The Biology of Fatigue: Omics in Human Disease

Definition
• Acquired feeling of overly tired, with low energy, and a strong desire to sleep that interferes with normal daily activities

Scope
• Acute or chronic

Causes
• Underlying disease – e.g., cancer, COPD, heart failure
• No underlying disease – e.g., sleep deprivation, heavy mental or physical exertion, “jet lag”, aging

Forms in which the disease manifests
• Physical – physically hard to do something, e.g., aging
• Mental – lack of motivation, e.g., depression
• Trained – form from the knowledge that if they exceed their tolerance, there will be consequences (e.g., exacerbation of symptoms or “crash”)

Chronic Fatigue
• Physical initially unrelated to deconditioning, but later confounded by deconditioning
• Pathophysiological mechanisms
• Stress initiated
• Disappointment or secondary depression
Clinical Study Features

• From a molecular perspective ("omics"), how does the human respond to an injury or stress that carries a 10 – 20% likelihood of death within days?

• This was a 10-year study involving > 100 NIH-funded investigators initially at 22 major academic centers with $100M support.

• To study inflammation in humans by monitoring multi-level information (genomics, proteomics, cell function, physiological and clinical) of injured patients in intensive care units.
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Generated by 20 years of team science focused on studying critically ill patients though public funding (U54 GM062119, R24 GM102656, R01 GM101401, R01 GM104481, and DARPA N66001-17-2-4035)

- Clinical information
  - Time course clinical, physiologic, outcomes data (~1,200 distinct fields)
  - 978 sampling patients, 1,633 additional epidemiology, 10 LPS, 199 controls

- Patient samples
  - 76,768 samples (blood, leukocytes, plasma, tissue, RNA, etc.)
  - samples from additional 160 patients as an independent validation cohort

- Time course genomics
  - Gene expression profiles of 2,518 WBC, muscle, fat and skin samples
  - Gene, exon, coding SNP profiles of 2,692 samples of T cells, monocytes and neutrophils

- Time course proteomics (flow cytometry and LC/MS)
  - MO and T-cell flow cytometry panels from 3,810 samples
  - High throughput mass spec proteomics data from 2,749 isolated MO, T-cell, and N samples
  - Plasma cytokine profile of 1,112 samples

- Comparisons with murine models
  - In vivo murine models of trauma, burns, and LPS
  - Time course gene expression profiles of WBC, T cells, monocytes and neutrophils of the murine models
  - Time course cytokine profiles of the murine models
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**ENDOTOXEMIA**
- Whole Blood
- Buffey Coat
- 2 ng/Kg LPS
- 10 Healthy Volunteers
- Affymetrix U133-2 plus

**MASSIVE BLUNT TRAUMA**
- Whole Blood
- Buffey Coat
- T-Cells
- Neutrophils
- 204 Trauma Patients
- 290 Trauma Patients
- Affymetrix U133-2 plus
- Affymetrix Human Transcriptome Array (HTA)

**BURN INJURY**
- Whole Blood
- Buffey Coat
- Muscle, Fat, Skin
- 484 Burns Patients
- 484 Burn patients with blood sampling
- 118 Burn epidemiology only

Total trauma and burns patients and healthy volunteers:
- 199 Healthy Controls for blood and other tissues
- 10 Healthy students with endotoxemia
- 494 Trauma with blood sampling
- 1515 Trauma epidemiology only
- 2009 Total Trauma Patients
- 484 Burn patients with blood sampling
- 118 Burn epidemiology only
- 602 Total Burn Patients
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Gene MMP8 in circulating leukocytes as an example
Findings – “A Genomic Storm”

- Using a false discovery rate (FDR) adjusted probability of < 0.001, 16,820 out of 20,720 ENTREZ genes were significantly different in the 167 trauma subjects when compared to the 37 control subjects, using EDGE or ANOVA, or >80% of the human genome.

- When the <0.001 false discovery was combined with a 1.5-fold change requirement, the number of genes that changed, declined to 10,001.

