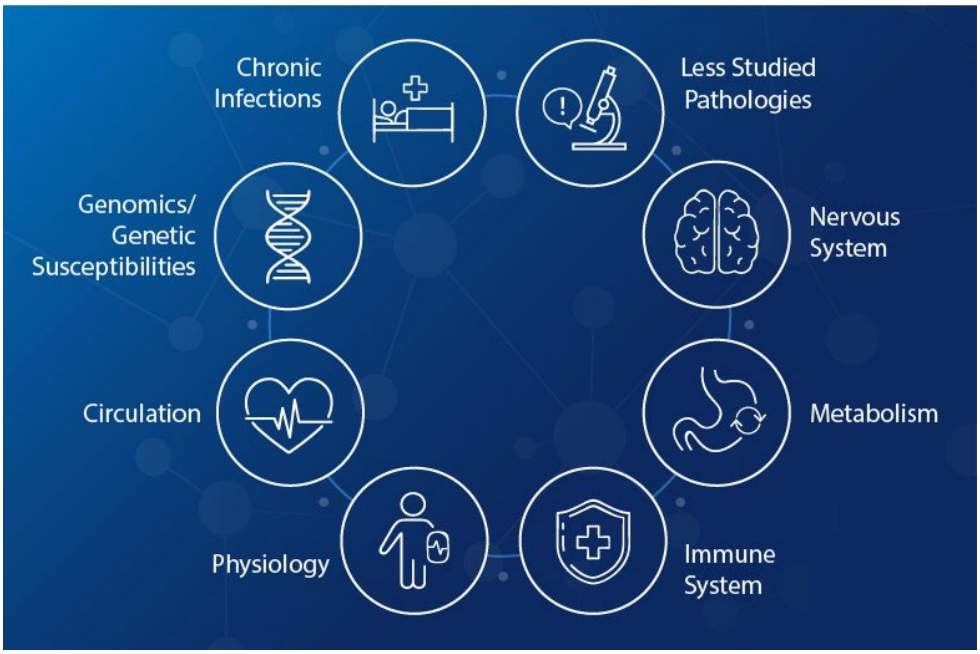

Report of the ME/CFS Research Roadmap Working Group of Council

May 15, 2024

Executive Summary

Myalgic encephalomyelitis, also known as chronic fatigue syndrome (ME/CFS), is a multifaceted, heterogeneous chronic disease that presents with a wide range of disabling symptoms that are exacerbated by exertion. At present, there are no FDA-approved treatments for the disease, and prognosis is poor, greatly limiting the lives of millions of individuals world-wide. To address this, the National Institutes of Health (NIH), led by the National Institute of Neurological Disorders and Stroke (NINDS), developed the Research Roadmap for ME/CFS to identify the highest priorities for research with emphasis on research that will lead to clinical treatment trials. Participants included individuals with lived experience of ME/CFS, representatives of non-profit advocacy and research organizations, researchers, and clinicians. In 2023 and 2024, eight chairpersons in association with a working group convened eight webinars that explored the following subtopics relevant to ME/CFS: chronic infections, the immune system, the nervous system, circulation, metabolism, physiology, less studied pathologies, and genomics/genetics. Each webinar group identified critical priorities for research into objective diagnosis and effective treatments. This document sets a foundation for advancing scientific understanding, improving diagnostic strategies, and developing personalized treatments for individuals affected by this challenging condition.

One of the major challenges of research in ME/CFS is the multisystem nature of the disease, with both overlap and interplay of multiple body systems contributing to disease presentation. For the purposes of this report, we created eight subtopics, but it is important to look at the big picture and understand how these subtopics interface with each other to create the pathophysiology and clinical presentation of the disease.

1. **Chronic infection:** This document addresses the potential role of chronic infections in ME/CFS, exploring the possibility of ongoing immune and metabolic abnormalities following a variety of implicated infectious triggers. Long COVID and other infection-associated chronic illnesses provide valuable insights into the potential link between ongoing or reactivated infections and symptoms in ME/CFS. Researchers have investigated various infectious agents, including enteroviruses, herpesviruses, and endogenous retroviruses, to unravel the complex interplay between chronic infections and immune dysfunction in ME/CFS.

2. **Immune system:** Research has highlighted chronic inflammation involving innate immune responses, upregulated antibody responses, and altered metabolic pathways in individuals with ME/CFS. These findings underscore the importance of understanding the role of infection in initiation of ME/CFS pathophysiology and identifying potential therapeutic targets. One of the primary focuses of recent studies on ME/CFS has been the identification of biomarkers indicative of intestinal damage and microbial translocation, which can trigger immune activation and neuroinflammation. Investigations into exercise-induced post-exertional malaise (PEM) have revealed insights regarding the interplay between physical activity and immune

function and are building a better understanding of the mediators of post-exertional relapse. Studies have also explored metabolic differences in response to exercise, aiming to elucidate molecular pathways underlying immune dysregulation in ME/CFS. Understanding the impact of exercise on immune function is crucial for developing tailored interventions to manage symptoms and improve quality of life in people with ME/CFS.

