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

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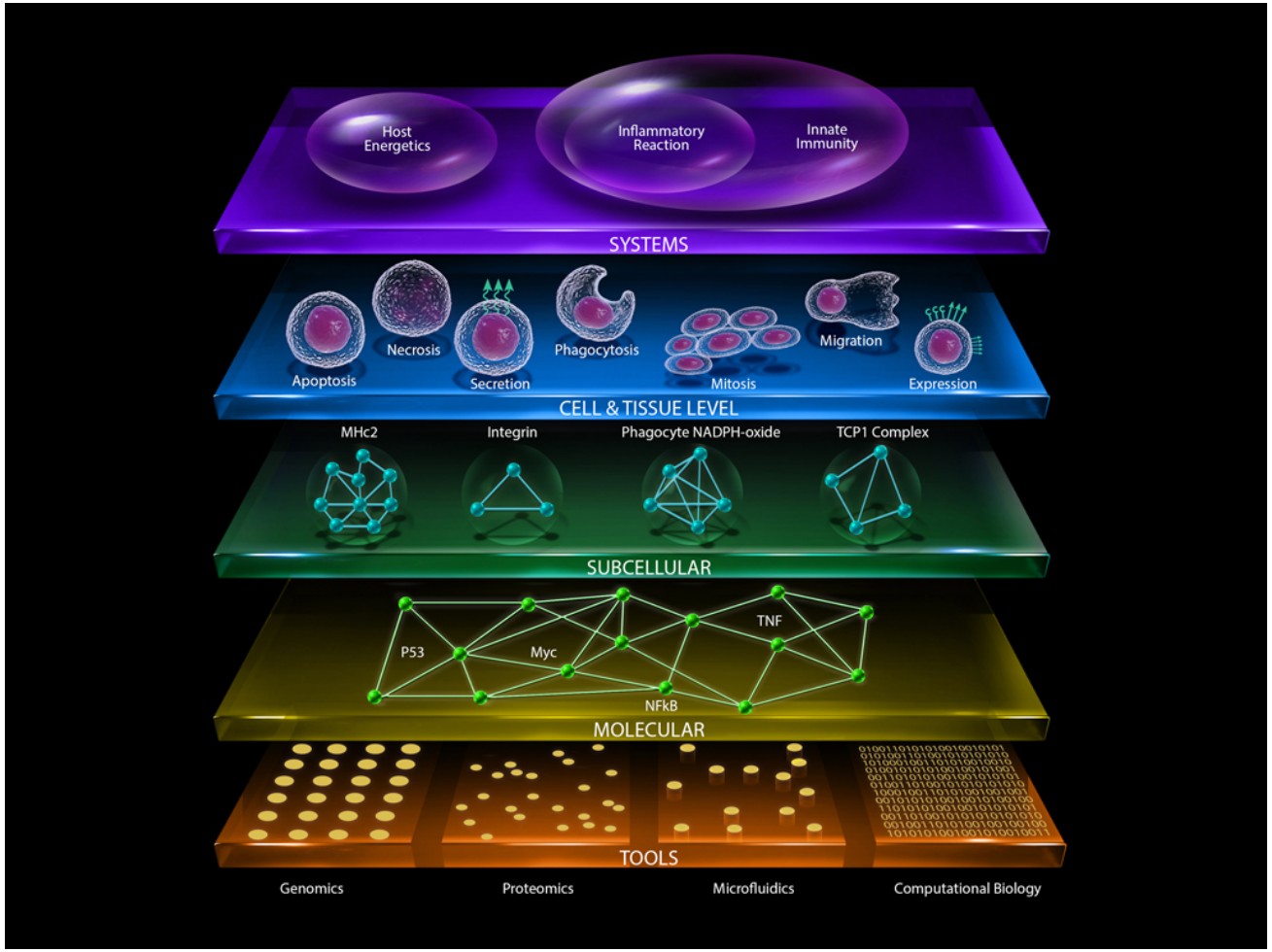
Clinical Study Features

