Request for Information: Soliciting Input for New Research Strategies for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) - Notice Number: NOT-NS-16-024

Sent from: ESPA Research
2A Hylton Park Road, Sunderland SR5 3HD, UK

Prof. Malcolm Hooper
Mr Paul Shattock
Mr Kevin Carr
Mrs Lynda Todd
Dr Paul Whiteley

Research Need:
Development of a potential bloodspot biomarker-based intervention for the treatment of phenotypes of myalgic encephalomyelitis (ME)

Comments:
Myalgic encephalomyelitis (ME) also known as chronic fatigue syndrome (CFS) and/or systemic exertion intolerance disease (SEID) represent a heterogeneous group of debilitating disorders markedly affecting various aspects of daily living and functioning. Ranked as ‘very poor’ in terms of their effects on health-related quality of life [1], further research efforts are required in order to establish roles for potential aetiological agent(s) and mechanisms pertinent to the onset of the condition(s) subsequent to providing reliable intervention and treatment options.

Encompassing a wide range of clinical presentations, evidence is emerging on the appearance of specific phenotypes covered under the umbrella term ME. A role for enteroviruses in cases of ME has been proposed [2] supported by clinical findings in both animal and human studies. The idea of a ‘smouldering infection’ due to inappropriate clearance of a pathogen or some immunerelated ‘burnout’ fits well with the known clinical course of some cases and preliminary corresponding research data on immune function [3]. Whether this enteroviral-related ME can be biologically ‘fingerprinted’ remains to be seen as does the utility of providing individual interventions to this specific patient group and beyond. We propose a 2-stage pilot metabolomic study based on the examination of a patient group diagnosed with ME with historical evidence of previous or current coexisting enteroviral infection. Based on the use of qToF (quadrupole time of flight) mass spectrometry, we propose analysing captured bloodspot data specifically linked to amino acid function and regulation onwards to detailing potential biomarkers and their usefulness in guiding intervention options. An
open trial of supplementary amino acids determined on the basis of their blood spot results would be subsequently initiated.

Initial (unpublished) data based on a small group (N=5) of patients has suggested that specific elements of amino acid metabolism may be affected in this patient grouping, specifically those compounds related to the metabolism of arginine, ornithine and citrulline. These amino acids are involved in various biological processes but of specific relevance to our proposal is their involvement in inflammatory and/or oxidative stress pathways. That some of these compounds and their biological relations have previously been linked to the attenuation of fatigue following exercise [4] is also particularly relevant to ME.

We have already developed mass spectrometric methods for the analysis of amino acids in dried blood spots as part of unrelated research work. Whilst such a sample medium is slightly more invasive than the collection of other media such as urine or saliva, the requirement for only several drops of blood over larger volumes collected via more traditional blood draws is an advantage. So too are the storage arrangements for such samples and if necessary, comparisons with other archived blood spot samples if available. Insofar as the analytical capacity of the proposed method, our use of an accurate mass spectrometric technique provides precise resolution and identification of target compounds. The automatic archiving of sample results as they are run on the analytical system also provides us with the means to reanalyze samples if other compounds of interest are detected.

Our proposal includes: (1) Recruitment of ‘enteroviral ME’ participants (n=50), ‘nonenteroviral ME’ (n=50) and asymptomatic age and gendermatched controls (n=50); (2) Baseline interviews/questionnaires about core ME symptoms and associated comorbidity will provide information on various clinical parameters; (3) Blinded analyses of fasting blood spot samples for all groups (more than one bloodspot from each participant may be analysed to provide data on intrasubject variability for example). We may also capture urine samples from participants in order to ascertain any loss of amino acids via such a medium; (4) Based on 2 analytical results, the use of specific supplementary amino acids will be provided accordingly to participants for a period of 2 weeks; (5) Baseline measures will be repeated and results analysed. Clinical trial ethic review, public registration and results publications will be undertaken. The chosen research design reflects the preliminary nature of this area of investigation. Should results indicate some usefulness of the approach suggested, subsequent studies based on a doubleblind, placebocontrolled methodology could be carried out. The primary outcome measures based on any indicated use of supplementary amino acids will be any change to bloodspot chemistry and any change to the behavioural (fatiguerelated) parameters captured.

The suggested use of metabolomics, specifically the use of qToF mass spectrometry, to ME research represents a novel application of this ‘goldstandard’ technology. Assuming positive results are obtained from this and any subsequent studies indicated, the application of this technology to largescale screening of patients with ME is relatively simple and costeffective (where techniques linked to the existing newborn screening program for inborn errors of metabolism could be adapted to serve such a screening function).

References


**Background**

Since 1997 Professor Malcolm Hooper has been associated as a scientist and advocate with people with ME. Much of his work until now has involved advocacy as an informed scientist, see [http://www.nameus.org/defintionspages/DefHooper.htm](http://www.nameus.org/defintionspages/DefHooper.htm) and [http://www.meauctionuk.org.uk](http://www.meauctionuk.org.uk) for summary. He first worked with Dr John 3 Richardson one of the great figures of the ME world who spent 50 years as a GP and carried out considerable research studies into entroviral ME. John died in 2002 and left behind a successor Dr Irving Spurr and the John Richardson Research Group an international group of interested scientists which includes Drs Byron Hyde and Betty Dowsett, now deceased.

For many years Malcolm has been involved with autism research and in supporting sick Gulf War Veterans from the first Gulf War, 19901. The work with autism has involved ESPA (European Services for People with Autism) Research. The emergence of sound biochemistry and analytical chemistry in the area of autism which includes curious similarities with some aspects of both ME and Gulf War Illness/Syndrome have been noticed. ESPA Research now has leading edge technology, qToF mass spectroscopy coupled to chromatographic techniques to investigate all these conditions that has resulted in the development of single blood spot analyses for many important amino acids and related metabolites, particularly those associated with the homocysteine cycle. Mr Kevin Carr & Dr Paul Whiteley run this facility and have a good publishing record in the field of autism.

Malcolm is also the scientific advisor to the Tyne and Wear ME group of 200 patients who have been rigorously assessed by Dr Spurr. We have begun initial studies on well characterised ME patients and found some very interesting results that point to possible marker(s) for ME using this established test procedure and also to some possible useful, simple and cheap treatments which are the basis of a grant application to the NIH which we intend to submit following the announcement by Dr Vicky Whittemore at the recent Invest in ME Conference in London, 3rd June 2016. Malcolm discussed this possibility with Dr Whitttemore following her Conference presentation.

---

Weir, William RC, FRCP FRCP (Edin).

[...] Subject: Successful treatment of ME/CFS with antiretroviral

I am an infectious disease physician with an interest in ME/CFS. I am retired from the UK National Health Service now but retain my interest in ME/CFS and continue to see and treat patients with this condition. I wish to report some preliminary observations.

I have prescribed the antiretroviral, tenofovir, in the treatment of ME/CFS. I have three patients with ME/CFS who recovered whilst being given this drug. Signs of recovery did not appear until the third/fourth month. Two of them had IRIS like features during the first three months of treatment reminiscent of what I have seen in the treatment of HIV infection. I have also had to stop the tenofovir in 3 other patients in whom there was no beneficial response after 5 months. One of these was also having severe IRIS like symptoms and felt unable to carry on with the drug. In the recovered patients, I think placebo response was an unlikely explanation insofar as there was no
beneficial effect until the 3rd/4th month. The occurrence of IRIS in two of them is highly suggestive to me of successful treatment of an immune-suppressing retrovirus. Could this be a HERV?

I now have 6 other patients on tenofovir, but all are within 3 months of starting the drug, and I do not expect any beneficial effects as yet. I intend to report on these when they have completed 4 months of treatment.

Of interest is that the apparent beneficial effects in Rituximab treated patients took a minimum of 16 weeks to appear. With the benefit of some (informed) guesswork I think that a retrovirus is the root cause of ME/CFS and that its preferred residence are CD20 cells, just as the preferred residence of HIV are CD4 cells. I am very excited by the response of the patients I have described and feel very strongly that a full double blind, randomised, controlled trial with close monitoring of immunological parameters is now warranted using antiretroviral therapy. As I am now retired I am not in a position to organise this but would be very happy to provide input based on my experience of seeing approximately 2,000 patients with ME/CFS.

Chia, John, MD

Subject: Re: NIH Request for Information (RFI) on ME/CFS

Please provide your perspective on the following issues as they relate to the Working Group's planning efforts:

Before you consider or dismiss enteroviruses as the cause(s) of ME/CFS, ask yourself the following questions: How many cases of common or serious enterovirus infections have I seen in the previous 1-5 years? How well do I know enteroviruses in my research and clinical training? Have I ever been taught how to recognize enterovirus infections? Do I have any knowledge concerning chronic enterovirus infection in human and in animal models? Do I know how to order and interpret sensitive and specific blood tests for different serotypes of enteroviruses? Do I know how many serotypes and genotypes of HEV (human enteroviruses) have been found?

If the answers are no and no ----, then you should read the following:

- Emerging needs and opportunities that should be considered as new ME/CFS research strategies are developed.

To further define the role of chronic enterovirus (EV) infection in ME/CFS by critically examining the manifestations of initial illnesses and epidemiologic information, perform or order sensitive and specific blood tests for enteroviruses, obtain and look at tissue biopsies for presence of persistent infections, develop better molecular or other diagnostic tests from the initial groundwork, and evaluate antiviral drugs in vitro and in animal models before clinical trial in ME/CFS patients.

Challenges or barriers to progress in research on ME/CFS.

The traditional belief and approach to chronic viral infection in ME/CFS was based on antibody testing for common, well-known Herpesviruses such as EBV, CMV and HHV6 which are known to
persist in the human hosts, even though the initial clinical syndrome was inconsistent with these virus infections. Do we remember some of the infectious diseases physicians/researchers who looked at these elevated antibody titers in symptomatic gay males in 1979-1980 and thought EBV and CMV had mutated and started lysing CD4+ T lymphocytes which led to AIDS? Once the technique of HIV culture was optimized, this cytopathic virus could be found in the blood of most, if not all the AIDS patients. ME/CFS was even more difficult challenge since coining of the term in 1984.

The most important evaluation of ME/CFS is when, how, and what happened with the initial illness and the epidemiologic clues vs. continuing dependence on useless blood tests or imaging techniques. One has to be familiar with the protean manifestations of acute and chronic enterovirus infections. The sometimes epidemic nature of ME/CFS, i.e. the Incline Village Outbreak, was a missed opportunity for the NIH/CDC scientists who had several misconceptions and erroneous assumptions, as we now know:

1. EBV, the usual cause of mononucleosis, a self-limited fatiguing illness, was the primary virus candidate for ME/CFS. Although reactivation of EBV is possible after another acute viral infection, the epidemic nature of the Lake Tahoe outbreak was much more consistent with EV infection: a. the epidemic started in the summer when people have much more contact with lake water. b. EVs are known to exist in the lake/river water since 1970's according to the elegant works by Dr. Joseph Melnick, c. there was a major sewage spill on the implicated side of the large lake within the prior year, as documented in court proceeding papers and people knowledgeable of the epidemic, which likely served as a major amplification step for growth of EV. d. EVs are known to be associated with more than 100 species of fresh water protozoans, according to Scandinavian investigators. e. many of the Incline village patients had diarrhea in the beginning of the illness before developing ME/CFS, according to treating physician Dr. Dan Peterson, which was extremely uncommon for well-documented acute EBV infection.

2. Although the double-blinded, placebo-controlled IV acyclovir trial published in NEJM in 1988 was flawless in its design, there were two major problems: a. the wrong virus, EBV, was treated with IV Acyclovir, a drug has no activities against enteroviruses, b. the level of improvement in the drug- and placebo-treated groups was set too low, and therefore about 30% of the patients in both groups had some minor improvement. The conclusion was “since significant number of placebo-treated patients had improvement, there had to be a significant psychogenic overlay”, instead of stating antiviral drug, Acyclovir, had no better effect than the placebo. This study was a major disservice to the field and further popularized ME/CFS as a psychological disease rather than pursuing a true organic cause(s).

3. Our continuing objection to the name ME and enterovirus theory proposed and studied by the British investigators -“the people on the other side of the Atlantic ocean”. Well-done, blinded studies in England have showed enterovirus RNA in 39% of blood samples taken from ME patients, as compared to 2% of blood samples from normal controls; and furthermore, EV RNA was demonstrated in muscle biopsies and also from various parts of the brain of a ME patient who committed suicide after 5 years of illness (published in Ann. Internal Medicine
British investigators objected to the name of CFS, and the name ME and CFS was only merged as one in 2007.

4. Chronic enterovirus infection in human should have continuing shedding of viruses in the stool and viremia, which has never been proven in animal models. In fact, in human with acute enterovirus infection, most of the stool samples would test negative after 3 weeks. Some exceptions did exist with asymptomatic poliovirus infection. EV RNA is detected in the blood at very low levels, as we have found in about 2005, which was never quantified in the 1980-90's. The sensitivity of the EV RNA testing was not clearly stated and may have been partly responsible for the discrepancy of results reported from different laboratories. Did we not remember many well-known laboratories had difficulty culturing HIV from the blood taken from asymptomatic HIV+ patients in the late 1980's and the initial sensitivity of HIV RNA test was 4000 copies/mL after many years of development instead of 20 copies in the recent years?

5. Persistent EV infection in a human host should continue to produce live viruses. No studies have actually documented cytopathic viruses from the blood and tissues of ME/CFS patients in the last 3 decades but EV RNA was found in these samples by a number of studies in human and animal model of chronic EV infections. Positive strand EV RNA and double-stranded RNA are potentially infectious in cell culture under optimal conditions. The concept of persisting, replication-competent double-stranded RNAs, which has been found in muscles and stomach of ME/CFS patients and pathogenic in SCID mice, should be further investigated and confirmed as the disease-causing mechanism by other laboratories.

Gaps and opportunities across the research continuum from basic through clinical studies.

Most, if not all of the patients from around the country, many of them were cared for by other ME/CFS experts, never had the appropriate testing for enteroviruses. There were no details of the primary infection that led to ME/CFS. All flu-like illnesses are not the same. Most of the serologic tests included EBV, CMV, HHV6, HSV1,2, VZV, which are not necessarily the etiologic agents of ME/CFS. Many of the patients were treated with Valacyclovir and Valganciclovir for 1-2 years without significant improvement. Physicians need to be better educated with knowledge of acute and chronic EV infection, before they can make better and independent assessment of the role of EV in ME/CFS.

1. The studies should focus on initial manifestation of acute enterovirus infections and learn how it leads to chronic persistent infection in human and in animal models.
2. Look for elevated neutralizing antibody for 11 of the 70+ serotypes of enteroviruses (Coxsackievirus B1-6 and echovirus 6, 7, 9, 11, 30, 11 of the most common enteroviruses in the past 30 years, tests need to be done at ARUP laboratory, Salt Lake City, Utah)
3. Look for the persistent viral infection in the tissues, i.e. stomach biopsies and muscle biopsies or other sites (i.e. the lymphoid follicles and swollen erythematous pharyngeal or tonsillar tissues).
4. Confirm and extend the finding of EV protein and RNA and host responses in brain samples of ME/CFS patients
5. Look for other laboratory techniques and imaging modalities to document the presence of chronic, persistent EV infection.
When commenting on a research need or scientific opportunity, The Working Group suggests that your comments contain the following for our full consideration:

Description of the need or opportunity

Prompt funding of Centers of Clinical Excellence for continuing research on the pathogenesis of chronic enterovirus infection based on past contributions to the understanding of chronic enterovirus infection and ME/CFS, and also for development of better diagnostics and effective antiviral strategies for these viruses. Many of the ME/CFS physicians/researchers are providing care to these highly complicated and needy patients and have little time to write papers and grants to continuing good scientific research. Governmental contracts should be given to the Centers instead of applying for grants, which are not funded until 18 months later. For examples: NIH and CDC contracts were given to the best HIV researchers (i.e. Dr. David Ho) to culture HIV from the blood of AIDS patients in 1988. Continuation of support will depend on progress reports.

Scientific rationale and potential public health impact

Based on clinical, epidemiologic, laboratory and pathological studies from the past 30 years, there is little doubt that chronic enteroviruses are the major causes of ME/CFS. The importance of establishing these viruses as the cause of acute infections can lead to chronic disease states can lead to:

1. Prevention of acute infection by limiting exposure to contaminated water and food.
2. Better prevention of spread of acute respiratory infections by masks and other means.
3. Establish better rapid molecular diagnostic testing for these viruses, as currently available for Influenza viruses with subtyping.
4. Establish an unmet medical need for faster antiviral drug development to treat acute and chronic infections.
5. Better government-sponsored education of ME/CFS patients and general public that chronic EV infections are the causes of this elusive disease.

Anticipated challenges that will need to be addressed

1. There are more than 70 serotypes and > 120 genotypes of enteroviruses found to date. Although molecular diagnostics are now available for testing of nasopharyngeal secretions from acutely infected patients, they are not validated and probably not useful for chronic infections.
2. A number of the enterovirus experts in the U.S. do not believe EVs cause chronic infections or ME/CFS but have done little to prove and disprove the association or causation. What is the mechanism for replicating a positive finding in order to moving the field forward?
3. Viral proteins and RNAs are more likely found in tissue samples rather than in blood. Better preservation and lysis of blood samples, i.e. Tempest or Paxgene-RNA tubes may be helpful.
4. Better sequencing of the viral RNA found in tissues or blood samples and overcome the difficulty of genotyping RNA found in tissues by staining and limited sequencing.
5. More monoclonal antibody needs to be developed for enteroviruses, especially directed against common proteins and highly conserved epitopes that do not have cross-reaction with
human peptide sequences. Large-scale purification of viral proteins or collaboration will be needed to accomplish this goal.

6. Better research to elucidate the mechanism of viral RNA persistence in cells and if any interaction with human DNA.

7. Prompt epidemiologic information from the enteric branch of the CDC concerning past and present enterovirus serotypes isolated in epidemics of respiratory and gastrointestinal infection in different geographic areas, as has been done to elucidate the EV D68 outbreak in 2014. An enterovirus other than CVB1-6 and echovirus 6, 7, 9, 11, 30 will have to be studied using laboratory-developed neutralizing antibody or specific molecular diagnostics. Better funding of enterovirus branch of CDC and strong collaboration will be needed.

Appropriate benchmarks for evaluating progress.

1. Production of monoclonal antibodies for highly conserved enteroviral proteins or peptide sequences for immunochemical studies.
2. Reproducible detection of EV proteins and RNA in infected tissues taken from symptomatic patients and not in control subjects.
3. Methods for genotyping of viruses in infected tissues
4. Detection of antigens and enzymatic activities of viral RNA dependent RNA polymerase from the infected tissue specimens, which will help validate the mechanism for viral RNA replication and provides a method for drug susceptibility testing
5. Establish a mouse model of chronic enteroviral infection for ME/CFS and to evaluate antiviral drug studies

For this RFI, the NIBIB is interested in the ideas for the development of new imaging and bioengineering technologies that could have the potential for a significant impact on ME/CFS research.

1. Look for technique to identify dsRNA in the brain of ME/CFS patients
2. Look at genetic changes and metabolic effect of chronic EV- infected neurons and glial cells and correlate with fMRI study or other imaging techniques to look for the abnormalities in the brain

The Trans-NIH ME/CFS Working Group also welcomes your general comments.

Do not forget the Semmelweiss reflex during your evaluation of this email/proposal.

Delany, Dudley

Subject: ME/CFS Research Suggestion

Hello,

Naltrexone is a drug approved in the mid-1980s by the United States Food and Drug Administration for the treatment of opioid addiction. It is currently a generic drug and is very inexpensive. In very low doses (less than 1/10 that approved by the FDA), some people afflicted
with ME/CFS are experiencing a great measure of symptomatic relief. It certainly deserves research. For more information, visit http://tinyurl.com/ldn-4-me-cfs

Underhill, Rosemary

Subject: Research opportunities

Dear Dr. Whittemore,

I am pleased to send my perspectives on gaps and opportunities across the ME/CFS research continuum. I include some suggestions for much needed research into ME/CFS (see attached). I would be grateful for any feedback about these suggestions.

Trans-NIH ME/CFS Working Group
Perspective on Gaps and Opportunities across the ME/CFS Research Continuum

R.A. Underhill MB BS, FRCS (Edin)
Independent Researcher

Summary of Background Studies
The etiology of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) has not been established. The hypothesis that the underlying cause of ME/CFS is an infectious agent is supported by clinical, immunological and epidemiological evidence.

- ME/CFS is endemic globally as sporadic cases and occasional cluster outbreaks (Carruthers BM, 2003; Parish G, 1992; Ramsey AM 1988). Cluster outbreaks imply an infectious agent.
- An abrupt flu-like onset, resembling an infectious illness, occurs in many sporadic patients and most cluster outbreak patients.
- Contagion has been shown by finding secondary cases in outbreaks (Gilliam AG, 1938; Crowley N, 1957), and it is suggested by a higher prevalence of ME/CFS in sporadic patients’ genetically unrelated close contacts (spouses/partners) than in the community (Underhill R, 2006).
- Immune responses in sporadic patients with ME/CFS resemble immune responses in other infectious diseases (Underhill RA, 2015).
- Some healthy contacts of ME/CFS patients show immune responses similar to patients’ immune responses (Abbott NC, 1994; De Merlier K, 2000; Grufferman S, 1994; Levine PH, 1998; Stewart C, 2003; Suhadolnik RJ, 2004), suggesting possible exposure to the same antigen (a putative pathogen).
- Exposure to patients with ME/CFS (a) by having a close family member with ME/CFS in both sporadic (Underhill R, 2006) and outbreak (Bell KM, 1991) cases and (b) occupational exposure of health care professionals in outbreaks (Crowley 1957; Gilliam AG, 1938) has been shown to increase the risk of developing the disease. This suggests that there may be a transmissible agent in ME/CFS.
- One study found that 6.5% of a large series of sporadic patients developed the illness a few days after a blood transfusion (De Becker, 2002), raising the possibility that some healthy blood donors might be silent carriers of a pathogen which can cause ME/CFS.
As yet, no known pathogen has been identified as the cause of ME/CFS. In the absence of evidence of active persistent replication of a causal infectious agent specific to ME/CFS patients, it is speculated that a causal pathogen might have been cleared by the patients’ immune systems, but a maladaptive host immunologic response might have triggered chronic symptoms (Levine PH, 1994). This speculation cannot be tested unless a causal pathogen can be found in acute cases of ME/CFS and a determination of its presence or absence in chronic cases is undertaken. More information on infective characteristics of ME/CFS can be found (Underhill RA. 2015).

**Suggestions for Research into ME/CFS**

1. Further study should be undertaken to confirm findings that showed that genetically unrelated close patient contacts (spouses/partners) have a higher prevalence of ME/CFS than the community (Underhill R, 2006). The results of this study suggest that patients with ME/CFS might be able to shed a transmissible agent. In this study, the patients had been diagnosed by a physician, but the diagnosis was not confirmed by examination of the patient. This study needs to be repeated in patients diagnosed by a knowledgeable physician.

2. Several studies have shown that some close contacts of patients show immune system findings similar to those of patients (Abbott NC, 1994; De Merlier K, 2000; Grufferman S, 1994; Levine PH, 1998; Stewart C, 2003; Suhadolnik RJ, 2004). When further immune studies are undertaken in patients with ME/CFS, both contact controls and non-contract controls should be recruited and the immune system findings compared in each group. If some contact controls are confirmed to have similar immune system findings to the patients, then this suggests that patient contacts might have been exposed to the same antigen as the patients. In the absence of evidence that a specific toxin has affected all patients everywhere with ME/CFS, this antigen is likely to be an infectious agent.

3. **Research should focus on identification of a causal pathogen for ME/CFS.** This may present a challenge. Since no known pathogen has, as yet, been found to be associated with the typical clinical pattern of symptoms in patients with either sporadic, or outbreak ME/CFS, the search should include novel as well as known pathogens. The putative pathogen may be difficult to culture, and may be present in the tissues, but not in the blood. E.g., it might be present in the throats of those ME/CFS patients who have recurrent sore throats.

From the clinical findings, a possible causal pathogen should be able to be transmitted by casual contact and it might be neurotrophic. From the immunological findings, a possible causal pathogen should be associated with a reduction in natural killer cell activity and cell mediated immunity; possibly able to become latent; possibly able to inhibit replication of poliovirus. Immunological findings suggest that a putative pathogen is likely to be viral but a bacterial infection (perhaps a cell wall deficient, L form) has not been excluded. Measurements of reverse transcriptase should be done to determine the possibility of a retrovirus infection.
Pathogens are more likely to be found when actively replicating. This usually occurs early in the disease, in a relapse and possibly in extremely debilitated patients. The search for a putative pathogen should involve requesting and arranging for blood/tissue samples from the more severely ill patients who may not be able to travel to a laboratory.

The presence of confounding, secondary, opportunistic or reactivated latent infections is less likely early in the disease. Early diagnosis is possible only in patients in cluster outbreaks. Physicians and public health authorities need to be educated about the characteristics of cluster outbreaks and encouraged to report suspected outbreaks to appropriate public health authorities. A new case definition for cluster outbreak patients should be created (Levine PH, 1994), as the various case definitions for sporadic ME/CFS patients are not useful. Patients may be very ill and it may not be possible e.g., to determine post-exertional symptoms. The requirement for symptoms to be present for six months is inappropriate.

Examination of the microbiome of the gastro-intestinal tract is currently under study. However, the immune system reactions of the patients suggest that it is likely that a putative pathogen is not confined to the interior of the gastro-intestinal tract, but is present in body tissues.

Finding a causal pathogen for ME/CFS could assist with diagnosis; help find a biomarker; enable the development of anti-microbial treatments; allow the development of a vaccine; suggest preventive measures; explain pathophysiological findings; and reassure patients about the validity of their symptoms.

References
A number of research psychiatrists posit that CFS and FM are both based on somatic amplification producing functional somatic syndromes varying only in degree. I have viewed this as a research question and have published data showing dramatic differences in several physiological parameters. But the way to answer this question directly is to work on developing biomarkers that either are the same or different for the two illnesses.

We have started on this avenue in two sets of studies -- the first in identifying the proteome of CFS in spinal fluid. The initial study did not stratify by presence or absence of FM and such a study needs to be done. The second study looked at brain biochemistry -- specifically at ventricular lactate and cortical glutathione. Further work using PET scanning to identify sites of central neuroinflammation are needed to determine mechanism for the brain chemical abnormalities we have identified. A third way of doing this which needs to be done in the future is to determine if there are unique brain signatures that discriminate CFS from FM using brain connectivity methodologies.
Finally CFS is a diagnosis of exclusion. However, about 14% of patients with major sleep complaints are found to fulfill diagnostic criteria for both CFS and for sleep disturbed breathing. [SDB] An additional subset of CFS patients appear to have a disorder of arousal. Critical research questions would include [1] answering the question of whether treating SDB would ameliorate the fatigue such that patients lose the diagnosis of CFS and [2] confirming the existence of a sleep disorder producing inability to sleep when very tired despite having severe fatigue and arriving at treatments for this disorder.

