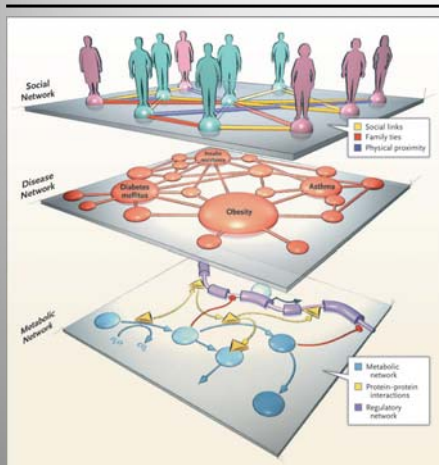*From Cytokines to Cells to Gene Expression: An Integrative Approach to the Study of Gulf War illness*

Gordon Broderick, Ph.D.
Associate Professor, Dept. of Medicine,
University of Alberta

Nancy Klimas, M.D., Miami Veterans Affairs Medical Center
Mary Ann Fletcher, Ph.D., University of Miami
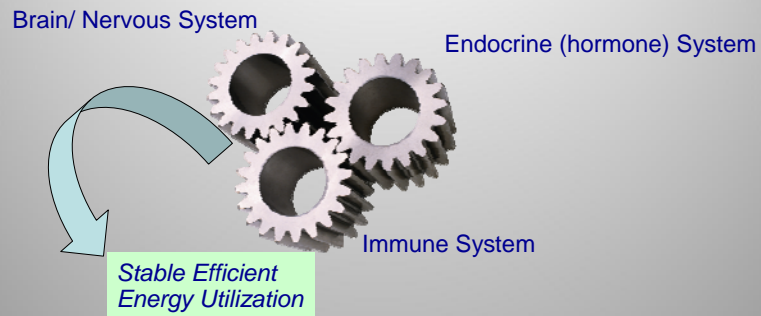Sol Efroni, Ph.D., Bar Ilan University

Systems Biology to Systems Medicine

- Key: **Integration and context**
  - Integration <u>and reconciliation</u> of *types of data*
  - Integration of *components* within /across <u>*functional levels*</u>.
- **Why?** <u>Context-sensitive environment</u>

Barabási A.-L. Network Medicine — From Obesity to the "Diseasome". N Engl J Med 2007; 357:4

Not Just a Collection of Parts

Tightly integrated and highly interactive components

Brain/ Nervous System

Endocrine (hormone) System

Immune System

Stable Efficient Energy Utilization

Basic premise: Illnesses will differ not only in the expression of markers but in their patterns of association or interaction.
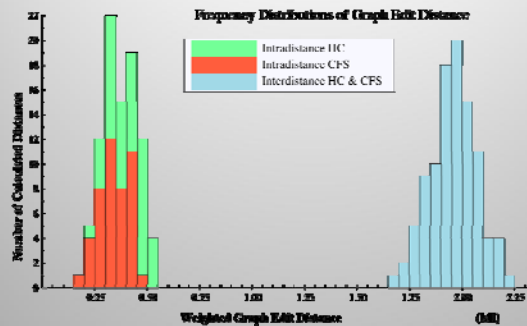
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Comparing Two Networks

Low   High

Network edit distance

(D)
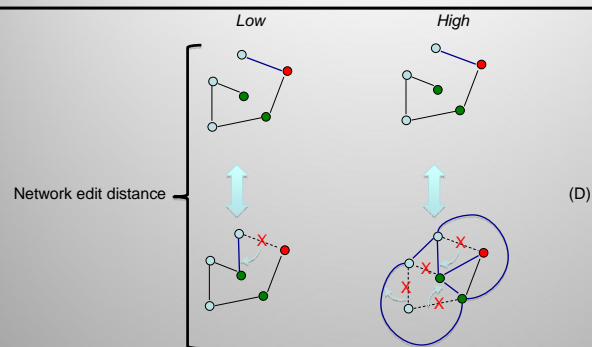
Compare based on changes needed to transform one into the other… Can do this at multiple scales
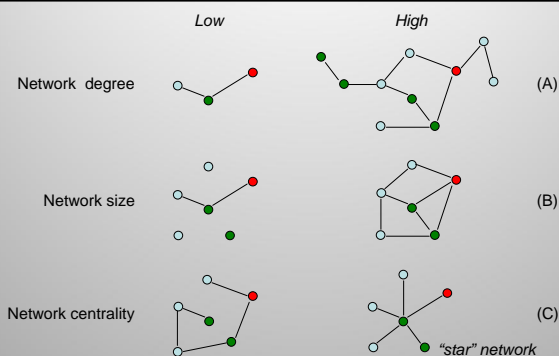
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Comparing several networks

