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End ME/CFS Project Biomarker Discovery: Severely Ill Big Data Study



Looking for a
diagnostic biomarker

INTRODUCTION

The IOM report on ME/CFS states “Myalgic encephalomyelitis (ME) and chronic fatigue syndrome (CFS) are serious, debilitating conditions that impose a burden of illness on millions of people in the United States and around the world. Somewhere between 836,000 and 2.5 million Americans are estimated to have these disorders.” The report further states: “Diagnosing ME/CFS in the clinical setting remains a challenge. Patients often struggle with their illness for years before receiving a diagnosis, and an estimated 84 to 91 percent of patients affected by ME/CFS are not yet diagnosed.”

This proposal will conduct a “BIG DATA” analysis on ME/CFS in search of a sensitive and specific molecular biomarker(s). Although many of the symptoms are neurological, molecular biomarker(s) may be found in the blood, saliva, sweat, urine and feces. Identification of biomarker(s) in these easily assayed fluids can be convenient, inexpensive and could be conducted on a bedbound patient. This BIG DATA set will be released to the scientific community and serve to provide a better understanding of the disease and lead to effective treatment and prevention. It is vital to find a molecular biomarker(s) for the diagnosis of ME/CFS. Switching from a symptom-based to a molecular-biomarker(s)-based diagnosis will remove doubt about the diagnosis from the patient, from the medical community and the medical researcher.

There are several problems in finding the biomarkers. The disease may be heterogeneous, which will require biomarkers for each disease type. We don’t know where to look. It could be in the DNA, RNA, protein, carbohydrate or metabolite or could be from an associated microbe in or on the body. The biomarkers need to be easily retrieved (in a home for the seriously ill) and thus should be in the blood, urine, saliva, stool, and cerebral spinal fluid, sweat or breathe.

The biomarkers need to be present at all severities of the disease. It would be helpful for the quantitative biomarkers that their intensity reflected the severity of the disease. The biomarkers need to uniquely identify ME/CFS and distinguish from all other diseases (e.g. Fibromyalgia, Lyme, depression, trauma, infection, etc.).

The heterogeneity of the disease may be evaluated by biomarker profiling patients with identical or nearly identical symptoms. A clustering of a unique set of biomarkers with symptoms may indicate a unique form of the disease. Another method of clustering is to include the immune repertoire DNA sequence. This might include information in the sequence that uniquely identifies the cause of the original stress or infection.

We could also use biomarkers to indicate most likely treatment. Patients could be extensively profiled for biomarkers prior to any treatment. Those patients that respond favorably to treatment could then be retrospectively analyzed for unique biomarker profile that would prescribe the best treatment options for new patients. The biomarkers need not be a single component or several components of the same type (i.e., several RNA species) but could be a mixture of different components (i.e., several proteins from blood, a metabolite from urine and several DNA alleles). Development of a mixed biomarker set would be greatly facilitated if the search for biomarkers is conducted on the same large clinical sample for both cases and controls.

The biomarkers contained in the DNA could be single nucleotide polymorphisms, structural rearrangements, unique methylation or demethylation, unique binding or unbinding of proteins and other unique arrangements of DNA. The cellular origin of the DNA could be any cell that gives a unique signal but most likely would be a cell from the blood or other easily accessible source. We must also be aware of the possibility of mosaicism in the origin of the DNA.

The use of RNA as a source of biomarkers is likely to be a quantitative determination. High precision and reproducibility is necessary to give the best resolution and accurate diagnoses and should be tested and demonstrated for biomarker discovery. A unique immune cell type is likely to be the best source for the RNA. In the past, a mixture of all cell types in the blood has been used for the search for biomarkers.

However, this approach is less likely to give clear biomarkers because the RNA quantity in each cell type is different and the number of each cell type is likely different in each patient. We should focus on Natural Killer (NK) cells because they are usually reduced and/or inactive in ME/CFS/SEID patients and could be a good source of biomarkers or a component of a set of biomarkers. Another approach is analyzing individual cells. This will not require separating individual cell types if we can analyze a very large number of cells.

The use of proteins as a source of biomarkers follows a classical approach. There are numerous antibody methods and other assays that allow easy fast analysis. Some of the newer methods allow extensive multiplexing that might be required for ME/CFS. The discovery phase could use various mass spectroscopy methods that are now quite advanced. Unique protein modifications could also be used.

Many physicians and researchers speculate that some microbe is the initiating event of ME/CFS. Although this supposed organism(s) may not continue to be present, we must exhaustively search for them. This can be done by standard microbiome DNA sequencing from all body fluids. We can increase sensitivity if we first disrupt all human cells followed by

DNase treatment. The DNase resistant DNA will be from DNA containing particles, such as viruses, bacteria, fungus or parasite.