Benjamin H. Natelson, MD  
Director, Pain & Fatigue Study Center, Mount Sinai Beth Israel  
Professor of Neurology, Icahn School of Medicine at Mount Sinai

Podell, Richard

Cc: Unger, Elizabeth (CDC/OID/NCEZID); Nancy Klimas; Lucinda Bateman; Daniel Peterson; Charles W. Lapp; Ben Natelson

Subject: Research finds for chronic fatigue syndrome

Some say that NIH should not create a usefully large budget for cfs because there are not many researchers in the field.

I think that puts it backwards. Cfs affects the immune system, the brain, the mitochondria, muscles, irritable bowel, irritable bladder, migraine, inflammation, increased lymphoma rates, epidemiology, genetics and more.

Were sufficient funds available researchers in all these fields would almost certainly discover research questions within their discipline that would make competing for cfs omits attractive.

Many more people suffer from cfs than who suffer from multiple sclerosis, yet NIH research funds for ms are many many times that for ms. Budget 100 million $ a year for cfs, and great research progress will certainly follow.

Richard podell md,mp  
Clinical professor  
Rutgers robert wood johnson  
Medical school

Spurgin, Maryann

 [...]  
Subject: Circulatory Impairment in Myalgic Encephalomyelitis: A Work Proposal Auto forwarded by a Rule

Circulatory Impairment in Myalgic Encephalomyelitis: A Work Proposal —by Maryann Spurgin, Ph.D.

January 16, 2016.
Note: I will be using "ME" in this proposal to distinguish it from the broader category of "fatigue" that has been so damaging in finding consistent research findings in the "CFS" Fukuda definition and Reeves/Oxford criteria. The findings I discuss here do not apply to the broader category of "fatigue."

I wrote this as an abstract, a proposal to try to obtain a position on a research team to explain, elucidate and integrate into research, or see if researchers can integrate what knowledge I bring, the pathophysiology of the circulatory impairment in ME that I, as well as numerous researchers, think is so crucial to understanding why patients with ME relapse at the slightest exertion.

While it may have been unrealistic to believe that a paid position would come out of this proposal, being too realistic and lacking ambition and imagination never advanced science. Apparently the proposal was good enough for some of its suggestions to have been adopted by research teams.

Here is my proposal:

https://docs.google.com/document/d/1y_SoLHW8NaxBr18bmBiY-ehBZfd3Ji7yttV50bG5qRY/mobilebasic?pli=1

Sincerely,
Dr. Maryann Spurgin
Sent via BlackBerry by AT&T

Dalgleish, Angus

[...]
Subject: ME/CFS

Dear Sir/Madam

I am an active researcher in Low Dose Naltrexone (LDN), and have shown that it uses TLR 9 as a receptor in addition to the opiate ones. ( Patent applied for and publication submitted)

I have also shown it enhances a gene set at low doses that are turned off at higher doses which may also explain the extraordinary clinical responses reported to date. Patent and in press( IJC, Liu et al).

My colleague Jonathon Brostoff reports that over half his patients are cured within 3 weeks with LDN treatment. The other half do not improve but I suspect they are vitD3 low as this appears vital for LDN clinical activity in other conditions especially Multiple Sclerosis.

I am very interested in your call for grant support.

Angus G Dalgleish MD FRCP FRACP FRCPath FMedSci
Professor of Oncology
St George’s, University of London.

[...]

14
Gottfries, Carl-Gerhard

Input for New Research Strategies for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS)
Notice Number: NOT-NS-16-024

ME/CFS is an unexplained disorder with molecular and immunological changes. One such finding of a potentially important change in ME patients is hypomethylation in a majority of certain immune cells and of DNA in genes associated with immune cell regulation. Although the reason for such hypomethylation can only be speculated upon, for the time being, it is interesting that the combined action of the vitamins B12 and folate (See enclosed figure) play a fundamental role in providing methyl groups to a number of substrates in various cell processes, including function of DNA (turning on/off genes), the immune system, neurotransmitters, and in detoxification, by having substantial antioxidant properties. Research should focus on B12/folate as a mean to improve well-being for patients with ME/CFS.

In 1997, we published an investigation on homocysteine and vitamin B12 (cobalamin) in the cerebrospinal fluid (CSF), drawn from patients who fulfilled the criteria of both FM and chronic fatigue syndrome, the former name for ME/CFS. In comparison with a large healthy control group, all eleven patients in the study had increased homocysteine levels in CSF, although the blood levels were usually not increased. The CSF-B12 level appeared to be generally low, and CSF-homocysteine and CSF-B12 levels correlated significantly with ratings of mental fatigue. The results suggested a blockage of B12 transport over the blood brain barrier.

Also in the late 1990s, we learned how to investigate genotypes of MTHFR (methylene-tetrahydrofolate reductase) as a mean to identify individuals who require a larger than usual dose of oral folic acid, in order to optimize the effect of vitamin B12. For the last fifteen years, we have been offering patients with ME/CFS to try out and see if they may benefit from vitamin B12 and folic acid supplements. Some patients can do well from oral treatment, but along the years we have recognized that patients with ME/CFS get the best of it with injective form of B12 therapy, and most of them require an injection at least once a week.

Frequent B12 injections in combination with unusually high doses of oral folic acid is not yet an established form of treatment. In recent years there was some debate on the possible risk for cancer with higher blood concentrations of folic acid. However, this last issue appears to have been settled when a very large meta-analysis was published last year: Folic acid treatment of 50 000 individuals in randomized trials, with an average treatment duration of 5.2 years, had no effect on the incidence of cancer.

We have reported our clinical studies: Regland et al Response to Vitamin B12 and Folic Acid in Myalgic Encephalomyelitis and Fibromyalgia Published: April 22, 2015 Plos One.

There is a great need worldwide for a controlled study of Vitamin B12 (metylcobalamine) in ME/CFS. It is also of interest to map out mutations in the MTHFR gen in patients with ME/CFS. Do
such mutations influence the response ME/CFS patients given to treatment with Vitamin B12 and folic acid. Our unit is prepared to do such studies if economically supported.

Carl-Gerhard Gottfries Professor
Gottfries Clinic AB Krokslätt Torg 5, S 431 37 Mölndal, Sweden

[...] Ph D Med Dr Head Physician
Gottfries Clinic AB
Associated with the University of Göteborg, Sweden.

Martin, W. John

Subject: Response to Notice NOT-NS-16-024; Request for Information

Dear Dr. Whittemore and the Trans NIH Working Group,

I welcome your open inquiry for input regarding NIH endeavors to address the chronic fatigue syndrome (CFS). I trust the following brief summary of my research will be of interest. I would be pleased to personally advise committee and NIH staff members by either phone, e-mail or a visit to NIH.
I began studying patients diagnosed as having the chronic fatigue syndrome in 1986. Initially, I sought evidence for infection with what is now known as human herpes virus-6 (HHV-6), using the polymerase chain reaction (PCR). Although authentic HHV-6 was only rarely detected, weak cross-reactive positive responses were found in approximately 1 in 4 blood samples from over a hundred tested CFS patients, but not in control blood samples from laboratory personnel. These results were presented at a 1990 international conference in Cambridge, England.

By further reducing the stringency of the PCR assay, it was possible to obtain positive results with all of the then known human herpes viruses. Using these conditions many more CFS patients tested positive. Similar data for active virus infection were obtained using blood and cerebrospinal fluid (CSF) samples from patients with a wide range of neurological illnesses. A positive reaction was also recorded on a brain biopsy obtained in 1990. Interestingly, the biopsy showed no inflammation. This led to the conclusion that the presumptive viruses had undergone a “stealth adaptation” as an immune evasion mechanism.

The virus from a PCR positive patient was successfully cultured in 1991. This allowed for further PCR characterization. A ~1,500 base pair product was clearly indicative of a cytomegalovirus. Additional DNA sequencing, indicated that the virus was unequivocally derived from African green monkey simian cytomegalovirus (SCMV).

Many additional positive virus cultures were obtained from CFS patients. The diversity of PCR reactivity of different cultures indicated a heterogeneous grouping of viruses in patients with a wide range of neuropsychiatric illnesses, including CFS. Even with culture positive CSF samples, there was typically no accompanying cellular response, confirming the concept of stealth adaptation.

Stealth adaptation is attributed to the loss or mutation of the relatively few genes coding virus components normally targeted by the cellular immune system. Evidence for this was obtained in the prototypic SCMV-derived stealth adapted virus. The virus was acutely pathogenic when inoculated into cats. The cats, nevertheless, clinically recovered in spite of a complete absence of inflammation. Along with other information, it is apparent that stealth adapted viruses can be suppressed through non-immunological means. This defense is attributed to an alternative cellular energy (ACE) pathway. Enhancing this pathway promises to provide symptomatic improvement to CFS patients, as well as to patients with other illnesses.

I would appreciate any opportunity to inform members of your committees or intramural researches on the methods for culturing stealth adapted viruses. NIH could also help oversee planned clinical trials. Kind regards, John (W. John Martin, MD, PhD., pathologist). [..]

Zeineh, Michael
[..]
Cc: Jose R Maldonado; Jose Montoya
Hi, please see below our perspective. Thank you.

Emerging needs and opportunities that should be considered as new ME/CFS research strategies are developed.
- Diagnostic and biomarker development with MRI-DTI and EEG
- Measuring inflammation in the brain with novel PET neuroinflammation tracers and/or intravenous iron-enhanced MRI
- Measurement of peripheral blood inflammation using high-throughput assays including multiplexed cytokines, gene expression, CYTOF
- Pathogen discovery addressing body compartments such as bone marrow and spinal fluid
- Assessing genetic predisposition through GWAS and HLA studies
- Analysis of human brain specimens from CFS patients

Challenges or barriers to progress in research on ME/CFS.
- Large single-institution studies
- Multi-institution studies
- A CFS Neuropathological Brain Bank

Gaps and opportunities across the research continuum from basic through clinical studies.
- Lack of published interdisciplinary studies
- Clinical therapeutic trials

Michael Zeineh
Jose Montoya
Jose Maldonado

Stanford University

On May 24, 2016, at 10:49 AM, Emr, Marian (NIH/NINDS) [...] wrote:

Good afternoon. The Trans-NIH Working Group has issued a Request for Information (RFI) to help identify research areas and topics to be included in strategies to advance research efforts on ME/CFS.

Through the RFI, the Trans-NIH ME/CFS Working Group invites input from anyone with an interest in the NIH’s research efforts on ME/CFS, including researchers, health care providers, patient advocates and health advocacy organizations, and scientific or professional organizations. You are invited to submit your comments to MECFSRFI@mail.nih.gov through June 24, 2016.

Please provide your perspective on the following issues:

- Emerging needs and opportunities that should be considered as new ME/CFS research strategies are developed.
- Challenges or barriers to progress in research on ME/CFS.
- Gaps and opportunities across the research continuum from basic through clinical studies.
The Trans-NIH ME/CFS Working Group also welcomes your general comments.

The members of the Trans-NIH ME/CFS Working Group will review and consider the comments received under this RFI as they develop strategies for ME/CFS research and research training.

For more information about NIH activities on ME/CFS, please visit www.nih.gov/mecfs.

Marian Emr
Director, Office of Communications and Public Liaison/NINDS
NIH Building 31, Room 8A07

Visser, Frans

Subject: NIH RFI

Dear madam, sir

Enclosed is a research proposal which, we think, may be interesting for the NIH research strategies on CFS/ME. I am not a US resident (living in the Netherlands), and therefore not familiar with the format of these requests, but I hope it will meet your requirements.

Sincerely yours
Frans C Visser
Cardiologist

Immune and neuro-immune activation during post-exercise malaise in CFS patients

One of the most characteristic and prominent symptoms in CFS/ME/SEID patients is post-exercise malaise (PEM) and is therefore used in the three currently used sets of diagnostic criteria: CFS, ME and SEID(1-3). PEM can be assessed by standard questionnaires, with its inherent limitations(4), by CPET on 2 consecutive days(5-8), by cognitive function tests, like the N-back test(9), and by pain threshold assessment(10). Therefore, the IoM concluded that “there is sufficient evidence that PEM is a primary feature that helps distinguish ME/CFS from other conditions”. The underlying pathophysiology in PEM is largely unknown but data suggest that an altered immune response to exercise is present in CFS/ME/SEID patients compared to healthy sedentary controls(11), but data are conflicting. Furthermore, immune abnormalities in the cerebral spinal fluid have recently demonstrated by Hornig et al.(12) in CFS/ME patients compared to healthy controls and to MS patients. Moreover, neuro-inflammatory abnormalities have been described by Nakatomi et al.(13) using PK11195 PET imaging.

Based on these findings, it is hypothesized that PEM following exercise is related to an altered immune response and to inflammatory changes in the brain. For this purpose 20 CFS/ME/SEID female patients (mild to moderate disease according to the ME criteria) will be studied and compared with 20 healthy sedentary controls, age and BMI matched. Methods: PK11195 PET imaging will be performed at baseline. Thereafter, a 2day CPET stress test will be performed with
blood sampling for analysis of cytokines, complement factors, oxidative stress and immune cell gene expression, prior to the first test and after the second stress test. Three days after the 2nd stress test the PK11195 PET study will be repeated together with blood sampling for immune factors. The DSQ questionnaire(14) will be used to quantify the degree of perceived PEM before and the stress tests, and after 3 days (before the second PET scan).

This study hopes to clarify a number of important questions:

1. Confirmation of the small study by Nakatomi et al.(13) that neuro-inflammatory abnormalities are present in CFS/ME/SEID patients
2. Confirmation of the hypothesis that PEM is associated with an increase in the neuro-inflammatory abnormalities
3. Further insight into the altered immune response following exercise
4. Assessment of a relation between neuro-inflammatory changes, immune abnormalities in blood and PEM symptoms of CFS/ME/SEID patients

Description of the need or opportunity: the pathophysiology of PEM is largely unknown and the debilitating nature of PEM is largely unknown to the general public

Scientific rationale and potential public health impact: insight into the pathophysiology of PEM. When the pathophysiology is clear, therapeutic interventions may be developed. Furthermore, the PET images may help the understanding of the general public.

Anticipated challenges that will need to be addressed: this is a complicated study involving PET centers with an in-house cyclotron, and the cooperation of specialized laboratories which analyze the different immune markers. The use of isotopes (although short-living) may limit the inclusion of healthy volunteers.

Appropriate benchmarks for evaluating progress: the most important benchmark is the inclusion rate of patients and healthy volunteers. Public announcements through appropriate media prior to the study, and during the study may overcome the potential problem with inclusion rates.

For this RFI, the NIBIB is interested in the ideas for the development of new imaging and bioengineering technologies that could have the potential for a significant impact on ME/CFS research: If the data of this study will confirm the previous Nakatomi study, and the above mentioned hypothesis, PET research with PK11195 and similar neuro-inflammatory tracers will considerably be stimulated.


Prof. Dr. F.C. Visser,
Cardiologist, specialized in ME/CFS
Planeteweg 5, 2132 HN Hoofddorp, the Netherlands
Cardiac Care Foundation

Camenzind, Mark
[...]
Subject: RFI: recommendations for future study for M.E.
1) Funding: based on disease burden, ME/CFS should receive $1B per year NIH R&D funds. For example, the disease burden graph for disability with blue dots per disease, can be modified to show and approx, somewhat arbitrary "fair funding line" and current, grossly discriminatory $6M/yr for 2015 added in Red, vs, requested $250M/year by MEACTION and MECFS coalition, vs. $1B which would be fair funding. NIH can spend Billions overnight for Ebola or Zika, yet has ignored 2 million ill in US incl my Severe ME 22 year old son, [...].

(Draft revision in Red, orange, green & arbitrary blue “fair funding” line by mjc)


**Figure 2: RCDC Funding vs. Global DALYs**

Given that ME/CFS is perhaps the worst, common, chronic disease based on quality of life scores, the lack of funding for this disease that has 75% afflicted being women, is gross discrimination against women and M.E.
Please fund ME/CFS R&D at much higher levels ASAP:

Fig 3. Unadjusted means and medians compared to different conditions.

EQ-5D 3L score: highest numbers correlate with best imaginable health and low numbers with worse imaginable health.
2) Please do not have NIH do R&D on its own, but enlist existing experts already engaged in R&D, esp:
- Ron Davis at Stanford
- Ian Lipkin and Mady Hornig at Columbia
- Jarred Young at UAB
- Leonard Jason at DePaul U
- Nancy Klimas
- Sue Levine
- Eric Gordon
- Dan Peterson

3) Recommend NIH start 12 centers of excellence for R&D, clinic trails, treating patients, each funded $2M per year with stable funding for 5 years, including at Stanford, UT, FL, NV, AL, IL, NY, MA, NC, TX, No Ca and So CA

4) www.diseasemaps.org has tracking of diseases, self reported, and CDC should develop a comprehensive map, to assess clusters, and start tracking ICD10 G93.3 to report 100% of cases and track these including also off label treatments, since no FDA approved treatments yet.

5) Hyperbaric oxygen may help some patients and can kill some anaerobic species that may not be recognized. Turkey did preliminary crude 16 patient study, and need to follow this up with longer term studies. (I can provide reference upon request)
6) For diagnostics, exhaled breath analysis is non-invasive, and has been found to be very
diagnostics for breast cancer, lung cancer, other diseases, based on exhaled hydrocarbon GC-MS
analysis, esp via MenssanaResearch.com in NJ, Dr. Michael Phillips. Dr Phillips has a current $2M
NIH contract to next study, exhaled breath analysis for 1000 TB patients, and should use same
controls and add on 200 severe ME/CFS patients for <$500,000 perhaps to assess if any unique HC
biomarkers for ME due to mitochondrial dysfunction, Reactive oxygen species etc. This can be a
common platform to diagnose and differentialte many diseases, using 2500 biomarkers that can
be detected in exhaled breath samples. The methods already work and justt need to try this for
M.E.

7) Exhaled breath analysis for NO, Nitric oxide, should be done, at low cost, since NO could
contribute to POTs that is common for most ME/CFS patients or be another probe for metabolic
dysfunction, either by going high or low. Aerocrine sells low cost NiOx Vero Handheld monitor
that costs only about $10 per test. Aisha Moore is there clinical coordinator in So CA that would
be happy to talk with NIH or other researchers.

8) Some people seem to be helped somewhat for their symptoms even by simple OTC drugs like
Clariten, incl my son, so controlled studies should be tried for these quite safe, common, low costs
drugs.

9) Transcranial and Vagus (Autonomic Specialists) nerve stimulation could be considered, since has
reportedly helped some patients

We appreciate NIH and CDC finally giving more attention to this serious and common disease, and
hope you can rapidly increase funding of key R&D goups to $250M. Anything less is gross
discrimination and taxation without NIH represenation.

Dr. Mark Camenzind, PhD, San Ramon, CA, R&D Advocate to Cure M.E., […]

Chu, Lily
[...]
Subject: Request for Information: Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS)

Dear Colleagues,

Thank you for the opportunity to contribute to this Request for Information. As directed, we, the
Board of the International Association for Chronic Fatigue Syndrome/ Myalgic Encephalomyelitis
(IACFS), have highlighted emerging opportunities, research needs, and continuing challenges in this
field in the attached document. This document is by no means exhaustive and, as such, is a snapshot
in time as the research continues to evolve.

We would appreciate it if you can confirm receipt of this e-mail. Please do not hesitate to contact
us at membership@iacfsme.org for further information or details.

Sincerely,
Dear Colleagues,

Thank you for the opportunity to contribute to this Request for Information. As directed, we, the Board of the International Association for Chronic Fatigue Syndrome/Myalgic Encephalomyelitis (IACFS), have highlighted emerging opportunities, research needs, and continuing challenges in the field of chronic fatigue syndrome/myalgic encephalomyelitis. This document is by no means exhaustive and, as such, is a snapshot in time as the research continues to evolve. Please do not hesitate to contact us at membership@iacfsme.org for further information or details.

Sincerely,

Lily Chu, MD, MSHS – Co-Vice-President
Fred Friedberg, PhD – President
Staci Stevens, MA – Co-Vice-President
Steve Krafchick, MPH, JD – Treasurer
Jon Kaiser, MD

Emerging Opportunities:

Currently, there is no easily available, well-established, objective diagnostic test for ME/CFS. Here are a few areas that may yield a diagnostic test or biomarker for ME/CFS. Some tests might not be suitable for clinical use but might provide a gold standard test for research purposes. This may also provide clues to the pathophysiology of this disease and even to future treatments.

1) **Low natural killer cell activity (NKCA) as a diagnostic marker:**

In a review of 17 studies, Strayer et al. (1) found that 88% of studies suggested ME/CFS patients tended to have lower natural killer cell activity than healthy controls. Furthermore, low natural killer cell activity has been shown to be correlated with functional status (2) and decreased in a dose-response manner in a family with multiple patients (3). Low NKCA was downgraded as a requirement for the Institute of Medicine’s SEID criteria for two main reasons: a) low NKCA can occur for reasons other than ME/CFS and b) it was not clear what percentage of ME/CFS patients have low NKCA. However, no diagnostic test in medicine is 100% sensitive and specific: clinicians just need a test to be sensitive and specific enough. Test results coupled with history, physical exam, and clinical course together make the diagnosis.
We encourage NIH to support research that a) can confirm or refute the finding of low NKCA in a larger population of ME/CFS patients and b) elucidate such a test’s operating characteristics (i.e. sensitivity, specificity, positive predictive value, negative predictive value).

2) **2-day repeated cardiopulmonary exercise testing:**
Post-exertional malaise is considered to be a cardinal and disabling symptoms of ME/CFS by both clinicians and patients. A major feature of post-exertional malaise is the inability to repeat the same frequency, intensity, or type of activity from one day to the next or repeatedly, regularly. 2-day CPET may be able to detect and quantify this symptom.

Healthy and even sick individuals are able to reproduce certain cardiometabolic parameters within a 7% margin of error when physically exercised on two separate days. Three separate groups (4-7) have shown this is not the case for ME/CFS subjects. One or more of 4 cardiometabolic parameters can decrease from 8% to 55% from the first day’s value to the second day’s value.

Further studies of 2-day repeated cardiopulmonary testing in larger populations can confirm if this test can be used as an objective marker of PEM and/or as a gold standard test for ME/CFS. Most studies have not specifically inquired subjects about presence, onset, and duration of PEM and linked cardiometabolic changes to the time course of symptoms so future studies should try to correct this gap. Researchers should be aware not every ME/CFS subject will have the physical capacity to undergo CPET nor be willing to risk the adverse effects of CPET. Tests which can detect PEM in patients unable to or concerned about undergoing CPET should be devised. For comparison purposes, both sick and healthy controls should be recruited for studies.

3) **Neuropsychological testing related to information processing:**
Neurocognitive symptoms are common in ME/CFS and may be more disabling than physical fatigue or pain. This manifests in patients’ lives in a variety of ways, for example, being unable to drive, understand/participate in a conversation, or read and remember a book. When tested formally, diminished information processing speed, appears to be a common characteristic that distinguishes subjects from healthy or sick controls. (8) Some subjects may perform normally with no time limits but abnormalities are particularly unmasked under time pressure. Furthermore, some researchers believe that slowed information processing speed is at the root of other neurocognitive symptoms such as poor attention and memory.

Validated tests of information processing that have already shown abnormalities in some subjects, such as the simple/ complex reaction time tests in the CANTAB (9) or CalCap (10) batteries, the Paced Auditory Serial Addition Test (PASAT), or visual information processing tests, could be applied to larger populations of subjects to confirm this finding. Objective findings should also be linked to
subjective symptoms. Confounding factors such as medication, sleep deprivation, and co-morbid depression will need to be accounted for in such studies (11). Finally, other than time restrictions, researchers should also consider testing subjects after they have experienced cognitive loading (e.g. a day of work or school) or chart the course of the cognitive performance over time. Patients report post-exertional malaise due to cognitive activity; in one study, subjects took an average of 57 hours to recover from a 3-hour cognitive battery whereas healthy controls had recovered their energy in 7 hours (11).

4) **Tilt table testing:**
Orthostatic intolerance (OI) is very common in ME/CFS patients, affecting up to 80% of patients (8, 12). Consequently, OI symptoms were included as part of the IOM definition. OI can also exacerbate post-exertional malaise and impacts function significantly and independently (13); if identified, there are effective treatments available. Tilt table testing is the gold standard for diagnosing different types of OI yet the full benefits of using tilt table testing to diagnose and help guide treatment in ME/CFS has not been fully explored.

Studies exploring the prevalence of OI symptoms, the prevalence of positive tilt table tests, and the correlation of symptoms with objective tilt table testing would be informative not only for diagnostic purposes but for treatment purposes.

5) **Neuroinflammation:**
ME/CFS patients experienced many symptoms that may be related to the neurological system. Aside from problems with cognition and sleep, experienced clinicians have also observed ataxia, muscle weakness, sensory hypersensitivity, visual issues, neuropathic pain, and bowel and bladder issues (14, 15). These symptoms may be related to infection and/or inflammation of nervous tissue: in a series of autopsies, patients were found to have inflammation of the dorsal root columns of the spinal cord (16) while a neuroimaging study with a small sample size showed widespread inflammation in the brain which was correlated with neuropsychological symptoms (17). These studies warrant further follow-up.

6) **Unrefreshing sleep, heart rate variability, and sympathetic predominance:**
Unrefreshing sleep is experienced by over 90% of ME/CFS patients and remains an issue even in patients who are able to fall and stay asleep, uninterrupted, for prolonged periods (8). Patients frequently report that upon awakening, they feel as if they had not slept at all. Routine polysomnography of has not revealed any striking differences between patients and healthy controls (8). In contrast, several studies show that the autonomic system of ME/CFS subjects, as measured by heart rate variability, might be overactivated and that this might be leading to poor sleep (18-20).

This is an area that would benefit from further investigation. Subjects selected for such studies should report current unrefreshing sleep despite, often with the use of
medications, no problems falling asleep or staying asleep. Natelson et al. found abnormalities primarily in those reporting being sleepier in the morning (21). Those who felt less sleepy in the morning were no or little different from healthy controls. Furthermore, physical activity the prior day improved sleep in the “less-sleepy-AM” group while it had no benefit or even disrupted the sleep of those in the “more-sleepy-AM” group (22).

7) **Familial studies:**
A number of past studies have shown that family members of patients are at higher risk than the general population for developing ME/CFS (23, 24, 25). These studies need to be replicated and followed up. Studies of pedigrees, coupled with technologies such as genetic analysis or human leukocyte antigen (HLA) testing, could help identify markers for the disease and lead to an understanding of its pathophysiology. Such studies could also stimulate work examining prevention of ME/CFS in at-risk family members or members of the general public.

8) **Energy metabolism issues and lactate processing in muscle and brain:**
Several studies suggest that patients have problems with energy metabolism. Four different studies of 2-day repeated cardiopulmonary exercise testing show that the anaerobic threshold, the time when production of lactic acid exceeds its removal, decreases by 7% to 26% on the second day in patients (4-7). Accumulation of lactic acid results in muscle pain and fatigue. Consequently, it is not surprising that patients report being able to accomplish an activity one day but not being able to repeat it again later.

Supporting this fall in anaerobic threshold are other studies documenting issues with lactate processing. Shungu et al. in a series of studies found high levels of lactate in the cerebrospinal fluid of subjects (26-28). Newton et al. found that, after an exercise challenge, the muscles of some ME/CFS subjects retained 20 times the acid amount of healthy controls (29). Her group also linked low cerebral blood flow, which might encourage anaerobic metabolism and higher levels of lactate, with muscle pH (30).