- 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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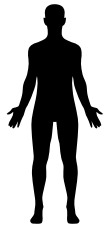
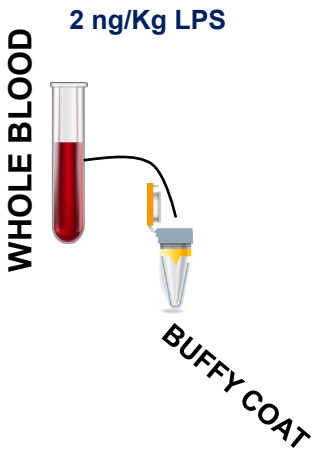
ENDOTOXEMIA

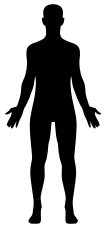


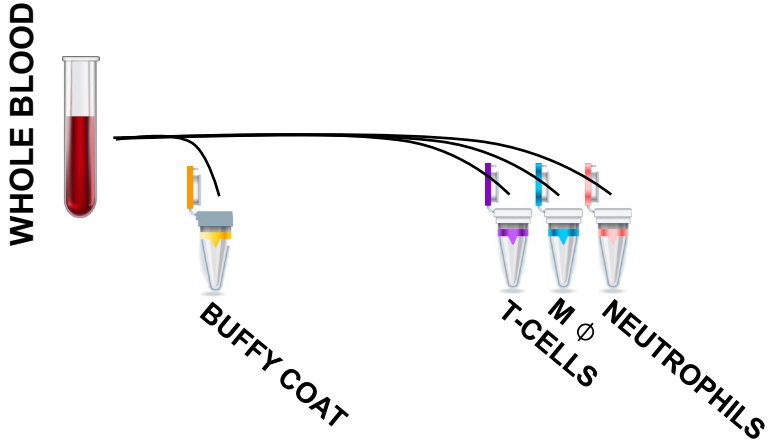


10 Healthy Volunteers

Affymetrix U133- 2 plus

MASSIVE BLUNT TRAUMA



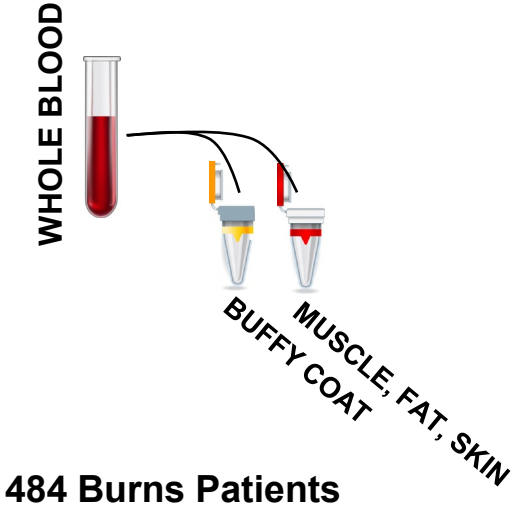


204 Trauma Patients **290 Trauma Patients**

Affymetrix U133- 2 plus

Affymetrix Human Transcriptome Array (HTA)

