Updates on Familial Interstitial Pneumonias

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Declarations of Interest

- Consultancy fees from BI, Roche/Intermune
- Speaker fees Roche
- Trustee Action for Pulmonary Fibrosis
Agenda

- Review familial interstitial pneumonias
- Genetic associations from familial studies and GWAS
  - Surfactant proteins
  - Telomere biology
  - MUC5B
- Role for genetics
  - Early diagnosis
  - Prognosis
  - Treatment
Usual Interstitial Pneumonia (UIP) pattern diagnostic of IPF
Familial Interstitial Pneumonia

- Idiopathic interstitial pneumonia in two or more affected family members (first degree)
- Autosomal dominant with variable penetrance; some are autosomal recessive
- Incidence variable
  - UK 0.5-2.2%
  - Finland 3.3-3.7%
  - Holland 10%
  - Lung transplant centre 20%
- Association with smoking (OR 3.6; 95% confidence interval 1.3–9.8)
- Complex genetic disorder
Radiological and Histological Heterogeneity of FIP

- 111 families
- Clinical evaluation – questionnaire, DLCO, CXR or HRCT (78.4%)
- 27.8% of affected individuals had lung biopsy

<table>
<thead>
<tr>
<th></th>
<th>Probable (n = 231)</th>
<th>Definite (n = 78)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPF/UIP</td>
<td>181 (78.4)</td>
<td>67 (85.9)</td>
</tr>
<tr>
<td>NSIP</td>
<td>12 (5.2)</td>
<td>8 (10.3)</td>
</tr>
<tr>
<td>COP</td>
<td>0</td>
<td>2 (2.6)</td>
</tr>
<tr>
<td>Centrilobular nodules</td>
<td>1 (0.4)</td>
<td>0</td>
</tr>
<tr>
<td>Unclassified ILD</td>
<td>37 (16.0)</td>
<td>1 (1.3)</td>
</tr>
</tbody>
</table>

Steele M et al Am J Respir Crit Care Med 2005
How to investigate genetics of pulmonary fibrosis?

- Study families
  - Smaller number
  - Linkage analysis
  - Rare alleles predominant, may detect common alleles

- Sporadic IPF
  - Large cohort (unbiased)
  - GWAS – common variants, whole exome or whole genome resequencing
    - identify associations between common variability and disease
    - unlikely to explain entire genetic predisposition to disease
    - obtain convincing statistical evidence
  - Common alleles
Studying families with IPF
<table>
<thead>
<tr>
<th>Histopathology</th>
<th>Age groups</th>
<th>Characteristics</th>
<th>Associated genetic mutations or acquired disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth abnormalities</td>
<td>Newborns, infants, and children</td>
<td>Alveolar simplification with poorly septated airspaces; malformed lobules</td>
<td>FOXF1, NKK2-1</td>
</tr>
<tr>
<td>Pulmonary alveolar proteinosis, neonatal onset</td>
<td>Newborns and infants</td>
<td>Intraalveolar accumulation of granular, eosinophilic, or PAS-positive lipoproteinaceous material, with or without accumulation of large, foamy alveolar macrophages hyperplastic alveolar type 2 cells, and septal thickening</td>
<td>SFTPB, SFTP, ABCA3, NKK2-1</td>
</tr>
<tr>
<td>Pulmonary alveolar proteinosis, pediatric and adult onset</td>
<td>Children and adults</td>
<td>Intraalveolar accumulation of granular, eosinophilic, or PAS-positive lipoproteinaceous material, with or without accumulation of large, foamy alveolar macrophages</td>
<td>CSF2RA, CSF2RB, anti-CSF2</td>
</tr>
<tr>
<td>Chronic pneumonitis of infancy</td>
<td>Newborns and infants</td>
<td>Septal thickening with mild lymphocytic inflammation and muscularization of the alveolar septa; intraalveolar accumulation of foamy macrophages; focal proteinosis and cholesterol clefts; hyperplastic type 2 cells</td>
<td>SFTP</td>
</tr>
<tr>
<td>Desquamative interstitial pneumonia, infantile</td>
<td>Newborns and infants</td>
<td>Intraalveolar accumulation of alveolar macrophages, with or without hyperplasia of alveolar type 2 cells</td>
<td>ABCA3, SFTP, SFTP, NKK2-1</td>
</tr>
<tr>
<td>Nonspecific interstitial pneumonia</td>
<td>Children and adults</td>
<td>Interstitial pneumonitis with diffuse lymphocytic infiltrates and varying degrees of fibrosis</td>
<td>SFTP, ABCA3, NKK2-1</td>
</tr>
<tr>
<td>Usual interstitial pneumonia</td>
<td>Children and adults</td>
<td>Alternating areas of normal lung with fibroblastic foci; progressing to dense fibrosis, with remodeling and scarring of the lung in adults</td>
<td>SFTP, ABCA3, SFTP, TERT, TERC</td>
</tr>
</tbody>
</table>
Importance of type II AEC in pathogenesis of pulmonary fibrosis

