MRI in healthy adult volunteers (HV).

Method
This was a single centre prospective observational study. HV were screened for eligibility. Informed written consent was obtained.

After an initial 1L test dose of non-polarised xenon and 30 minutes of observation, we proceeded to 129Xe-MRI. The inhaled volume of hyperpolarised 129Xe ranged 0.6-1.0L. There was 30 minutes of observation after each scan. Each visit comprised of a maximum of four scans.

Results
Eight subjects (male:female 7:1, aged 20-34) underwent 22 scan visits. 129Xe-MRI was well-tolerated, with no serious adverse events.

There was no significant change in oxygen saturations (mean difference 30minutes post-scan (MD30PS) 0.13, standard deviation (SD) 1.26, confidence interval (CI) -0.19 to 0.45, p=0.34) or blood pressure (MD30PS -0.18, SD 7.90, CI -2.27 to -1.91, p=0.06). A statistically significant difference was noted in heart rate (MD30PS -3.47, SD 7.29, CI -5.57 to 01.82, p<0.0001), not deemed clinically significant.

We achieved good image quality. Spectroscopy distinguished lung tissue-dissolved xenon from blood-dissolved. Dissolved phase imaging (DPI) was obtained. The technique was reproducible.

Discussion
The data demonstrates satisfactory feasibility and tolerability of 129Xe-MRI. DPI can image regional gas exchange. 129Xe-MRI may be used to develop biomarkers of disease progression, and assess drug efficacy, to personalise medicine, improving patients' quality of life, reduce healthcare costs, and lower cost and duration of drug development.

Small airway epithelial cells show distinct growth requirements compared to large airway epithelial cells

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Rationale
The bronchial epithelium is functionally impaired in asthma. Less is known about the small airway epithelium (internal diameter <2mm) as sampling is difficult. We aimed to characterise cells obtained via bronchoscopy from both large airways (bronchial epithelial cells (HBECs)) and small airways (small airway epithelial cells (SAECs)) by means of i) growth characteristics and ii) gene expression profiling.

Methods
Normal SAECs (n=2) and HBECs (n=2) were stained for cytokeratin 14 (CK14) using immunofluorescence. Cells were cultured in both small airways growth medium (SAGM™, Lonza) and bronchial epithelial growth medium (BEGM™, Lonza) and growth monitored using MTT cell viability assay and cell counting. A panel of qPCR assays for SAEC markers; SCGB1A1 (CCSP), SCGB3A1, SLPI and BPIFB1 (1) were measured.

Results
Cells stained positive for CK14 demonstrating epithelial lineage. We found that SAECs cultured for 96 hours in BEGM were 76% less viable and proliferative than those cultured in SAGM (SAGM median optical density (OD) 0.17, BEGM median OD 0.04, $p<0.0001$ n=4). We did not observe this medium preference for HBECs (BEGM median OD 0.22, SAGM median OD 0.18, $p=0.67$ n=4). SCGB1A1, SCGB3A1, SLPI and BPIFB1 mRNA levels were at the limit of detection however did show some enrichment in SAECs.

Conclusions
These preliminary data suggest that SAECs and HBECs have distinct growth requirements and are intrinsically different, providing some confidence in our bronchoscopy technique. These data also highlight the difficulty of identifying molecular markers differentiating SAECs and HBECs with certainty however greater numbers of donors are required prior to conclusions.

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Genome-wide association study of PEF and FEF_{25-75} in UK Biobank: an investigation of the utility of studying lung function flow measures

