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UNIVERSITY OF MIAMI

Immune Function in Gulf War Illness

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University of Alberta

Miami VA Medical Center

Miami VAMC/UM GWI and CFS Research and Clinical Center– Research Protocols

- GWI and CFS Gene Array studies (VA)
- GWI longitudinal study – biomarker discovery (VA)
- Dynamic Modeling in GWI (DOD)
- Isoprinosine in GWI Phase 2 placebo control study (submission pending – VA)
- Telehealth SMART Energy Study CFS (CBT) (NIH)
- Pathogenesis of NK cell defect in CFS (NIH)
- Natural history of CFS (Foundation)
- CFS Biomarker discovery project (NIH)

Collaborators

University of Miami Research Team Leaders:
CO PI: Mary Ann Fletcher, PhD Immunology/Biomarker
Barry Hurwitz, PhD Autonomic
Mike Antoni, PhD CBT/Stress response
Arthur LaPerriere, PhD Exercise Physiology

Gordon Broderick, PhD Computational Biology, U Alberta

University of Miami Genomics Institute Margaret Vance, Lubov Nathanson
CDC CFS genomics lab Bill Reeves, Suzanne Vernon, Toni Whistler, Will Lonehan
Funding: VA, DOD, NIH ORWH /transNIH CFS team, private donations and CFIDS Association of America

GWI and CFS : Comparisons

* Both defined by symptoms which overlap
* Significant overlap in research findings
* Study of GW veterans showed a 16 fold increase risk of CFS, but no other increased risk over controls
* Issues surrounding the study of a multisymptom illness with a multisystem pathogenesis are the same

Gulf War Illness	**Chronic Fatigue Syndrome*** **
Fatigue	Disabling fatigue
Depression	Exercise induced relapse
Arthralgia	Arthralgia
Myalgia	Myalgia
Sleep disturbance	Non restorative sleep
Cognitive dysfunction	Cognitive dysfunction
Headache	Headache
Diarrhea, intermittent	Sore throat
Wheezing, **Cough,** **Chest pain,** **Shortness of breath***	Tender lymph nodes
Weight loss, low grade fever**	

Immune abnormalities in CFS

Immune Activation

- DR, CD26 expression
- TH2 cytokine shift
- Proinflammatory cytokines expression TNF-a, IL-1, IL6

Functional defects

NK Cell dysfunction

CD8 abnormalities

perforins, granzymes

Macrophage abnormalities

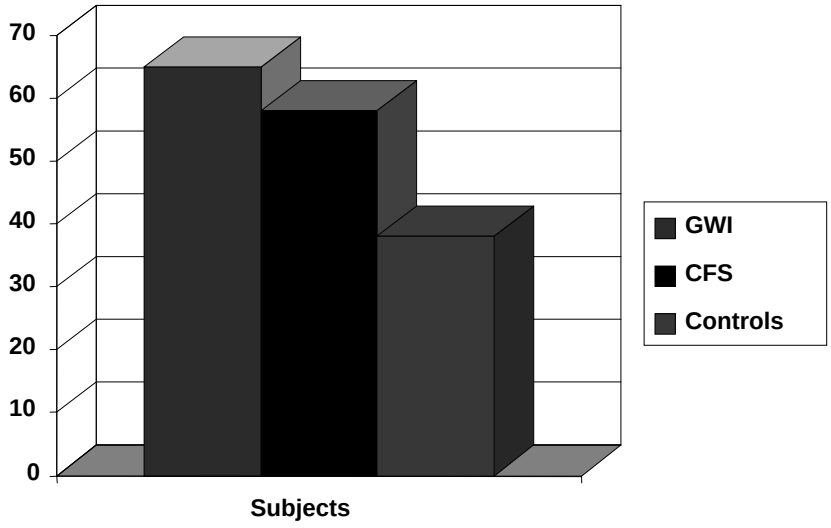
Antibody production

CD26 (dipeptidyl peptidase IV) is involved in the activation of T cells, and is expressed on antigen-reactive memory T cells.

As reported by the Miami CFS research group, the percentage and number of CD26+ lymphocytes is elevated in CFS and GWI.

Quantification of CD26 per cell is reduced in both conditions.

Lymphocyte Activation in GWI and CFS: Percent of CD2+CD26+ Lymphocytes



The GWI & CFS patients had reduced amount of sDPPIV/CD26 in plasma.

BIOMARKER	GWI	CFS	HC
sDPPIV/CD26 (ng/ml)	319**	641*	874

*Significantly different from HC (p<.05)
**Significantly different from HC (p<.0001)

DPPIV/CD26 plays a key role in T cell-mediated immune response by modification, processing and/or inactivation of biologically active peptides in the signaling process of lymphocytes. Several cytokines, chemokines, other growth factors and psyiologiclly important peptides have DPPIV susceptible N-terminal sequences.

