ADVANCING FATIGUE RESEARCH THROUGH CLINICAL AND BIOLOGIC INVESTIGATIONS

Leorey N. Saligan, PhD, CRNP, RN

National Institute of Nursing Research

National Institutes of Health

Intramural Research Program

Our Research Changes Lives

one program
many people
infinite possibilities

Department of Health & Human Services · USA

Unmet Needs for Improved Fatigue Management

- Up to 96% of patients with cancer experience fatigue (National Cancer Institute, 2008). > 42% of breast cancer survivors have moderate to severe forms of fatigue (Meeske et al., 2007).

- $9.1 billion lost productivity due to Chronic Fatigue Syndrome (Fukuda et al., 1994).

- Clinical management of fatigue is compromised by unknown etiologic mechanisms and the complexity of diagnosing fatigue syndromes (Mayou & Sharpe, 1997; Chew-Graham et al., 2008).

Limitations in Practice Guidelines

NCCN guideline for CRF

Non-pharmacologic

- activity enhancement
- psychosocial interventions
- attention-restoring therapy
- nutrition
- sleep therapy

Pharmacologic

- attention restoring agent

CDC guideline for CFS

Non-pharmacologic

- professional counseling
- cognitive behavioral therapy
- graded exercise therapy
- alternative therapies
- support groups
- orthostatic instability treatment

Pharmacologic

- sleep medicine
- pain therapy
- antidepressants

Research Directions

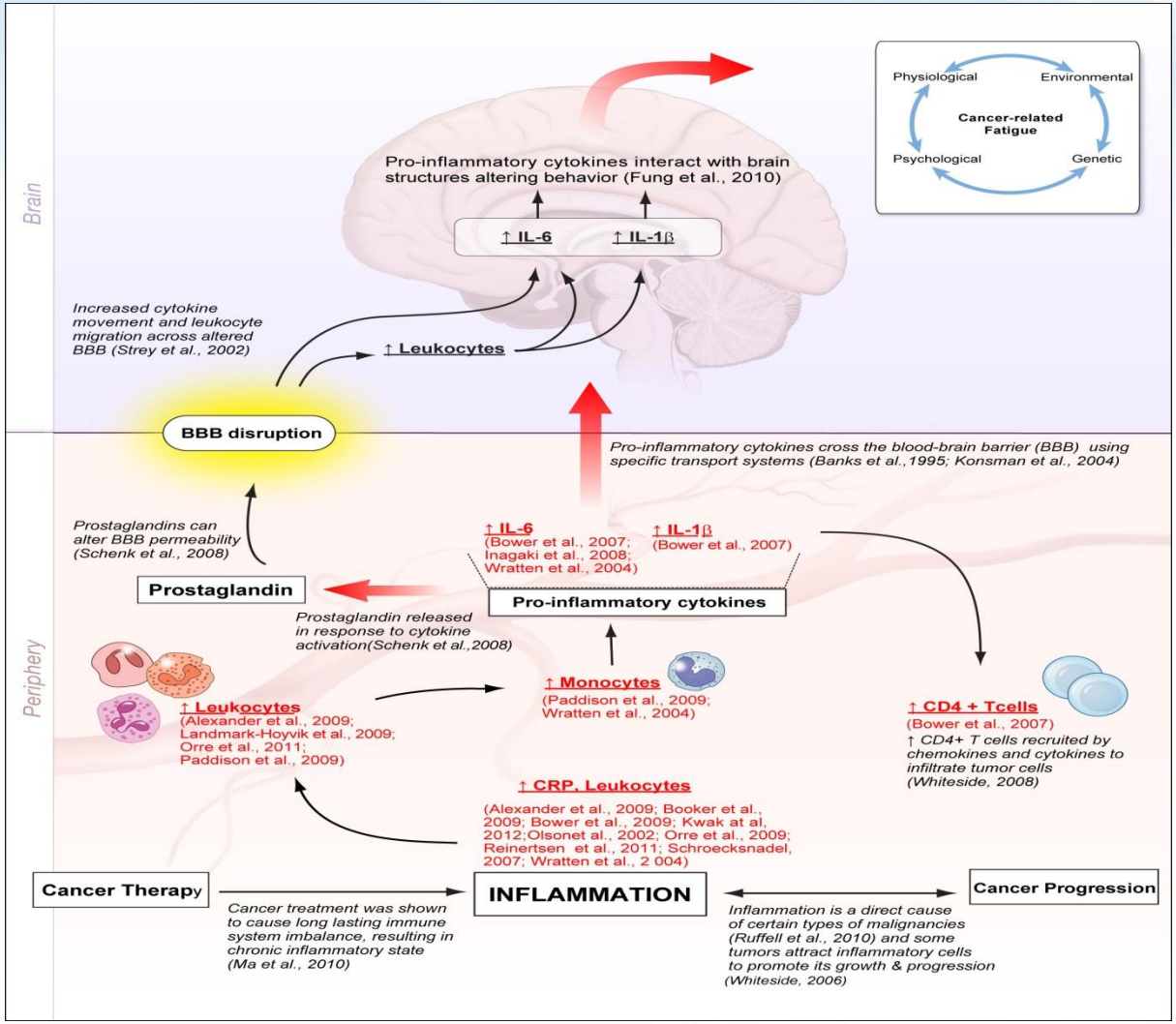
- Describe behavioral, molecular, and genetic correlates of fatigue in different populations.
- Investigate potential interventional targets (peripheral and central) for fatigue.
- Conduct clinical trials to manage fatigue.
- Address limitations of practice guidelines based on scientific evidence.

Genomic Studies in CFS and FM

- No differences in expression of genes specific to adrenergic receptors, ion channel receptors, and cytokine/immune cell production, using PCR between CFS (with or without co-morbid FM) and healthy controls (Light et al., 2011).

- Glutamatergic neurotransmission (*GRIK2*) and circadian rhythm (*NPAS2*) genes were differentially expressed using SNPs and microarray in CFS patients compared to healthy controls (Smith et al., 2011).

