



**Abstract T2 Figure 1** The association of total testosterone (TT) and sex hormone-binding (SHBG) with FVC (A) and FEV<sub>1</sub>/FVC (B) in MVMR analyses separately per sex. Effect estimates show the difference in lung function (mL or %) per log increase in hormone level for 4 MR methods: Two-Stage Least Squares regression (2SLS), fixed-effect (F-IVW) and random-effect (R-IVW) inverse variance-weighted (IVW) meta-analysis; and MR-Egger.

### T3 OCCUPATIONAL EXPOSURES AND RESPIRATORY HEALTH: THE BURDEN OF OBSTRUCTIVE LUNG DISEASE (BOLD) STUDY RESULTS

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**Introduction and Objectives** It has been estimated that 15% of the population burden of chronic obstructive pulmonary disease population is attributable to occupational factors. Most of the evidence comes from studies conducted in high-income countries (HICs). Our aim was to examine the relationship between occupational exposures and respiratory health in both HICs and Low- and middle-income countries (LMICs) participating in the multinational, population-based, cross-sectional BOLD study.

**Methods** We analysed data from 28,823 adults aged  $\geq 40$  years who completed respiratory and occupational questionnaires and had acceptable and repeatable post-bronchodilator spirometry measurements. Occupational exposures comprised three categories (organic dust; inorganic dust; fume) and 11 high-risk occupations (farming; flour, feed or grain milling; cotton or jute processing; hard-rock mining; coal mining; sandblasting; working with asbestos; chemical or plastics manufacturing; foundry or steel milling; welding; and firefighting). The associations of respiratory symptoms and lung function with occupational exposures were estimated using multivariable regression models adjusted for potential confounders for each BOLD site and then pooled using meta-analysis. Sensitivity analyses by sex, national gross national income and smoking status were also performed.

**Results** We found that people working in any of three categories of occupational exposures and the 11 high-risk occupations under consideration were more likely to report respiratory symptoms than those who do not work in any of those occupations. Overall, we found no consistent associations between the occupational exposure categories and high-risk occupations and measures of lung function. Nevertheless, in sensitivity analyses, men in HICs exposed to organic dusts in the workplace for at least 20 years (median) had significantly decreased FEV<sub>1</sub>/FVC ( $\beta = -0.34\%$ ; 95% CI -0.42% to -0.27%) and decreased FVC ( $\beta = -0.18L$ ; 95% CI -0.32L to -0.04L). Men in LMICs exposed to fumes for at least 11 years had significantly decreased FEV<sub>1</sub>/FVC ( $\beta = -0.29\%$ ; 95% CI -0.41% to -0.16%).

**Conclusions** In a large global study, we found respiratory symptoms to be associated with 11 high-risk occupations. The associations between occupational exposures and lung function varied by gross national income groups; more research is needed to understand these differences. Meanwhile, preventive measures and respiratory health surveillance should be enhanced among exposed workers.

### T4 THE RESPIRATORY MICROBIOME AND METABOLOME IN IDIOPATHIC PULMONARY FIBROSIS

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Idiopathic pulmonary fibrosis (IPF) is a progressive and fatal fibrotic lung disease of unknown aetiology. There is growing evidence that the lung microbiota may play a role in IPF. However, no study has investigated the functional impact of the short-chain fatty acids (SCFAs) on primary bronchial epithelial cells (PBECS) and disease pathogenesis. Therefore, we investigated the influence of acetate, propionate, and butyrate on PBECS from healthy controls and subjects with IPF.

Subjects diagnosed with IPF ( $n=201$ ) and healthy controls ( $n=40$ ) were prospectively recruited and underwent bronchoalveolar lavage. Bacterial DNA was isolated and 16S rRNA gene sequencing undertaken to characterise bacterial communities. Untargeted <sup>1</sup>H nuclear magnetic resonance spectroscopy-based metabolomics and targeted gas chromatography-mass spectrometry captured the metabolic profile of these samples. PBECS from healthy controls and subjects with IPF were differentiated at air-liquid interface (ALI) and either left untreated or exposed to the SCFAs.

The IPF microbiota was less diverse ( $P<0.01$ ) and had increased proportions of *Firmicutes* ( $P<0.01$ ) compared to healthy controls. *Streptococcus* and *Staphylococcus* were more abundant in IPF cases than controls ( $P<0.05$ ). Metabolomics analysis revealed distinct differences between the cohorts. Relative concentrations of the SCFAs were increased in IPF compared to healthy controls, and in IPF, propionate positively correlated with bacterial burden ( $\rho=0.47$ ,  $P=8 \times 10^{-5}$ ). Exposure of healthy and IPF PBECS cultured at ALI to 1 mM of the SCFAs did not impact cell viability. Treatment of

PBECs from IPF subjects but not healthy controls with the SCFAs led to morphological changes, a dose-dependent release of pro-inflammatory mediators in the cell supernatant, and a decrease in transepithelial electrical resistance (TEER) over time. Specifically, compared to baseline, exposure of IPF PBECs to 1 mM of propionate led to a 40% reduction in TEER and a 2-fold increase in the secretion of IL-6.

Subjects with IPF display an altered microbiome which is associated with a distinct metabolic signature in the lower airways. Differences in specific bacterial genera and an increased bacterial burden in IPF results in changes in the SCFAs in the airways. *In vitro* work demonstrates the potential of these SCFAs to shape immunological responses in the lung, mediating the pathogenesis of fibrosis.

