Case #1

- 68 yo WF referred by UTHSC in Tyler for evaluation of ILD
- Gradual worsening of cough and dyspnea x 1 year
- Never smoker
- >20 years of raising 250,000+ chickens in East Texas (1971-1995) but no exposures since
- Family history of lung fibrosis (affected father and uncle, possibly affected paternal grandfather)
Case #1

- Exam notable for loud dry inspiratory crackles over lower posterior lung fields, 95% O2 saturation at rest

- Pulmonary function testing:
  
<table>
<thead>
<tr>
<th>May 2011</th>
<th>January 2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC</td>
<td>FEV1</td>
</tr>
<tr>
<td>1.56 L</td>
<td>1.81 L</td>
</tr>
<tr>
<td>(57%)</td>
<td>(65%)</td>
</tr>
<tr>
<td>FEV1</td>
<td>Ratio</td>
</tr>
<tr>
<td>1.27 L</td>
<td>88%</td>
</tr>
<tr>
<td>(63%)</td>
<td>85%</td>
</tr>
<tr>
<td>Ratio</td>
<td>BDR</td>
</tr>
<tr>
<td>88%</td>
<td>None</td>
</tr>
<tr>
<td>85%</td>
<td></td>
</tr>
<tr>
<td>BDR</td>
<td>TLC</td>
</tr>
<tr>
<td>None</td>
<td>2.60 L</td>
</tr>
<tr>
<td></td>
<td>(56%)</td>
</tr>
<tr>
<td>TLC</td>
<td>DLco</td>
</tr>
<tr>
<td>2.60 L</td>
<td>3.02 ml/min/mmHg</td>
</tr>
<tr>
<td>(56%)</td>
<td>(30%)</td>
</tr>
<tr>
<td>DLco</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>(56%)</td>
</tr>
</tbody>
</table>

- 6-MWD: 110 m, desaturation to 83% on room air

HRCT with Probable UIP pattern
Genetics of Pulmonary Fibrosis:  
Time for Genetic Screening?

Case #1

- Labs notable for mild anemia (Hgb 11)
- ANA 1:80 speckled pattern, ENA, RF and CCP negative
- Diagnosis of IPF by Multi-disciplinary Discussion
- Died from rapid progression of pulmonary fibrosis at outside hospital
- Can genetics help us with her management?

Personalized Medicine: Role of Genetics

Will genetics explain WHY some people develop ILD?
Will genetics explain HOW people are affected?
Will genetics inform WHAT drugs to use to treat patients with ILD?
Genetics of Pulmonary Fibrosis: Time for Genetic Screening?

**ILDs: Interstitial Lung Diseases**
- Complex disease
- Effects of age and environment
- Heterogeneous collection of >100 different diseases
- Non-neoplastic, non-infectious chronic lung diseases
- Similar clinical, radiographic and physiologic features
- Characterized by inflammatory-fibrotic infiltration

---

**PF: Genetic-Allelic Spectrum**

<table>
<thead>
<tr>
<th>Effect Size</th>
<th>Frequency</th>
<th>Variant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very Rare</td>
<td>MAF &lt;0.1%</td>
<td>SFTPC, SFTPA1/A2</td>
</tr>
<tr>
<td>MAF &gt; 5%</td>
<td>Common</td>
<td>MUC5B, TERT, DSP, TOLLIP</td>
</tr>
<tr>
<td>MAF &lt; 0.1%</td>
<td>Rare</td>
<td>TERT, DKC1, TINF2, NAF1</td>
</tr>
</tbody>
</table>

- **Personal Genome**
  - Next-Generation Sequencing (NGS)
- **Genome-wide Association Studies (GWAS)**
Genetics of Pulmonary Fibrosis: Time for Genetic Screening?

**Common Variants**

- Variant is found in a sizable proportion of the population
- Modest effect
- In general, the high prevalence of CVs in the general population in comparison with disease limits prognostication.

---

**MUC5B Promoter Variant and Rheumatoid Arthritis with Interstitial Lung Disease**

- Fingerlin et al. Nature Genetics 2013
- Jude et al. NEJM 2018
Genetics of Pulmonary Fibrosis: Time for Genetic Screening?

Rare Variants

- Variant is found very rarely in the population
- Majority of variants in genome
- In general, RVs may provide more prognostication especially for individual families.

PARN Mutations Shared by All Affecteds

PARN IVS4 -2a>g

Stuart et al. Nature Genetics 2015
## Genetics Pathways in Lung Disease

### RV Genes:
- ABCA3
- SFTPB
- SFTPC
- SFTPA1/2
- NKX2.1
- CSF2RA/B
- SFTPC
- HSP1/4
- COPA
- PARN
- NAF1
- DKC1
- TINF2
- TERT
- RTEL1
- TERC

### CV Genes:
- MUC5B
- TERT
- TERC
- OBFC1

### Pathways:
- Lung Homeostasis
- ER Stress
- Telomere Shortening

## Telomerase Maintains Chromosomal Ends

- Telomeres
- Centromere
Genetics of Pulmonary Fibrosis: Time for Genetic Screening?