Cancer-Related Fatigue

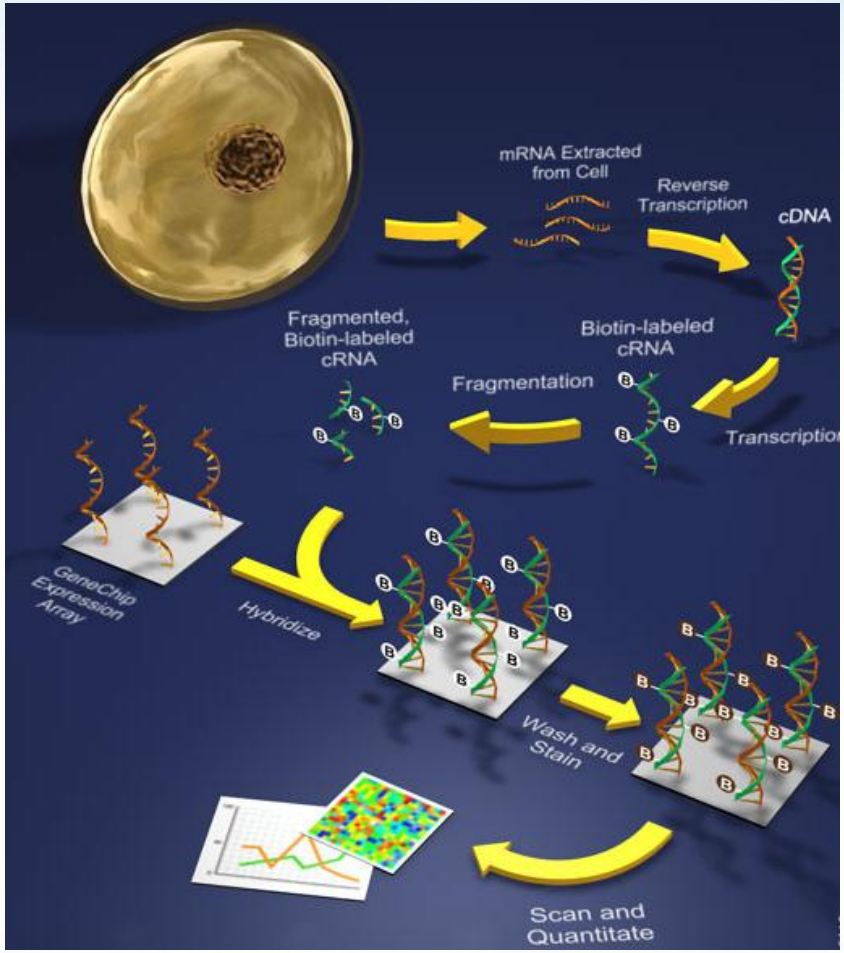


Gene Expression

Whole blood collected using Paxgene tube

Extracted RNA

Microarray



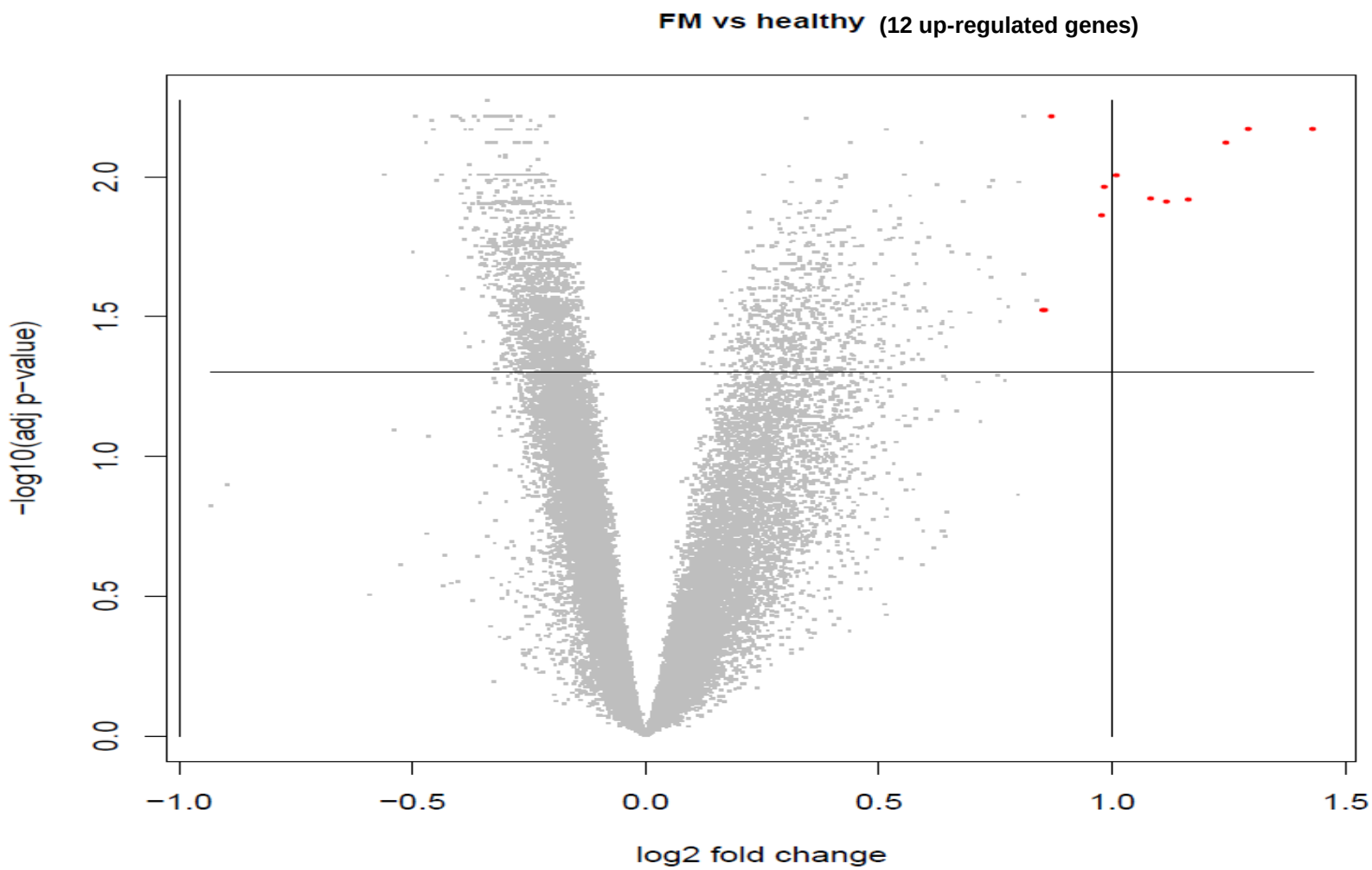
Demographics

- 29 Caucasian, female diagnosed with Fibromyalgia (based on ACR 2010 FM diagnostic criteria)

- 20 Caucasian, healthy women

- Ages: from 30-55 years old
  - Mean FM = 44.10 (± 7.02)
  - Healthy control = 40.40 (±7.47)

Differential Gene Expression

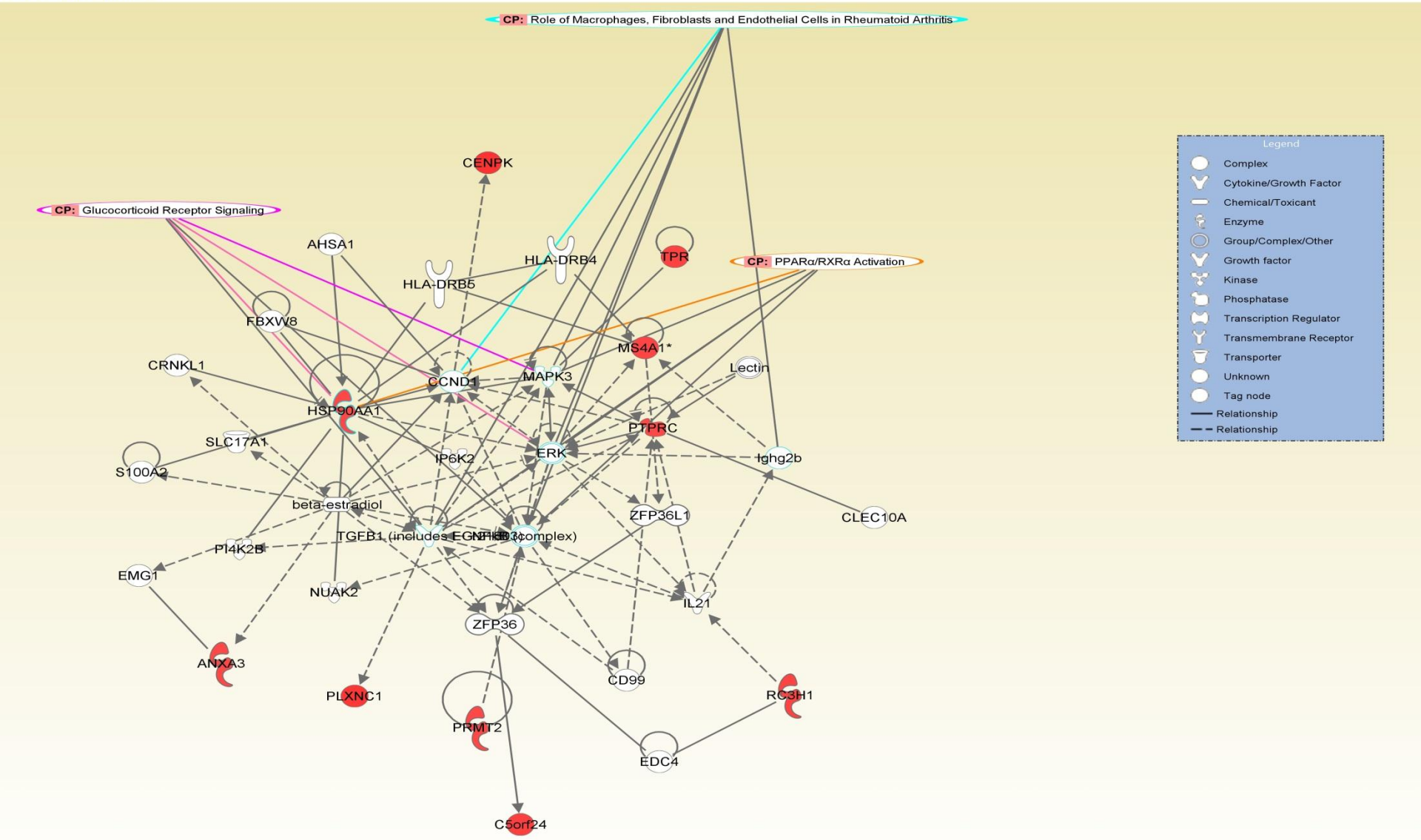


Differentially Expressed Genes

Gene Symbol	Gene Title	Function	p-value	Fold-Change
CENPK	centromere protein K	Maintain kinetochore function	0.00068	2.03111
PLXNC1	plexin C1	Modulate immune response	9.26E-06	1.98653
C5orf24	chromosome 5 open reading frame 24		4.76E-06	1.90018
TPR	translocated promoter region	mRNAs nuclear export	6.65E-06	1.87403
RC3H1	ring finger and CCCH-type domains 1	mRNA translation and stability	0.000112	1.85571
MS4A1	membrane-spanning 4-domains, subfamily A, member 1	Development & differentiation of B-cells	7.69E-05	1.84318
HSP90AA1	heat shock protein 90kDa alpha (cytosolic), class A member 1	Inducible molecular chaperone	1.59E-08	1.82075
ANXA3	annexin A3	Regulation of cellular growth	0.001305	1.81675
PTPRC	protein tyrosine phosphatase, receptor type, C	Regulate cellular process	5.43E-05	1.81098

Network Analysis

Path Designer Network 1

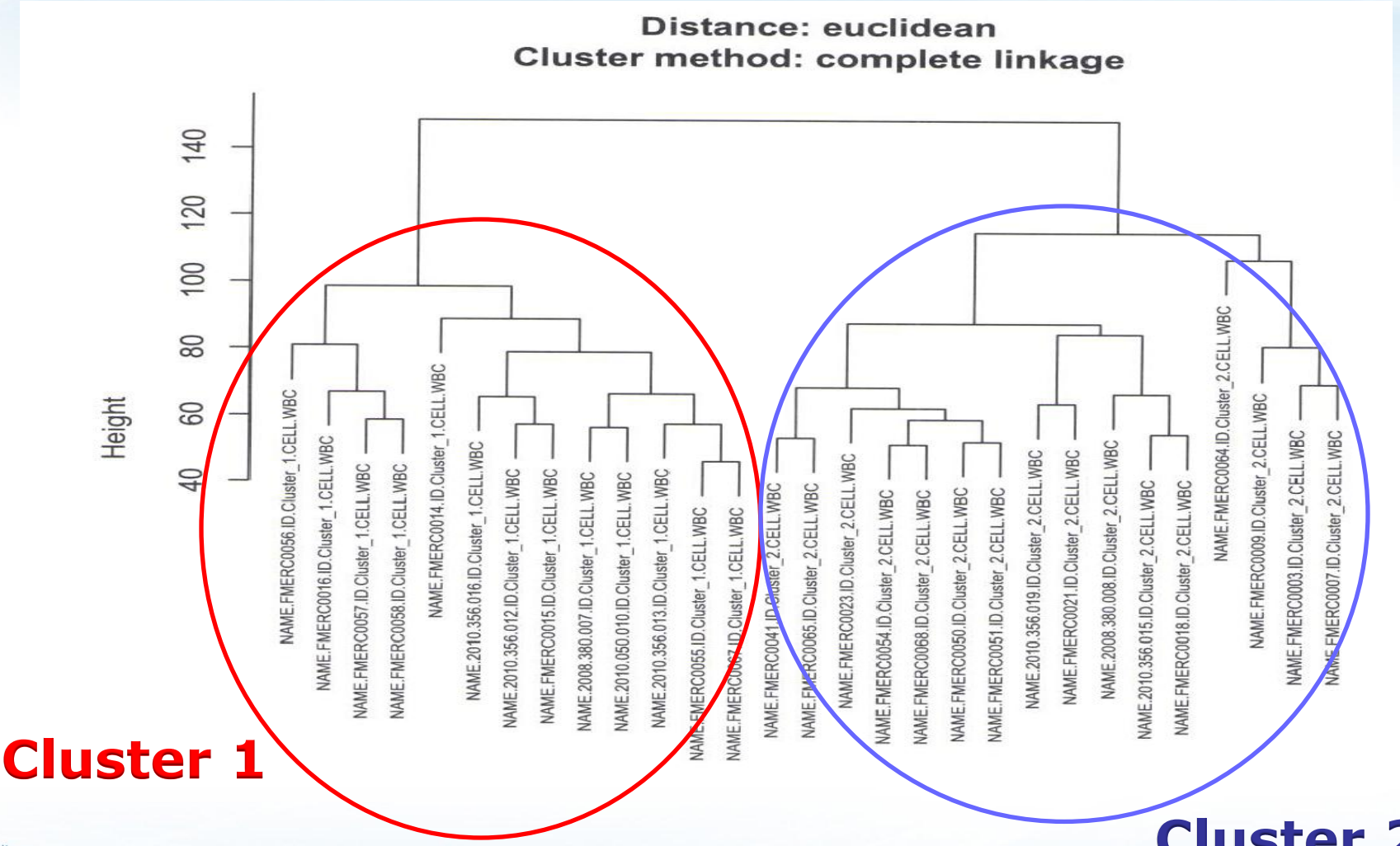


© 2000-2012 Ingenuity Systems, Inc. All rights reserved.