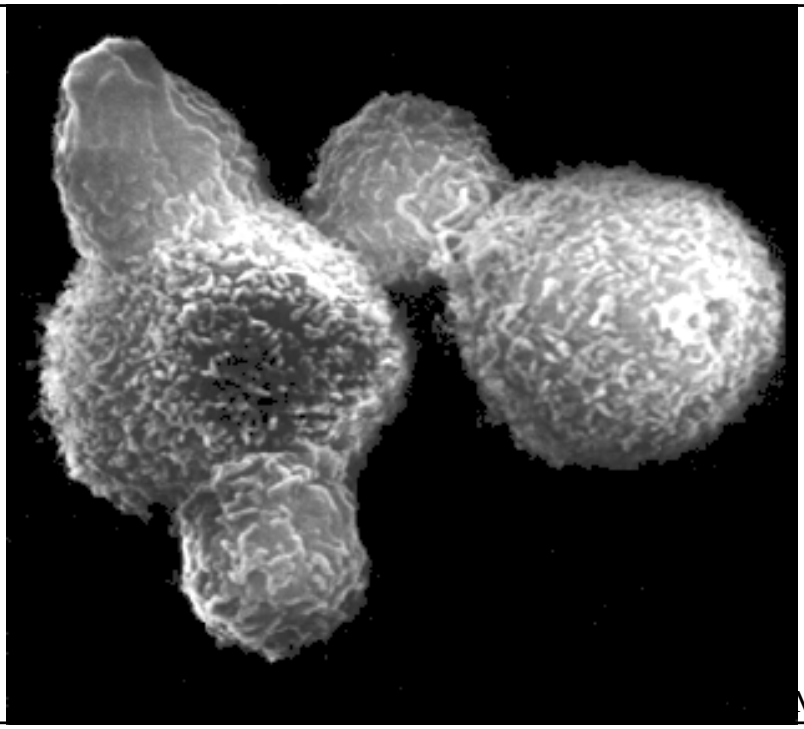
Neuropeptide-Y (NPY) is a 36 amino acid peptide, which participates in the regulation of a large number of physiological and pathophysiological processes in the cardiorespiratory system, immune system, nervous system and endocrine system.

NPY is stored in sympathetic nerve terminals and is released along with catecholamines during stress-induced activation. Only a few peptidases are capable of cleaving NPY due to its unique 3-diminsional structure. DPPIV/CD26 is one such peptidase.

In the GWI patients, we found a reduced amount of NPY in plasma.

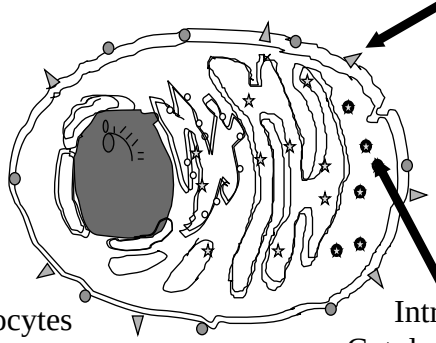
BIOMARKER	GWI	CFS	HC
NPY (pmol/L)	37*	41	52

***Significantly different from HC (p<.05)**



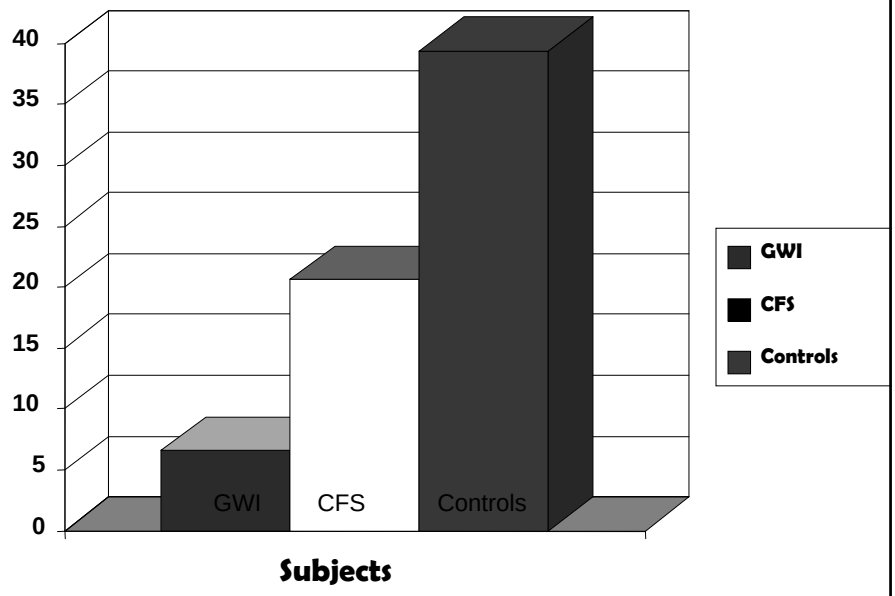
Martz

Natural Killer Cells

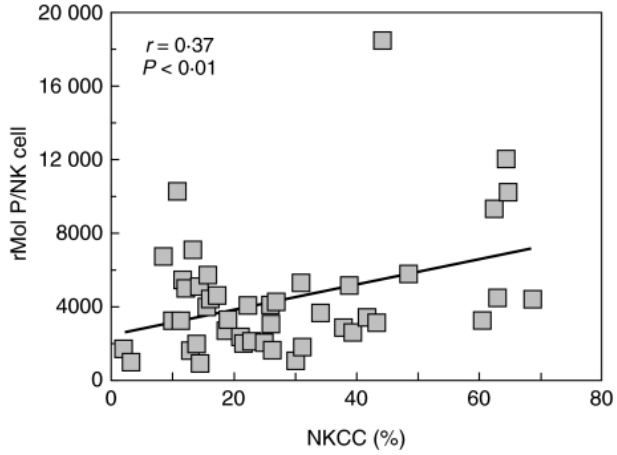


* 5 - 15% of Lymphocytes
* Antigen non Specific
* Not “MHC “ restricted
* Inhibitory Receptors Bind Class I MHC
* Kill Virus Infected and Tumor Cells

