

Is occupational asbestos exposure an under-recognised cause of idiopathic pulmonary fibrosis?

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A thesis presented for the degree of
Doctor of Philosophy

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I, Carl Jonathan Reynolds confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

The question of whether occupational asbestos exposure is an under-recognized cause of idiopathic pulmonary fibrosis arises because it is clinically plausible, epidemiologically plausible, and consistent with fibre studies and case-control data. This thesis examines the question by means of a literature review and a novel hospital based case-control study, the idiopathic pulmonary fibrosis job exposures study (IPFJES).

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Abbreviations

- **IPF** Idiopathic pulmonary fibrosis.
- **MUC5B** Mucin 5B gene.

Chapter 1

Introduction to thesis

1.1 Occupational asbestos exposure as an underrecognised cause of idiopathic pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is a progressive, fibrotic lung disease which in 2012 was the recorded cause of death for c.4000 people in England and Wales. Its incidence, currently around 7.5/100,000 person-years, has increased by 5% pa since 2000.[1] The pathophysiology of IPF is complex, the outcome of host susceptibility factors, epithelial injury, and a dysregulated repair process. Several gene polymorphisms which result in a vulnerable alveolar epithelium have been characterized; they include abnormalities in mucin genes (eg MUC5B), surfactant protein genes, and telomerase genes (eg TERT and TERC).[2][3][4] The median age of onset is 70 years and the condition is more common in men (M:F ratio 1.6), manual workers, and those living in industrial areas[1], patterns that are not unique to the UK.[3] The prognosis is poor, with a median survival of three years.[5][6]

These epidemiological distributions of IPF are consistent with a long-latency response to occupational dust exposure; in particular, the incidence of IPF correlates strongly (if ecologically) with historic asbestos use.[7] Clinical, radiological and histopathological findings in asbestosis and IPF are similar[8][9]. Mineralogical studies support the concept of asbestosis-IPF mis-

classification by revealing high fibre burdens in the lung tissue of patients diagnosed with ‘IPF’ and revision of the diagnosis to ‘asbestosis’.[10][11][12][13]

Identification of occupational asbestos fibre exposure as an under-recognised cause of IPF is important to improve our understanding of the aetio-pathophysiology of IPF and the accuracy of prognostic information. It would have implications for compensation and impact on the current restrictions on individual treatment. Importantly, it would inform evidence-based workplace exposure policies in the UK and internationally, particularly in the many countries with continuing high levels of asbestos use.

1.2 Aims and objectives

My overall aim is to characterize and measure asbestos exposure as an occupational determinant of IPF; additionally, I will determine host-exposure interactions mediated by candidate susceptibility polymorphisms (in particular MUC5B promoter polymorphism rs35705950).

My specific research questions are:

1. Does a dose-response relationship exist for occupational asbestos exposure and IPF?
2. Does the presence of asbestos exposure modify the association between IPF and rs35705950?

1.3 Data sources

- For the literature review and meta-analysis of occupational exposures in IPF I consider all published IPF case-control studies reporting on occupational exposures.
- For the mortality analysis I use data obtained from the Office of National Statistics, Health and Safety Executive, and the World Health Organisation Mortality Database.

- Brief reviews of asbestos exposure assessment and genetic susceptibility in IPF rely on the published literature.
- Primary case-control data collected during my PhD as part of the idiopathic pulmonary fibrosis job exposures study (IPFJES) is used to analyze asbestos exposure in IPF. (?include navaratum case control jobs data that was shared)

1.4 Outline of thesis

This chapter (Chapter 1) describes the problem studied, aims and objectives, and approach. Chapter 2 is a literature review and meta-analysis of IPF case-control studies that report on occupational exposure. Chapter 3 is an analysis of IPF and asbestos related disease mortality data. Chapter 4 is a review of asbestos exposure assessment methodology. Chapter 5 is a review of genetic susceptibility in IPF. Chapter 6 describes the idiopathic pulmonary fibrosis job exposures study including results and analysis arising from it. Chapter 7 concludes the thesis by summarising it and suggesting future work.

Chapter 2

Literature review and meta-analysis: how much IPF is attributable to occupational exposures?

2.1 Introduction

Idiopathic pulmonary fibrosis (IPF) is a diagnosis of exclusion. It is made in the presence of a usual interstitial pneumonitis (UIP) pattern on high resolution CT scan or biopsy. The diagnosis requires that known causes of interstitial lung disease (such as drug toxicity, connective tissue disease, domestic, and occupational or environmental exposures) be excluded.[14]

Attributing a disease process to a specific exposure can be difficult. Disease processes are frequently complex or multifactorial, depending on the interaction of genetic and environmental components. Well-studied and relatively frequent entities such as chronic obstructive pulmonary disease, ischaemic heart disease and diabetes lend themselves to epidemiologic investigation, delineating the major risk factors for disease and their relative contributions to risk at the population level. IPF presents an additional challenge to attribution; because of its relative infrequency, epidemiologic study of the disease

is largely limited to case-control studies.[15] Studying specific occupational exposures also presents its own challenges; co-exposure is common, occupational hygiene data is frequently limited and self-reported exposure is prone to recall bias.

I identified several review articles of the epidemiology of interstitial lung disease that do not necessarily focus on IPF and only briefly mention occupational factors (e.g Ley2013[3]). Here I consider review articles that specifically deal with occupational factors in IPF and cite the case-control studies used.

Turner-Warwick (1998) discusses potential difficulties in establishing attribution and causality in IPF. She observes that there is variation in clinical practice with respect to the standard applied to exclude IPF; some clinicians exclude IPF when exposure to a potential cause is identified, others only when there is clear exposure to an established cause. She explains that diagnosis based on radiologic and clinical findings, and not on lung biopsy or bronchioalveolar lavage, may result in initiating agents for disease being overlooked. Further, that exposures such as asbestos, silica, coal, graphite, hard metal, and avian proteins, may result in disease that can not be differentiated from IPF.[16]

Reviewing the epidemiology of IPF and case-control studies to date Hubbard (2001) describes the association of IPF with occupational exposures to metal and wood and estimates that 10% of IPF cases may be due to occupational metal exposure and 5% of cases to wood.[17]

Taskar and Coultas (2006) review and carry out a meta-analysis of six case-control studies investigating environmental and occupational exposures in IPF. They report population attributable risk percentages for agriculture and farming (20.8%), livestock (4.1%), wood dust (5%), metal dust (3.4%), stone/sand/silica (3.5%), and smoking (49.1%).[18]

Gulati and Redlich's (2015) review of exposures causing usual interstitial pneumonia highlights that asbestosis may appear indistinguishable from IPF and summarises previous case-control studies but did not pool studies to perform a meta-analysis.[19]

I sought to identify and meta-analyze all IPF case-control studies dealing with occupational exposures.

2.2 Method

Pubmed, embase, and google scholar search engines were searched for combinations of the terms ‘idiopathic pulmonary fibrosis’, ‘occupation’, ‘case-control study’ and synonyms. When a relevant papers was identified papers referenced and papers citing the paper were reviewed. Medline ranker[20] and bespoke pubmed ‘mining’ techniques[21] were also used.

Two investigators independently reviewed and abstracted data for five exposure categories common to the identified case-control studies: “vapors, gases, dusts, and/or fumes (VGDF)”, “metal dust”, “wood dust”, “silica dust”, and “agricultural dust”. We calculated PAF as follows: $PAF = pc(OR - 1)/OR$, where pc is the proportion of cases exposed and OR is the risk estimate.

We calculated pooled OR and pooled PAF for occupational exposures using fixed effects models and random effects models in Stata. When there was results of the models differed substantively, we used the results of the fixed effects model, which were more conservative. The pooled PAF relied on the ratio of attributable cases to all cases underlying each risk estimate.

2.3 Results

We found (as of May 2017) 15 case-control studies looking at occupational exposures in IPF the most recent review article covers only eight of them. Associations with metal, wood, silica, and agricultural dust were reported. [22] [23] [24] [25] [26] [27] [28] [29] [30] [31] [32] [33] [34] [35] [36]

40 risk estimates from 12 publications (1326 IPF cases in total) were used (Table 3.1)[22] [24] [25] [26] [27] [28] [29] [31] [32] [33] [34] [36]

Three studies were not used, one because data was not collected on the proportion of cases with specific occupational exposures[23], one because of methodological differences in exposure assignment[30], and one because it reported data for pulmonary fibrosis rather than IPF.[35]

Each exposure category was assessed with 6-11 risk estimates (Table 3.2).

2.4 Discussion

Our results support the case for a proportion of IPF cases being attributable to occupational exposures.

Pooled ORs were significantly elevated for VGDF, metal dust, wood dust, agricultural dust, and silica dust; the pooled PAF estimates by category ranged from 4-23%. This is an important finding for an otherwise idiopathic disease which carries significant morbidity and mortality; identifying causal occupational agents would permit remediation and prevention.

Associations between IPF and wood, metal, and agricultural dust were previously reported in a meta-analysis of six case-control studies by Taskar and Coultas. [18] While our findings are similar we found a smaller effect size for agricultural exposure and a large effect size for non-specific vapours, gases, dust, and fumes (VGDF), see Table 3.2.

Funnel plot asymmetry using Egger’s test, which may be due to publication bias, was present for VGDF ($p = 0.04$) and metal dust ($p = 0.03$) but not for wood dust ($p = 0.09$), silica dust ($p = 0.2$), and agricultural dust ($p = 0.6$). However, the number of studies included is small and funnel plot asymmetry may be due to chance rather than bias.

There are several limitations to the meta-analysis that arise from the case-control studies included.