Glucocorticoid Receptor (GR) and CFS

- Dysregulation of glucocorticoid signaling as evidenced by:
  - hypocortisolism (Jason et al., 2010; Cleare, 2003; Gaab et al., 2002)
  - lack of HPA responsiveness to challenge (Dinan et al., 1997)
  - glucocorticoid resistance (Kavelaars et al., 2000)
  - decreased gene expression of GR (Jason et al., 2010)
  - increased histone deacytelase expression, a marker of immune activation (Jason et al., 2011).

Cluster Analysis of Differentially Expressed Genes



Behavioral Characteristics of Clusters

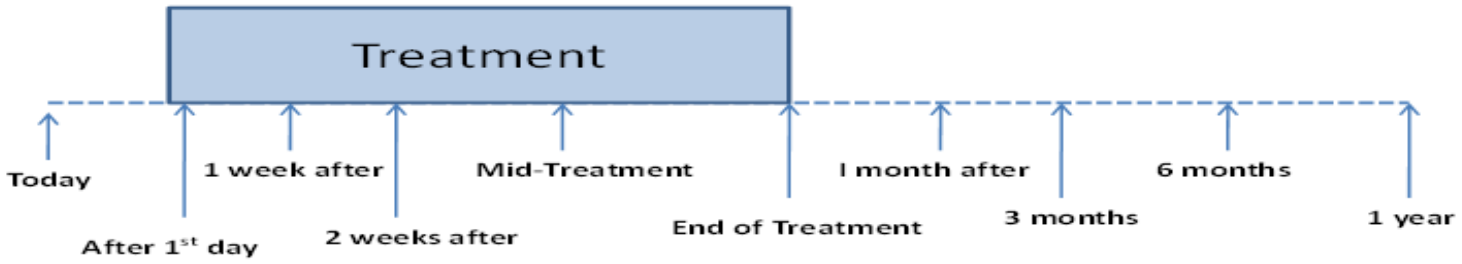
Variables	Mean (SD)		T-test P value
	Cluster 1	Cluster 2	
Age	47.0 (11.9)	45.7 (6.51)	0.68
Pain severity	4.3 (1.6)	4.8 (2.2)	0.46
Pain interference	4.0 (1.8)	6.0 (2.5)	0.01**
Fatigue	71.6 (12.5)	73.9 (11.5)	0.57
Catastrophizing	14.1(10.2)	17.8(9.1)	0.28
Anxiety	7.4 (4.1)	9.9 (4.1)	0.08
Depression	5.2 (3.1)	8.2 (3.8)	0.02**
Pain threshold	2.8 (1.4)	2.3(0.9)	0.26
Fibromyalgia Impact Questionnaire	52.5 (10.9)	55.9 (13.0)	0.40

* *Significant independent T-test (p value < 0.05)*

Natural History of Fatigue in Cancer

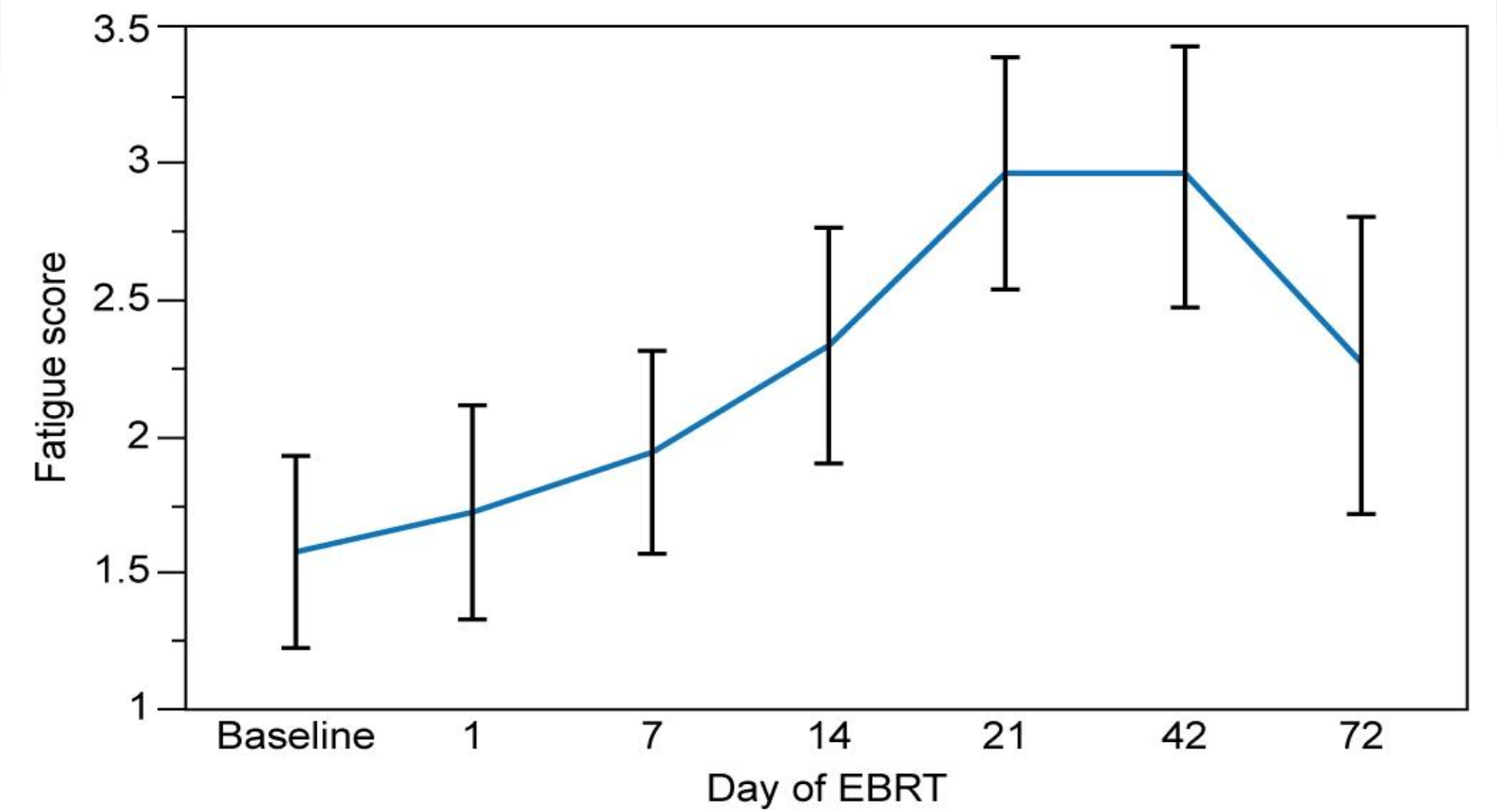
Design: prospective, descriptive, observational study

Methods: questionnaires, computerized cognitive tests, portable hand-grip test, physical activity monitor, 16 mL of blood