NK Cytotoxicity: % K562 Cells Killed at Target to Effector Cell Ratio of 1:1



Significant correlation between natural killer (NK) cell activity (NKCC) and amount of intracellular perforin (rMolP).



Maher KJ, Klimas NG, Fletcher MA. Clin Exp Immunol. 2005 Dec;142(3):505-11.

Dynamic Modeling in CFS/ME

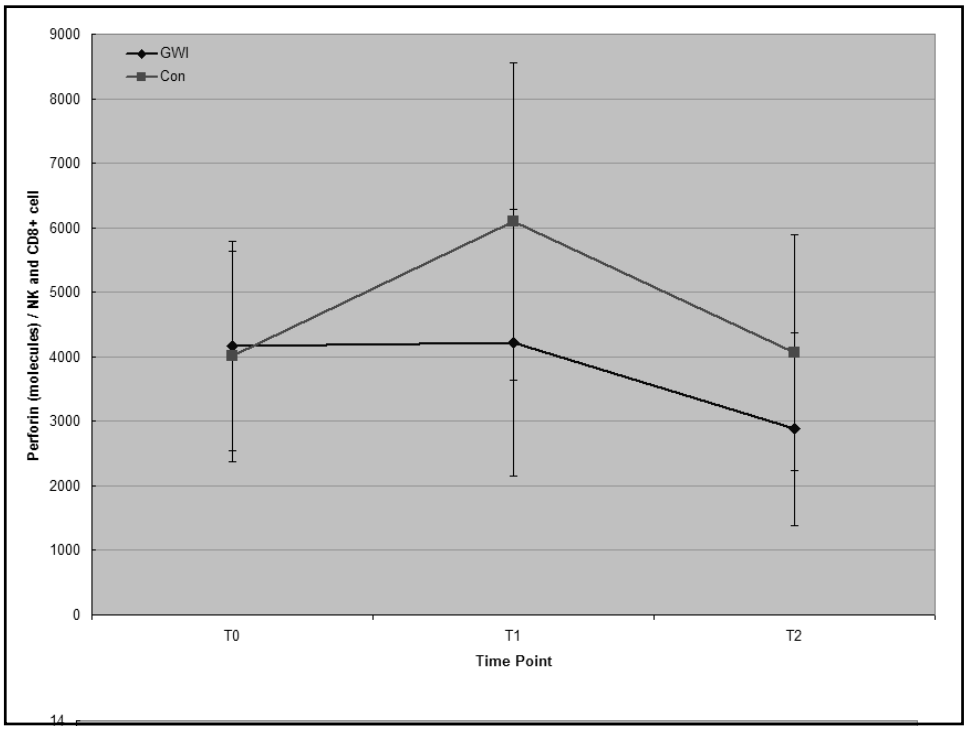
* Gulf War Illness, CFS/ME and healthy controls matched for sex, age, BMI and duration of illness.
* Exercise stressor model, sampling before, peak and 4 hours post exercise challenge, using an exercise bike and VO2 submax challenge.
* Measuring flow cytometric immune markers of activation and apoptosis, soluble mediators including cytokines, neuropeptide Y, cortisol, gene expression microarrays