These studies suggest an underlying mechanism that could account for both physical and cognitive fatigue in patients. As with neuropsychological testing and sleep research, subjects who are recruited for such studies need to endorse these symptoms and not merely be enrolled based on fitting a specific criterion.

9) **Post-infectious triggers:**
Up to 80% of patients report a post-infectious trigger close to the beginning of the onset of their illness. Prospective studies have shown that, following infection with a wide variety of microbes (e.g. Epstein-Barr virus, Coxiella Burnetti, Girardia, influenza), approximately 10% of patients come down with ME/CFS (31-33).
Because current diagnostic criteria require that patients must be sick for at least 6 months prior to diagnosis, researchers may be missing the early stage of the illness. One study already suggests that the immune system of patients who have been sick less than 3 years differs from those who have been sick longer (34).

Encouraging studies to follow patients who come down with known ME/CFS-associated infections prospectively might yield answers to the etiology, pathophysiology, prognosis, and even early interventions that might prevent or ameliorate the severity of this disease.

**Treatment:**

Currently, not one well-established, FDA-approved treatment exists for ME/CFS. Thus, it is urgent that this area be pursued.

1) **Rituximab**

In 3 decades of ME/CFS research, no treatment has shown as much promise as rituximab. Three studies have been published supporting the effectiveness of rituximab in treating ME/CFS with two of those being trials showing about two-thirds of subjects responding positively (35-37) with no major side effects. Some individuals sustained substantial improvement, enough to return to work or school.

These studies however have consisted of small sample sizes so replication is needed. The approximately 30% of ME/CFS subjects who fit Canadian Consensus Criteria, have higher levels of immunoglobulin G subsets 1-3, and possess antibodies to certain cholinergic or muscarinic receptor might be most likely to benefit (38). Questions to be answered include what dose, frequency, and duration of rituximab works best and what types and severity of short-term/ long-term side effects are expected and how to mitigate them. Future trials should also include more detailed subjective outcome measure and objective outcome measures (e.g. 2-day CPET, pedometers to measure steps walked, return to work/ school).

2) **Anti-virals for targeted groups:**

Up to 80% of ME/CFS patients identify an infectious precipitant associated with their condition yet most do not have objective tests documenting active or acute infection. However, a subset of subjects do. Subjects with persistence of Epstein-Barr virus IgM and or evidence of chromosomally integrated human herpes virus 6 respond positively to the appropriate anti-virals (39-41). Yet, trials of anti-virals in undifferentiated groups of subjects might obscure any positive results. Replication of these studies would confirm or refute prior results.

3) **Self-management – pacing:**
Graded exercise therapy (GET) has been touted as a treatment for ME/CFS subjects. Yet in the largest trial of GET to date, in spite of lenient recovery criteria (42, 43), only 22% of subjects were considered recovered compared to 15% of subjects receiving usual care (44). Additionally, outside of trials, both experienced clinicians and up to 50% of patients report worsening with GET (45). In contrast, pacing, a method of self-management involving balancing activity with rest and monitoring/modifying activities to match energy levels, is recommended by many experts (46) and endorsed as the most effective treatment currently by patients in managing their condition (47, 48) with no or minimal side effects.

Yet, hardly any trials of pacing have been carried out. The UK PACE trial (49) claimed that pacing (termed “APT”) conferred no benefits above that of usual care but some would contend their version of pacing is different from the type employed by patients or some clinicians. Two other studies (50, 51) suggest pacing may help decrease symptoms and increase ability to carry out activities of daily living but replication in much larger samples are needed. Identifying which components of a pacing program are most effective, who would implement such an intervention, and how pacing could complement other treatments are also other areas that warrant exploration.

4) Isoprinosine
Isoprinosine, an older antiviral and immunomodulator with minimal side effects, has been used for years by ME/CFS specialists for patients who present with abnormally low natural killer cell activity. It has been shown to elevate NK cell activity (52) and to improve function in some patients (53). These results need to be explored and further replicated.

One obstacle to studying isoprinosine is that it is not currently FDA-approved for any medical condition in the US. It was originally FDA-approved for subacute sclerosing panencephalitis (SSPE) but because this is a rare condition, the manufacturers did not seek to renew their approval after several years. It is obtainable in countries like Canada, Ireland, and New Zealand with a prescription.

5) Low dose naltrexone
Naltrexone, a medication traditionally used for the treatment of opioid and alcohol dependence, has been used off-label in low doses to treat pain, sleep, and inflammation in ME/CFS subjects for years with minimal side effects. There is some evidence naltrexone may work by decreasing the production of pro-inflammatory cytokines and neurotoxic superoxides by microglial cells (54). Low-dose naltrexone also reduces pain substantially in some patients with fibromyalgia, a common co-morbid condition in ME/CFS (55, 56).

Studies of naltrexone in ME/CFS subjects with and without fibromyalgia would be helpful to confirm the effectiveness and safety of this drug in ME/CFS.
6) **Intravenous immunoglobulin for targeted groups:**

In patients who come down with ME/CFS following a documented parvovirus infection, early treatment with IVIG in some cases has been shown to cure ME/CFS (57, 58). Similarly, a 1997 trial in Australia showed that IVIG increased function 25% above that of placebo in adolescents (59). The few IVIG trials in adults have shown mixed results; however, a recent German study (60) demonstrated that about a quarter of ME/CFS subjects had lower IgGs whereas another 25% had elevated immunoglobulins. It is possible that the former group may benefit from IVIG whereas IVIG may have no benefit for the latter.

**Research Needs:**

1) **Basic Epidemiology** –

Basic facts about ME/CFS are missing due to a lack of community-based and longitudinal studies. There have only been a few large community-based studies in the US. Most study subjects have been drawn from tertiary care centers or from already diagnosed or self-identified ME/CFS subjects. Thus, we do not have as good a grasp on prevalence and incidence. Secondly, there are very few long-term studies of ME/CFS despite recognition that it is a chronic condition. The studies that do exist generally track people for less than 5 years and are not detailed in their collection of subjective or objective data. Thus, we have a mediocre grasp of co-morbidities, natural history, and prognosis.

Misconceptions about ME/CFS have also influenced the paucity of data. ME/CFS is not viewed as a fatal condition even though there have been cases of people dying with this illness (61) and three studies link it with development of cancer (62-64). Mortality data needs to be tracked and explored. ME/CFS is also not viewed as a condition associated with outbreaks even though up to 80% of cases are reported to be associated with an infectious onset and there have been reports of outbreaks in the past (65) and more recently (33). Thus, any potential outbreak-associated ME/CFS cases would not be noticed and reported.

Community-based studies are especially important for this condition, not only because studies in tertiary care might distort the true picture of this condition, but also because up 91% of people affected are estimated to be undiagnosed (66). A community-based study with a surveillance component would pick up these subjects even if they were not diagnosed by their treating clinicians.

2) **Neglected/ special subgroups:**

The overwhelming majority of subjects studied have been self-identified, middle-aged, Caucasian women of higher socio-economic backgrounds being seen in tertiary care clinics. Subject-associated factors, such as access to healthcare or a positive view of research, might account for some of this bias but clinician/ researcher-associated
factors, such as preconceptions about who might be affected or convenience sampling, might also have contributed.

Researchers should be encouraged and incentivized to recruit for subjects beyond this group. We know from other medical conditions that characteristics like age, sex, or ethnicity can affect diagnostic test characteristics, prognosis, and treatment recommendations.

a) Children/younger people/elderly: There appears to be two age peaks of ME/CFS onset, in the 10-20 age range and in the 30-40 age range (67). Yet the average age of most ME/CFS studies is around 50. Since the full recovery rate of ME/CFS is estimated at 5% (68), many people remain sick for years to decades. Despite this, there is only one study of patients focusing on patients over age 65 (63).

b) Men: In most studies, men account for about 25%-30% or less of subjects. Some of this might come down to biology and ME/CFS affecting the sexes disproportionately but rural studies and pediatric studies suggest the ratio is closer to 1:1 (67, 68). Studies can confirm or refute these ratios.

c) Homebound/Bedridden patients: 25% of patients are estimated to be homebound or bedridden at any one point and up to 93% of patients experience these statuses at some point during their illness (47). Yet, except for two studies (69-71), almost all have required subjects to be able to go to a clinic, often multiple times, and few have formally included accommodations to recruit the severely ill. NIH could include supplements for technology, travel expenses, or additional staff that allow this group to be reached.

d) Ethnic minorities: The few studies specifically including ethnic minorities show that ME/CFS might affect some groups more commonly and more severely than even Caucasian groups (66). Yet most studies do not actively recruit or focus on these groups exclusively. Future studies are needed to confirm past studies’ results and also to provide equity of care.

e) Lower socio-economic classes: Similar to ethnic minorities, some studies suggest the poor are disproportionately affected by ME/CFS than wealthier groups (66).

f) Pregnancy – Many women affected by ME/CFS are of child-bearing age yet there has only been one study of pregnancy in ME/CFS (72). A review article relied partly on clinician experience to give recommendations (73). Consequently, women, their families, and their providers are left with no or little answers during this critical stage of their lives. Some women also report near remission of ME/CFS during some stages of pregnancy and this could be an interesting phenomenon to study.

3) Palliative treatment and treatment for co-morbid conditions: Research targeting treatments that effectively palliate common symptoms and co-morbid conditions would be valuable. For example, currently, practitioners are instructed to treat the sleep problems inherent to ME/CFS the same way as they treat...
sleep problems in the general population, with behavioral measures mostly. Yet, this may not be optimal as many patients do not have bad sleep habits to begin with. Experienced ME/CFS clinicians also utilize certain sleep medications, like trazodone and zolpidem (74), yet there is no research on what may work best for patients. Similarly, ME/CFS patients with orthostatic intolerance are treated the same way as those with OI only yet some treatments for that condition, such as physical exercise, might have to be titrated more cautiously in those affected by both conditions.

4) **Pain:**

Pain is a very common symptom of ME/CFS and up to 70% of patients may have co-morbid fibromyalgia. Yet, with the exception of a few papers (75,76), there is hardly any research on the types of pain (muscle, gut, headaches, neuropathic, sore throat, etc.) patients experience, the severity of pain, its impact on function, and what treatments work best (e.g. for neuropathic pain, anecdotally, some patients have experienced relief with anti-virals). Rather studies on pain tend to conflate the different types of pain into one category, which does not provide much illumination into the symptom. Much more work needs to be done in this area.

5) **Post-exertional malaise:**

Post-exertional malaise (PEM) is a common, disabling, and hallmark symptom of ME/CFS yet relative to its importance, not enough research has examined this symptom in detail. Most research has focused on only one PEM symptom, fatigue, yet patients and clinicians report exacerbation of multiple symptoms as part of PEM. The few studies that have examined other symptoms suggest that patients have a paradoxically abnormal response to physical activity. Rather than improving their mood, sleep, pain thresholds, or energy, as it does for healthy people or even people with other chronic illnesses, physical activity worsens these domains (8). Secondly, most studies have focused on or introduced a physical activity stressor yet patients report that cognitive activity, emotional distress, poor sleep, orthostatic distress, infections, and even weather can affect PEM (8, 77). Thirdly, PEM studies usually last only a few days when patients report PEM can start at and last for days after a trigger is introduced. Finally, most studies have utilized only subjective outcomes yet studies with objective outcomes have found a range of abnormalities, from immunologic to autonomic (8). Future studies of PEM should consider these under-researched aspects and incorporate them into the research design.

6) **Outcome measures relevant to ME/CFS patients:**

In 2012, Haywood et al. (78) performed a systematic review of patient-reported outcome measures (PROMs) used in ME/CFS studies and concluded that a) psychometric traits like validity, reliability, responsiveness etc. had not been established in ME/CFS patients for the outcomes used and b) researchers had not integrated the input of patients in selecting or designing outcome measures. While generic measures such as the Short Form-36 (SF-36) are valuable for their usefulness in comparing ME/CFS patients to patients with other conditions, the lack of any
ME/CFS-specific PROMs means that many aspects of ME/CFS are overlooked. For example, current sleep PROMs do not focus on unrefreshing sleep and there are no PROMs covering the phenomenon of PEM comprehensively. Furthermore, objective measures that correspond with subjective improvement need to be established for ME/CFS. It is not certain how well common measures such as the 6-minute walk test work for ME/CFS. Finally, concrete, measurable functional outcomes such as number of hours worked need to be considered. Downstream, the lack of good established outcome measures hinders the Food and Drug Administration’s ability to draw the attention of and to guide pharmaceutical companies (79).

7) **Validated questions, questionnaires, and physical examination findings for bedside diagnosis:**
   Although the Institute of Medicine published a Clinician Guide with interview questions, questionnaires, and physical examination findings that healthcare providers can use to diagnose ME/CFS now (80), many of those items were based on expert opinion and consensus. Consequently, the IOM recommended, as an urgent need, that “a toolkit [comprising such elements and] appropriate for screening and diagnosing patients with myalgic encephalomyelitis/chronic fatigue syndrome” (8) be developed and tested. NIH should especially prioritize diagnostic questions, questionnaires, and procedures that can be performed easily and inexpensively at the bedside in a busy clinic environment.

8) **Other symptoms experienced by ME/CFS subjects:**
   ME/CFS patients and clinicians report a host of other symptoms that have not been or are only rarely studied by researchers. For example, in a survey of 561 subjects (47), over 40% reported flu-like symptoms, gastrointestinal problems, temperature intolerance (feeling hotter or colder than others), or hypersensitivity to various stimuli as “major problems” independently yet little formal research has been published on these symptoms. The Canadian Consensus Criteria and Myalgic Encephalomyelitis – International Consensus Criteria are two other resources that list under-studied symptoms.

**Continuing Challenges:**

1) **Some case definitions may be too broad and all case definitions need validation:**
   Although the Fukuda 1994 criteria has been the most widely used criteria in the last 3 decades, many in the ME/CFS community are concerned that it may be overly broad and select for subjects who are quite different from another and/or are actually affected by another medical condition. Therefore, a number of case definitions have been with the most recent one being the Institute of Medicine’s Systemic Exertion Intolerance Disease (SEID). To clarify this situation, studies validating existing definitions, comparing how they perform head-to-head, relating them to one another (e.g. are definitions recruiting subjects with entirely different conditions or variations
of the same condition?), and linking them with objective findings would be invaluable.

In the absence of solid evidence, we advise NIH to defer from requiring investigators to use any one specific definition. Rather, they should be encouraged to assist the ME/CFS community in finding the most valid definition(s) possible.

2) **Heterogeneity generated by case definitions and lack of subgrouping lead to conflicting/contradictory study results and decrease comparability across studies:**
Currently all the case definitions for ME/CFS involve polythetic criteria, that is, subjects may qualify for any case definition via different symptom combinations. To decrease the heterogeneity this creates both within and across studies, NIH should request that researchers elaborate on the specific symptoms used to recruit subjects or that their subjects experienced. For example, regardless of the definition used, all subjects were affected by PEM, unrefreshing sleep, fatigue and slow information processing speed OR that X% of subjects in this study experienced sleep, and Y% experienced problems thinking, etc. This would allow grant reviewers and readers a better sense of who exactly constituted a study’s subjects and permit easier comparisons between studies.

Another way to reduce heterogeneity is for researchers to focus on specific subgroups, as defined, for example, by demographic traits, illness onset type, duration of illness, lack of or presence of certain co-morbidities, and/or specific test abnormalities like low natural killer cell activity or inability to reproduce cardiometabolic parameters.

3) **Current study subject recruitment strategies are biased towards the 10% of patients who already have a diagnosis:**
Our understanding of ME/CFS is limited and may even be distorted by the clinic- or convenience-based (e.g. support-group based) subject recruitment strategies used in almost all studies. Up to 91% of people affected by ME/CFS may not have received any diagnosis or a correct diagnosis from their regular physician and of the ones who do, multiple physician visits and months-years often pass before they obtain an answer (8). Although expensive, resource-demanding, and time-consuming, community-based studies that actively and randomly find cases are necessary to give a clear picture of ME/CFS. Certain groups, such as men, ethnic minorities, and the poor, should be oversampled intentionally to assure adequate representation.

4) **Collection and publication of different types of data at varying levels of detail by researchers impedes creation of a clear, accurate picture of ME/CFS and decreases comparability among studies:**
In March of 2012, several members of the US CFS Advisory Committee collaborated with the Centers for Disease Control and Prevention to publish a paper describing minimal and additional data elements that should be included in every
ME/CFS study (81). That paper should be used as a starting point to standardize data collection and publication in this field.

5) **Collaboration and sharing of data, resources, and expertise among different institutions will accelerate progress:**
We applaud the National Institute of Neurological Disorders and Stroke’s recent decision to create a research consortium of universities and institutions to study ME/CFS. The lack of funding, resources, and career support, both externally and from their own institutions, has often meant researchers cannot study this medical conditions as broadly or as deeply as they would prefer. A consortium spanning multiple institutions and groups across the US would help accelerate progress in this field by, for example, recruiting adequate numbers of subjects so that solid conclusions can be made and exploiting resources and expertise unique to each site to answer questions across sites.

6) **To decrease confounding, studies need to take into account the impact co-morbidities may have on results:**
Many ME/CFS patients are affected by other medical conditions. Common co-morbidities include fibromyalgia, hypothyroidism, obstructive sleep apnea, orthostatic intolerance, depression, anxiety, and irritable bowel syndrome. Yet, most studies have not considered if and how these co-morbidities may affect results. For example, Natelson et al. found, counterintuitively, that ME/CFS subjects without depression experienced more cognitive problems than those with depression (83). This finding supported the conclusion that ME/CFS is not the same disease as depression. NIH should encourage researchers to think about the effect of co-morbidities in study design, analysis, and interpretation. Conversely, researchers studying co-morbidities frequently found in ME/CFS could also be encouraged to screen their subjects for ME/CFS. This latter strategy could increase the number of investigators interested in this condition and generate new research avenues to explore.

7) **Studies using only healthy controls will not help clinicians to distinguish ME/CFS patients from other patients with similar clinical presentations nor help researchers separate out epiphenomena from the true effects of ME/CFS:**
Many ME/CFS studies have recruited age- and sex-matched healthy controls. These studies show how ME/CFS patients differ from healthy people yet, in the clinic, the question healthcare providers usually face is not whether the patient is healthy or not but rather what medical condition(s) or situation(s) is causing distress for the patient. Thus, NIH should support studies that also compare ME/CFS subjects to subjects who might present similarly at the doctor’s office. Examples might include subjects who have delayed recovery from a variety of infections, who have chronic fatigue after undergoing cancer chemotherapy successfully, who are healthy but physically
sedentary, or who are healthy but suffer from primary insomnia. In the latter two cases, such controls can help account for findings that are epiphenomena, i.e. existing because of physical inactivity or insomnia itself, rather than uniquely due to ME/CFS.

8) **Lack of awareness and appropriate education about ME/CFS results in challenges recruiting and retaining researchers and attracting funding to this field.**

Only about a third of medical schools (84) even mention ME/CFS superficially and only about 40% of medical textbooks (85) include any information. Furthermore, misconceptions and biases about the condition continue to exist. Researchers will not be drawn to a field if they have never heard of it, learn inaccurate disparaging information about it, are discouraged (86,87) from pursuing it by their peers and superiors, and cannot find a strong support system providing them with potential mentors/ collaborators, resources, and support. While a dedicated cadre of researchers exists currently, there are not enough of them and many are aging or retiring without an adequate number of replacements. Likewise, funders, whether governmental, nonprofit, for-profit, or pharmaceutical often are not familiar with ME/CFS. For example, even in recent years, other than the Office for Research in Women’s Health, few NIH institutes have stepped up to the plate to claim ME/CFS for their research portfolios and some institutes, like the National Institute on Aging, have even withdrawn support even though there is a pressing need for research on aging and ME/CFS.

We applaud the positive steps NIH has taken, especially over the last year, but hope these are not only continued but expanded. Extramurally, for example, NIH could educate researchers about and mention ME/CFS as an under-researched, emerging area in conferences, online webcasts, newsletters, listservs, or other media they control or are involved in. Even if NIH cannot cover all the complexity or details of ME/CFS, just mentioning ME/CFS might pique the interest of readers, watchers, and listeners. Training and career development grants targeting both early stage as well as mid-career investigators are needed. The former would assist junior scientists to jumpstart their careers and the latter would allow more experienced scientists to contribute their expertise to this field. Loan forgiveness programs are yet another avenue to attract professionals.

Intramurally, the awareness and education of NIH-associated staff, including program officers, institute senior officials, and grant reviewers, probably reflects that of the mainstream scientific community. Consequently, it is vital NIH staff are appropriately and regularly educated about ME/CFS, especially with staff turnover or changes. Otherwise, grant submissions may not be given adequate guidance, directed to the appropriate review section, or receive a fair and thorough review. The State-of-the-Knowledge Conference held by NIH in 2011 to promote discussion and learning between NIH staff and extramural researchers/ clinicians was very useful and some version of it could be repeated in the future.
9) http://www.calcaprt.com/calcap.htm
10) http://www.cambridgecognition.com/academic/products


52) Vera MA, et al. Clinical Immunomodulatory effects of Isoprinosine in CFS. Presented at the 10th IACFS/ME scientific conference; 2009; Reno, NV, USA.


64) Darakjy S. Chronic fatigue syndrome and co-morbid and consequent conditions: evidence from a multi-site clinical epidemiology study. Presented at the International Association for Chronic Fatigue Syndrome/ Myalgic Encephalomyelitis 11th Biennial Scientific Conference; March 2014; San Francisco, CA, USA.

Lemle, Marian

Subject: Input for New Research Strategies for ME/CFS Working Group

To: Trans-National Institutes of Health ME/CFS Working Group
From: Marian Lemle, Independent Researcher

Re: Input for New Research Strategies for ME/CFS

The following submission is intended to interest the Working Group in the hypothesis that ME/CFS represents a “hibernative” state shaped by the dysregulation of the third known gasotransmitter, hydrogen sulfide (H2S), a theory I first published in 2007. Our understanding of the diverse roles and actions of this gas, both beneficial and harmful, has increased significantly in the last decade. Many of the findings in other diseases have direct bearing on symptoms and research findings in ME/CFS and suggest that H2S may play a key role as an underlying mechanism in the pathophysiology of ME/CFS.

I am currently writing a new paper tying together recent findings regarding hydrogen sulfide gas with symptoms and findings in ME/CFS with the purpose of raising awareness that leads to focused future research and testing of the critical role H2S may play in CFS/ME and related illnesses. I am not looking for funding from NIH for myself.

Because the following observations are untested and unpublished, there is a limit to what I can write with scientific certainty. However, I have decided to include it anyway because I hope the findings will engage the attention of the Working Group. I hope to present the full paper at the IACFS/ME Conference in Fort Lauderdale, Florida at the end of October 2016. [...]

Additionally, although not directly tied to ME/CFS, per se, the findings described in “Mitochondria-Related Gene Expression Changes Are Associated With Fatigue in Patients With Nonmetastatic Prostate Cancer Receiving External Beam Radiation Therapy” (Drs. Hsiao, Wang, Saligan, National Institute of Nursing Research, Dr. Kaushal, National Cancer Institute) deserve further attention.

A further general comment is that it is not clear whether there are any functional medicine specialists on the committee. Many have found hope and answers through the tests employed by these doctors that are not generally part of the traditional physician’s toolkit.

Attached is my original 2007 paper and my abbreviated hypothesis published in 2008. I am also attaching a brief bio that provides some personal background, as well as my October 2008 testimony before the CFSAC Committee. I hope that you will take the time to read at least the longer 2007 hypothesis and the shortened 2008 version. I appreciate your attention and consideration of the subject for inclusion in your research plans.

Sincerely,

Marian Dix Lemle

TESTIMONY TO THE
DHHS CHRONIC FATIGUE SYNDROME ADVISORY COMMITTEE
by Marian Dix Lemle
October 28, 2008

Good afternoon, Chairman Oleske, members of the CFSAC Committee, and concerned members of the CFS community. My daughter’s life, and by extension, our family’s world—were turned upside down four and half years ago when our beautiful daughter, who had been President of her graduating class with a very bright future ahead of her, came down with what was then an unspecified virus, and was later diagnosed with the absurdly named disease “Chronic Fatigue Syndrome.”

For ten years prior to her getting sick, I had on the table in the living room this little artist’s
book called “The Blind Men and the Elephant”. In this well-known parable, one blind man touches the elephant’s side and is certain he is touching a wall. The second blind man grabs a tusk and is certain he is holding a spear. The third touches a squirming trunk, and thinks it is a snake, and on it goes…

I cherished this book because it so simply and elegantly illustrated how our conceptual framework, our view of a problem, can limit our ability to see the larger whole, particularly when combined with unwarranted certainty. It reminded me of the importance of thinking more broadly about a problem and maintaining an open mind, something that I, in turn, will ask of you here today, as I discuss a hypothesis I developed on CFS/ME.

I have been working in a very promising new area of research, akin to the discovery of nitric oxide in its importance, for which the Nobel Prize was awarded. Very few scientists or physicians are familiar with it. I should add that I am not looking for any research money, but instead, I am hoping to interest researchers in this idea. I could not have gotten to this point without the support of Dr. Carl Peck, a former Assistant Surgeon General, who, early on, felt that I had made a discovery and guided me through the process of writing the hypothesis, which was e-published ahead of print in September by the Journal of Medical Hypotheses.

Almost two years ago to the day, I attended a lecture by a scientist who was able to induce a state of suspended animation in mice using the gas hydrogen sulfide, or H2S. As I listened to him, I was struck by the similarities between what happened to the mice, i.e., a decrease in core body temperature, an apnea-like sleep state, reduced heart and respiration rates, and a severe metabolic drop, and what happens to people with CFS/ME.

Out of that idea, grew my hypothesis that CFS/ME is caused by dysregulation of hydrogen sulfide metabolism. Further I postulate that the multi-system disturbances in the homeostasis of endogenous H2S result in mitochondrial dysfunction. Research on H2S – the gas that causes the characteristic smell of rotten eggs – dates to the 1700’s. At high concentrations, H2S is instantaneously deadly, on a par with cyanide. At low concentrations, some evidence suggests that H2S has beneficial effects and can act as an endogenous biological mediator. In fact, the brain, pancreas and the gastrointestinal tract produce H2S. Endogenous H2S plays a role in regulating blood pressure, body temperature, vascular smooth muscle, cardiac function, cerebral ischemia, and in modulating the hypothalamus/pituitary/adrenal axis. It even has been called a “master metabolic regulator”.

We refer to CFS/ME as a systemic disease, but no unifying thread has been found. H2S directly affects the neurologic, endocrine and immunologic systems—the very systems most involved in CFS.

In persons with CFS/ME, one plausible etiology is an increase in the activity of endogenous H2S, thereby inhibiting mitochondrial oxygen utilization. In this view, fatigue and the other CFS/ME symptoms could be due to diminished physiological and cellular energy due to reduction in the capacity of mitochondria to utilize oxygen and synthesize ATP. Specifically, H2S binds to the mitochondrial enzyme cytochrome c oxidase, which is part of Complex IV of the electron transport chain, and attenuates
oxidative phosphorylation and ATP production.