Fatigue Scores

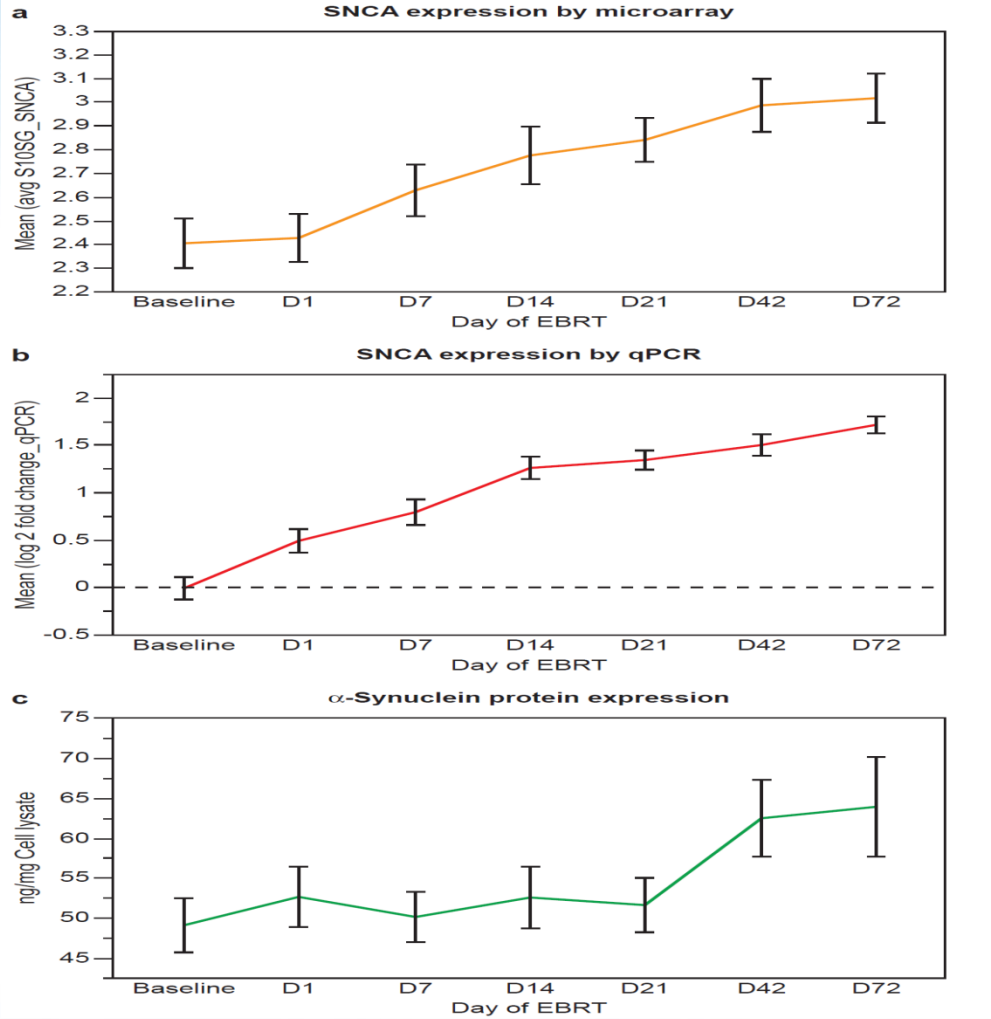
N = 20



Top 10 Up- and Down-Regulated Genes

Top Upregulated Genes			Top Downregulated Genes		
Genes Symbol	Gene Name	Expression Value	Genes Symbol	Gene Name	Expression Value
IFI27	Interferon alpha-inducible protein 27	0.774	**MS4A1**	B-lymphocyte antigen CD20	-0.821
CA1	Carbonic anhydrase 1	0.705	**IGHM**	Ig mu chain C region	-0.816
HBD	Hemoglobin subunit delta	0.640	**PAX5**	Paired box protein Pax-5	-0.791
XK	X-linked Kx blood group	0.534	**FCRLA**	Fc receptor-like A	-0.669
HBG2	Hemoglobin subunit gamma-2	0.513	**TTC3**	Tetratricopeptide repeat protein 3	-0.647
RHCE/RHD	Blood group Rh(CE) polypeptide	0.507	**NSUN5C**	NOP2/Sun domain family, member 5C	-0.642
AHSP	Alpha hemoglobin stabilizing protein	0.496	**POU2AF1**	POU domain class 2-associating factor 1	-0.636
GYPB	Glycophorin B	0.483	**CCR7**	C-C chemokine receptor type 7	-0.632
SNCA	Alpha synuclein	0.470	**FAIM3**	Fas apoptotic inhibitory molecule 3	-0.613
ISCA1	Iron-sulfur cluster assembly 1 homolog	0.464	**BLK**	B lymphoid tyrosine kinase	-0.612

SNCA Expression Overtime

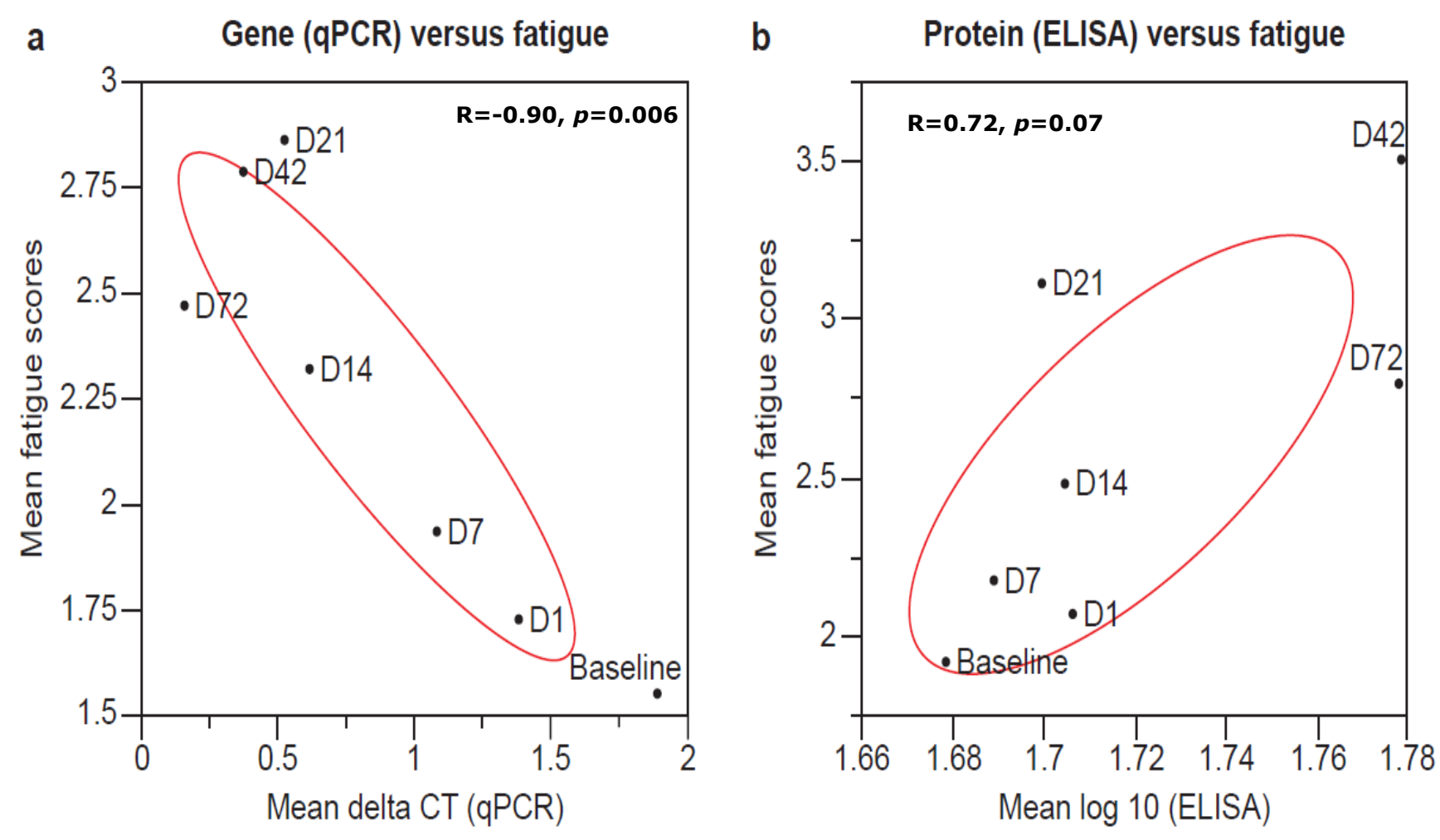


N=10, p<0.0001

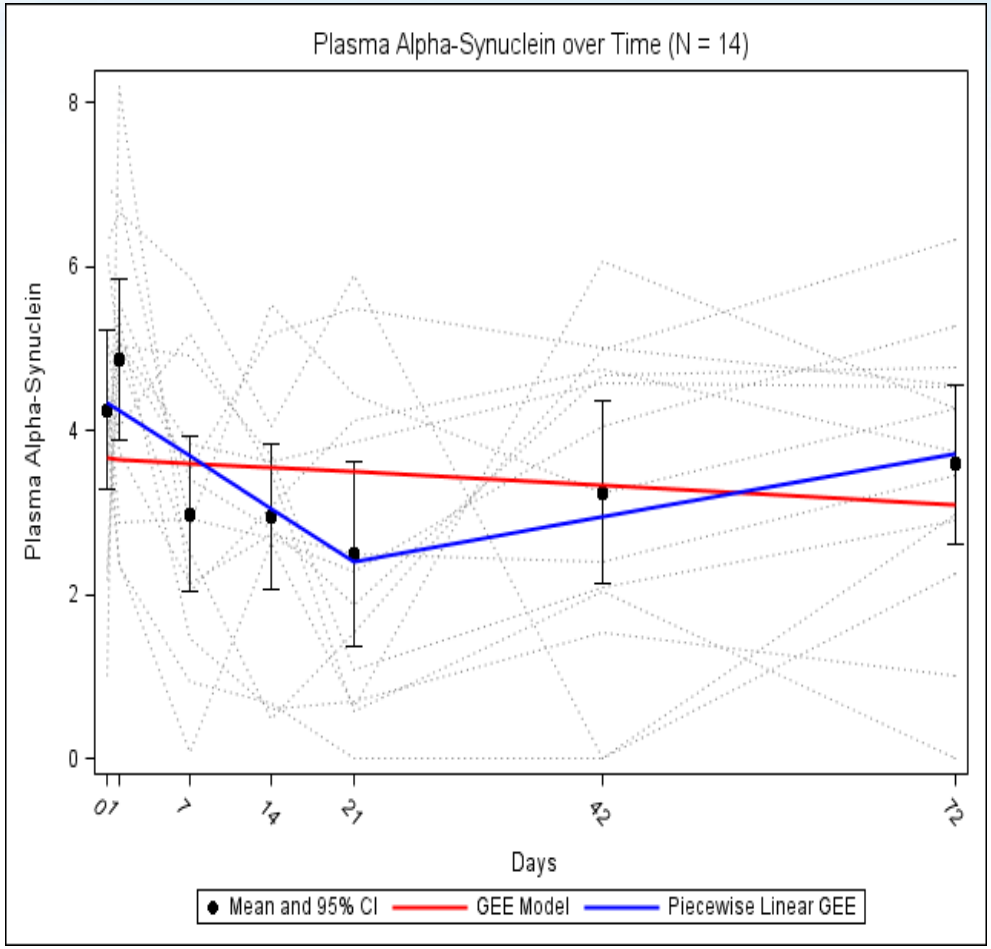
N=20, p<0.0001

N=20, p=0.0005

Correlation between *SNCA* and Fatigue



α-Synuclein Expression in Plasma



Piecewise GEE model, a-synuclein decreased before day 21 ($\beta \leq 21 = -0.09$, $p < 0.0001$), and then increased through the end of the study period ($\beta < 21 > 21 = 0.03$, $p = < 0.001$).