BURN INJURY



484 Burns Patients

Affymetrix U133- 2 plus

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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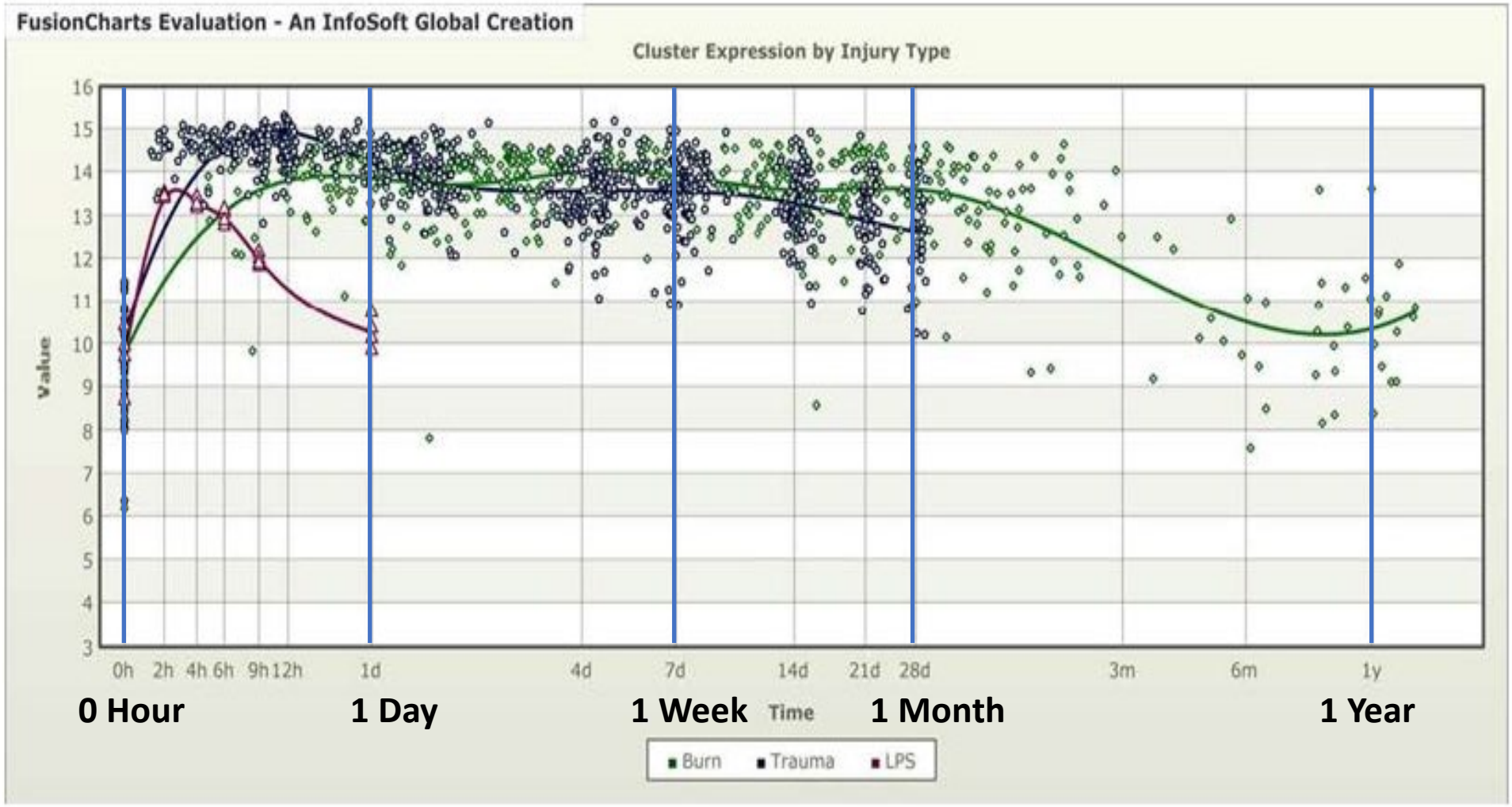
Gene MMP8 in circulating leukocytes as an example



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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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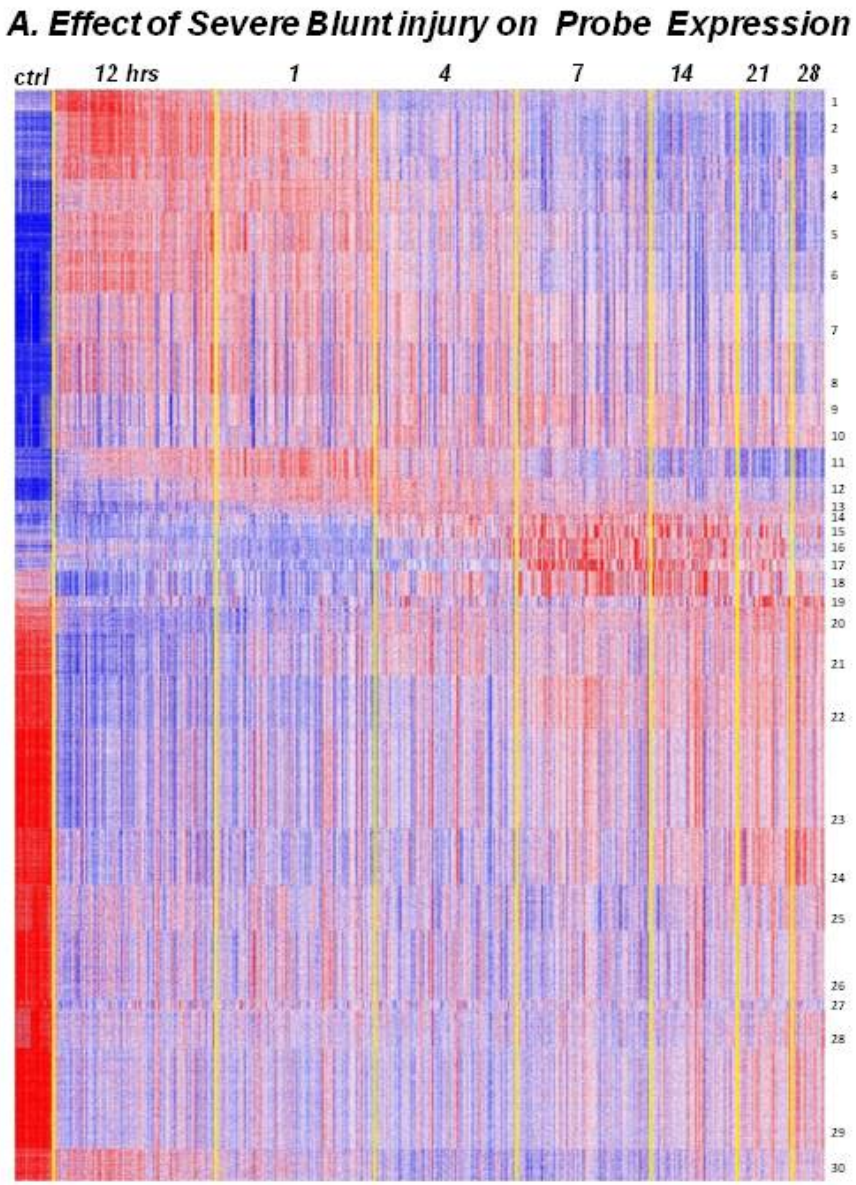

