S89

SOLUBLE ADAM33 AUGMENTS THE PULMONARY IMMUNE RESPONSE PROMOTING ALLERGIC AIRWAY SENSITIVITY

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Rational A *disintegrin and metalloproteinase 33* (ADAM33) was discovered in 2002 as an asthma susceptibility gene. Genetic associations have been made between ADAM33 polymorphisms and asthma disease severity, bronchial hyperresponsiveness (BHR) and rate of lung function decline in both adults and children. A soluble, catalytically active form of the protein (sADAM33) has been identified in the bronchoalveolar lavage fluid of patients, levels of which correlate with disease severity (Lee JY et al, AJRCCM 2006). To study the role sADAM33, a lung specific, doxycycline (Dox) inducible, transgenic mouse, expressing the human pro and metalloproteinase domains of the full-length protein was generated (Ccsp-rtTA/Otet-ADAM33-Pro-MP). Induction of sADAM33, followed by house dust mite (HDM) sensitisation and challenge, resulted in increased BHR and airway inflammation (Davies ER et al, JCI-Insight 2016). The mechanisms by which ADAM33 promotes this susceptibility are unclear. The aim of this work is to identify pathways that are augmented by the induction of sADAM33, which promote increased sensitivity to allergen.

Methods RNA samples from whole lung of adult mice, where sADAM33 had been induced for 4 or 8 weeks, were analysed by next generation RNA sequencing. Identified genes were confirmed across experimental time points (72 hour, 7 day, 4 and 8 weeks on Dox) in wider sample cohorts of Ccsp-rtTA/Otet-ADAM33-Pro-MP and control mice through RTqPCR.

Results The predominant signal from the RNAseq output was for modulation of immune response genes at 4 weeks of sADAM33 expression (GO:0006955 Immune response: 31 genes, 58.49% coverage, FDR p value=7.09 E-22). Genes associated with an immune activation signature (*Ccl5*, *Irgm1*, *Gm12250*, *Gzmb*, *Ncr1*) were validated at least one time point. By 8 weeks of sADAM33 expression genes associated with negative regulation of the response were also validated (*Ido1*, *Cd274*).

Conclusion Induction of sADAM33 in murine lungs, without allergic sensitisation, augmented underlying immune processes in this transgenic mouse model, which may contribute to increased susceptibility to allergic airway inflammation and BHR when challenged with allergen. Further work is required to delineate how sADAM33 affects immune cell populations and their behaviour in the lung.

S90

THE EFFECT OF LONG ACTING BETA-AGONISTS ON GLUCOCORTICOID RECEPTOR AND IMPORTIN-7 NUCLEAR TRANSLOCATION IN AIRWAY SMOOTH MUSCLE CELLS

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Introduction and Objectives 5%–10% of asthmatic patients display relative insensitivity to the therapeutic benefits corticosteroids and are termed severe asthmatics. This has been attributed, in severe asthma (SA) airway smooth muscle cells (ASMCs), to defective corticosteroid-induced nuclear translocation of the glucocorticoid receptor (GR). Importins, including importin-7, mediate GR nuclear translocation. Studies report increased importin-7-GR interaction in response to corticosteroids in ASMCs. Long-acting beta agonists (LABAs) augment corticosteroid-induced GR nuclear translocation in COPD macrophages. The primary aim of this project was to determine whether the LABA salmeterol could reverse impaired corticosteroid (dexamethasone)-induced GR nuclear translocation in severe asthmatic ASMCs through an importin-7-dependent mechanism.

Methods ASMCs derived from bronchial biopsies from patients with non-severe asthma (NSA) (n=4) and SA (n=4) were cultured and treated with dexamethasone (10–8 M) in the absence or presence of salmeterol (10–10 M or 10–8 M). 30 min post-stimulation, nuclear and cytoplasmic protein fractions underwent western blot analysis to determine levels of importin-7 and GR. RNA extraction was conducted at 4 hours post-stimulation followed by RT-qPCR to investigate the levels of the GR-activated genes, mitogen activated protein kinase phosphatase-1 (MKP-1) and glucocorticoid leucine zipper mRNA levels (GILZ).

Results The Results show a reduced dexamethasone-induced GR nuclear translocation in SA when compared to NSA ASMCs, which was increased with co-treatment with dexamethasone and salmeterol. Co-treatment with dexamethasone and salmeterol also increased GR-activated gene expression leading to an increase in the expression of MKP-1 in SA ASMCs compared to dexamethasone alone. Nuclear importin-7 levels in NSA and SA ASMCs were not increased by co-treatment compared to dexamethasone alone.