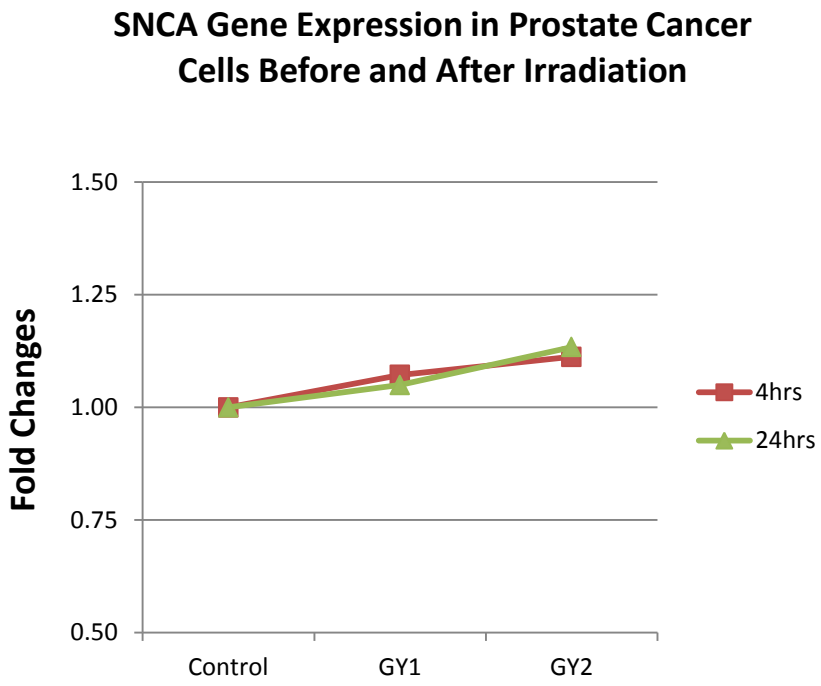
No significant association between fatigue and plasma a-synuclein levels ($p = 0.33$).

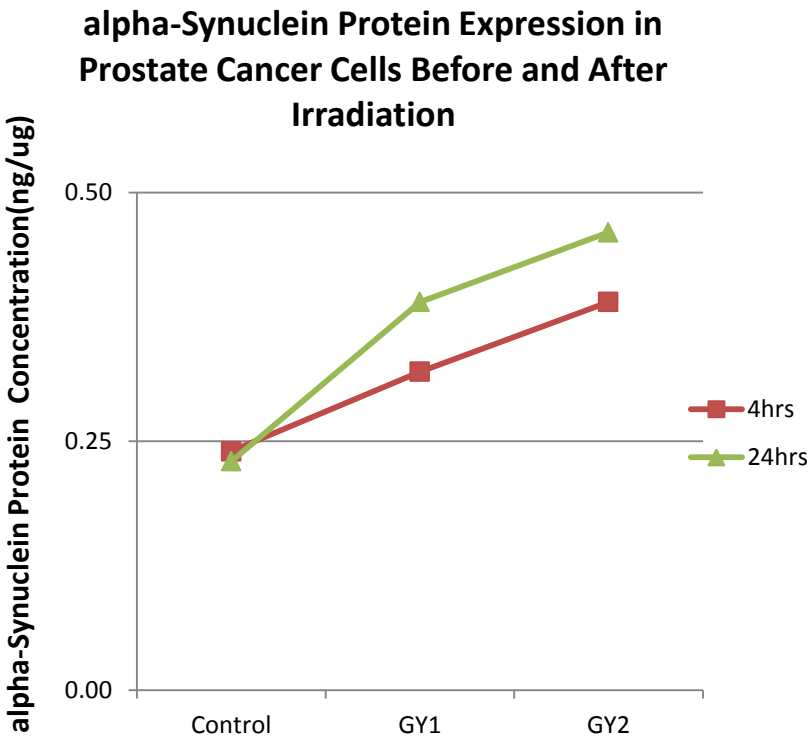
α-Synuclein

- Overexpression of α-synuclein inhibits catecholamine release from adrenal chromaffin cells (Larsen, Schmitz, Troyer, et al., 2006).

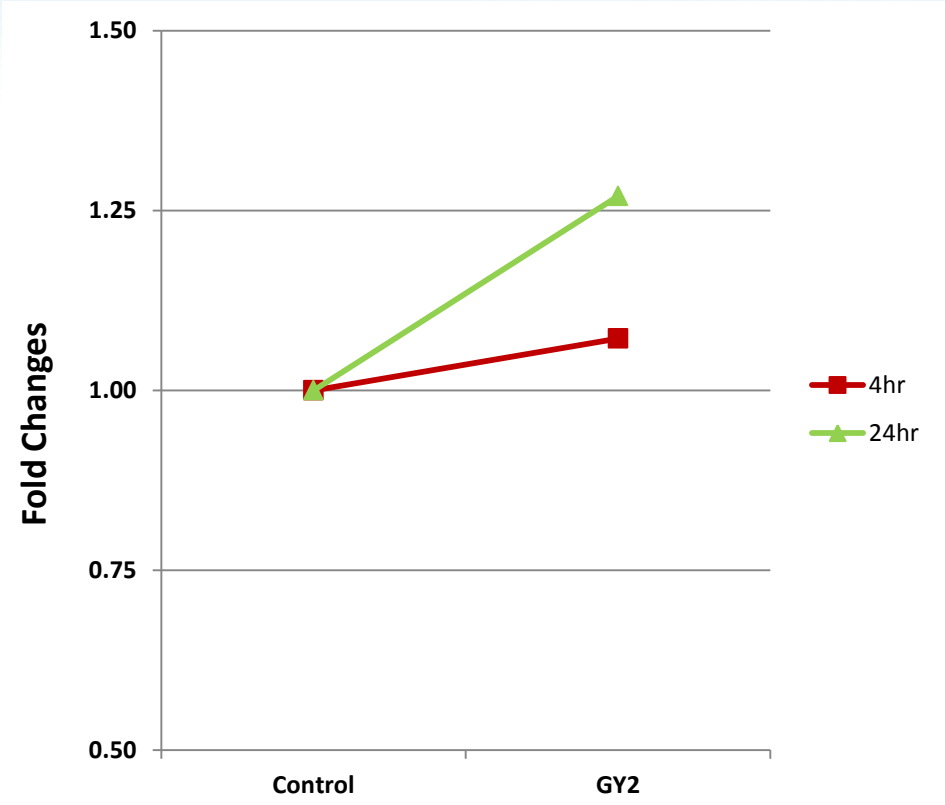
- Elevations of α-synuclein in cellular cytoplasm is a measure of resilience against oxidative stress and cellular protection from toxic insults (Hashimoto et al., 2002).

In-Vitro Confirmation (Prostate Cancer Cells)

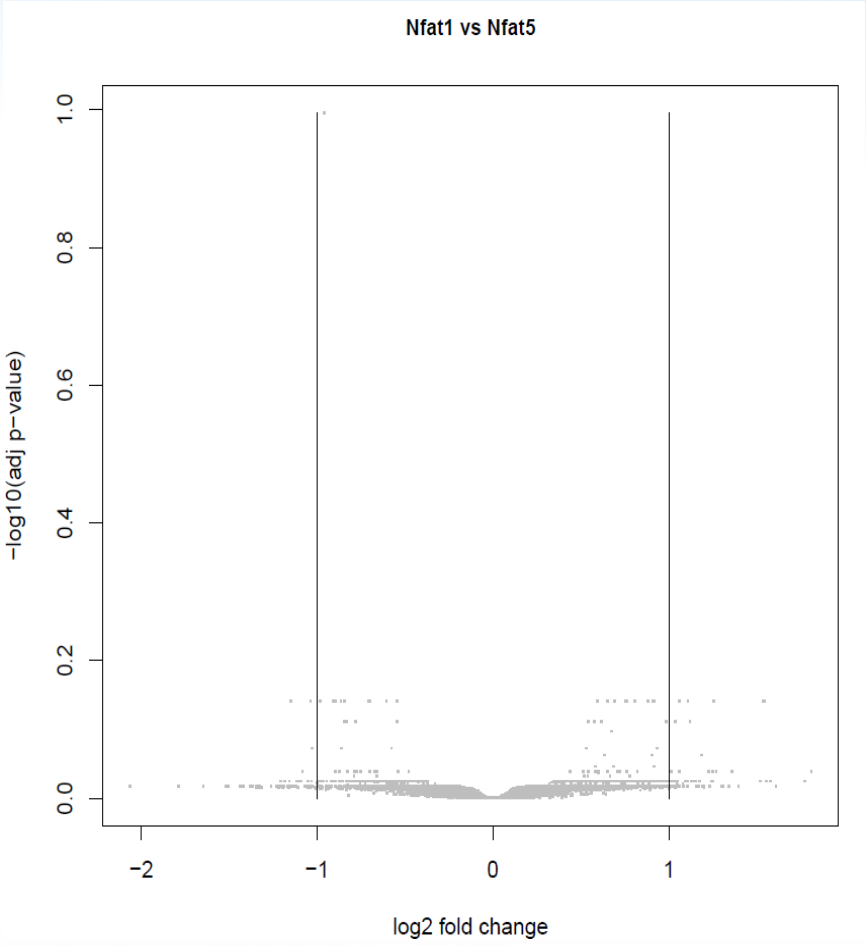


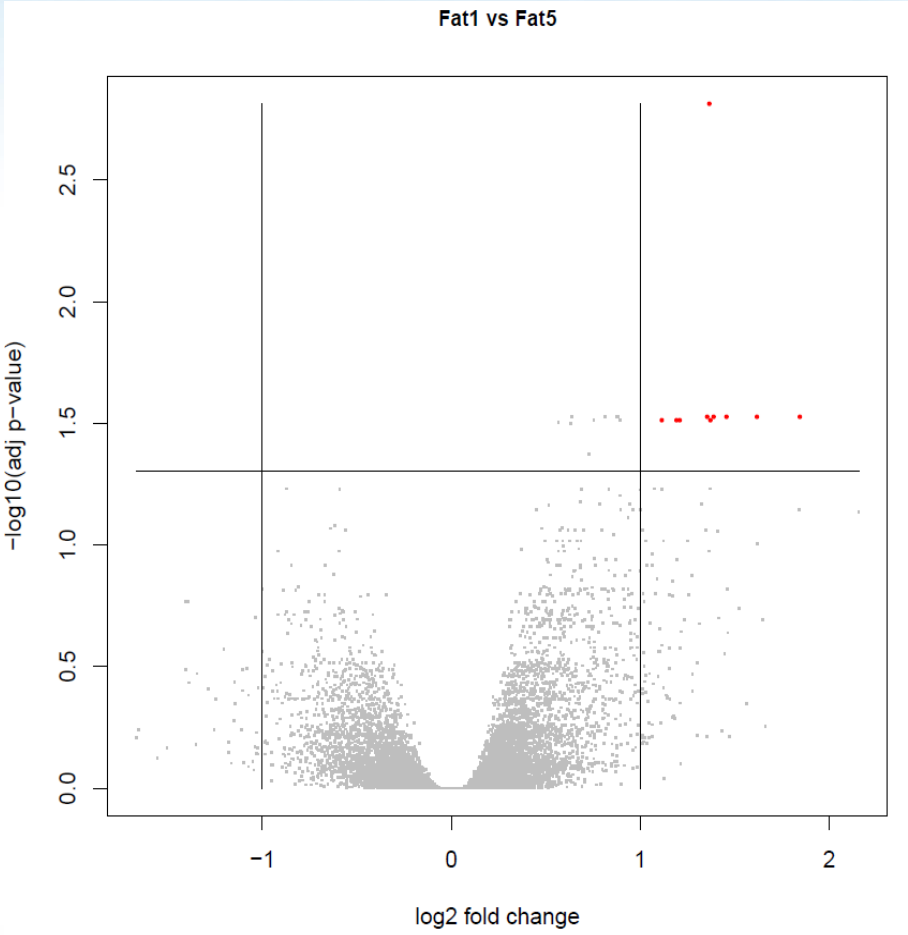


In-Vitro Confirmation (Monocytes)