3. **Central nervous system (CNS) and peripheral nervous system (PNS):** Advances in imaging and diagnostic testing have documented changes in both the central and peripheral nervous system in infection-associated chronic illness, including ME/CFS. The CNS and PNS intimately interact with every bodily system through afferent and efferent signaling that ultimately impacts inflammation, circulation, and endocrine function. Improved imaging of the brain, including structure, perfusion, the blood brain barrier and glymphatic flow, plus imaging of the spinal cord as well as assessment of the autonomic nervous system will help advance research in ME/CFS.

4. **Cardiovascular circulation:** Circulation and perfusion are high priority topics. Areas of interest include endothelial inflammation and dysfunction, microclots, and adequate perfusion of the brain and peripheral tissues. Many additional factors may play a role, including blood volume, neurovascular dysregulation, endothelial integrity, and characteristics of red blood cells.

5. **Metabolism:** The metabolomics webinar highlighted the impact of metabolic abnormalities related to possible mitochondrial dysfunction on individuals with ME/CFS and identified research gaps and unmet needs in the field. Understanding the metabolic profiles of people with ME/CFS is essential for developing targeted treatments and improving clinical outcomes. Recommendations were formulated to enhance the clinical research landscape for ME/CFS, with a focus on addressing treatment limitations and advancing scientific understanding of metabolic dysregulation in the condition.

6. **Physiology:** The physiology of ME/CFS is complex and encompasses all systems. There is optimism regarding whole body positron emission tomography (PET) scanning as well as understanding cellular responses to infection, inflammation, enervation, and energy production. Several hypotheses that were developed to explain the disturbed physiology and metabolism in ME/CFS need to be tested, including the metabolic trap and the itaconate shunt. Little information is available to explain the basis of the unrefreshing sleep that occurs in the disease.

7. **Less studied pathologies:** In some individuals, ME/CFS is associated with hypermobility and connective tissue disease, spinal compression syndromes, neuroendocrine disorders, problems in reproductive systems, and allergic disorders, all of which need further study. The reason for the higher incidence of ME/CFS in females versus males remains unknown. The glymphatic system has not yet received attention in ME/CFS.

8. **Genomics/Genetic susceptibility:** ME/CFS is not a monogenic disease, but individuals may be predisposed to the illness due to genetic or epigenetic factors. A combination of multiple alleles may be exerting an effect in susceptible individuals. Large well-characterized cohorts are needed to identify factors that may explain differential responses of individuals to

environmental insults, including infections or physical stress. Identifying susceptibility alleles may reveal pathways that can be targeted for treatment.

Conclusion and Future Directions

The ME/CFS Research Roadmap Report highlights the significance of identifying infectious triggers, understanding immune and metabolic interactions, querying the central and peripheral nervous systems, exploring circulatory and perfusion abnormalities, delving into the physiology in every system, utilizing genetics to understand risk and utilizing genomics to find biomarkers, and gaining insight into the relationship of comorbid conditions, along with exploring novel therapeutic targets. Understanding how these factors combine to impair activity and create post-exertional malaise is the ultimate goal. The roadmap emphasizes the need for robust biomarkers and development of additional objective markers. Due to the debilitating and severe nature of the disease, there are urgent recommendations for additional research, small exploratory trials, larger controlled trials, international collaboration, and involvement of people with lived experience in research. There is sufficient scientific rationale to utilize some re-purposed drugs in ME/CFS clinical trials. Basic mechanistic studies and targeted treatments are essential for guiding future research and improving outcomes for individuals with ME/CFS. A biobank of tissue specimens, as well as blood fractions and cerebrospinal fluid, could greatly facilitate ME/CFS research. A biobank that is accompanied by standardized measures of illness would both enable and encourage new researchers to enter the field.

In summary, the ME/CFS Research Roadmap Report represents a cross-sectional, multidisciplinary discussion of current ME/CFS research, encompassing potential biomarkers related to acute and chronic infections, immune responses, central and peripheral nervous system, metabolism, circulation, genetic and genomic science, and comorbid conditions, plus the importance of involvement of people with lived experience, clinical trial recommendations, and future research directions. By addressing the complex interactions between these systems, the document sets a foundation for advancing scientific understanding, improving diagnostic strategies, and developing personalized treatments for individuals affected by ME/CFS.

Respectfully submitted,

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Introduction

Charge and Process

Charge

In 2019, the National Advisory Neurological Disorders and Stroke (NANDS) Council Working Group for myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) presented the Report of the NANDS Council Working Group for ME/CFS Research. In 2022, as part of the strategic planning process outlined in the report, NINDS announced the development of a Research Roadmap for ME/CFS, which will identify research priorities to move the field toward translational studies and clinical trials.

> "The roadmap will be informed by a Working Group, which will include ME/CFS basic and clinical experts from the research community, leaders of ME/CFS non-profit advocacy and research organizations, as well as people who are living with ME/CFS, have a family history of ME/CFS, are caregivers/care partners of people living with ME/CFS, and/or identify as ME/CFS advocates. The Working Group will meet regularly in 2023 to discuss and develop a Research Roadmap for ME/CFS.
>
> Members of the Trans-NIH ME/CFS Working Group and staff from the NINDS Office of Science Policy and Planning and NINDS Office of Neuroscience Communications and Engagement will coordinate the activities of the ME/CFS Research Roadmap Working Group. The roadmap will be presented at the NINDS Advisory Council meeting on February 14-15, 2024 (amended to May 15, 2024)."

