# From lab bench to work bench: Do genetic variants within the NLRP3 inflammasome alter the risk of IPF in patients with occupational silica and asbestos exposure? A mendelian randomisation approach.

Idiopathic pulmonary fibrosis (IPF) is a progressive, fibrotic lung disease which in 2016 was the recorded cause of death for approximately 5000 people in England and Wales. Its incidence, currently around 7.5/100,000 person-years, has increased by 5% per annum in the period 1979-2016.[1][2] The diagnosis is made when there are characteristic honeycomb cystic changes, called usual interstitial pneumonia (UIP), on high resolution CT scan or biopsy and known causes of interstitial lung disease (such as drug toxicity, connective tissue disease, domestic, and occupational or environmental exposures) have been excluded.[3] However, a recent meta-analysis of epidemiological studies estimated 26% of cases may be attributable to occupational exposures, including to metal, silica, wood, and agricultural dusts.[4] Asbestosis, silicosis, chronic hypersensitivity pneumonitis, and rheumatoid arthritis associated ILD can all give rise to UIP[5][6] and the potential for misdiagnosis as IPF is recognised.[7][8] In light of this there have been calls for the systematic capture of key occupational and environmental data to elucidate specific causal exposures and improve diagnostic specificity.[9]

The Idiopathic Pulmonary Fibrosis Job Exposures Study (IPFJES) collected lifetime occupational histories, DNA, and serum for 494 IPF cases and 466 hospital controls to investigate asbestos exposure in the aetiology of idiopathic pulmonary fibrosis. IPFJES was designed to overcome the risk of bias present in previous case-control studies due to low control participation rates and the use of binary self-reported exposure measures, and to permit investigation of gene-environment interaction. Smoking appeared to interact with occupational asbestos exposure to increase risk of IPF in participants carrying one or more copies of the minor allele of rs35705950 (manuscript in preparation), this common variant is the strongest identified genetic risk factor for IPF; minor allele frequency > 0.1 in Caucasian populations, OR 4.84 (95%CI 4.37-5.36, p=1.18x10-203) in a recent GWAS meta-analysis (total 2,668 IPF cases and 8,591 controls).[10] Its main effect is to increase airway expression of a distal airway glycopeptide called MUC5b (>30-fold).[11] MUC5b is a dominant feature of the honeycomb cysts that characterise IPF.[12] It has recently emerged that rs3505950 is also a risk factor for asbestosis[13], chronic hypersensitivity pneumonitis, and rheumatoid arthritis associated ILD.[14] In a transgenic mouse model over-expression of MUC5b was associated with impaired mucociliary clearance and enhanced lung fibrosis.[15] In a mouse bleomycin model fibrosis was attenuated in germ free mice[16]; in humans, IPF (and honeycombing in particular) is associated with an increased airway microbial burden, and immunosuppressive drugs shorten life.[17] Together these suggest that MUC5b overexpression results in greater susceptibility to infection as part of the disease process.

There is strong intuitive sense for occult occupational inhalation of silica or asbestos fibres being an under-recognised causes of IPF since both are known to be fibrogenic and are frequently used in animal models of pulmonary fibrosis.[18] Interestingly, both silica and asbestos exposure also result in production of IL-1beta, via the NLRP3 inflammasome in a process that appears to be dependant on reactive oxygen species (ROS). IL-1beta is known to be a key proinflammatory cytokine in IPF and a potent stimulus for MUC5b expression.[19] Smoking cigarettes is thought to increase the risk of asbestosis, silicosis, and IPF.[20][21] Of note, the lungs are thought to be an initiating site of rheumatoid arthritis.[22] Occupational exposure to respirable crystalline silica is associated with an increased risk of rheumatoid arthritis in men[23], and rheumatoid arthritis associated ILD (which causes UIP) is more common in men despite rheumatoid arthritis being more common in women.[24] Genetic variants in the NLRP3 inflammasome (e.g rs35829419) have been found to be associated with increased risks of rheumatoid arthritis[25], asbestosis[26], coal workers pnemoconniosis[27] (which is also associated with a MUC5b promoter SNP[28]), and susceptibility to infection.[29][30]