Several studies [22] [37] [26] [29] [32] used population controls but do not provide details on participation rates. Participation rates can be low for community controls; a recent UK case-control study investigating prothrombotic factors in IPF reported a response rate of 28% for community con-

Table 2.1: Summary of IPF case-control studies investigating occupational exposures

Reference (n cases)	OR; 95% CI					PAF %				
	vgdf*metalwoodag si					vgdf*metalwoodag si				
Scott 1990(40)[?]	1.3; 0.8, 2.0	11.0; 2.3, 52.4	2.9; 0.9, 9.9	10.9; 1.2, 96.0	1.6; 0.5, 4.8	17	12	10	12	5
Hubbard 1996(218)[?]	1.7; 1.1, 2.7	1.7; 1.0, 2.9				10	6			
Mullen 1998(15)[?]	2.4; 0.7, 8.4	3.3; 0.4, 25.8	11; 1.1, 115			20	7			20
Baumgartner 2000(248)[?]	2.0; 1.0, 4.0	1.6; 0.8, 3.3	1.6; 1.0, 2.5	3.9; 1.2, 12.7		5	3	7	2	
Hubbard 2000(22)[?]	1.1; 0.4, 2.7					5				
Miyake 2005(102)[?]	5.6; 2.1, 17.9	9.6; 1.7, 181.1	6.0; 0.3, 112.4	0.6; 0.2, 41.9	1.8; 0.5, 7.0	26	11	4	0	5
Gustafson 2007(140)[?]	1.1; 0.7, 1.7	0.9; 0.5, 1.6	1.2; 0.7, 2.2	1.4; 0.7, 2.7		6	0	3		3
Garcia-Sancho Figueroa 2010(97)[?]	1.2; 0.8, 1.9					9				
Garcia-Sancho 2011(100)[?]	2.8; 1.5, 5.5					50				
Awadalla 2012 men (95)[?]	1.6; 0.7, 3.6	2.7; 1.1, 6.8	1.0; 0.4, 2.3	1.1; 0.5, 2.7		6	9	0	1	
Awadalla 2012 women (106)[?]		4.3; 0.8, 22.1	3.3; 1.2, 10.1				6	14		
Paolocci 2013 soft wood (abstract only)(65)[?]	2.8; 1.1, 7.2	1.1; 0.4, 3.3	2.0; 0.9, 4.4			9	0			11
Paolocci 2013 hard wood (abstract only)(n/a)[?]		0.9; 0.3, 2.8	8			0				
Kassam 2013 men (100)[?]	2.7; 0.7, 10.0	5.0; 1.5, 17.0	2.5; 1.2, 5.0	1.2; 0.5, 3.0		25	22	5		5

trols. [38] This approach is vulnerable to non-responder bias. One study[27] used employee occupational records and death certificates from pension-fund records for a single company and was only able to locate the occupational records for 40% of cases and 38% of controls.

Nearly all studies relied on self-reported exposures rather than life time occupational histories to assess exposure; an approach that is prone to recall bias and does not permit examination of dose-response relationships.

Reliance of self-reported exposures also means that studies are potentially vulnerable to confounding as a result of co-exposure. For example, several studies have described strong associations between metal work and IPF and specify sheet metal workers[23][22][27], a group who are frequently exposed to dust containing asbestos fibres[39] and who in a recent UK study, had the highest risk of mesothelioma.[40]

Case definitions and sources for cases varied between studies. For example Scott (1990)[22] used a case definition which included a chest radiograph showing bilateral interstitial shadowing whereas most other studies relied on high resolution CT. Four studies used mortality data [23][30][29][27] to identify cases and one study[29] used a national register of patients receiving oxygen therapy. Differences in healthcare coverage and coding practices can result in selection bias.[41]

2.5 Conclusion

The observed excess risk could represent disease misclassification of pneumoconiosis or hypersensitivity pneumonitis, but this is unlikely to fully explain the observed effects. Our analysis supports an etiologic role for occupational exposures in IPF, potentially explaining up to 23% of the burden of disease and highlighting a role for workplace exposure reduction in disease prevention.

Chapter 3

Mortality analysis: do mortality trends support an occupational cause?

3.1 Introduction

The incidence of Idiopathic pulmonary fibrosis (IPF) has been increasing at a rate of 5% per annum since 2000. By definition, the diagnosis of IPF is not made in the presence of an identifiable cause. However, the distribution of the disease in the population (more common in men, manual workers, and those living in more industrial areas of the country) suggests a causal contribution from an occupational or environmental source.

It is hypothesised that a proportion of Idiopathic Pulmonary Fibrosis (IPF) cases are due to occult environmental or occupational exposures to asbestos dust. This would be expected to result in a spatio-temporal association between IPF, Mesothelioma, and Asbestosis mortality patterns coinciding with asbestos exposure. It would also be expected to produce a birth cohort effect.

Our aim was to examine trends in IPF, Mesothelioma, and Asbestosis mortality data for evidence of cohort effect and association.

3.2 Method

Regional age and sex stratified mortality data for IPF, Mesothelioma, and Asbestosis were obtained for England and Wales from the Office of National Statistics for the period 1974–2012. Data were age-standardised and visualised using the Python Pandas data analysis library and matplotlib.

3.3 Results

IPF mortality continues to rise. Female:Male is approximately 1:1.6. There are more IPF deaths in the North West and South East of England. IPF mortality does appear to correlate with mesothelioma mortality (Figure 3.1). There is evidence of a cohort effect with age-specific IPF death rates increasing in successive cohorts, most clearly seen from age 60 (Figure 3.2). While overall rates were higher for men but there were not marked sex differences in cohort mortality trends.

3.4 Discussion

icd coding chat

This is the discussion. Duis ultrices tempor sem vitae convallis. Pellentesque lobortis risus ac nisi varius bibendum. Phasellus volutpat aliquam varius. Mauris vitae neque quis libero volutpat finibus. Nunc diam metus, imperdiet vitae leo sed, varius posuere orci.

3.5 Conclusion

Conclusions: The birth cohort effect we observed is consistent with a proportion of IPF cases being due to an occupational or environmental exposure with latency and further research is needed.

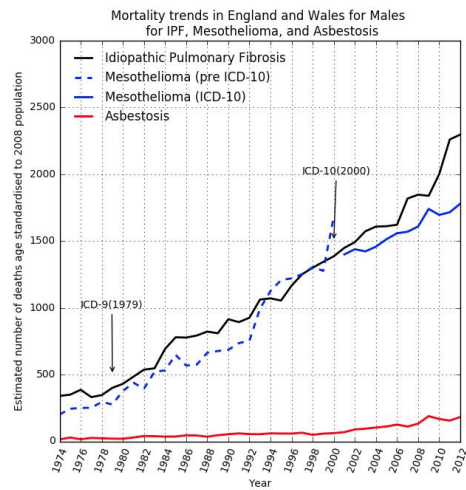


Figure 3.1: IPF, mesothelioma, and asbestosis mortality trends

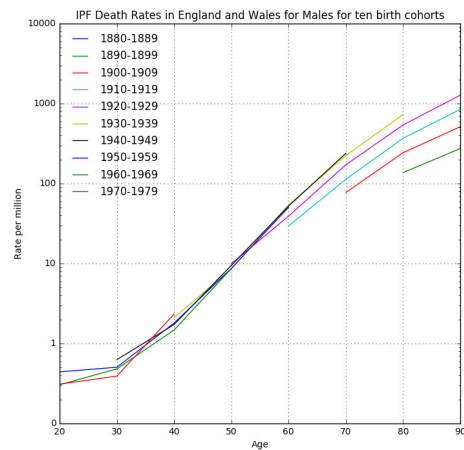


Figure 3.2: IPF male birth cohorts

Chapter 4

Historic asbestos exposure assessment: can it be done?

4.1 Introduction

Asbestos related respiratory disease is initiated by inhalation of asbestos fibres. In the UK clinically significant asbestos exposure is largely occupational and, as a result of asbestos control legislation, historic.

Occupational asbestos exposure can be assessed quantitatively by sampling ambient air at a workplace and calculating a fibre count using microscopy. Alternatively, because inhaled asbestos fibres persist in the lung they can be sampled by lung biopsy, bronchoalveolar lavage, or at autopsy.

Historic workplace measurements are a valuable resource for assessing exposure but are limited in several ways. Measurements are not available for many occupations, where measurements are available they are dependant on working practices and measurement technique at the time of assessment; they do not necessarily generalize well.

Measurement of asbestos fibres in lung tissue by means of biopsy or bronchoalveolar lavage is invasive and both procedures carry the risk of serious complication including death. Additionally, the biopersistence of asbestos fibres is variable, counts are sensitive to techniques used, and establishing

appropriate references ranges is challenging.[42]

Expert assessment and exposure modelling approaches integrate historic workplace measurements with simulated workplace measurements and an individuals recollection of job processes he or she has carried out during their working life.[43]

Job-exposure matrices (JEMs) are widely used in occupational epidemiology studies to assess exposure to potential hazards. These assign levels of exposure to health hazards on the basis of job title.

Finally, self-reported exposures are a subjects direct report of what they have been exposed to, these are usually elicited by questionnaire or at interview.

The asbestos exposure assessment literature presents difficulties for review because it is large and recognised to be at risk of bias as a result of its economic importance to powerful industrial and medicolegal actors[44].

Here we critically review different means of historic asbestos exposure assessment and consider their clinical and research utility.

4.2 Method

We searched pubmed and google scholar for combinations and synonyms of “asbestos”, “exposure assessment”, together with terms for modes of assessment including “lung biopsy”, “bronchoalveolar lavage”, “exposure reconstruction”, and “job-exposure matrix”. When a relevant papers was identified, papers referenced, and papers citing, the paper were reviewed.

4.3 Results

4.3.1 LUNG BIOPSY AND BRONCHOALVEOLAR LAVAGE

The first report of fibrosis of the lung due to asbestos dust[45] included a description of the post mortem microscopic appearances of the lungs which

showed abundant asbestos fibres in areas of fibrosis.

