Neuro-endocrine and Immune Network Re-modeling in Chronic Fatigue Syndrome: An Exploratory Analysis

Jim Fuite[1], Suzanne D Vernon[2], Gordon Broderick[1 *]
[1] Department of Medicine, University of Alberta, Edmonton, AB, Canada
[2] CFIDS Association of America, Charlotte, NC, USA [†]

Introduction.

Chronic fatigue syndrome (CFS) is a neuro-immune disorder linked to chronic immune activation [1] and dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis [2,3]. For example CFS patients are reported to have significantly fewer CD3+/CD25- T cells and significantly more CD20+/CD5+ B cells [4]. In addition greater numbers of CD16+/CD3- NK cells are documented albeit with impeded cytotoxicity [5]. Upsets in immune demographics are reflected in cell-cell signaling and elevated levels of pro-inflammatory cytokines such as INF-α and TNF-α in CFS [6]. The HPA axis is central in modulating this inflammatory response through the synthesis of cortisol via a cascade involving adrenocorticotropic hormone (ACTH) and corticotropin-releasing hormone (CRH) [2]. Binding immune cell glucocorticoid receptors, cortisol signals a downregulation of pro-inflammatory cytokine production in turn captured by cytokine receptors located along the HPA axis [7]. Accordingly HPA axis dynamics are tightly coupled with those of the immune system through a variety of feedforward and feedback mechanisms. CFS patients inhabit a stable hypocortisolic state [3] highly conducive to the emergence of chronic inflammatory immune signaling. On this basis we propose that CFS involves not only a modulation but an emergent restructuring of neuro-immune signaling networks.

Only recently has CFS been examined from a network perspective [8]. Linear correlation of gene sets was used to create undirected graphs in non-fatigued and CFS patients from microarray data. We extend this work in several important ways. First we establish gene sets that are representative of immune cell subset activity enabling comparison of results with published flow cytometry findings. Second we incorporate neuro-endocrine data describing HPA and thyroid axis status in the analysis. Furthermore, mutual information (MI) rather than Pearson coefficient is used to capture nonlinear patterns of association [9]. Finally comparative measures of network topology are extended beyond global edit distance to include local measures of node centrality.

Data.

Association networks were constructed from the Wichita Clinical dataset [10] using neuro-endocrine measurements and gene expression in peripheral blood mononuclear cells (PBMC). A final group of 111 female subjects was obtained by excluding male subjects and subjects with confounding medical or psychiatric conditions. Diagnostic classification adheres to the CFS research case definition [11] resulting in 39 CFS and 37 non- fatigued (NF). Collection and processing of PBMCs including microarray hybridization are found in [10]. Details of the data preprocessing including normalization, outlier detection and false discovery correction are available in [12].

Methods.

Gene sets. Using data from Lyons [13], gene sets were constructed a priori from Affymetrix expression profiles of CD4+ T cells, CD8+ T cells, CD19+ B cells, CD14+ monocytes and CD16+ neutrophils isolated from peripheral blood. Of 12,022 genes

[†] Chronic Fatigue and Immune Dysfunction Syndrome

2,641 were differentially expressed across cell lines and 268 were present on the Wichita study microarrays. Profiles were dissected into discrete non-overlapping sets composed of genes at least 2-fold induced or 2-fold repressed preferentially in each lineage. Sets were also defined for NK and regulatory T cells. Set expression was computed as the mean of the Ln-transformed expression of member genes.

Mutual information networks. Association networks were constructed using mutual information criteria (MI) as implemented in the ARACNe software [9]. The null probability of MI values was computed by sub-sampling the data with replacement. Networks for each diagnostic class were generated from a consensus of at least 1000 sub-sampled networks. Indirect associations were removed using data processing inequality (DPI). General topological differences in networks were evaluated using graph edit distance [14] generalized for continuously weighted graphs. Significance of edit distance was estimated using equal-sized random networks created by edge shuffling: (i) conserving distribution of edge weight [15] and (ii) through multi-graphs conserving distribution in node degree [16]. Finally a null distribution was also computed from reference networks generated by random sub-sampling of non-fatigued subjects.

Results.