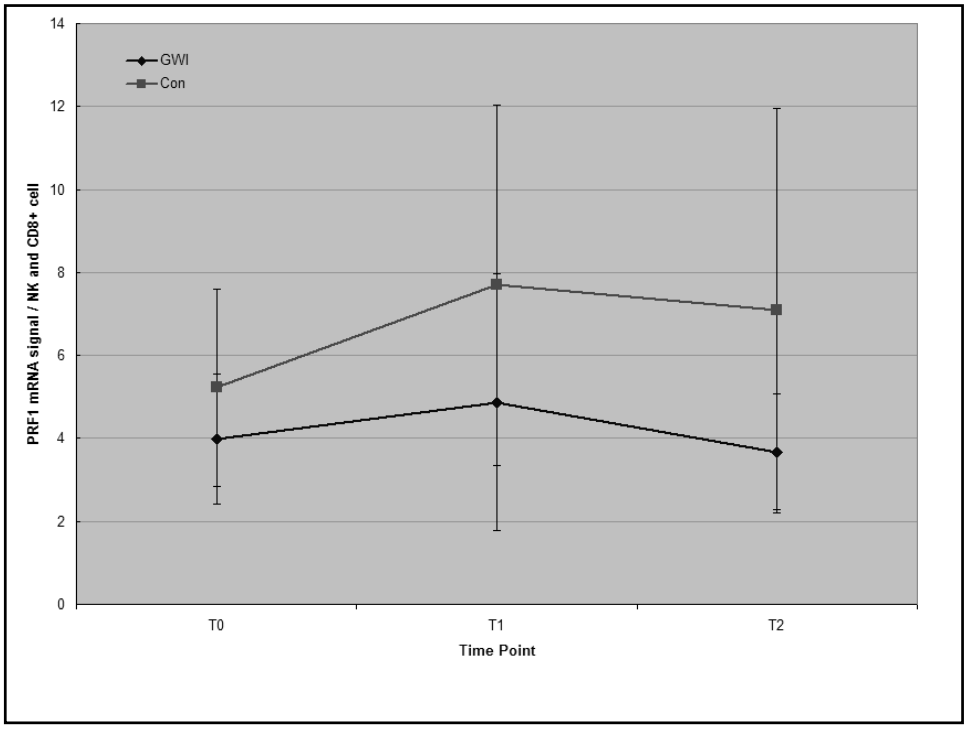
*Computational Biology –

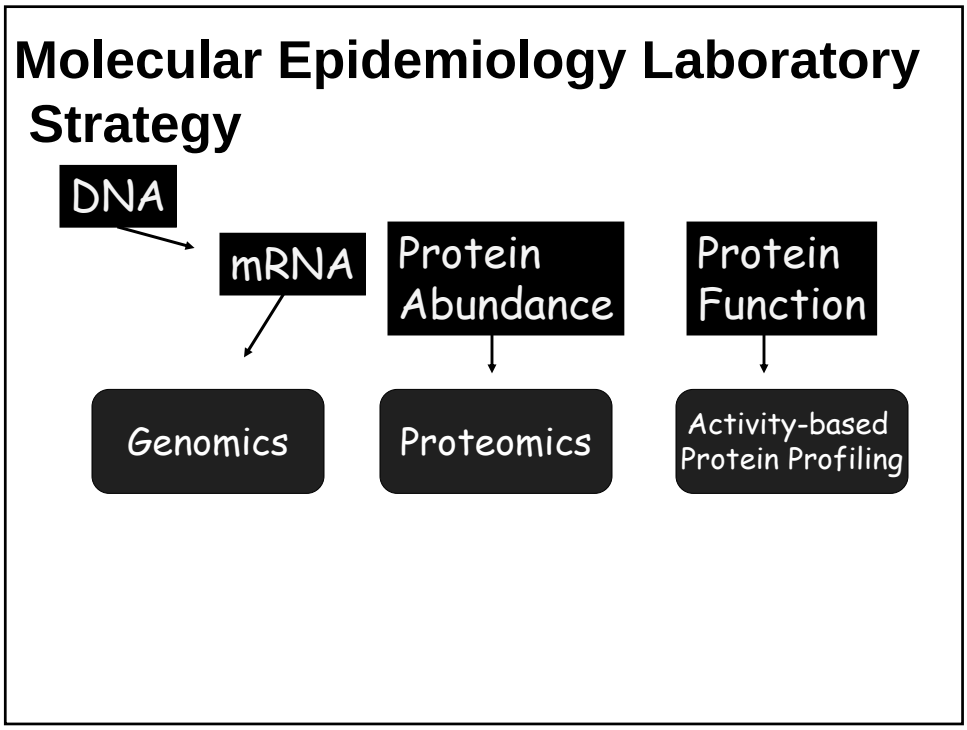
* an approach to better understanding complex networks and modeling potential interventons
* Method: Using the CDC population based study of CFS a comprehensive data set of genomic, laboratory and clinical data set was made available to competing teams to develop innovative analytical approaches. This grew into new collaborative relationships, and strategies,
* Gordon Broderick and colleagues –

University of Alberta in collaboration with the CDC, Suzann Vernon and University of Miami led to this study of dynamic change with exercise challenge in CFS and GWI

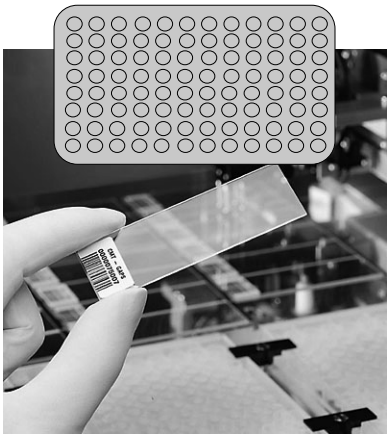
Immune Measure	Group	T0	T1	T2	Interaction (Time x Group) F Value	Interaction (Time x Group) p-value
NK cytotoxocity	GWI	5.38 (4.02)	9.50 (2.95)	NA	0	0.9524
	Con	17.60 (3.00)	22.02 (2.20)	NA		
CD3- CD56+	GWI	127.00 (21.46)	554.22 (85.87)	131.44 (32.29)	4.09	**0.0367**
	Con	169.20 (20.36)	790.70 (81.47)	234.00 (30.64)		
CD3- CD16+	GWI	115.44 (24.86)	509.78 (97.21)	130.89 (35.75)	3.35	*0.0608*
	Con	176.70 (23.59)	806.40 (92.22)	237.40 (33.92)		
CD3- CD16+ CD11a+	GWI	99.78 (22.40)	469.67 (93.67)	117.56 (34.69)	3.48	*0.0557*
	Con	160.00 (21.25)	768.50 (88.86)	224.40 (32.91)		
Perforin molecules/NK and CD8+ cells	Con	4009.46 ± 515.33	6092.4 ± 777.5	4063.90 ± 579.32	1.51	0.1929
	GWI	4259.13 ± 581.92	4057.3 ± 673.11	3213.73 ± 391.27		




