Genetics in COPD

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Edwin K. Silverman: Conflicts of Interest

1) Personal financial relationships with commercial interests relevant to medicine, within past 3 years:
   - Consulting: GlaxoSmithKline, Merck
   - Lecture Fees (Honoraria): Merck, Novartis

2) Personal financial support from a non-commercial source relevant to medicine, within past 3 years:
   - No relationships to disclose

3) Personal relationships with tobacco industry entities:
   - No relationships to disclose
Overview of Complex Disease Genetics

From Network Medicine: Complex Systems in Human Disease and Therapeutics, edited by Loscalzo/Barabasi/Silverman
COPD: Evidence for Genetic Determinants

- Development of COPD in smokers is highly variable (Burrows 1977).


- Twin study of 22,422 Danish and 27,668 Swedish twin pairs estimated COPD heritability ~60% (Ingebrigtsen 2010).

- A small percentage of COPD patients inherit severe alpha-1 antitrypsin deficiency.
Are People with PI MZ at Increased Risk for Lung Disease? (Hersh, Thorax 2004)

• Case-control studies suggest an increased risk for lung disease in PI MZ individuals

• General population surveys do not find reduced lung function in PI MZ individuals

• Unclear if PI MZ subjects are at increased risk for COPD
### Multivariable Models for PI MZ Genotype in 8,271 Non-Hispanic Whites (239 MZ) and African Americans (22 MZ)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Odds (CI) or Estimate (se) in NHW</th>
<th>PI MZ p-value in NHW</th>
<th>Odds (CI) or Estimate (se) in AA</th>
<th>PI MZ p-value in AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>COPD Affection Status</td>
<td>1.4 (1.05 – 1.9)</td>
<td>0.02</td>
<td>2.0 (0.8- 5.4)</td>
<td>0.2</td>
</tr>
<tr>
<td>Log (Emphysema)</td>
<td>0.3 (0.1)</td>
<td>0.001</td>
<td>0.5 (0.3)</td>
<td>0.08</td>
</tr>
<tr>
<td>Log (Gas Trapping)</td>
<td>0.13 (0.07)</td>
<td>0.05</td>
<td>0.3 (0.2)</td>
<td>0.3</td>
</tr>
<tr>
<td>FEV₁ (% pred)</td>
<td>-5.4 (1.7)</td>
<td>0.001</td>
<td>-13.0 (5.0)</td>
<td>0.01</td>
</tr>
<tr>
<td>FEV₁/FVC</td>
<td>-0.03 (0.01)</td>
<td>0.003</td>
<td>-0.10 (0.03)</td>
<td>0.02</td>
</tr>
</tbody>
</table>
International COPD Genetics Consortium

COPD GWAS (Hobbs/Cho, Nat Genet 2017)

- Included discovery in 15,256 COPD cases and 47,936 controls from 26 studies with genotyping of select top results ($P < 5 \times 10^{-6}$) in 9,498 COPD cases and 9,748 controls from UK-BiLEVE
UK-BiLEVE Collaborative Lung Function GWAS (Wain/Tobin, Nat Genet 2017)

- GWAS of spirometry with discovery in 48,943 subjects selected from lung function extremes, with follow-up in 95,375 subjects
- Identified 97 GWAS loci for lung function, many of which showed at least nominal association with COPD
COPD Genetic Risk Scores Are Associated with Lung Function in the International COPD Genetics Network

(R. Busch, AJRCMB, In Press)

After adjustment for covariates, each additional risk allele predicted 1.9% (95% CI 1.2 to 2.5) decrease in FEV\(_1\) (% predicted)
Exome Sequencing Analysis in Severe, Early-Onset Chronic Obstructive Pulmonary Disease
(D. Qiao, AJRCCM 2016)

- 69 genes had rare variants segregating in at least two pedigrees
- No genes had segregating rare variants in more than three pedigrees
- Four genes with segregating rare variants in EOCOPD pedigrees had nominal significance in COPDGene: ALCAM, RARS, DNAH8, and GBF1
- Rare coding variants could influence COPD susceptibility, but they likely are found in multiple different genes
Preliminary COPD TOPMed Single Variant Analysis of Whole Genome Sequencing in 956 NHW Subjects

(M. Cho/D. Prokopenko)
Moving from Gene Discovery to Gene Localization to Functional Validation