Undirected graphs computed for non-fatigued and CFS subject groups are visibly different in topology as shown in Figure 1. They are separated by a graph edit distance ~1.62 which is 83 standard deviations (0.02) above the expected distance between two networks constructed from randomly sampled non-fatigued subjects (~0.08). It is 4 standard deviations (~0.06) above the expected separation between two multi-graphs (~1.38) conserving node degree. However this separation is 5 standard deviations (~0.09) below the expected distance separating two naively shuffled networks (~2.07). The way in which this re-modeling occurs at the level of individual nodes is described through changes in node degree centrality, a measure of direct node connectivity, and eigenvector centrality, a measure of indirect connectivity (Table 1). Results indicate that immune function nodes are most altered in their association with the remainder of the network. In CFS, nodes associated with monocyte, NK cell and T regulatory activity are less connected both directly and through their immediate neighbors while nodes associated with T cell and neutrophil activity gain in interaction with the environment. The preferentially up-regulated genes in B cells abandon associations and the network shifts association towards lymphocyte functions that are preferentially suppressed in B cells. Neuropeptide Y also increases in node degree. All differences in node degree centrality are at least $p<0.01$ significant except in the case of estradiol, free testosterone, TSH and urine volume. In terms of eigenvector centrality, nodes for epinephrine and its metabolite metanephrin both dramatically increase their sphere of influence in CFS by finding first neighbors that are very highly connected. Eigenvector centrality is also dramatically increased in the case of C reactive protein. Again all changes in eigenvector centrality are significant at the $p<0.01$ level with the exception of creatinine and urinary cortisol. It is interesting to notice that regardless of this restructuring average direct and indirect connectivity is virtually conserved between non-fatigued and CFS networks.

Discussion.

The reported changes in connectivity of immune functional nodes align well with observations of altered immune activity in CFS. In particular monocytes, neutrophils and B cells are known players in chronic inflammation [17]. Neuropeptide Y (NPY)

receptors are present in most immune cells. Oddly in CFS NPY loses significant association with neutrophil activity and aligns instead with C reactive protein (CRP)- an acute phase reactant increased dramatically during inflammation. In CFS, the CRP node shifts association from monocyte and T regulatory nodes towards NPY and T cell nodes. This would suggest an alternate involvement of NPY in the inflammatory process. Although immune cell nodes are highly connected hubs and central to both non-fatigued and CFS networks direct connections between these nodes were not retained by the network identification process. This result indicates that immune cell subsets are more strongly associated to the neuroendocrine environment (i.e. cortisol) then they are to one another and that immune cell communication occurs principally through intermediaries. Further examination of the gene set design and the effects of network pruning with DPI are required to fully substantiate this observation.

Conclusions

We have successfully constructed association networks demonstrating the key role of immune function in CFS. Neuroendocrine immune networks differ significantly in topology between CFS and controls while being much more closely related than random networks.

References.

[1] U. Tirelli, D. Bernardi, S. Improta, A. Pinto, Immunologic abnormalities in chronic fatigue syndrome, J Chronic Fatigue Syndrome 2(1) (1996) 85-96.

[2] C.L. Raison, A.H. Miller, When Not Enough Is Too Much: The Role of Insufficient Glucocorticoid Signaling in the Pathophysiology of Stress-Related Disorders, Am J Psychiatry 160 (2003)1554–65.

[3] S.Gupta, E. Aslakson, B. M. Gurbaxani, S. D. Vernon, Inclusion of the glucocorticoid receptor in a hypothalamic pituitary adrenal axis model reveals bistability, Theor Biol Med Model 4 (2007) 8.

[4] M.J. Robertson, et al., Lymphocyte subset differences in patients with chronic fatigue syndrome, multiple sclerosis and major depression, Clin Exp Immunol 141(2) (2005) 326-32.

[5] M. Caligiuri, et al., Phenotypic and functional deficiency of natural killer cells in patients with chronic fatigue syndrome, J Immunol 139(10) (1987) 3306-3313.

[6] R.B. Moss, A. Mercandetti, A. Vojdani, TNF-alpha and chronic fatigue syndrome, J Clin Immunol 19(5) (1999) 314-316.

[7] M.N. Silverman, B.D. Pearce, C.A. Biron, A.H., Review: Immune Modulation of the Hypothalamic-pituitary-adrenal (HPA) axis during viral infection, Viral Immunol 18(1) (2005) 41–78.