Conclusion We have demonstrated impaired dexamethasone-induced GR nuclear translocation in SA ASMCs, which can be reversed by the addition of the LABA salmeterol in combination with dexamethasone. No differences were observed in importin-7 nuclear localisation after co-treatment compared to dexamethasone alone in SA ASMCs. This could suggest that importin-7 is either rapidly recycled back into the cytoplasm after entry into the nucleus, or that the effect of LABAs are not mediated through Importin-7 in severe asthmatic patients. Further real-time experiments would elucidate the role of importin-7 in LABA mediated GR nuclear translocation.

Cystic fibrosis: disease trajectory and evolving therapies

S91

EARLY GROWTH TRAJECTORIES IN CYSTIC FIBROSIS

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Introduction Within CF-EpiNet; Harnessing Data to Improve Lives (a CF Trust funded Strategic Research Centre) we will

investigate relationships of lung function in early childhood with growth patterns (here considered as change in weight) before the age of five years. We use flexible longitudinal modelling methods to describe early growth trajectories, and identify factors associated with suboptimal growth.

Methods Growth measurements from diagnosis to five years were extracted from two national CF registries: UK (n=2999, years 2007–2015) and Canada (n=2690, years 1990–2013). SITAR (super-imposition by translation and rotation) was used to model weight (kg) over the 5 years. Output parameters were average growth curve, summaries of growth velocity and overall weight. Associations of growth and growth velocity with sex, genotype and new born screening (NBS) were investigated.

Results Most children in the UK had been diagnosed early in life (median age of diagnosis 0.06 years; inter quartile range 0.03 to 0.1) by NBS. 52% were homozygous for deltaF508. Despite similar initial average weight in boys and girls, males were heavier than females over the first five years. Children homozygous for deltaF508 were lighter than other children. No tested factor was associated with velocity of weight gain. Only 10% of the Canadian children were diagnosed by NBS, and, overall, the age at diagnosis was later (median 0.17 years; inter quartile range 0.08 to 0.53). Over the first five years, Canadian CF children were lighter than those in the UK (figure 1). Associations of weight with sex and genotype were similar to those seen in the UK. In addition, we observed that those diagnosed by NBS were heavier than those who were not.

Conclusions In children with CF there are sex differences in weight during the first 5 years, despite similar initial weight. In Canada, NBS has a positive impact on early growth. SITAR allows exploration of growth patterns in CF patients, but time independent characteristics were not associated with velocity of growth. Statistical modelling incorporating time dependent factors (e.g., infections, treatments) are required to explain variability of growth trajectories.

Abstract S91 Figure 1 Average growth curves for children with CF.

S92

GENOMIC INVESTIGATION UNMASKS EVIDENCE OF TRANSMISSION ACROSS MYCOBACTERIUM ABSCESSUS CYSTIC FIBROSIS PATIENTS

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Background *Mycobacterium abscessus* (*Mabs*) is a critical respiratory pathogen in Cystic Fibrosis (CF), with significant challenges in diagnosis and treatment. Recent whole genome sequencing (WGS) studies have challenged the assumption that acquisition in CF is primarily from soil and water systems.¹ We sought to investigate potential transmission of *Mabs* within and between CF centres through WGS analysis of CF isolates from the Scottish Mycobacteria Reference Laboratory.

Methods 64 isolates were recovered and sequenced from CF samples positive for *Mabs* between 2008 and 2016. Multilocus sequence typing (MLST), and phylogenetic analysis based on core genome Single Nucleotide Polymorphisms (SNPs) was used to identify dominant circulating clones. Probable transmission clusters were defined by <20 SNPs between isolates, as suggested by previous studies. This work was carried out as part of a public health investigation.

Results MLST analysis of the 64 confirmed *Mabs* isolates demonstrated the following: 8 strain types (ST) associated with *Mabs* subspecies *abscessus*, including ST9 (n=18), ST24 (12), ST5 (7), ST26 (4), ST21 (3), and ST10 (2); 11 identified as subspecies *massiliense* associated with ST4 (4), ST6 (3), ST7 (2) and ST3 (2); 4 identified as subspecies *bolletii*.

Phylogenomic analysis provided higher resolution and identified significant relatedness between and within clonal complexes. The most prevalent subspecies *abscessus* clone was ST9, of which 16 isolates were distinguished by <20 SNPs, 12 of which from one hospital region. Five of the ST9 isolates were indistinguishable at the SNP level, and yet originated from different Health Board regions and different hospitals. ST5, ST24 and ST26 also contained isolates distinguished by <20 SNPs and originated from several geographically distinct regions.

Conclusion WGS has provided strong evidence of the circulation of dominant clones between and within CF centres across geographically diverse settings over an 8 year period. The high genomic relatedness provides strong evidence of transmission between CF patients, even though the precise mechanisms of transmission remain uncertain. Further studies are underway to understand the mechanism of transmission in this clinical context.

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