• **Discovery**
  – Genetic Association Analysis

• **Localization**
  – Fine Mapping
  – Long-range Genetic Interactions
  – Regions containing functional activity

• **Functional Validation**
  – Cell-based models
  – Animal models
Relationship of Genetics Research to Cell/Molecular Biology Studies

Genetics Researchers

- GWAS Associations

Cell/Molecular Biologists

- No thanks, we have our own ideas of what to study
- We don’t believe that what you found is important or useful
COPD GWAS Locus Near *HHIP*  
(Wilk, PLoS Genetics 2009)
Long-range Interaction Detected Between COPD GWAS Region and *HHIP* Promoter (Zhou, *Hum Mol Genet*, 2012)

*Chromosome conformation capture*

![Graph showing interaction frequency between Beas-2B and MRC5 cells.](image)

- Interaction frequency on the y-axis.
- Chromosome conformation capture on the x-axis.
- An asterisk indicates *p* < 0.01.

**Figure a:**
- *Beas-2B* and *MRC5* interaction frequency bars.
- *rs12504628* and *rs13118928* SNP regions.
- 7kb SNP region highlighted.
- Anchor point indicated.

**Chromosome conformation capture:**
- Chromosome 4 (Chr4: 145Mb).
- Scale bars for SNP positions.
- NHGRI Catalog of Published Genome-Wide Association Studies.

**References:**
**Hhip\(^{+/-}\) Mice: Cigarette Smoke Effects**  
(T. Lao/X. Zhou, Genome Med 2015)

C57BL/6 --- Gill staining

Hhip\(^{+/-}\)  
Hhip\(^{+/-}\)

Mean Chord Length

Air  
Smoke

**Alveolar Chord Length**

- Hhip\(^{+/-}\) Mice: Cigarette Smoke Effects (T. Lao/X. Zhou, Genome Med 2015)
Hhip\(^{+/-}\) Mice: Cigarette Smoke Effects
(T. Lao/X. Zhou, Genome Med 2015)

Note: Lymphoid aggregates contain mainly CD8+ T cells and B cells
Aging-Related Emphysema in Hhip+/- Mice Without Smoke Exposure
(Lao, PNAS 2016)
Fam13a<sup>-/-</sup> Mice: Cigarette Smoke Effects
(Z. Jiang/X. Zhou, AJRCCM 2016)

Note: Fam13a knockout is protected from emphysema development
**FAM13A in COPD: Biological Mechanism**
(Z. Jiang/X. Zhou, AJRCCM 2016)

Complex of FAM13A, PP2A, and Beta-catenin

Beta-catenin Inhibitor Reverses Fam13a KO Mouse Emphysema Protection
*Ireb2*<sup>−/−</sup> Mice Are Resistant to Emphysema Development

(S. Cloonan/A. Choi, Nature Medicine, 2016)
Ireb2 Influences COPD Susceptibility by Regulating Lung Mitochondrial Iron (S. Cloonan/A. Choi, Nature Medicine 2016)

Smoke-induced mitochondrial abnormalities are reduced in Ireb2\(^{-/-}\) mice

Smoke-induced increases in COX, a key regulator of mitochondrial iron, are reduced in Ireb2\(^{-/-}\) mice
Overlap of Murine Emphysema Model Genes and COPD GWAS Region Genes

Murine Emphysema Model Genes ~109

COPD/Emphysema GWAS Region Genes ~48

HHIP
FAM13A
IREB2
MMP12
MMP1

Total Genes ~20,000
## Functional Validation of COPD GWAS Genes in Murine Models

<table>
<thead>
<tr>
<th>Gene</th>
<th>Reference</th>
<th>Model</th>
<th>Phenotype</th>
<th>Postulated Biological Effect/Pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP1</td>
<td>D’Armiento (1992)</td>
<td>Transgenic</td>
<td>Increased Emphysema</td>
<td>Collagenase Activity</td>
</tr>
<tr>
<td>MMP12</td>
<td>Hautamaki (1997)</td>
<td>Knock-out</td>
<td>Decreased Emphysema</td>
<td>Metalloelastase Activity</td>
</tr>
<tr>
<td>HHIP</td>
<td>Lao (2015)</td>
<td>Heterozygous Knock-out</td>
<td>Increased Emphysema</td>
<td>Lymphocyte Activation</td>
</tr>
<tr>
<td>IREB2</td>
<td>Cloonan (2016)</td>
<td>Knock-out</td>
<td>Decreased Emphysema and Airway Disease</td>
<td>Mitochondrial Iron</td>
</tr>
<tr>
<td>FAM13A</td>
<td>Jiang (2016)</td>
<td>Knock-out</td>
<td>Decreased Emphysema</td>
<td>Wnt/Beta Catenin</td>
</tr>
</tbody>
</table>
COPDGene Cluster Analysis
(Castaldi/Cho, Thorax 2014)