[8] F. Emmert-Streib, The Chronic Fatigue Syndrome: A Comparative Pathway Analysis. J Comp Biol 14(7) (2007) 961–972.

[9] A.A. Margolin, et al., ARACNE: an algorithm for the reconstruction of gene regulatory networks in a mammalian cellular context, BMC Bioinformatics 7(Suppl.1) (2006) S7.

[10] S.D. Vernon, W.C. Reeves, The challenge of integrating disparate high-content data: epidemiological, clinical and laboratory data collected during an in-hospital study of chronic fatigue syndrome, Pharmacogenomics 7(3) (2006) 345-54.

[11] W.C. Reeves et al., Chronic fatigue syndrome - A clinically empirical approach to its definition and study, BMC Medicine 3 (2005) 19.

[12] G. Broderick, et al., Identifying illness parameters in fatiguing syndromes using classical projection methods, Pharmacogenomics 7(3) (2006) 407-419.

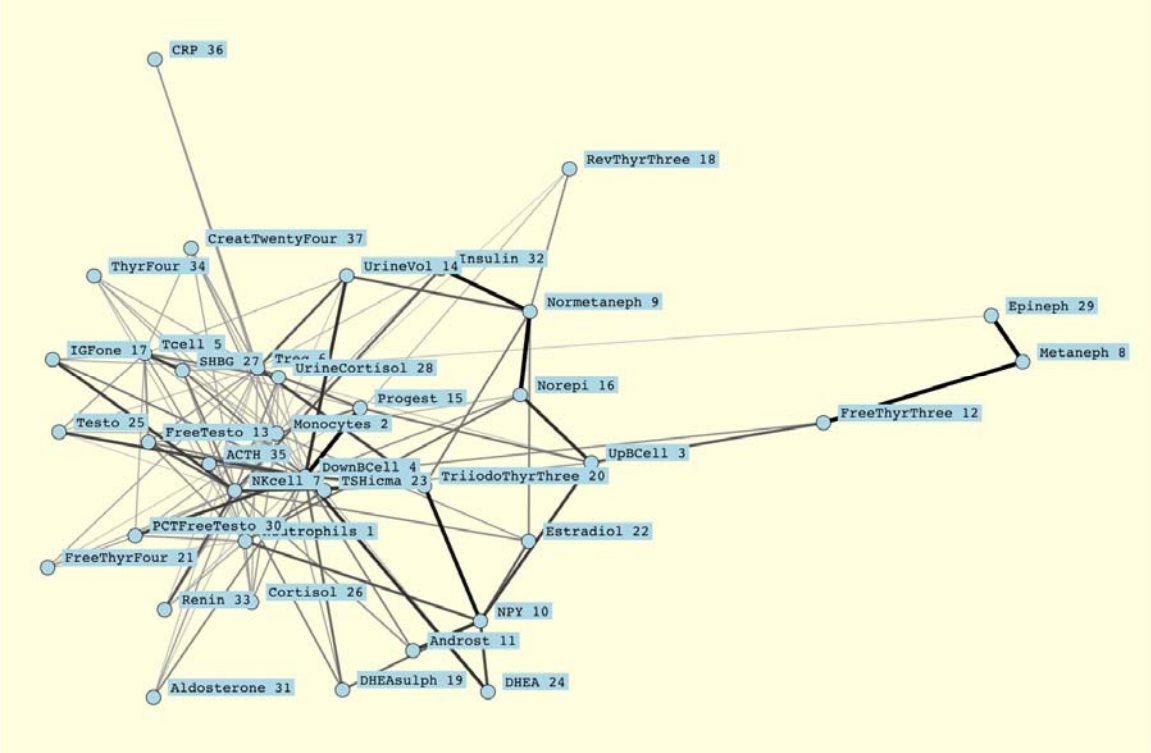
[13] P.A. Lyons, et al., Microarray analysis of human leucocyte subsets: the advantages of positive selection and rapid purification, BMC Genomics 8 (2007) 64.

[14] Bunke, H.: Graph matching: Theoretical foundations, algorithms, and applications, in Proc. Vision Interface 2000, Montreal, 2000, 82–88.

