ARDS – NEW TREATMENTS

• Genotyping most Important

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Scientist, Keenan Center and Li Ka Shing Knowledge Institute
Scientist, Institute of Medical Sciences and Collaborative Program in
Genome Biology and Bioinformatics, University of Toronto
Disclosures

CIHR OCN 126573
CIHR MOP 106545
CIHR MOP 130331
CIHR MOP 140242
MRI Early Researchers Award
Physician Services Incorporate (PSI 0-35)
Stem Cell Network (SCN-72)
Brain-Canada (Z-BRAIN)
Ontario Thoracic Society

Tissue Regeneration Therapeutics (TRT)
Northern Therapeutics Inc.
Genotyping in ARDS

Goals:

• To identify the **genetic cause(s)** of the syndrome
• Determine the **genetic variants** that modulate risk and outcomes
• Widespread implications not only for individuals but also for the health care system
Overview

- Traditional approaches to understanding genetics of ARDS
- Recent advances in technology
- How these technologies have allowed us to better understand ARDS
Traditional Genetic approaches used in complex diseases

- **Linkage**

  Relies of related individuals
  No ‘a-priori’ hypothesis

- **Association**

  Candidate Gene Required
Assessing the quality of studies supporting genetic susceptibility and outcomes of ARDS

Marialbert Acosta-Herrera\textsuperscript{1,2,3}, Maria Pino-Yanes\textsuperscript{1,2,4}, Lina Perez-Mendez\textsuperscript{1,2}, Jesús Villar\textsuperscript{1,3,5} and Carlos Flores\textsuperscript{1,2,6*}

![Histogram comparing quality control scores of association studies in ARDS published from 1996 to 2008 (taken from Flores et al., 2008) and from 2008 until present. Statistically significant improvements affected criteria relevant to study design (LD exploration), study reproducibility (polymorphism identification) and statistical analysis (multiple testing adjustments). *\textit{p-value} \leq 0.05; **\textit{p-value} \leq 0.001.](image)
Case and Cohort Gene Association Studies

41 Genes
The human genome

First announcement
In June 2000: first announcement of a working draft (haplotype!) with the Nature and Science papers in February 2001

James Kent (UCSC)
Eugene Myers (Celera)


In June 2001: finished chromosome 20, with others following until finishing of chromosome 1 in May 2006

Gregory et al. (2006), Nature, 441, 315-321
**General Work Flow for Genome Wide Association Analysis**

1. **Large cohort of cases and controls** ($n > 1000$)
   - Matched for confounding variables, such as race, ethnicity and sex
   - Stratified in order to maximize signals

2. **Microarray-based SNP genotyping**
   - ~1 million random marker SNPs or
   - ~25,000 risk-enhancing SNPs (for example, nsSNPs)

3. **Derivation of haplotypes**
   - Predicated on International HapMap

4. **Detection of association signals**
   - $\chi^2$ or similar test
   - Uncorrected $P < 10^{-7}$ or false discovery rate-like correction

5. **Fine mapping of association signal** (see FIG. 2)
   - Directed genotyping of additional SNPs in region
   - Fine mapping of LD in region of association
   - Empirical derivation of haplotypes
   - Examination of effect of stratification, if available

6. **Replication of association**
   - Large independent cohort of cases and controls ($n > 1000$)
   - Genotyping of nominated candidate SNPs (<20)
   - $\chi^2$ or similar test; replication of initial signal

7. **Biological validation of association**
   - Identification of risk-enhancing variant
   - Examination of functional consequence of variant
   - Determination of mechanism of risk-enhancement

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**Genotypes**

<table>
<thead>
<tr>
<th></th>
<th>CC</th>
<th>AA</th>
<th>CA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases observed</td>
<td>59</td>
<td>27</td>
<td>98</td>
<td>184</td>
</tr>
<tr>
<td>Controls observed</td>
<td>60</td>
<td>89</td>
<td>36</td>
<td>185</td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
<td>116</td>
<td>134</td>
<td>369</td>
</tr>
</tbody>
</table>
The pace of genome-wide association study (GWAS) publications has increased dramatically over 7 years and shows no signs of slowing. The figure is based on data from the US National Human Genome Research Institute Catalog of Published Genome-Wide Association Studies.
Whole genome association studies

P-value

the probability of seeing your data or more extreme data if the null hypothesis is true.

By chance, with 1,000,000 statistical tests:

• a threshold of $p = 0.05$
  would show 50,000 “significant” associations

• a threshold of $p = 0.05/1,000,000 (5 \times 10^{-8})$
  would show 0.05 “significant” associations
Everybody who went to the moon has eaten chicken!