- *Cytokine networks* differ significantly in _distribution_ and strength of associations D_{edit} =1.96
- >10 std dev. over separation of networks from randomly sampled HC subjects (D_{edit} ~0.18)
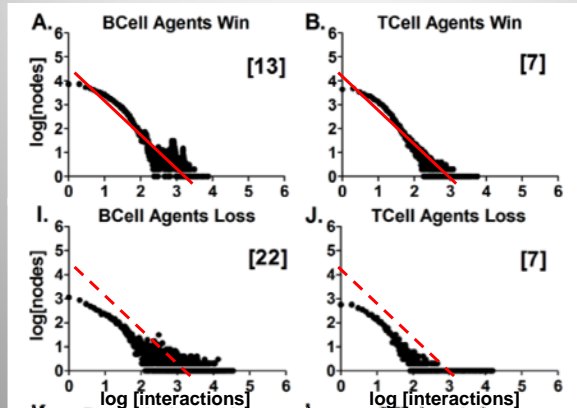
Describing Basic Network Structure

Others include *path-dependent measures* such as:
- Average *path length*, network *diameter*, etc…
- Edge *betweeness centrality*, *modularity* index, etc…
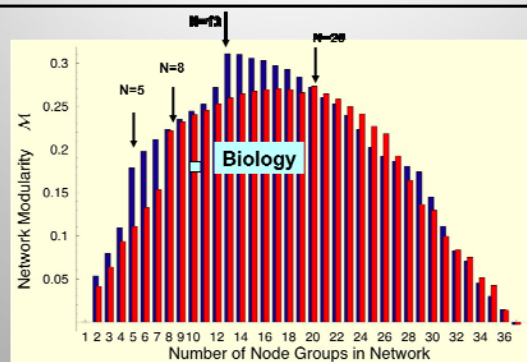
A Scalable Design in Biology

Facebook or immune networks[1]: scale free *hub-centric* networks are ubiquitous across scales in biology…

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[1] Folcik N, Broderick G, et al. Using an agent-based model to analyze the dynamic communication network of the immune response. Theor Biol Med Model. 2011 Jan 19;8:1.

A Granular Composition

Modularity with number of sub-networks controls (red) and CFS (blue).

- Significant differences at aggregation thresholds: N=5 and 13 cliques.
- CFS shows very early separation of immune nodes i.e. N=5 cliques

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[1] Fuite J, Vernon SD, Broderick G. Understanding chronic fatigue using comparative cross-scale analysis of information networks. Sys Biol: Global Regulation of Gene Expression, CSHL, Long Island, NY, Mar 27-30, 2008.

Altered Immune Communication [1]
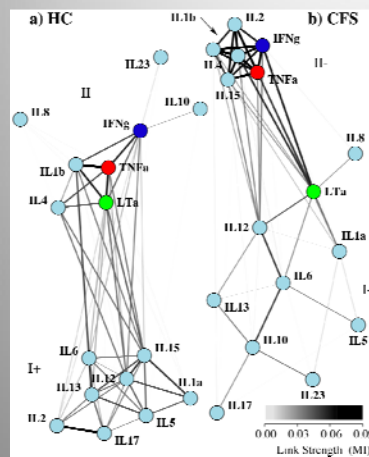
Cohort of adult female CFS patients

- Immune network wiring looks different; *altered immune homeostasis*.

- Highly attenuated Th1 and Th17 responses.

- High Th2 expression and interactions pointed to allergic inflammation.

- Indirect evidence of diminished NK cell responsiveness to IL-12 and Lta .

Similar *in latent viral infection...*

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[1] Broderick G, et al.. A formal analysis of cytokine networks in Chronic Fatigue Syndrome. Brain Behav Immun. 2010 May 4

GWI Study

Participants
- Comparing veterans with GWI to veterans with CFS
- Using standard *Graded eXercise Test* (GXT) to question the system
- *Initial set* of n=10 GWI and 11 healthy veterans

Measuring:
- Immune signaling proteins, stress hormones, immune cell populations and gene expression in immune cells
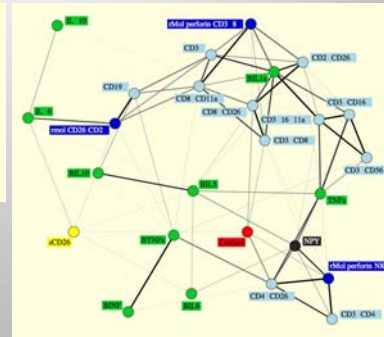- Pre-exercise, peak effort (max VO_2), and 4 hours post-exercise