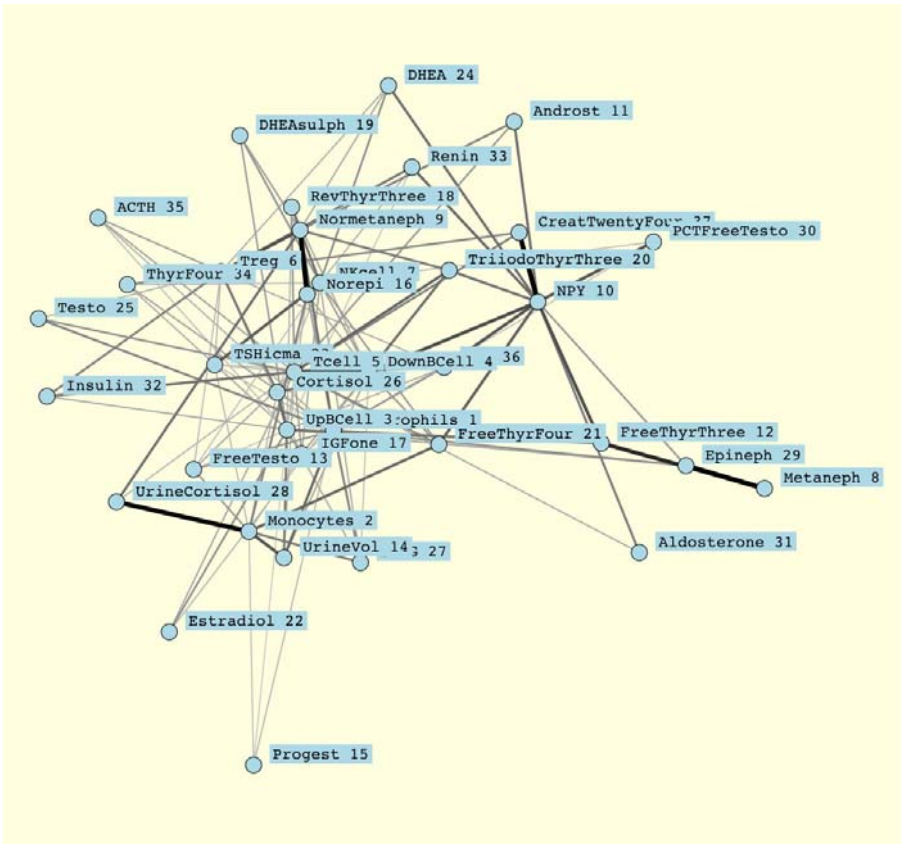
[15] R. Milo, N. Kashtan, S. Itzkowitz, M.E.J. Newman, U. Alon, On the uniform generation of random graphs with prescribed degree sequences, arXiv:cond-mat/0312028v2 [cond-mat.stat-mech] (2004).

[16] M.E.J. Newman, Analysis of weighted graphs, Phys Rev E 70(5) (2004) 56131-1-56131-9.

[17] Lefkowitz DL, Lefkowitz SS. Macrophage–neutrophil interaction: A paradigm for chronic inflammation revisited. Immunology and Cell Biology79 (2001) 502–506.



(A)



(B)

Figure 1. Mutual information networks of association between neuroendocrine measures and immune cell gene sets in non-fatigued controls (panel A) and CFS patients (panel B).

Table 1. Immune function nodes alter connectivity. Details of total weight and total number of edges linked at each node as well as edges acquired through first neighbors (Eigenvector centrality). Monocytes and NK cell nodes shed associations while T cell and neutrophil nodes acquiring new connections in CFS. Suppressed B cell genes are disavowed while up-regulated genes increase interaction with the neuroendocrine environment.

