

## The Promise of the Proteome

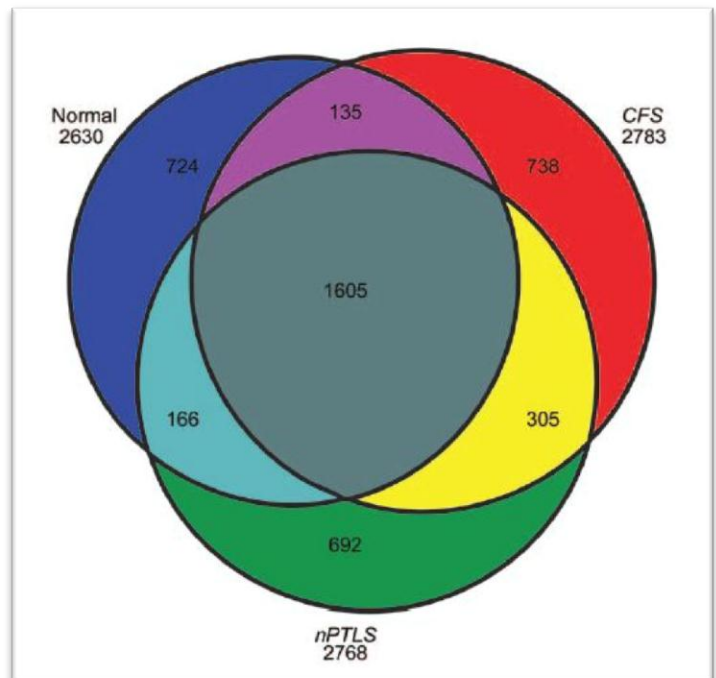
*Analysis of the PLoS ONE paper*

Today, February 23, 2011, in the online journal *PLoS ONE*, researchers from six institutions published a tantalizing study that uses powerful discovery technology to catalogue proteins in chronic fatigue syndrome (CFS) and neurologic post-treatment Lyme disease (nPTLS) spinal fluid samples. The exciting work is the product of a large team led by Steven Schutzer at the University of Medicine and Dentistry of New Jersey and Thomas Angel and Tao Liu of the Pacific Northwest National Laboratory.

In an article titled, "[Distinct Cerebrospinal Fluid Proteomes Differentiate Post-Treatment Lyme Disease and CFS](#)," Schutzer and colleagues tested samples collected by lumbar puncture from 43 CFS subjects carefully evaluated using [1994 Fukuda criteria](#), 25 subjects who met [CDC surveillance criteria for Lyme disease](#) and who had completed at least three weeks of intravenous antibiotic therapy at least four months earlier, and 11 healthy controls. The disease comparison groups were chosen because of the overlap of central nervous system (CNS)-related symptoms and lack of medical explanation for either CFS or nPTLS. They write, "Specific abnormalities found in cerebrospinal fluid (CSF) relating to CFS and nPTLS would suggest CNS involvement, and could facilitate mechanistic understanding."

The investigators combined two powerful technologies called mass spectroscopy and liquid chromatography to analyze the CSF samples. [Mass spectroscopy](#) is a tool that measures the mass of particles and thus the composition of a sample. [Liquid chromatography](#) measures the relative proportions of particles in a mixture. This combination of methods was chosen for its ability to cast a wide "discovery" net in the analysis of complex biological specimens, without having to define in advance what proteins might be present. The group recently established a comprehensive [normal "proteome"](#) as a baseline for comparison to disease samples. The term proteome refers to the entire set of proteins found in a biological sample; in this case, cerebrospinal fluid. Proteins are made up of smaller particles called peptides.

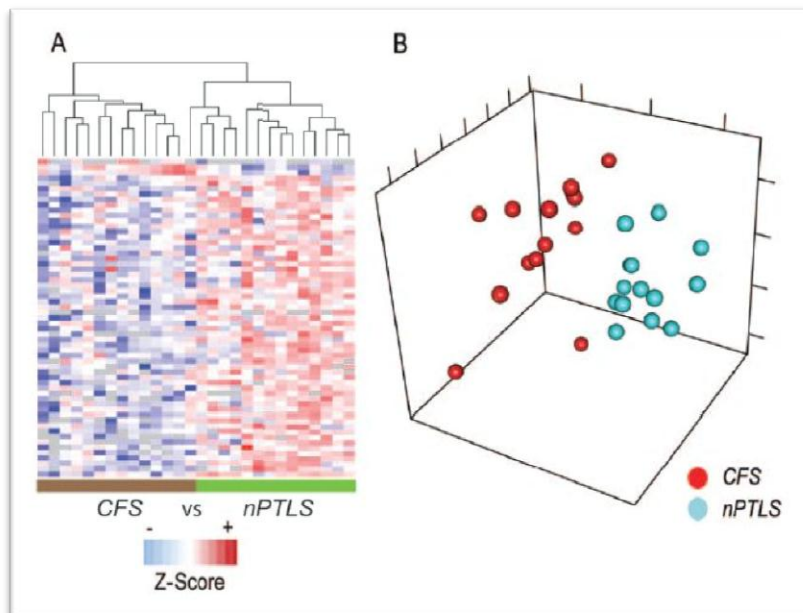
Using these tests the team was able to generate a comprehensive list of 30,000 peptides in the samples pooled from subjects in each disease group. Of these 30,000 peptides, 738 proteins were found only in CFS subjects (Figure 1). The nPTLS samples had 692 unique proteins and the normal controls had 724 unique proteins. Differences in the amounts of various proteins were detected between groups and CFS and nPTLS had more proteins in common than with the healthy controls.