Whitsett et al. Annu Rev Pathol 2014
Surfactant Protein C Mutations

- Autosomal dominant

- 25% FIP in Dutch cohort
  - UIP
  - Cystic change

- 1% sporadic IPF

- SFTPC mutations with combined emphysema and UIP pulmonary fibrosis in non-smokers

- SPC -/- spontaneous ILD

- BRICHOS domain mutations
  - Conformational change in protein folding
  - Cells show increased ER stress and apoptosis
  - No detectable SPC in lungs

- Non-BRICHOS domain mutations
  - Impair processing of Pro-SPC
  - Activates CD4+ T cells and neutrophils

- Unclear how mutations cause pulmonary fibrosis
  - Alveolar epithelial cell injury, abnormal lamellar bodies
  - Innate immune response to infections

Van Moorsel et al Am J Respir Crit Care Med 2010
Lawson et al Thorax 2004
Thomas et al Am J Respir Crit Care Med 2002
Surfactant Protein A mutations associated with FIP and lung cancer

- Whole genome linkage analysis
- Families with IPF and lung cancer
- Identified mutations in SFTPA1 and SFTPA2 within highly conserved regions
- Disrupt protein folding and ER retention
- No change in BAL levels of SPA

Wang Y et al, Am J Human Genetics 2009; 84:52
Maitra M et al, J Biol Chem 2010; 285:22103
ABCA3 Mutations

- Transporter protein involved with inward transport of lipids into lamellar bodies
- 150 mutations described
- Autosomal recessive, compound heterozygous mutations in adult
- Spectrum of clinical disease and severity
  - Neonates – fatal severe respiratory distress
  - NSIP and DIP – milder, childhood
  - UIP – young adult
- Modifier gene for SFTPC
- Disorderd lipid composition and lamellar body organisation

Kumar A et al, PLOS One 2014;9(9):e106744
Dyskeratosis Congenita and FIP

- Characterised by skin hyperpigmentation, nail dystrophy, oral mucosal leukoplakia, bone marrow failure (aplastic anaemia), pulmonary fibrosis (20%), liver fibrosis
- Associated with short telomeres
- X-linked – DKC1
- Autosomal dominant – TERT (15%), TERC (<5%), TINF2
- Avoid pulmonary toxic drugs and minimise radiation exposure for bone marrow transplant
Sporadic IPF and FIP have short telomeres in alveolar epithelium

Alder et al, PNAS 2008; 105
Mutations TERT and TERC (TR) associated with familial interstitial pneumonia.

- Mutations associated short telomeres.
- Short telomeres in 25% familial and sporadic IPF without coding mutation.
- Anticipation – earlier, more severe onset with successive generations.

