

## Understanding Airways and Blood Vessels in the Lung

### P234 SPUTUM CYTOKINES AND CLINICAL BIOMARKERS IN SEVERE ASTHMA

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10.1136/thoraxjnl-2016-209333.377

**Introduction** Emerging treatments for type-2 high asthma such as anti-IL-5 (mepolizumab) and anti-IL-4 and IL-13 (dupilumab) target specific cytokine pathways resulting in type-2 inflammation. Whether patients with type 2 inflammation respond equally to both treatment or have distinct IL-13 and IL-5 profiles is currently unclear. We have tested the hypothesis that these pathways may function independently of each other and that simple biomarkers can help differentiate IL-13 and IL-5 high patients.

**Methods** Patients with well characterised, severe asthma were evaluated with the blood eosinophil count and fractional exhaled nitric oxide (FeNO). Patients also had paired measurements of type-2 cytokines in induced sputum samples. Sputum cytokines were measured using a Luminex assay.

**Results** We found that there was no relationship between the blood eosinophil count and FeNO. There was a positive correlation between FeNO and sputum IL-13 ( $r = 0.51$ ,  $p < 0.01$ ) and blood eosinophils and sputum IL-5 ( $r = 0.47$ ,  $p < 0.01$ ).

**Conclusions** These findings suggest that readily available, non-invasive biomarkers may be able to differentiate sub-phenotypes in type-2 high asthma. Post-hoc analysis of clinical trial data of anti-IL-5 and anti-IL-4 and IL-13 treatments based on the predominant clinical biomarker would be of interest to see if these predict response to treatment. Simple biomarkers may be of use in deciding which of the emerging biological treatments to use in severe, type-2 high asthma.

### P235 EPIGENETIC LANDSCAPE OF THE ASTHMATIC AIRWAYS

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10.1136/thoraxjnl-2016-209333.378

The airway epithelium of asthmatics exhibits distinct genomic and phenotypic characteristics. However the mechanisms underlying the establishment and chronicity of these characteristics remains unknown. We investigated if epigenetic changes underpin the genomic characteristics of the asthmatic airways by determining the chromatin landscape of bronchial epithelial cells (BECs) in healthy and asthmatic adults.

We employed ChIP-seq of histone H3 acetylation (H3K27ac) to determine the chromatin landscape in *ex vivo* cultured BECs from healthy and allergic-atopic asthmatics ( $n = 3$  donors each). Regions of differential enrichment were identified (MEDIPS) and associated genes and pathways determined (GREAT). Gene expression profiles were investigated by microarray (Illumina) and differential analysis conducted (Partek Genome Suite). Super enhancers (SEs) were identified (ROSE) and enrichment of transcription factor motifs (MEME) and their tissue distribution (protenatlas.org) determined.

We identified 33,744 differentially enriched regions (DERs) of H3K27ac between asthma and healthy BECs. DERs were associated with genes (e.g. SERPINB2, TSLP) and pathways (e.g. leukotriene synthesis, antiviral response) previously implicated in asthma and had little overlap with known glucocorticoid receptor binding sites (1.7% of total). DERs occurred up to 100kb from gene promoters and gain or loss of H3K27ac was associated with increased and decreased gene expression in asthmatics respectively. Using a comparative approach, we identified SEs that were common (i.e., present across all donors) and distinct to health and asthma. In addition to established asthma genes (e.g. CLCA1) and transcription factors (e.g. TP63), asthma-SEs encompassed non-coding RNAs (up to 32% of genes) and epithelial-specific transcription factors (e.g. GCM2) previously unreported in asthma.

Our data indicates that asthma influences the chromatin landscape of BECs and suggests the genomic differences observed in the asthmatic airway epithelium are underpinned by established epigenetic mechanisms.

### P236 THE ROLE OF HISTONE ARGININE METHYLATION IN GENE EXPRESSION OF AIRWAY SMOOTH MUSCLE CELLS IN ASTHMA

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10.1136/thoraxjnl-2016-209333.379

**Introduction and objectives** Asthma is estimated to affect at least 300 million people globally. About 25% of the patients do not respond to therapy; therefore we need to develop novel treatments. ASM cells have a crucial role in asthma, contributing to airway remodelling, inflammation and airflow obstruction. We have previously shown that epigenetic histone modifications, particularly histone lysine acetylation and methylation regulate the secretion of inflammatory mediators from ASM cells. Here we tested the hypothesis that histone arginine changes are also involved. Protein arginine N-methyltransferases (PRMTs) are the enzymes which catalyse histone arginine methylation (HRme, the addition of a methyl group to arginine residues on the N-terminal tails of histones), and inhibiting them represents a strategy to reduce the secretion of inflammatory mediators from ASM cells.

**Methods** Studies were performed in cultured human ASM cells from asthmatic and non-asthmatic donors at passage 6. PRMT expression in human ASM cells was investigated by qPCR. Protein levels of four PRMTs in human ASM cells were investigated by western blotting. As PMRT1 has previously been suggested to play a role in mouse asthma models, we studied the association of PRMT1 with eotaxin, IL-6, IP-10 and CXCL8 promoters in healthy ASM cells, under basal conditions and following stimulation with TNF- $\alpha$  (1ng/ml), by chromatin immunoprecipitation (ChIP). IgG was used as a negative control, while acetylated histone H4 (AcH4) was used as a positive control.