This new NANDS Council Working Group was charged with the development of a Research Roadmap for ME/CFS to provide scientific guidance to the NANDS Council on how best to advance research on ME/CFS. Consistent with that charge, the Working Group was to assess current ME/CFS research activities and identify opportunities and gaps in ME/CFS research to identify targets for the development of treatments.

Process

NINDS, the lead institute for ME/CFS-related research within the NIH, initiated discussions around the process for development of a research roadmap in September 2019 and was prepared to begin in March 2020 when the COVID-19 pandemic hit the United States. The effort was put on hold until September 2022 and was initiated in February 2023.

Working Group and Planning Group Formation

NINDS established a Working Group of Council (WGC) (see *Appendix 4: Rosters*) that included health care providers, researchers, leaders of non-profit advocacy and research organizations, and people with lived experience of ME/CFS (PWLE ME/CFS) to oversee the development of the research roadmap. To better represent PWLE ME/CFS, NINDS developed a self-nomination form that was posted publicly

and open to any PWLE of ME/CFS interested in being part of the research roadmap development process. More than 90 self-nominations were received and 21 individuals with lived experience were selected to participate in the research roadmap process, with an additional 6 individuals serving on the Working Group of Council.

The WGC members were responsible for several tasks and deliverables, including:

- High-level planning for the ME/CFS Research Roadmap, which included providing input on topics, organization, approach, and structure.
- Participation in at least one topic-specific webinar.
- Serving as webinar leads. Not all WGC members served as leads, but those who did were responsible for the coordination of the webinar and development of the research recommendations for that topic area.
- Development of the ME/CFS Research Roadmap research priorities and draft report.

This Working Group also acknowledged early on that the community of PWLE ME/CFS would be critical partners in the planning process. Therefore, the Working Group designed the process with multiple opportunities to engage and co-develop the research priorities with PWLE ME/CFS and incorporate feedback from the community.

Another early consideration for the development of the Research Roadmap was how to properly represent the various symptoms associated with ME/CFS, as well as the possible causes and contributing factors. To help focus the effort, the WGC co-chairs held early meetings to develop a list of topic areas. These topics would serve as the focus areas for half-day webinars during which each could be addressed and discussed to develop specific, actionable recommendations for how best to advance research on ME/CFS in that area.

The Working Group formed eight topic-specific "webinar planning groups", which included researchers, clinicians, advocates, and PWLE ME/CFS (see *Appendix 4: Rosters*). Two to three PWLE ME/CFS with unique expertise, experience, and perspectives were selected to serve on each planning group. Researchers were identified as Chairs for each of the webinar planning groups on the following eight topics: chronic infection, immune system, nervous system, circulation, metabolism, physiology, less studied pathologies, and genomics/genetic susceptibilities.

PWLE ME/CFS Inclusion

There was an extensive effort to include PWLE ME/CFS (*i.e.*, individuals with ME/CFS, those with a family history of ME/CFS, caregivers/care partners, and/or advocates) as partners throughout the process. PWLE ME/CFS were included as members of the Working Group of Council and on each webinar planning group. These group members were included in all communications and meetings equal to scientists and clinicians. Each webinar included time to hear from a PWLE ME/CFS about each subtopic and the publicly open webinars included many questions, comments, and discussion from PWLE of ME/CFS.

Additional efforts were made to ensure that PWLE could participate in the process, including an identified NINDS staff personnel with PWLE engagement expertise, a weekly update email for transparency and clarity throughout the entire process, and opportunities to provide feedback on participation, including through interviews with the NINDS PWLE engagement expert. There were periodic checks with the PWLE ME/CFS members of the Working Group of Council and webinar planning groups, including a mid-process check-in and interviews. The key takeaways from those are summarized in *Appendix 2*.

In addition to inclusion of those PWLE ME/CFS that served directly as subject matter experts on the WGC and webinar planning groups, we included the broader community of PWLE ME/CFS by developing a digital crowdsourcing platform to collect input and facilitate online conversations with anyone with lived experience of ME/CFS, along with the scientists and health care providers. The NINDS team developed specific assistance for using the platform and offered an email alternative for submitting feedback to allow for maximum accessibility. An ME/CFS email listserv was utilized to help NINDS reach any PWLE ME/CFS wanting to be included in discussions. The quarterly ME/CFS telebriefing also helped bring more PWLE ME/CFS to the conversation and equip them to participate in the research roadmap process.

Webinar Series: Information Gathering and Discussion

Each planning group organized a virtual webinar during which the speakers were asked to address the following questions:

1. What do we know in this area of ME/CFS research?
2. What don't we know/or what do we think we know but needs replication?
3. What do we need to know to move the field toward translational studies and clinical trials?

The webinars featured scientific and clinical experts in ME/CFS and were open to the public via Zoom, a video conferencing platform. Each open webinar had a Q&A session with attendees and was followed by a closed discussion with the speakers, panelists, NINDS staff, members of the WGC and PWLE ME/CFS who helped plan the webinars. The webinars attracted about 250 attendees, with the largest totaling over 400 people. The video and transcript from each webinar are archived on the NINDS website.

Public Crowdsourcing: Communication with the Broader PWLE ME/CFS and Research Communities

During each webinar, questions and comments were received from the audience. Many were addressed during the open or closed webinar sessions and others were raised afterward to advance working group discussions and add to the roadmap.