### T5 TOLL-LIKE RECEPTOR 2 HAS A TUMOUR SUPPRESSOR FUNCTION IN NON-SMALL CELL LUNG CANCER VIA REGULATION OF THE SENESENCE ASSOCIATED SECRETORY PHENOTYPE

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**Background** Lung cancer is the leading cause of cancer related deaths worldwide. The value of targeting early stage disease has been widely recognised to improve survival. Oncogene-induced senescence (OIS) is a stress response instigated following the activation of oncogenes and is a well-known tumour suppressor mechanism. OIS is abundant in pre-invasive lesions in murine lung cancer models, however is lost during the progression to malignancy. We previously identified a regulatory role for Toll-like receptor 2 (TLR2) in OIS and expression of the senescence-associated secretory phenotype (SASP), however the functional relevance of this has yet to be established.

**Methods** We used genetically engineered mouse (GEM) models of lung cancer (*Kras*<sup>LSL-G12D/+</sup> and *Kras*<sup>LSL-G12D/+</sup>; *TP53*<sup>fl/fl</sup>) on both wild-type and *Tlr2* null backgrounds. Lung specific activation of mutant *Kras*<sup>G12D</sup> and *TP53* loss was achieved upon intranasal infection with Cre-recombinase expressing adenovirus. Tumour burden, senescence markers and SASP expression were assessed by immunohistochemistry. Immune cell recruitment was measured using flow cytometry on whole lung single cell suspensions from tumour bearing mice. The expression of *Tlr2* and associated SASP components were measured in human pre-invasive lung cancer samples that either progressed to invasive malignancy or regressed to normal epithelium.

**Results** *Tlr2* loss was associated with an increased tumour burden and reduced survival in our GEM model. Furthermore, *Tlr2* loss caused significantly reduced epithelial expression of key senescence markers and SASP factors. However, immune cell recruitment was not affected suggesting this effect was cell intrinsic. Inhalational administration of a synthetic TLR2 agonist (Pam2CSK4) significantly reduced tumour burden in our GEM model. Molecular profiling of bronchoscopic biopsies of human pre-invasive lung lesions revealed increased TLR2 and SASP expression in samples that did not progress to invasive malignancy, suggesting a tumour suppressor role for TLR2-SASP signalling in human lung cancer.

**Conclusions** We have identified TLR2 as a potential tumour suppressor in lung cancer via regulation of the SASP.

Furthermore, we have highlighted a novel therapeutic strategy for the treatment of early stage lung cancer. SASP factors are released into the bloodstream and are ideal candidate biomarkers of pre-invasive disease and thus could potentially aid in stratifying lung cancer screening populations.

### T6 SPUTUM PROTEOMICS IDENTIFIES MECHANISMS OF DISEASE SEVERITY AND TREATMENT RESPONSE IN BRONCHIECTASIS

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**Introduction** Neutrophil extracellular traps (NETs) are a form of antimicrobial defence which have been implicated in multiple inflammatory diseases. This study investigated the role of NETs in bronchiectasis, a neutrophilic disease which lacks therapies that directly target neutrophilic inflammation, through a series of UK and international studies.

**Methods** LC/MS proteomics was used to compare protein profiles in sputum between severe and mild bronchiectasis (20 vs 20 patients). Microbiome changes, using 16S rRNA sequencing, clinical characteristics and NET levels using a validated histone-elastase immunoassay were analysed. Results were validated in an independent European cohort. Proteomics was used to identify proteins associated with treatment response of acute exacerbations of bronchiectasis in 20 patients treated with intravenous antibiotics for 14 days. Two studies of long-term macrolide treatment, one in bronchiectasis and a post-hoc analysis of the AMAZES trial in asthma, investigated the effect of macrolide treatment on NETs.

**Results** 96 proteins were differentially expressed in sputum between severe and mild bronchiectasis, with known NET proteins being the most abundant and discriminating. The relationship between NETs and associated proteins were validated in two independent cohorts, a UK cohort (n=175) and a European cohort (n=275). Sputum NETs were associated with BSI (p<0.0001), a history of severe exacerbations (p=0.0089), quality of life (p<0.0001), time to first exacerbation (p<0.0001) and mortality (p=0.009). High NET levels were associated with reduced microbial alpha-diversity, measured by Shannon-Weiner, and microbial dysbiosis (p<0.0001, PERMANOVA) and elevated IL-8, IL-1beta, TNF-alpha, interferon-gamma and GM-CSF in sputum. Antibiotic treatment (n=20) significantly altered the expression of 39 sputum proteins, with the 'neutrophil degranulation' pathway being most strongly implicated in response. Patients with *P. aeruginosa* infection had a blunted proteomic and clinical response to treatment. Treatment with azithromycin was associated with a significant reduction in sputum NETs over 12 months in both bronchiectasis (n=52, p=0.016) and asthma (n=47, p<0.0001).

**Conclusion** NET-associated proteins are elevated in bronchiectasis sputum and are associated with disease severity, bacterial infection and mortality. Treatment response is linked to successfully reducing NET levels with intravenous antibiotic or macrolide therapies suggesting that NETs may be an important therapeutic target in bronchiectasis.