The recent advances in mass spectroscopy make searching for biomarkers among the small molecule metabolites a feasible approach. The biological source is likely to be blood, urine, saliva or cerebral spinal fluid although all bodily fluids should be evaluated.

The physiological state of the patient is likely to have a major impact on revealing suitable biomarkers. Because post exercise malaise is a major phenotype of ME/CFS this is the state that is most likely to contain unique biomarkers. Working with the most severely affected patients is also likely to give good biomarker signatures although they probably will not be able to enter a state of post exercise malaise unless they are constantly in this state.

Conducting all of these molecular investigations with state-of-the-art methodologies on well-phenotyped patients will be a daunting task. It will require significant resources and considerable coordination and cooperation within the scientific and medical communities.

RESEARCH

Project 1 Genomic Biomarkers of ME/CFS

Below is a list of assays that we plan to perform.

Blood:

- Whole Genome sequence
- Exome DNA sequence
- Mitochondrial DNA sequence
- Cell free RNA and DNA
- DNA methylation of all immune cells
- Metabolomics

Saliva:

- Whole Genome sequence
- Metabolomics

Urine:

- Metabolomics

Feces

- DNA sequence of microbes
- Metabolomics

Project 2 Immunology Biomarkers of ME/CFS

Blood:

Isolation of individual immune cell types. Especially NK cells
 Cy-TOF of many immune cell types
 Karyotype of immune cells
 Measure cytokine levels
 Activity of NK cells and gene expression

Project 3 Novel approaches to Biomarkers of ME/CFS

Blood:

HLA DNA sequence
 KIR DNA sequence
 Immune repertoire of all immune cells
 Gene expression of a mix of all immune cells on Affymetrix exon array
 Gene expression on individual immune cell types. Especially NK cells
 Gene expression on individual cells by molecular bar coding and sequencing
 Evaluation of alternative splicing from exon array
 Magnetic levitation profile of all immune cells
 DNA sequence all particles (virus, bacteria, fungus, & parasites)
 PCR assay for common viruses (EBV, HHV6, CMV, enterovirus,
 MS/antibody assay for mycotoxins
 Cu concentration and other metals
 Development of software for Hypothesis Generator using our data and all literature

Saliva:

DNA sequence all particles (virus, bacteria, fungus, & parasites)
 PCR assay for common viruses (EBV, HHV6, CMV, enterovirus,
 MS/antibody assay for mycotoxins

Sweat: In real time remotely by wearable electronics

Na, K, glucose, lactate and cytokines

Urine:

DNA sequence all particles (virus, bacteria, fungus, & parasites)
 PCR assay for common viruses (EBV, HHV6, CMV, Enterovirus)
 MS/antibody assay for mycotoxins
 Cu concentration and other metals

Feces

DNA sequence all particles (virus, bacteria, fungus, & parasites)
 PCR assay for common viruses (EBV, HHV6, CMV, enterovirus)
 MS/antibody assay for mycotoxins

PATIENT POPULATION

The first patient population that will be studied is the severely ill and bedbound patients. Being the most ill, they should show the strongest molecular biomarker signal. The signals that are seen will hopefully come from the disease but could also come from inactivity and being largely prone and confined to a bed. To some extent, we can control for the molecular biomarker signature coming from the physical environment by profiling other groups that are bedbound but clearly don't have ME/CFS/SEID or similar disease (Lyme, fibromyalgia, etc.). We will be required to travel to the patient. We will have to develop the equipment and protocols to process the samples on site.

ANALYSIS

We will conduct an extensive bioinformatics analysis on the data sets. We will search for the best combination to generate a diagnostic biomarker set. Because of the large data sets, false discovery is of serious concern. Therefore, we will evaluate our diagnostic molecular biomarker set on different patients with and without ME/CFS to determine sensitivity and specificity. Another problem is the possibility that ME/CFS is heterogeneous and no biomarker set can be found. We will then attempt to cluster the patient population into groups and search for a biomarker set in each group. The immune repertoire of all immune cells may be a useful data set to achieve biological meaningful clustering. It is possible that the repertoire will reflect the initiating event. This approach has been successful for other diseases.

This BIG DATA set will be useful toward understanding the disease and may suggest various treatments. It also might suggest methods of prevention. There is the remote possibility that nothing useful is found. In this case because we focused on using the best and latest technology and focused on best practices we can move on. The experiments conducted here will not have to be repeated. Also because the data will be in the public domain other investigators can explore other analysis and uses without the expense of doing additional experimentation. If BIG DATA of accessible body fluids does not yield useful results then we will probably have to investigate the brain, which will be more difficult and expensive.

BUDGET

Our goal and plan for this initial study is to raise \$1 million. To run this extensive data set, \$25,000 for each patient will be needed for logistical and supply costs. We will run the tests on as many patients as we can with the funds that we raise.

This proposal was written by OMF Scientific Advisory Board Director, Ronald W. Davis, PhD. 4-27-15.