The webinars were followed by a public crowdsourcing "campaign" to gather more feedback from the community. The IdeaScale platform allowed individuals to provide their comments and feedback on the draft research priorities, comment on other posted discussion items, and identify any missing research priorities. Individuals could also provide feedback via email and comments were posted on their behalf on IdeaScale. A summary of the feedback received via IdeaScale and email is in *Appendix 3*.

Writing the Research Roadmap

The Working Group used information collected from each of the eight webinars, follow-up closed session discussions with webinar speakers, and pre-webinar planning discussions to develop the research priorities for all eight of the topic areas discussed. The Chair of each webinar planning group was tasked with working with their group to refine the research priorities and draft their section of the research roadmap report. Feedback from the community was utilized to help inform components of the final report.

NINDS staff helped facilitate and coordinate the Working Group and webinar planning groups, and a contractor assisted with webinar logistics, technical support, and website maintenance. The contractor also provided a scientific writer to capture the discussions and research priorities identified by each speaker in the webinars.

The Co-Chairs of the Working Group of Council, Maureen Hanson, PhD, and Lucinda (Cindy) Bateman, MD, compiled and edited the draft reports from the eight webinar planning groups and wrote the Executive Summary. Anthony Komaroff, MD wrote the Background section of the report. The draft report to Council was reviewed and edited by the Working Group of Council and PWLE ME/CFS before submission to the NANDS Council for their review and recommendation.

Background

The Disease and its Burden

Contemporary interest in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS)—previously, and sometimes still, called either "ME" or "CFS"—began in the 1980s. Initially, no specific abnormalities were identified on physical examination or on commonly ordered diagnostic laboratory tests. It was unclear how prevalent the illness was, and what kind of economic burden it represented for society.

In the decades since, new technologies have gradually revealed abnormalities involving multiple organ systems, including the central and autonomic nervous systems, as described in this report. Much of this research has been funded by NIH, particularly by NINDS and National Institute of Allergy and Infectious Disease (NIAID). Many of these abnormalities have been replicated by multiple laboratories.

Most, but not all, individuals report their ME/CFS onset followed an "infectious-like" illness characterized by respiratory and/or gastrointestinal symptoms, fatigue, adenopathy, myalgias and sometimes low-grade fevers. Because the initial illness has been assumed by person with ME/CFS and doctor to be just another self-limited viral infection, however, usually no attempt has been made to identify an infectious agent. Some individuals report ME/CFS symptoms followed immediately after the initiating illness, while others describe slow or stuttering onset. Those individuals who are unable to identify an obvious triggering event often ascribe the onset to be due to some physical or emotional stress, but asymptomatic infection or viral reactivation cannot be ruled out.

While some of the signs and symptoms of the initial acute illness (e.g., fever, adenopathy) usually wane, people are left with a chronic debilitating illness characterized by fatigue, post-exertional malaise, cognitive problems, orthostatic intolerance, disrupted sleep, and often many other symptoms. Several stressors—exercise, prolonged upright position, cognitive and emotional stressors—typically produce a worsening of all the symptoms of the illness. This condition, called post-exertional malaise, is a cardinal feature of the illness[1].

For most individuals with ME/CFS the symptoms are cyclic, with some relatively "good" days and frequent "bad" days. People with ME/CFS are more functionally impaired than people with congestive heart failure and major depression[2,3]. The majority find it difficult to maintain full-time employment, and the most severely affected are bed-ridden or housebound, and unable to work. It is notable that up to 75% of individuals with ME/CFS are biologically female.

There is no solid evidence that individuals with ME/CFS are more likely than the general population to exhibit a psychiatric disorder, as these are prevalent in the general population. Mental health scores on common surveys such as the Short-Form 36 are often similar between ME/CFS cases and controls. In addition, when present, psychiatric disorders have typically developed *after* the onset of ME/CFS and are no more frequent in the years *before* the onset of the illness than they are in the community at large[4]. A well-accepted biomarker of major depression—upregulation of the hypothalamic-pituitary-

adrenal axis—is absent in people with ME/CFS[5-7]. Finally, the antidepressant fluoxetine does not improve mood or fatigue in individuals with ME/CFS[8,9].

Many investigators believe that ME/CFS reflects a dysfunctional immune and metabolic response triggered by some infectious agents. While it may not be caused by a single, novel agent, it is also unlikely that every known pathogen is able to incite ME/CFS. Whether the same infectious agents that trigger the illness also contribute to perpetuating the chronic symptoms is not clear.

The Societal Burden of the Disease

In the U.S., prior to 2020, ME/CFS was estimated to affect up to 3.1 million people and generated direct and indirect expenses of approximately $36-51 billion annually, according to research from the U.S. National Academy of Medicine (NAM), the Centers for Disease Control and Prevention (CDC) and several other studies[1,10,11]. The illness also has been reported in many countries around the world, with an estimated prevalence of as many as 67 million people, worldwide[12].