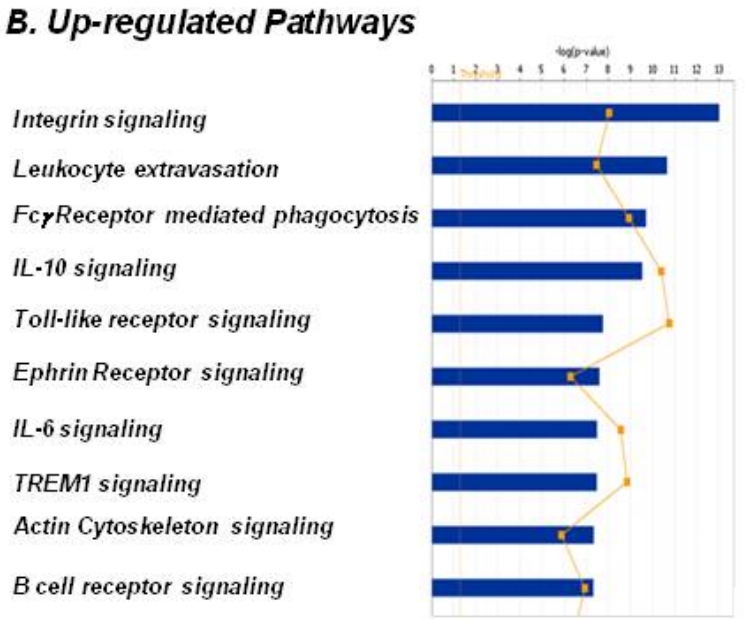

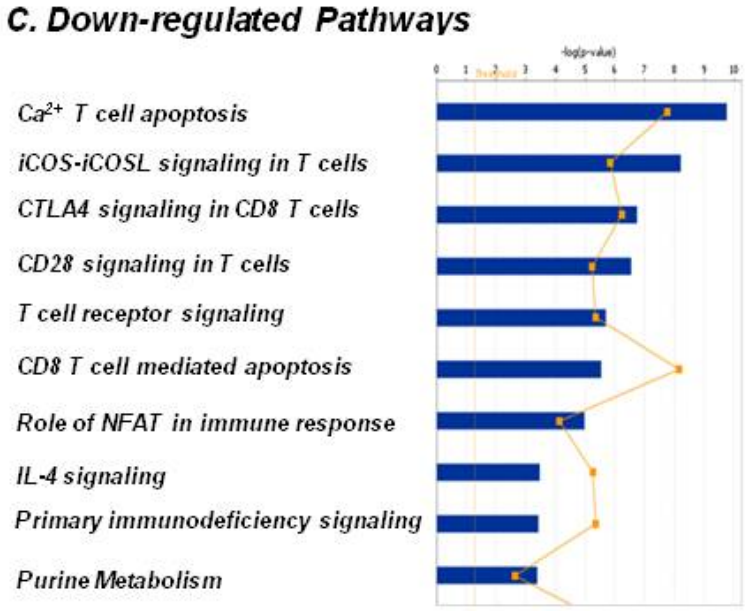

Xiao et al., J Exp Med. 2011.