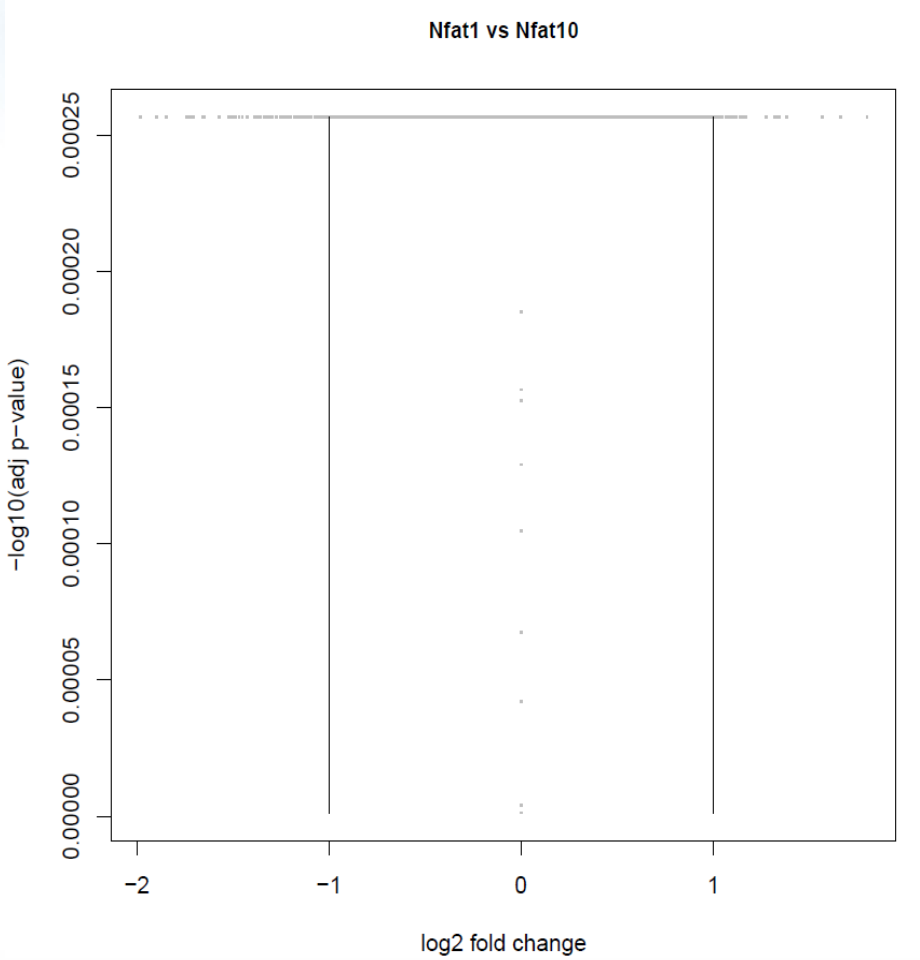


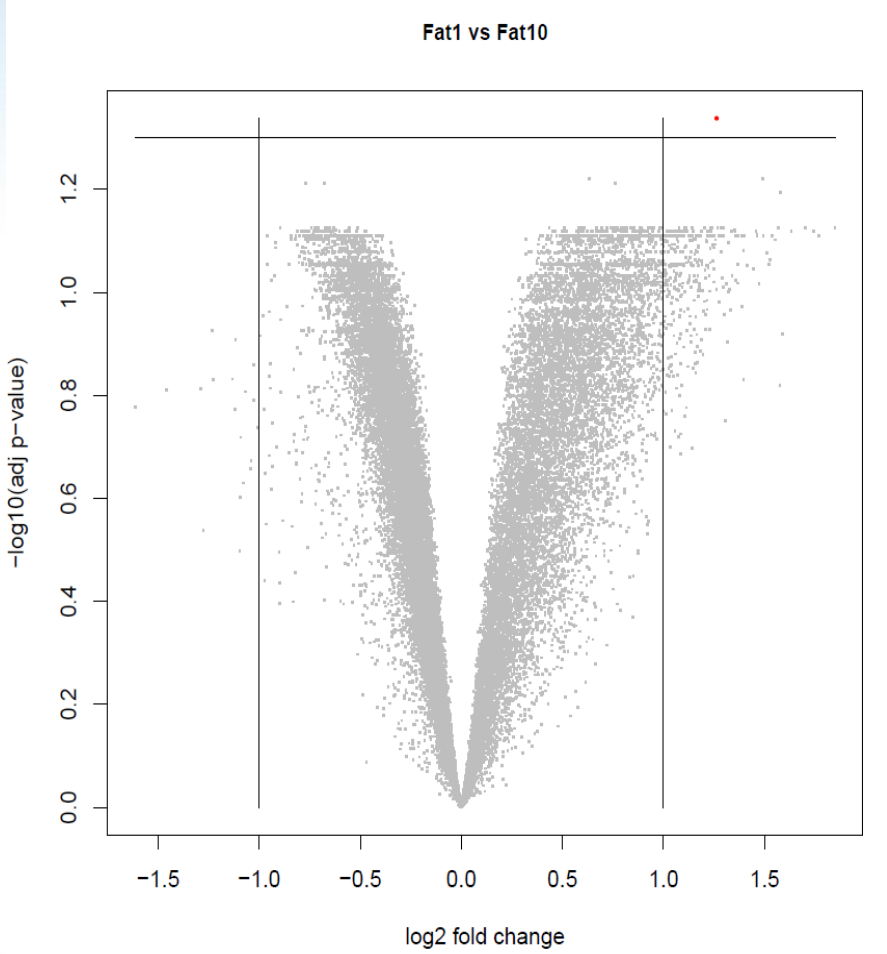
Differentially Expressed Genes
High Fatigue vs Low Fatigue (Mid-EBRT)





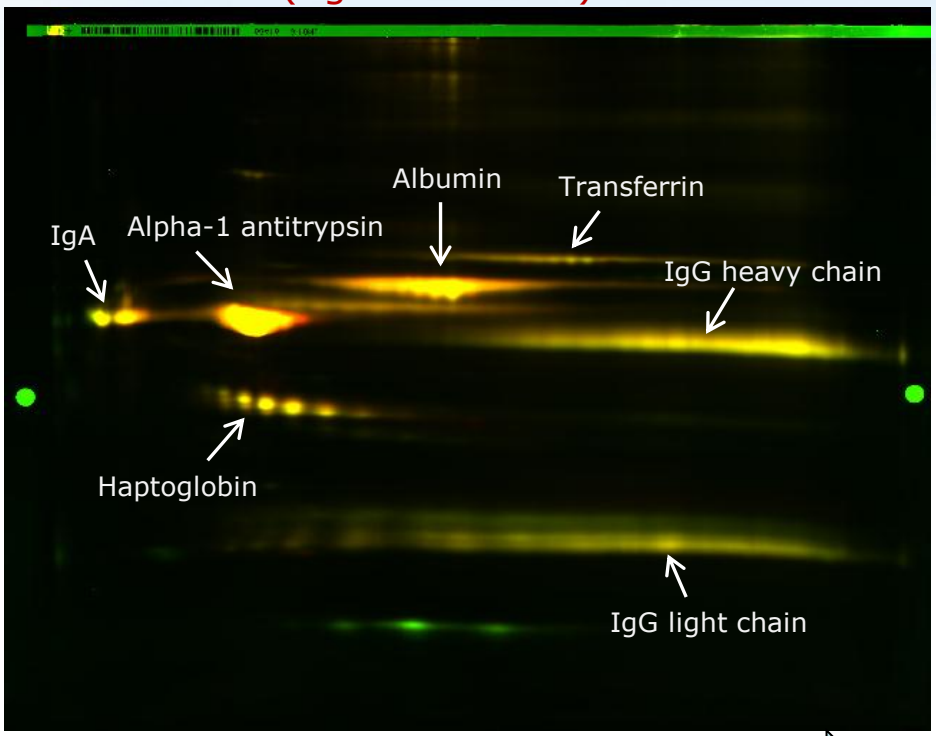
Differentially Expressed Genes
High Fatigue vs Low Fatigue (1 yr post EBRT)

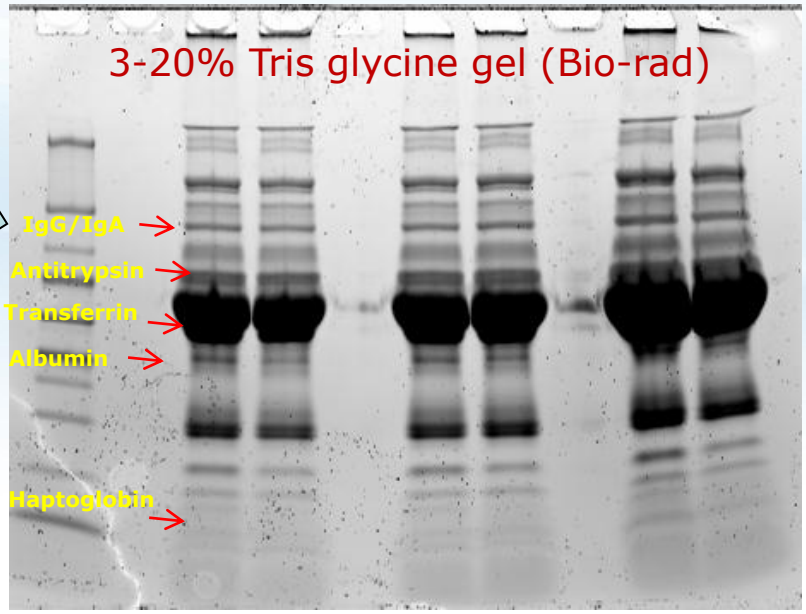




Proteomics

Top-6 depleted proteins
(Agilent column)