Epidemic Forms of ME/CFS

An illness clinically similar to ME/CFS has been reported in epidemic form in multiple outbreaks, around the world, over the past century[13-19]. The individuals often were young or middle-aged adults. These outbreaks were reported before any of the case definitions of ME/CFS had been published. For the outbreaks occurring before the 1950s, the details on symptoms, physical examination findings and laboratory test results make it difficult to determine how similar these illnesses were to more recent cases of ME/CFS. Many of the outbreaks occurred during the "polio era" from the 1920s-1950s. Indeed, the poliovirus and other enteroviruses were suspected in many of the outbreaks. Another group of outbreaks occurred in the 1980s during the NIH/AIDS epidemic, although few of the affected individuals were infected with HIV. These outbreaks clearly led to ME/CFS, and many victims are still ill today. While no infectious agent was definitively linked to any outbreak, the technology for identifying infection during these outbreaks was rudimentary compared to what is available today.

Post-acute Infection Syndromes

An illness with similar symptoms to those of ME/CFS has been reported to follow acute infection by a variety of infectious agents: viruses (e.g., Epstein-Barr virus, human herpesvirus 6, enteroviruses); bacteria (e.g., *Borrelia burgdorferi)*; and protozoa (e.g., *Giardia lamblia*) [20,21].

The most recent example of a chronic condition following an acute infectious illness is the development of post-acute COVID-19 syndrome, also called "Long COVID". It has been estimated that post-COVID conditions may cost the U.S. economy as much as $2.6 trillion in medical care, lost productivity, and disability payments in the coming years[22]. A meta-analysis of 57 studies involving over 250,000 people found that ongoing symptoms impairing functional mobility persisted in 43% of people with acute COVID-19 for at least *six months* after acute infection[23]. Among people with persistent, debilitating symptoms following acute COVID-19, an estimated 13-45% meet the 2015 Institute of Medicine (IOM) case definition for ME/CFS[24-27]. Individuals fulfilling the IOM case definition who become ill after 2020 may have acquired their illness either from SARS CoV-2 or from a pathogen that resulted in the pre-2020 ME/CFS outbreaks and sporadic cases. Such pathogens did not disappear with the advent of SARS CoV-2, complicating the study of both Long COVID and ME/CFS.

The pathophysiology of most of these post-acute infection syndromes has not been carefully studied. However, research over the past three years has found a remarkable number of similar pathophysiological abnormalities in ME/CFS and Long COVID[28]. Both ME/CFS and Long COVID are examples of a broader group of syndromes—post-acute infection syndromes—that may share not only similar symptoms but similar pathophysiology.

Pathophysiology

In this brief Introduction, we can cite only a few of the many published studies and will mention only those abnormalities that have been replicated by multiple investigators. In all these studies, ME/CFS cases are compared to matched healthy controls, and sometimes also to people with other chronic diseases characterized by fatigue. Additional information about pathophysiology is available in subsequent chapters of this Report that describe individual webinar topics. A recent review article cites nearly 600 publications reporting abnormalities of the nervous system, immune system, metabolism and cardiovascular system[28]. In the subsections, below, on the Nervous System, Immune System and Circulation, readers are referred to this published review for details. Many references to the literature on pathophysiology are also found in each of the chapters in this report.

Nervous System

Formal psychometric testing has revealed cognitive deficits, primarily in attention and reaction time, that worsen after physical and cognitive exertion and that are not explained by concomitant mood disorders. Reduced cerebral blood flow has been demonstrated by single-photon emission computed tomography (SPECT), magnetic resonance imaging (MRI) and trans-cranial Doppler flow imaging. In addition, reductions in cerebral blood flow have been shown to correlate with symptom severity. Multiple studies have reported abnormalities of several hypothalamic-pituitary axes: adrenal, prolactin, growth hormone and thyroid.

Increased MRI signal in the white matter has been reported by many investigators. Multiple studies have found a reduced pain threshold that is made worse by orthostatic stress. Functional MRI has revealed an impaired response to cognitive, motor, visual and auditory challenges. Several studies, employing PET and magnetic resonance spectroscopy (MRS), have demonstrated widespread activation of glial cells. Impaired connectivity has been found, particularly involving the brainstem.

Finally, autonomic dysfunction has been repeatedly demonstrated, and confirmed in a meta-analysis. Autoantibodies against multiple targets in the central and autonomic nervous system have repeatedly been reported, and sometimes correlated with symptom severity.

Immune System

Multiple phenotypic and functional immunologic abnormalities have been reported. The most robust finding is decreased natural killer cell function. In addition, multiple laboratories have found increased proportions of naïve or activated B cells, as well as activated CD8+ cytotoxic T cells. There is evidence for T cell exhaustion and immune senescence among people with long-term illness.

In addition, many investigators have found increased levels of circulating pro-inflammatory cytokines, particularly in the first three years of illness. Finally, there are multiple reports of different types of autoantibodies, although *in vitro* studies to determine if these autoantibodies have functional agonistic or antagonistic properties often have not been performed.

Metabolism

About 25 years ago, it was proposed that an individual who feels a lack of "energy" might, in fact, have *cellular* deficits in the ability to generate and/or utilize ATP. While appearing simplistic, at first, that hypothesis has gained credibility following publication of many studies demonstrating deficits in generating ATP from oxygen (through oxidative phosphorylation), glucose (through both glycolysis and the tricarboxylic acid cycle), fatty acids and amino acids.