Consistent with this finding, recent research on low level H2S toxicity points to increased formation of free radicals and depolarization of the mitochondrial membrane, a condition that would decrease ATP synthesis. If poisoning renders mitochondria inefficient, one would expect cells to shift to anaerobic mechanisms, a shift that has been reported for CFS patients. Also consistent with this hypothesis is the fact that mitochondria are organelles descended from ancient eukaryotic sulfur-utilizing microbes. Thus, it is not surprising that they show a very high affinity for sulfide. In other words, they have retained the ancient capability of utilizing this gas.

Given a predisposing genetic background, H2S may lead to genomic instability or cumulative mutations in the mitochondrial DNA. Alternatively, the effects of H2S could be initially mediated by changes in the redox potential of cells or changes in their sulfur metabolism.

Of importance, H2S plays a pivotal role in both aerobic and non-aerobic organisms as a signaling molecule. Bacteria in the gut both produce H2S and utilize it as a substrate alternative to oxygen. This is of particular relevance in the gastrointestinal tract, where unusually high levels of gram-negative bacteria, which increase intestinal permeability have been found in patients with CFS/ME. In addition to bacteria, many of the foods and substances people with CFS are sensitive or allergic to produce H2S under certain conditions, such as mold, milk, eggs, wine, corn syrup and the ever-ubiquitous yeast.

CFS/ME is a model disease for multi-system disturbance. It is my hypothesis that the mitochondria in patients with CFS/ME, organelles required by every cell to sustain life, are unable to adequately utilize oxygen. This mitochondrial disturbance could be due to
the combined effects of anaerobic conditions known to occur in CFS and associated low-level H2S toxicity. This increase in H2S alters fine signaling necessary for body homeostasis and, in my hypothesis, causes CFS/ME.

New discoveries on H2S are being made every day. I would encourage you to go to PubMed or Google and type in your area of research and “H2S”. If you are interested in cardiac function, you will find last week’s article in Science Daily about John Hopkins’ Solomon Synder’s finding that H2S controls blood pressure. If you are interested in catecholamines, you can read about the inhibitory action of H2S donors on norepinephrine. If you are interested in immune function, you will find that exogenous hydrogen sulfide induces functional inhibition and cell death of cytotoxic lymphocyte subsets of CD8 (+) T cells and NK cells. If you are prescribing vitamin B-12 to your patients, you will see evidence supporting hydroxocobalamin as an antidote against H2S poisoning, and so on.

My hypothesis paper does not address the fact that H2S is increasing in the environment as a result of global warming, natural gas and crude oil refining, centralized animal feeding operations, and chemical processes. It seems logical, though, that external levels could affect internal levels, just as oxygen does. Just yesterday I came across an article in last week’s Science magazine about the role green sulfur bacteria played in the Permian-Triassic extinction, as H2S levels rose and oxygen decreased in the oceans.

In summary, I ask you to keep an open mind and support this idea. A simple program could be undertaken. There are several genetic polymorphisms and enzyme deficiencies related to H2S and sulfur metabolism that should be investigated, after which H2S and associated chemicals such as thiols and glutathione levels in the body could be assessed. As H2S cuts across disciplines and appears to be implicated in several diseases, I think it would be an important focus for NIH. I feel confident that such a program would lead to discoveries.

It may well provide a unifying lens through which to view the diverse manifestations of this complex disease. With determination and resources, we may discover that the disparate parts so many dedicated people have been researching for two decades can begin to add up to a new understanding of this very complex disease. Thank you.
Hypothesis: Chronic Fatigue Syndrome, Mitochondrial Hypo-function, and Hydrogen Sulfide

Marian Dix Lemle, M.B.A.

[...]

Copyright 2007

ACKNOWLEDGEMENTS
The author wishes to express deep gratitude to Carl Peck, M.D., Ph.D., for his devoted mentoring, critical insights, and unfailing support in the development of the hypothesis and in the preparation of this paper. Any errors are solely the responsibility of the author.
Disease conditions evident in Chronic Fatigue Syndrome (CFS) reflect mitochondrial hypo-function, resulting in multi-system hypo-function. Mitochondrial hypo-function can result from disturbances of hydrogen sulfide homeostasis. CFS involves mitochondrial hypo-function related to disturbed hydrogen sulfide homeostasis in the body. Disturbed hydrogen sulfide homeostasis leads to systemic changes at the molecular level. Understanding the role of hydrogen sulfide in the body may provide a unifying lens through which to view the diverse manifestations of this complex disease known as Chronic Fatigue Syndrome.

HYPOTHESIS: CHRONIC FATIGUE SYNDROME, MITOCHONDRIAL HYPO-FUNCTION, AND HYDROGEN SULFIDE
Introduction

Defining the debilitating disease known as Chronic Fatigue Syndrome is a challenge. According to the International Association for Chronic Fatigue Syndrome, CFS, (also known as Myalgic Encephalomyelitis, or ME), is an immune-related illness with immune activation or dysfunction resulting from a number of possible root causes, including triggers by infectious agents, environmental sensitivities, genetic factors and/or physical stressors.

Many U.S. clinicians treating CFS patients have found the 1994 CDC definition too ambiguous for diagnostic purposes, preferring to use a more specific case definition, formally adopted by the Canadian government in 2003. Under that definition, a patient with ME/CFS must meet the following criteria for a minimum of six months: unexplained (after excluding known causes) debilitating fatigue, post-exertional malaise and/or fatigue, sleep dysfunction, and pain, and have two or more specific neurological/cognitive manifestations, and one or more symptoms from two of the categories of autonomic, neuroendocrine, and immune disorders.

A multi-factorial systems approach may provide the greatest opportunity for understanding the complex web of relationships in this disease. I propose that CFS involves an external trigger, possibly infectious and/or environmental, possibly in conjunction with a genetic predisposition, leading to systemic changes at the molecular level. The occurrence of periodic outbreaks is consistent with an external trigger. Understanding the role of hydrogen sulfide in the body may provide a uniform basis in which to view this complex disease.

Data on the interaction of environmental exposures with genetic factors to alter normal biological function are sparse, although this topic recently has become a focus of research at the National Institutes of Health. The systematic integration of diverse data, such as that proposed by the CDC, will certainly broaden our base of knowledge.

Mitochondria

The primary manifestation of CFS is a severe and highly debilitating deficit of energy. Energy is generated at the cellular level in the mitochondria, the “power plants” of the body’s oxygen-based aerobic cellular respiration. Adenosine triphosphate (ATP), the molecule the cell uses for the bulk of its energy needs, is produced here. Cells requiring large amounts of ATP, such as muscle cells, have many mitochondria.
Mitochondria are cellular organelles descended from ancient eukaryotes. The eukaryotes had a physiologically diverse range of oxygen requirements, ranging from aerobic, facultatively anaerobic to strictly anaerobic. The study of evolutionary metabolic pathways, particularly with respect to substrate flexibility and bacteria/organelle interface, may provide insight into CFS.

Mitochondrial Disease

Mitochondria contain their own DNA (mtDNA). Protein products of this mtDNA join with nuclear DNA-coded proteins in the electron transport chain, where damaged (either through genetic inheritance or through environmental insult) nuclear DNA can result in damage to mtDNA. In addition to defects of mtDNA which can occur as a result of inheritance, damage to DNA can be sustained through environmental injury, e.g., such as that arising from methyl mercury or manganese exposure. Thus there are several possible ways in which damage to the mitochondrial DNA can occur.

Mitochondrial disease leads to impaired respiratory chain function and reduced ATP production. Because mitochondria exist in almost all cells of the body, multi-system dysfunction and phenotypic variability are hallmarks of mitochondrial disease. The range of mitochondrial injury and resulting illness is extensive, for example, from severe, irreversible pathogenic early-onset disease such as Leber’s optic neuropathy, which leads to blindness in young adults, to lesser dysfunction associated with metabolic pathways, such as oxidative phosphorylation (OXPHOS) diseases. The understanding of mitochondrial encephalomyopathies is quickly evolving.

Exogenous Hydrogen Sulfide

H₂S, the gas with a rotten egg odor, is generally considered to be an environmental toxin, on the level of cyanide. At a level of 1000 parts per million, breathing H₂S is lethal. In 1999, nearly 130 public health and related groups called for the EPA to list hydrogen sulfide as a hazardous air pollutant. According to their report, demonstrable symptoms of chronic exposure included pronounced deficits in balance and reaction time, as well as such ailments as dizziness, insomnia, and overpowering fatigue, as well as abnormal neurobehavioral functioning and altered mood states (e.g., depression, fatigue, tension, vigor). Further, the report indicated that numerous CNS-brain effects occur including: changes in brain density, headache, memory loss, reduced sense of smell, loss of balance, dizziness, sleep difficulties, and fatigue.

The traditional explanation of the toxic effects of hydrogen sulfide is based on its property as a chemical asphyxiate; it binds to the mitochondrial enzyme cytochrome c oxidase (iron-containing protein), blocking oxidative phosphorylation and ATP production. In rats, the gas causes an increase of blood lactate concentration and the lactate/pyruvate ratio, leading to anaerobic glycolysis and inhibition of lipid peroxidation.
Recent research has indicated that exogenous hydrogen sulfide induces functional inhibition and cell death of cytotoxic lymphocyte subsets for CD8 (+) T cells and NK cells. Lowered CD8+ T cells and poorly functioning NK cells are among the most robust immunological abnormalities found in CFS.

Endogenous Hydrogen Sulfide

Within the last decade, interest has been directed towards the role of endogenous H2S as the “third” gaseous mediator involved in natural biological function, after carbon monoxide and nitric oxide. Homeostatic abnormalities of the gas in the body have been identified in several disorders, including ulcerative colitis, Alzheimer’s disease, Down’s syndrome and possibly in diabetes and sudden infant death syndrome.

Of particular interest to the CFS community is that, at certain oxygen tensions, H2S has been used to produce a (reversible) hibernative state of reduced metabolic activity in mice, not dissimilar from the disease conditions that exist in patients with Chronic Fatigue Syndrome. Physiological responses induced in the mice included a decrease in core body temperature, an apnea-like sleep state, reduced heart and respiration rates, and severe metabolic changes, with possible vagus nerve involvement. Oxygen consumption dropped by ~50% and carbon dioxide output dropped by ~60% within the first five minutes. By inhibiting cytochrome c oxidase and oxidative phosphorylation, it was theorized that the gas had “switched off” the cell’s utilization of oxygen. It recently has been reported that hydrogen sulfide could protect mice in the hibernative state from lethal hypoxia and that H2S serves as an oxygen sensor/transducer, mitigating effects of hypoxia.

Hydrogen Sulfide and Chronic Fatigue Syndrome

The physical responses of hibernation induced by the gas are not unlike the symptoms and torpor experienced by CFS/ME patients. A similar mechanism is postulated to play a role in CFS patients.

H2S is apparently active in many of the same systems involved in CFS. It is produced from cysteine in the brain by two enzymes, cystathionine beta-synthase and cystathionine gamma lyase, in response to neuronal excitation. There it alters hippocampal long-term potentiation, initiates calcium waves, and regulates the release of corticotrophin-releasing hormone from the hypothalamus. H2S may also play a role in the control of the neuroendocrine stress axis.

H2S plays an important role in the cerebrovascular system. It serves as an oxygen sensor/transducer in vertebrate hypoxic vasoconstriction and hypoxic vasodilation. Low concentrations of H2S cause arterial vasoconstriction, reverse NO-mediated vasorelaxation and cause an NO-dependent pressor effect in vivo. It is reported to be a mediator of cerebral ischemic damage. H2S is believed to affect vasoactivity in an
oxygen-dependent manner,⁴⁹ and to regulate the availability of nitric oxide in the vascular system.⁵⁰

Genetic evidence of mitochondrial involvement in CFS has been found, including in genes related to fatty acid metabolism, apoptosis, mitochondrial membrane function,⁵¹ protein production in mitochondria,⁵²,⁵³ and others.⁵⁴,⁵⁵ Two studies have found evidence of cytochrome c oxidase gene involvement.⁵⁶ The metabolic processes associated with the production of energy, reactive oxygen species (ROS), otherwise known as free radicals, and the accumulation of mtDNA damage, have been suggested as underlying pathophysiological mechanisms of CFS.⁵⁷,⁵⁸,⁵⁹,⁶⁰

The mechanisms involving reduction and utilization of oxygen (redox) and those involving ATP, as well as fatty acid metabolism, are all of relevance to mitochondrial processes.

The cell’s ability to utilize oxygen in the process of creating ATP is critical. Too much or too little oxygen can be deadly.⁶¹ Mitochondria adapt to hypoxia, or more precisely, to differing oxygen tensions, by altering mitochondrial oxygen consumption.⁶²,⁶³,⁶⁴,⁶⁵ Further, the role of reactive sulfur species⁶⁶ may be important in the oxidation process and balance of CFS patients. Preferential retention of sulfur amino acids occurs during an inflammatory response, suggesting an increased requirement for cysteine and a higher level of glutathione turnover during sepsis in rats.⁶⁷,⁶⁸

H₂S affects the cell’s ability to utilize oxygen by inhibiting Level IV of the electron transport chain. The inhibition of mitochondrial complex IV may lead to secondary loss in complex II-III activity, which may lower reactive oxygen species formation.⁶⁹ In addition, hydrogen sulfide binds to hemoglobin in red blood cells, interfering with oxygen transport. Decreased levels of reactive oxygen species can improve cell viability and, in doing so, limit cellular damage induced by homocysteine.⁷⁰ Recent research on the molecular mechanisms of H₂S toxicity points to reactive oxygen species formation and mitochondrial depolarization.⁷¹

Mitochondria show a very high affinity for sulfide that permits its use as an energetic substrate at low micromolar concentrations, hence, below the toxic level. However, if the supply of sulfide exceeds the oxidation rate, poisoning renders mitochondria inefficient and an anaerobic mechanism involving partial reversion of Krebs cycle already known in invertebrates may take place.⁷² Given a predisposing genetic background that compromises DNA repair or “hyper-susceptibility”, H₂S may lead to genomic instability or cumulative mutations.⁷³,⁷⁴,⁷⁵

H₂S plays a pivotal role in both aerobic and anaerobic organisms as a signaling mediator.⁷⁶ It contributes significantly to chronic intestinal disorders that are dependent upon gene-environment interactions.⁷⁷,⁷⁸,⁷⁹ Impaired butyrate oxidation and raised counts of sulfate-reducing bacteria in the colon of patients with ulcerative colitis indicate that the disease may be induced or aggravated by hydrogen sulfide toxicity.⁸⁰ H₂S induces direct radical-associated DNA damage, highlighting the possible role of sulfide as an environmental insult that, given a predisposing genetic background, may lead to genomic instability or the cumulative mutations characteristic of colorectal cancer.⁸¹
Metal toxicity and glutathione depletion also appear to be affected by H₂S. Both also have also been mentioned as underlying causes of CFS. It is theorized that H₂S can reduce intracellular bound ferric iron to form unbound ferrous iron, which activates iron. Bacteria in the gut produce H₂S, which when combined with ferrous iron, produces insoluble heavy metal sulfides.  

Additionally, H₂S can increase hepatocyte formation of reactive oxygen species. H₂S cytotoxicity also involves a reactive sulfur species, which depletes glutathione (GSH) and activates oxygen to form ROS. Glutathione-depleted hepatocytes have been shown to be susceptible to NaHS cytotoxicity, indicating that GSH detoxifies NaHS (a H₂S source) or H₂S in cells. H₂S also plays a role in cellular proliferation and apoptosis.  

Recently, gram-negative bacteria, which are increased in gut-intestinal permeability, were implicated in the etiology of CFS, and similarly, in AIDS activation. The relationship between bacteria, other microorganisms and hydrogen sulfide in the context of chronic fatigue syndrome should be investigated.

Conclusion

All of these systems described in the foregoing have a role in CFS. It is proposed that focused research will demonstrate that the mitochondrial hypo-function in CFS can result from abnormalities of hydrogen sulfide homeostasis. Understanding the role of hydrogen sulfide in the body may provide a unifying lens through which to view the diverse manifestations of this complex disease.
REFERENCES


7 Dyall SD, BrownMT, Johnson PJ, Ancient Invasions: from endosymbionts to organelles, Science, 2004 Apr. 9; 304(5668): 253-7. PMID: 15073369


12 DiMauro S, Mitochondrial Function and Disorders, Basic and Clinical Neurosciences Dept. Columbia University
http://neuroscienceupdate.cumc.columbia.edu/speakers/speaker_dimauro.html

13 Mori N, Yasutake A, Hirayama K, Comparative study of activities in reactive oxygen species production/defense system in mitochondria of rat brain and liver, and their susceptibility to methylmercury toxicity. Arch. Toxicol. 2007 April 27, PMID: 17464500


18 Berdanier, C, editor, Mitochondria in Health and Disease (Oxidative Stress and Disease), CRC Press, Taylor & Francis Group, 2005, p. 53


25 Sierra Club Press Release, January 25, 1999


28 Komaroff, AL, HHV-6 Biology, Viruses and Immunology in Chronic Fatigue Syndrome, paper presented at the IACFS Conference, January 12-14, 2007 www.iacfs.net

29 Bhatia M, Hydrogen sulfide as a vasodilator. IUBMB Life. 2005 Sep;57(9):603-6 PMID: 16203678


33 Kimura H, Hydrogen Sulfide Is Severely Decreased in Alzheimer Disease Brains, Molecular Neurobiology of Alzheimer Disease and Related Disorders, 2004, pp 79-83

34 Eto K, Asada T, Arima K, Makifuchi T, Kimura H. Brain hydrogen sulfide is severely decreased in Alzheimer’s disease, Biochem Biophys Res Commun. 2002 May 24;293(5):1485-8 PMID: 12054683


48 Qu K, Chen CP, Halliwell B, Moore PK, Wong PT. Hydrogen sulfide is a mediator of cerebral ischemic damage. Stroke. 2006 Mar;37(3):889-93. PMID: 16439695


52 “Chronic fatigue is not all in the mind”, 21 July 2005 http://www.newscientist.com/article.ns?id=mg18725093.700&print=true

53 The most comprehensive study of genetics in CFS is being undertaken at the Department of Cellular and Molecular Medicine, St. George’s University of London, Imperial College, London. So far, the principal investigator has found three genes related to mitochondrial function in his gene expression study. For general information see, Current research priorities in chronic fatigue syndrome/myalgic encephalomyelitis:disease mechanisms, a diagnostic test and specific treatments. J Clin Pathol. 2007 Feb;60(2):113-6 PMID: 16935968


56 Ibid., Vernon, et al

57 Ali M, The Cause of Fibromyalgia: the respiratory-to-fermentative shift (the DysOx State) in ATP production, J. Integrative Medicine 2003;8:135-140


61 Adler, J, To Treat the Dead: The new science of resuscitation is changing the way doctors think about heart attacks-and death itself. Newsweek, May 7, 2007, p. 56


72 Goubert M, Andriamihaja M, Nubel T, Blachier F, Bouillaud F, Sulfide, the first inorganic substrate for human cells, FASEB J. 2007 Feb 23. PMID: 17314140


74 Attene-Ramos et al, Hydrogen Sulfide Induced Direct Radical-Associated DNA Damage, Mol. Cancer Res. 2007 PMID: 17475672


81 Attene-Ramos MS, Wagner ED, Gaskins HR, Plewa MJ, Hydrogen Sulfide Induces Direct Radical-Associated DNA Damage Mol Cancer Res. 2007 May 2; [Epub ahead of print] PMID: 17475672

82 Wang L, Warner NE, Sherrod AE, Pathological Quiz Case: A 79-Year Old Woman with a Black, Ulcerated Cecal Tumor and 3 Negative Guaiac Test Results, Archives of Pathology and Laboratory Medicine: Vol. 129, No. 1, pp. 113-114 PMID: 15628891


85 Maes M, Mihaylova I, Leunis JC, Increased serum IgA and IgM against LPS of enterobacteria in chronic fatigue syndrome(CFS): indication for the involvement of gram-negative enterobacteria in the etiology of CFS and for the presence of an increased gut-intestinal permeability, J. Affect Disord. 2007 Apr;99(1-3):237-40 PMID: 17007934

Hypothesis: Chronic fatigue syndrome is caused by dysregulation of hydrogen sulfide metabolism

Chronic fatigue syndrome (CFS), which is also known as myalgic encephalomyelitis (ME), is a debilitating, multi-system disease whose etiology is unclear, and for which there are as yet no reliable treatments. Here the hypothesis is advanced that the multi-system disturbances in CFS/ME are caused by disturbances in the homeostasis of endogenous hydrogen sulfide (H₂S) and result in mitochondrial dysfunction.

Research on H₂S – the gas that causes the characteristic smell of rotten eggs – dates to the 1700’s and has shown a remarkable range of effects in both animals and humans. At high concentrations, H₂S has a variety of biological toxicities including being instantaneous deadly; at low concentrations some evidence suggests that H₂S has beneficial effects and can act as an endogenous biological mediator – the third such gaseous mediator discovered (after nitric oxide and carbon monoxide). The brain, pancreas and the gastrointestinal tract produce H₂S. Endogenous H₂S plays a role in regulating blood pressure, body temperature, vascular smooth muscle, cardiac function, cerebral ischemia, and in modulating the hypothalamus/pituitary/adrenal axis. It even has been called a “master metabolic regulator”.

Recent research has demonstrated that at low, non-toxic doses, exogenous H₂S produces a reversible state of hibernation-like deanimation in mice, causing a decrease in core body temperature, an apnea-like sleep state, reduced heart and respiration rates, and a severe metabolic drop [1]. These characteristics are not unlike the symptoms and extreme “de-animation” experienced by CFS/ME patients. Moreover, H₂S affects biological networks that are disrupted by CFS including neurologic, endocrine and immunologic systems. Therefore, a plausible etiology of CFS is an increase in the activity of endogenous H₂S, thereby inhibiting mitochondrial oxygen utilization.

H₂S and Mitochondria

In this view, fatigue and the other CFS/ME symptoms could be due to diminished physiological and cellular energy due to reduction in the capacity of mitochondria to utilize oxygen and synthesize ATP. Specifically, H₂S binds to the mitochondrial enzyme cytochrome c oxidase, which is part of Complex IV of the electron transport chain, and attenuates oxidative phosphorylation and ATP production.

Consistent with this finding, recent research on low level H₂S toxicity points to increased formation of free radicals and depolarization of the mitochondrial membrane, a condition that would decrease ATP synthesis [2]. If poisoning renders mitochondria inefficient, one would expect cells to shift to anaerobic mechanisms, a shift that has been reported for CFS patients. Also consistent with this hypothesis is the fact that mitochondria are organelles descended from ancient eukaryotic sulfur-utilizing microbes. Thus, it is not surprising that they show a very high affinity for sulfide.

Of course, H₂S or sulfide may not directly affect mitochondria by binding to them. Genomic changes could mediate some of the effects of H₂S. Some studies have found evidence for the involvement of the cytochrome c oxidase gene in CFS/ME. Also, investigators have found CFS abnormalities in genes related to fatty acid metabolism, apoptosis, mitochondrial membrane function, and protein production in mitochondria. Given a predisposing genetic background, H₂S may lead to genomic instability or cumulative mutations in the mitochondrial DNA [3].

Alternatively, the effects of H₂S could be initially mediated by changes in the redox potential of cells or changes in their sulfur metabolism, especially in glutathione. Another possible mechanism is a direct effect of H₂S on the immune system. Recent research indicates that exogenous hydrogen sulfide induces functional inhibition and cell death of cytotoxic lymphocyte subsets of CD8 (+) T cells and NK cells.

Finally, H₂S plays a pivotal role in both aerobic and anaerobic organisms as a signaling molecule. Bacteria in the gut both produce H₂S and utilize it as a substrate alternative to oxygen. This is of particular relevance in the gastrointestinal tract, where unusually high levels of gram-negative bacteria, which increase intestinal permeability, have been found in patients with CFS/ME [4]. In addition to bacteria, yeast, mold and other fungi also emit H₂S.

CFS/ME is a model disease for multisystem disturbance. It is my hypothesis that mitochondria, organelles required by every cell to sustain life, are unable to adequately utilize oxygen. This mitochondrial disturbance could be due to the combined effects of anaerobic conditions known to occur in CFS and associated low-level H₂S toxicity. This increase in H₂S alters fine signaling necessary for body homeostasis, and causes CFS. Understanding the role of H₂S in the body, and, in particular, in mitochondrial function, may provide a unifying lens through which to view the diverse manifestations of this complex disease.

References


Marian Dix Lemle
3135 Elliot Street, NW
Washington, DC 20008, United States
Tel.: +1 202 537 6344; fax: +1 202 775 0045
E-mail address: mdlemle@yahoo.com
doi:10.1016/j.mehy.2008.08.004
Marian Dix Lemle

Marian Lemle is a citizen scientist. When her daughter suddenly became ill with CFS/ME, Marian worked hard to discern the possible causes. While attending a lecture on induced hibernation in mice at the annual meeting of the White House Fellows in 2006 (where she had lectured the previous year on creative thinking), she had a spark of inspiration— that people with CFS/ME didn’t need an injection of hydrogen sulfide gas (a focus of the mouse study)—they were already in a hibernative state. That insight led to the development of the “hibernation” theory of illness in CFS/ME. She produced a paper on the topic in 2007, published a short hypothesis in 2008 and testified before the CFSAC Committee that same year. She also presented a poster on her theory at the IACFS/ME Conference in 2009. Since then, she has carefully monitored developments in the field and recently decided that it was timely to publish her findings.

Early on in her career, Marian worked in policy planning and development at the White House Office of Telecommunications Policy. She has served on four non-profit educational boards.

She holds an M.B.A. from the Wharton School, University of Pennsylvania, where she was the Edward Shils Teaching Assistant in Venture Initiation and Entrepreneurial Development, and a Certificate in Painting and Drawing from the Corcoran College of Art + Design, where she was awarded the Vera Lester Memorial Award for Painting. Recently, she descended over 2000 feet below sea level in a small submarine to witness firsthand life forms that thrive in an environment of diminished oxygen and total darkness.

Rowe, Peter

[...] 

Subject: RFI comments

Attached is my response to the RFI. Thanks for this opportunity.

Sincerely,

Peter C. Rowe, MD
Johns Hopkins University

June 24, 2016

Trans-NIH ME/CFS Working Group

To Whom It May Concern:
I appreciate the opportunity to respond to the RFI. My background is as a clinical researcher and pediatric clinician. I have been engaged in the clinical care of adolescents and young adults with CFS since 1993, and had several suggestions that I hope are germane to the Working Group’s task

**Emerging needs/Gaps and opportunities**

The following seem to me to be important areas of emerging scientific need in this field.