Familial Pulmonary Fibrosis: Rare Variants in TERT Lead to Reduced Telomerase Activity

Armanios et al. NEJM 2007
Tsakiri et al. PNAS 2007
Cronkhite et al. AJRCCM 2008
Diaz de Leon et al. PLoS One 2010

Rare Variants in IPF
FPF Family Members with Rare Pathogenic Variants have Short Telomere Lengths

Stuart et al. Nature Genetics 2015

Balancing Genetic Mechanism

Heterozygous Rare Variant
Telomerase Germline Mutations

Telomerase
Genetics of Pulmonary Fibrosis:
Time for Genetic Screening?

Balancing Genetic Mechanism

- **Heterozygous Rare Variant**
  - Telomerase Germline Mutations
- **Acquisition of Somatic Promoter Telomerase Mutations in Cis with WT allele Found in WBC**

Senescence

Telomerase

Cancer

Maryoung et al JCI 2017

Short Telomere Syndromes

<table>
<thead>
<tr>
<th>Time</th>
<th>DC</th>
<th>Blood</th>
<th>Lung</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inheritance</td>
<td>Age of onset</td>
<td>Inheritance</td>
<td>Age of onset</td>
</tr>
<tr>
<td></td>
<td>XLR, AD, AR</td>
<td>1-30</td>
<td>AD, AR</td>
<td>AD, AR</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>DKC1</td>
<td>TERT</td>
<td>TERT</td>
<td>TERT</td>
</tr>
<tr>
<td></td>
<td>TERC</td>
<td>TERT</td>
<td>TERT</td>
<td>TERC</td>
</tr>
<tr>
<td></td>
<td>TERLI*</td>
<td>PARP*</td>
<td>PARP*</td>
<td>PARP*</td>
</tr>
<tr>
<td></td>
<td>TERT*</td>
<td>TERT</td>
<td>TERT</td>
<td>TERT</td>
</tr>
<tr>
<td></td>
<td>RTEL1*</td>
<td>NAF1</td>
<td>NAF1</td>
<td>NAF1</td>
</tr>
<tr>
<td></td>
<td>RTEL1*</td>
<td>TINF2</td>
<td>TINF2</td>
<td>TINF2</td>
</tr>
<tr>
<td></td>
<td>PARN*</td>
<td>TINF2</td>
<td>TINF2</td>
<td>TINF2</td>
</tr>
<tr>
<td></td>
<td>NOLA3</td>
<td>TINF2</td>
<td>TINF2</td>
<td>TINF2</td>
</tr>
<tr>
<td></td>
<td>NOP10</td>
<td>TINF2</td>
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<td>TINF2</td>
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<tr>
<td></td>
<td>ACB1</td>
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<td>TINF2</td>
<td>TINF2</td>
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<td>TINF2</td>
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<td>NOP10</td>
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<td></td>
<td>TCAB1</td>
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<tr>
<td></td>
<td>ACD</td>
<td>TINF2</td>
<td>TINF2</td>
<td>TINF2</td>
</tr>
</tbody>
</table>

- **Short telomeres**
  - Yes

* Gene dosage effect; Biallelic mutations in DC patients; Heterozygous mutations in PF patients
Telomerase Mutations and Dyskeratosis Congenita Related Phenotypes

- More bone marrow failure of ILD patients with telomerase mutations post-lung transplantation
- More occult hematologic disease
- Elevated LFTs
- Higher rate of infection and allograft dysfunction
- More immunosuppression medication changes
- More impaired CMV immunity

Silhan et al Eur Respir J 2014
Borie et al J Heart Lung Transpl 2014
Tokman et al J Heart Lung Transpl 2015
George et al Chest 2015
Popescu et al AJRCCM 2018
Genetics of Pulmonary Fibrosis: Time for Genetic Screening?

**ILD Associated with Pathogenic or Likely Pathogenic Telomere-Related Variants**

64 families: *TERT*(43), *TERC*(6), *RTEL1*(7), *PARN*(8); n=115
Multidisciplinary Diagnosis (MDD) of 77 cases:
- IPF 46%
- NSIP 3%
- DIP 1%
- PPFE 10%
- Unclassifiable 20%
- Chronic HP 12%
- CTD-ILD 3%
- IPAF 6%

80% of family members with identical mutation had discordant ILD diagnoses.

**“Monogenic” Short Telomere ILDs**

Wide spectrum of ILD Diagnoses

Newton et al ERJ 2016
Genetics of Pulmonary Fibrosis: Time for Genetic Screening?