Another feature of ME/CFS seems to be redox imbalance. Increased levels of pro-oxidants and decreased levels of antioxidants have repeatedly been reported, as has evidence of considerable nitrosative stress. Oxidative stress in the brain has been revealed by magnetic resonance spectroscopy. Several reports indicate low levels of a large number of metabolites in the circulation in ME/CFS, as well as some in the central nervous system, which has been interpreted as hypometabolism.

Genomics/Epigenetics

Studies of dizygotic and monozygotic twins discordant for ME/CFS indicate that the heritability of a state of chronic fatigue (not necessarily meeting criteria for ME/CFS) ranges from 40-50%[29,30]. Investigators report finding specific polymorphisms in genes involved in pathways important in the function of the brain[31-33] and immune system[34-36]. There also are multiple reports of distinctive differences in *gene expression,* as determined by gene expression microarrays, microRNA studies and gene methylation studies[37-43]. These reports have studied serum, plasma, extracellular vesicles, cerebrospinal fluid, and muscle.

Chronic Infections

An "infectious-like" syndrome often occurs at the onset of ME/CFS, suggesting that infectious agents trigger and possibly perpetuate the chronic illness. No evidence has emerged of a single, novel infectious agent that causes all cases of the illness. However, infection has been linked to ME/CFS in at least two ways.

Particularly, in apparent epidemics of ME/CFS, enteroviruses have long been suspected; however, with the limited diagnostic technology available at the time, the identities of outbreak agents have not usually been documented[13-19]. More recently, one investigator has reported strong evidence of enteroviral infection in the gut of endemic cases[44].

In ME/CFS, there also is evidence for reactivation of latent, lifelong infection with herpesviruses—particularly Epstein-Barr virus and human herpesvirus 6[45-48]. Reactivation of these neurotropic viruses in the brain would be a plausible cause of the symptoms of the illness, but this possibility has not been carefully studied. Also, reactivation of herpesviruses could be an epiphenomenon, reflecting immune dysregulation but having only indirect connection to and little impact on the illness' symptoms.

Finally, there is evidence of gut microbiota dysbiosis—a low-grade pro-inflammatory state caused by an excess of species producing inflammatory molecules and by a deficit of species producing anti-inflammatory molecules such as butyrate[49,50]. This leads to breaches in the gut-blood barrier, with spillage of bacterial endotoxins and even gut bacteria into the circulation—a stimulus to systemic inflammation[51] which, in turn, is a stimulus to neuroinflammation[52].

Physiology and Less Studied Pathologies

Several specific processes have been suggested as playing a role in the pathophysiology of ME/CFS: mast cell activation syndrome[53-55]; the cell danger response[56]; the itaconate shunt hypothesis; the indolamine-2,3-dioxygenase (IDO) metabolic trap hypothesis[57]; the major tissue trauma hypothesis[58]; an increased number of perineural cysts in the brain[59]; connective tissue damage and disorders, including Ehlers-Danlos syndrome, a disease affecting connective tissue[60,61]; craniocervical instability and other spinal conditions[62]; abnormalities in ion channels[63]; reproductive health conditions, neuroendocrine dysfunction, gastrointestinal dysfunction, and abnormalities in the metabolism of the kynurenine pathway that could affect both serotonin biology and the generation of ATP[57,64]. It also has been suggested that the unrefreshing sleep and cognitive impairment seen in ME/CFS might reflect a failure to adequately "flush" the brain's glymphatic circulation during sleep[65].

Circulation

As summarized in a recent review[28], exercise testing reveals diminished exercise capacity and ventilatory efficiency, impaired oxygen extraction by multiple cell types, reduced venous return, increased oxidative stress, and dysautonomia. In addition, many studies report endothelial dysfunction with associated platelet abnormalities and coagulopathies.

Research Agenda

The substantial increase in knowledge about the underlying pathophysiology of ME/CFS that has been amassed over the past 40 years still leaves important questions to be addressed by future research. These include:

- For those multi-system abnormalities that have been robustly documented by many laboratories:
  - What are the potential causes of each abnormality?
  - How might different abnormalities reinforce each other, bidirectionally, and thereby contribute to a "vicious cycle" that perpetuates the illness?
  - How do the abnormalities lead to the generation of the symptoms of the illness? Is there a "final common pathway" in the brain that, when triggered, leads to the symptoms? Is neuroinflammation the primary trigger? Do some individuals have persistent infections by an inciting pathogen?
- For those potentially important abnormalities that have been identified by only a few studies, do additional attempts at replication confirm them? Do they contribute to progress toward successful clinical trials?
- Is the pathophysiology seen in both ME/CFS and Long COVID also found in other post-acute infection syndromes (e.g., post-mononucleosis and post-Lyme disease syndromes)?
- What are the best research criteria for ME/CFS, and if we lack optimal criteria, what should be done next to complement existing clinical diagnostic criteria?

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REPORTS

Overarching ME/CFS Research Priorities

The overarching themes of the ME/CFS Research Roadmap Report revolve around collaboration, innovation, individual-centered care, and the pursuit of precision medicine in the field of ME/CFS. These themes are interwoven throughout the document, shaping the approach to research, clinical trials, and treatment strategies for individuals with ME/CFS.

