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=$1.18\text{x}10^{-203}$) 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 $1 \cdot 04$–$1 \cdot 18$, p=$0 \cdot 0009$) and Staphylococcus OTU 1348 OR $1 \cdot 16$ (95%CI $1 \cdot 03$–$1 \cdot 31$, p=$0 \cdot 012$). 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]

References

1 Navaratnam V, Fleming K, West J *et al.* The rising incidence of idiopathic pulmonary fibrosis in the uk. *Thorax* 2011;**66**:462–7.

2 Navaratnam V, Hubbard RB. The mortality burden of idiopathic pulmonary fibrosis in the united kingdom. *American journal of respiratory and critical care medicine* 2019;**200**:256–8. doi:10.1164/rccm.201902-0467LE

3 Raghu G, Remy-Jardin M, Myers JL *et al.* Diagnosis of idiopathic pulmonary fibrosis. An official ats/ers/jrs/alat clinical practice guideline. *American journal of respiratory and critical care medicine* 2018;**198**:e44–68. doi:10.1164/rccm.201807-1255ST

4 Blanc PD, Annesi-Maesano I, Balmes JR *et al.* The occupational burden of nonmalignant respiratory diseases. An official american thoracic society and european respiratory society statement. *American journal of respiratory and critical care medicine* 2019;**199**:1312–34. doi:10.1164/rccm.201904-0717ST

5 Arakawa H, Johkoh T, Honma K *et al.* Chronic interstitial pneumonia in silicosis and mix-dust pneumoconiosis: Its prevalence and comparison of ct findings with idiopathic pulmonary fibrosis. *Chest* 2007;**131**:1870–6. doi:10.1378/chest.06-2553

6 allen. Differential diagnosis of usual interstitial pneumonia: When is it truly idiopathic? *European respiratory review : an official journal of the European Respiratory Society* 2014;**23**:308–19. doi:10.1183/09059180.00004914

7 Monsó E, Tura J, Pujadas J *et al.* Lung dust content in idiopathic pulmonary fibrosis: A study with scanning electron microscopy and energy dispersive x ray analysis. *Br J Ind Med* 1991;**48**:327–31.

8 Morell F, Villar A, Montero M-Á *et al.* Chronic hypersensitivity pneumonitis in patients diagnosed with idiopathic pulmonary fibrosis: A prospective case-cohort study. *Lancet Respir Med* 2013;**1**:685–94. doi:10.1016/S2213-2600(13)70191-7

9 Nett RJ, Wood JM, Blackley DJ. Collecting occupational exposure data would strengthen idiopathic pulmonary fibrosis registries. *American journal of respiratory and critical care medicine* Published Online First: October 2019. doi:10.1164/rccm.201908-1631LE

10 Allen RJ, Guillen-Guio B, Oldham JM *et al.* Genome-wide association study of susceptibility to idiopathic pulmonary fibrosis. *American journal of*

respiratory and critical care medicine Published Online First: November 2019. doi:10.1164/rccm.201905-1017OC

11 Evans CM, Fingerlin TE, Schwarz MI *et al.* Idiopathic pulmonary fibrosis: A genetic disease that involves mucociliary dysfunction of the peripheral airways. *Physiological reviews* 2016;**96**:1567–91. doi:10.1152/physrev.00004.2016

12 Seibold MA, Smith RW, Urbanek C *et al.* The idiopathic pulmonary fibrosis honeycomb cyst contains a mucocilary pseudostratified epithelium. *PloS one* 2013;**8**:e58658. doi:10.1371/journal.pone.0058658

13 Platenburg MGJP, Wiertz IA, Vis JJ van der *et al.* The muc5b promotor risk allele for idiopathic pulmonary fibrosis predisposes to asbestosis. *The European respiratory journal* Published Online First: January 2020. doi:10.1183/13993003.02361-2019

14 Namba N, Kawasaki A, Sada K-E *et al.* Association of muc5b promoter polymorphism with interstitial lung disease in myeloperoxidase-antineutrophil cytoplasmic antibody-associated vasculitis. *Annals of the rheumatic diseases* 2019;**78**:1144–6. doi:10.1136/annrheumdis-2018-214263