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Bringing It Together: An Immune Response Network
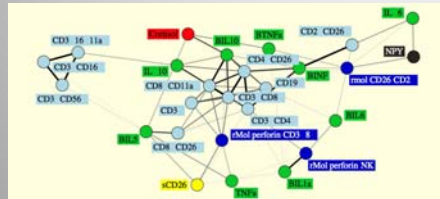
1(a).Ctrl at rest (t0)

- Cell abundance
- Cytokines
- Cortisol
- NK cytotoxicity

1(b). GWI at rest (t0)

Edit D(t1) =3.89 (0.036); 44 pooled std error

Bringing It Together: An Immune Response Network

- Cell abundance
- Cytokines
- Cortisol
- NK cytotoxicity

1(c). Ctrl at peak effort (t1)

1(d). GWI at peak effort (t1)

Edit D(t2) = 4.65 (0.038); 60 pooled std error

Bringing It Together: An Immune Response Network
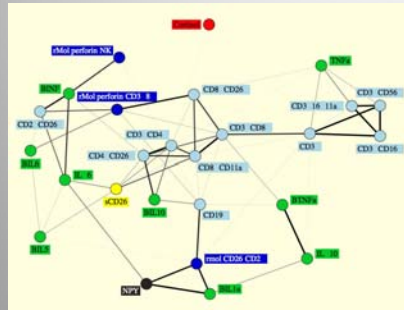
1(e). Ctrl 4hrs post-exercise (t2)

- Cell abundance
- Cytokines
- Cortisol
- NK cytotoxicity
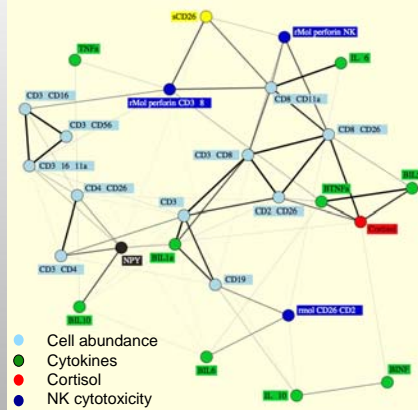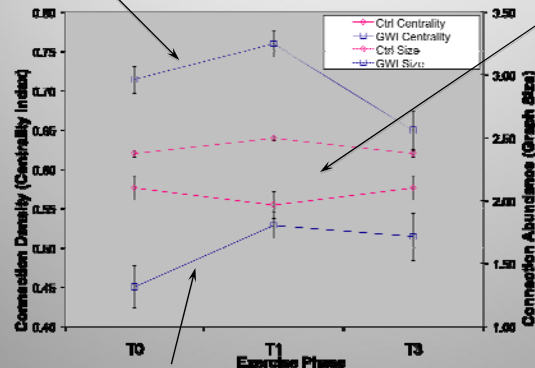
1(f). GWI 4 hrs post-exercise (t2)

Edit D(t3) =3.75 (0.046); 42 pooled std error

GWI: A very Different Immune Response Strategy

GWI: more abundant active connections

HC: Not much change in general architecture

GWI: more diffuse, less organized, fewer hubs

Agents of Change

2(b). GWI - Ctrl at t1

IL-5, sCD26 mounting B cell response with IL-6 Th17?

Altered NPY, TNFa CD26 energy usage?

2(a). GWI - Ctrl at t0

Delayed IL-6 response to exercise (insulin sensitive)

2(c). GWI - Ctrl at t3

Propagation through Time of IL-1a [3]

Post-exercise (t2)

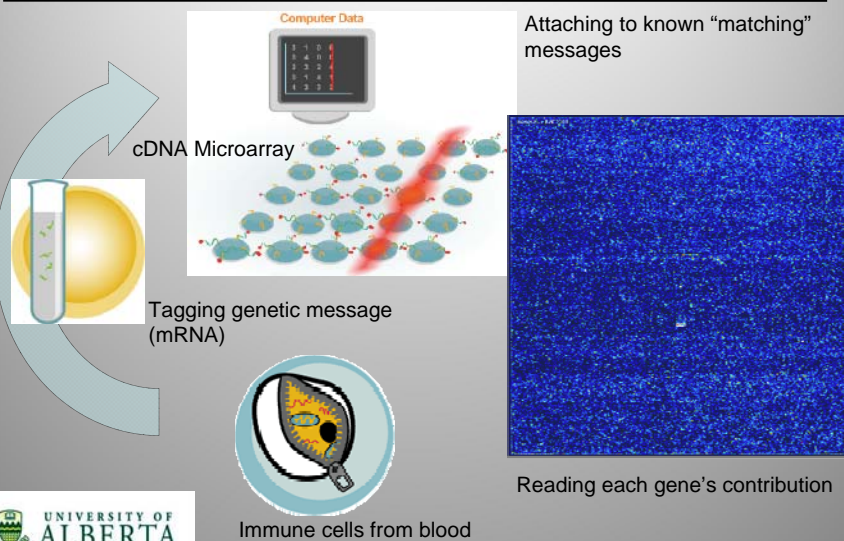Peak effort (t1)

At rest (t0)

Healthy Controls

GWI Patients

[3] Broderick G, Kreitz A, Fuite J, Fletcher MA, Vernon SD, Klimas N. A pilot study of immune network remodeling under challenge in Gulf War Illness. Brain Behav Immun. 2011 Feb;25(2):302-13. .