Collaboration
A central theme of the report is the importance of collaboration among researchers, clinicians, people with ME/CFS, and advocacy groups in advancing ME/CFS research. The document emphasizes the need for multidisciplinary and interdisciplinary approaches, international cooperation, data sharing when possible, and involvement of people with ME/CFS in clinical trials to drive progress in understanding the complex nature of the disease and developing effective interventions.

A great deal of lived experience by people affected by the disease and clinicians exists in those communities. Individual reports related to the onset and time-course of ME/CFS, various treatments and their efficacies, the progression of the syndrome, along with any remissions or sustained recoveries are extremely useful in guiding hitherto unexplored therapeutic avenues. Although these reports are “anecdotal,” n-of-1 studies have sometimes provided important information that can lead to further study of possible treatments.

Innovation
The report highlights the role of innovation in exploring novel diagnostic markers, therapeutic targets, and research methodologies for ME/CFS. From metabolomics and immune responses to molecular pathways and chronic infections, innovative approaches are essential for unraveling the underlying mechanisms of ME/CFS and identifying personalized treatment options. Innovation in the formulation of new integrative hypotheses should be encouraged, i.e., “connecting the dots” in terms of the inter-systemic linkages that produce the constellation of cardinal symptoms of ME/CFS.

Urgency
Although some types of studies inherently require longer time frames, obtaining and reporting actionable research results as soon as possible should be a priority. Time is life, especially where life-diminishing diseases are concerned. Many participants cited the decades lost to this disabling disease.

Biobanks
Lack of access to appropriate samples for studying ME/CFS was often described as a limitation for research studies. Biobanks that can be accessed by researchers who are unable to acquire samples themselves could expand the number of scientists studying the disease. In particular, it was noted that most studies have been done with easily obtained biofluids such as blood, saliva, or urine, while other types of biological samples are necessary to pursue many of the research priorities. These include

tissues obtained by neurosurgery, muscle and nerve biopsies, gastrointestinal and reproductive biopsies, and cerebrospinal fluids. Furthermore, acquisition of samples from severely ill people with ME/CFS is particularly difficult and often requires home visits. Banking such samples could expand the participation of those whose illness would otherwise prevent participation in studies.

Individual-Centered Care

Involvement of people affected by ME/CFS is a recurring theme throughout the report, emphasizing the importance of engaging these individuals in research, clinical trials, and treatment decision-making. By prioritizing PWLE ME/CFS perspectives, needs, and experiences, the report advocates for an individual-centered approach to care that fosters empowerment, inclusivity, and improved outcomes for those living with ME/CFS.

Precision Medicine

The pursuit of precision medicine is a key theme in the report, highlighting the importance of tailoring interventions to the individual people with ME/CFS given the biological diversity and clinical heterogeneity of the illness. The report advocates for a targeted and individualized approach to managing ME/CFS. Standardized measures of illness combined with biomarkers to subgroup individuals will be important to improve clinical outcomes.

Clinical Trials

There is urgent need for effective ME/CFS treatments. Expert clinicians who have treated individuals with ME/CFS for decades provide care based on clinical experience rather than robust scientific evidence specific for the disease. As these clinicians age out of the profession, much expertise will be lost, and little guidance is available to those who will take over responsibility for the large population of people with ME/CFS. All who contributed to this ambitious project emphasize that a top research priority should be to move more rapidly toward validated effective treatments.

Clinical trials can also facilitate research into basic mechanisms of ME/CFS pathophysiology. By comparing subjects before an intervention and afterward, and examining characteristics of responders vs. non-responders, valuable insights may be made into the disease. Furthermore, by comparing the same individual over time as their condition changes (or does not), insights can be gained that would elude detection when a case cohort is compared to a control cohort.

Successful clinical trials rely on good study design, and a critical first step is inclusion of correctly diagnosed subjects and having tools to reliably measure outcomes. In addition to low diagnosis rates, two important barriers to treatment advances in ME/CFS historically have been lack of objective markers and inadequate scientific understanding of underlying pathophysiology driving the symptoms, impairment, and post-exertional malaise. We emphasize that standardized and validated tools for collecting individual-reported data exist that can be utilized now. The report summarizes considerable scientific progress since 2015 and presents recommendations to help overcome these remaining barriers so that more effective treatments can be developed.

A highlight of the report are the “less studied” pathologies, which open the door to additional treatment approaches, and provide insights about features of ME/CFS shared with comorbid conditions.

Common clinical trial themes emerged throughout the project: 1) We should promptly identify known drugs that can be repurposed to target aspects of known pathophysiology based on emerging science. 2) Larger and higher quality clinical trials are needed. 3) Establishing clinical trial networks or expanding existing networks such as NeuroNEXT will facilitate more efficient completion of treatment trials. 4) There are promising objective markers that can be used *now*, or further developed, to complement individua reported outcomes. 5) Researchers are encouraged to access guidance from people with Lived Experience and from experienced ME/CFS clinicians. Scientists proposing clinical trials should be familiar with the U.S. ME/CFS Clinician Coalition published expert consensus recommendations[1] on diagnosis and treatment in 2021. It should be noted that some Lived Experience participants expressed acceptance of higher risk drug trials because of urgent need.

Overall, the report's overarching themes of collaboration, innovation, individual-centered care, and precision medicine underscore a collective commitment to advancing ME/CFS research, improving diagnostic strategies, and enhancing the quality of life for individuals affected by this challenging condition. By embracing these themes and working together towards a common goal, the ME/CFS research community can make significant strides in unraveling the complexities of ME/CFS and ultimately improving outcomes for individuals with the disease worldwide.