• 10,192 enrolled smokers (GOLD 0-4 and GOLD-U)
• 8,128 with complete data for all potential clustering variables and outcomes
• Split into training and test sample
  – training = 4187
  – test = 4101
• Clustering based on FEV$_1$, Emphysema, Emphysema Distribution, and Airway Wall Area
K-means, Four Clusters: Training Set
(Castaldi/Cho, Thorax 2014)

<table>
<thead>
<tr>
<th></th>
<th>Training Sample</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>C1:Mean</td>
</tr>
<tr>
<td>N (#)</td>
<td>1597</td>
</tr>
<tr>
<td>Age</td>
<td>58.9</td>
</tr>
<tr>
<td>Race (% African-American)</td>
<td>30</td>
</tr>
<tr>
<td>Gender (%female)</td>
<td>44</td>
</tr>
<tr>
<td>FEV1, percent of predicted</td>
<td>95.3</td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>0.76</td>
</tr>
<tr>
<td>BMI</td>
<td>28.7</td>
</tr>
<tr>
<td>Pack Years</td>
<td>38</td>
</tr>
<tr>
<td>Emphysema at -950HU</td>
<td>2.6</td>
</tr>
<tr>
<td>Segmental Airway Thickness</td>
<td>58.8</td>
</tr>
<tr>
<td>Upper/Lower Emphysema Ratio</td>
<td>0.69</td>
</tr>
<tr>
<td>Upper/Lower Emphysema Difference</td>
<td>-1</td>
</tr>
<tr>
<td>Gas Trapping</td>
<td>12.9</td>
</tr>
</tbody>
</table>

Cluster 1 = Resistant Smokers
Cluster 2 = Mild Upper Lobe Predominant Emphysema
Cluster 3 = Airway Predominant Disease (High BMI, Less Emphysema)
Cluster 4 = Severe Emphysema
### What Are the Relationships of COPD GWAS Variants to the the Four Clusters? (Castaldi/Cho, Thorax 2014)

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Exacerbations</td>
<td>2.27</td>
<td>5.80E-09</td>
<td>3.16</td>
<td>4.60E-23</td>
<td>8.93</td>
<td>1.00E-82</td>
</tr>
<tr>
<td>MMRC</td>
<td>2.81</td>
<td>1.30E-30</td>
<td>3.38</td>
<td>1.40E-57</td>
<td>10.87</td>
<td>2.80E-177</td>
</tr>
<tr>
<td>BODE</td>
<td>3.36</td>
<td>7.20E-38</td>
<td>4.62</td>
<td>9.00E-80</td>
<td>66.44</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Hospitalizations/ER Visits</td>
<td>4.06</td>
<td>1.40E-12</td>
<td>5.04</td>
<td>1.50E-20</td>
<td>11.81</td>
<td>2.30E-48</td>
</tr>
<tr>
<td>rs7671167 (FAM13A)</td>
<td>0.94</td>
<td>5.00E-01</td>
<td>0.85</td>
<td>2.40E-02</td>
<td>0.83</td>
<td>9.10E-03</td>
</tr>
<tr>
<td>rs1980057 (HHIP)</td>
<td>0.64</td>
<td>3.10E-06</td>
<td>0.91</td>
<td>1.90E-01</td>
<td>0.78</td>
<td>3.90E-04</td>
</tr>
<tr>
<td>rs13180 (Chr15q)</td>
<td>0.81</td>
<td>2.30E-02</td>
<td>1.04</td>
<td>5.90E-01</td>
<td>0.82</td>
<td>5.70E-03</td>
</tr>
<tr>
<td>rs8034191 (Chr15q)</td>
<td>1.32</td>
<td>2.70E-03</td>
<td>1.02</td>
<td>8.30E-01</td>
<td>1.43</td>
<td>8.90E-07</td>
</tr>
<tr>
<td>rs7937 (Chr 19q)</td>
<td>1.28</td>
<td>6.30E-03</td>
<td>1.15</td>
<td>4.30E-02</td>
<td>1.18</td>
<td>1.90E-02</td>
</tr>
</tbody>
</table>
## Current Top Genes for COPD Susceptibility (March 2017)