The demonstration of asbestos fibres on lung biopsy in the context of pulmonary fibrosis is clearly supportive of the diagnosis of asbestosis. However, a failure to demonstrate fibres can not be used to rule out asbestos exposure because fibres, particularly chrysotile fibres, may be cleared from the lung and counting methods have a significant false-negative rate.[42]

Despite this recent 2014 Helsinki guidelines[46] and UK Royal College of Pathologists guidelines appear to suggest that a clear history of substantial occupational asbestos exposure is insufficient for diagnosis and that the absence of asbestos bodies or fibre counts above a certain threshold might be used to rule out the diagnosis. The shortcomings of such an approach highlighted above are also described by responses to the Helsinki guideline.[47][48][49]

Lung biopsy carries significant health risks, particularly for patients who already have compromised lung function and it can not be justified solely on medico-legal grounds.[48] Therefore, the clinical utility of lung biopsy and bronchoalveolar lavage is limited to ruling in asbestosis when a suggestive exposure history and radiology are lacking.

In a research context lung biopsy and bronchoalveolar lavage have provided valuable population level insights. Lung biopsy asbestos fibre counts have been examined in a UK case-control study where mesothelioma cases were compared with lung cancer controls. Fibre counts were found to be higher in groups with greater occupational risk (as defined by PMR), providing additional support for the pre-eminence of an occupational history.[40][50] In a follow up study asbestos fibre counts from unselected surgically treated pneumothorax patients were used to demonstrated that population amphibole burden is falling and is proportional to mesothelioma mortality.[51]

A similar correlation with occupational exposure history, overall downward trend in fibre counts, and a significant false negative rate has been observed in a recent Belgian study of patients undergoing bronchoscopy with bronchoalveolar lavage sampling for asbestos fibre quantification.[52]

4.3.2 HISTORIC WORKPLACE MEASUREMENTS

Occupational hygienists have recorded a large numbers of workplace measurements of asbestos in different settings, at different times, using a variety of different means. These measurements reside in national databases such as the HSE National Exposure Database[53], and EV@LUTIL[54], in the published literature, and in unpublished company records.

The use of different means of making workplace assessments results in difficulties with respect to the accuracy and comparability of measurements. For example, instruments that count particles rather than asbestos fibres have been used and there is no established conversion factor.[55] Phase contrast microscopy has also been used which is less sensitive than scanning electron microscopy, which is in turn less sensitive than transmission electron microscopy and energy-dispersive x-ray analysis.[56]

Where era and task specific workplace exposure data matching a particular patient occupational history is available and readily deniable it is a valuable means of assessing exposure history. Unfortunately, in practice measurements are usually limited to the subset of jobs thought to be potentially harmful “high” exposure jobs at the time of measurement. As awareness of the sources and harm of asbestos exposure has developed overtime the available data, until the use of asbestos was banned in the UK, is also skewed to more recent times.[57][58]

Measurements have found greater utility in a research setting where they can help to quantify risk and inform regulatory policy and compliance in specific workplace settings, for example, in car mechanics[59] or skilled craftsmen.[60]

4.3.3 EXPOSURE RECONSTRUCTION

Sahmel et al[58] propose a seven-step framework (see Figure 4.1) which they use to enumerate and critique exposure reconstruction approaches.

Reconstruction techniques may be quantitative, semi-quantitative, or qualitative. Quantitative exposure reconstruction bases exposure estimates on

data from similar (historic or current) exposure scenarios or simulation studies. Semi-quantitative exposure reconstruction bases exposure estimates on exposure data matrices (using a job-exposure matrix) and/or exposure determinants (using an exposure model). Qualitative exposure reconstruction bases exposure estimates on the expert judgement of an industrial hygienist and self reported exposures.[58]

4.3.3.1 Job-exposure matrices

Several job-exposure matrices that deal with asbestos have been reported. Pannett et al's 1985 job-exposure matrix for use in population studies in England and Wales[61] found good agreement between job-title assigned categories of exposure (none, low, moderate, high) for asbestos and direct review of the original occupational history by an expert.

Rake et al[40] assigned categories risk of exposure (low, medium, high) using occupational mortality statistics for pleural mesothelioma. Because pleural mesothelioma in men is nearly entirely attributable to occupational asbestos exposure, pleural mesothelioma is rapidly fatal, and death certificates record occupation in addition to cause of death, the proportional mortality ratio for pleural mesothelioma (number of deaths due to pleural mesothelioma/total number of deaths) can serve as proxy for average asbestos exposure in a particular occupation. This approach has been validated in the same cohort by amphibole fibre counts.[50]

DOM-JEM[62] was developed for use in population based multi-centre lung



Figure 4.1: Seven step framework for exposure reconstruction

cancer case-control study. It assigns job titles one of three categories of asbestos exposure (no exposure, low exposure, high exposure) based on the consensus of three independent expert raters. DOM-JEM showed poor agreement with expert assessment ($\kappa = 0.17$) but less heterogeneity. In a study applying DOM-JEM to the Netherlands Cohort Study (NCS) DOM-JEM showed poor agreement with expert assessment ($K = 0.29$).[63]

The Finish Information System on Occupational Exposure (FINJEM)[64] covers exposure to 84 different agents, including asbestos, for 311 jobs across 9 periods spanning 1945-2015. Era-specific estimates of the mean level of asbestos exposure are available for 27 jobs based on expert assessment and measurement data; the exact details of the grounds for estimates are kept in a proprietary FINJEM database which is not freely available. FINJEM showed poor agreement with expert assessment of asbestos exposure ($\kappa = 0.23$) but reasonable identification of mesothelioma risk when evaluated using the NCS.[63][65]

AsbJEM[66] was developed in Australia by an expert panel of three industrial hygienists using all available exposure data. It is based on FINJEM and provides quantitative estimates of annual exposure for 224 occupations across three time periods spanning 1943 to 2004. It also showed poor agreement with expert assessment of asbestos exposure ($\kappa = 0.10$)

SYN-JEM[67] describes a JEM developed for four carcinogens. It provides quantified asbestos exposure estimates based on 27958 personal measurements (spanning 1971-2009), a mixed effects statistical model, and a priori categorical assessment of exposure (none, low, high). Cherrie et al[68] point out that SYN-JEM provides little contrast in the modelled exposure level between categories as the geometric mean for low jobs was 0.061 fibres/ml and for high jobs 0.074 fibres/ml and that there are wide variations in regional estimates that are difficult to explain.

JEMS are generally taken to be superior to direct questions about exposures because they are cheaper, have greater validity, and are less vulnerable to differential recall. This is because recall of occupations is not influenced by disease status, coding of occupation is blind to case-control status, and translation of codes into exposure is standardized and can not be influence

by disease status of a subject.[69][70][71]

Orlowski et al[72] compared two JEMs with a structured job specific questionnaire (SQ) in a lung cancer case-control study. They found that agreement between the JEMs and the SQ was poor ($\kappa = 0.23 - 0.27$) and suggested that the sources of error for JEMs were loss of information due to the use of job codes as surrogates for job task descriptions and the insufficiency of published data on occupational asbestos exposure.

JEMs are not routinely used in clinical practice because they are not usually available or accessible for specific patients. In a research setting they are frequently helpful though in addition to the strengths and weaknesses outlined about the desirability of reusing an existing JEM vs developing a study specific JEM must be considered.

4.3.3.2 Exposure modelling approaches

Exposure modelling approaches modify existing measurement data on the basis of knowledge of the determinants of exposure. They may be viewed as the formalization of professional decision criteria used by hygienists in their assessment of workplace exposures.[57]

A common conceptual framework for this is the source-receptor model source receptor model[73][57] whereby inhalation exposure is considered in terms of an exposure source, a pathway from source to receptor, and the receptor. The model is then used to propose modifying factors such as activity emission potential, substance emission potential, localized control, worker behavior, surface contamination and respiratory protection.[73].

In the hands of some hygienists assessment of historic asbestos exposure based on interview can correlate well with amphibole fibre counts.[74] By extension, exposure modelling approaches, using industrial hygienist methods, might be expected to be useful. Exposure modelling approaches make strong intuitive sense; it is known that there is significant within-worker and between-worker variability in occupational exposures[75] and, for example, room size and ventilation have been empirically shown to affect the concen-

tration of airborne chemical exposures.[76] Further, mathematical exposure models that take account of known exposure modifying factors to estimate past exposures have shown a good correlation with measured values.[43]

A quantified validated historic asbestos exposure model[68] has recently been developed and proposed as a means of for risk stratifying asbestos exposed workers to optimize mesothelioma screening efforts. The approach has the advantage, compared with job-exposure matrices, of providing a more granular quantified exposure assessment, sensitive to the exposure circumstances of the individual. However, the approach is limited by the fact that the individual must recall that they must recall their exposure circumstances which due to the latency of asbestos related disease may have occurred over 30 years ago. The approach is also limited by the relatively small number of industry-specific data points used for validation, though is unavoidable because of the scarcity of exposure measurement data.

Exposure modelling approaches to assessing asbestos exposure have research and clinical utility notwithstanding the limitations outlined above together with the requirement that assessors be appropriately trained.

4.3.3.3 Self-reported exposure

Self-reported exposures are a subjects direct report of what they have been exposed to. Typically this is elicited by asking about a specific exposure via questionnaire or interview. Differential recall of self-reported exposures according to disease status is a concern but few studies have found evidence of this and it appears to be less of an issue when prompted responses, rather than volunteered, responses about occupational exposures are used.[77]

Most studies comparing self-reported exposures to industrial hygiene measurements have found significant associations but with wide variation in the proportions of variance explained by the self reports. This is not surprising given that it is known there is significant within-worker and between-worker variability in occupational exposures.[70][75]

Studies comparing self-reported exposures to expert assessment find highly

variable levels of agreement ($\kappa = 0.05 - 0.94$) with a median $\kappa 0.6$. In two studies comparing self-reported exposures with JEMs, self-reported exposures were more sensitive and of similar or worse specificity.[70]

Self-reported exposures have been shown to be more accurate for easily sensed exposures such as solvents with a strong smell, dusts with larger particle sizes, and vibrations that can be felt. Providing a reference point, for example using well known machines from a workplace to gauge noise category also improves accuracy.[70]

Self-reported exposures have clinical utility in that they can suggest or support consideration of an occupational cause for disease. Ideally such self-reports are combined with the clinicians knowledge of the likely occupational exposures given the occupational history and other available data to strengthen or weaken the case as appropriate. Similarly, they have utility in a research setting where they may augment other means of assessment.