GWI
Con
PRF1 mRNA signal / NK and CD8+ cell
14
12
10
8
6
4
2
0
T0
T1
T2
Time Point


Molecular Epidemiology Laboratory Strategy
DNA
mRNA
Protein Abundance
Protein Function
Genomics
Proteomics
Activity-based Protein Profiling

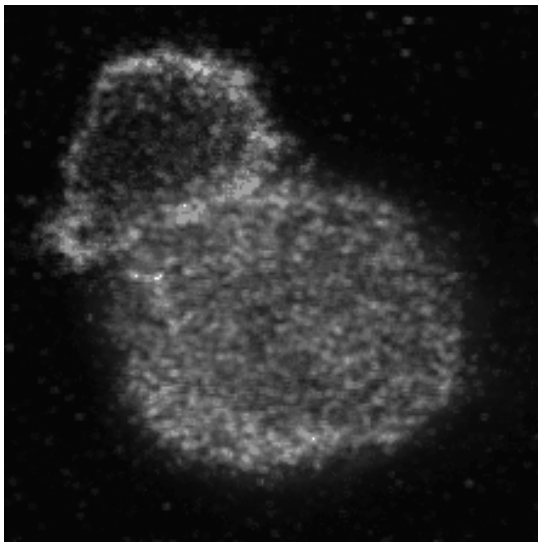
Microarray Technology



* A tool to measure the expression (mRNA) of genes
* An ordered array of spots (that represent genes) on a glass microscope slide

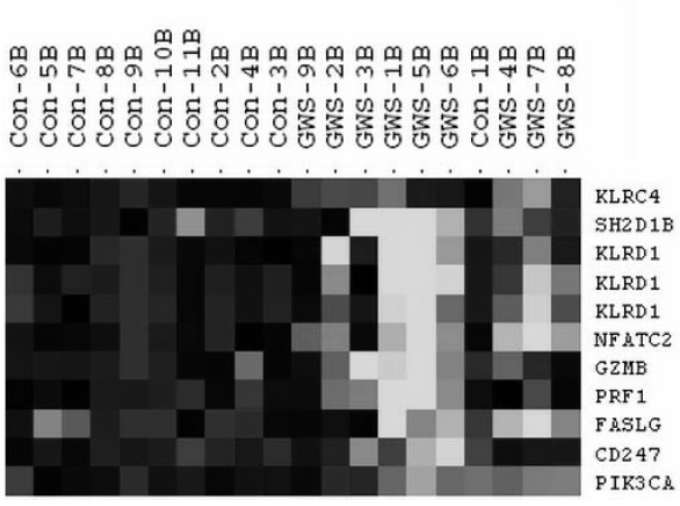
Genetic studies in CFS

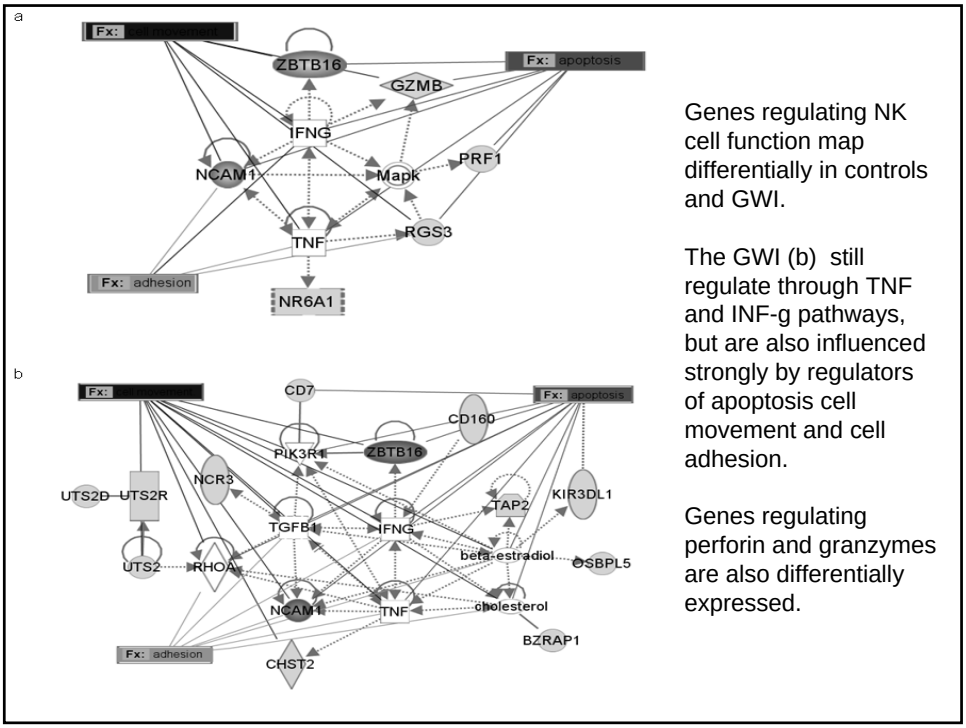
* Differential expression of 35 genes of 9522 tested. T Cell activation, neuronal and mitochondrial regulatory abnormalities Kaushik J Clin pathol 2005 58(8):826
* Abnormalities of Immune response genes in post-infection fatigue suggest genetic variations in susceptibility to persistent fatigue. Helbig JM 2005 98(8):565
* Pre-post exercise challenge gene studies saw differences in genes that regulate ion transport, intracellular cell functions. Challenge studies such as these may be more useful than single cross sectional studies. Whistler et al BMC Physiol 2005 24;5(1):5