**Results** We found that ASM cells express the PRMT1, PRMT2, PRMT3, CARM1, PRMT5, PRMT6, PRMT7 and FBX011 mRNA and PRMT1, CARM1, PRMT5, and PRMT6 protein. The analysis showed no difference in the levels of expression between cells isolated from asthmatic and non-asthmatic donors.

Under basal conditions, PRMT1 was associated with all of the promoters and association increased following 1 hour stimulation with TNF- $\alpha$ .

**Conclusions** ASM cells express a number of PRMTs at mRNA and protein levels. PRMT1 associates with a number of chemokine and cytokine promoters after TNF- $\alpha$  stimulation. PRMTs may have an important role in regulating chemokine production from ASM cells in asthma, and are a promising target for future investigations in asthma.

### P237 LUNG FUNCTION DECLINE IS ASSOCIATED WITH SERUM PERIOSTIN LEVEL BUT NOT FRACTIONAL EXHALED NITRIC OXIDE OR BLOOD EOSINOPHILS IN SEVERE ASTHMA

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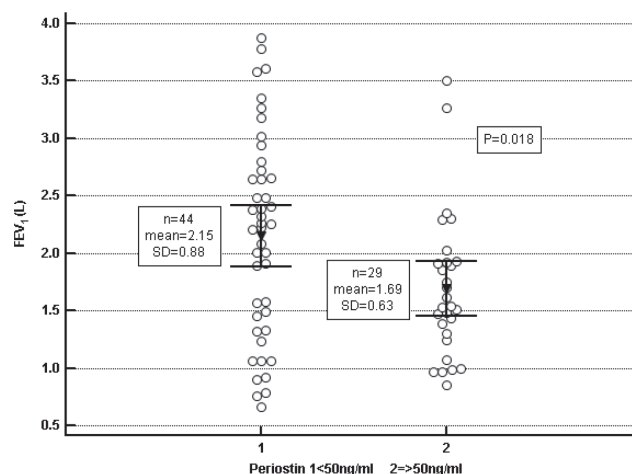
10.1136/thoraxjnl-2016-209333.380

**Background** In the airways, periostin encoded by the POSTN gene is up-regulated by IL13-IL4-TGF- $\beta$  axis. It is produced by structural cells such as epithelial cells and fibroblasts and inflammatory cells such as eosinophils and macrophages. Consequently it has been linked to airways remodelling, mucus production and subepithelial fibrosis. However, an association between periostin and lung function impairment in severe asthma has not been confirmed.

**Methods** Unselected patients attending severe asthma centre were clinically characterised using systematic protocol and undergone lung functions, serum periostin, fraction exhaled nitric oxide (FeNO) and peripheral blood eosinophils (PBE) measurement. Correlation analysis and one way analysis of variance were undertaken to explore the relationships.

**Results** 127 patients consented to the study (mean age 45.5 yrs [range 17–70], 88 [69%] females), 72/103 (69%) were atopic. The mean FEV1 was 2 L, mean%predicted FEV1 68.1, and mean FEV1/FVC ratio was 71.3. The mean inhaled daily corticosteroids dose was 1.65mg/day and 56.3% were on maintenance oral corticosteroids. Periostin measurement was available in 78 patients who had a mean level of 49.5 ng/L (SD  $\pm$  18.1). Using 50 ng/L as a cut-off point, 30/78 (36%) patients had high periostin and 48/78 (62%) had low periostin. The mean FEV1 in the periostin high group was 1.69 L Compared to 2.15 L in the low group ( $p = 0.018$ ) (see Figure). We also observed significant correlation between serum periostin and% predicted FEV1 ( $r = 0.36$ ,  $p = 0.0017$ ). In contrast the association analysis between FeNO and PBE with FEV1 were both non-significant ( $p = 0.8$  and  $p = 0.35$  respectively).

**Conclusion** Raised serum periostin is associated with low lung function in this cohort of severe asthma but not FeNO or PBE. Further research is required to confirm this relation and explore the role of periostin as predictor of decline in lung function and airway remodelling.



Abstract P237 Figure 1 Periostin and FEV1

### P238 INVESTIGATING GENOME WIDE DNA METHYLATION IN AIRWAY SMOOTH MUSCLE CELLS FROM ASTHMATIC AND NON-ASTHMATIC DONORS

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10.1136/thoraxjnl-2016-209333.381

**Rationale** Genetic mechanisms fail to fully explain asthma pathogenesis and environmental factors are considered to play an important role. Environmental factors may lead to permanent changes in epigenetic patterns and contribute to asthma. Epigenetics is the study of heritable changes in gene expression that are not due to changes in DNA sequence. DNA methylation is a reversible modification of DNA structure in which a methyl group is added to cytosine residues. Parental smoking affects the methylation of buccal cell DNA from children and children with early onset wheeze have an altered blood DNA methylation profile to healthy individuals. No studies have compared DNA methylation profiles in the disease relevant cell type of airway smooth muscle (ASM) cells.

**Methods** DNA was isolated from ASM cells at passage 5 and bisulphite treated to convert epigenetic information into sequence-based information. Site specific, quantitative genome wide methylation was determined using the Illumina 450K Infinium Methylation BeadChip array. Hits were validated by Pyrosequencing. RNA was extracted simultaneously for mRNA expression analysis by real time PCR.

**Results** There were no independent CpG sites associated with asthmatic status of ASM cells following multiple test correction. Without correction over 13000 CpG sites showed a significant difference in methylation (linear modelling,  $p$  value  $>0.05$ )