4.4 Discussion

The accuracy of historic asbestos exposure assessment, by any means, is limited by the paucity of occupational asbestos measurement data, measurement technique limitations, within and between worker exposure variability, and participant recall. There does not exist a universally agreed “gold standard” against which to evaluate methods. Accurate quantified assessment of historic exposure, where evidence is scarce, may be an impossible task.[78]

Nonetheless, clinically, historic asbestos exposure assessments must be made for attribution. Specifically, to inform whether the required threshold of asbestos exposure (as assessed by various means) has been crossed so it is possible to say that, for example, scarring of the lung with an usual interstitial pneumonia pattern in an individual patient is caused by asbestos exposure. This carries medicolegal in addition to scientific importance and has not been well established by any assessment method.

In the context of mesothelioma case-control studies fibre-counts do at least provide an objective means of assessing historic asbestos exposure against

which other means can be compared. It is encouraging that industrial hygienist assessment and assessment using job title and PMR correlates strongly with fibre counts.[71][50] Further and more generally, it is encouraging that estimates from explicit asbestos exposure modelling systems such as Cherrie et al's[68], show good correlation with measurement data.

4.5 Conclusion

Quantitative estimates of historic occupational asbestos exposures will generally have high uncertainty. However, less precise measures, such as relative difference in exposure among epidemiological groups may be quite certain even though the numerical estimates are only approximate. This is invaluable in studies examining aetiological hypothesis.[57]

Chapter 5

MUC5b + environmental insult = IPF?

5.1 Introduction

5.1.1 MUCUS, MUCINS, MUC5B: STRUCTURE, FUNCTION AND EVOLUTIONARY IMPORTANCE

Mucus is an essential part of the innate immune system, considered to be universal within most phyla of both aquatic and terrestrial metazoans. It plays a pivotal role in the prevention of disease by serving as an antimicrobial barrier, it also has physiological functions including allowing the exchange of oxygen, carbon dioxide, nutrient and metabolites, lubricating surfaces and reducing damage due to sheer, reducing dehydration of the epithelia and providing the polymeric matrix which enables ciliary-mucus particle transport. Mucus barriers are essential for the separation and protection of an organism from its external environment, and likely a prerequisite for the exclusion of bacteria from bodily tissues and evolution of gastrointestinal and respiratory tracts. The importance of mucus barriers is further underlined when one considers the energy investment continuous mucus production and release requires; for example, corals use mucus to trap particles and transport them towards their mouths and the reef-building coral *Acropora acuminata*

is thought to dedicate up to 40% of its daily net carbon fixation to this task alone.[79] Mucins are a key component of mucus, they are highly evolutionary conserved large glycoproteins that date back around 600 million years to *Nematostella vectensis*, the starlet sea anemone, which is an early marine invertebrate. The earliest human mucin analogue is found in *Xenopus tropicalis*, the African clawed frog, which evolved about 300 million years ago and mucins are the likely explanation for the observation that frogs show such great resistance to infection during dissection and it has been shown that knockdown of mucin in the skin mucus barrier of *Xenopus tropicalis* tadpoles leads to susceptibility to infection by the opportunistic pathogen *Aeromonas hydrophila*. [80]

The mucin family is composed of proteins that contain tandem repeat structures with a high proportion of prolines, threonines, and serines; the PTS domain. It is further defined by extensive glycosylation of the PTS domain through N-Acetylgalactosamine O-linkages at the threonine and serine residues.[81] The resultant oligosaccharide chains and polymeric structure create the viscoelastic properties of mucus which confer its barrier properties and play an important role in storage and secretion. [79] Mucins are 50-90% carbohydrate and they are anionic because most of their terminal sugars contain carboxyl or sulphate groups. Mucin glycan helps to sequester pathogen by acting as a ‘decoy’ and providing sites for microbial adhesins to bind; for example, human salivary MUC5b interacts with streptococcal species, and patterns of glycosylation change during inflammation.[82][83] Mucin barriers can be subverted by pathogens, strategies include production of enzymes to degrade mucin core proteins and mucin carbohydrates, and evolution of effective motility through mucus gels - many mucosal bacterial pathogens are flagellated for this reason. There is evidence that degradative enzymes are required for pathogenesis in species such as *Vibrio cholerae* and that flagella are required for infectivity in species such as *Helicobacter pylori*. [82] Intracellular gel-forming mucins are stored in a compact and condensed form in granules within mucus-secreting cells. They are stored in solution with a high concentration of calcium ions and protons which is thought to be necessary to mask the anionic charge and prevent electrostatic repulsion, upon secretion mucins expand 1000-3000 fold taking up water to form a gel as

calcium is exchanged for sodium and the pH rises.[79] One consequence of mucins being stored in such a compact form is that when they're released they can obstruct the airway which in mouse models appears necessary for the clearance of helminth infection[83] and may provide a clue to their evolution.

Normal human airway mucus is a hydrogel composed of approximately 98% water, 0.9% salt, 0.8% globular proteins, and 0.3% high-molecular-weight mucin polymers.[84] Mucin hypersecretion may increase the concentration of solids up to 15% resulting in viscous elastic mucus that is not easily cleared.[85] 17 genes encode mucins in the human genome of which the gene products of seven are secreted and the remainder are membrane bound. Five of the secreted mucins have terminal cysteine rich domains that can form disulfide bonds resulting in polymers that impart the properties of a gel. MUC5AC and MUC5B, two secreted gel-forming mucins, are strongly expressed in the human respiratory tract. MUC5AC is predominantly expressed in the conducting airways and MUC5B is predominantly expressed in the respiratory airways (muc5b is also expressed in salivary glands, cervix, gallbladder, seminal fluid, and middle ear epithelium). Secreted mucins are large glycoproteins (up to 3×10^6 D per monomer), ranking among the largest molecules encoded in mammalian genomes, and their expression induces and requires an endoplasmic reticulum stress response.[86] Mucin production and secretion are regulated by distinct mechanisms. Production is highly regulated at transcriptional level. The ErbB family of proteins contains four receptor tyrosine kinases, structurally related to the epidermal growth factor receptor (EGFR), its first discovered member. ErbB-receptor signaling appears important for MUC5AC production since inhibition blocks MUC5AC up-regulation by diverse stimuli. Interleukin-13 (IL-13) is a cytokine secreted by T helper type 2 (Th2) cells, CD4 cells, Natural killer T cell, Mast cell, Basophil cells, Eosinophil cells and Nuocyte cells. IL-13 is a central regulator in IgE synthesis, goblet cell hyperplasia, mucus hypersecretion, airway hyperresponsiveness, fibrosis and chitinase up-regulation. It is a mediator of allergic inflammation and different diseases including asthma. IL-13 appears important because it increases MUC5AC expression (IL-1 beta appears to be an important stimulus for MUC5b expression[83]).

Basal levels of production and secretion of MUC5AC and MUC5B change as part of an allergic response. The production of MUC5AC can increase 40-200 times as high as normal levels in humans with similar findings in mice, MUC5B increases more modestly, 3 to 10 times in mice. The most important stimulus for secretion appears to be ATP which acts on apical membrane purinergic (P2Y₂) receptors. Once secreted mucus gel is propelled in a proximal direction towards the mouth, by ciliary beating as part of the mucociliary escalator, where is expectorated or swallowed. [85]

5.1.2 MUC5B RS3570950 AND RESPIRATORY DISEASE

Expression and localisation of MUC5AC and MUC5B is different in patients with lung disease compared with health controls. MUC5AC expression is increased in asthma for example, while MUC5B expression is increased in COPD[87] and IPF. In COPD MUC5b expression occurs in more proximal airways, whereas in IPF it localised to the bronchiole.[88] MUC5b appears to be particularly important in IPF.

The gain of function promoter variant rs5270590, 3.5 kb upstream of the mucin 5b (MUC5B) transcriptional start site, is the strongest identified risk factor (genetic or otherwise) for the development of either sporadic or familial IPF. The largest study to date (1616 non-hispanic white patients with fibrotic interstitial pneumonias and 4683 controls) estimated that the odds of developing pulmonary fibrosis for those with one copy of the risk allele were 4.5 times (95% CI: 3.9, 5.2) the odds of those with no copies and that the odds for those with two copies are 20.2 times those with no copies (95% CI: 15.2–27.0).[89] The strength of association is substantially higher than for most other common risk variants for complex disease with the exception of the human leukocyte antigen (HLA) region for some autoimmune diseases such as type-1 diabetes mellitus and systemic lupus erythmatosis which have OR greater than 10. The association between rs35705950 has been replicated in 3 genome wide association studies (GWAS) and a total of 10 independent cohorts including a Mexican cohort and two Asian cohorts and is thought to account for about a third of IPF cases.[90] However, penetrance is low with up to 20% of non-Hispanic whites having a least one copy of the variant yet

