Heterogeneity in the sepsis response

LAB MEETING - 5TH MAY 2015
KATIE BURNHAM
Background

SEPSIS AND THE GAINS STUDY
Sepsis

**Sepsis**: systemic inflammatory response following infection

**Treatment options?**

**Clinical Impact**: 46% all ICU bed days in the UK

28-50% mortality
Immune Dysregulation

Xiao et al., 2011
Heterogeneity in Sepsis

**Clinical Heterogeneity:** Poorly understood
Stratify patients

**Origin of sepsis:** Underlying infections/site of infection

**Genetic factors:** susceptibility, outcome

Buras et al 2005
Heterogeneity in Sepsis

Clinical Heterogeneity: Poorly understood
Stratify patients

Origin of sepsis: Underlying infections/site of infection

Genetic factors: susceptibility, outcome

Candidate gene studies: e.g. TLRs, NOD2, IRAK1
TNF, IL6

Figure 1. Probability of Dying before a Given Age from Causes (Panel A) or from Infection (Panel B) for Adopted Least One Biologic Parent Who Died from the Same Causes before the Age of 50 (Parent Dead) and for Whose Biologic Parents Were Both Alive at That (Parents Alive).

Sorensen et al 1988
Genomic Advances in Sepsis (GAinS)

Impact of genetics: susceptibility and outcome

Previous studies: limited power, heterogeneous cohorts, confounding effects

Aims: large collection for adequately powered genomics research
   Well-characterised homogeneous cohort
   GWAS, functional studies
   New insights, targets, tailored therapy
Genomic Advances in Sepsis (GAInS)

**CAP**: Community Acquired Pneumonia

**FP**: Faecal Peritonitis

**34 collection sites – now 7**

Clinical Information

- **Day 1, 3 & 5 (if possible):**
  - Buffy coat: DNA
  - Blood samples x2
  - LeukoLock filter: RNA
  - Urine x2
  - Plasma x4
Genome-wide association study of survival from sepsis due to pneumonia: an observational cohort study

# Sepsis Cohorts

<table>
<thead>
<tr>
<th>GAinS: Radhakrishnan</th>
<th>Patients</th>
<th>Samples</th>
<th>Data type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>73 CAP</td>
<td>127 CAP</td>
<td>Microarray</td>
</tr>
<tr>
<td></td>
<td>64 FP</td>
<td>94 FP</td>
<td></td>
</tr>
<tr>
<td>GAinS: Davenport</td>
<td>265 CAP</td>
<td>265 CAP</td>
<td>Microarray</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Genotyping</td>
</tr>
<tr>
<td>GAinS: Burnham</td>
<td>106 CAP</td>
<td>106 CAP</td>
<td>Microarray</td>
</tr>
<tr>
<td></td>
<td>54 FP</td>
<td>54 FP</td>
<td></td>
</tr>
</tbody>
</table>
Sepsis due to CAP

REGULATORY VARIANTS AND SEPSIS RESPONSE STATE
eQTL analysis

Resolve variants that modulate immune response

Context specificity of regulation

Innate Immune Activity Conditions the Effect of Regulatory Variants upon Monocyte Gene Expression

Benjamin P. Fairfax,1* Peter Humburg,2† Seiko Makino,2† Vivek Naranbhai,1 Daniel Wong,1 Evelyn Lau,1 Luke Jostins,1 Katharine Plant,1 Robert Andrews,2 Chris McGee,2 Julian C. Knight1†
eQTL analysis

644,390 SNPs

Cis: 17,347 probes

First 30 PCs

3,795 genes FDR<0.05
Pathway Analysis

Peak cis eQTL FDR<0.01 analysed with IPA

Enriched for disease relevant canonical pathways

- antigen presentation
- mitochondrial dysfunction
- PI3K signalling in B lymphocytes
Pathway Analysis

Peak cis eQTL FDR<0.01 analysed with IPA

Upstream regulators are key mediators of immune response
- FAS: cell surface death receptor
- HNF4A: transcription factor
- IFN\(\gamma\)
- TNF
- TP53: apoptotic regulator
- BID: cell death regulator
Sepsis-specific eQTLs

Westra et al

Whole blood eQTL

1,377 context specific (36.3%)
Sepsis-specific eQTLs

Westra et al
Whole blood eQTL
1,377 context specific (36.3%)
Sepsis-specific eQTLs

Disease relevance
- viral respiratory infections
- viral infection
- cellular immune response
Genomic context of eQTLs

Distance to transcriptional start site
Mann-Whitney test: $p=9.4 \times 10^{-38}$

Sepsis-specific more distal
Genomic context of eQTLs

Overlap with epigenetic marks

BLUEPRINT data: LPS-stimulated monocytes

Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity

Sepsis due to CAP

REGULATORY VARIANTS AND SEPSIS RESPONSE STATE
FactoMineR based on 10% most variable probes in dataset (2,619)

- SRS 1 = 108 patients
- SRS 2 = 157 patients
Clinical Relevance?