Probability Legend:

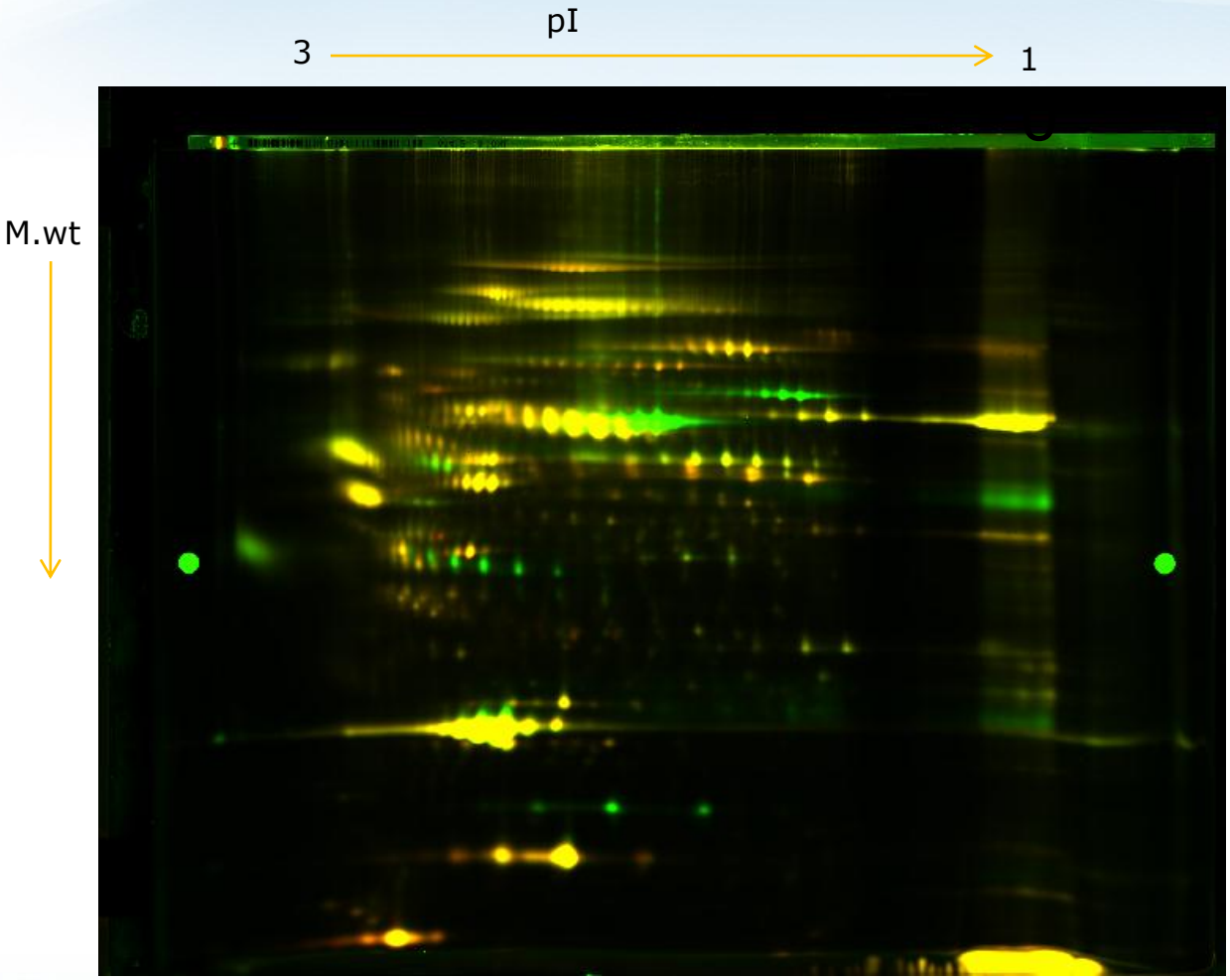
over 95%
80% to 94%
50% to 79%
20% to 49%
0% to 19%

Bio View:
Identified Proteins (107)
Including 0 Decoys

#	Visible?	Starred?		Accession Number	Molecular Weight	Protein Grouping Ambiguity	1stru... Dep D0 (1strun_DepletedA)	1stru... Dep D21 (1strun_DepletedB)	Elutio... EL D0 (ElutionA_1strun)	Elutio... EL D21 (ElutionB_1strun)
1	☑	☆	RecName: Full=Serum albumin; Fl...	ALBU_HUM...	69 kDa		1	3	62	65
2	☑	☆	RecName: Full=Complement C3; A...	CO3_HUMAN	187 kDa		115	113	6	2
3	☑	☆	RecName: Full=Alpha-2-macroglo...	A2MG_HUM...	163 kDa		68	70	7	3
4	☑	☆	RecName: Full=Ceruloplasmin; Alt...	CERU_HUM...	122 kDa		38	39	1	
5	☑	☆	RecName: Full=Hemopexin; AltNa...	HEMO_HUM...	52 kDa		21	21	5	4
6	☑	☆	RecName: Full=Apolipoprotein B-...	APOB_HUM...	516 kDa		93	114	2	
7	☑	☆	RecName: Full=Serotransferrin; S...	TRFE_HUMAN	77 kDa				34	33
8	☑	☆	RecName: Full=Alpha-1-antichym...	AACT_HUM...	48 kDa		18	17	2	2
9	☑	☆	RecName: Full=Apolipoprotein A-I...	APOA1_HU...	31 kDa		23	24	2	
10	☑	☆	RecName: Full=Ig gamma-1 chain...	IGHG1_HU...	36 kDa	★			14	14
11	☑	☆	RecName: Full=Complement C4-B;...	CO4B_HUM...	193 kDa	★	50	51	4	3
12	☑	☆	RecName: Full=Apolipoprotein A-I...	APOA2_HU...	11 kDa		9	8	2	1
13	☑	☆	RecName: Full=Alpha-1-antitrypsi...	A1AT_HUM...	47 kDa			1	14	15
14	☑	☆	RecName: Full=Complement facto...	CFAB_HUM...	86 kDa		19	20		
15	☑	☆	RecName: Full=Alpha-1-acid glyc...	A1AG1_HU...	24 kDa	★	10	11	2	2
16	☑	☆	RecName: Full=Inter-alpha-trypsi...	ITIH4_HUM...	103 kDa		24	26	1	
17	☑	☆	RecName: Full=Ig gamma-2 chain...	IGHG2_HU...	36 kDa	★			6	8
18	☑	☆	RecName: Full=Vitamin D-binding ...	VTDB_HUM...	53 kDa		23	23	3	4
19	☑	☆	RecName: Full=Haptoglobin; Cont...	HPT_HUMAN	45 kDa	★		2	13	14
20	☑	☆	RecName: Full=Inter-alpha-trypsi...	ITIH2_HUM...	106 kDa		19	19		
21	☑	☆	RecName: Full=Complement facto...	CFAH_HUM...	139 kDa	★	30	27		
22	☑	☆	RecName: Full=Ig alpha-1 chain C ...	IGHA1_HU...	38 kDa				8	10
23	☑	☆	RecName: Full=Inter-alpha-trypsi...	ITIH1_HUM...	101 kDa		17	15		
24	☑	☆	RecName: Full=Complement C5; A...	CO5_HUMAN	188 kDa		16	20		
25	☑	☆	RecName: Full=Alpha-2-HS-glyco...	FETUA_HU...	39 kDa		11	10	1	
26	☑	☆	RecName: Full=Ig lambda-2 chain...	LAC2_HUM...	11 kDa				4	3
27	☑	☆	RecName: Full=Ig kappa chain C r...	IGKC_HUMAN	12 kDa				8	6
28	☑	☆	RecName: Full=Transthyretin; Alt...	TTHY_HUM...	16 kDa		10	10		
29	☑	☆	RecName: Full=Antithrombin-III; ...	ANT3_HUM...	53 kDa		14	17	1	2
30	☑	☆	RecName: Full=Gelsolin; AltName: ...	GELS_HUMAN	86 kDa		13	15	1	
31	☑	☆	RecName: Full=Plasma protease ...	IC1_HUMAN	55 kDa		11	12	2	2
32	☑	☆	RecName: Full=Prothrombin; AltN...	THRB_HUM...	70 kDa		15	16		
33	☑	☆	RecName: Full=Heparin cofactor 2...	HEP2_HUM...	57 kDa		10	16		
34	☑	☆	RecName: Full=Alpha-1B-glycopr...	A1BG_HUM...	54 kDa		11	10	1	1

Proteomics

(8-16% gel for 2nd dimension)