IPF occurring only rarely. The rs35705950 variant is a G-to-T transversion that occurs in an area of the MUC5B 5' flanking region, a region which has characteristics of being an enhancer subject to epigenetic control via DNA methylation and histone modification.[88] An enhancer is a sequence of DNA that functions to enhance transcription. A promoter is a sequence of DNA that initiates the process of transcription. A promoter has to be close to the gene that is being transcribed while an enhancer does not need to be close to the gene of interest. Publicly available data through the Encyclopedia of DNA Elements (ENCODE) suggest MUC5b promoter site is a complex area of the genome with many transcriptional factors showing evidence of binding.[91] In other words MUC5b expression likely a function of genetic and non-genetic factors.[90] In addition to IPF, rs35705950 has been found to be positively associated with interstitial lung abnormalities (ILA), chronic hypersensitivity pneumonitis (CHP), rheumatoid arthritis associated interstitial lung disease (RA-ILD), and myeloperoxidase-antineutrophil cytoplasmic antibody-associated vasculitis associated interstitial lung disease (AAV-ILD).[92] It has also been found to not be associated with cutaneous systemic sclerosis interstitial lung disease (SSc-ILD), sarcoidosis, and myositis-ILD. [93]

5.1.2.1 Potential role in IPF pathogenesis (and normal function inc make the point penetrance low need something else too e.g occ exposure and bring in recent review and coal dust)

The rs5270590 variant is associated with a 34 fold increase in expression of MUC5b compared with wild type in healthy control populations and a 5 fold increase in patients with IPF (see figure 1).[90] In IPF patients distal airway MUC5b is expressed preferentially, compared with MUC5Ac. MUC5b also expressed in honeycomb cysts, a defining characteristic of the usual interstitial pneumonia CT pattern typically seen in IPF.[94]

Proposed mechanisms for the role of the rs5270590 variant in the pathogenesis of IPF include:

1. excessive production of MUC5B by stem cells that attempt to regenerate injured bronchiolar and alveolar epithelium could disrupt normal development pathways and highjack normal reparative mechanisms of the distal lung resulting in fibroproliferation and honeycomb cyst formation.
2. excessive MUC5B production leads to reduced mucociliary function, retention of particles, and enhanced lung injury.
3. interaction between MUC5b and motile cilia since distinct cilium gene expression in IPF lung has been observed.
4. excessive MUC5b production inducing endoplasmic reticulum stress and the unfolded protein response.[90]

Muc5b has been studied in mice. A Muc5b knockout mouse study found that muc5b is essential for mucociliary clearance, for controlling airway and middle ear infections, and maintaining immune homeostasis in the lungs. Knockout mice had airflow limitation and died from infection by multiple bacterial species, including *Staphylococcus aureus*. [95] A transgenic muc5b mouse model of muc5b overexpression found that overexpression causes mucociliary dysfunction and enhances lung fibrosis on response to bleomycin. [96] Intriguingly, in recent bleomycin lung fibrosis model studies lung fibrosis was attenuated and mortality reduced in both germ-free mice and IL-17B deficient mice supporting the concept that fibrosis in response to epithelial injury is mediated by interaction of the immune system with microbiota. [97][98]

5.1.3 INFECTION/IMMUNITY

The frequency of the disease associated allele at rs35705950 exceeds 10% in European populations (<https://www.ncbi.nlm.nih.gov/snp/rs35705950>) but is less than 1% in African and East Asian populations. Clearly the rs35705950 variant is not subject to negative selection due to IPF risk since onset is well after the reproductive age begins [90]; the variation in frequency observed is consistent with strong positive selection. The increased MUC5b expression in the airways associated with the rs35705950 variant may have conferred a survival advantage by providing protection

against lung infection. [86][83] A relation between the rs35705950 variant, disease risk, and infection is also supported by the observation that in a prospective study of 65 IPF patients have higher bacterial loads than COPD and healthy controls and within IPF patients those with homozygous (TT) for variant had significantly lower bacterial loads ($P=0.01$), measured by 16S rRNA quantitative polymerase chain reaction of bronchoalveolar lavage samples. Within IPF those with higher bacterial loads were also at increased risk of death.[99] These findings are consistent with observation that the rs35705950 variant is associated with improved survival in IPF[100] and fewer acute respiratory disease events in the COPD Gene cohort with interstitial features.[101] However, these studies are vulnerable to index event bias, by which selection of subjects according to disease status creates biased associations if common causes of incidence and prognosis if not properly accounted for.[102] For example, it is known that the rs35705950 variant is associated with interstitial lung abnormalities[103], since the diagnosis of IPF relies heavily on radiological appearances individuals with the variant might tend to be diagnosed earlier in the course of their disease giving the false impression, when comparing them to IPF patients without the disease variant that is associated with survival. Further support for the importance of infection in IPF provided by the observation that immunomodulatory therapies such as interferon gamma, etanercept, prednisolone, azathioprine and N-acetylcysteine have failed to prolong survival in IPF[104] to prolong survival in IPF, from a small ($N = 181$) double blinded randomized controlled study which found reduced symptom burden and improved survival associated with cotrimoxazole[105], as well as evidence from genetic and animal studies. IPF GWAS have identified single nucleotide variants associated with disease susceptibility in the Toll interacting protein (TOLLIP) gene, for example rs111521887. TOLLIP is an inhibitory adaptor protein within Toll-like receptors (TLR) and part of the innate immune system recognising pathogen associated molecular patterns (PAMPs)[106] and, intriguingly, in a mouse bleomycin lung fibrosis model the absence of a microbiome protected against mortality.[97]

5.1.4 INORGANIC OCCUPATIONAL STIMULI

While the frequency of the disease associated allele at rs35705950 exceeds 10% in European populations(<https://www.ncbi.nlm.nih.gov/snp/rs35705950>), its penetrance is low. The median prevalence of IPF for men and women in Europe is approximately 3.75 per 100000 for the period 2001-2013[107], other genetic and/or environmental factors must be at play. In addition to responding to PAMPs as outlined above the innate immune system also responds to damage-associated molecular patterns (DAMPs) which can result from inhalation of inorganic respirable toxins such as silica or asbestos.[108] Secretion of the inflammatory cytokine IL-1beta (which is also a stimulus for MUC5b expression) is elevated in alveolar macrophages of patients with ILD, including IPF, sarcoidosis, silicosis, RA-ILD, and asbestosis.[109][110] Inflammasome are multiprotein intracellular complexes that detect pathogenic microorganisms (PAMPs) and sterile stressors (DAMPs). The NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) inflammasome is an intracellular sensor that detects a broad range of PAMPs and DAMPs leading to caspase 1-dependent release of the pro-inflammatory cytokines IL-1 beta and IL-18, as well as to gasdermin D-mediated pyroptotic cell death.[111] Interestingly the NLRP3 inflammasome appears to be implicated, albeit with differing activation patterns[112], in all of these conditions, interaction between smoking (a risk factor for IPF) and the NLRP3 inflammasome is recognised, and recent work has shown age-dependent susceptibility to pulmonary fibrosis in a bleomycin-induced lung injury mouse model.[113] Occupational risk factors such as metal, wood, and stone dust exposure are well recognised in IPF, accounting for up to 8% of cases the basis of a meta-analysis of case-control data[114] and its likely that innate immune system activation via the NLRP3 inflammasome and other means by occupational exposures mediates this risk.

5.2 Conclusion

The apparently complex interplay between exposure to organic and inorganic respiratory toxins, the mucus barrier, respiratory epithelium and resident

cells such as alveolar macrophages in idiopathic pulmonary fibrosis remains incompletely characterised but genetic, epigenetic, gene-expression, and epidemiological studies are beginning to fill in the gaps. Gene-environment interaction between the rs5270590 variant and occupational inorganic respiratory toxins such as asbestos may modulate IPF risk and help to explain the incomplete penetrance observed. Studies to date which have selected patients on the basis of a diagnosis of IPF and then stratified by MUC5b genotype are at risk of index-event bias. A large case-control study of IPF which captures details of occupational exposures, genotype, and potential confounders, whilst also measuring factors likely to affect disease pickup such as disease severity and radiographic changes is required.

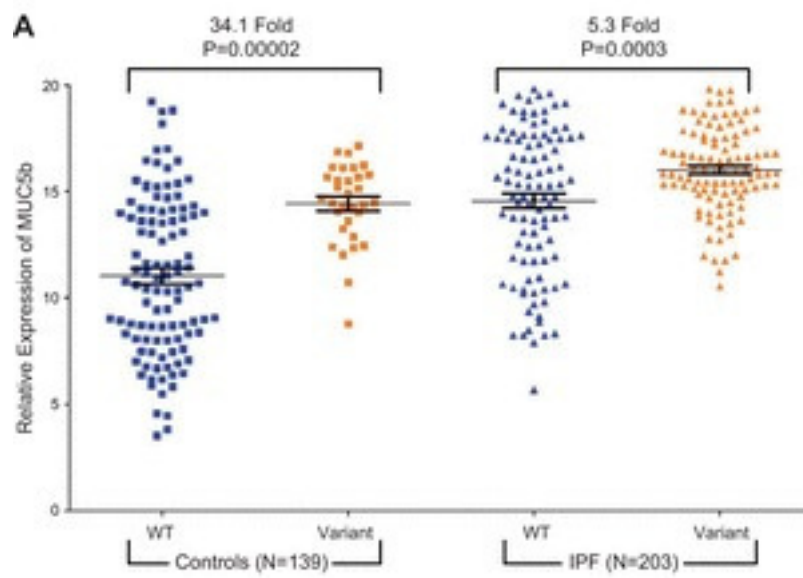


Figure 5.1: MUC5b expression (Evans 2016)

Chapter 6

Idiopathic pulmonary fibrosis job exposures study (IPFJES): Is occupational asbestos exposure an under-recognised cause of IPF?

6.1 Introduction

My study will be a multi-centre, hospital-outpatient, incident case-control study. Participants will be recruited from a UK network of six confirmed centres. Cases will be men who present, between 07.2017 and 07.2019, with a new diagnosis of IPF consistent with standard criteria[115]; they will be identified monthly by the MDT coordinator of participating centres.[116]

For each case, four controls, frequency-matched on age, will be randomly selected from incident outpatient attendances (not confined to respiratory) who do not have a diagnosis of IPF and are from the hospital as the case. Monthly lists of outpatient attendances will be obtained using the patient administration systems of participating centres. 120 cases and 480 controls will be recruited over two years with four participants enrolled and interviewed

per day.