<table>
<thead>
<tr>
<th>Patient characteristic</th>
<th>Cohort</th>
<th>Cluster 1</th>
<th>Cluster 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>265</td>
<td>108</td>
<td>157</td>
</tr>
<tr>
<td>Mean age (± SD)</td>
<td>62 years (± 16)</td>
<td>60 years (± 15)</td>
<td>63 years (± 17)</td>
</tr>
<tr>
<td>Male</td>
<td>55%</td>
<td>56%</td>
<td>54%</td>
</tr>
<tr>
<td>Median APACHE II score (range)</td>
<td>18 (6-42)</td>
<td>18 (6-42)</td>
<td>17 (6-34)</td>
</tr>
<tr>
<td>Median SOFA score (range)</td>
<td>6 (0-21)</td>
<td>6 (1-21)</td>
<td>5 (0-14)</td>
</tr>
</tbody>
</table>
Association with Mortality

\[ p = 0.032 \]
Differential Gene Expression
Pathway Analysis

- T cell activation
- Cell death: apoptosis, necrosis
- Cytotoxicity
- Phagocyte movement
- Inflammatory response
- Bacterial infection
- Viral respiratory infection
- Severe acute respiratory syndrome
Endotoxin Tolerance

Pena et al 2014
ROAST

Overlap in same direction: $p=1 \times 10^{-5}$

ROAST: rotation gene set tests for complex microarray experiments

Di Wu$^{1,2}$, Elgene Lim$^{1}$, François Vaillant$^{1}$, Marie-Liesse Asselin-Labat$^{1}$, Jane E. Visvader$^{1,2}$ and Gordon K. Smyth$^{1,2,\ast}$

$^{1}$The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria 3052 and $^{2}$The University of Melbourne, Victoria 3010, Australia

Associate Editor: Jonathan Wren
Comparison to Clusters

PBMCs from healthy volunteers

- No treatment
- LPS

Primary LPS response

Tolerant LPS response

LPS

398 genes

Comparison to Clusters

3045 genes

p = 4.36 \times 10^{-15}
Comparison to Clusters

- PBMCs from healthy volunteers
  - No treatment
  - LPS

Primary LPS response
Tolerant LPS response

398 genes
3080 genes
Comparison to Clusters

PBMCs from healthy volunteers

- No treatment
- LPS

Primary LPS response

- LPS
- Tolerant LPS response

398 genes

ET

- 208 genes
- 123 genes

2118 genes

p<1x10^{-5}

3080 genes
SRS Prediction
Validation Dataset

Age and sex matched survivors and non-survivors

106 CAP Patients

Using 7 gene prediction model:
- Cluster 1 = 37 patients
- Cluster 2 = 69 patients
Association with Mortality

p=0.032

p=0.0036
Basis of Clusters?

Genetically predetermined difference in response?

Capture of different point in disease progression?

SRS2 Variants

- Leukocyte activation
  - Cytokines
  - Proteases
  - Reactive oxygen species
  - NETs
- Complement activation
- Coagulation activation
- Necrotic cell death

SRS1 Variants

- Septic response
- Impaired function of immune cells
  - Apoptosis of T, B and DCs
  - Expansion of Tregs and myeloid suppressor cells
  - Impaired phagocytosis
- Neuroendocrine regulation
- Inhibition of proinflammatory gene transcription
  - Anti-inflammatory cytokines
  - Soluble cytokine receptors
  - Negative regulators of TLR signaling
  - Epigenetic regulation

Proinflammatory response
Excessive inflammation causing collateral damage (tissue injury)

Imune suppression
Enhanced susceptibility for secondary infections and late mortality
Sepsis Response State and eQTLs

2,699 DE genes with cis eQTL

SIRT1 histone deacetylase

Hypoxia network
- HIF1A + EPAS1
- Modulates glycolysis genes
- metabolic switch
Sepsis Response State and eQTLs

eQTL analysis for each group separately

25 PCs

FDR<0.01 & >0.05

124 SRS1

766 SRS2
Genome-wide association for SRS

Restricted SNP set:
- Genes DE between clusters
- Evidence of an eQTL in sepsis or
- Evidence of an eQTL with LPS stimulation
- $r^2$ threshold of 0.1
- 1620 SNPs
Multiple SNP model

TRIM44: enhances viral response
LAX1: negative regulator of T cell activation
DDX24: modulates IRF7 activity
VAMP4: required for granule exocytosis in NK cells
Testing the model

\[ D^2 = (\text{Null deviance} - \text{Residual deviance}) \]

Null deviance

\[ D^2 = 0.232 \]

Adjusted deviance

\[ \text{Adj. } D^2 = 1 - \frac{(n-1)}{[1 - D^2]} \]

\[ (n-p) \]

\[ \text{Adj. } D^2 = 0.199 \]
Testing the model

\[ D^2 = (\text{Null deviance} - \text{Residual deviance}) \]

Null deviance

\[ D^2 = 0.232 \]

Adj. \[ D^2 = 1 - \frac{(n-1)[1 - D^2]}{(n-p)} \]

Adj. \[ D^2 = 0.199 \]
CAP vs FP
Clusters in FP?

Same approach as in CAP derivation cohort
148 samples from Radhakrishan and Burnham cohorts

Include serial samples from same patients

\[ p = 0.0458 \]
Clusters in FP?

p=0.751
HETEROGENEITY IN THE SEPSIS RESPONSE

- Genetic Background
- Changes over time
- Causative Infection
- Previous exposures and environment
- Epigenetics
Acknowledgements

**Wellcome Trust Centre for Human Genetics**

Emma Davenport
Julian Knight
Jayachandran Radhakrishnan
Peter Humbug
The Knight lab

**Barts and the London**

Charles Hinds

**The GAinS Collaborators**

The research nurses
The patients

**John Radcliffe Hospital**

Paula Hutton
Thank you!