- Using a <0.001 false discovery rate and a 2.0-fold change requirement, the number of genes that were different between healthy control subjects and the trauma patients was 5,136.
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A. Effect of Severe Blunt Injury on Probe Expression

B. Up-regulated Pathways
- Integrin signaling
- Leukocyte extravasation
- FcR Receptor mediated phagocytosis
- IL-10 signaling
- Toll-like receptor signaling
- Ephrin Receptor signaling
- IL-6 signaling
- TREM1 signaling
- Actin Cytoskeleton signaling
- B cell receptor signaling

C. Down-regulated Pathways
- Ca²⁺ T cell apoptosis
- iCOS-iCOSL signaling in T cells
- CTLA4 signaling in CD8 T cells
- CD28 signaling in T cells
- T cell receptor signaling
- CD8 T cell mediated apoptosis
- Role of NFAT in immune response
- IL-4 signaling
- Primary immunodeficiency signaling
- Purine Metabolism

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Time course studies of 167 patients up to 28 days after severe blunt trauma and 244 patients up to 1 year after burn injury.

A

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<th>Significant Genes</th>
<th>Burn</th>
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<td>Trauma</td>
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B

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Genomic Response are Similar in Patients of Different Severe Acute Inflammatory Diseases
Transcriptome Changes after Burns are Tissue Specific

- **Fat**: 2181 genes up-regulated and 1012 down-regulated after burns.
- **Muscle**: 1297 genes up-regulated and 2058 genes down-regulated after burns.
• Genes that are regulated can be tissue specific
• Gene perturbations can persist (e.g., months, years, lifetime)
• At the tissue level, never return to pre-injury state
Skeletal Muscle Dysfunction: 95 skeletal muscle samples using RNA-seq systems by NovoGene

There were two studies of healthy aging subjects:

- 2 weeks bedrest [younger (18-30 years) vs. older (55-65 years)]
  - In the older cohort with short term bedrest, the older subjects had tremendous genomic response compared to the younger cohort
  - The pathways involved were consistent with immobility
  - Two weeks of physical rehabilitation reversed these genomics

- 2 months bedrest (young 34 ± 1.8 years)
  - In younger subjects, a comparable genomic response mirrored those changes seen in the two-week study in the older cohort
  - These genomic changes were consistent with those seen after two weeks of bedrest in the older cohort

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<th>Age Group</th>
<th>Genes Upregulated</th>
<th>Genes Downregulated</th>
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<tr>
<td>Old (55-65 yrs)</td>
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<tr>
<td>Young (18-30 years)</td>
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Multi-omics iCPET Exercise Intolerance:

- Two plasma components (radial and pulmonary arterial): (1) inflammasome (cytokine response to exercise), (2) metabolome by Metabolon
  - The inflammasome has shown an enhanced pro-inflammatory cytokine response in ME/CFS patients.
  - There appears to be a further exaggeration of this response within the oxygenated arterial system.

Heart Failure in ME/CFS:

- Two forms of heart failure identified in ME/CFS patients: preload failure and poor oxygen extraction
  - Preload failure consistently shows a reduced aerobic capacity along with a reduced right atrial pressure (RAP) – low flow form with potential autonomic dysregulation.
  - The poor oxygen extraction patients routinely also show a reduced aerobic capacity and unexpectedly high pO2 in the mixed venous blood (pav O2) – high flow form with either mitochondrial oxidation or peripheral shunting dysfunctions.
  - In these preload failure patients, the metabolomic response to exercise appears comparable or potentially exaggerated in the ME/CFS patients depending upon the computational approach.
Summary & Conclusions

• Many forms of fatigue (i.e., mental and physical)
• A form “trained” not simply mental but also physical (e.g., chronic fatigue, Long-COVID)
• Acquired post-stress from infections, injuries, ...
• At least 80% of the human genome can be perturbed after massive stress (up to 20 – 30% in individual tissues)
• Genomics are comparable whether initiated by injuries, infections, and other insults (e.g., bedrest)
• Study of “omics” has allowed discovery of forms of preload heart failure without primary heart or lung parameters being abnormal

Chronic Fatigue

• Stress initiated with “omics” failure to recover fully in each and every tissue (i.e., CNS...)