Eligible participants will be contacted by telephone and invited to participate. An interviewer will collect data on demographics, lifetime occupational history, hobbies, family medical history, and smoking using a structured web-based questionnaire designed by us to collect lifetime occupational histories. This approach will facilitate coding, allow input validation, and permit questions to be tailored to pre-specified conditions. The questions will be developed in collaboration with an international expert in asbestos exposure measurement, Dr John Cherrie of the IOM. Participants will be invited to provide a venous blood sample for genetic analysis.

Cases and controls will be genotyped using a panel of 15 pre-defined candidate susceptibility SNPs including rs35705950. Genotyping will be undertaken using Q-PCR and Taqman assays on DNA isolated from whole blood samples.

For the primary analysis unconditional logistic regression will be used to analyse ‘any’ vs ‘no’ asbestos exposure and categories of cumulative exposure adjusting for age and smoking status. Prior data[40] indicate that the probability of exposure among controls is 0.29. If the true OR for disease in exposed subjects relative to unexposed subjects is 2.0, I will need to recruit 94 case patients and 376 control patients to be able to reject the null hypothesis that this odds ratio equals 1 with $\beta = 0.2$ and $\alpha = 0.05$ [117]; my planned sample size includes a margin for model stability and incomplete data.[118]

Secondary (exploratory) analysis will investigate gene-environment interaction. The global minor allele frequency of MUC5B rs35705950 is 0.05.[119] With an estimated prevalence of IPF of 20/100000[1] and with ORs 2.0 for asbestos exposure and 6.8 for rs35705950[120], 113 cases would be required to detect a minimum interaction OR of 4.0.[121] While I acknowledge that this exploratory analysis will have the power to detect only a large effect size it seems a valuable opportunity to examine an unexplored area in IPF research.

6.2 Method

Genotyping

Genotypes of the MUC5B SNP rs35705950 were determined using TaqMan assays (Life Technologies, Carlsbad, CA). Reactions were performed in 384-well plates, and fluorescence was read using an Applied Biosystems Viia7 Sequence Detection System.

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6.3 Results

These are the results. Curabitur vulputate nisl non ante tincidunt tempor. Aenean porta nisi quam, sed ornare urna congue sed. Curabitur in sapien justo. Quisque pulvinar ullamcorper metus, eu varius mauris pellentesque et. In hac habitasse platea dictumst. Pellentesque nec porttitor libero. Duis et magna a massa lacinia cursus.

6.4 Discussion

possibility of missed chronic HP [122]

6.5 Conclusion

This is the conclusion to the chapter. Nulla sed condimentum lectus. Duis sed tempor erat, at cursus lacus. Nam vitae tempus arcu, id vestibulum sapien. Cum sociis natoque penatibus et magnis dis parturient montes, nascetur ridiculus mus.

Chapter 7

Conclusion

7.1 Thesis summary

In summary, pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Nunc eleifend, ex a luctus porttitor, felis ex suscipit tellus, ut sollicitudin sapien purus in libero. Nulla blandit eget urna vel tempus. Praesent fringilla dui sapien, sit amet egestas leo sollicitudin at.

7.2 Future work

chronic hp

Appendix 1: IPF epidemiology code

IPF epidemiology

Appendix 2: IPFJES study documentation

IPFJES study documentation

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 Maher TM. Idiopathic pulmonary fibrosis: Pathobiology of novel approaches to treatment. *Clin Chest Med* 2012;**33**:69–83. doi:10.1016/j.ccm.2011.11.002
- 3 Ley B, Collard HR. Epidemiology of idiopathic pulmonary fibrosis. *Clinical epidemiology* 2013;**5**:483.
- 4 Spagnolo P, Grunewald J, Bois RM du. Genetic determinants of pulmonary fibrosis: Evolving concepts. *The Lancet Respiratory Medicine* 2014;**2**:416–28.
- 5 Hubbard R, Johnston I, Britton J. Survival in patients with cryptogenic fibrosing alveolitis a population-based cohort study. *CHEST Journal* 1998;**113**:396–400.
- 6 Vancheri C, Failla M, Crimi N *et al.* Idiopathic pulmonary fibrosis: A disease with similarities and links to cancer biology. *Eur Respir J* 2010;**35**:496–504. doi:10.1183/09031936.00077309
- 7 Barber C, Wiggans R, Young C *et al.* UK asbestos imports and mortality due to idiopathic pulmonary fibrosis. *Occup Med* 2015;kqv142.
- 8 Corrin B, Dewar A, Rodriguez-Roisin R *et al.* Fine structural changes in cryptogenic fibrosing alveolitis and asbestosis. *The Journal of pathology* 1985;**147**:107–19. doi:10.1002/path.1711470206
- 9 Copley SJ, Wells AU, Sivakumaran P *et al.* Asbestosis and idiopathic pulmonary fibrosis: Comparison of thin-section ct features. *Radiology* 2003;**229**:731–6. doi:10.1148/radiol.2293020668
- 10 Monso E, Tura JM, Marsal M *et al.* Mineralogical microanalysis of idiopathic pulmonary fibrosis. *Arch Environ Health* 1990;**45**:185–8. doi:10.1080/00039896.1990.9936714
- 11 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.

12 Glazer C, Maier L. Occupational interstitial lung disease. *Eur Respir Monograph* 2009;**46**:265–86.

13 Ghio A, Sangani R, Roggli V. Expanding the spectrum of particle-and fiber-associated interstitial lung diseases. *Turk Toraks Derg* 2014;**15**:1–8.

14 Travis WD, Costabel U, Hansell DM *et al.* An official american thoracic society/european respiratory society statement: Update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. *American journal of respiratory and critical care medicine* 2013;**188**:733–48.

15 Reynolds CJ, Blanc PD. Organising pneumonia and other uncommon interstitial disorders. In: *Parkes' occupational lung disorders, fourth edition.* 2018.

16 Turner-Warwick M. In search of a cause of cryptogenic fibrosing alveolitis (cfa): One initiating factor or many? *Thorax* 1998;**53**:S3–9.

17 Hubbard R. Occupational dust exposure and the aetiology of cryptogenic fibrosing alveolitis. *Eur Respir J* 2001;**18**:119s–21s.

18 Taskar VS, Coultas DB. Is idiopathic pulmonary fibrosis an environmental disease? *Proc Am Thorac Soc* 2006;**3**:293–8.

19 Gulati M, Redlich CA. Asbestosis and environmental causes of usual interstitial pneumonia. *Current opinion in pulmonary medicine* 2015;**21**:193–200. doi:10.1097/MCP.0000000000000144

20 Fontaine J-F, Barbosa-Silva A, Schaefer M *et al.* MedlineRanker: Flexible ranking of biomedical literature. *Nucleic acids research* 2009;**37**:W141–6. doi:10.1093/nar/gkp353

21 Reynolds C, De Matteis S, Cullinan P *et al.* Pubmed mining for occupational idiopathic pulmonary fibrosis papers. 2017.

22 Scott J, Johnston I, Britton J. What causes cryptogenic fibrosing alveolitis? A case-control study of environmental exposure to dust. *BMJ* 1990;**301**:1015.

23 Iwai K, Mori T, Yamada N *et al.* Idiopathic pulmonary fibrosis. Epidemiologic approaches to occupational exposure. *Am J Respir Crit Care Med* 1994;**150**:670–5. doi:10.1164/ajrccm.150.3.8087336

24 Hubbard R, Lewis S, Richards K *et al.* Occupational exposure to metal or wood dust and aetiology of cryptogenic fibrosing alveolitis. *The Lancet* 1996;**347**:284–9.

25 Mullen J, Hodgson MJ, DeGraff CA *et al.* Case-control study of idiopathic pulmonary fibrosis and environmental exposures. *J Occup Environ Med* 1998;**40**:363–7.

- 26 Baumgartner KB, Samet JM, Coultas DB *et al.* Occupational and environmental risk factors for idiopathic pulmonary fibrosis: A multicenter case-control study. Collaborating centers. *Am J Epidemiol* 2000;**152**:307–15.
- 27 Hubbard R, Cooper M, Antoniak M *et al.* Risk of cryptogenic fibrosing alveolitis in metal workers. *The Lancet* 2000;**355**:466–7.
- 28 Miyake Y, Sasaki S, Yokoyama T *et al.* Occupational and environmental factors and idiopathic pulmonary fibrosis in japan. *Ann Occup Hyg* 2005;**49**:259–65.
- 29 Gustafson T, Dahlman-Höglund A, Nilsson K *et al.* Occupational exposure and severe pulmonary fibrosis. *Respir Med* 2007;**101**:2207–12.
- 30 Pinheiro GA, Antao VC, Wood JM *et al.* Occupational risks for idiopathic pulmonary fibrosis mortality in the united states. *Int J Occup Environ Health* 2008;**14**:117–23.
- 31 García-Sancho Figueroa MC, Carrillo G, Pérez-Padilla R *et al.* Risk factors for idiopathic pulmonary fibrosis in a mexican population. A case-control study. *Respir Med* 2010;**104**:305–9.
- 32 García-Sancho C, Buendía-Roldán I, Fernández-Plata MR *et al.* Familial pulmonary fibrosis is the strongest risk factor for idiopathic pulmonary fibrosis. *Respiratory medicine* 2011;**105**:1902–7. doi:10.1016/j.rmed.2011.08.022
- 33 Awadalla NJ, Hegazy A, Elmetwally RA *et al.* Occupational and environmental risk factors for idiopathic pulmonary fibrosis in egypt: A multicenter case-control study. *Int J Occup Environ Med* 2012;**3**:107–16.
- 34 Paolocci G, Nicolici V, Folletti I *et al.* Risk factors for idiopathic pulmonary fibrosis in southern europe: A case-control study. 2013.
- 35 Ekstrom M, Gustafson T, Boman K *et al.* Effects of smoking, gender and occupational exposure on the risk of severe pulmonary fibrosis: A population-based case-control study. *BMJ open* 2014;**4**:e004018.
- 36 Koo J-W, Myong J-P, Yoon H-K *et al.* Occupational exposure and idiopathic pulmonary fibrosis: A multicentre case-control study in korea. *The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease* 2017;**21**:107–12. doi:10.5588/ijtld.16.0167
- 37 Hubbard R, Johnston I, Coultas DB *et al.* Mortality rates from cryptogenic fibrosing alveolitis in seven countries. *Thorax* 1996;**51**:711–6.
- 38 Navaratnam V, Fogarty AW, McKeever T *et al.* Presence of a prothrombotic state in people with idiopathic pulmonary fibrosis: A population-based case-control study. *Thorax* 2014;**69**:207–15. doi:10.1136/thoraxjnl-2013-203740

