Genetic Insights to Acute Lung Injury Pathogenesis

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• Personal financial relationships
  – None

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Figure 1. Queen Victoria's family tree.
A Case for Heritability

Moss and Mannino Crit Care Med 2002;30:1679; Sorensen TI NEJM 1988;318:727
A Case for Heritability

- No family pedigrees of ALI
- Discordant ALI, sepsis outcomes by ethnicity

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A Case for Heritability

• No family pedigrees of ALI
• Discordant ALI, sepsis outcomes by ethnicity
• Strong evidence for heritability in response to infection
• Premature death from infection: Inherited Risk
  – RR 5.81 (2.47 – 13.7) if parent died before age 50
  – Significantly larger than for vascular disease, cancer

Moss and Mannino Crit Care Med 2002;30:1679; Sorensen TI NEJM 1988;318:727
Evolutionary Pressure on Injury Response
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- Sickle Cell Disease: heterozygote advantage
- Duffy antigen/DARC: CXCL receptor
Evolutionary Pressure on Injury Response

- Sickle Cell Disease: heterozygote advantage
- Duffy antigen/DARC: CXCL receptor
- Complement biology: Macular degeneration
  - CFH, BF, C2, C3
- Caspase 12: African private SNP → longer, less active proenzyme, ↑ Sepsis susceptibility, mortality
ALI as a Complex Genetic Trait
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- Genetic predisposition, Environmental stress
- Few validated ALI genetic risk variants: SFTPB, IL-6, SOD3, ACE, MBL2, IL10, VEGF, FAS, MYLK, PBEF, ANGPT2
- Difficult to validate
  - Candidate gene selection
  - Effect size
  - Heterogeneity of ALI
  - Variable control populations
  - Population structure / racial admixture
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Genome Wide Investigations Limited by Power and Heterogeneity

• Trauma ALI SNP Consortium (TASC)

• Identification of SNPs Predisposing to Altered ALI Risk Acute (iSPAAAR)

Christie JD PLoS One 2011 in press; Wurfel MM AJRCCM 2011 183; A5535
Genome-Wide Significance ($5 \times 10^{-8}$), strong LD signal
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Does not meet classical GWA significance; candidate for further study
Genome Wide Investigations Limited by Power and Heterogeneity

- Trauma ALI SNP Consortium (TASC)
  - 600 ALI Cases, 2200 Healthy controls
  - Strongest replicated associations $p \sim 1.5 \times 10^{-7}$

- Identification of SNPs Predisposing to Altered ALI Risk Acute (iSPAAAR)
  - Phase I: ALI DNA (1184) vs at-risk controls (1246)
  - Strongest associations: $p \sim 2 \times 10^{-7}$

Christie JD PLoS One 2011 in press; Wurfel MM AJRCCM 2011 183; A5535
Candidate Gene Approach

• Maximize power by limiting number of tests
  – Candidate Gene Chip: “IBC Chip”
  – ~50,000 SNPs in ~2000 Genes
• Diminish Heterogeneity
  – Single at-risk inciting event for discovery (Trauma)
  – Cohort study with at-risk controls
• Less conservative Discovery stage p-value
  – $p < 5 \times 10^{-4}$
ALI Candidate Gene 1: ANGPT2

<table>
<thead>
<tr>
<th>SNP</th>
<th>Stage I OR (95% CI)</th>
<th>Stage I p-value</th>
<th>Stage II OR (95% CI)</th>
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<tbody>
<tr>
<td>rs1868554</td>
<td>2.60 (1.66 – 4.09)</td>
<td>3.34E-05</td>
<td>1.22 (1.06 – 1.40)</td>
<td>0.017</td>
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<tr>
<td>rs2442598</td>
<td>2.73 (1.71 – 4.35)</td>
<td>2.52E-05</td>
<td>1.16 (1.01 – 1.33)</td>
<td>0.038</td>
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Discovery in African ancestry, Replication in European ancestry

Common variant: rs1868554 minor allele frequency 0.27
Association of chromosome 8 with trauma – associated ALI: Stage I

ANGPT2: chromosome 8, positions 6357K – 6424K

- rs1868554: p = 3.68e-05
- rs2442598: p = 2.54e-05

Mini-Manhattan plot: Association (y axis) vs Genomic locus (x axis)

Meyer NJ AJRCCM 2011
Association of chromosome 8 with trauma – associated ALI

ANGPT2: chromosome 8, positions 6360K – 6400K

rs1868554
p=0.0083

rs7825407
p=0.0019

Consistent genomic region associated with ALI: adjacent to the variably spliced 2\textsuperscript{nd} exon
Huang H Nat Rev Cancer 2010
ANGPT–TIE Axis in ALI and Sepsis

Parikh PLoS Med; Bhandari Nat Med; McCarter AJRCCM; Mei PLoS Med; Gallagher Shock; Fremont J Trauma 2010; Fang J Biol Chem 2010
ANGPT–TIE Axis in ALI and Sepsis

- Exogenous ANG2 disrupts endothelium
- Rescued by ANG1
- ANGPT2−/− mice protected from inhaled LPS

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Exogenous ANG2 disrupts endothelium
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WT mice given ANG2 → lung injury
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- ANG1 contributes to alveolar fluid clearance in MSC cultured medium

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## Candidate Gene 2: IL1RN

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<th>Stage III: Mixed ICU Cohort OR (95% CI)</th>
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<td>rs315952 syn-coding</td>
<td>0.37 (0.22 – 0.95) p = 0.00019</td>
<td>0.67 (0.52 – 0.88) p = 0.0023</td>
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- 2 IL1RN SNPs in linkage disequilibrium associate with decreased risk of ALI in 3 ICU populations
• Meta-analysis of > 7000 ambulatory subjects
  – 1000 MI survivors
• rs315952 associated with IL1RA level (pQTL)
  – p = 1.5 x 10^{-11}
  – Explained the largest genetic variance of IL1RA among MI survivors (~5% variance)
• rs315952 associates with mRNA IL1RA
Implications
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- Subset of patients whose ALI predisposition may be mechanistically tied to ANGPT2
  - Candidates for anti-ANG2 / pro-ANG1 therapy?
- Subpopulation of critically ill patients who are protected from ALI by virtue of IL1RN genotype (potentially through increased IL1RA?)
  - Highlights role of IL1RA homeostasis in ALI development
  - May resurrect the potential of rhIL1RA therapy
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