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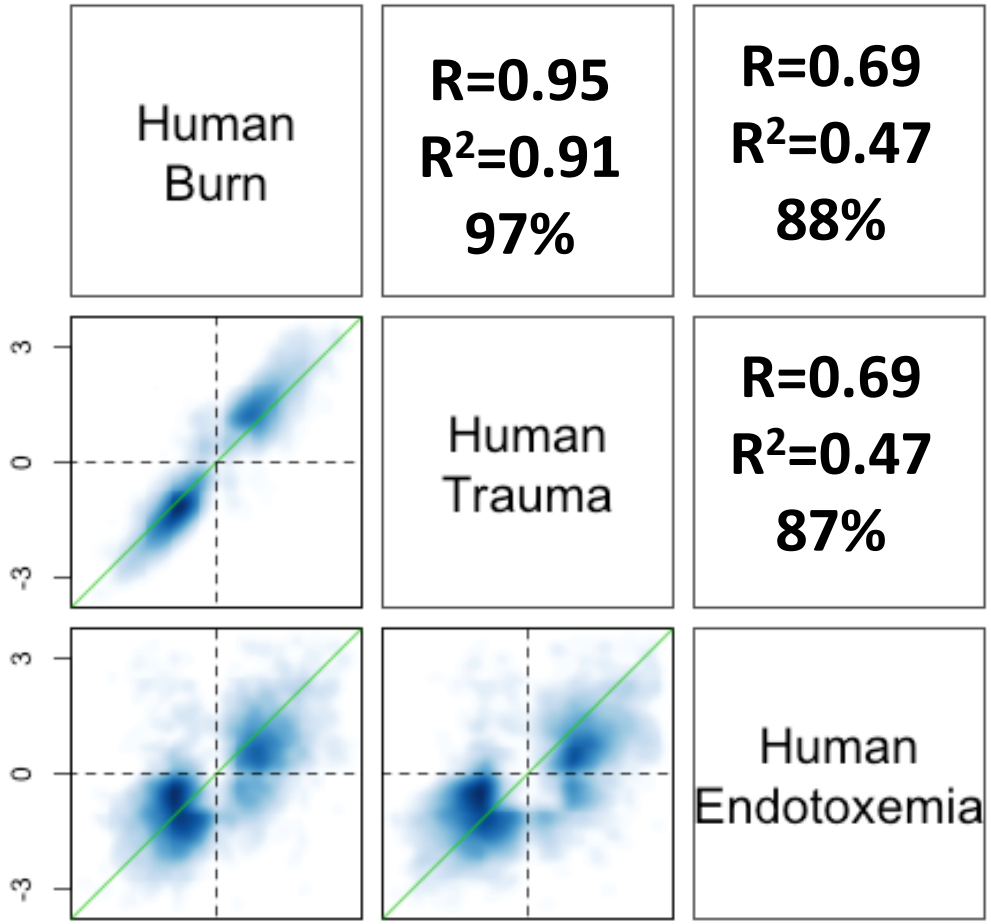
Time course studies of 167 patients up to 28 days after severe blunt trauma and 244 patients up to 1 year after burn injury.