**Investigation of the role of auto-immunity in ME/CFS**, including:

i. Is Rituximab as effective as was suggested by the intriguing results of the pilot Rituximab trial in Norway (Fluge 0, et al, 2011)? I am not aware of formal RFPs on this topic at the NIH level, despite the potential for this kind of approach to both improve patient care and improve understanding of the mechanisms of the illness.

ii. There is some interesting preliminary work on antibodies directed against autonomic receptors in POTS and CFS (see for example work by Kem D, 2014; Loebel M, 2015). Further study of these issues and of the efficacy of IVIG in this subset would be warranted. We know IVIG had a mixed effect when offered to unselected adult participants with CFS in the past, but reducing the heterogeneity of the sample by enrolling only those with autonomic autoantibodies would be a more efficient study design.

iii. Katherine Rowe (1997) published a randomized placebo-controlled trial of IVIG that enrolled 71 Australian adolescents with ME/CFS. There was a significant improvement in overall function after at 6 months of follow-up in those who had received IVIG in a dose of 1 gram/kg (max 60 grams) monthly for 3 months. Cell-mediated immunity was abnormal in 52 percent of ME/CFS participants at baseline. Given the scientific strength of the randomized controlled trial design, the relatively larger sample size, and the reported benefit of IVIG for pediatric ME/CFS patients, further investigation of IVIG in the pediatric ME/CFS population is warranted.

**Investigation of the interaction of connective tissue laxity and the circulatory abnormalities in CFS:**

i. Ehlers Danlos syndrome and joint hypermobility are risk factors for both orthostatic intolerance and CFS. This observation was made by our group in 1999 and extended in the years to come (Barron D, et al, 2002). Subsequent work has confirmed the high prevalence of orthostatic and autonomic dysfunction among those with joint hypermobility or EDS (Gazit, 2004), de Wandele (2013, 2016). These observations have been relatively neglected in the studies on CFS, despite a substantial overlap in symptoms. For example, EDS is known to have a high prevalence of chronic fatigue and chronic pain. A much better understanding of the prevalence and impact of connective tissue laxity on the phenotype of CFS seems warranted. In study designs, a failure to
stratify or subgroup participants on the presence or absence of EDS would completely muddy the study results.

ii. Mast cell activation syndromes have emerged in recent years as overlapping with joint hypermobility, POTS, and allergic disorders (Molderings G, 2011; Afrin L, 2014; Lyons JJ, 2014). Lyons in the Joshua Milner group at the NIAID reported hereditary tryptasemia among individuals with joint hypermobility, POTS, neuropsychiatric symptoms, and allergic problems. The symptoms of MCAS overlap a great deal with CFS, and a subset of those with POTS have mast cell disorders (Shibao C, et al, 2005). Apart from some early speculative papers (Theoharides, 2005) there has been very little investigation of this overlap, despite the potential for improved understanding of the mechanisms of symptoms, and some very practical therapeutic options for those with ME/CFS.

iii. There is emerging evidence of circulatory dysfunction associated with anatomic abnormalities in the cranio-cervical junction, especially among those with ligamentous laxity. This warrants further study, especially among the most impaired individuals with ME/CFS.

Investigation of orthostatic intolerance as a treatable contributor to ME/CFS symptoms:

Twenty years ago our group reported a high prevalence of treatable orthostatic intolerance (OI) in those with CFS. Our screening of 160 individuals in the Florinef RCT conducted with Steven Strauss’s group at the NIH showed that > 95% of those who were tested felt worse in response to orthostatic stress, even though only 64% had objective hemodynamic abnormalities. Gaps in this literature relate to:

i. Lack of studies on orthostatic stress as a cause of post-exertional worsening. Even when not accompanied by hemodynamic changes, orthostatic stress typically has been associated with a provocation or exacerbation of characteristic CFS symptoms. CFS symptoms and hemodynamic abnormalities with orthostatic stress can be reversed upon application of external lower-body compression (Streeten, 2000). Little has been written about the potential for orthostatic stress to be considered a consistent method of provoking prolonged exacerbations in symptoms (akin to exercise as a stressor). We missed an opportunity to address this in our early tilt testing studies: patients reported a several day exacerbation of CFS symptoms following tilt testing. We responded to this by providing IV saline boluses of 1-2 L after the tilt test, which was then followed by a marked improvement. This would warrant more formal investigation, including such studies as examining whether the same gene expression changes as observed by Light and colleagues after exercise are also seen in response to orthostatic stress.

ii. Paucity of studies on the interaction of cognitive dysfunction and orthostatic stress. Cognitive testing is often similar at baseline in those with CFS and healthy controls. Significant differences and worse performance emerge if cognitive challenges are combined with orthostatic stress (Ocon, 2012). This important work has yet to be replicated.
iii. Paucity of work on the optimal treatment of OI in CFS and in specific subgroups. Apart from the trials examining Florinef in adult CFS and clonidine in pediatric CFS, there have been very few studies examining the response to treatment of OI in ME/CFS.

iv. Relatively little study of the pathophysiological mechanisms of OI (connective tissue laxity, auto-antibodies, low blood volume, biomechanical and neuro-anatomic contributors). This has the potential to improve understanding and treatment of this common problem.

Challenges

Lack of funding for treatment trials: There has thus far been little support at the NIH level for treatment trials. Treatment studies may be a "back-door" method of improving our understanding of the pathophysiology of symptoms. I believe an emphasis on enrichment strategies to better understand whether commonly used treatments are working (randomized withdrawal of medications that are thought to be helping, after the patient has had a run-in period to control other co-morbid conditions) would provide a better evidence base for treating patients. For example, injectable vitamin B12 is commonly used by many CFS practitioners (Regland B, 2015). A crossover trial of the randomized withdrawal of B12 in those who report a benefit of B12 has the potential to clarify matters, but would need a fairly large commitment to a center that sees a large number of patients, has pharmacist and clinical trials support, etc.

The paucity of academic clinical sites: In the US there is a striking mismatch between the number of patients needing care and the number of experienced providers. Because insurers in this country do not reimburse in a manner commensurate with complexity of CFS, there are very few hospital/university clinics dedicated to CFS. We have no CFS training grants or federally funded research or treatment centers to help advance and translate knowledge to the primary care physicians. Patients are often left doing their best working with a beleaguered generalist, or paying inordinate sums to those who work in private practice specialty CFS clinics, thereby ensuring that only the wealthy have access to the best care. I think a better linkage between clinical care and scientific investigators is critical to advancing understanding.

Attracting and retaining young investigators for pediatric CFS: Offering an estimated 1 million annually in NIH funds targeting children with CFS is not enough to create a critical level of interest in attracting and retaining qualified young investigators into the field, and it does not make CFS seem like a viable option to the new pediatric CFS researcher. Foundation grants cannot be expected to make up the difference, as they are relatively small, and most foundation grants carry no option for salary support. A person trained to do research can always re-tool and move to other fields where NIH funding is less capricious and more predictable (e.g., obesity, asthma, HIV).

I'll end there to ensure this gets submitted on time. I wish the Working Group good energy and good fortune in dealing with all of the comments, and appreciate your efforts on behalf of this patient population.
Sincerely,

Peter C. Rowe, MD
Professor of Pediatrics
Director, Chronic Fatigue Clinic,
Johns Hopkins Children’s Center
Sunshine Natural Wellbeing Foundation Professor of
Chronic Fatigue and Related Disorders

Lipkin, Ian
[...]
Subject: NOT-NS-16-024 Response

Dear Trans-National Institutes of Health (NIH) ME/CFS Working Group,
Attached please find our response to notice NOT-NS-16-024, “Request for Information: Soliciting Input for New Research Strategies for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS)”. Best,

W. Ian Lipkin, MD
John Snow Professor of Epidemiology,
Mailman School of Public Health

Professor of Neurology and Pathology,
College of Physicians and Surgeons

Director, Center for Infection and Immunity

Director, NIAID Center for Research in Diagnostics and Discovery

Columbia University
www.cii.columbia.edu
Re: Response to notice NOT-NS-16-024, “Request for Information: Soliciting Input for New Research Strategies for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS)”

Dear Trans-National Institutes of Health (NIH) ME/CFS Working Group,

We deeply appreciate the opportunity to share our thoughts on research strategies to address the challenge of ME/CFS. Our overarching objectives are to understand the causes and mechanisms of disease, establish reliable diagnostic tests and find treatments to reduce the morbidity of ME/CFS.

Toward this end, we have formed a group of leading ME/CFS clinicians and researchers under the auspices of “Center for Solutions for ME/CFS (CfS for ME/CFS)”. Members of this group include:

- **Lucinda Bateman, MD**
  Founder & Chief Medical Officer
  Bateman Horne Center
  Salt Lake City, Utah

- **Mady Hornig, MD**
  Associate Professor of Epidemiology
  Mailman School of Public Health, Columbia University
  New York, NY

- **Nancy Klimas, MD**
  Director, Institute for Neuro Immune Medicine
  Nova Southeastern University
  Miami, FL

- **Anthony Komaroff, MD**
  Professor of Medicine
  Harvard Medical School
  Boston, MA

- **Susan Levine, MD**
  Founder, Medical Office of Susan M. Levine, M.D.
  New York, NY

- **Eugene Major, PhD**
  NIH/NINDS Laboratory of Molecular Medicine & Neuroscience
  Bethesda, MD

- **Jose G. Montoya, MD**
  Professor of Medicine, Division of Infectious Diseases
  Stanford University Medical Center
  Palo Alto, CA

- **Daniel Peterson, MD**
  President, Sierra Internal Medicine
  Incline Village, NV

- **Beth Unger, PhD, MD**
  Branch Chief, Chronic Viral Diseases Branch
  Centers for Disease Control and Prevention
  Atlanta, GA

The team came together in 2011 at the request of NIH director, Francis Collins, to rigorously review evidence linking XMRV to ME/CFS. After XMRV was found to be a laboratory contaminant we continued under the auspices of the Hutchins Family Foundation- Chronic Fatigue Initiative and a patient-led crowdfunding initiative, The Microbe Discovery Project, to build an integrated, multicenter program focused on pathogen discovery, microbiome research, proteomics, metabolomics and epigenetics. With the assistance from the ME/CFS community we have amassed detailed questionnaire data for epidemiology and hundreds of blood, stool and saliva samples for microbiome, proteomic and metabolomic analyses. Our team has already identified differences between short- and long-term illness duration subsets of patients based on CSF cytokine data and has early findings that we believe will impact the lives of thousands of patients.
In 2015, we began a one-year NIH-funded study to recruit and enroll an additional 125 ME/CFS cases and 125 healthy controls across five geographically distinct clinical sites. Each of these subjects are rigorously screened by clinical experts in ME/CFS. A wide range of biological samples are to be collected at four time points to account for seasonal variation. We have already collected 25% of the projected questionnaire data and samples; thus, we are on track to have the world’s most comprehensive ME/CFS database and sample repository by early 2017. We meet in monthly teleconferences to review progress in meeting study objectives and to focus our limited analytical resources on the most urgent research questions. Drs. Major and Unger provide linkage to the National Institutes of Health and the Centers for Disease Control, respectively, thereby insuring integration of extramural and intramural efforts.

The following list briefly describes gaps in knowledge that we have discussed as highest priority. I have also shared this list independently with the Director of the NINDS, Walter Koroshetz.

**Microbiome (bacteriome, mycobiome, virome)**
- Characterize the microbiome of the blood, oropharynx and gastrointestinal tract in an effort to identify potential triggers of immune response or metabolic dysfunction.

**Proteomics**
- Identify biomarkers in blood and cerebrospinal fluid that can be used for diagnosis, prognosis, and to follow the course of disease and response to interventions. Biomarkers may also provide insights into potential therapeutic targets.

**Metabolomics**
- Identify potential diagnostic and therapeutic targets.

**Immunology**
- Identify biomarkers for diagnosis, prognosis as well as potential therapeutic targets; determine history of exposure to infectious agents that may trigger onset or exacerbation of ME/CFS.

**Genetics/Epigenetics**
- Identify gene variants that may be associated with specific clinical presentations or phenotypes that may predict course of illness or response to varying therapeutic interventions.
- Identify epigenetic signatures that may be associated with ME/CFS and that may correlate with infectious or other triggers.

**Brain Imaging**
- MRI and fMRI studies to enhance understanding of the neural circuitry and inflammation associated with ME/CFS.

To meet these urgent research needs, the CfS for ME/CFS will prioritize the following components in our “center without walls”:

1. Patient Registry
2. Biobank
3. Clinician Training Program
4. Clinical Trials

**Patient Registry**
The Patient Registry will provide ready access to the pre-qualified, well-characterized and consented cases and controls required to obtain samples, test hypotheses, validate assays, assess the safety and efficacy of therapeutic interventions and pursue exploratory research. Questionnaires provide a wealth of qualitative and quantitative data. This registry would include instruments to assess quality of life, cognitive dysfunction, medical history, medications, and other metrics. Standardization will be key to comparing results across various studies. To maximize the registry’s utility, common data elements must be identified and incorporated and diagnostic criteria must be standardized across studies. Existing study subjects tend to be demographically homogenous, which may skew scientific findings. To diversify the sample set, studies must actively recruit underrepresented groups, such as males and ethnic minorities.

**Biobank**
A biological sample repository should be readily available to researchers across all disciplines and geographic locations. Samples will represent a wide range of human tissues and be acquired and processed at all sites in strict adherence to established protocols. Aliquots will be created in pre-designated sizes for each sample type in anticipation of sample size requirements for downstream analyses to minimize excess thaw/refreeze cycles, ensure that banked samples are of optimal yield and integrity and to preserve the utility of the Biobank for long-term studies, including prospective investigations. Diverse tissue samples, including biopsy material acquired during routine or clinically-indicated endoscopy/colonoscopy, examinations of suspicious thyroid growths as well as bone marrow biopsies will be included. Eventual integration of the biobank and patient registry would provide an unparalleled comprehensive resource for epidemiologic and physiologic investigations.

Clinician Training Program
Given the reticence of the medical community to accept ME/CFS as a biological illness, it is no surprise that medical schools do not include ME/CFS in their curricula and that there is no existing specialization program. Accordingly, the CfS for ME/CFS is designing a two-year Masters-level training program that will include rotations in neurology, immunology, rheumatology, clinical epidemiology, statistics and health services training to provide the interdisciplinary clinical and research knowledge needed to quickly diagnose and administer treatment to ME/CFS patients and to expand the pool of potential recruits for research studies.

Clinical Trials
As this group and others discover more clues to the biological underpinnings of this disease, a clinical trials program will enable research to be rapidly translated into clinical practice. A Clinical Trials Unit should be positioned to rigorously examine interventions, including probiotic/nutritional, biological (e.g., immune regulators; anti-cytokine antibodies), medication and potentially, microbiome-related (e.g., fecal microbiome transplantation, other) approaches. Subject recruitment would be enabled by the Patient Registry.

Addressing the challenges of ME/CFS will require the insights and resources of the best and brightest of clinicians and investigators in the United States and abroad. We are optimistic that the field is poised for breakthroughs in understanding that will impact how we diagnose, treat and prevent ME/CFS.

Sincerely,

W. Ian Lipkin, MD
Dear colleagues:

As I suspect it may have for a number of your respondents, this RFI struck close to home for me. Just over four years ago, while living overseas in Southeast Asia, I was stricken with a "mystery illness" that carried with it a number of symptoms, but most predominantly a crushing degree of physical and mental fatigue. Fortunately, in my case, the situation is now almost entirely resolved, but finding the solution took an incredible amount of effort, and much of that effort came from my own personal legwork, as I found the medical establishment (both overseas, and in the United States once I returned in 2013) relatively ill-equipped to deduce potential causes of, and treatments for, my syndrome. This is no criticism of the establishment per se -- the solutions just don't seem to be out there right now. Hence, I presume, the need for this RFI.

Simply from talking to acquaintances and colleagues, this does not appear to be an uncommon affliction among academic researchers, and several of my fellow academics that I spoke to had embarked upon similar journeys of self-study and background research in attempts to go beyond the ineffective treatments that the medical status quo was able to provide. Although I am not a physician or clinical researcher by trade (my research is in neuroimaging of healthy cognition), I was lucky to have a sufficiently deep background education in the biological sciences to read the scientific literature, suggest candidate treatments to my various doctors, and have my suggestions taken seriously. Of course, most individuals who suffer from similar afflictions are not so lucky, and although many also make attempts at self-treatment, the majority of them do not have the educational background necessary to make informed deductions. Complicating the matter is the fact that so many quack remedies and theories are offered on the Internet, and given that even the more solid scientific literature on the topic remains somewhat speculative, it can be difficult for the average sufferer to make the distinction. So, clearly, there is a critical need for a clearer diagnostic and treatment pathway for the majority of patients who rely on their physicians for a solution.

In my view, the biggest challenge to the study of ME/CFS stems from the non-specificity of the symptom of chronic fatigue (and associated symptoms; in my case, things like abdominal pain and bloating, extreme sensitivity to alcohol, and fluctuations in real and perceived body temperature), and the large number of potential etiologies for any particular symptom set. Various physicians I saw considered options as widely varying as food sensitivities, hernia, parasites, and cancer. Ultimately, in my case, we were able to suss out the most likely cause/mechanism for my symptoms (bacterial overgrowth in the GI tract, compounded by numerous abdominal adhesions causing partial obstructions), and my medical team and I were able to assemble, piece-by-piece, the treatment regimen (probiotics, the gut-specific antibiotic rifaximin, and physical therapy to break up adhesions) that has gradually led to my almost-complete recovery. However, at every step of the way, the various alternative diagnoses under consideration seemed plausible to everyone involved, and one of the biggest pieces of the puzzle (the rifaximin treatment) came from a relatively obscure source.
Given my personal experience, as well as the growing interest by the research community in the human microbiome (both in general, and specifically with regard to ME/CFS), I naturally am inclined to suggest further work on the role of gut flora as a significant area of focus. There are clear challenges to this; obviously, the microbiome is incredibly complex and shows enormous variation between individuals, making it a difficult target for focused inquiries. Such complex scenarios are often well-suited for analysis by contemporary "big data" analytic approaches, but attaining the sample sizes necessary for those kinds of analyses (and/or making gut flora analysis a more practical tool for everyday medical diagnostic usage) may also require the development of more time- and cost-efficient techniques (e.g., improvements in shotgun metagenomic approaches) for characterizing and quantifying individual human beings’ microbiomes. However, even if such techniques are developed/improved, there remain challenges -- for example, studies of human microbiota most frequently focus on analysis of fecal samples (as do treatment approaches, e.g. fecal transplantation) due to the relative ease of collection, but the composition of gut flora may differ significantly (and in ways relevant to health) at earlier stages in the digestive tract, where sample collection presents a much greater challenge. On the other hand, despite any challenges, any advances in the study of the human microbiome would have potential benefits not only for the study of ME/CFS, but for a number of other conditions that have been shown to have linkages with gut flora, some of which have demonstrated comorbidities with ME/CFS (e.g., depression, irritable bowel syndrome).

Although I do believe that the microbiome is relatively understudied and poorly understood (both in general, and with regard to ME/CFS), I certainly also believe that other etiologies could produce similar symptom profiles, with these varying underlying conditions ultimately being collapsed under the current catch-all label of ME/CFS. Distinguishing between these causal alternatives in the absence of any obvious symptoms/signs is clearly a significant challenge for the field, both for medical practice (differential diagnosis and suggestion of appropriate treatments) and for guiding future research (into the causal mechanisms and for the development of new treatments). To me, it seems that two (related) approaches would help in resolving ambiguities:

1) More detailed profiling of individual patients (and/or research participants). The symptoms commonly used to diagnose and characterize ME/CFS remain relatively vague and non-specific, but more detailed analyses of individual patients may yield clues into their underlying conditions. For example, in my own case, it was only after detailed study of my own patterns of maximal and minimal fatigue over time that I was able to determine a concrete linkage between my eating patterns and my fatigue symptoms -- the worst fatigue typically occurred between 30 minutes and an hour after eating a meal, and often persisted for several hours. Given that I, like most humans, typically eat at least 2-3 times a day with only a few hours' separation between meals, it was only after extended occasions of fasting (prior to various medical appointments) that I began to notice the distinct improvement in my physical fatigue that occurred after 5-6 hours of fasting or more. This, in turn, became a key data point in favor of the bacterial overgrowth hypothesis that eventually led to my successful treatment. This level of self-study took months and went far beyond the degree of observation is possible to achieve in either typical doctors' appointments or most clinical research studies, but similar degrees of specificity in recording patients' patterns of symptoms, behaviors, and attempted interventions may indeed prove to be necessary for deducing the underlying cause of their suffering. Fortunately, modern technology makes it easier than ever to record detailed data on individual human beings, both in terms of personal
health recording devices (e.g., Fitbits, Apple Watches, and more specialized medical devices like glucose meters, blood pressure monitors, and thermometers) and in terms of using online interfaces to record detailed self-reports from the patients themselves (e.g., web-based forms for daily journals of activities and symptom severity, Skype interviews for brief but more frequent check-ins between patients and doctors/researchers). The data produced by such detailed study of individual patients could be overwhelming for any individual doctor or research team to collect and process effectively, but it could be more practically handled by:

2) A consortium approach to collecting and analyzing detailed data from large samples of ME/CFS patients. Having both detailed data on each patient as well as a large sample size would not only allow automated "big data" analyses of patterns (which might identify sub-groups of patients most likely to share etiologies and benefit from similar treatments), but would also enable individual researchers and teams to focus on elements of the dataset that are most relevant to their expertise while benefiting from a larger sample size than might typically be available to them. Researchers would also have access to a network of colleagues working in different disciplines, which would facilitate brainstorming and novel, cross-disciplinary approaches to a problem that has historically shown relatively slow progress from the study of small clinical samples by isolated research teams.

I realize that this is not my domain of primary research expertise, and my suggestions should probably be taken with a grain of salt. However, I do have a relatively broad interdisciplinary background in computer science, psychology, and neuroscience as well as an extensive (if highly focused) knowledge of the literature that was relevant to the understanding and treatment of my own chronic fatigue. I also have a personal interest in putting some of the knowledge I have gained to good use and helping other patients with similar problems receive effective treatment. The focus areas of my own academic research (neuroimaging of cognitive processes such as attention, memory, and visual perception, along with sophisticated machine-learning approaches to analyzing neuroimaging data) would, I believe, be potentially useful to future studies of ME/CFS. Thus, I would certainly be interested in learning more about any future research programs or activities that may result from this RFI, and contributing in any way I can.

Please feel free to contact me with any questions, comments, or updates.

Best wishes,
Matt Johnson

Matthew R. Johnson, Ph.D.
Assistant Professor
Department of Psychology &
Center for Brain, Biology and Behavior
University of Nebraska-Lincoln

Montoya, Jose

Cc: Whittemore, Vicky (NIH/NINDS) [E]; Jill Anderson; Michael Zeineh;
Jose R Maldonado; LaMoria Roberts
Subject: Request for Information: Soliciting Input for New Research Strategies for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS)

Notice Number: NOT-NS-16-024

From the Stanford CFS Team:

Dear Trans-National Institutes of Health (NIH) ME/CFS Working Group:

We previously sent a summary of our perspective (see below e-mail from our colleague, Dr. Michael Zeineh [...] sent to you on Mon 6/20/2016 9:51 PM.

In our view, CFS is the greatest scientific and medical challenge of this century. Below please also see additional perspective on our underlying, unfolding and evolving scientific view of CFS.

1. Given the complexity of CFS (chronic, protean nature of symptoms, wide spectrum in severity and duration) and the fact that elucidating the pathogenesis of the disease has eluded researchers for decades, probably the best approach to the disease should be: 1) implementation of collaborative research involving several disciplines (e.g. immunology, infectious diseases, neurology, neuroradiology, endocrinology, statistical and programming sciences, genetics); 2) use of novel technologies, thoughtfully and carefully chosen, in these studies 3) rigorous application of the scientific method (e.g. study and design, sample size); 4) in addition to having the underlying hypothesis drive the work, allow for new data to drive subsequent research; 5) creation of centers of excellence driven by a thoughtful overarching vision but at the same time allows candid and “outside the box” thinking; 6) infuse healthy levels (hundreds of millions of dollars nationwide) of funding with a significant component being unrestricted so that data driven research can take place swiftly and more expeditiously than current funding mechanisms allow.

2. CFS patients experience, often for years, numerous and a wide spectrum of symptoms, some times, involving several organ-systems. This is suggestive of two candidate organs/body sites, being significantly involved: the central nervous system and the bone marrow. These two sites can be the initial target of the disease or the site(s) that is secondarily involved and becomes the “headquarters of the disease”. A corollary of this is that studies should focus on these body sites as “primary” and/or “perpetuator” sites for CFS. Immunological, genetic, pathogen discovery studies ought to be implemented exploring these sites.

3. The presence of ongoing or fluctuating flu-like symptoms, arthralgias, myalgias, autonomic disturbances, and a striking hypersensitivity to stimuli among other symptoms in many CFS patients leads to the suspicion that this is an inflammatory or immunological disorder. Surprisingly, conventional markers of inflammation commonly used in the daily practice of medicine (e.g. erythrocyte sedimentation rate, C-reactive protein) are seldom elevated in CFS patients. This is suggestive that immunological studies should have priority particularly those using novel technologies that allow the investigation of the human immune system in hundreds of CFS patients and controls, both cross-sectionally and longitudinally. NK cells are often claimed as abnormal in CFS (numerically and functionally). Immune studies should focus on NK cell biology and function in these patients. If it is correct that NK cells are abnormal in CFS patients, is their dysfunction genetic, environmental or infectious in origin?
There are several immune related questions “begging” to be answered in this fascinating disease:

Could CFS be the result of **uncontrolled activation of the innate immune system** such as antigen presenting cells (i.e. monocytes/macrophages, dendritic cells)? Could CFS be the result of an imbalance in the monocyte-macrophage/neutrophil phagocytic efforts at the tissue level where a pathogen persists?

Could CFS be the result of an **over activation of the adaptive immune system** such cytotoxic and/or helper T cells or dysregulation of regulatory T cells?

Could CFS be the result of an **over activation of the mucosa associated lymphoid tissue**?

Could CFS be the result of central abnormalities in the **microfold or M cells** (Peyer’s patches)?

Thank you for considering our input.

Michael Zeineh
Jose R Maldonado
Jose G Montoya

José G. Montoya, MD, FACP, FIDSA
Professor of Medicine
Division of Infectious Diseases and Geographic Medicine
Stanford University School of Medicine
Stanford, CA 94305

 [...]

 [...]

Director,
Palo Alto Medical Foundation Toxoplasma Serology Laboratory
National Reference Center for the Study and Diagnosis of Toxoplasmosis
Palo Alto, CA 94301

 [...]

Website: [www.pamf.org/serology/](http://www.pamf.org/serology/)

From Dr. Michael Zeineh’s e-mail:
Mon 6/20/2016 9:51 PM
To: 
[MMECFSRFI@mail.nih.gov](mailto:MMECFSRFI@mail.nih.gov)
Cc: 
Jose R Maldonado;
Jose G Montoya

Emerging needs and opportunities that should be considered as new ME/CFS research strategies are developed.
- Diagnostic and biomarker development with MRI-DTI and EEG
- Measuring inflammation in the brain with novel PET neuroinflammation tracers and/or intravenous iron-enhanced MRI
- Measurement of peripheral blood inflammation using high-throughput assays including multiplexed cytokines, gene expression, CYTOF
- Pathogen discovery addressing body compartments such as bone marrow and spinal fluid
- Assessing genetic predisposition through GWAS and HLA studies
- Analysis of human brain specimens from CFS patients

Challenges or barriers to progress in research on ME/CFS.
- Large single-institution studies
- Multi-institution studies
- A CFS Neuropathological Brain Bank

Gaps and opportunities across the research continuum from basic through clinical studies.
- Lack of published interdisciplinary studies
- Clinical therapeutic trials

Michael Zeineh
Jose Montoya
Jose Maldonado

VanElzakker, Michael
[..]
Subject: Suggested ME/CFS research priorities Auto forwarded by a Rule

Hello -

Attached is the description of a specific and testable hypothesis on the cause of ME/CFS (see PDF). This hypothesis is based in basic research and empirical observations, described in the paper. Since its publication, evidence continues to mount in support of the hypothesis. This includes structural and functional imaging of the brainstem (e.g., Barnden et al., 2011; 2016), where neuroimmune nerves enter the central nervous system and PET imaging demonstrating glial activation within the central nervous system (Nakatomi et al. 2014).