Additional evidence for the importance of common pathways in UIP related disease is provided by the recent demonstration of the effectiveness of the anti-fibrotic nintedanib, a treatment licensed for IPF, in non-IPF UIP[31] and the observation that it is also effective in animal models of silicosis.[32] It is also intriguing that IBD-like colitis has been observed as a side effect of antifibrotic agents; NLRP3 inflammasome variants are strongly implicated in IBD.[33]

Gastro-oesophageal reflux disease (GORD) has long been recognised to be associated with idiopathic pulmonary fibrosis but whether this is a result of confounding or reverse causation is unclear. A recent meta-analysis of eighteen case-control studies including 3,206 patients with IPF and 9,368 controls found that GORD is associated with IPF, OR 2.94 (95%CI 1.95-4.42, p < 0.0001), but there was no significant association in a metaregression when adjusting for smoking.[34] A recent GWAS identified a number of genetic loci for GORD susceptibility, several of which were known to also be associated with smoking.[35] A study of bronchoalveolar lavage (BAL) lung microbiome and disease progression in IPF found disease progression was significantly associated with increased relative abundance of two operational taxonomy units (OTUs), Streptococcus OTU 1345 OR 1.11(95% CI 1·04–1·18, p=0·0009) and Staphylococcus OTU 1348 OR 1·16 (95%CI 1·03–1·31, p=0·012). Both OTUs were also significantly associated with GORD.[36] Streptococcus species were also found to associated with IPF in another BAL microbiome study[37] and to exacerbate lung fibrosis by secretion of pneumolysin in a mouse model.[38] Access to iron by Streptococcus species is necessary for virulence and host defence mechanisms limit access to iron as part of the acute phase response.[39] There is differential gene expression in Streptococcus pneumoniae in response to various iron sources, and is thought Streptococcus species are able to access ferritin following its release from cells damaged or lysed by virulence determinants or may utilize serum or secreted ferritin.[40] It has very recently been shown that transferritin receptor CD71 is significantly down-regulated in alveolar macrophages from IPF patients and that this is associated with impaired phagocytosis and enhanced expression of profibrotic genes.[41]

In summary there appears to be recognition that a proportion of IPF is associated with occupational exposure, convergence on the importance of excessive MUC5b expression in several respiratory diseases that can cause UIP, some of which e.g silicosis, asbestosis, are occupational, and a plausible biological mechanism for this; increased IL-1beta expression via alveolar macrophage NLRP3 inflammasome activation, leading to increased MUC5b expression, impaired mucociliary clearance, greater alveolar epithelial exposure to injurious agents (infectious or otherwise), and sustained inflammation and scarring.

Mendelian randomization (MR) is a technique that uses randomly distributed genetic variants as natural experiments to provide evidence about putative causal relations between modifiable risk factors and disease.[42] MR has the advantage that because of its use of genetic variance it can overcome problems of confounding and reverse causality. MR can be used within a case-control study design to help triangulate suspected causal associations.[43] It could be usefully applied to IPFJES to investigate interactions between occupational silica and asbestos exposure, smoking, and NLRP3 inflammasome variants, with respect to IPF risk, in order to better understand the aetiology of IPF and potentially identify new therapeutic targets.

During my fellowship I will pioneer the use of novel methods in occupational lung disease beginning with mendelian randomisation in IPF to investigate causal occupational exposure-response pathways.

Research plans:

* Occupational phenotyping of IPF; in particular assessing occupational asbestos and silica exposure through well validated quantitative means
* Exome sequencing IPFJES cohort
* Performing the largest GWAS meta-analysis of IPF to date
* Carrying out the first two-sample MR studies of smoking, gastro-oesophageal reflux disease, iron status, and cytokine profiles in IPF
* Colocalisation study of GWAS NRLP3 inflammasome loci across asbestosis, silicosis, RA, CHP, and IPF

My research plans are deliberately non-sequential. Occupational phenotyping for asbestos and silica will make use of already collected lifetime occupational histories coded to the standard occupational classification 1990 which will permit application of validated job-exposure matrices. DNA has already been extracted for the IPFJES cohort, we would arrange exome sequencing externally subject to securing adequate funding. Participants in IPFJES consented for future research including GWAS and collaboration, and data sharing with, commercial organisations. Instruments for performing two-sample MR studies of smoking, GORD, iron status, and cytokine profiles in IPF are readily available from exposure GWAS.[44][35][45][46] Variant summary data from a recent pooled meta-analysis of IPF GWAS is available for academic research on application.[10]

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