A

Significant Genes		Burn	
		Up	Down
Trauma	Up	2,066	19
	Down	9	3,042

B

Significant Genes		LPS	
		Up	Down
Trauma	Up	1,649	436
	Down	177	2,874

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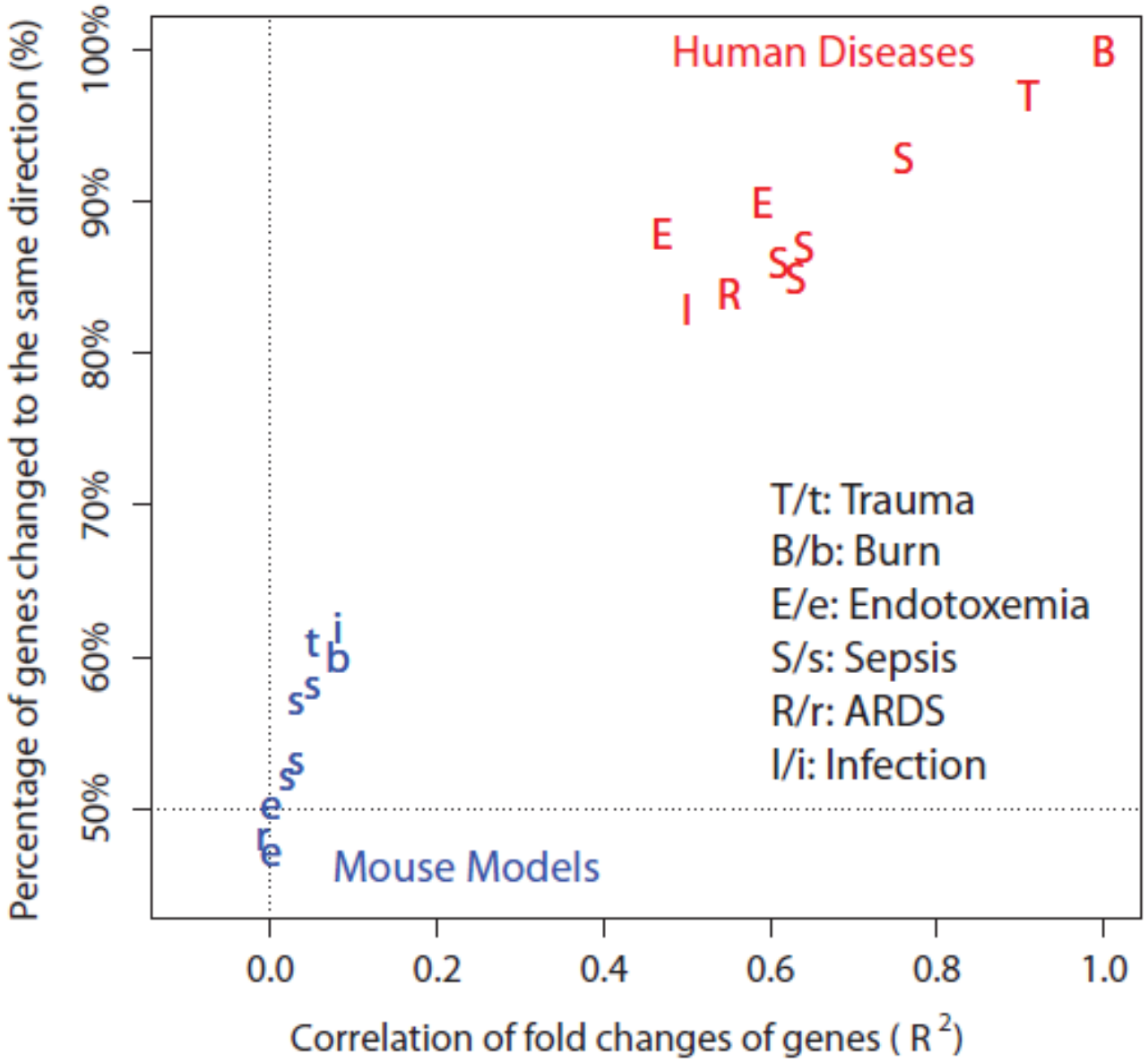
Genomic Response are Similar in Patients of Different Severe Acute Inflammatory Diseases