Propagation through Time of CD2+/26+
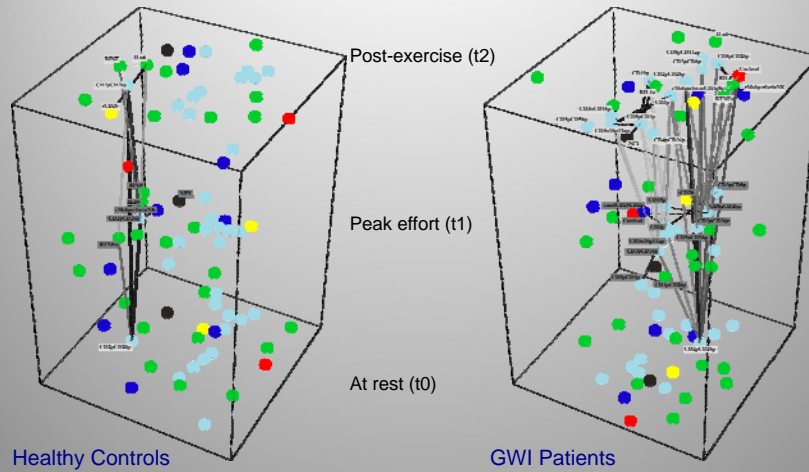
Post-exercise (t2)

Peak effort (t1)

At rest (t0)

Healthy Controls

GWI Patients

[3] Broderick G, Kreitz A, Fuite J, Fletcher MA, Vernon SD, Klimas N. A pilot study of immune network remodeling under challenge in Gulf War Illness. Brain Behav Immun. 2011 Feb;25(2):302-13. .

Looking Within Immune Cells

Computer Data

Attaching to known "matching" messages

cDNA Microarray

Tagging genetic message (mRNA)

Reading each gene's contribution

Immune cells from blood

Survey of Lymphocyte Gene Expression

- Surveyed whole genome in *duplicate samples* using the Affymetrix GeneChip Human Genome (HG) U133 Plus 2.0 Array *

- All probe data was RMA background-adjusted and quantile normalized using the Affymetrix Power Tools (APT) platform.

- Sample set extended to include:
  - ➢ n= 20 GWI subjects
  - ➢ n= 7 CFS subjects
  - ➢ n= 22 healthy control subjects

- Sampled at rest prior to exercise, at maximum effort and 4 hours post-exercise.

_____
* Dr. Lubov Nathanson; Hussman Institute for Human Genomics, Univ. of Miami.
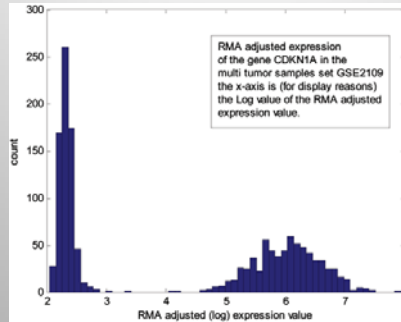
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Lymphocyte Gene Expression at Peak Effort
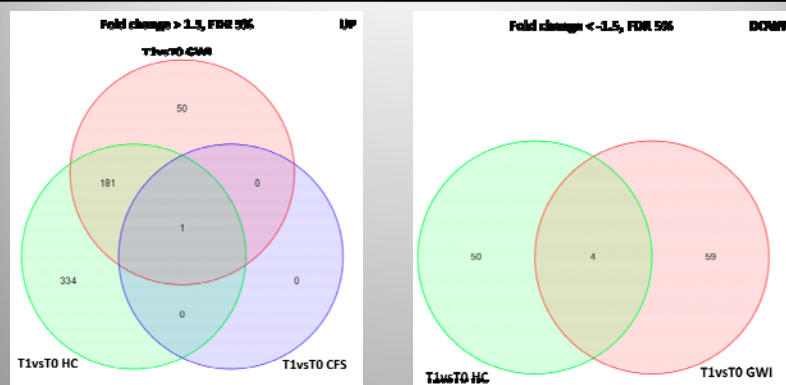
- 50 genes uniquely *over-expressed* under effort in GWI at FDR<0.05
- 59 genes uniquely *down-expressed* in CFS, none in GWI…