“Monogenic” Short Telomere ILDs

Similar transplant-free survival regardless of gene mutation or ILD diagnosis.
Genetic classification trumps clinical diagnosis

Newton et al ERJ 2016
Genetics of Pulmonary Fibrosis: Time for Genetic Screening?

IPF & FPF Enriched for Short Telomeres & RVs

Normal Population

~10% Qualifying Rare Variants
~35% TL < 10th percentile

~25% Mutations
~45% TL < 10th percentile

IPF

FPF

Telomere Lengths and IPF Survival in Dallas Cohort (n=149)


Cronkhite et al. AJRCCM 2008
Alder et al. PNAS 2008
Stuart et al. Nature Genetics 2015
Petrovski et al. AJRCCM 2017
Dressen et al. LRM 2018
Genetics of Pulmonary Fibrosis:
Time for Genetic Screening?

Telomere Lengths and IPF Survival

Stuart et al Lancet Resp Med 2014

Telomere Lengths and CHP Survival

Ley et al Lancet Resp Med 2018
Genetics of Pulmonary Fibrosis: Time for Genetic Screening?

Telomere Lengths and Transplant Outcomes

Newton et al JHLT 2017

Telomere Lengths and Immunosuppression

Newton et al Under Review
Genetics of Pulmonary Fibrosis: Time for Genetic Screening?

Telomere Lengths and Immunosuppression

A. PANTHER-IPF, LTL <10th percentile

B. PANTHER-IPF, LTL >10th percentile

Summary

- Rare Variants in genes confer a strong risk toward developing pulmonary fibrosis
- Different RV pulmonary fibrosis genes maintain Telomere integrity
- Telomere pathway identifies ILD patients with a clinical subtype that is characterized by rapid progression and worse survival
- Knowledge of leukocyte telomere length may help personalize ILD clinical care
Genetics of Pulmonary Fibrosis: Time for Genetic Screening?

Case Presentations

Classification of Genetic Variants

- Benign
  - MAF too high for disease
  - Silent variant
  - Nonsegregation
  - Normal telomere lengths
  - No deleterious effect

- Likely Benign
  - Least
  - Clinically
  - Useless

- VUS
  - Nonsegregated
  - Normal telomere lengths

- Likely Pathogenic
  - Rare MAF
  - Predicted LOF variant
  - Cosegregation with ILD
  - Very short telomere lengths

- Pathogenic
  - Most
  - Clinically
  - Useful
Genetics of Pulmonary Fibrosis:  
Time for Genetic Screening?

**Case #2**

- 45 yo never smoker WF referred for evaluation of familial pulmonary fibrosis
- Paternal grandfather died at age 68 of PF, Father died at age 58 of PF (genetic anticipation)
- Premature graying of hair (started age 20, completely gray by age 45)
- Macrocytosis (MCV 101) without anemia
- HRCT with features of PPFE and UIP
Genetics of Pulmonary Fibrosis: 
Time for Genetic Screening?

Case #2

- Genetic counseling
- Sequential CLIA-certified gene sequencing
- TERT gene normal
- TERC r.234c>g heterozygous
- Classification: VUS
- Not in ExAC database
- Predicted to cause change in 2° structure
- Blood telomere lengths <1st percentile

Case #2

- Followed closely for progression of pulmonary fibrosis
- Pirfenidone started in 2015 for worsening restriction and reduction in DLco
- Biopsy of painless oral (tongue) ulcer revealed HNSCC
- Surgically staged as T1 lesion, 10 mm depth with peri-neural invasion, negative lymph nodes
- Cancer free x 2 years; progressive lung disease
Case #3

- 54 yo HF referred for evaluation of familial pulmonary fibrosis (2 affected brothers)
- Diagnosed with PBC by liver biopsy 12 years prior, not on treatment
- Positive ANA (1:2560, anti-centromere), macrocytosis, thrombocytopenia;
- Gray hair noted at age 21, now all gray
- PFTs with pulmonary restriction, decreased DLco
- HRCT with findings inconsistent with UIP and cirrhotic liver

HRCT inconsistent with UIP (uniform distribution, GGO, no honeycombing)
Genetics of Pulmonary Fibrosis: Time for Genetic Screening?

Case #3

- Genetic counseling of patient
- CLIA-certified gene sequencing revealed homozygous PARN variant p.Arg444Cys
  ExAC frequency of 0.00018; gnomAD frequency of allele 7-fold higher in Hispanics than Europeans
- Classification: VUS
- Unclear genetic mechanism confounds genetic counseling of at risk family members

Zhang et al Under Review
Genetics of Pulmonary Fibrosis: Time for Genetic Screening?

Case #3

- Parents equivalent to 3rd degree relatives
Genetics of Pulmonary Fibrosis:
Time for Genetic Screening?