Good grief. Chicken makes you go to the moon!
ANGPT2 Genetic Variant Is Associated with Trauma-associated Acute Lung Injury and Altered Plasma Angiopoietin-2 Isoform Ratio

Nuala J. Meyer¹, Mingyao Li², Rui Feng², Jonathan Bradfield³, Robert Gallop², Scarlett Bellamy², Barry D. Fuchsl¹, Paul N. Lanken¹, Steven M. Albedla¹, Melanie Rushefski⁴, Richard Aplenc²,³,⁵, Helen Abramova¹, Elena N. Atochina-Vasserman¹, Michael F. Beers¹, Carolyn S. Calfee², Mitchell J. Cohen⁶, Jean-Francois Pittet⁷, David C. Christiani³,⁹, Grant E. O’Keefe⁶, Lorraine B. Ware¹¹, Addison K. May¹², Mark M. Wurfel¹³, Hakon Hakonarson³, and Jason D. Christie¹,²


Genetic Variation in the FAS Gene and Associations with Acute Lung Injury

Bradford J. Glavan¹, Tarah D. Holden¹, Christopher H. Goss², R. Anthony Black³, Margaret J. Neff¹, Avery B. Nathens⁴, Thomas R. Martin⁵, Mark M. Wurfel¹ and ARDSnet Investigators

¹Section of Pulmonary/Critical Care Medicine, Harborview Medical Center, Seattle, Washington; ²Division of Pulmonary/Critical Care Medicine, and ³Biomedical Informatics Core of the Institute of Translational Health Sciences, University of Washington, Seattle, Washington; ⁴Department of Surgery, St Michael’s Hospital, University of Toronto, Toronto, Canada; and ⁵Medical Research Service, Veterans Affairs Puget Sound Medical Center, Seattle, Washington

Am J Respir Crit Care Med. 2011 Feb 1;183(3):356-63.

IL1RN Coding Variant Is Associated with Lower Risk of Acute Respiratory Distress Syndrome and Increased Plasma IL-1 Receptor Antagonist

Nuala J. Meyer¹, Rui Feng², Mingyao Li², Yang Zhao³, Chau-Chyun Sheu³,⁴, Paula Tejera³, Robert Gallop², Scarlett Bellamy², Melanie Rushefski⁵, Paul N. Lanken¹, Richard Aplenc²,³,⁵, Grant E. O’Keefe⁶, Mark M. Wurfel⁷, David C. Christiani³,⁸, and Jason D. Christie¹,²

Am J Respir Crit Care Med. 2013 May 1; 187(9): 950–959.
• Initial GWAS subjects at risk for ARDS (AJRCCM 2011; 183:A5536)
• Validation in an independent data set of at risk patients
• Used top 1st percentile of associations from GWAS
• Generated a custom Illumina iSelects BeadChip (5,000 SNPs)
• Profiled iSPAAR data base (identification of SNPs altering ALIRisk (Caucasian patients N=1,577)
• After quality control retained 784 ARDS and 769 at risk controls
• Performed tests for SNP-level associations (and gene level associations)
• Identified FARP1 as top gene associated with risk of ARDS
Distinct and replicable genetic risk factors for acute respiratory distress syndrome of pulmonary or extrapulmonary origin

Paula Tejera, Nuala Meyer, Feng Chen, Rui Feng, Yang Zhao, D. Shane O'Mahony, Lin Li, Chau-Chyun Sheu, Rihong Zhai, Zhaoxi Wang, Li Su, Ed Bajwa, Amy M. Ahasic, Peter Clardy, Michelle N. Gong, Angela J. Frank, Paul N. Lanken, B. Taylor Thompson, Jason D. Christie, Mark Wurfel, Grant O'Keefe, and David C. Christiani

<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene</th>
<th>Minor allele</th>
<th>Human G10-quad</th>
<th>MAF Case/Control</th>
<th>Odds Ratio 95% CI</th>
<th>p value</th>
</tr>
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<tbody>
<tr>
<td>rs3244207</td>
<td>FAAH</td>
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<td>Typed</td>
<td>0.23/0.16</td>
<td>1.59 (1.10–2.23)</td>
<td>0.0131</td>
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SNPs not replicated in Stage II