Here are suggestions for CFS research funding priorities:

- Focus on neuroinflammation (the "M.E." of ME/CFS) - Less emphasis on investigating circulating blood, as not all immune responses that affect subjective experience and behavior via the central nervous system are detectable in peripheral blood (see paper for explanation and background). No studies should have circulating markers as their only dependent variable - Functional neuroimaging following exercise - not cognitive - challenge - Physiologic measures after exercise challenge, especially measures of vagal tone and VO2MAX - Neuroimaging studies utilizing inflammation-specific PET radioligands such as PBR28 and PK11195 - Imaging studies should be encouraged to use brainstem-specific spatial registration, as standard techniques register by neocortex - Animal models on long-term viral infection of the vagus and trigeminal neuroimmune nerves
Please feel free to follow-up with me for clarifications or questions.

thanks very much,
Mike.

=-=-=-=-=-=-=-=-=
Michael VanElzakker, PhD
Research Fellow, Psychiatric Neuroscience Division Harvard Medical School & Massachusetts General
Hospital Instructor, Tufts University Psychology

The information in this e-mail is intended only for the person to whom it is addressed. If you believe this
e-mail was sent to you in error and the e-mail contains patient information, please contact the Partners
Compliance HelpLine at http://www.partners.org/complianceline. If the e-mail
Chronic fatigue syndrome from vagus nerve infection: A psychoneuroimmunological hypothesis

Michael B. VanElzakker *

Tufts University Psychology, Massachusetts General Hospital Psychiatric Neuroscience, 490 Broadway Avenue, Medford, MA 02155, USA

ARTICLE INFO
Article History:
Received 25 July 2013
Accepted 23 May 2013

ABSTRACT
Chronic fatigue syndrome (CFS) is an often-debilitating condition of unknown origin. There is a general consensus among CFS researchers that the symptoms seem to reflect an ongoing immune response, perhaps due to viral infection. Thus, most CFS research has focused upon trying to uncover that putative immune system dysfunction or specific pathogenic agent. However, no single causative agent has been found. In this speculative article, I describe a new hypothesis for the etiology of CFS: infection of the vagus nerve. When immune cells of otherwise healthy individuals detect any peripheral infection, they release proinflammatory cytokines. Chemoreceptors of the sensory vagus nerve detect these localized proinflammatory cytokines, and send a signal to the brain to initiate sickness behavior. Sickness behavior is an involuntary response that includes fatigue, fever, myalgia, depression, and other symptoms that overlap with CFS. The vagus nerve infection hypothesis of CFS contends that CFS symptoms are a pathologically exaggerated version of normal sickness behavior that can occur when sensory vagal ganglia or paraganglia are themselves infected with any virus or bacteria. Drawing upon relevant findings from the neuropathic pain literature, I explain how pathogen-activated glial cells can bombard the sensory vagus nerve with proinflammatory cytokines and other neuroexcitatory substances, initiating an exaggerated and intractable sickness behavior signal. According to this hypothesis, any pathogenic infection of the vagus nerve can cause CFS, which resolves the ongoing controversy about finding a single pathogen. The vagus nerve infection hypothesis offers testable hypotheses for researchers, animal models, and specific treatment strategies.

© 2013 Elsevier Ltd. All rights reserved.

Introduction

Chronic fatigue syndrome (CFS) is an often-debilitating state of constant intense exhaustion that is unmitigated by rest or sleep. A diagnosis of CFS is given in the absence of alternative diagnoses, and the United States Center for Disease Control definition of this syndrome is based entirely upon subjective symptom self-report [1,2]. Prognosis is poor [3]. The cause of CFS is unknown and is the source of considerable contentious debate. Previous studies of CFS patients have reported a diverse array of viral and even bacterial agents (e.g. [4–11]), as well as many immune system abnormalities (e.g. [12,13]). These findings have led most researchers to assume a role for pathogen-induced immune system activation in CFS. However, inconsistent and contradictory results between (and even within) studies have left the field at a loss to explain the causal mechanisms. No single pathogen has emerged as the common etiological agent.

In this article, I describe a hypothesis that integrates many of the general observations in CFS and explains some of the conflicting observations. Rather than continuing the search for one specific virus or bacteria as the root cause of CFS, this hypothesis focuses on the location of an infection, along the sensory (afferent) vagus nerve. The Vagus Nerve Infection Hypothesis (VNHI) of CFS is as follows: While the sensory vagus nerve normally signals the body to rest when it senses a peripheral infection, that fatigue signal is pathologically exaggerated when an infection is located on the vagus nerve itself. More specifically: Immune cells, including neuroimmune cells called glial cells, sense infection and launch the same basic neuroexcitatory response regardless of infection type. When the glial cells that envelop the sensitive vagus nerve are activated by any viral or bacterial infection, their neuroexcitatory secretions escalate different vagus nerve signaling, which is misinterpreted by the brain as evidence of a severe peripheral infection. The brain then initiates sickness behavior, which includes fatigue and many other CFS symptoms (see Key Terms Table). Because of the way that glial cell activation may persist in a pathological positive feedback loop (as it does in neuropathic pain conditions), these CFS symptoms can persist for many years.
Key Terms Table

Glia: Neuroimmune cells that contain astrocytes and oligodendrocytes in the central nervous system or satellite glial cells, Schwann cells, and enteric glia cells in the peripheral nervous system. Glial cells are in close proximity to nerve cells and release neurotoxic substances when they encounter a foreign pathogen. These substances include proinflammatory cytokines, glutamate, nerve growth factor, prostaglandin, nitric oxide, and reactive oxygen species.

Neurotropic virus: A virus that has particular affinity for nerve tissue. Herpesviruses are neurotropic, frequently associated with CIS, and are characterized by their tendency to lay latent in nerve tissue until reactivated by stress or illness. CIS symptoms often begin following a period of stress or illness.

Paraganglia: Ganglia of the sensory vagus nerve that are embedded in or near most trunk organs. These immunoprivileged and glial-rich sites are potential sites for viral infection to cause glial signaling of the vagus nerve.

Proinflammatory cytokine: A class of neuroendocrine innate immune system proteins that includes IL-1beta, IL-6, and TNF-alpha. Proinflammatory cytokines are released locally by immune cells, including glial cells, when these cells encounter a pathogen.

Sensory vagus nerve: The afferent division of the tenth cranial nerve. The sensory vagus nerve innervates every major trunk organ, especially tissues that are likely to contact pathogens. It is sensitive to proinflammatory cytokines, and upon contact signals the brain to begin sickness behavior.

Sickness behavior: Involuntary behavioral changes, such as fatigue, that are triggered by innate immune system activation. Sickness behavior is brain-based and triggered by cytokine signaling of the vagus nerve. The vagus nerve infection of hypothesis states that CIS is a pathological version of normal sickness behavior (see Table 1).

The study of phenomena such as sickness behavior - that sit at the intersection of behavior, brain biology, and immunology - is a relatively new field of study known as psychoneuroimmunology [14]. Psychoneuroimmunology spans several scientific domains, and readers may not be familiar with them at all. I will give ample background for each. To understand the VNH, one must understand each part of the connection between behavior ("psycho"), the nervous system ("neuro") and the innate immune system ("immunology"). In this speculative article, I will begin with a discussion of neurotropic viruses as a model pathogen for CIS, and explain how an active virus can trigger a localized immune response. I will then describe how one class of molecules, proinflammatory cytokines, turns this local immune response into an organism-wide immune response, which includes involuntary behaviors such as fatigue. I will explain the vagus nerve's vital role in this process, which is the epi of the VNH. I will then use existing neuropathic pain literature as a template for explaining how an infection on the vagus nerve could lead to ongoing CIS symptoms. Finally, I will suggest how the VNH of CIS might be empirically evaluated with patient studies and animal models, and I will also describe potential treatment strategies.

A caveat: Because fatigue and many other symptoms associated with CIS are part of the general innate immune response to infection, and because there are currently no definitive diagnostic tests for CIS, it is unlikely that all CIS cases have the same etiology. Thus, the VNH is not intended to be an all-inclusive explanation for every case of intractable fatigue. Rather, I merely intend to hypothesize a mechanism by which many -- and possibly most -- cases of CIS may arise.

Neurotropic viruses

The association of many different types of infection with CIS is currently an inconsistency in the literature. These seemingly conflicting findings may instead provide evidence of a chronic neuroimmune activation (described in more detail in later sections) that can be caused by any pathogen, including viruses or bacteria. The suggestion that the location of infection matters more than the specific infection type is at the core of the VNH of CIS. However, neurotropic viruses are the type of pathogen most commonly associated with CIS. Because the VNH of CIS is based on the infection of nerve tissue, this is likely not a coincidence: neurotropic viruses are characterized by their affinity for invading neural tissue, especially afferent sensory nerves [15]. As a large and widely permeating afferent sensory nerve that highly innervates the organs that are most likely to come into contact with foreign pathogens, the afferent vagus nerve and associated glial cells are prominent targets for neurotropic virus infection and the subsequent general immune response. I will briefly review some relevant information about neurotropic viruses, however it is important to point out that those viruses and bacteria which are not classically considered to be particularly neurotropic could actually be the cause of CIS if they infect the vagus nerve.

Neurotropic viruses implicated in CIS include the eight human herpesvirus types [16], especially human herpesvirus type 6 (HHV-6) [47,10,17], and HHV-5 (cytomegalovirus) [5]. Although it is immunotrophic more often than neurotropic [it can be both, and the vagus nerve directly (virus with immune cells), HHV-4 (Epstein-Barr virus) is also commonly associated with CIS [10,18,19]. Herpesviruses are characterized by their ability to become latent, especially in the ganglia of nervous and lymphoid tissues [20]. Even though initial infection may have occurred within the first 10 years of life [15], neurotropic viruses such as herpesvirus can be reactivated even in the healthiest adults [21]. As these viruses tend to remain latent until reactivation during stress or illness, it follows that CIS patients usually report that their symptoms began during a period of stress or with a normal cold or flu [22].

While latency tends to occur within nerve tissue, upon reactivation, the viral infection spreads to the extracellular space. There, satellite glial cells envelop the viral particles [15]. These satellite glial cells proliferate and activate, releasing neuroactive mediators such as immune proteins called proinflammatory cytokines, and other substances which are described below [13,24]. The release of proinflammatory cytokines is a general response by glia and other immune cells like interleukin-producing cells (white blood cells) to encountering any virus or bacteria anywhere in the body. These locally-released cytokines are detected by the nearest sensory vagus nerve chemoreceptors, causing an afferent signal to the brain. The brain then initiates fatigue and several other symptoms that overlap with CIS (see Table 1). The premise of the VNH of CIS is that when a neurotropic virus or any other pathogen infects the vagus nerve itself, cytokines are released directly onto sensitive vagus nerve receptors and this normal immune response becomes pathologically intense. Here, I will provide some background and detail to the general immune response and how it relates to CIS symptoms.

Proinflammatory cytokines, the innate immune system, and sickness behavior

Over one hundred years ago, Kuniomi Shimori, a Japanese physiologist, made an important discovery about the biological
Many of the most fundamental chronic fatigue syndrome (CFS) symptoms are also proinflammatory cytokine-mediated aspects of the normally adaptive acute phase response and sickness behavior. (See Table 1.)

Table 1

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Part of acute phase response?</th>
<th>Proinflammatory cytokine-mediated?</th>
<th>Common CFS symptoms?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache; headaches</td>
<td>Yes [14, 33]</td>
<td>Yes [14, 25]</td>
<td>Yes [1]</td>
</tr>
<tr>
<td>Hypersomnia</td>
<td>Yes [14, 35]</td>
<td>Yes [14, 12, 54]</td>
<td>Yes [17, 18]</td>
</tr>
</tbody>
</table>

The vagus nerve is a sensitive detector of proinflammatory cytokines.

Vagus nerve dysfunction has been found in CFS patients. The vagus nerve is a key means of communication for the parasympathetic nervous system. As such, the level of control that the parasympathetic nervous system exerts over the sympathetic nervous system is known as vago-vagal balance. Vagal activity is one way to reduce symptoms associated with chronic illness and is often considered a physiological response to stress.

When otherwise healthy individuals become sick with almost any form of illness or infection, they are likely to exhibit the physiological changes described above. However, in the case of CFS, these changes become pathological. As depicted in Table 1, there is a striking overlap between the set of behavioral changes related to sickness behavior and the symptoms of CFS. The NIH of CFS is based on the idea that CFS symptoms are an inappropriately strong and long-lasting expression of normally adaptive sickness behavior. Understanding the manner in which cytokines cause this behavior is the focus of the next section.
sickness behaviors including fatigue

Fig. 1. A highly simplified schematic of vagus nerve anatomy. Circles represent ganglia and paraganglia, which contain both glial cells and sensory vagus nerve fibers. A viral or bacterial infection within any ganglia or paraganglia causes glial activation, leading to the release of proinflammatory cytokines and other neurotransmitters. The resulting abnormal signal can alter the brain at the nucleus tractus solitarius (NTS) and trigger sickness behaviors. When normal glial cell activation becomes pathological as it does in neuropathic pain conditions, the signal is intensified and intractable, leading to CFS.

evolved to be sensitive to small amounts of cytokine: as a key neuroimmune link, some vagal terminals form direct synapse-like connections with proinflammatory cytokine-producing lymphocytes [77]. An additional factor is the close proximity of the vagus nerve to another type of cytokine-producing cell: glial cells.

Glial cells (e.g., astrocytes and oligodendrocytes in the central nervous system or satellite glial cells, Schwann cells, and enteric glial cells in the peripheral nervous system) were once thought to be nothing more than scaffolding for neurons and nerves (glia is Greek for “glue”). Recent research demonstrates that this is far from the case, and that glia are a vital part of, if not all, nervous system signaling [78,79]. It follows that glial dysfunction can be an important factor in disorders of the nervous system, and the VNH postulates that pathogen-activated glial cells cause pathologically strong vagus nerve signaling to the brain. This pathological signaling occurs when pathogen-activated glial cells release neuroexcitatory substances such as proinflammatory cytokines, excitatory amino acids (e.g., glutamate) nitric oxide, nerve growth factor, reactive oxygen species, and prostaglandins [35,80] onto the vagus nerve’s sensory terminals. The VNH of CFS advances the novel idea that, while normal immune cell cytokine signaling leads to appropriate sickness behavior, inappropriate glial cell signaling can lead to CFS. Here, evidence of the vagus nerve’s involvement in sickness behavior is reviewed.

Cytokine to vagus nerve to brain communication induces sickness behavior

When immune cells such as glial cells or monocytes detect a pathogen, they release proinflammatory cytokines. The sensory terminals of the afferent vagus nerve that detect those cytokines send a signal to the brain, synapsing in prominent ganglia such as the jugular (superior) and nodose (inferior) ganglia, and then entering the central nervous system at the nucleus tractus solitarius (NTS) in the medulla oblongata [81]. There is good evidence from animal research that this signaling pathway from proinflammatory cytokine to vagus nerve to brain is the cause of each aspect of sickness behavior listed in Table 1 (see also [2,3,5,3]). This is important for the VNH of CFS because an infection anywhere along this pathway could cause the exaggerated sickness behaviors seen in CFS.

Rodent animals that have had their vagus nerve cut do not “act” sick: rodent studies have demonstrated that the vagus nerve is critical for the expression of sickness behavior in response to peripheral infection [82]. In rats, injection of peripheral cytokines causes vagus nerve electrical activity [82,83] and increases the activity in the nodose ganglia [84]. Furthermore, when otherwise healthy rodents are injected with proinflammatory cytokines, pathogens, or lipopolysaccharide (LPS, a molecule that activates the immune system by mimicking foreign pathogens), they show the type of sickness behaviors that are seen in CFS. However, these responses are blocked or attenuated by transection of the abdominal vagus nerve. This includes significantly reduced social interaction and exploration [84–86] and sleep stage architecture changes [87] as well as other responses relevant to CFS, such as fever and hyperalgesia in rats [87–90].

The experimental conditions described above, proinflammatory cytokines are in vivo very high circulating concentrations, modeling a response to a severe systemic infection. However, even at relatively low concentrations of endogenous proinflammatory cytokines seen during a normal, more localized peripheral infection, the vagus nerve sends the message to the brain to involuntarily cease non-essential energy use, engaging in sickness behavior. So what would happen if, instead of sensing proinflammatory cytokines in low concentrations in the periphery, vagus nerve receptors were directly and ceillessly bombarded with these cytokines? The symptoms of sickness behavior would be severe and intractable, and could occur even in the absence of evidence of peripheral infection, just like in CFS. Such a state may require two conditions to be met: (1) vagus nerve proximity to cytokine-producing cells, and (2) pathological overproduction of cytokines by those cells. In the following sections, I review evidence that (1) vagus nerve chemoreceptors are uniquely exposed to glial cell cytokine signaling and that (2) there is strong evidence from the neuropathic pain literature that cytokine production from glial cells can become pathological.

The vagus nerve is enveloped in glia

The gross vagus anatomy described above maximizes sensitive chemoreceptors’ chances for contact with cytokines released in response to peripheral infection. The cellular anatomy of vagus ganglia and paraganglia also makes the vagus nerve particularly sensitive to cytokine signaling from activated glia. The vagus nerve is densely eneveloped in satellite glial cells [94] which produce proinflammatory cytokines and other neuroexcitatory mediators
when activated, and in each of the many vagal paraganglia are chemo-
receptors for cytokines [75]. While vagal parasympathetic paraganglia are not well characterized, they are thought to be
fairly similar in structure to sympathetic paraganglia, having a
very small (~20 nm) space between satellite glial cells and neu-
nets, giving glia tight control over the paraneuronal space [74]
and allowing for even minute quantities of proinflammatory cy-
kine released into this space to greatly increase relative concen-
trations available to vagal chemoceptors. Given that these sensitive
chemoceptors can initiate sickness behavior after detecting rela-
tively sparse proinflammatory cytokines released by circulating
white blood cells, the concentration of cytokine response to activated
glia within a paraganglion is quite likely to cause sickness behav-
ior. The neuropathic pain literature offers a specific mechanism
by which this normal signaling can become pathological, leading
a normal sickness behavior response to become CPS.

Neuropathic pain as a mechanistic model for dysfunctional

glia signaling

Much progress has been made in elucidating the crucial role
of glial cells' cytokine signaling in neuropathic pain states
[35,30,29,39]. The VHNH simply contends that the same process
that causes pathologically exaggerated pain in pain-transmitting
nerves (such as virus infection in cranial nerve 5, the regen-
ernal nerve, leading to trigeminal) would cause pathologically
elevated sickness behavior in the nerve that transmits the signal for sickness behavior (calciated nerve 10, the vagus nerve).

Types of response to injury include hyperglycemia (exaggerated pain) or allodynia (interpreting non-painful stimuli as painful),
which are normally adaptive mechanisms to protect a site of infec-
ion. Injury to the injury can activate glial cells ensheathing syn-
apses in the dorsal horn of the spinal cord, increasing postsynaptic sensitivity to incoming nociceptive information from the
periphery. In neuropathic pain states, activated glial release of
neurotransmitter substances such as proinflammatory cytokines, glutamate, nitric oxide, nerve growth factor, reactive oxygen spe-
cies, and prostaglandins leads to an amplified pain response and
subjective hyperalgesia or allodynia of the individual [35,68]. It
follows that release of these substances directly onto the afferent
vagus nerve could lead to amplified sickness behaviors. In pain-
transmitting nerves, there is a point at which the protective
and adaptive pain function becomes pathological, intractable hyper-
algia or allodynia results when proinflammatory cytokine release
operates as a feed-forward loop. For example, the release of IL-1
stimulates more IL-1, and activated glial cells tend to activate other
glial cells [35,94]. This is a general property of glia and there is no
reason to suspect that sensory nerve-associated glia would function
differently than pain nerve-associated glia. Indeed, the neuropathic
pain state of fibromyalgia and CRPS are frequently confused or
coupled, and concomitantly may reflect a general predisposition
to dysfunctional glial signaling. Thus, hyperglycemia and allodynia
in neuropathic pain as with sickness behavior in CPS, glial activa-
tion causes a normally adaptive and protective response to become
persistent and debilitating state. A normal signal in a pain-trans-
mitting nerve leads to subjective pain. When that signal is en-
hanced by activated glia, it may lead to neuropathic pain. The
VHNH then states that a normal signal in sensory vagus nerve leads
to sickness behavior and when that signal is enhanced by activated

glia it may lead to CPS.

In an elegant series of experiments characterizing the mecha-
nism by which central nervous system viral infection can lead
to neuropathic pain, the Miligan, Maier, and Watkins group re-
ported several findings that can be directly applied to the VHNH
of CPS, and help us to account for several apparent inconsistencies
in the CPS literature (see Table 2 for a list of inconsistencies re-
olved by the VHNH). In a rat model, recombinant gp10, the gly-
protein of the human immunodeficiency virus-1 (HIV-1) viral
envelope, was delivered by intrathecal injection at the level of
the lumbar spinal cord [95,96]. Gp10 is the component of
HIV-1 that activates glial cells. From these studies there are three
major lessons relevant to the VHNH of CPS:

1. Not all cytokine responses that affect the central nervous
system are measurable in blood. Central nervous system viral
infection leads to a proinflammatory cytokine response, caused
by glial activation, which is measurable in the infected tissue
and in cerebrospinal fluid sampled from near the site of infec-

tion. However, the proinflammatory cytokine response is not
detectable in cerebrospinal fluid sampled a distance from the
site of infection or in peripheral blood. This general property
is found elsewhere in the cytokine literature as well, for exam-
ple, virus infections induced in mouse lung led to acute phase
responses, and proinflammatory cytokine increases were found
in lung lavage fluid but not peripheral blood [97]. This principle
is essential to understanding why there are inconsistencies in
cytokine studies of CPS patients (e.g., [57,98]); cytokines
responding to local infection stay local. The cytokine profile of
a given CPS patient would depend upon where along the vagal
pathway the infection is, and whether blood or cerebrospinal
fluid was analyzed. For example, if CPS were caused by a viral
infection in one of the many abdominal vagal paraganglia that
are near or embedded in their target organ or by an infection
in the jugular (superior) or nodice (inferior) ganglia in the
cervical cord, the cytokine response would likely not be
detectable in cerebrospinal fluid and may or may not be detect-
able in peripheral blood. If CPS were caused by a viral infection
within the NPS where the vagus nerve enters the brainstem,
proinflammatory cytokines may or may not be detectable in
cerebrospinal fluid, but would likely not be detectable in
peripheral blood.

2. Cytokine profiles are dynamic. Milligan et al. demonstrate
why it may be fruitful to focus on particular cytokine
or to attempt to find a "cytokine profile" for CPS diagnosis.
The initial glia-mediated proinflammatory cytokine response
to viral infection occurs in an interesting and dynamically timed
cascade that changes hourly (cytokine-cytokine interactions are
reviewed by Turin and Pata-Salim [52]). Furthermore, other
studies have shown that evanescent cascade of hour-
to-hour changes has fluctuating rhythms. For example, in
fibromyalgia patients as well as in healthy controls, cytokine profiles
are characterized by ultradian bursts [99]. Add to that the fact
that even in healthy individuals, cytokines have a circadian
rhythm [100] and it becomes apparent that cytokine studies
of single-timepoint peripheral blood samples are likely to pro-
vide inadequate information. Many CPS patient studies have
ignored these first two basic properties of cytokines: they are
released locally, and their levels change in ultradian bursts
within circadian rhythms.

3. Inhibiting glial cells can improve symptoms. In the Milligan et
al. model of peripheral infection, intrathecal injection of glial
inhibitors attenuated viral-induced glial activation, pro-
inflammatory cytokine response, and subsequent allodynia
[95,96]. This is key to one potential treatment option for CPS
patients, to be discussed in the treatment strategies section
below.

Implications of the hypothesis: research

The VHNH of CPS lends itself to modeling, testable hypotheses,
and treatment strategies. Three main goals of related research
Table 1
A list of conflicting observations in the current chronic fatigue syndrome (CFS) literature and their resolution by the Vagus Nerve Infection Hypothesis (VNIH) of CFS.