15 'd. Muc5b overexpression causes mucociliary dysfunction and enhances lung fibrosis in mice. *Nature communications* 2018;**9**:5363. doi:10.1038/s41467-018-07768-9

16 O'Dwyer DN, Ashley SL, Gurczynski SJ *et al.* Lung microbiota contribute to pulmonary inflammation and disease progression in pulmonary fibrosis. *American journal of respiratory and critical care medicine* 2019;**199**:1127–38. doi:10.1164/rccm.201809-1650OC

17 Dickson RP, Huffnagle GB, Flaherty KR *et al.* Radiographic honeycombing and altered lung microbiota in patients with idiopathic pulmonary fibrosis. *American journal of respiratory and critical care medicine* 2019;**200**:1544–7. doi:10.1164/rccm.201903-0680LE

18 B Moore B, Lawson WE, Oury TD *et al.* Animal models of fibrotic lung disease. *American journal of respiratory cell and molecular biology* 2013;**49**:167–79. doi:10.1165/rcmb.2013-0094TR

19 Dostert C, Pétrilli V, Van Bruggen R *et al.* Innate immune activation through nalp3 inflammasome sensing of asbestos and silica. *Science (New York, NY)* 2008;**320**:674–7. doi:10.1126/science.1156995

20 Baumgartner KB, Samet JM, Stidley CA *et al.* Cigarette smoking: A risk factor for idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 1997;**155**:242–8. doi:10.1164/ajrccm.155.1.9001319

21 Mossman BT, Churg A. Mechanisms in the pathogenesis of asbestosis and silicosis. *Am J Respir Crit Care Med* 1998;**157**:1666–80.

22 Catrina AI, Joshua V, Klareskog L *et al.* Mechanisms involved in triggering rheumatoid arthritis. *Immunological reviews* 2016;**269**:162–74.

doi:10.1111/imr.12379

23 Stolt P, Yahya A, Bengtsson C *et al.* Silica exposure among male current smokers is associated with a high risk of developing acpa-positive rheumatoid arthritis. *Annals of the rheumatic diseases* 2010;**69**:1072–6. doi:10.1136/ard.2009.114694

24 Rosenman KD. Rheumatoid arthritis and pneumoconiosis. *American journal of respiratory and critical care medicine* 2012;**185**:1024; author reply 1025–1024; author reply 1026. doi:10.1164/ajrccm.185.9.1024a

25 Mathews RJ, Robinson JI, Battellino M *et al.* Evidence of nlrp3-inflammasome activation in rheumatoid arthritis (ra); genetic variants within the nlrp3-inflammasome complex in relation to susceptibility to ra and response to anti-tnf treatment. *Annals of the rheumatic diseases* 2014;**73**:1202–10. doi:10.1136/annrheumdis-2013-203276

26 Kukkonen MK, Vehmas T, Piirilä P *et al.* Genes involved in innate immunity associated with asbestos-related fibrotic changes. *Occupational and environmental medicine* 2014;**71**:48–54. doi:10.1136/oemed-2013-101555

27 Ji X, Hou Z, Wang T *et al.* Polymorphisms in inflammasome genes and risk of coal workers' pneumoconiosis in a chinese population. *PloS one* 2012;**7**:e47949. doi:10.1371/journal.pone.0047949

28 Ji X, Wu B, Jin K *et al.* MUC5B promoter polymorphisms and risk of coal workers' pneumoconiosis in a chinese population. *Molecular biology reports* 2014;**41**:4171–6. doi:10.1007/s11033-014-3100-2

29 Pontillo A, Oshiro TM, Girardelli M *et al.* Polymorphisms in inflammasome' genes and susceptibility to hiv-1 infection. *Journal of acquired immune deficiency syndromes (1999)* 2012;**59**:121–5. doi:10.1097/QAI.0b013e3182392ebe

30 Abate E, Blomgran R, Verma D *et al.* Polymorphisms in card8 and nlrp3 are associated with extrapulmonary tb and poor clinical outcome in active tb in ethiopia. *Scientific reports* 2019;**9**:3126. doi:10.1038/s41598-019-40121-8