Cronkhite JT et al Am J Respir Crit Care Med 2008; 178
Armanios M et al PNAS 2005
Short telomere length occurs in family members without telomerase germline mutation – epigenetic inheritance.
Exome Sequencing FIP identified novel mutations RTEL1 and PARN

Stuart BD et al Nature Genetics 2015
TERT mutation carriers outcome similar to IPF

Diaz de Leon et al PLoS ONE 2010
Clinical Characteristics of Telomere Syndrome

- Premature greying
- Bone marrow dyscrasias
  - Macrocytosis
  - Low platelet count
  - FHx of aplastic anaemia
- Liver fibrosis
- Pulmonary fibrosis (UIP)
- Hypothyroid
- TERT/TERC mutations up to 15% of FIP and 3-5% sporadic IPF
- RTEL1 mutations 7% FIP
MUC5B identified from genomewide linkage scan

82 multiplex families with FIP (deCODE linkage panel)

Identified strongest evidence for linkage on chromosome 11

Fine mapping using 306 tagging SNPs

145 subjects with FIP

152 subjects with sporadic IPF

233 controls

Confirm results

83 subjects with FIP

492 subjects with IPF

492 controls

MUC5B is increased in IPF

Detected in secretory columnar cells of bronchi
Metaplastic epithelia in honeycomb cysts and mucus plugs
Independent of smoking
MUC5B rs35705950 is associated with increased risk for FIP and IPF

One copy of minor T allele - 4.5 times risk (95% CI 3.9, 5.2)
Two copies of T allele - 20.2 times risk (95% CI 15.2, 27)
Risks similar for FIP and sporadic IPF

Evans CM et al, Physiol Rev 2016; 96:1567
How have GWAS helped?
542 IPF patients and 542 matched controls

- Identified 20 loci
- 2 replication cohorts
- 6 SNPs with genome-wide significance
- MUC5B rs35705950 - confirmed
- TOLLIP – host defence
- SPPL2C

Noth et al Lancet Respir Med 2013
GWAS identified 7 novel targets for idiopathic interstitial pneumonia

Fingerlin TE et al, Nat Genet 2013;45:613–620
Novel loci and genes associated with idiopathic interstitial pneumonia

- MUC5B and **ATP11A** – host defence
- TERT, TERC, **OBFC1** – DNA repair
- **DSP** and **DPP9** – barrier function
- **FAM13A** – Wnt pathway activation
- **Chromosomal regions 7q22 and 15q14-15**
- **No difference in odds ratio for disease between familial and sporadic IPF**
  - Genetic risk factors for fibrotic IIP similar
  - Familiar and sporadic IPF have similar genetic backgrounds
- **Genome-wide variants account for 30–35% of the risk of familial and sporadic pulmonary fibrosis**

Fingerlin TE et al, Nat Genet 2013;45:613–620
Mathai SK et al, AJRCCM 2016; 193: 1151-1160
Genetic variants and risk for IPF

Evans CM et al, Physiol Rev 2016; 96:1567
What are the risks for family members?
HRCT abnormalities are present in asymptomatic family members

<table>
<thead>
<tr>
<th>TABLE 1. SUBJECT CHARACTERISTICS</th>
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<tbody>
<tr>
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<tr>
<td>HRCT Findings</td>
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<tr>
<td>Normal HRCT (n = 53)</td>
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<tr>
<td>Nonspecific Changes (n = 59)</td>
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<tr>
<td>Asymptomatic ILD (n = 31)</td>
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<tr>
<td>Familial IPF (n = 21)</td>
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<tr>
<td>Smoking history, % (n)</td>
</tr>
<tr>
<td>23 (12)</td>
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<td>25 (15)</td>
</tr>
<tr>
<td>45 (14)*</td>
</tr>
<tr>
<td>67 (14)†</td>
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<tr>
<td>Age, yr, mean (SE)</td>
</tr>
<tr>
<td>35 (±1.7)</td>
</tr>
<tr>
<td>40 (±1.6)</td>
</tr>
<tr>
<td>46 (±2.1)†</td>
</tr>
<tr>
<td>67 (±2.7)†</td>
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<tr>
<td>Race, % (n)</td>
</tr>
<tr>
<td>White</td>
</tr>
<tr>
<td>100 (53)</td>
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<tr>
<td>91 (54)</td>
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<tr>
<td>97 (30)</td>
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<tr>
<td>100 (21)</td>
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<tr>
<td>Hispanic</td>
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<tr>
<td>0 (0)</td>
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<tr>
<td>9 (5)</td>
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<tr>
<td>3 (1)</td>
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<tr>
<td>0 (0)</td>
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<tr>
<td>Female sex, % (n)</td>
</tr>
<tr>
<td>48 (25)</td>
</tr>
<tr>
<td>59 (35)</td>
</tr>
<tr>
<td>48 (15)</td>
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<tr>
<td>52 (11)</td>
</tr>
</tbody>
</table>