Natural Killer Cell Attacks a Cancer Cell

Heatmap depicting the expression levels of genes separating GWI cases from controls immediately after the exercise challenge



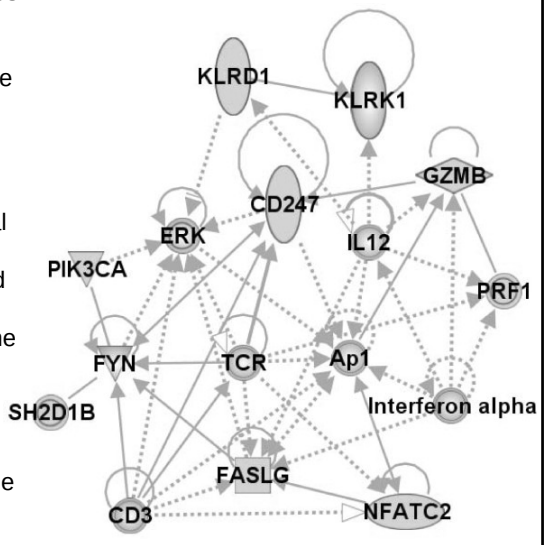


Genes regulating NK cell function map differentially in controls and GWI.

The GWI (b) still regulate through TNF and INF-g pathways, but are also influenced strongly by regulators of apoptosis cell movement and cell adhesion.

Genes regulating perforin and granzymes are also differentially expressed.

Functional network of 9 NK genes differentiating cases from controls. The genes were overlaid onto a global molecular network The genes added to the network as connecting molecules are colored grey. The node shape denotes transmembrane receptor (vertical oval), transcription factor (horizontal oval), cytokine (square), kinase (triangle), peptidase (diamond), and a group or complex (double ringed circle). The edges stand for the gene relationship; solid lines indicate a direct interaction, a dashed line an indirect interaction. A solid arrow head between two nodes denotes gene A at arrow base "acts on" gene B at arrow head. Green node color indicates protein correlated to NK cell subset by QTA that differentiates GWI cases from controls.



Peak of Exercise (10 minutes)

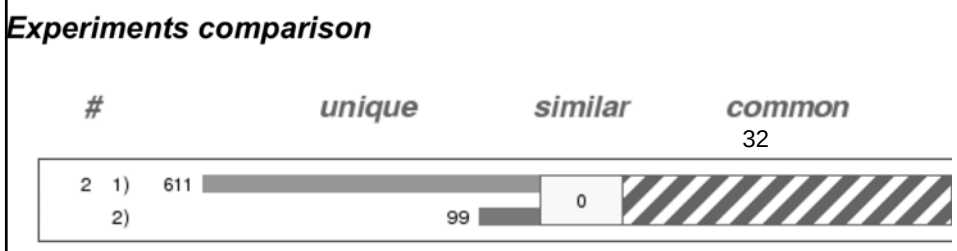


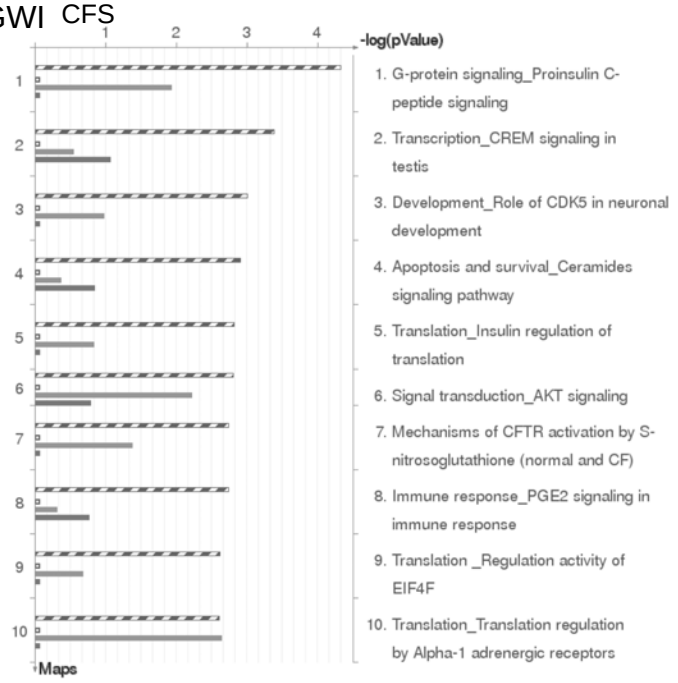
Figure 2. The gene content is aligned between all uploaded experiments. The intersection set of genes is defined as 'common' and marked as a blue/white striped bar. The unique genes for the experiments are marked as colored bars. The genes from the 'similar' set are present in all but one (any) file. The parameters for comparison are set as above.