			Non-fatigued			CFS			Δ Connectivity		Δ Eig. Connectivity	
Node Name	Variable	Source	Weighted Node Degree	Node Degree Ceiling	Eigenvector Centrality	Weighted Node Degree	Node Degree Ceiling	Eigenvector Centrality	Edges	(%)	Edge Weight	(%)
Monocytes	monocyte activity	immune	0.19 (0.001)	22.9 (0.10)	0.23 (0.002)	0.13 (0.002)	10.6 (0.22)	0.20 (0.008)	-12	-54%	-0.03	-13%
DownBCell	suppressed B cell activity	immune	0.31 (0.002)	22.8 (0.20)	0.44 (0.002)	0.12 (0.003)	12.0 (0.30)	0.20 (0.006)	-11	-47%	-0.24	-54%
NKcell	NK cell activity	immune	0.22 (0.001)	19.2 (0.13)	0.29 (0.001)	0.12 (0.002)	13.2 (0.29)	0.16 (0.002)	-6	-31%	-0.12	-44%
Treg	T regulatory activity	immune	0.18 (0.001)	18.4 (0.16)	0.23 (0.001)	0.13 (0.002)	13.0 (0.30)	0.20 (0.002)	-5	-29%	-0.03	-14%
UrineCortisol	urine_free_cortisol_24h	adrenal cortex	0.06 (0.000)	7.0 (0.00)	0.14 (0.001)	0.05 (0.001)	3.6 (0.16)	0.14 (0.004)	-3	-49%	0.01	4%
Progest	progesterone	ovaries	0.10 (0.000)	6.0 (0.00)	0.27 (0.002)	0.02 (0.001)	2.9 (0.18)	0.05 (0.003)	-3	-52%	-0.23	-83%
ACTH	adrenocorticotropic hormone	pituitary gland	0.06 (0.000)	6.0 (0.00)	0.14 (0.001)	0.03 (0.002)	3.9 (0.23)	0.07 (0.004)	-2	-35%	-0.07	-48%
CreatTwentyFour	urine creatinine 24h	muscle	0.04 (0.000)	5.0 (0.00)	0.11 (0.001)	0.05 (0.000)	3.0 (0.00)	0.11 (0.002)	-2	-40%	0.00	0%
Aldosterone	aldosterone	adrenal cortex	0.04 (0.000)	4.0 (0.00)	0.10 (0.000)	0.02 (0.001)	2.1 (0.10)	0.05 (0.002)	-2	-48%	-0.05	-51%
Renin	plasma renin activity	adrenal cortex	0.05 (0.000)	5.0 (0.00)	0.12 (0.001)	0.03 (0.001)	3.1 (0.10)	0.08 (0.002)	-2	-38%	-0.04	-36%
PCTFreeTesto	procalcitonin / free testosterone	adrenal cortex / gonads	0.05 (0.002)	4.7 (0.15)	0.14 (0.002)	0.03 (0.001)	2.9 (0.18)	0.07 (0.002)	-2	-38%	-0.08	-53%
Androst	androstenedione	adrenal cortex / gonads	0.06 (0.002)	4.5 (0.17)	0.13 (0.003)	0.04 (0.000)	3.0 (0.00)	0.11 (0.001)	-2	-33%	-0.03	-20%
Testo	testosterone	adrenal cortex / gonads	0.05 (0.001)	4.9 (0.10)	0.14 (0.002)	0.04 (0.001)	3.9 (0.10)	0.09 (0.002)	-1	-20%	-0.05	-36%
SHBG	sex hormone-binding globulin	liver	0.07 (0.000)	6.0 (0.00)	0.17 (0.001)	0.05 (0.000)	5.0 (0.00)	0.10 (0.001)	-1	-17%	-0.06	-37%
ThyrFour	thyroxin T4	thyroid gland	0.04 (0.000)	5.0 (0.00)	0.09 (0.001)	0.04 (0.000)	4.0 (0.00)	0.08 (0.001)	-1	-20%	-0.02	-16%
TriiodoThyrThree	triiodothyronine T3	thyroid gland	0.09 (0.000)	5.0 (0.00)	0.21 (0.001)	0.06 (0.001)	4.4 (0.16)	0.16 (0.004)	-1	-12%	-0.05	-24%
UrineVol	urine volume 24h		0.07 (0.001)	4.9 (0.10)	0.16 (0.002)	0.06 (0.002)	4.4 (0.22)	0.13 (0.004)	-1	-10%	-0.03	-20%