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Identifying Active Genes in Individual Patients[1]

RMA adjusted expression of the gene CDKN1A in the multi tumor samples set GSE2109 the x-axis is (for display reasons) the Log value of the RMA adjusted expression value.
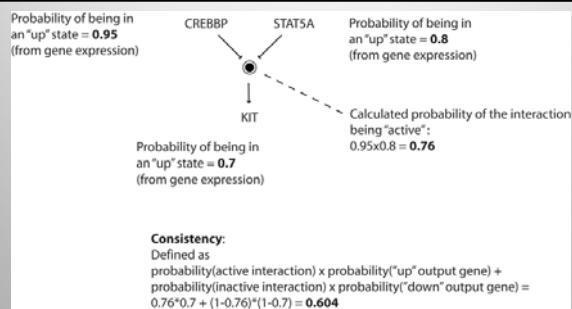
- Map to Up/Down discrete states for every gene *in each individual sample*
- Model expression values to a mixture of gamma distributions;

[1] Efroni S, Schaefer CF, Buetow KH.Identification of key processes underlying cancer phenotypes using biologic pathway analysis. PLoS One. 2007 May 9;2(5):e425.

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Mapping Active Genes to Active Pathway Elements[1]

Probability of being in an "up" state = **0.95** (from gene expression)

CREBBP   STAT5A

Probability of being in an "up" state = **0.8** (from gene expression)

KIT

Calculated probability of the interaction being "active": 0.95x0.8 = **0.76**

Probability of being in an "up" state = **0.7** (from gene expression)

Consistency:
Defined as
probability(active interaction) x probability("up" output gene) + probability(inactive interaction) x probability("down" output gene) = 0.76*0.7 + (1-0.76)*(1-0.7) = **0.604**
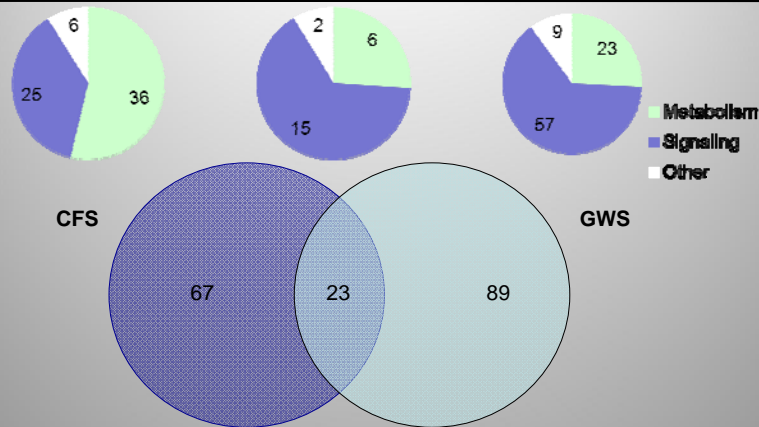
- Gene expression supports *directed functional interactions*
- Pathway elements in NCI-Nature Pathway Interaction Database (PID)[1]
- Assess if combination of *gene activity* levels support rule *consistently*

[1] http://pid.nci.nih.gov/

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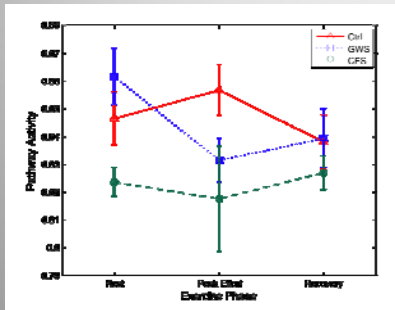ALBERTA

Differences in Pathway Activity in PI-CFS

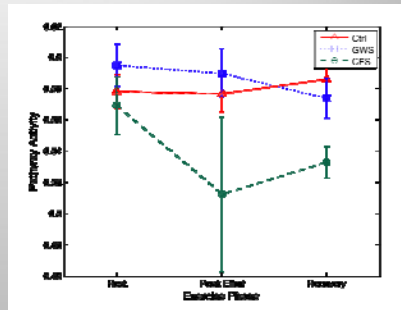- 112 GWS, 90 CFS /585 pathways show *group effects* with FDR < 0.05

Differences in Metabolic Pathway Activity in CFS

Purine metabolism (KEGG)
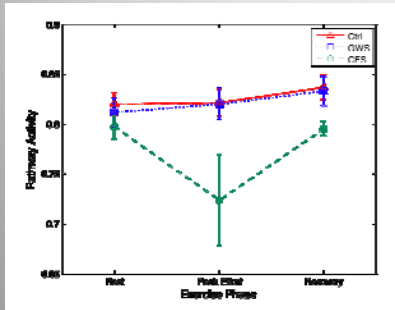
Cell cycle (KEGG)