Case #3

• Genetic counseling regarding risk of pulmonary fibrosis to family members not possible without molecular characterization of this family
• Hispanic-specific risk factor?
• Patient died of S. aureus sepsis while waiting for genetic work-up

Summary

1. Spectrum of possible genetic test results
2. CLIA-certified testing often yields VUS
3. For pathogenic or likely pathogenic variants, more information than just the allele and its frequency are needed (telomere length, clinical pattern of inheritance, co-segregation with short telomere phenotype)
4. High suspicion for related phenotypes
5. Need to know pattern of inheritance in order to accurately counsel patients and family members
6. More research needed for individual variants!
Genetics of Pulmonary Fibrosis:  
Time for Genetic Screening?

Acknowledgements

Lab Members:
Chad Newton
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Jerry Shay
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Imre Noth

ILD Care Team:

Special Thanks to:
Patients and Families
Referring Physicians
Genetics of Pulmonary Fibrosis:  
Time for Genetic Screening?

Case #2

- 40 yo never smoker WF referred for evaluation of familial pulmonary fibrosis
- Mother died at age 54 of PF
- HRCT shows upper lobe fibrosis
Case #2

- Surgical biopsy reveals Pleuroparenchymal fibroelastosis (PPFE)
- Premature graying of hair (started age 24, completely gray by age 40)
- HELLP diagnosed during pregnancy
- Macrocytosis (MCV 104) without anemia

Case #2

- Genetic counseling
- CLIA-certified gene sequencing
- \textit{TERT} gene with heterozygous change c.416T>G, p.Leu139Arg
- Ultra rare; not reported in ExAC database
- Classification: \textbf{VUS}
### Genetics of Pulmonary Fibrosis: Time for Genetic Screening?

#### Case #2
- Blood telomere length <1st percentile
- Followed closely for progression of PF
- Over last 10 years with progressive restriction and reduction in DLco
- Died while awaiting lung transplant

#### Monogenic Short Telomere ILDs

<table>
<thead>
<tr>
<th>Condition</th>
<th>TERC (n=7)</th>
<th>TERT (n=75)</th>
<th>RTE1 (n=14)</th>
<th>PARN (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telomere Length</td>
<td>-0.7±0.25</td>
<td>&lt;-0.58±0.27</td>
<td>&lt;-0.51±0.13</td>
<td>&lt;-0.36±0.14</td>
</tr>
</tbody>
</table>

**Age of ILD Dx**
- 51±11 < 58±10 < 60±11 < 64±8

*Age at diagnosis correlates with telomere lengths.*

<table>
<thead>
<tr>
<th>Condition</th>
<th>TERC (n=7)</th>
<th>TERT (n=75)</th>
<th>RTE1 (n=14)</th>
<th>PARN (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemia</td>
<td>71%</td>
<td>27%</td>
<td>21%</td>
<td>21%</td>
</tr>
<tr>
<td>Macrocytosis</td>
<td>29%</td>
<td>25%</td>
<td>29%</td>
<td>16%</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>43%</td>
<td>8%</td>
<td>0%</td>
<td>0%</td>
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<tr>
<td>Thrombocytopenia</td>
<td>43%</td>
<td>9%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>AA/MDS</td>
<td>29%</td>
<td>3%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Any</td>
<td>71%</td>
<td>48%</td>
<td>50%</td>
<td>32%</td>
</tr>
</tbody>
</table>

*Higher incidence of severe hematologic comorbidities found in TERC mutation carriers.*

*Newton et al ERJ 2016*
Genetics of Pulmonary Fibrosis: Time for Genetic Screening?

Rare Novel \textit{RTEL1} and \textit{PARN} Variants in Familial Pulmonary Fibrosis

Novel Damaging + Missense: \begin{figure}
\centering
\includegraphics[width=0.4\textwidth]{parn_rtel1}
\end{figure}

Novel Damaging: \begin{figure}
\centering
\includegraphics[width=0.4\textwidth]{parn}
\end{figure}

Activating Telomerase Promoter Mutations

\begin{figure}
\centering
\includegraphics[width=0.4\textwidth]{blood}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=0.4\textwidth]{ut248}
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Bridget Stuart, MD, PhD; Chao Xing PhD, Jerry Shay, PhD Rick Lifton, MD, PhD; YCGA Stuart et al Nature Genetics 2015

Maryoung et al JCI 2017
Activating Telomerase Promoter Mutations

Maryoung et al JCI 2017

Activating Telomerase Promoter Mutations

Maryoung et al JCI 2017
Genetics of Pulmonary Fibrosis:  
Time for Genetic Screening?

**Balancing Genetic Mechanism**

- Noncoding variants mitigate the presence of a genetic disease
- Hyperactivating mutations are not sufficient to drive the development of cancer
- Promoter mutations may be better tolerated in certain cell types (Granulocytes, B cells)
- Selected by self-renewal and proliferative advantage
- Persistence for 10 years in one patient