Definition of abbreviations: HRCT = high-resolution computed tomography; ILD = interstitial lung disease; IPF = idiopathic pulmonary fibrosis.

* P = 0.02.
† P < 0.001 estimated using Fisher’s exact test.

Rosas IO et al. Am J Respir Crit Care Med 2007
Phenotype individuals at risk for FIP

- Interstitial abnormalities on HRCT thorax
- Shortened telomeres
- Increased frequency of minor allele of MUC5B
- Plasma biomarkers – increase in MMP7 and SPD
- Transbronchial lung biopsies demonstrated ER stress in AEC

Kropski et al. Am J Respir Crit Care Med 2015
Advice for family members

- Genetic testing should be considered
  - NHS England SFTPC
  - 100,000 Genome Project

- Vigilant for respiratory symptoms

- Avoid environmental and occupational pneumotoxic exposures

- Smoking cessation
IPF has an unpredictable course

King TR et al, Lancet 2011
Can genetics help with early diagnosis?
How common are early interstitial lung abnormalities?

Putman RK et al. Am J Respir Crit Care Med 2014
MUC5B is associated with early interstitial lung abnormalities

Table 2. Association between Interstitial Lung Abnormalities and MUC5B Genotype in the Framingham Heart Study.*

<table>
<thead>
<tr>
<th>Status of Interstitial Lung Abnormalities</th>
<th>No. of Patients</th>
<th>MUC5B Genotype (rs35705950)</th>
<th>Adjusted Odds Ratio (95% CI)†</th>
<th>P Value</th>
<th>Adjusted Odds Ratio with Covariates (95% CI)‡</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absence of interstitial lung abnormalities</td>
<td>1370</td>
<td>1113 (81) 247 (18) 10 (&lt;1)</td>
<td>1.0</td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Presence of interstitial lung abnormalities</td>
<td>177</td>
<td>115 (65) 56 (32) 6 (3)</td>
<td>2.3 (1.6–3.1)</td>
<td>&lt;0.001</td>
<td>2.8 (2.0–3.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Definite fibrosis§</td>
<td>47</td>
<td>26 (55) 20 (43) 1 (2)</td>
<td>3.0 (1.8–5.0)</td>
<td>&lt;0.001</td>
<td>6.3 (3.1–12.7)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

At-Risk Population (Normal Chest CT)

**MUC5B** variant 19% NHWs
*NEJM* 2011; 364:1503

**MUC5B** variant OR=6.3 per allele
*NEJM* 2013; 368:2192

Preclinical Pulmonary Fibrosis (Abnormal Chest CT)

**MUC5B** variant OR=6.8-9.0 per allele
*NEJM* 2011; 364:1503

Prevalence 1.8% ≥ 50 yrs
*NEJM* 2013; 368:2192

Survival (%)

- No ILA
- ILA Without Progression
- ILA With Progression

HR=3.7 (1.3-10.7)
*AJRCCM* (Ref.3)