Insulin	serum insulin	pancreas	0.05 (0.000)	4.0 (0.00)	0.11 (0.001)	0.04 (0.001)	3.6 (0.16)	0.08 (0.003)	0	-10%	-0.03	-25%
Estradiol	estradiol	Adrenal cortex / ovaries	0.05 (0.000)	4.0 (0.00)	0.09 (0.001)	0.03 (0.002)	3.7 (0.15)	0.07 (0.004)	0	-8%	-0.02	-18%
TSHicma	thyroid-stimulating hormone	anterior pituitary gland	0.06 (0.000)	7.0 (0.00)	0.13 (0.001)	0.06 (0.001)	6.9 (0.10)	0.12 (0.002)	0	-1%	-0.01	-9%
DHEAsulph	DHEA sulfate	adrenals	0.05 (0.000)	4.0 (0.00)	0.13 (0.001)	0.04 (0.000)	4.0 (0.00)	0.10 (0.001)	0	0%	-0.03	-22%
Metaneph	metanephrine	adrenal medulla	0.05 (0.000)	2.0 (0.00)	0.02 (0.002)	0.07 (0.000)	2.0 (0.00)	0.10 (0.004)	0	0%	0.07	292%
FreeTesto	free testosterone	thyroid gland	0.07 (0.001)	5.8 (0.13)	0.16 (0.002)	0.05 (0.001)	6.0 (0.21)	0.11 (0.004)	0	3%	-0.06	-36%
DHEA	Dehydroepiandrosterone	adrenals / gonads	0.05 (0.000)	3.0 (0.00)	0.12 (0.001)	0.04 (0.001)	3.4 (0.16)	0.08 (0.006)	0	13%	-0.04	-32%
FreeThyrThree	free triiodothyronine T3	thyroid gland	0.05 (0.001)	3.3 (0.15)	0.08 (0.004)	0.06 (0.001)	3.8 (0.13)	0.11 (0.005)	1	15%	0.04	47%
Norepi	norepinephrine	Adrenal medulla	0.09 (0.000)	5.0 (0.00)	0.16 (0.002)	0.11 (0.001)	5.8 (0.13)	0.39 (0.007)	1	16%	0.23	142%
FreeThyrFour	free thyroxin T4	Adrenal cortex / gonads	0.04 (0.000)	5.0 (0.00)	0.10 (0.001)	0.07 (0.001)	5.9 (0.10)	0.15 (0.003)	1	18%	0.05	45%
Normetaneph	normetanephrine	Adrenal medulla	0.13 (0.003)	6.9 (0.18)	0.16 (0.005)	0.16 (0.003)	8.2 (0.25)	0.42 (0.008)	1	19%	0.26	164%
RevThyrThree	reverse T3	thyroid gland	0.02 (0.002)	2.3 (0.21)	0.04 (0.006)	0.04 (0.002)	4.1 (0.18)	0.10 (0.007)	2	78%	0.06	158%
Cortisol	serum cortisol	Adrenal cortex	0.05 (0.001)	5.1 (0.10)	0.12 (0.001)	0.07 (0.000)	7.0 (0.00)	0.15 (0.002)	2	37%	0.03	27%
Epineph	epinephrine	Adrenal medulla	0.04 (0.002)	1.9 (0.23)	0.03 (0.006)	0.08 (0.001)	4.4 (0.16)	0.13 (0.006)	3	132%	0.10	386%
CRP	C reactive protein	liver	0.02 (0.000)	2.0 (0.00)	0.04 (0.000)	0.06 (0.000)	5.0 (0.00)	0.14 (0.003)	3	150%	0.10	230%
IGFone	insulin-like growth factor 1	liver	0.05 (0.000)	4.0 (0.00)	0.14 (0.001)	0.06 (0.000)	7.0 (0.00)	0.12 (0.001)	3	75%	-0.01	-11%
Tcell	T cell activity	Immune	0.13 (0.001)	12.2 (0.13)	0.17 (0.001)	0.17 (0.002)	16.4 (0.22)	0.21 (0.005)	4	34%	0.03	19%
Neutrophils	neutrophil activity	Immune	0.12 (0.003)	11.5 (0.27)	0.15 (0.004)	0.16 (0.002)	16.2 (0.25)	0.25 (0.006)	5	41%	0.09	60%
NPY	neuropeptide Y	brain	0.12 (0.000)	7.0 (0.00)	0.16 (0.001)	0.18 (0.001)	11.9 (0.10)	0.23 (0.006)	5	70%	0.06	39%
UpBCell	Promoted B cell activity	Immune	0.09 (0.003)	6.5 (0.17)	0.14 (0.005)	0.17 (0.002)	19.5 (0.27)	0.22 (0.004)	13	200%	0.08	60%
Average			0.08	6.86	0.15	0.07	6.48	0.14	0	0	0	0