- 39 Welch LS, Michaels D, Zoloth SR. The national sheet metal worker asbestos disease screening program: Radiologic findings. National sheet metal examination group. *Am J Ind Med* 1994;**25**:635–48.
- 40 Rake C, Gilham C, Hatch J *et al.* Occupational, domestic and environmental mesothelioma risks in the british population: A case-control study. *Br J Cancer* 2009;**100**:1175–83. doi:10.1038/sj.bjc.6604879
- 41 Caminati A, Madotto F, Cesana G *et al.* Epidemiological studies in idiopathic pulmonary fibrosis: Pitfalls in methodologies and data interpretation. *European respiratory review : an official journal of the European Respiratory Society* 2015;**24**:436–44. doi:10.1183/16000617.0040-2015
- 42 De Vuyst P, Karjalainen A, Dumortier P *et al.* Guidelines for mineral fibre analyses in biological samples: Report of the ers working group. European respiratory society. *The European respiratory journal* 1998;**11**:1416–26.
- 43 Cherrie JW, Schneider T. Validation of a new method for structured subjective assessment of past concentrations. *Ann Occup Hyg* 1999;**43**:235–45.
- 44 Nemery B, Nuyts V, Nackaerts K. Quantifying asbestos in lung tissue: What debate? *The European respiratory journal* 2017;**49**. doi:10.1183/13993003.00861-2017
- 45 Cooke WE. FIBROSIS of the lungs due to the inhalation of asbestos dust. *British medical journal* 1924;**2**:147–140.2.
- 46 Wolff H, Vehmas T, Oksa P *et al.* Asbestos, asbestosis, and cancer, the helsinki criteria for diagnosis and attribution 2014: Recommendations. *Scandinavian journal of work, environment & health* 2015;**41**:5–15. doi:10.5271/sjweh.3462
- 47 Hammar SP, Abraham JL. Commentary on pathologic diagnosis of asbestosis and critique of the 2010 asbestosis committee of the college of american pathologists (cap) and pulmonary pathology society’s (pps) update on the diagnostic criteria for pathologic asbestosis. *American journal of industrial medicine* 2015;**58**:1034–9. doi:10.1002/ajim.22512
- 48 Baur X, Frank AL, Budnik LT *et al.* Collegium ramazzini: Comments on the 2014 helsinki consensus report on asbestos. *American journal of industrial medicine* 2016;**59**:591–4. doi:10.1002/ajim.22595
- 49 Baur X, Weitowitz H-J, Budnik LT *et al.* Asbestos, asbestosis, and cancer: The helsinki criteria for diagnosis and attribution. Critical need for revision of the 2014 update. *American journal of industrial medicine* 2017;**60**:411–21. doi:10.1002/ajim.22709
- 50 Gilham C, Rake C, Burdett G *et al.* Pleural mesothelioma and lung cancer risks in relation to occupational history and asbestos lung burden. *Occupational and environmental*

medicine 2016;**73**:290–9. doi:10.1136/oemed-2015-103074

51 Gilham C, Rake C, Hodgson J *et al.* Past and current asbestos exposure and future mesothelioma risks in britain: The inhaled particles study (tips). *International Journal of Epidemiology* 2018.

52 Nuyts V, Vanhooren H, Begyn S *et al.* Asbestos bodies in bronchoalveolar lavage in the 21st century: A time-trend analysis in a clinical population. *Occupational and environmental medicine* 2017;**74**:59–65. doi:10.1136/oemed-2016-103710

53 Burns D, Beaumont P. The hse national exposure database—(nedb). *The Annals of occupational hygiene* 1989;**33**:1–14.

54 Orlowski E, Audignon-Durand S, Goldberg M *et al.* EV@LUTIL: An open access database on occupational exposures to asbestos and man-made mineral fibres. *American journal of industrial medicine* 2015;**58**:1059–74. doi:10.1002/ajim.22498

55 Peto J. Problems in dose response and risk assessment: The example of asbestos. In: *Epidemiology and quantitation of environmental risk in humans from radiation and other agents*. Springer 1985. 175–85.

56 Toxic Substances A for, (ATSDR). DR. Agency for toxic substances and disease registry (atsdr). 2001. Toxicological profile for asbestos. U.S. Department of Health; Human Services, Public Health Service. 2001. <https://www.atsdr.cdc.gov/toxprofiles/TP.asp?id=30&tid=4#bookmark16>

57 Smith TJ, Hammond SK, Hallock M *et al.* Exposure assessment for epidemiology: Characteristics of exposure. *Applied Occupational and Environmental Hygiene* 1991;**6**:441–7.

58 Sahmel J, Devlin K, Paustenbach D *et al.* The role of exposure reconstruction in occupational human health risk assessment: Current methods and a recommended framework. *Critical reviews in toxicology* 2010;**40**:799–843. doi:10.3109/10408444.2010.501052

59 Blake CL, Dotson GS, Harbison RD. Assessment of airborne asbestos exposure during the servicing and handling of automobile asbestos-containing gaskets. *Regulatory toxicology and pharmacology : RTP* 2006;**45**:214–22. doi:10.1016/j.yrtph.2006.04.007

60 Williams PRD, Phelka AD, Paustenbach DJ. A review of historical exposures to asbestos among skilled craftsmen (1940-2006). *Journal of toxicology and environmental health Part B, Critical reviews* 2007;**10**:319–77. doi:10.1080/10937400601034191

61 Pannett B, Coggon D, Acheson ED. A job-exposure matrix for use in population based studies in england and wales. *British journal of industrial medicine* 1985;**42**:777–83.

62 Peters S, Vermeulen R, Cassidy A *et al.* Comparison of exposure assessment methods for occupational carcinogens in a multi-centre lung cancer case-control study. *Occupa-*

- tional and environmental medicine* 2011;**68**:148–53. doi:10.1136/oem.2010.055608
- 63 Offermans NSM, Vermeulen R, Burdorf A *et al.* Comparison of expert and job-exposure matrix-based retrospective exposure assessment of occupational carcinogens in the netherlands cohort study. *Occupational and environmental medicine* 2012;**69**:745–51. doi:10.1136/oemed-2011-100556
- 64 Kauppinen T, Toikkanen J, Pukkala E. From cross-tabulations to multipurpose exposure information systems: A new job-exposure matrix. *American journal of industrial medicine* 1998;**33**:409–17.
- 65 Offermans NSM, Vermeulen R, Burdorf A *et al.* Occupational asbestos exposure and risk of pleural mesothelioma, lung cancer, and laryngeal cancer in the prospective netherlands cohort study. *Journal of occupational and environmental medicine* 2014;**56**:6–19. doi:10.1097/JOM.000000000000060
- 66 Oyen SC van, Peters S, Alfonso H *et al.* Development of a job-exposure matrix (asbjem) to estimate occupational exposure to asbestos in australia. *The Annals of occupational hygiene* 2015;**59**:737–48. doi:10.1093/annhyg/mev017
- 67 Peters S, Vermeulen R, Portengen L *et al.* SYN-jem: A quantitative job-exposure matrix for five lung carcinogens. *The Annals of occupational hygiene* 2016;**60**:795–811. doi:10.1093/annhyg/mew034
- 68 Cherrie JW, McElvenny D, Blyth KG. Estimating past inhalation exposure to asbestos: A tool for risk attribution and disease screening. *International journal of hygiene and environmental health* 2018;**221**:27–32. doi:10.1016/j.ijheh.2017.09.013
- 69 Ahrens W, Jöckel KH, Brochard P *et al.* Retrospective assessment of asbestos exposure-i. Case-control analysis in a study of lung cancer: Efficiency of job-specific questionnaires and job exposure matrices. *International journal of epidemiology* 1993;**22 Suppl 2**:S83–95.
- 70 Teschke K, Olshan AF, Daniels JL *et al.* Occupational exposure assessment in case-control studies: Opportunities for improvement. *Occup Environ Med* 2002;**59**:575–93; discussion 594.
- 71 Gramond C, Rolland P, Lacourt A *et al.* Choice of rating method for assessing occupational asbestos exposure: Study for compensation purposes in france. *Am J Ind Med* 2012;**55**:440–9. doi:10.1002/ajim.22008
- 72 Orlowski E, Pohlabein H, Berrino F *et al.* Retrospective assessment of asbestos exposure-ii. At the job level: Complementarity of job-specific questionnaire and job exposure matrices. *International journal of epidemiology* 1993;**22 Suppl 2**:S96–105.
- 73 Tielemans E, Schneider T, Goede H *et al.* Conceptual model for assessment of in-