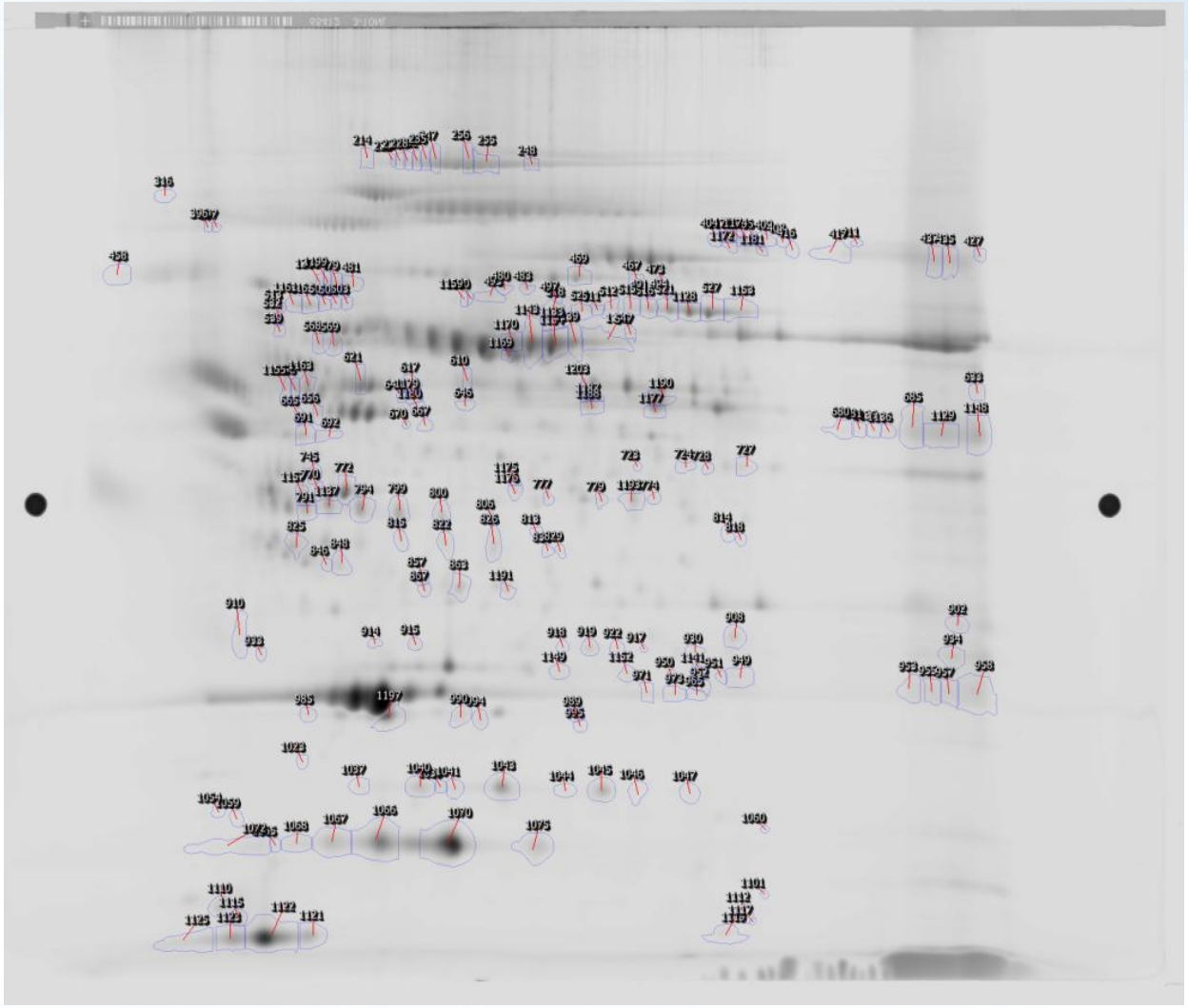


Yellow: No change in protein in control and treatment

Green: Proteins are down-regulated with treatment

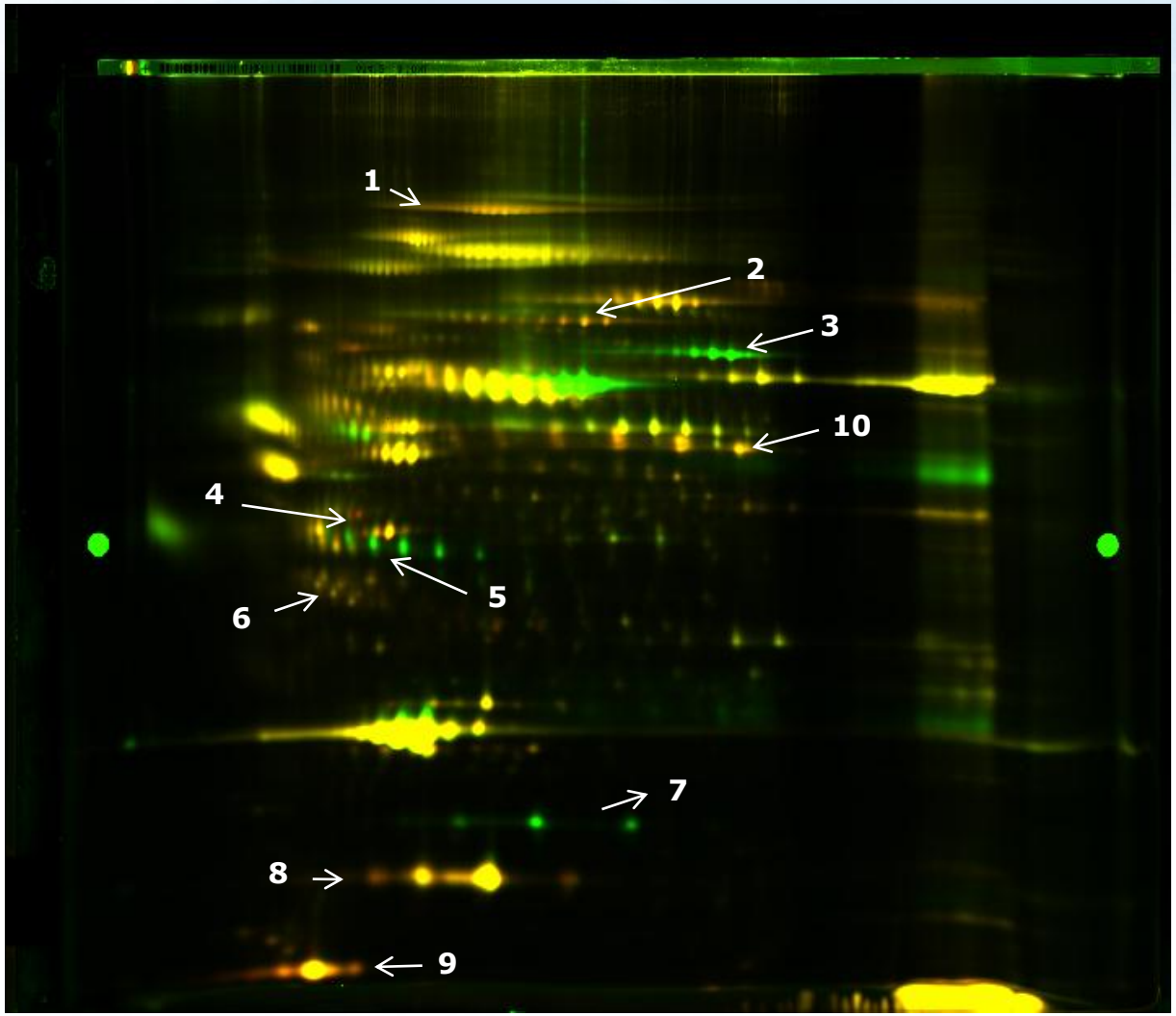
Red: Proteins are up-regulated with treatment

Proteomics



There were ~200 spots that indicate up/down regulation of proteins (1.5 fold change or more)

Proteomics



1. Complement factor H
2. Gelsolin
3. Serotransferrin
4. Serum paraoxonase/
5. Arylesterase 1
6. Haptoglobin
7. Clusterin
8. Haptoglobin related-protein
9. Transthyretin
10. Apolipoprotein A-II

Proteomics in Prostate Cancer

- Zn-alpha2-glycoprotein (ZAG), **haptoglobin** and apolipoprotein C-III increased in prostate cancer patients with bone metastasis (Zhang, Shen, Xianyu, 2010).

- **α2-macroglobulin (α2M)** is decreased in prostate cancer and functions as a carrier protein of IL-6 (Kanoh et al., 2011).

Proteomics in Fatigue

- Urinary levels of **retinol binding protein (RBP), prealbumin (PA),** and **transferrin (TFN)** are biochemical indicators of post operative fatigue syndrome (Mao, Chen, Zhao, 2002).

- Proteins found in CSF of patients with chronic fatigue syndromes (Baraniuk et al., 2005)
  - **α2-macroglobulin (α2M)**
  - **Gelsoline**
  - **Ig lambda**
  - **A-1-Antichymotrypsin**
  - **Haptoglobin**
  - **Hemopexin**
  - **Complement C3**

Peripheral versus Central Phenomenon

- HIGH (n = 14) and LOW (n = 24) fatigue groups based on the change in fatigue scores while receiving EBRT (N = 38).
- HIGH fatigue group had weaker MVC than LOW fatigue ($p = 0.04$).
- HIGH fatigue had longer reaction times with STROOP test than LOW fatigue ($p = 0.02$).

Summary

- Our findings suggest that fatigue may have both central and peripheral mechanisms.

- Differential expression of genes and proteins observed in this study suggest that pathways associated with stress and neuro-inflammation may explain mechanisms behind fatigue.

Follow-up Study

Ongoing activities investigating mechanisms to support peripheral and central causes of fatigue through

1. **Brain imaging: tagging α-synuclein**
2. **Expanding study to other types of clinical populations**

First Interventional Study

Exercise intervention

1. **Continuous training**
   - **target heart rate: 60-70% more than resting**

2. **High impact intensity training**
   - **target heart rate: >90% more than resting**

Hypothesis: improving fatigability will reduce overall fatigue

Acknowledgements

Chao-Pin Hsiao, PhD, RN
Nada Lukkahatai, PhD, RN
Michelle Millwood, BSN, RN
Maria Araneta, BS
Christina Yetter, BS
Benjamin Majors, BS
Dan Wang, PhD
Xiao-Min Wang, MD, PhD
Lena St. John, MD
Hyung Suk Kim, DDS, PhD
Raymond A. Dionne, DDS, PhD

CIT Collaborators:

Jennifer Barb, PhD
Peter Munson, PhD

NLM Collaborators:

Gelio Alves, PhD

NCI Collaborators:

Deborah Citrin, MD, PhD
Aradhana Kaushal, MD
Karl Haglund, MD, PhD
Ms. Debbie McNally, RN

Georgetown University Hospital Collaborators:

Hang Yuan, PhD
James Baraniuk, MD
Brian Walitt, MD, MPH

NIA Collaborator:

Heather Lucas, PhD

Rehab Medicine Collaborators:

Randy Keyser, PhD
Lisa Chin, PhD
Joshua Woostenhulme, PhD
Bart Drinkard, MS, PT