Unique genes expressed

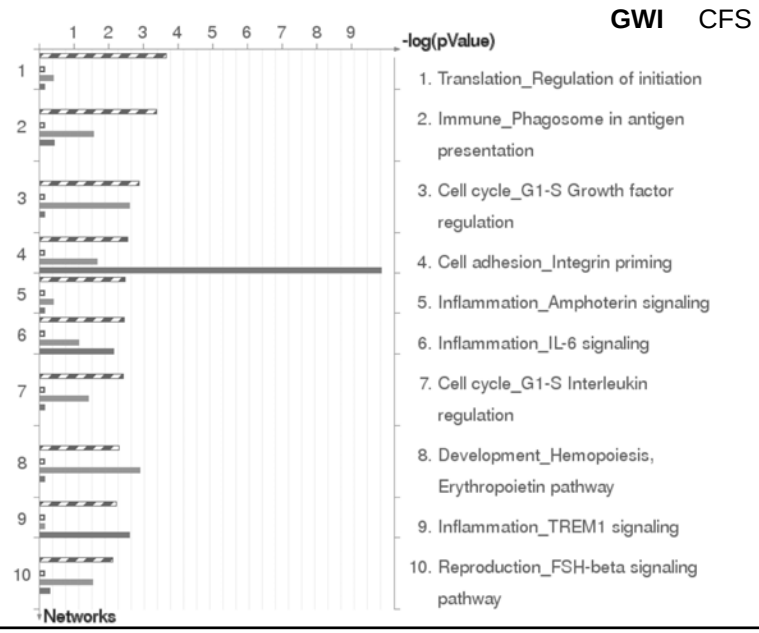
… …	…t0 (rest)	t1 (exercise)	t2(4h post)
CFS	25	99	13
GWI	429	611	740

Peak of Exercise GWI CFS

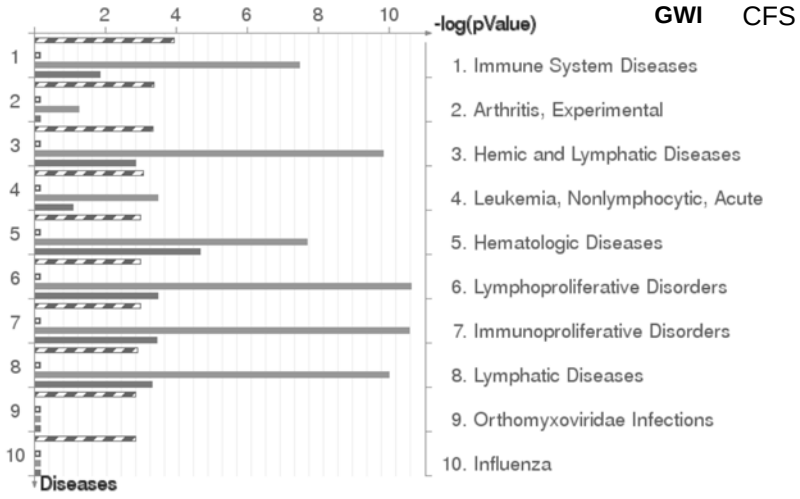
GeneGo
Pathway Maps
Of 650



Peak of Exercise (10 minutes)
GeneGo Process Networks (of 110)

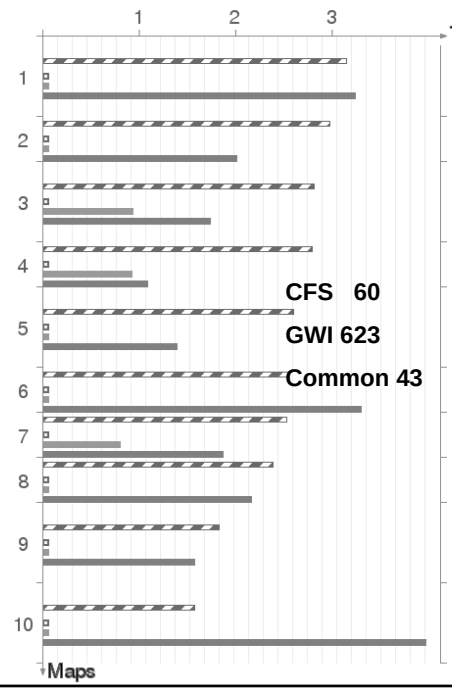


GeneGo Disease Comparison (of 500 human diseases)