<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene</th>
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<th>p value</th>
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<tbody>
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<td>rs198977</td>
<td>KLK2</td>
<td>T</td>
<td>Typed</td>
<td>0.36/0.22</td>
<td>1.23 (0.89–1.70)</td>
<td>0.2019</td>
</tr>
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<td>rs9645765</td>
<td>VWF</td>
<td>G</td>
<td>Imputed</td>
<td>0.08/0.08</td>
<td>0.98 (0.60–1.61)</td>
<td>0.942</td>
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<tr>
<td>rs2889490</td>
<td>SFRS16</td>
<td>G</td>
<td>Imputed</td>
<td>0.48/0.49</td>
<td>1.03 (0.77–1.37)</td>
<td>0.8319</td>
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<tr>
<td>rs3128126</td>
<td>ISG15</td>
<td>G</td>
<td>Imputed</td>
<td>0.33/0.36</td>
<td>0.77 (0.52/1.16)</td>
<td>0.2112</td>
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<td>rs16903496</td>
<td>ADRBK2</td>
<td>A</td>
<td>Imputed</td>
<td>0.07/0.09</td>
<td>1.05 (0.69–1.66)</td>
<td>0.8509</td>
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<tr>
<td>rs2070887</td>
<td>VWF</td>
<td>G</td>
<td>Typed</td>
<td>0.08/0.08</td>
<td>1.14 (0.69–1.88)</td>
<td>0.5928</td>
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<tr>
<td>rs10490072</td>
<td>BCL11A</td>
<td>C</td>
<td>Imputed</td>
<td>0.24/0.23</td>
<td>1.09 (0.78–1.52)</td>
<td>0.6125</td>
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</table>

SNPs associated with extrapulmonary injury-related ARDS in Stage I and tested for validation in Stage II using a trauma-related ALI population (Population I, UW trauma Cohort)

SNPs replicated in Stage II

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<thead>
<tr>
<th>SNP</th>
<th>Gene</th>
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<tr>
<td>rs1902867</td>
<td>POPDC3</td>
<td>C</td>
<td>Imputed</td>
<td>0.14/0.20</td>
<td>0.65 (0.46–0.90)</td>
<td>0.0094</td>
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SNPs not replicated in Stage II

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<tr>
<td>rs7807760</td>
<td>PRKAG2</td>
<td>A</td>
<td>Imputed</td>
<td>0.42/0.39</td>
<td>1.08 (0.83–1.38)</td>
<td>0.5082</td>
</tr>
<tr>
<td>rs7801616</td>
<td>PRKAG2</td>
<td>T</td>
<td>Imputed</td>
<td>0.42/0.40</td>
<td>1.08 (0.83–1.37)</td>
<td>0.5195</td>
</tr>
<tr>
<td>rs9960450</td>
<td>TNFRSF11A</td>
<td>C</td>
<td>Imputed</td>
<td>0.05/0.03</td>
<td>1.64 (0.91–3.00)</td>
<td>0.1009</td>
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<tr>
<td>rs2254358</td>
<td>HSPG2</td>
<td>C</td>
<td>Imputed</td>
<td>0.32/0.31</td>
<td>0.90 (0.75–1.28)</td>
<td>0.8977</td>
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<td>rs732821</td>
<td>HTR2A</td>
<td>A</td>
<td>Imputed</td>
<td>0.46/0.47</td>
<td>0.99 (0.78–1.25)</td>
<td>0.9171</td>
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<td>rs970522</td>
<td>PRKAG2</td>
<td>G</td>
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<td>0.5847</td>
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<tr>
<td>rs3887893</td>
<td>ABCC1</td>
<td>G</td>
<td>Imputed</td>
<td>0.39/0.56</td>
<td>1.32 (0.97–1.83)</td>
<td>0.0912</td>
</tr>
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SNPs associated with pulmonary injury-related ARDS in Stage I and tested for validation in Stage II using a pneumonia/pulmonary sepsis-related ARDS population (Population II, MGH/ARDS net)

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Associations between single nucleotide polymorphisms in the FAS pathway and acute kidney injury

Pavan Bhatraju, Christine Hsu, Paramita Mukherjee, Bradford J. Glavan, Amber Burt, Carmen Mikacenic, Jonathan Himmelfarb and Mark Wurfel

Table 4  Top 15 Fas/Fas ligand pathway polymorphisms in the Fluid and Catheter Treatment Trial associated with acute kidney injury (AKI) (stage 2–3 vs. stage 0) in Caucasians (stage 1 excluded)