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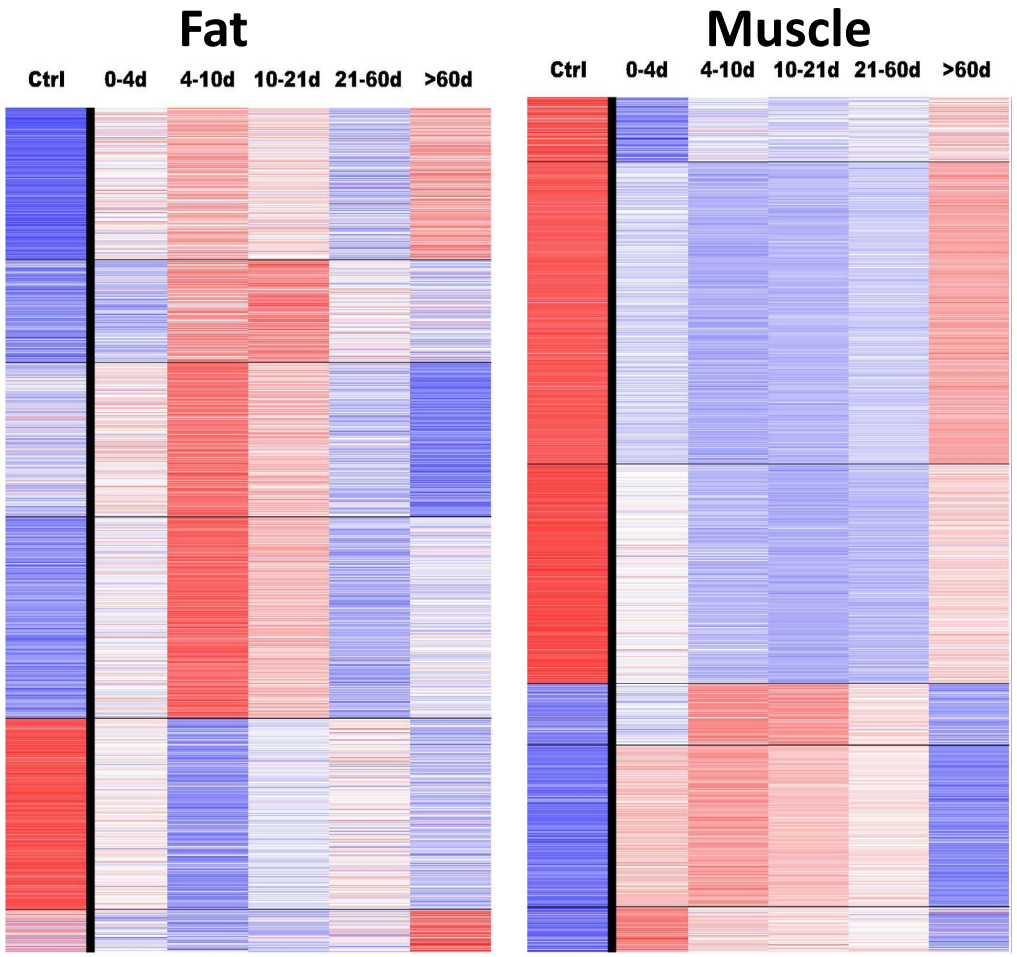
Transcriptome Changes after Burns are Tissue Specific

2181 genes up-regulated and 1012 down-regulated after burns.