31 Flaherty KR, Wells AU, Cottin V *et al.* Nintedanib in progressive fibrosing interstitial lung diseases. *The New England journal of medicine* 2019;**381**:1718–27. doi:10.1056/NEJMoa1908681

32 Wollin L, Wex E, Pautsch A *et al.* Mode of action of nintedanib in the treatment of idiopathic pulmonary fibrosis. *The European respiratory journal* 2015;**45**:1434–45. doi:10.1183/09031936.00174914

33 Fiddler CA, Simler N, Parfrey H *et al.* Severe colitis associated with pirfenidone use in idiopathic pulmonary fibrosis. *Annals of the American Thoracic Society* 2016;**13**:1430–2. doi:10.1513/AnnalsATS.201602-118LE

34 Bédard Méthot D, Leblanc É, Lacasse Y. Meta-analysis of gastroesophageal reflux disease and idiopathic pulmonary fibrosis. *Chest* 2019;**155**:33–43. doi:10.1016/j.chest.2018.07.038

35 An J, Gharahkhani P, Law MH *et al.* Gastroesophageal reflux gwas identifies risk loci that also associate with subsequent severe esophageal diseases. *Nature communications* 2019;**10**:4219. doi:10.1038/s41467-019-11968-2

36 Han MK, Zhou Y, Murray S *et al.* Lung microbiome and disease progression in idiopathic pulmonary fibrosis: An analysis of the comet study. *The Lancet Respiratory medicine* 2014;**2**:548–56. doi:10.1016/S2213-2600(14)70069-4

37 Molyneaux PL, Cox MJ, Willis-Owen SAG *et al.* The role of bacteria in the pathogenesis and progression of idiopathic pulmonary fibrosis. *American journal of respiratory and critical care medicine* 2014;**190**:906–13. doi:10.1164/rccm.201403-0541OC

38 Knippenberg S, Ueberberg B, Maus R *et al.* Streptococcus pneumoniae triggers progression of pulmonary fibrosis through pneumolysin. *Thorax* 2015;**70**:636–46. doi:10.1136/thoraxjnl-2014-206420

39 Parrow NL, Fleming RE, Minnick MF. Sequestration and scavenging of iron in infection. *Infection and immunity* 2013;**81**:3503–14. doi:10.1128/IAI.00602-13

40 Gupta R, Shah P, Swiatlo E. Differential gene expression in streptococcus pneumoniae in response to various iron sources. *Microbial pathogenesis* 2009;**47**:101–9. doi:10.1016/j.micpath.2009.05.003

41 Allden SJ, Ogger PP, Ghai P *et al.* The transferrin receptor cd71 delineates functionally distinct airway macrophage subsets during idiopathic pulmonary fibrosis. *American journal of respiratory and critical care medicine* 2019;**200**:209–19. doi:10.1164/rccm.201809-1775OC

42 Davies NM, Holmes MV, Davey Smith G. Reading mendelian randomisation studies: A guide, glossary, and checklist for clinicians. *BMJ (Clinical research ed)* 2018;**362**:k601. doi:10.1136/bmj.k601

43 Lawlor DA, Tilling K, Davey Smith G. Triangulation in aetiological epidemiology. *International journal of epidemiology* 2016;**45**:1866–86. doi:10.1093/ije/dyw314

44 Wootton RE, Richmond RC, Stuijfzand BG *et al.* Evidence for causal effects of lifetime smoking on risk for depression and schizophrenia: A mendelian randomisation study. *Psychological medicine* 2019;1–9. doi:10.1017/S0033291719002678

45 Gill D, Benyamin B, Moore LSP *et al.* Associations of genetically determined iron status across the phenome: A mendelian randomization study. *PLoS medicine* 2019;**16**:e1002833. doi:10.1371/journal.pmed.1002833

46 Georgakis MK, Gill D, Rannikmäe K *et al.* Genetically determined levels of circulating cytokines and risk of stroke. *Circulation* 2019;**139**:256–68. doi:10.1161/CIRCULATIONAHA.118.035905