<table>
<thead>
<tr>
<th>Conflicting observation in CFS literature</th>
<th>VNIH resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Many different viruses and even bacteria have been associated with CFS (4-11)</td>
<td>Type of infection is less important than location because the same innate immune system response occurs regardless of infection type. Location and severity of infection leads to different cytokine profiles in the cerebrospinal fluid and peripheral blood of individual patients (55-57) (see text for details).</td>
</tr>
<tr>
<td>Cytokines are not in concert (54-57)</td>
<td>The anti-HIV-1 and HIV-1 (system) drug valganciclovir only helps some patients with elevated HIV-1 and HIV-4 antibodies (101).</td>
</tr>
<tr>
<td>The anti-HIV-1 and HIV-4 (system)-innate drug valganciclovir only helps some patients with elevated HIV-1 and HIV-4 antibodies (101).</td>
<td>Antiviral drugs are not effective on infections within immunoprivileged sites such as vagnal paranganglia (15). These paranganglia are hypothesized to be the most likely location for CFS-causing infection.</td>
</tr>
<tr>
<td>Antiviral drugs are not effective on infections within immunoprivileged sites such as vagnal paranganglia (15). These paranganglia are hypothesized to be the most likely location for CFS-causing infection. In addition to being ineffective for meta-ganglia infections, antivirals are made for a specific type of virus. CFS can be caused by different virus types, and every virus location of infection along the afferent vagus nerve pathway results in different symptoms and levels of severity. (The vagus nerve is structurally and functionally similar to meta-ganglia) (107-108). The modulation of vagus afferents of sickness behavior-related hypoglycemia is carboxylic dimorphogen (111).</td>
<td></td>
</tr>
</tbody>
</table>

Women are much more likely than men to have CFS, with reported female-to-male ratios of 2:1-4:1, depending on the clinical definition used (113-108). | Should be experimental support for the VNIH, the development of diagnostic tools, and the development of treatments. Basic research in support of these goals should involve animal models as well as investigative patient studies. Research using animal models may have the advantage of controlling the type, location, and severity of experimental vagus nerve infection. For example, Blessing et al. demonstrated that it is possible to conduct rat survival surgeries in which vagal ganglia are deliberately virally infected in a targeted fashion (111). In that study, behavioral measures were not taken because the infections were very severe, causing significant sickness in the model and mortality within 1-4 days (personal communication with W. Blessing, October 10, 2011). Future studies should use less denaturing viral load and should include behavioral measures of the sickness response. For example, initial studies could target prominent affrent vagal paranganglia and ganglia for experimental infection with active virus. After recovery, a forced-swim paradigm followed by measures of voluntary wheel running could serve as a model of post-exertional malaise. Rodents experiencing post-exertional malaise following forced swim would be expected to engage in significantly less voluntary wheel running. If this model works, it could be used to answer specific questions about CFS sequelae, for example, the VNIH would explain exaggerated post-exertional malaise as being the result of a normal post-exertional increase in proinflammatory cytokine (114,115) leading to an enhanced feed forward loop of vagus nerve cytokine signaling. Therefore, one testable hypothesis would be increased vagus nerve electrophysical activity or increased NTS activity in a vagus ganglia infected rat after forced swim. |

For reasons reviewed above, systemic measures in human CFS patients such as peripheral blood cytokine levels may not be particularly diagnostic or informative. With no blood test for CFS forthcoming, two human studies are difficult. The current gold standard of direct evidence to support the VNIH may be CFS patient cadaver studies consisting of immunohistochemical staining for activated glia, inflammation, and active virus infection within vagus nerve, its paranganglia and ganglia, or MFS. However, the most common marker for glial activation, glial fibrillary acidic protein (GFAP), may not be present in paranganglionic satellite glial cells (74). Furthermore, given the likely difficulty in finding suitable cadavers, the fact that CFS infection could be caused by any number of neurotropic viruses (some of which the majority of humans already harbor), and the difficulty of dissecting out all possible infection locations in the long and highly branched vagus nerve, other models and approaches should also be considered. |

In patients, magnetic resonance imaging (MRI) after injection of gadolinium can be used to detect viral lesions in tissue within the central nervous system (116). This can only be accomplished within the central nervous system because gadolinium contrasting delineates a disruption of the blood–brain barrier and not a viral lesion per se. Use imaging of an infection in peripheral vagus paranganglia would be more difficult. In vivo electrophysiological recordings of vagus nerve are possible (117) but invasive. A new line of research should seek to develop novel protocols for testing state and functional imaging of vagus nerve and branchient NTS in CFS patients. In addition, the use of transducer protein radioligands in positron emission tomography (PET) imaging has shown promise as a method of imaging microglial activation in neurodegenerative disorder-induced neuroinflammation (118), and may prove valuable in CFS research. Such methods could provide both support for the general hypothesis and important information that could inform individual treatment strategies. |

One significant ongoing barrier to human CFS research is the difficulty recruiting the most severely symptomatic patients, who are often unable to get out of bed on their own and who recognize that even the minor physical activity associated with traveling to a research facility would likely lead to a severe and sustained post-exertional malaise. Given normal individual differences in means of vagal tone and immune physiology, studies attempting to contrast vagus function in mildly symptomatic patients versus controls may become underpowered. It is important for any CFS study to include patients with the most severe symptoms, and as such neither IFR approval for home visits should be included in grant proposals when feasible. |

**Implications of the hypothesis: treatment strategies**

**Pharmacological, neurotherapeutic & surgical treatment strategies**

According to the VNIH of CFS, possible treatment strategies include gial inhibitors, specific antivirals, vagus nerve stimulation (VNS), and vagotomy. If infection-induced glial activation within vagus nerve is the central underlying cause of most CFS symptoms, then glial inhibitors could be a particularly effective treatment strategy. Gial inhibitors have shown promise as an adjunct medication for treating neuropathic pain states (119) and, given that some drugs have relatively minor side effects, use of glial inhibitors could become the standard treatment for CFS caused by CNS vagus nerve infection.

For example, ibudast (also known as AV411 of Min515) inhibits the production of proinflammatory cytokines, by inhibition of a proinflammatory cytokine called macrophage–migration inhibitory factor (MFI) (120). In a series of experiments, Alexander et al. demonstrated the critical role of MFI in the establishment, severity, and duration of neurogenic signaling in pain transmitting nerves (121). Given the mechanistic overlap between neuropathic pain...
and the VNHI of CPS, their data demonstrate that an MIF inhibitor such as ibudilast could be an effective method for reducing pathologic vagus nerve signaling. They found that MIF increased transcription of proinflammatory cytokines such as IL-1β, IL-6, and TNF-alpha within rat microglia, and treatment with MIF inhibitor led to reduction of proinflammatory cytokine transcription within rat microglia. Furthermore, MIF led to localized structural plasticity and neuroexcitability in areas prone to inflammation in the CNS, and increased the production of the proinflammatory gas, nitric oxide. In addition to acting as an MIF inhibitor, ibudilast also acts as a phosphodiesterase inhibitor or to inhibit production of the proinflammatory cytokine TNF-alpha by glial cells [124]. TNF-alpha is a key proinflammatory cytokine in the initial cytokine cascade and acts synergistically with other proinflammatory cytokines [52], meaning that its inhibition may also inhibit the production and efficacy of other proinflammatory cytokines. Furthermore, blocking glial TNF-alpha increases uptake and metabolism of glutamate by glial cells [121], which would attenuate a direct mechanism of vagus nerve excitation, as the terminals and ganglia of vagal afferents contain glutamate receptors [124] and ibudilast can also prevent glial activation of microglia [125], and is safe for human use. Ibudilast is already frequently prescribed in Japan as an anti-asthmatic [126] and in Australia it is undergoing clinical trials for use in neuro-pathic pain states. There are also several other general glial inhibitor drugs, such as minocycline, pentoxifylline and propentofylline, all with slightly different mechanisms but often with undesirable side effects. It is likely that, just as glial inhibitors are being combined with traditional opioids for treatment of neuropathic pain states, glial inhibitors may need to be combined with appropriate antioxidants for effective treatment of CPS.

Even on their own, antisense have shown promise in treating select groups of CPS patients. For example, in patients with elevated HMW-6 and HMW-4 (Epstein-Barr) antibody titers, vagus nerve stimulation significantly improves fatigue symptoms in a majority of patients [101]. The lack of efficacy in some patients could reflect the fact that, after neurotropic viruses have been taken up into sensory ganglia, they are protected from antisense drugs and antibodies [35], as it could also reflect the fact that the vagus nerve was not infected by the types of virus best treated by valganciclovir, but rather by a different pathogen. According to the VNHI of CPS, many different pathogens could cause CPS, making individualized medicine crucial for proper patient care. Identifying the specific infectious agent in each patient will be critical, giving antiviral drugs to someone whose symptoms are caused by a non-reiter virus such as HHV-6 will do more harm than good. If the VNHI of CPS proves to be accurate, individualized treatment should include tests for each patient to identify the particular viruses infecting them, which can be demanding because most humans are infected with common viruses in childhood, so blood tests for these viral antibodies are likely to be positive. However, the specific location of infection rather than the mere presence of infection may be the causal factor for CPS. Future CPS research could benefit from a more focused approach and use radiolabeled antibodies to localize clusters of specific virus types in vivo.

More basic research supports the vagus infection hypothesis of CPS, VNS is another potential CPS treatment that may merit investigation. Traditional VNS is invasive and involves stimulation of the cervical branch of the vagus nerve within the carotid sheath. VNS has shown promise in conditions that overlap with CPS such as depression [129] and chronic backache [129]. There is also some evidence that VNS could treat symptoms related to an ongoing acute phase response. Bronzivico et al. reported that VNS with acetylcysteine reduced the systemic inflammatory response in rats, including reducing inflammation in proinflammatory cytokines [130]. In that same study, direct electrical stimulation of peripheral vagus during acute lesion inhibited TNF-alpha synthesis and peak plasma levels. However, the mechanism of action for the effect of VNS is not entirely understood and if different vagus stimulation is the cause of CPS, VNS could make symptoms worse. In rat pain models, hyperalgesia severity changes with proinflammatory cytokine levels and, depending on the strength of stimulation, VNS can either increase or decrease baseline nociceptive thresholds [119-130]. Careful calibration of vagus nerve stimulation may be an important factor and it is likely that individual differences would play a substantial role in the effects of a given level of VNS on CPS symptoms. A newer and less invasive form of VNS involves transcutaneous stimulation of the afferent auricular branch (see Fig. 1) of the vagus nerve [134]. While this method is not as well studied as traditional cervical VNS, its effects seem to be similar and as such may be an attractive, less invasive treatment option.

More radically, vagotomy has been used in animal models to experimentally block several aspects of sickness behavior after peripheral infection (reviewed above), and may be an option for the most severe cases of CPS. However, in rats, bilateral cervical vagotomy is fatal [68], pointing to the necessity of a targeted vagotomy. Such targeting depends on the detection of an isolated acute lesion within the afferent vagus nerve system, and this is currently not feasible. Again, this is potentially a very important problem for basic biomedical research to solve.

**Psychological and behavioral treatment strategies and the false dichotomy**

The debate over the etiology of CPS has been rife with a questionable dichotomy between mind and body. It has been argued that CPS is a psychological disorder caused by psychological mechanisms such as classical conditioning or learned helplessness (e.g. [135,136]). Strong evidence for the vagus nerve hypothesis of CPS would contradict this assumption of a purely psychological etiology to CPS. On the other side of the dichotomy lies the idea that CPS is a central neural, caused by a purely physical event, and is in need of a purely physical cure. In some patients who have had a central lesion or a central nervous system injury, they could also reflect the fact that the vagus nerve was not infected by the types of virus best treated by valganciclovir, but rather by a different pathogen.

According to the VNHI of CPS, many different pathogens could cause CPS, making individualized treatment null. If the VNHI of CPS proves to be accurate, individualized treatment should include tests for each patient to identify the particular viruses infecting them. This of course may prove challenging because most humans are infected with common viruses in childhood, so blood tests for these viral antibodies are likely to be positive. However, the specific location of infection rather than the mere presence of infection may be the causal factor for CPS. Future CPS research could benefit from a more focused approach and use radiolabeled antibodies to localize clusters of specific virus types in vivo.

More basic research supports the vagus infection hypothesis of CPS, VNS is another potential CPS treatment that may merit investigation. Traditional VNS is invasive and involves stimulation of the cervical branch of the vagus nerve within the carotid sheath. VNS has shown promise in conditions that overlap with CPS such as depression [129] and chronic backache [129]. There is also some evidence that VNS could treat symptoms related to an ongoing acute phase response. Bronzivico et al. reported that VNS with acetylcysteine reduced the systemic inflammatory response in rats, including reducing inflammation in proinflammatory cytokines [130]. In that same study, direct electrical stimulation of peripheral vagus during acute lesion inhibited TNF-alpha synthesis and peak plasma levels. However, the mechanism of action for the effect of VNS is not entirely understood and if different vagus stimulation is the cause of CPS, VNS could make symptoms worse. In rat pain models, hyperalgesia severity changes with proinflammatory cytokine levels and, depending on the strength of stimulation, VNS can either increase or decrease baseline nociceptive thresholds [119-130]. Careful calibration of vagus nerve stimulation may be an important factor and it is likely that individual differences would play a substantial role in the effects of a given level of VNS on CPS symptoms. A newer and less invasive form of VNS involves transcutaneous stimulation of the afferent auricular branch (see Fig. 1) of the vagus nerve [134]. While this method is not as well studied as traditional cervical VNS, its effects seem to be similar and as such may be an attractive, less invasive treatment option.

More radically, vagotomy has been used in animal models to experimentally block several aspects of sickness behavior after peripheral infection (reviewed above), and may be an option for the most severe cases of CPS. However, in rats, bilateral cervical vagotomy is fatal [68], pointing to the necessity of a targeted vagotomy. Such targeting depends on the detection of an isolated acute lesion within the afferent vagus nerve system, and this is currently not feasible. Again, this is potentially a very important problem for basic biomedical research to solve.

1. While the VNHI of CPS posits a clearly non-psychological etiology, patients with other clearly non-psychological conditions also see physical benefits from psychological and behavioral interventions. For example, Folstein et al. reviewed evidence that such interventions could improve biomarkers for the non-psychological disorders type 2 diabetes, AIDS, and cancer [138]. Medications improved both blood pressure and insulin sensitivity in individuals with type 2 diabetes. In individuals with HIV, cognitive behavioral stress management, even when controlling for the effect of medication adherence, resulted in both lower viral load and greater immune T-cell count. Individuals undergoing adjuvant therapy for breast cancer who were also undergoing cognitive behavioral therapy showed improve-
ments in an indicator of immune function (lymphocyte proliferative response to challenge) relative to those who were not undergoing cognitive behavioral therapy. No one would argue that breast cancer reflects a weakness of character and yet psychological interventions help physical symptoms.

2. Both cognitive behavioral therapy and graded exercise therapy can convey to understandability and help individuals suffering from CFS that recovery is possible. Furthermore, graded exercise therapy can help overcome the atrophy of long-term muscle deconditioning, provided that post-exertional malaise does not worsen symptoms long-term. These two benefits are not directly related to vague neuron function; however, both are crucial for recovery. The ardent refusal of some patients to engage in psychological or behavioral treatment strategies should be challenged—whether empiric, logic, and intervention is medically unavoidable.

Thus, previous research indicates that the best approach for combating CFS symptoms caused by vague neuron infection may be some combination of the above strategies, for example a cocktail of glial inhibitors with an appropriate specific anti-viral agent and with cognitive behavioral therapy and graded exercise therapy. Careful clinical research should be undertaken before such a regimen is attempted.

Conclusion

The VHN offers CFS researchers and patients a specific mechanism for explaining symptoms, and it offers testable hypotheses and treatment strategies. According to this hypothesis, the major symptoms experienced by CFS patients represent pathologically exaggerated sickness behavior caused by infection-induced glial signaling somewhere along the afferent vagus nerve system. Several researchers have advanced theories that align with the VHN of CFS. Many groups have pointed out that CFS symptoms are consistent with viral infection and ongoing immune activation. More specifically, Shapiro theorized that CFS could be caused by the common neurotropic herpesvirus varicella-zoster infecting the peripheral nervous system [39]. Maes has pointed out the overlap between inflammation, depression, and CFS [140]. The vague nerve hypothesis provides an exact mechanism to these hypotheses, as well as an explanation for many of the inconsistencies in the literature (see Table 2).

According to the VHN, both qualitative and quantitative variation in CFS symptoms between patients could be explained by the following related and intersecting factors:

1. Location of infection along the vague nerve pathway
2. Severity and duration of the body's sickness behavior response
3. Severity and duration of inactivity
4. Infection type, location of any infection outside of vague nerve, and severity of infection

Eliciting one of these four factors is likely to be critical for understanding individual patients' symptoms and determining individualized management and treatment strategies.

Research into the VHN of CFS should involve several avenues. These include animal models utilizing deliberate vague neuron infection, and human case studies staining for viral infection and activated glia in vagal ganglia and paraganglia. Utilizing basic biomedical imaging research to discover a successful method for localizing active viral infection along the vagal path from peripheral to central nervous system would be of great importance for both testing the hypothesis and determining effective clinical treatment. Functional studies of the vague nerve should compare highly symptomatic patients to healthy controls; in patients, the effectiveness of viral inhibitors can be tested, but these may not be effective in the absence of concurrent emotional and behavioral treatment. Animal studies should only be given if the specific type of virus causing the infection has been determined. VNS and vagotomy are theoretical treatment options that may benefit from validation in animal models before human studies are attempted.

Conflict of Interest

Grant support comes from a National Defense Science and Engineering Graduate (NDSEG) fellowship to M.R.B. and the Tufts University Psychology Graduate Program. Funding sources had no role in the content of this manuscript. The author declares no conflicts of interest.

Acknowledgements

My sincere gratitude to Robin N. Durham, Devan J. Harrison, Gina K. Kuperberg, and Julie L. Wieszler for illuminating discussions and comments on an earlier version of this manuscript. Thanks to the Shl Psychopathology Neuroimaging Lab for help with proofreading and editing.

References

Mcnamara, Mary

[...]

Subject: Input to ME/CFS RFI Notice Number: NOT-NS-16-024

June 22, 2016

REFERENCE: Request for Information: Soliciting Input for New Research Strategies for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) Notice Number: NOT-NS-16-024

Dear Working Group Members,

Thank you for inviting patient input to NIH’s planning process re: identifying ME/CFS research areas and setting priorities. A long-time ME patient with an engineering/scientific computing background, I am pleased the RFI has a focus on imaging and bioengineering technologies the application of which will most certainly have a positive impact on the diagnosis and treatment of this complex multisystem disease.

Emerging Needs

The 2015 IOM Report: Between 836,000 and 2.5 million Americans suffer from ME/CFS, and an estimated 84 percent to 91 percent of people with ME/CFS are not diagnosed. The disease’s symptoms can be treated, even though a cure does not exist. Major barriers to ME/CFS diagnosis and treatment include: too few ME/CFS physicians/specialists; lack of medical school training programs; too few clinics; patients often too debilitated to seek care; and the inherent complexity of the disease, i.e., heterogeneity and symptom overload both of which can overwhelm clinicians.

1. Imaging & Bioengineering Opportunities

Dr. Shirley Ann Jackson, President, Rensselaer Polytechnic Institute addressed “new” computing technologies and how they can be applied to complex healthcare problems at an RPI Cognitive Colloquium in November 2015. The advent of “cognitive computing” in 2011 plus these factors were cited: digitization of enough data; increases in computing power; self-learning systems that use data mining; systems that can reason; machine vision; and the ability to pull information off the Internet. ME/CFS opportunities include:

- **Big Data Analytics**: Dr. Ron Davis, Stanford University, and his team are already using this technology to study twenty severely ill patients searching for biomarkers. At the liME Conference in London on June 3, 2016 he described how metabolomics and personalized medicine are being successfully applied to ME/CFS with promising results. They may dovetail with new insights in the field of metabolic regulation of immunity [AAAS Webinar, April 27, 2016]. Personal omics profiling research involving longitudinal tracking of immune system-
infection interaction is also warranted in ME/CFS [see Stanford geneticist Michael Snyder’s iPOP study [PMC3341616]. Much more “omics” and microRNA research is needed. Technologies are currently available to make significant progress. The challenges are twofold: substantial increases in funding for Big Data Analytics are required and highly skilled technical resources are needed to get the work done.

- **Cognitive Computing**: Self-learning systems like Cleveland Clinic’s WatsonPaths and WatsonEMR Assistant address ME/CFS’s complexity. In medical schools they could help train students in the differential diagnosis of ME/CFS and in clinics assist physicians with diagnosis and treatment. They should be an integral part of NIH’s ME/CFS research strategy. From academia and industry, system designers and developers, IBM Watson Technologies representatives, an ME/CFS International Consensus Panel and others should collaborate on system implementation. Existing International Consensus guidelines such as the *Myalgic Encephalomyelitis International Consensus Primer for Medical Practitioners*; the historical *The Clinical and Scientific Basis of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome*; other practice guidelines; Pubmed articles; case studies; and on-going research studies provide the knowledge base to get started.

- **Telemedicine**: Telemedicine technology can enable housebound/bedbound ME/CFS patients to participate in research studies as Dr. Nancy Klimas’ Genetics Study at Nova Southeastern University recently demonstrated. Importantly, Telemedicine combined with mobile sensing devices and applications can provide and track treatment to housebound and bedbound patients, patients in rural areas, and geriatric patients easing the burden on both patients and caregivers.

- **Imaging Systems**: Dr. Jarred Younger at the University of Alabama, Birmingham is doing innovative ME/CFS brain imaging research, for example exploring the use of MRI spectroscopy to measure brain temperature. His goal is to develop a cost effective diagnostic test. Non-invasive imaging tools that do in vivo imaging of biological processes including gene expression and infections should be utilized in ME/CFS. Interestingly immunoprofiling is used in cancer to visualize the quantity and location of various immune cell types (T-cell, B-cell, NK cell, etc.) in tissue samples revealing the tumor microenvironment [AAAS Webinar, April 1, 2015]. Perkin Elmer has indicated this technology could also be applied in Infectious Diseases, for example to Dr. John Chia’s ME/CFS stomach tissue samples. Imaging research in general is critical in ME/CFS. MRI and SPECT scans are used to confirm the acquired brain injury (ABI) Gudrun Lange PhD described in the CDC PCOCA conference call on February 25, 2014.

**Benchmarks for Progress**

There are quantitative and qualitative ways to measure the success of how the above four technologies will increase understanding of the pathophysiology of ME/CFS; improve medical education; facilitate timely patient diagnosis and treatment; reduce misdiagnoses; decrease health care costs; and deliver more precise and personalized care.
• **Big Data Analytics:** A goal is to uncover a “total systems” view of ME/CFS per patient, a first step toward personalized medicine. Identification of disease activity biomarkers and subgroups recently achieved in a “Personalized Immunomonitering” Lupus study [PMID 27040498] would be a major milestone if that type of technology were to be successfully applied in ME/CFS.

• **Cognitive Computing:** Periodically surveying the number of medical schools, medical students, clinicians, Centers of Excellence, and private clinics using these systems as well as evaluating feedback from users would give direct indicators of progress.

• **Telemedicine:** Periodically surveying the number of ME/CFS patients using Telemedicine and evaluating user satisfaction would indicate progress.

• **Imaging Systems:** A major goal is to discover disease biomarkers that can be turned into tests for diagnosing and tracking treatment effectiveness as well as for identifying new therapies. Retrospectively using new higher resolution systems to re-examine findings like hypoperfusion and unidentified bright objects (UBOs) would be helpful.

2. **Other Research Areas**

A broad, comprehensive ME/CFS research strategy is required encompassing neurology, immunology, infectious disease, cardiology, genetics, bioengineering, and other disciplines. That said, the following understudied research areas warrant inclusion in the plan:

• **Interferon** — More than two decades ago Wakefield and Lloyd noted the symptoms of ME/CFS bear a startling resemblance to those of interferon poisoning. In 2008 interferon genes topped the list in the Kerr, et al. gene expression study [PMID 18462164]. In 2013 new insights into the immunoregulatory properties of the interferon pathway during acute or chronic infections were published [Science, 12 April 2013 “An Interferon Paradox”]. Interferon signatures have been used to predict rituximab response in autoimmune disease [PMID 22540992.] And on June 3, 2016 at the iIME Conference in London Dr. Jo Cambridge, UCL, mentioned interferon may be involved in positive patient response to rituximab. It’s time to better understand the role of interferon and interferon signatures in ME/CFS, currently a gap in the research continuum.

• **Cardiac Abnormalities**— Very serious cardiac abnormalities like malignant hypertension are known to occur in ME/CFS and need to be studied. There are no diagnostic or treatment guidelines. ER physicians and clinicians are uninformed. At least one ME specialist has warned about trying to treat this type of hypertension in ME/CFS due to adverse events. The entire area of cardiac and circulatory abnormalities involving blood pressure, chest pain, arrhythmias, inflammation and blood volume represents a large gap in the research continuum.

• **Immune System Dysfunction**— More than two decades ago Immunologist Dr. Jay Levy observed chronic immune activation in ME/CFS. Dr. Ian Lipkin and his Columbia University team have been searching for what might be its cause — infection or autoimmune — which is key to its treatment and prevention. Carl Nathan’s article “From Transient Infection to Chronic Disease” Science, 9 October 2015 [PMID 26450196] presents new insights into the hit-and-run theory long thought to apply to ME/CFS. The possibility an infection can “scar” the immune system stresses the importance of Dr. Lipkin’s infectious disease, immune, and microbiome research. Dr. Lipkin has consistently observed hypergammaglobulinemia in ME/CFS patients
which is a research gap as is the need to search for infectious triggers, co-infections, and hidden viral reservoirs.

- **MCS:** Patient drug, food and chemical sensitivities are a challenge for any future pharmacological treatment. With pharmacogenetics and nutrigenomics testing, they need to be researched and mitigated for optimum clinical trial outcomes. In addition, anesthesia is problematic for many patients and reactions to it need to be researched with clinical guidelines developed. This entire area is another large gap in the research continuum.

- **Geriatric Patients:** How “age-related” immune, metabolic and cognitive changes impact elderly ME/CFS patients as they interact with their pre-existing “disease-related” immune, metabolic and cognitive dysfunction is another gap in the research continuum. Development of care guidelines for elderly patients and occupational therapy recommendations would be helpful.

**Research Priorities**

Developing and implementing an ME/CFS research strategy will be a multi-year journey optimally undertaken with a sense of urgency. Priorities are:

- **Funding**—increasing levels of ME/CFS funding to support a comprehensive research strategy
- **Technical and project management skills**—staffing with personnel experienced in neurology, immunology, infectious diseases, genetics and bioengineering sciences
- **Illness severity**—overcoming logistical challenges enabling severely ill patients to participate in studies; focusing on the most severe symptoms first: energy production on demand; PEM/PENE; cardiac abnormalities; acquired brain injury; and drug and chemical sensitivities
- **Illness complexity**—recalling that in the past, to be manageable, the scope of an ME/CFS research study was often scaled back by disregarding symptoms and comorbidities. However, it is the complexity, the combined acquired dysfunctions across multiple bodily systems that is a hallmark of this disease, and wherein the answers to its cause will most likely be found. RPI’s Dr. Jackson highlighted the arrival of a new era of computing whereby systems excel at complexity. Let’s make sure their full power and the latest medical technologies are used in ME/CFS research to relieve the suffering of millions of people worldwide.

Should you have questions or wish additional information, kindly contact me at: [...]

Best regards,

Mary E. McNamara
IEEE, Senior Member, Emeritus
AAAS, Member, Emeritus
New Jersey MECFS Association, Inc., Co-Founder

Keller, San
Subject: Response to Request for Information: Soliciting Input for New Research Strategies for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS)

San Keller
San Keller, PhD
Managing Research Scientist, American Institutes for Research
Principal Investigator, PROMIS Network Center
100 Europa Drive, Chapel Hill, NC

24 June 2016
Vicki Whittmore, PhD
National Institute of Neurological Disorders and Stroke (NINDS), the National Institutes for Health (NIH)

Dear Dr. Whittmore:

Thank you so much for the opportunity to provide information to support an NIH research agenda for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS). There is an urgent need for research on this disease, and a historically appalling neglect despite the prevalence of ME/CFS and the enormous human and societal costs. In 2013, the Centers for Disease Control and Prevention (CDC) estimated that the prevalence of ME/CFS among the U.S. population was more than 1 million\(^1\): nearly as high as the number who suffer from HIV.\(^2\) An economic study published in 2008 estimated that the total direct and indirect costs of ME/CFS to the U.S. economy was between 17 and 24 billion dollars (including costs for office visits, prescriptions, lost wages, and disability claims, and lost tax revenue).\(^3\) The prevalence of ME/CFS is estimated to be twice that of MS\(^3,4\) a similarly disabling condition, yet, NIH funding for ME/CFS research was approximately $6 million in 2015—just 6.4 percent of that for MS!\(^5\) I, and other researchers working in this field, as well as the patient advocates I know, are heartened by NIH’s recent increase in attention to ME/CFS, and by this request.