1297 genes up-regulated and 2058 genes down-regulated after burns

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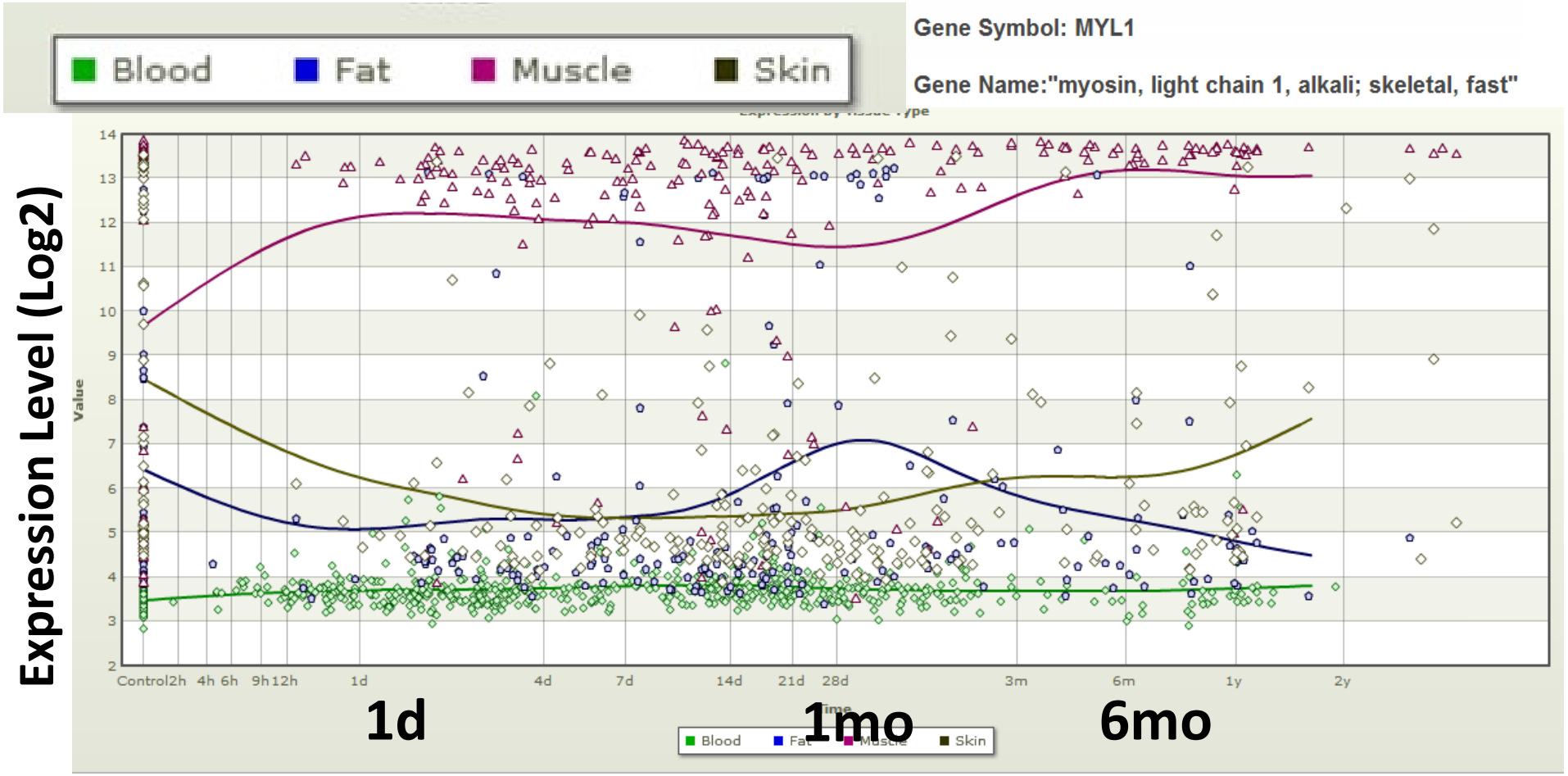

- Genes that are regulated can be tissue specific
- Gene perturbations can persist (e.g., months, years, lifetime)
- At the tissue level, never return to pre-injury state

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Skeletal Muscle Dysfunction: 95 skeletal muscle samples using RNA-seq systems by NovoGene

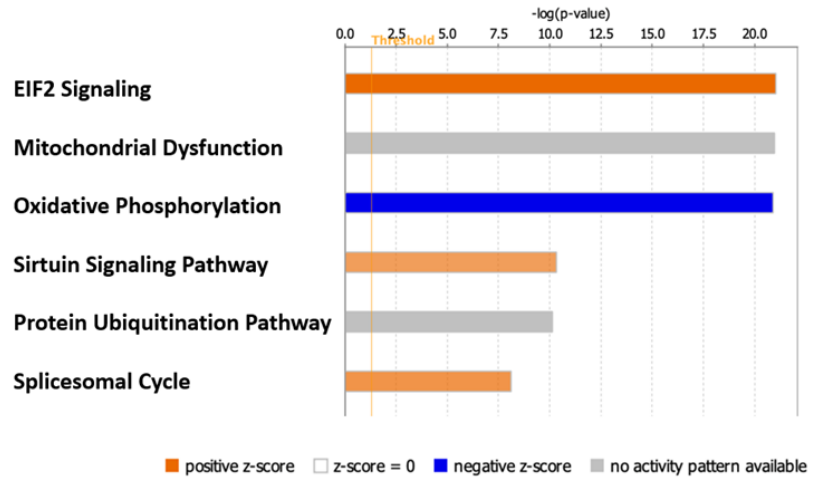
There were two studies of healthy aging subjects:

- 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

Table . Numbers of Genes Significantly Altered in Expression Levels in the Muscle after Bedrest

Age Group	Genes Upregulated	Genes Downregulated
Old (55-65 yrs)	4,053	285
Young (18-30 years)	5	16



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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.

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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…)