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>OR (95% CI)*</th>
<th>P value</th>
<th>FDR</th>
<th>MAF</th>
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<tbody>
<tr>
<td>NFKBIA</td>
<td>rs1050851</td>
<td>4.00 (2.10–7.62)</td>
<td>1.05 × 10^-5</td>
<td>&lt;0.003</td>
<td>0.18</td>
</tr>
<tr>
<td>NFKBIA</td>
<td>rs2233417</td>
<td>4.03 (2.09–7.77)</td>
<td>1.88 × 10^-5</td>
<td>&lt;0.003</td>
<td>0.15</td>
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<tr>
<td>BAX</td>
<td>rs4645878</td>
<td>0.12 (0.02–0.90)</td>
<td>0.004</td>
<td>0.41</td>
<td>0.12</td>
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<tr>
<td>TIRAP</td>
<td>rs3802814</td>
<td>2.45 (1.28–4.72)</td>
<td>0.008</td>
<td>0.55</td>
<td>0.14</td>
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<tr>
<td>BCL2L1</td>
<td>rs6058381</td>
<td>0.13 (0.02–1.02)</td>
<td>0.008</td>
<td>0.48</td>
<td>0.09</td>
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<tr>
<td>BAX*</td>
<td>rs2387583</td>
<td>0.20 (0.05–0.89)</td>
<td>0.009</td>
<td>0.42</td>
<td>0.13</td>
</tr>
<tr>
<td>NFKBIA</td>
<td>rs7157810</td>
<td>2.19 (1.22–3.94)</td>
<td>0.009</td>
<td>0.38</td>
<td>0.18</td>
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<tr>
<td>BAX</td>
<td>rs1010103</td>
<td>0.21 (0.05–0.92)</td>
<td>0.01</td>
<td>0.38</td>
<td>0.13</td>
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<tr>
<td>LY96</td>
<td>rs16938758</td>
<td>2.22 (1.21–4.09)</td>
<td>0.01</td>
<td>0.34</td>
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<tr>
<td>ALS2CR1</td>
<td>rs17468277</td>
<td>2.36 (1.15–4.88)</td>
<td>0.02</td>
<td>0.67</td>
<td>0.10</td>
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<tr>
<td>DAXX</td>
<td>rs2239839</td>
<td>2.05 (1.10–3.82)</td>
<td>0.02</td>
<td>0.61</td>
<td>0.26</td>
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<tr>
<td>CASP8</td>
<td>rs1045485</td>
<td>2.32 (1.12–4.78)</td>
<td>0.03</td>
<td>0.64</td>
<td>0.11</td>
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<tr>
<td>CASP9</td>
<td>rs1820204</td>
<td>1.84 (1.04–3.27)</td>
<td>0.03</td>
<td>0.77</td>
<td>0.47</td>
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<tr>
<td>BCL2</td>
<td>rs1542578</td>
<td>0.55 (0.31–0.97)</td>
<td>0.04</td>
<td>0.72</td>
<td>0.49</td>
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<tr>
<td>TIRAP</td>
<td>rs8177343</td>
<td>1.98 (1.03–3.79)</td>
<td>0.04</td>
<td>0.79</td>
<td>0.16</td>
</tr>
</tbody>
</table>

*Risk of AKI (stage 1 or above) with each additional copy of the minor allele.

**Risk nominally associated with stage 2–3 AKI in African-Americans (p <0.05).
SNP: single nucleotide polymorphism; OR: odds ratio; FDR: false discovery rate; MAF: minor allele frequency.
Functional Characterization of Polymorphisms in the Peptidase Inhibitor 3 (Elafin) Gene and Validation of Their Contribution to Risk of Acute Respiratory Distress Syndrome

Paula Tejera¹*, D. Shane O’Mahony²*, Caroline A. Owen³,⁴, Yongyue Wei¹, Zhaoxi Wang¹, Kushagra Gupta³, Li Su¹, Jesus Villar⁵,⁶, Mark Wurfel², and David C. Christiani¹,⁷

Elafin: neutrophil serine proteinase inhibitor- important role in preventing excessive tissue injury during inflammatory events

↑ Risk of ARDS
Prevention of LPS-Induced Acute Lung Injury in Mice by Mesenchymal Stem Cells Overexpressing Angiopoietin 1

Shirley H. J. Mei¹,³, Sarah D. McCarter¹, Yupu Deng¹, Colleen H. Parker¹, W. Conrad Liles²,³,⁴,⁵, Duncan J. Stewart¹,³,⁴,⁵*
The Tie2-agonist Vasculotide rescues mice from influenza virus infection


B

X31 influenza (H3N2)

Survival (%)

Days

Control

Flu

Flu/VT0

Flu/VT24

Flu/VT48

Flu/VT72

Flu/VT96

C

Oxygen saturation (%)

Control

Flu

Flu/VT48

D

Wet/dry ratio

Control

Flu

Flu/VT0

E

Ejection fraction

Control

Flu

Flu/VT48

F

PR8 influenza (H1N1)

Survival (%)

Days

Control

Flu

Flu/VT48

Flu (6)
Thank You

Questions