- halation exposure: Defining modifying factors. *The Annals of occupational hygiene* 2008;**52**:577–86. doi:10.1093/annhyg/men059
- 74 Rödelberger K, Jöckel KH, Pohlabeln H *et al.* Asbestos and man-made vitreous fibers as risk factors for diffuse malignant mesothelioma: Results from a german hospital-based case-control study. *American journal of industrial medicine* 2001;**39**:262–75.
- 75 Symanski E, Maberti S, Chan W. A meta-analytic approach for characterizing the within-worker and between-worker sources of variation in occupational exposure. *The Annals of occupational hygiene* 2006;**50**:343–57. doi:10.1093/annhyg/mel006
- 76 Cherrie JW. The effect of room size and general ventilation on the relationship between near and far-field concentrations. *Applied occupational and environmental hygiene* 1999;**14**:539–46. doi:10.1080/104732299302530
- 77 Teschke K, Smith JC, Olshan AF. Evidence of recall bias in volunteered vs. Prompted responses about occupational exposures. *American journal of industrial medicine* 2000;**38**:385–8.
- 78 Burstyn I. The ghost of methods past: Exposure assessment versus job-exposure matrix studies. *Occup Environ Med* 2011;**68**:2–3. doi:10.1136/oem.2009.054585
- 79 Bakshani CR, Morales-Garcia AL, Althaus M *et al.* Evolutionary conservation of the antimicrobial function of mucus: A first defence against infection. *NPJ biofilms and microbiomes* 2018;**4**:14. doi:10.1038/s41522-018-0057-2
- 80 sei. Functional characterization of the mucus barrier on the , javax.xml.bind.JAXBElement@461bc6e7, skin surface. *Proceedings of the National Academy of Sciences of the United States of America* 2018;**115**:726–31. doi:10.1073/pnas.1713539115
- 81 Kufe DW. Mucins in cancer: Function, prognosis and therapy. *Nature reviews Cancer* 2009;**9**:874–85. doi:10.1038/nrc2761
- 82 Linden SK, Sutton P, Karlsson NG *et al.* Mucins in the mucosal barrier to infection. *Mucosal immunology* 2008;**1**:183–97. doi:10.1038/mi.2008.5
- 83 Jaramillo AM, Azzegagh Z, Tuvim MJ *et al.* Airway mucin secretion. *Annals of the American Thoracic Society* 2018;**15**:S164–70. doi:10.1513/AnnalsATS.201806-371AW
- 84 Boucher RC. Muco-obstructive lung diseases. *The New England journal of medicine* 2019;**380**:1941–53. doi:10.1056/NEJMra1813799
- 85 Fahy JV, Dickey BF. Airway mucus function and dysfunction. *The New England journal of medicine* 2010;**363**:2233–47. doi:10.1056/NEJMra0910061
- 86 Dickey BF, Whitsett JA. Understanding interstitial lung disease: It's in the mucus. *American journal of respiratory cell and molecular biology* 2017;**57**:12–4.

doi:10.1165/rcmb.2017-0116ED

87 Kesimer M, Ford AA, Ceppe A *et al.* Airway mucin concentration as a marker of chronic bronchitis. *The New England journal of medicine* 2017;**377**:911–22. doi:10.1056/NEJMoa1701632

88 Helling BA, Gerber AN, Kadiyala V *et al.* Regulation of muc5b expression in idiopathic pulmonary fibrosis. *American journal of respiratory cell and molecular biology* 2017;**57**:91–9. doi:10.1165/rcmb.2017-0046OC

89 Fingerlin TE, Murphy E, Zhang W *et al.* Genome-wide association study identifies multiple susceptibility loci for pulmonary fibrosis. *Nat Genet* 2013;**45**:613–20. doi:10.1038/ng.2609

90 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

91 Selman M, Pardo A, Barrera L *et al.* Gene expression profiles distinguish idiopathic pulmonary fibrosis from hypersensitivity pneumonitis. *American journal of respiratory and critical care medicine* 2006;**173**:188–98. doi:10.1164/rccm.200504-644OC

92 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

93 Integrating genomics into management of fibrotic interstitial lung disease. *Chest* 2019;**155**:1026–40. doi:10.1016/j.chest.2018.12.011

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

95 Roy MG, Livraghi-Butrico A, Fletcher AA *et al.* Muc5b is required for airway defence. *Nature* 2014;**505**:412–6. doi:10.1038/nature12807

96 Hancock LA, Hennessy CE, Solomon GM *et al.* Muc5b overexpression causes mucociliary dysfunction and enhances lung fibrosis in mice. *Nature communications* 2018;**9**:5363. doi:10.1038/s41467-018-07768-9

97 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

98 Yang D, Chen X, Wang J *et al.* Dysregulated lung commensal bacteria drive interleukin-

- 17B production to promote pulmonary fibrosis through their outer membrane vesicles. *Immunity* 2019;**50**:692–706.e7. doi:10.1016/j.immuni.2019.02.001
- 99 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
- 100 Peljto AL, Zhang Y, Fingerlin TE *et al.* Association between the muc5b promoter polymorphism and survival in patients with idiopathic pulmonary fibrosis. *JAMA* 2013;**309**:2232–9.
- 101 Ash SY, Harmouche R, Putman RK *et al.* Association between acute respiratory disease events and the , javax.xml.bind.JAXBElement@24470b74, promoter polymorphism in smokers. *Thorax* 2018;**73**:1071–4. doi:10.1136/thoraxjnl-2017-211208
- 102 Dudbridge F, Allen RJ, Sheehan NA *et al.* Adjustment for index event bias in genome-wide association studies of subsequent events. *Nature communications* 2019;**10**:1561. doi:10.1038/s41467-019-09381-w
- 103 Hunninghake GM, Hatabu H, Okajima Y *et al.* MUC5B promoter polymorphism and interstitial lung abnormalities. *N Engl J Med* 2013;**368**:2192–200. doi:10.1056/NEJMoa1216076
- 104 Warheit-Niemi HI, Hult EM, Moore BB. A pathologic two-way street: How innate immunity impacts lung fibrosis and fibrosis impacts lung immunity. *Clinical & translational immunology* 2019;**8**:e1065. doi:10.1002/cti2.1065
- 105 Shulgina L, Cahn AP, Chilvers ER *et al.* Treating idiopathic pulmonary fibrosis with the addition of co-trimoxazole: A randomised controlled trial. *Thorax* 2013;**68**:155–62. doi:10.1136/thoraxjnl-2012-202403
- 106 Noth I, Zhang Y, Ma S-F *et al.* Genetic variants associated with idiopathic pulmonary fibrosis susceptibility and mortality: A genome-wide association study. *Lancet Respir Med* 2013;**1**:309–17. doi:10.1016/S2213-2600(13)70045-6
- 107 Marshall DC, Salciccioli JD, Shea BS *et al.* Trends in mortality from idiopathic pulmonary fibrosis in the european union: An observational study of the who mortality database from 2001-2013. *The European respiratory journal* 2018;**51**. doi:10.1183/13993003.01603-2017
- 108 book. Innate immune activation through nalp3 inflammasome sensing of asbestos and silica. *Science (New York, NY)* 2008;**320**:674–7. doi:10.1126/science.1156995
- 109 Byrne AJ, Maher TM, Lloyd CM. Pulmonary macrophages: A new therapeutic pathway in fibrosing lung disease? *Trends in molecular medicine* 2016;**22**:303–16. doi:10.1016/j.molmed.2016.02.004

- 110 Howrylak JA, Nakahira K. Inflammasomes: Key mediators of lung immunity. *Annual review of physiology* 2017;**79**:471–94. doi:10.1146/annurev-physiol-021115-105229
- 111 Swanson KV, Deng M, Ting JP-Y. The nlrp3 inflammasome: Molecular activation and regulation to therapeutics. *Nature reviews Immunology* 2019;**19**:477–89. doi:10.1038/s41577-019-0165-0
- 112 Lasithiotaki I, Giannarakis I, Tsitoura E *et al.* NLRP3 inflammasome expression in idiopathic pulmonary fibrosis and rheumatoid lung. *The European respiratory journal* 2016;**47**:910–8. doi:10.1183/13993003.00564-2015
- 113 Stout-Delgado HW, Cho SJ, Chu SG *et al.* Age-dependent susceptibility to pulmonary fibrosis is associated with nlrp3 inflammasome activation. *American journal of respiratory cell and molecular biology* 2016;**55**:252–63. doi:10.1165/rccb.2015-0222OC
- 114 Blanc PD, Annesi-Maesano I, Balmes JR *et al.* The occupational burden of non-malignant 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
- 115 Raghu. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management, author=Raghu, Ganesh and Collard, Harold R and Egan, Jim J and Martinez, Fernando J and Behr, Juergen and Brown, Kevin K and Colby, Thomas V and Cordier, Jean-François and Flaherty, Kevin R and Lasky, Joseph A and others. *Am J Respir Crit Care Med* 2011;**183**:788–824.
- 116 NICE. Idiopathic pulmonary fibrosis: The diagnosis and management of suspected idiopathic pulmonary fibrosis. 2013.<https://www.nice.org.uk/guidance/cg163>
- 117 Dupont WD, Plummer WD. Power and sample size calculations: A review and computer program. *Control Clin Trials* 1990;**11**:116–28.
- 118 Agresti A. Building and applying logistic regression models. *Categorical Data Analysis, Second Edition* 2007;211–66.
- 119 Cariaso M, Lennon G. SNPedia: A wiki supporting personal genome annotation, interpretation and analysis. *Nucleic Acids Res* 2012;**40**:D1308–12. doi:10.1093/nar/gkr798
- 120 Seibold MA, Wise AL, Speer MC *et al.* A common muc5b promoter polymorphism and pulmonary fibrosis. *N Engl J Med* 2011;**364**:1503–12. doi:10.1056/NEJMoa1013660
- 121 Gauderman WJ. Sample size requirements for association studies of gene-gene interaction. *Am J Epidemiol* 2002;**155**:478–84.
- 122 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