<table>
<thead>
<tr>
<th>Approach</th>
<th><strong>Definite COPD Genes or Regions</strong></th>
<th><strong>Probable COPD Genes or Regions</strong></th>
<th><strong>Possible COPD Genes or Regions</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mendelian Syndromes</td>
<td><strong>SERPINA1, ELN, FBLN5</strong></td>
<td></td>
<td><strong>LTBP4</strong></td>
</tr>
<tr>
<td>COPD GWAS or Exome Chip</td>
<td><strong>HHIP, FAM13A, HTR4, DSP, RIN3, ADAM19, GSTCD, THSD4, EEFSEC, IL27, ADGRG6, MMP12/MMP1, IREB2/CHRNA5</strong></td>
<td><strong>TGFB2, CYP2A6/EGNL2, SFTPD, RARB, MTCL1, PID1, ARMC2, CFDP1, TET2</strong></td>
<td></td>
</tr>
<tr>
<td>GWAS of COPD-related Phenotypes</td>
<td><strong>AGER</strong></td>
<td><strong>DLC1</strong></td>
<td>Multiple Genes</td>
</tr>
<tr>
<td>Lung Function GWAS</td>
<td>Several Genes now replicated in multiple COPD studies (e.g., HHIP)</td>
<td>Multiple Genes associated with lung function and COPD</td>
<td>Multiple Genes associated with lung function but not COPD</td>
</tr>
</tbody>
</table>
Significant Insights From COPD Genetic Studies

• Alpha-1 antitrypsin PI MZ genotype is a significant risk factor for COPD in smokers
• 24 genomic regions have been shown to contain genetic determinants that influence COPD and/or emphysema susceptibility by GWAS and/or Exome Chip analysis
• A functional genetic variant upstream from HHIP has been found in the COPD GWAS region on chromosome 4q31
• Several novel COPD susceptibility genes identified by GWAS have been supported by animal models of emphysema
• Functional studies of COPD GWAS genes are implicating key biological pathways in COPD pathogenesis
• Identification of COPD genetic determinants has the potential to provide insights into COPD heterogeneity
Collaborators

- **Transcontinental COPD Genetics Study (Korea):** Young Soo Shim, JJ Yim, Woo Jin Kim, DK Kim, Sei Won Lee, Myung Goo Lee
- **International COPD Genetics Consortium (Korea):** JJ Yim, Woo Jin Kim, DK Kim, Yeon Mok Oh, Mi Kyeong Lee, Sang Do Lee
- **ECLIPSE Genetics Study:** Michael Cho, DK Kim, Wayne Anderson, Sreekumar Pillai, Xiangyang Kong, David Lomas, ECLIPSE Steering/Scientific Committees
- **Norway Case-Control Study:** Per Bakke, Amund Gulsvik, Sreekumar Pillai, Craig Hersh, Dawn DeMeo, Michael Cho
- **Functional Genetics of COPD:** Xiaobo Zhou, Augustine Choi, Suzanne Cloonan, Dawn DeMeo, Craig Hersh, Jarrett Morrow, Jeanine D’Armiento, John Quackenbush, Kimberly Glass, John Platig, Amitabh Sharma, Yang-Yu Liu, Caroline Owen, Mark Perrella, Bart Celli, Miguel Divo, Zhiqiang Jiang, Taotao Lao, Raphael Bueno, Gerard Criner
- **COPDGene:** James Crapo, Barry Make, John Hokanson, Doug Everett, Terri Beaty, Michael Cho, Peter Castaldi, David Lynch, George Washko, Raul San Jose Estepar, James Ross, Merry-Lynn McDonald, Craig Hersh, Dawn DeMeo, Emily Wan, Brian Hobbs, Robert Busch, Lystra Hayden, Adel El-Boueiz, Megan Hardin, Jin Hwa Lee, Sung Ho Won, and 21 Clinical Centers
- **Funding:** NIH R01 HL089856 and R01 HL089897 (COPDGene), R01 HL111759 (COPD Networks), P01 HL105339 (COPD Functional Genetics PPG), P01 HL114501 (IPF/COPD PPG), R01 HL086936 (D’Armiento), and GlaxoSmithKline (ECLIPSE)