Node degree: 0 in CFS, 31 in Ctrl

Node degree: 2 in CFS, 11 in Ctrl

- 36 pathways specific to CFS vs GWS and Ctrl related to cell *metabolism*
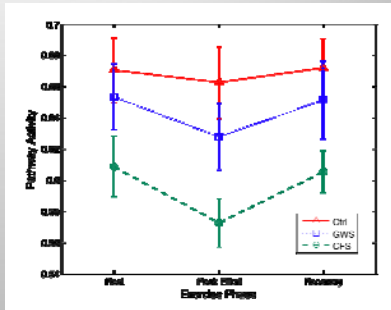- Purinergic (cw alanine/aspartate), amino, nucleic acid metabolism

Differences in Signaling Pathway Activity in CFS

Toll like receptor signaling (KEGG)   Rac1 cell motility signaling (Biocarta)

Node degree: 0 in CFS, 30 in Ctrl   *Node degree: 5 in CFS, 22 in Ctrl*

- 25 pathways specific to CFS vs GWS and Ctrl related to cell *signaling*
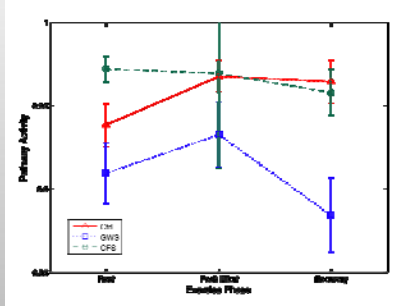- Suggest disengagement of innate immune signals and lymphocyte repair

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Differences in Metabolic Pathway Activity in GWS

Nicotinate/ nicotinamide metabolism   Pentose/glucuronate interconversions (KEGG)
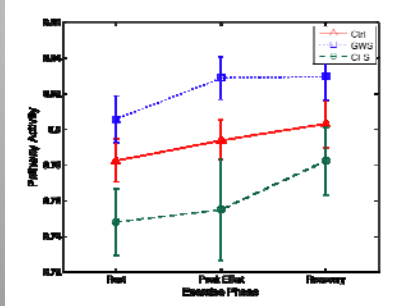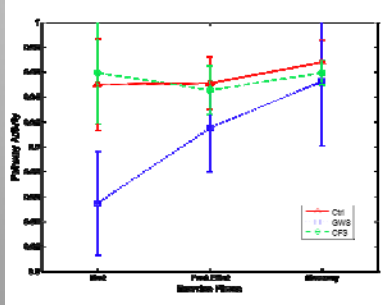

Node degree: 0 in GWS, 1 in Ctrl   *Node degree: 20 in GWS, 9 in Ctrl*

- 23 pathways specific to GWS vs CFS and Ctrl related to *metabolism*
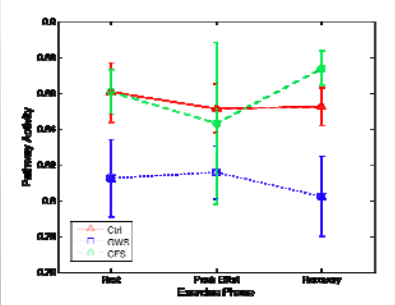- Imbalanced redox pathways - lowered defense against oxidative stress

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Differences in <u>Immune</u> Signaling Pathway Activity in GWS

TNF/stress related signaling (Biocarta)   B cell receptor signaling pathway (Biocarta)

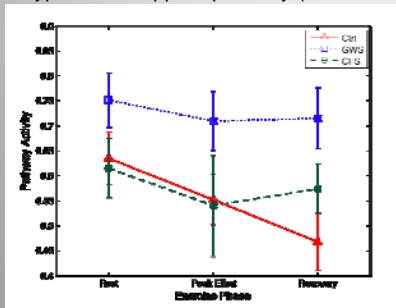*Node degree: 28 in GWS, 16 in Ctrl*   *Node degree: 46 in GWS, 22 in Ctrl*

- 57 pathways specific to GWS vs CFS and Ctrl related to cell *signaling*
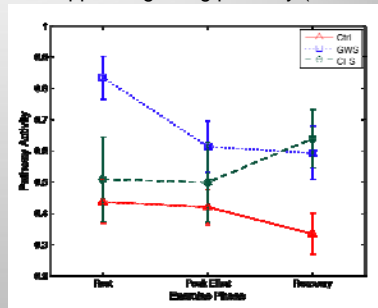- Engagement into network of *dampened* immune signaling (adaptive)

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Differences in <u>Immune</u> Signaling Pathway Activity in GWS