Victoria Jackson

Victoria E Jackson, Louise V Wain, David P Strachan, Ian P Hall, Martin D Tobin

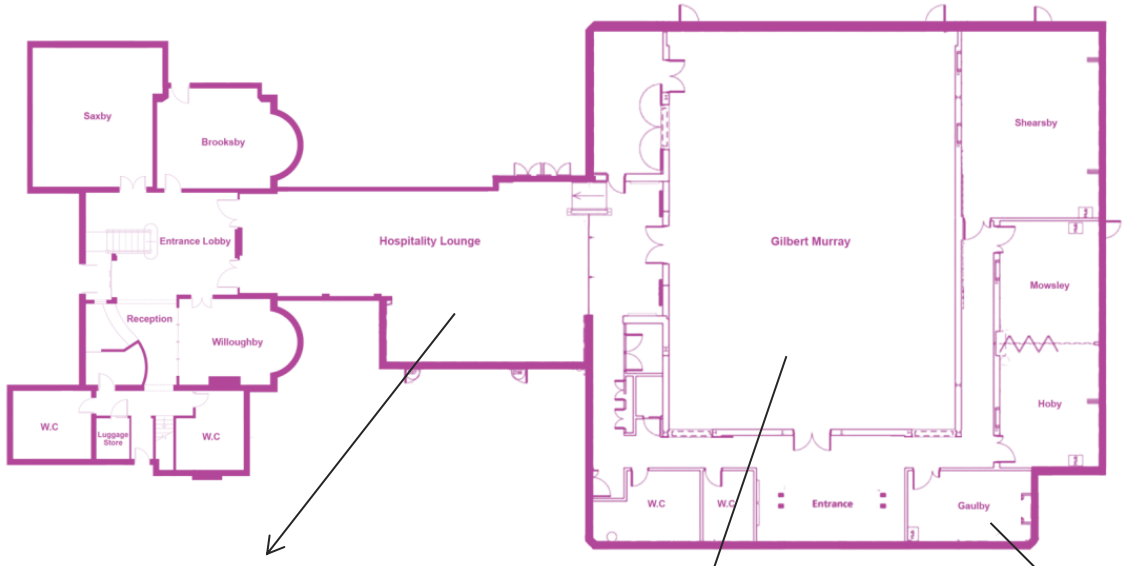
Genome-wide association studies (GWAS) of lung function to date have generally focussed on three volumetric lung function measures: forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC) and the ratio of FEV_1 to FVC (FEV_1/FVC). Other measures that may be derived from spirometry include measures of flow, such as the peak expiratory flow (PEF) and the forced expiratory flow between 25% and 75% of vital capacity (FEF_{25-75}). PEF and FEF_{25-75} are correlated with each other and with FEV_1, FVC and FEV_1/FVC; however each measure varies in terms of clinical significance. We will present the largest GWAS of lung function flow measures carried out to date with the aim of determining whether investigating these measures of lung function can reveal any regions of the genome which might be influencing lung function, which would not be identified through studies of FEV_1, FVC and FEV_1/FVC alone.

These analyses utilise a subset of individuals from UK Biobank with high quality spirometry measures and genotype data imputed to a combined 1000 Genomes and UK10K imputation panel. After undertaking stringent quality control checks on the phenotypes and genotype data, 14,527,158 genetic variants from 102,929 individuals were analysed. We identified a substantial number of variants associated with both PEF and FEF_{25-75}, a large proportion of which were low frequency or rare. Most of the variants associated with one or both of PEF and FEF_{25-75} were also found to be associated with at least one volumetric measure of lung function (FEV_1, FVC and FEV_1/FVC). However there were 10 variants which were identified as showing association with PEF ($P<5x10^{-8}$), but no other lung function trait with $P<5x10^{-5}$. We shall describe the overlap of trait associations and highlight some of the “PEF-specific” signals.

The Venue

Stamford Court

Leicester Conferences
Stamford Court
Manor Road
Leicester
LE2 2LH
(0116) 223 1680
http://www2.le.ac.uk/offices/conference/meeting-spaces-1/oadby-conference-centre-1/stamford-court-1



Refreshments and lunch are served in the Hospitality Lounge

Event will be held in the Gilbert Murray Room

Please leave coats and bags in the Gaulby Room

Essentials

Nearest Train Station: Leicester (LEI)

Sat Nav: LE2 2LH

Wifi: Free wifi available at the venue

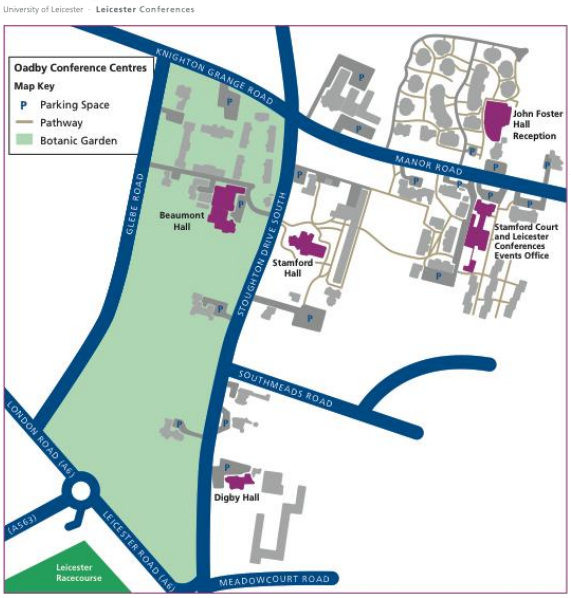
Directions

Car

From Leicester Town Centre: Take A594/Welford Road
Turn left onto A563/ Palmerston Way, 2nd exit onto Leicester Road
Turn left onto Stoughton Drive South and turn right onto Manor Road

From M1: Leave at Exit 21 and take A5460
4th exit onto Narborough Road South/B4114. Left onto Soar Valley Way/A563
2nd exit onto Leicester Road.
Turn left onto Stoughton Drive South and turn right onto Manor Road

Parking



Free Parking at Stamford Court

Bus

Route 80/A: City Centre via Leicester Train Station to Oadby Conference Centres.
Route 31/A: City Centre via Leicester Train Station to Leicester Road (A6) followed by a 5-10 minute walk to the Oadby Conference Centres.
Go to www.travelineeastmidlands.co.uk for public transport information

Taxi

Please ask to go to Oadby Conference Centres – University of Leicester, Stamford Court and not the main campus.
ABC – 0116 255 5111
Swift Fox Cabs – 0116 262 8222
Club Taxis – 0116 231 3001