NIH funding to support the development of reliable and valid consensus measures of ME/CFS symptoms and functioning (i.e. patient-reported outcome measures) will address a critical gap in research and development required to combat this disease. There is no cure for ME/CFS or FDA-approved treatment for ME/CFS. Patients suffer for decades, often dying prematurely.\(^6\) There has been little supplementation of public research dollars for this disease by private industry, because companies are reluctant to invest in clinical trials of therapy for a condition that lacks U.S. Food and Drug Administration (FDA)-recommended or qualified assessments of clinical endpoints. At the same time, misunderstanding of the effects of ME/CFS based in part

---


on inferior assessments of outcome, is widespread, leading to inappropriate treatment of patients and inability to interpret research results.7

Patients are desperate for progress toward development of effective therapies for ME/CFS.8 They describe fatigue that is “bone crushing” and “exhaustion to the point where speaking is not possible.”8 Co-occurring symptoms include impaired memory and concentration, musculoskeletal pain, and sleep abnormalities.9 ME/CFS causes irregularities in multiple body systems, including neurological, immune, and endocrine; and so many with this disease also suffer flu-like symptoms, orthostatic intolerance, hypersensitivity to sensory input, gastrointestinal problems, muscle weakness, and lack of coordination, among several other effects.9 Those with mild disease may be able to fulfill one or two social roles—but have no remaining physical resources for anything else. Those with moderate to severe disease may have lost many of their social roles and be house bound. Those with very severe disease are mostly bedbound and cannot visit a provider for therapy, as illustrated by this quote from a patient: “...exertion of daily toileting…sends me back to bed struggling for breath and feeling like I just climbed a mountain.”8 Due to misunderstanding of ME/CFS, providers prescribe exercise regimens that result in profound pain and disability for persons with ME/CFS; or they prescribe psychiatric therapies that have no impact on symptoms other than to increase the patients’ sense of hopelessness, alienation, and stigmatization.10

In March of 2014, the FDA released for comment, draft drug development guidance to industry that calls for the use of patient-reported outcome measures as primary endpoints in clinical trials of therapy for ME/CFS.11 This guidance also recommended the use of Patient-Reported Outcomes (PROs) as primary endpoints in clinical trials of therapy for ME/CFS. But the guidance acknowledged a gap in basic research to develop valid and reliable consensus methods of documenting, tracking, and describing outcomes of therapy and quantifying the effect of ME/CFS on the functioning and symptoms of patients. The author has been participating in an interagency clinical outcomes assessments working group for ME/CFS including representatives from NIH and grantees, FDA and CDC. This working group is interested in exploring the

---

potential of the NIH’s Patient Reported Outcome Measurement Information System (PROMIS®) Fatigue Item Bank as a measure of outcome for ME/CFS. As a result of earlier interest in the PROMIS Fatigue Item Bank as a measure of fatigue outcome for Rheumatoid Arthritis, the author and colleagues conducted an extensive review of the gray and peer-reviewed literature relevant to the reliability and validity of that measure. This review showed that the PROMIS Fatigue Item Bank requires substantial supplemental research and development before it can be used as a measure of outcome in FDA-approved clinical trials of therapy. For example, the PROMIS initiative’s qualitative research with patients included just one patient with CFS and it is not possible to tie any patient’s focus group comments back to a specific diagnosis.

In sum, the search for effective therapies to treat or alleviate the suffering and declining functioning of persons with ME/CFS is held hostage to the development of reliable and valid clinical endpoint assessments. Achievement of consensus assessments of signs and symptoms of ME/CFS is an essential first step in developing widely available therapies for this disease. NIH funding for research to develop PRO assessments for ME/CFS is critical.

Again, thank you for the opportunity to comment. Please let me know if I can be of further assistance to you in your important work regarding this terrible and misunderstood disease.

Sincerely yours,

San Keller
San Keller, PhD
Managing Research Scientist,
American Institutes for Research
100 Europa Drive, Chapel Hill, NC

Subject: Input for New Research Strategies for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS)

In response to the above-cited request, pleased find attached the suggestions being offered by myself and Drs. Gurwitt and Underhill.

Our "organization" affiliations is as follows: We three have served as the author steering committees of the ME/CFS Primer revision, published by the IACFS/ME, and of the Pediatric and Adolescent ME/CFS Primer which is nearing completion.

We thank you for the consideration.

Sincerely,

Kenneth J. Friedman, Ph.D.

--
Kenneth J. Friedman, Ph.D.
Board Member, NJME/CFSA
Board Member, ImmuneDysfunction.org
Associate Professor of Pharmacology and Physiology, New Jersey Medical School (retired)

-----------

Recommendations for Advancing ME/CFS Research
Kenneth J. Friedman, Ph.D., Alan Gurwitt, M.D., Rosemary Underhill, MB BS, MRCOG, FRCS (Edin).

Barriers to research into ME/CFS

- ME/CFS is not accepted as an organic medical problem.
- ME/CFS has been, and still is, stigmatized by many healthcare providers, educators, and many of the general public.
- Many medical schools refuse to include ME/CFS in their medical school curricula.
- Not one medical society has “adopted” ME/CFS as an illness within their purview, treatment management, and oversight.
- Failure to recognize ME/CFS as a serious medical condition has generated a very serious funding deficiency for ME/CFS research, in comparison to the funding levels of comparable illnesses.
- Failure to accept ME/CFS as a serious medical condition has resulted in difficulty in publishing ME/CFS research papers in peer-reviewed journals. Many peer reviewed journals reject papers on ME/CFS, e.g., The New England Journal of Medicine.
- University faculty are, “punished,” for pursuing ME/CFS research. As documented in the N.I.H. State of Knowledge Workshop, faculty have been told to stop performing ME/CFS if they wish to be promoted in academic rank, faculty have failed to receive promotion because their research has been in the field of ME/CFS, faculty have been forbidden to pursue ME/CFS using University resources because such activity is deemed, “unprofessional.”
Needs, Gaps and Opportunities

- ME/CFS needs to be accepted and declared an organic illness and not a psychosomatic disorder.
- Mandate equal access of ME/CFS patients to patient care, and equal access of ME/CFS researchers and clinicians to professional opportunity by announcing that ME/CFS is an illness to be treated as all others and denial of care and/or research is against the goals of the U.S. government subject to withdrawal of federal funding from any organization or institution found to have discriminated against ME/CFS patients, healthcare providers or researchers.
- There is a need for both medical and lay public ME/CFS education, there is the opportunity to develop materials, coursework, and personnel and distribute them, and institute educational programs.
- Dramatically increase funding and encouragement for more ME/CFS researchers.
- Develop a career path for creating ME/CFS specialists.
- Declare ME/CFS a health emergency and provide emergency funding, such as is being done for for the potential Zika virus epidemic.
- Treat ME/CFS as an infectious and/or post-infectious illness and provide better characterization of the illness.
- Establish a national ME/CFS epidemiology or health surveillance system.
- Establish a national ME/CFS prevention research program.
- Establish a national ME/CFS vaccine development program as has been done for other contagious illnesses and proposed for the Zika virus.
- Establish a national healthcare provider and general public education mechanism which will provide current information and be routinely reviewed and updated based upon new research and clinical findings.
- Include severely affected as well as ambulatory patients in research studies and clinically trials.
- Promote the establishment of a national, research collaborative effort such as ME/CFS Centers of Excellence
- Include ME/CFS research articles published in the Journal of Chronic Fatigue Syndrome in the knowledge base of ME/CFS. These articles have been overlooked in the IOM literature review because the journal is not a Medline-indexed journal. This journal should be added to the list of journals indexed for Medline.

Aiyar, Raeka

Subject: Input for new ME/CFS Research Strategies (sent on behalf of Dr. Ronald Davis)

To whom it may concern:

We are grateful for the opportunity to provide input to the Trans-National Institutes of Health (NIH) ME/CFS Working Group as they develop strategies to guide NIH’s research efforts and priority setting for research on ME/CFS. Our mission at the Stanford Chronic Fatigue Syndrome Research Center is to discover causes, a molecular diagnosis, and treatment options for ME/CFS. Through our research efforts, collaborations with the ME/CFS research and clinical community, and extensive engagement with patients, we have defined several elements of importance for future ME/CFS research programs.
A key consideration in ME/CFS research efforts is the complex and multisystemic nature of this disease, and we are happy to see the involvement of several NIH institutes in developing this plan. Because the causative factors driving the disease remain unknown, and because work from our team and others has indicated effects on neurology, metabolism, immunity, and more, it will be crucial that calls for proposals allow for open, unbiased, multifaceted, and systematic research. Broadening the scope of ME/CFS research will create opportunities for engaging researchers in other disciplines. Similarly, investigating numerous organ systems and biological pathways perturbed in ME/CFS may well reveal informative parallels to other diseases – for example, we and others have observed symptomatic, transcriptomic, and metabolic overlap between ME/CFS and neurodegenerative disorders like Parkinson’s Disease. It is important not to limit research to single organs like the brain, and to integrate results from many different organs and molecular processes so that they can be understood at the systems level. Big data approaches and high-throughput, large-scale molecular profiling should therefore be prioritized. Such efforts hold promise to identify key genes or pathways underlying ME/CFS. Similarly, large-scale in vitro drug screening efforts would help point to a variety of molecules and molecular processes as therapeutic targets.

Understanding the molecular etiology of ME/CFS is another important opportunity. A long-standing belief in the field is that an infectious agent causes the disease, and that the pathogenicity of the as-yet-undiscovered organism is responsible for the severity of the illness. An equally plausible explanation is that a stressor such as trauma, infection, or genotoxic stress may trigger a series of events that lead to a hypometabolic state. This model is observed in children with congenital mitochondrial disorders, where the phenotype does not present itself until after a serious viral infection. This shift in thinking opens up the possibility that ME/CFS has strong genetic and environmental associations, which may also explain the extensive heterogeneity in its presentation, progression, and recovery across patients. The search for novel infectious agents should continue, but research efforts should also focus on understanding individual host susceptibility and response to infection. For example, it may not be a particular infectious agent that results in the disease, but rather a particular host state as a function of numerous biological and external factors that governs an individual’s susceptibility. This perspective mirrors the NIGMS-funded Glue Grant on Inflammation and Host Response to Injury, which used an integrated omics approach to define variable responses to infection and trauma. Characterizing host responses to infection and understanding the mechanisms of the long-term sequelae may reveal insights into ME/CFS that are relevant to numerous other diseases of infectious origin, such as Chronic Lyme Disease and Post-Ebola Syndrome (Mattia et al., 2016). Moreover, such precision medicine approaches would build a more comprehensive understanding of ME/CFS and offer richer opportunities for therapeutic intervention.

Another major challenge is our lack of understanding of the prevalence and landscape of ME/CFS, which is largely due to the difficulty in diagnosing the disease. The search for precise molecular biomarkers is a great opportunity afforded by this research program, which would be accelerated through multi-omics approaches in large patient cohorts. Current estimates of the prevalence of ME/CFS vary widely (800,000 to 2.5 million cases in the US) due to varying diagnostic and data collection methods. There is an opportunity here to improve these estimates based on modernized methods and community-defined standards, including criteria specified in the 2015 Institute of Medicine Report, and by considering questionnaire-based responses like the Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort in the United Kingdom (Collin et al., 2016).
Because of these complex scientific challenges, ME/CFS research presents an excellent opportunity for developing and piloting novel methods and technologies in discovering biomarkers, elucidating disease mechanisms, and revealing therapeutic possibilities. The methods we need to understand this complex disease may very well not exist yet. Engineering and technology development efforts towards highly sensitive, quantitative molecular profiling and/or measuring novel cellular properties, as well as novel computational analyses that integrate multiple datatypes to define disease mechanisms, should be encouraged. Again, it is highly likely that such efforts will prove useful in the study of other diseases, be they infectious, genetic, or complex in origin.

Beyond scientific considerations, we would like to note several programmatic considerations that we believe are key for rapid progress. Long-term studies of patients are absolutely essential. Such a mechanism has proven effective in the NIGMS Glue Grants described above. Moreover, maintaining an open structure in RFAs will allow scientists to develop and refine their hypotheses as research progresses, as appropriate for the unknown/uncertain nature of the field. As highlighted in several places above, the opportunities for collaborative efforts within and beyond the ME/CFS research community to understand and treat this disease are numerous. There are numerous experts spread across the world, each taking their own approaches based on their own expertise. We believe future funding programs should not only encourage, but establish frameworks for highly collaborative data sharing and strategizing that bring together researchers and clinicians. All data should be made publicly available as early as possible (even before publication), in both raw and accessible formats. This will not only facilitate collaboration (for example by encouraging biocomputing experts to engage with the data) and integrative analyses, but also empower patients to understand more about their disease and what progress is being made. As we have all seen, the ME/CFS patient community is extremely active, engaged, and eager for actionable results.

We thank you once again for the opportunity to provide input on this matter, and look forward to the new strategies for ME/CFS research efforts put forth by this working group.

Yours sincerely,

Ronald W. Davis, Ph.D.
Professor of Biochemistry and Genetics, Stanford University
Director, Stanford Chronic Fatigue Syndrome Research Center and Stanford Genome Technology Center
On behalf of the Stanford Chronic Fatigue Syndrome Research Center and Stanford Genome Technology Center

References:


Raeka Aiyar, Ph.D. | Communications Director
Stanford Genome Technology Center
Reynolds, Leigh

Cc: 'Suzanne Vernon'; Cindy Bateman
Subject: Bateman Horne Center Response to ME/CFS Research Priorities RFI

Please find attached the response prepared by the Bateman Horne Center concerning the Trans-National Institutes of Health (NIH) ME/CFS Working Group’s RFI concerning new strategies to guide NIH’s research efforts and priority setting for research on ME/CFS.

If you have any questions, please do not hesitate to contact me.

Leigh A Reynolds
Bateman Horne Center
Communications Director
• Determine how to objectively measure the diagnostic criteria recommended by the IOM, since they represent the core underlying physiology. Get these methods and measures into the hands of primary care physicians. Use these objectively defined diagnostic criteria as outcome measures to interest pharma in pursuing clinical trials for ME/CFS.

**Challenges or barriers to progress in research on ME/CFS**

• Catch 22 – not enough funding dollars and therefore not enough scientists. Without enough funding for scientists, the chance of discovering biomarkers decreases.

• ME/CFS biomarkers may be evasive because scientists aren’t studying the “same” ME/CFS; some are looking for biomarkers in sudden onset, others in post-infection ME/CFS, others in severe ME/CFS and others cast a broad net and study all ME/CFS patients at once. Biomarker research has been hampered because we have not consistently studied gold standard patients – that is, patients that have been rigorously evaluated and followed by clinical experts.

• There are not enough clinical collaborators to make the clinically evaluated, “gold standard” patients available to research. There must be parallel efforts to fund research investigators and to increase expertise in clinical diagnosis.

**Gaps and opportunities across the research continuum from basic through clinical studies**

• In her 2012 presentation to DHHS and the CFS Advisory Committee Dr. Sandra Kweder said, “For progress to be made in ME/CFS we must define the core signs, symptoms and decrements in specific functioning”. The core signs, symptoms and decrements in specific functioning have been defined in the 2015 Redefining ME/CFS IOM report and are summarized here:

  o **Reduced functional capabilities** on a global scale—physical, cognitive, etc. This alone should be easily approachable with what is already known—orthostatic intolerance, possible mitochondrial or cellular dysfunction, altered immunity and possible chronic viral reactivation, etc. Every study should be taking this into account.

  o **Post exertional consequences** that parallel illness severity and the degree of effort/activity. Every study should involve measurements before, during and after an activity/exertion stressor, and should track at least to resolution of symptoms in all participants.

  o **Sleep** is very abnormal, but variable across the population, resulting in unrefreshing sleep. Current methods of sleep assessment have been inadequate because light disturbed sleep is considered “sleep artifact” on polysomnography. New innovative ways of assessing sleep disturbance over longer period of time and in the home should be implemented.

  o **Orthostatic intolerance** is an often overlooked aspect of illness that can be easily measured by a bedside 10 min stand/lean test of blood pressure, pulse and
symptoms. This alone could easily subset groups of patients to target for research and clinical trials.

- Dysautonomia, POTS, orthostatic hypotension, etc, already have a significant base of research and a growing group of clinical specialists and we should learn from their experiences and leverage this knowledge to ME/CFS

  - **Cognitive impairment.** While it is often difficult to measure cognitive impairment, there is an ample evidence base to investigate altered cerebral perfusion from chronic dysautonomia and neuroimmune illness altering brain tissue directly. Innovative cognitive testing should be implemented in research such as that used in TBI. For example, a 5-minute cognitive testing module can be implemented before and after a 10 minute lean test.

- Other important areas of exploration:
  - **Centrally mediated pain amplification, hyperalgesia, central sensitivity.** There is a rich literature in fibromyalgia that should be considered in all ME/CFS patients who meet FM criteria, which are symptom based and easy to apply to this population.
  - **Neuroendocrine function.** There are very few high quality studies that directly assess hypothalamic and pituitary function, yet the presentation of illness and the known science clearly suggests “downstream” endocrine issues—low normal cortisol, a high rate of thyroid disease, significantly altered gynecologic health, low testosterone in male subjects, etc.
  - **Immune dysfunction.** Clear evidence but needs larger sample sizes and more robust assays to validate as diagnostic and treatment biomarkers.
    - **Autoimmunity.** There is growing evidence of autoimmunity involving the central and peripheral nervous system, high prevalence of thyroid disease, high rate of celiac disease, B12 deficiency, etc.

**General Comments:**

Research in ME/CFS has been lean and under-funded for several reasons, including the shortage of accurately diagnosed patients available for research studies. The Bateman Horne Center – a clinic specializing in ME/CFS, with a large number of patients who have been carefully and accurately diagnosed and continue to be treated effectively- is committed to bringing ME/CFS into the mainstream of clinical and medical science. Identifying biomarkers is a critical first step. BHC is a unique example of an independent non-profit integrative health center where medical care informs research, and research informs medical care. Efforts should be made to encourage the development of additional centers of excellence for ME/CFS in order to create collaborations between well-qualified patients and research partners. NIH grantees should be required to include clinical collaborators as a part of their grant submission.
Need: An optimized clinical trial consortium for identifying the most promising ME/CFS treatments.

Description of the need or opportunity
Physicians currently have few tools available for treating ME/CFS, with no FDA-approved pharmaceutical medications. While many clinical trials of potential ME/CFS treatments have been conducted, the extreme heterogeneity in sample size, duration, outcomes assessed, scientific controls, inclusion criteria, disease severity, and statistics makes it impossible to contrast results and determine which treatment options are particularly promising. There is therefore a need to accelerate testing of potential treatments to be used by physicians or further developed by industry.

The opportunity is to create a consortium of independent, clinical research laboratories that can apply a standardized protocol to systematically test a prioritized set of potential treatments. This process would involve querying clinicians, patients, scientists, and industry groups to develop a prioritized list of treatments. A standardized testing protocol would then be developed, with the aim of creating highly rigorous, interpretable, and efficient clinical trials. A number of sites would then implement the trials so that results of different treatments could be directly compared and contrasted. This process would yield a subset of treatments that showed enough promise to warrant further testing or development.

Scientific rationale and potential public health impact
The ME/CFS clinical trial literature has a low impact on science and medicine because of the poor and/or idiosyncratic designs of many studies. By improving the methodology of ME/CFS trials, the scientific community could have a more direct impact on public health by providing more viable treatment options. Because the process of developing and approving new drugs can take well over 10 years, trials of existing pharmaceuticals could yield quicker benefits for millions of individuals who are not receiving adequate treatment.

Anticipated challenges that will need to be addressed
- Determining the ranking of medications in a prioritized list would be controversial.
- Gaining consensus on a standard testing protocol can be difficult.
- Ensuring integrity of research protocols across multiple study sites requires significant resources.

Appropriate benchmarks for evaluating progress
1. Creation of core scientific/medical team and participating sites.
2. Query of stakeholders for medication suggestions, and creation of a ranked list of potential treatments.
3. Development of a standardized testing and analysis protocol.
5. Conduction of the clinical trials.
6. Analysis of trial results and identification of most promising treatments.

Thanks for copying me in Mark
This is very interesting and certainly represents a reasonable hypothesis. As you say we have been looking at muscle function and found some interesting abnormalities. Our PLoS one paper - Brown et al., might also be of interest.

J

Sent from my iPhone

On 24 Jun 2016, at 22:03, [...] wrote:

Dear Trans-NIH ME/CFS Working Group and Dr Whittemore,

I'm a Doctor who has been bedridden with severe ME/CFS for a long time after graded exercise therapy caused a severe relapse from which I haven't recovered and I will only get my health and independence back if I get treated with effective medication which isn't available yet as a review of the PACE trial has now clearly shown that CBT and GET are ineffective for this debilitating disease [1].

I am writing to you in relation to your "NOT-NS-16-024: Request for Information: Soliciting Input for New Research Strategies for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS)".

I would like to ask you to focus more research into the underlying problems in the severely affected i.e. viral, Lyme and co-infections like Bartonella etc, metabolic and energy problems, auto antibodies and treatment trials with (for example) Ampligen, antiviral medications, ARV's and medication that works for (other) autoimmune diseases.

The reason to concentrate on the severely affected is the worse the disease the more likely it is that patients have ME and not something else.

I also have a more detailed RESEARCH PROPOSAL and that's why I have taken the liberty to copy this email to Professor Julia Newton who has done a lot of research on the energy production problems in this disease. She was involved in the paper by Jones et al. [2] and like Lane et al. [3] they demonstrated a left shift in the anaerobic threshold in about 40% of patients.

I have done some research on myself and found a big left shift as well and last year I published a paper about it [4] in which I demonstrate that if I eat less than 3 hours before I just walk to the toilet and back, this left shift will get a lot worse and the same applies to the delay in releasing a second batch of lactic acid after the exercise. Even though walking to the toilet and back is trivial, eating has the same effect as it did in the past when I would go for a long run i.e. I would need to make sure that I had eaten 3 hours or more before I did go for the Run. If I do that now I have "more power" and less pain afterwards.

In the paper I show that it's very likely that the oxygen uptake into my muscle cells or mitochondria is impeded and my RESEARCH PROPOSAL therefore is the following: Take 10 to 15 ME/CFS patients with a left shift of the anaerobic threshold and the same number of healthy controls, let them do exercise which they can do which produces a large quantity of lactic acid but doesn't cause a relapse and let them inhale radioactive oxygen to see if the oxygen distribution into the muscles is the same as in the healthy controls to find out if a severely reduced oxygen uptake is the reason for the severe problem with the oxidative phosphorylation hence we have to rely on the glycolysis, produce lots of lactic acid for trivial exercise which results in a left shift.
The reason why I have taken the liberty to include Professor Newton in this email is that she might be interested to do that study and maybe you could then Finance it.
(Due to the severity of my illness I had to use my energy for this email and haven't been able to contact Professor Newton about it. So I do apologize for that).

I didn't attach my article because I wasn't sure if you would open an email from an unknown person with an attachment. The full article is available here:
The Aerobic Energy Production and the Lactic Acid Excretion are both Impeded in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome http://dx.doi.org/10.16966/2379-7150.112

Thank you for your time.

Sincerely,

[...]
(Family Physician)

References


Hanson, Maureen
[...]
Subject: Response to RFI

Please find attached.

Sincerely,
Maureen Hanson
Liberty Hyde Bailey Professor
Cornell University
[...]

[522x40]109
To: The Trans-NIH ME/CFS Working Group

From: Maureen Hanson, Liberty Hyde Bailey Professor

Date: June 24, 2016

I would like to highlight several important issues. I would be glad to elaborate on these further upon request.

1. Access to samples.
One of the primary issues in recruiting new research groups to ME/CFS research is the difficulty of obtaining well-documented experimental samples. Every new research study usually requires a period of sample collection, as there are few biobanks from which one can obtain samples, and a limited number of physicians expert in diagnosis who have large numbers of ME/CFS patients. Several such physicians have retired or are reaching retirement age and have not trained successors. There are also problems with some biobanks, which restrict who can obtain samples and limit what a researcher may do with the samples.

Several studies—for example, the NIH intramural study and the Stanford Severely Ill study, are going deep instead of wide. In other words, a small number of patients will be intensively analyzed. Such studies do not require an extensive biobank. But there are others that must go wide—requiring large number of samples. Some studies that could provide very valuable insights—for example, GWAS—require a large number of samples. There appear to be subgroups of patients with different symptom clusters, and for some such studies, larger samples sizes are needed to determine whether various parameters correlate with the subset status. Funding for readily accessible biobanks, with appropriate documentation and adequate amounts of samples, could greatly stimulate research on the disease.

2. Uniformity of survey information. One issue that has made use of some biobanks or sample collections problematic is the lack of a uniform set of questionnaires that provide adequate information about patient history, results of prior tests, diet, drug and supplement intake, and detailed symptomology. Having a standard set of questionnaires would allow more studies to be readily compared to one another. This does not mean that researchers should not sometimes have special questionnaires appropriate to their particular studies, but having a basic set of questionnaires so that survey data can be readily compared, would allow comparisons of studies that are not possible now.

I would note that my own lab could not have been able to correlate symptoms with mitochondrial SNPs (http://translational-medicine.biomedcentral.com/articles/10.1186/s12967-016-0771-6) if detailed information on symptoms and their severity had not been gathered for the Chronic Fatigue Initiative biobank. Without such data, the study would have been merely a negative report, as we saw no correlation of mere disease vs. healthy status with any sequence variation.

3. Epidemiology. The field needs an assessment of the true numbers of individuals ill with ME/CFS in the US, and determination of whether the disease has increased beginning in the 1980s. While
some estimates of the patient population have been made, no such estimates have been made with the
diagnostic criteria recommended by the IOM. To understand the true burden of disease in the U.S. and
the economic cost, the number of individuals in different categories—bedbound or housebound vs
able to work part-time—is important to determine.

There is anecdotal evidence that ME/CFS incidence increased in the 1980’s, though outbreaks have
been recorded prior to that time. It is important to examine data on incidence retrospectively, to find
out whether ME/CFS is actually an emerging illness, as many patients and some medical personnel
suspect. The patient community is quite angry about prior actions of CDC—but most individuals
involved in the issues surrounding CDC’s response to ME/CFS in the 1980s are either retired or
deceased. I believe the CDC should set these past problems aside and investigate whether the disease
did, in fact, increase in the 1980’s, as having such information will alter researchers’ views of the
potential causes of the illness. Furthermore, because of the valuable research opportunity a new
outbreak would provide, the CDC should be particularly attentive to any reports that might suggest
that a new outbreak is occurring.

4. Analysis of responders to Ampligen. As this drug is one of few which have resulted in dramatic
improvements in some patients’ conditions, it merits further investigation not only in clinical trials,
but for the research opportunity it can provide. There is no question that there is a subset of
individuals who have extremely strong positive responses to this drug. While clinical trials are
certainly needed, why not examine a set of known responders to determine what
molecular/physiological/neurological changes when a patient who is quite disabled receives the drug
and becomes able to greatly increase the level of daily activities? Such a study to give insights into
the disruption that results in the disabling cognitive problems, fatigue, and post-exertional malaise that
individuals in the responding subset and their physicians have reported are greatly ameliorated on the
drug.

5. Request for applications. Evidently NIH will be issuing an RFA for ME/CFS Centers of
Excellence. I believe an RFA is needed for R01/R21s as well, as good ideas may come from
researchers who have previously not carried out research in this area. Many talented researchers were
attracted to the AIDS field once RFAs were made available, and a similar phenomenon is currently
happening for Zika virus because of its obvious increase in incidence. Presently, potential researchers
are discouraged by low levels of funding and the unrecognized importance of ME/CFS, stigmatized
by its name and years of erroneous assignment of the illness as psychological. An RFA for
investigator-initiated research projects will signal that research on this disease has a future and bring
new scientists into the field.