Atypical NF-kappa b pathway (NCI/Nature) NF-kappa b signaling pathway (Biocarta)

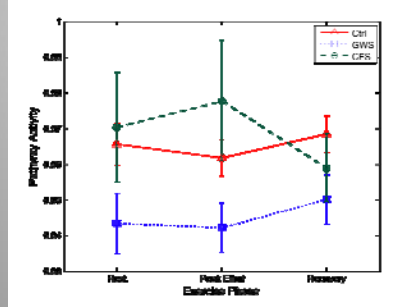
Node degree: 7 in GWS, 9 in Ctrl   *Node degree: 12 in GWS, 11 in Ctrl*

- Stable immune hubs heightened in activity but down-modulated by effort
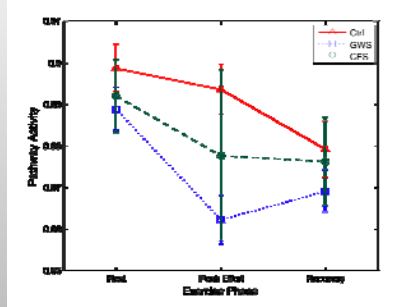- Broad regulator of immune response chronically active

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Ripple Effect in <u>Other</u> Signaling Pathways in GWS

Thromboxane a2 rec signaling (NCI/Nature)   EGR (NGF) control pathway

Node degree: 11 in GWS, 9 in Ctrl   *Node degree: 1 in GWS, 3 in Ctrl*

- Stable hemodynamic hub decreased in activity, *opposite to CFS*
- Early growth response (EGR) lowered under effort like CFS but more so

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Differences in Pathway Activity in GWS and CFS

- First general observations

  o Immune *metabolic function* majority of disrupted pathways in CFS

  o Irregular immune and associated signaling main theme in GWS

  o Different aspects of *alanine-aspartate / phenylalanine metabolism* affected in both CFS and GWS

  o Suppression of *1- and 2-methylnaphthalene degradation* pathway common to both illnesses, similar discriminator.

- Another part of the picture

  o Increased *recruitment* of these pathway segments into larger network in GWS vs disengagement in CFS…

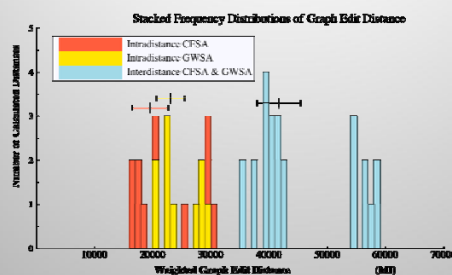UNIVERSITY OF ALBERTA

Comparing transcription factor (TF) networks
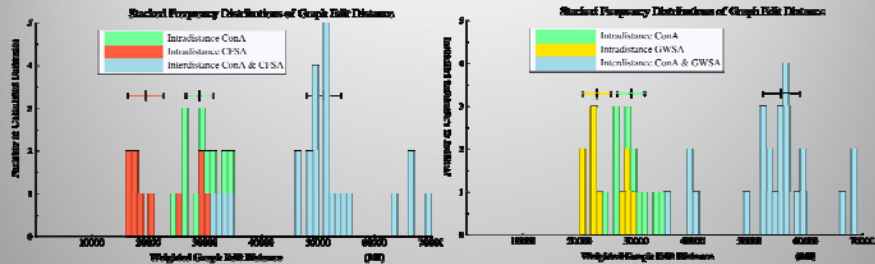
Transforming one TF network into another…

- CFS and GWI TF networks differ significantly from that of controls
- TF network for GWI is more distant from control than CFS