**Figure 1: Venn diagram illustrating distribution of proteins identified in the three groups**

Looking at the biological pathways implicated by the different proteins identified, the team found that proteins in the [complement cascade](#) were elevated in abundance in the pooled nPTLS and CFS samples compared to controls, but at different levels for the disease groups. The complement system is a part of the immune system that helps to clear infectious pathogens. Interestingly, in a [2005 proteomics study](#) conducted by James N. Baraniuk and published in *BMC Neurology*, the complement system proteins were also found in cerebrospinal fluid from CFS patients and differentiated CFS patients from healthy controls. Alterations in the complement cascade pathways have been a relatively consistent abnormality in CFS patients (Sorensen B, et al., [2003](#) and [2009](#)). This convergence of data implicates biomarkers and pathways that should be prioritized for verification and validation.

Proteins involved in the CDK5 signaling pathway were significantly enriched in the CFS samples. Alterations in the CDK5 signaling pathway have been linked to [Parkinson's disease](#) and [Alzheimer's disease](#). Proteins relevant to specific neurological functions were lower in CFS than nPTLS, and both disease groups had lower levels than the healthy controls. The authors recognized that the clinical significance of the proteins and protein levels identified in the sample groups is "difficult to determine in the discovery phase."



**Figure 2: Comparative analyses of the individual CFS and nPTLS proteomes**

They also examined individual CSF samples (rather than pooled samples) from CFS and nPTLS subjects. In doing so, they identified a set of proteins that distinguished the two disease states (Figure 2A and 2B). By analyzing individual CSF samples, they determined that nPTLS patients are distinct from CFS patients. The ability to distinguish CFS and nPTLS on the basis of these proteins has important diagnostic implications. Because the etiologic agent for Lyme disease is known to be *Borrelia burgdorferi*, this finding suggests distinct pathophysiologies for these two clinically similar diseases. It also suggests different

treatment approaches may be warranted. Some have proposed that nPTLS represents a subset of CFS; however, the authors of this paper conclude that their data does not support that concept.

In the final discussion section, the authors state, "CSF proteome analysis may provide important and meaningful insights into the biological processes modulated as a function of disease and facilitate the identification of protein candidates for further investigation...Distinguishing CFS and nPTLS will have etiologic implications which could lead to novel diagnostics and therapeutic interventions." They suggest that by uncovering these candidates in cerebrospinal fluid, a targeted search in blood for these proteins is now possible.

This team of researchers provided the comprehensive list of proteins they identified as part of the open access paper. This will enable these and other investigators to explore and interrogate the data further. This list of proteins can immediately be put in the context of other findings in the CFS literature using cutting-edge computational and text mining tools. It could rapidly accelerate the identification of the most promising proteins and pathways for generating objective diagnostic assays and targeted treatments.

The Association's scientific director, Suzanne D. Vernon, PhD, notes, "I am particularly excited about this study and the new avenues it opens. Pairing this treasure trove list of proteins with the biological and clinical resources in the Association's [SolveCFS BioBank](#) will quicken the pace at which these biomarkers can be verified and validated, hopefully shortening the pipeline from benchside discovery to bedside application. The contributions made by this group to understanding the biology of CFS and nPTLS could not have come at a more timely moment for the field and the patient community." The firestorm generated by last week's publication of the U.K.'s injurious [PACE Trial](#) and uncertainty about the role of [XMRV/MLVs](#) in CFS have taken a toll on patients, advocates and researchers alike.

The study is a model for partnership across multiple institutions and funding agencies. It was supported by the National Institutes of Health (National Institute of Allergy and Infectious Diseases, National Institute on Drug Abuse, National Institute of Neurologic Diseases and Stroke and the National Center for Research Resources), the Swedish Research Council, Uppsala Berzelii Technology Center for Neurodiagnostics, SciLifeLab-Uppsala, Time for Lyme, Lyme Disease Association and the Tami Fund. The Pacific Northwest National Laboratory is a national scientific user facility sponsored by the Department of Energy. CFS patients were identified and evaluated by Benjamin Natelson at Albert Einstein School of Medicine. Lyme patients were identified and evaluated by Brian Fallon at Columbia University. Jonas Berquist of Uppsala University in Sweden assisted with data analysis. The collaborative effort, use of new technologies for discovery and willingness to openly share data to advance the field represent an inspiring 21<sup>st</sup> century research initiative worthy of high hopes.

The CFIDS Association of America is committed to advancing research that leads to the early detection, objective diagnosis and effective treatment of CFS. The scientific and medical communities are obligated to understand the biological roots of CFS so that targeted and effective treatments can be made available to the millions of people around the world whose lives have been derailed by CFS.

#### **References:**

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