Idiopathic Pulmonary Fibrosis

Prevalence < 1/1000
But MUC5B is not associated with all ILD

<table>
<thead>
<tr>
<th>Study</th>
<th>Diseases or Abnormalities Included</th>
<th>Study Type</th>
<th>Minor Allele Frequency (%)</th>
<th>Effect Size (Allelic Odds Ratio)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seibold et al., 2011</td>
<td>IPF, FPF (also NSIP, COP, RB-ILD, and unclassified IIP)</td>
<td>Genome-wide linkage study with fine mapping</td>
<td>34</td>
<td>8.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Zhang et al., 2011</td>
<td>IPF</td>
<td>Case-control replication</td>
<td>34</td>
<td>4.2*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stock et al., 2013</td>
<td>IPF</td>
<td>Case-control replication</td>
<td>36</td>
<td>4.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Systemic sclerosis</td>
<td></td>
<td>12</td>
<td>1.2</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>Sarcoidosis</td>
<td></td>
<td>11</td>
<td>1.1</td>
<td>0.60</td>
</tr>
<tr>
<td>Peljto et al., 2012</td>
<td>IPF</td>
<td>Case-control replication</td>
<td>11</td>
<td>1.1</td>
<td>0.80</td>
</tr>
<tr>
<td>Borie et al., 2013</td>
<td>IPF</td>
<td>Case-control replication</td>
<td>42</td>
<td>6.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Systemic sclerosis</td>
<td></td>
<td>10</td>
<td>1.0</td>
<td>0.64</td>
</tr>
<tr>
<td>Noth et al., 2013</td>
<td>IPF (discovery)</td>
<td>Genome-wide association study</td>
<td>14</td>
<td>1.6*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fingerlin et al., 2013</td>
<td>IPF and FIP (also NSIP, COP, DIP, RB-ILD, and unclassified IIP)</td>
<td>Genome-wide association study</td>
<td>NR</td>
<td>3.1-3.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hunninghake et al.,</td>
<td>ILA (all)†</td>
<td>Case-control replication</td>
<td>19</td>
<td>2.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2013 (16)</td>
<td>ILA (definite fibrosis)‡</td>
<td></td>
<td>23</td>
<td>6.3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Putman RK et al. Am J Respir Crit Care Med 2014
Challenges in early diagnosis

- What does early IPF look like?
- How long does it take to transform to progressive or end-stage IPF?
- Can interventions targeted at these early stages prevent disease progression?
Can genetics predict survival?
MUC5B promoter polymorphism affects survival

Minor T allele had survival advantage

Independent of age, sex, FVC, DLCO, MMP7 or treatment

Mechanism unclear – enhance mucosal defence or regulate wound repair

Peljto AL et al JAMA 2013
TOLLIP minor allele associated with increased mortality

TOLLIP rs5743890 minor allele G
Reduced susceptibility to IPF
Heterozygote had worse outcome
Regulate TLR and TGFβ

TLR3 polymorphism associated with increased mortality

- TLR3 L412F is associated with
- Accelerated disease progression
- Increased mortality
- Impairs functional responses
- Reduced IFNβ
- Dysregulates fibroproliferative responses

O’Dwyer et al. Am J Respir Crit Care Med 2013
Will genetics determine treatment choice?
TOLLIP genotype may influence response to N-acetylcysteine therapy

- TOLLIP mutations associated with IPF susceptibility and survival
- Involved with lung defence, sensitive to oxidative signalling
- CC phenotype have worse prognosis with NAC treatment
- TT phenotype have better prognosis with NAC treatment

Oldham JM et al, AJRCCM 2015; 192:1475
Telomerase mutations are associated with increased complications after lung transplantation

- 8 patients with IPF (diagnosed 47 yrs)
- Median age 52 years at transplantation
- Mutations in TERT or TERC
- Increased complications
  - 50% required dialysis for acute tubular necrosis (renal tubular injury and calcineurin inhibitor toxicity)
  - 88% haematological abnormalities
  - Increased risk of adverse events with immunosuppression

Will genes affect anti-fibrotic treatment responses?

- No evidence that genetic data should determine selection of approved anti-fibrotic treatments

- But future clinical trials will need to take into account potential genotypic variation and primary outcomes especially for genes that modify survival
Summary

- Significant developments in identifying genetic variants associated with FIP and sporadic IPF
- MUC5B is the strongest known risk factor for IPF
- Key role of environmental modifiers - smoking
- Novel insights to the pathogenesis
  - Host defence, barrier function, role of distal airway
- Develop new therapeutic strategies
  - Mucus clearance