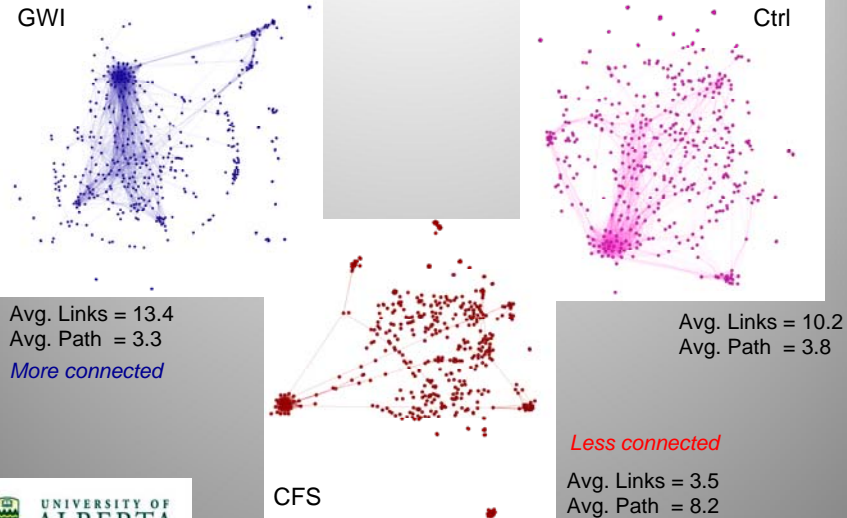Comparing transcription factor (TF) networks
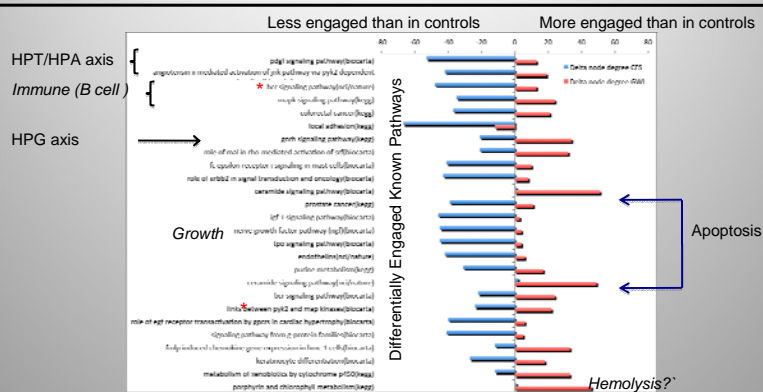
Transforming one TF network into another…

- TF network for GWI is structurally different from the TF network for CFS
- They are more similar to one another than either is to the control TF network

Mapping Active Genes to Active Pathway Elements

Mapping Active Genes to Active Pathway Elements[1]

- Disengagement of growth factor signaling and tissue repair in ME/CFS
- Opposite true in GWI; distinct *porphyrin (heme) and ceramide (apoptosis) signaling* (neither is differentially active**)

Mapping Active Genes to Active Pathway Elements[1]

Inflammatory/ tissue repair hub
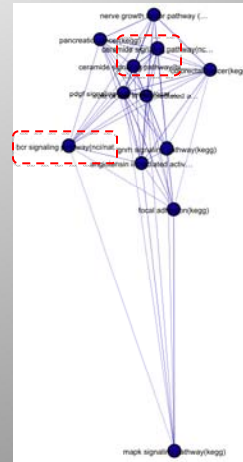
GWI Network of pathway elements

High node degree sub-net
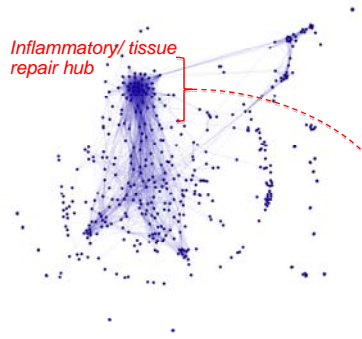
- Ceramide: inflammatory *hub among hubs*…
- Embedded with immune (B cell receptor, focal adhesion, MapK), repair/growth (PDGF, NGF), broad signaling (angiotensin, GnRH)

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## Synopsis

A. Activity of individual pathway segments
- Several pathways differ significantly in activity during exercise
- Majority are unique to either GWS or CFS

B. Interaction of pathways segments
- Significant differences in patterns of association
- GWS recruiting new associations
- CFS shedding normally active associations

C. Flow of regulatory information
- Ongoing. Requires the construction and analysis of directed graphs.

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Accelerating the Design of Treatment Trials [1]
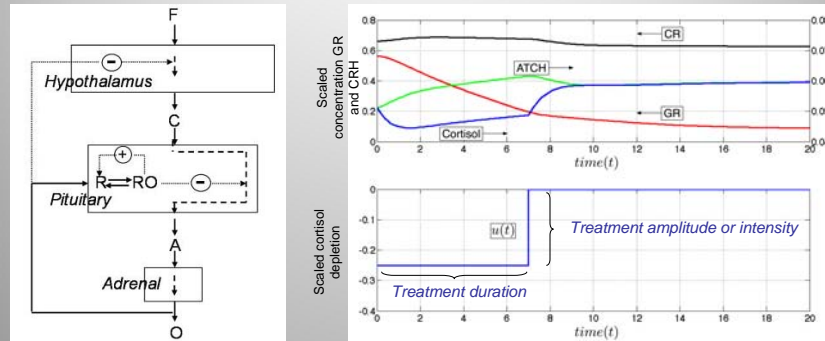
- Early models point to manipulation of bound cortisol for HPA reset

[1] Ben-Zvi A, Vernon SD, Broderick G, PLoS Comput Biol. 2009 Jan;5(1):e1000273

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Acknowledgement of Funding

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