4 hours post exercise

T0 to T2 comparison

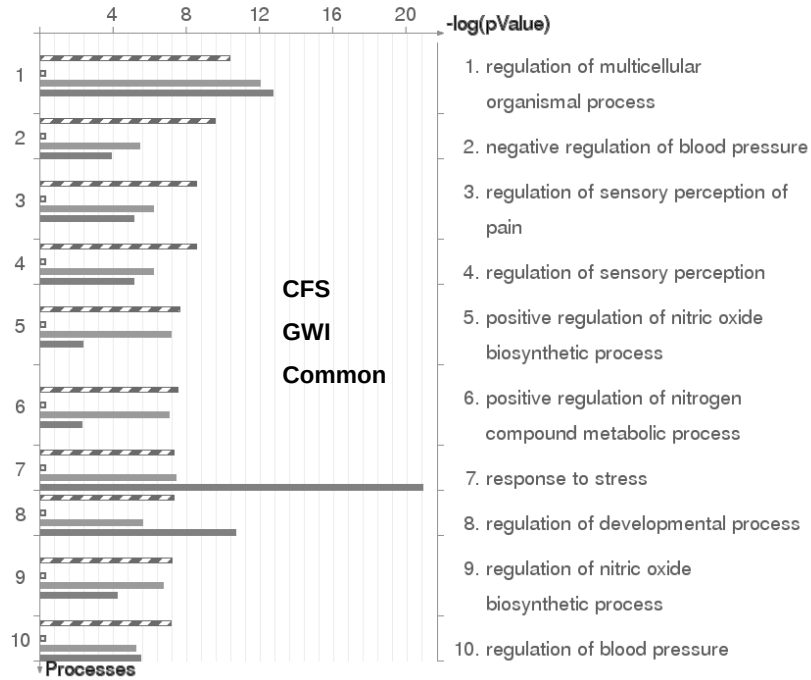


From 650 potential pathways

1. Immune response_IL-3 activation and signaling pathway
2. Development_ERBB-family signaling
3. Immune response_NF-AT signaling and leukocyte interactions
4. Development_HGF signaling pathway
5. Immune response _Immunological synapse formation
6. Development_EGFR signaling pathway
7. Immune response_CD40 signaling
8. Chemotaxis_Leukocyte chemotaxis
9. Immune response_Antigen presentation by MHC class II
10. Immune response _MIF-mediated glucocorticoid regulation

Top GeneGo Process Networks

	name	pValue
*	Development_Regulation of angiogenesis	7.904e-05
	Cell adhesion_Leucocyte chemotaxis	5.560e-04
	Development_Blood vessel morphogenesis	9.184e-04
	Proliferation_Negative regulation of cell proliferation	1.934e-03
	Transcription_Chromatin modification	9.082e-03
	Chemotaxis	1.050e-02
	Reproduction_FSH-beta signaling pathway	1.632e-02
	Reproduction_GnRH signaling pathway	1.919e-02
	Cell cycle_G0-G1	2.483e-02
	Signal transduction_ERBB-family signaling	2.882e-02



Top GeneGo Processes GWI T0T2

	name	pValue
	regulation of multicellular organismal process	5.082e-11
	negative regulation of blood pressure	3.656e-10
	regulation of sensory perception of pain	3.640e-09
	regulation of sensory perception	3.640e-09
	positive regulation of nitric oxide biosynthetic process	3.115e-08
	positive regulation of nitrogen compound metabolic process	4.041e-08
	response to stress	6.203e-08
	regulation of developmental process	6.445e-08
	regulation of nitric oxide biosynthetic process	8.186e-08
	regulation of blood pressure	9.707e-08

Top GeneGo Diseases (by Biomarkers) GWI T0T2

Warts	2.772e-12
Papillomavirus Infections	1.905e-11
Psoriasis	4.692e-10
Hepatitis, Chronic	5.092e-10
Aortic Aneurysm, Abdominal	5.389e-10
Skin Diseases, Papulosquamous	1.585e-09
Diabetic Retinopathy	2.281e-09
Cicatrix (scar tissue)	6.785e-09
Aortic Aneurysm	7.496e-09
Tumor Virus Infections	9.405e-09

Conclusions

GWI and CFS patients have similar immune dysfunction.

GWI and CFS were low compared to HC in intracellular perforin and in ability of NK cells to kill K562 tumor cell targets. Deficiencies even more pronounced in GWI than in CFS.

GWI and CFS had elevated lymphocyte activation compared to HC as indicated by expression of CD26 on most T cells. Amount of soluble CD26 in plasma was low in both CFS and GWI. In CFS, # of molecules of CD26 per T cell was low.

Plasma NPY is low in GWI.

Conclusions

* Differential expression is helpful but by itself can not show a complete picture of complex systems interactions. Methods that look at structural reorganization can help better understand the dynamics of the networks
* Time course data allows us to look at differences in the rate of information flow through these networks. Models that that challenge the dynamics (e.g. exercise challenge) can be used to evaluate associations and speed of information transmission
* Using the models demonstrated using these computational approaches, a new understanding of homeostasis emerges.
* We hope to test models that shift homeostasis in an unwell population towards that seen in a well population.

* Thank you.