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Chronic Infection

Introduction

Three types of chronic infections are relevant to ME/CFS: 1) a new primary infection in which the infectious agent (or its nucleic acids and/or antigens) persist in the body, eliciting an ongoing immune and metabolic response; 2) reactivation of latent infections in which the primary infection occurred long before; 3) inheritance of viral genomes, present in every cell, of which the best known examples are the endogenous retroviruses. Many individuals with ME/CFS indicate their illness onset immediately followed a flu-like illness, a "flu that never went away." [1]. Such a clinical scenario suggests the first type of chronic infection, but it also might occur with the other two.

With most sporadic cases of ME/CFS that began with a flu-like illness, doctors typically have not attempted to diagnose that initial "flu", since it was assumed to be just a self-limited illness. Thus, information critical in identifying the infectious etiology is lacking. Finding the responsible infectious agent in ME/CFS outbreaks, most of which occurred in the 1980s or before, was hampered by a different problem: the technology to detect the pathogen that was causing the outbreak lacked sensitivity compared to technology available today. Thus, although it is reasonable to assume that each outbreak must have been caused by a single pathogen, most likely a virus, agents were not conclusively linked to most of the epidemics.

Another problem in identifying the triggers of ME/CFS is that numerous viruses, including SARS-CoV-2[2], frequently result in asymptomatic cases, obscuring the presence of a past and possibly still present infection. For example, 70% of individuals infected with poliovirus exhibit no symptoms, 25% have mild illness, and less than 1% become paralyzed[3].

The best-known example of latent infections that can produce immune responses, and symptoms, during reactivation are the herpesviruses. In developed countries, people usually become infected with herpesviruses in childhood, adolescence or early adulthood[4,5]. As for endogenous retroviruses, all humans carry ancient retroviral sequences integrated into their genomes. Some of these endogenous retroviruses (ERVs) are expressed, can regulate expression of other genes, and are transcribed following treatment with epigenetic modifier drugs[6] or after infection with exogenous viruses[7,8].

Another controversy about ME/CFS is whether the symptoms of the illness are caused by the direct cytopathic effect of the infectious agent, or whether instead ME/CFS represents an unusual immune and metabolic response to infection. The purpose of this webinar was to consider the evidence for, and research needed concerning the possibility of these three different types of infection. The view that the host response to an infectious agent could result in ongoing immune and metabolic abnormalities will be covered in the respective immunology and metabolism webinars.

The fact that Long COVID, an illness with many symptoms similar to ME/CFS, arose following SARS-CoV-2 infection, provides a new example of an infection-associated chronic illness. At the Chronic Infection webinar, Michael Peluso (U. Cal San Francisco) provided current evidence for long-term infection with SARS-CoV-2 following COVID-19[9,10]. Maureen Hanson (Cornell) described historical information about

past ME/CFS outbreaks and the evidence for chronic infection with enteroviruses. Anthony Komaroff (Harvard Medical School) gave an overview of the substantial literature about the role of herpesviruses in ME/CFS. Finally, Simon Carding (Quadram Institute, UK) explained what is known about endogenous retroviruses and their possible involvement in disease.

Background and Summary of Webinar and Closed Session

RNA Viruses

In cases of ME/CFS prior to 2020, particularly in multiple epidemics of an ME/CFS-like illness, enteroviruses were often the suspected inciting agents[11-14]. A wide variety of RNA viruses can persist following an acute infection[15]. While a thorough screen for viruses in pre-2020 ME/CFS using blood, saliva, and feces was negative, the study is limited by the sensitivity of virus detection and the absence of tissue/organ samples[16]. Hence, to truly determine whether all or a subset of people with ME/CFS have a chronic RNA virus infection, tissue samples must be acquired, banked, and made available for analysis[14]. Another possible strategy to identify viruses that incite or persist in people with ME/CFS are global examinations of antibody reactivity to virus antigens, using such methods as protein or peptide arrays[17] or bacteriophage display[18]. However, a major difficulty in comparing the presence of antibody reactivity in ME/CFS cases vs controls is the fact that some viruses implicated in ME/CFS, especially enteroviruses and herpesviruses, frequently infect much of the population with no obvious sequelae. Thus, detection of unusual variant of a common virus that might cause ME/CFS in a susceptible individual may not be feasible in an ME/CFS cohort because the control population will also have been recently exposed to the same viruses.

Recent findings of SARS-CoV-2 or viral protein persistence in blood as well as in many different tissue types underscore the potential for an inciting virus to remain in locations not readily accessible for analysis[9,10,19-23]. Whether chronic SARS-CoV-2 infection is behind all cases of Long COVID that fulfill ME/CFS diagnostic criteria is currently unknown. However, there is an intriguing report that three individuals with Long COVID recovered rapidly after infusion of a monoclonal antibody directed at the SARS- CoV-2 variant that had infected them[24]. The fact that their ME/CFS-like symptoms were most likely caused by continued presence of the inciting virus provides strong support for research to identify the viruses that initiate ME/CFS and to develop drugs to combat them. A few drugs are known that affect replication of most RNA viruses (e.g., remdesivir) and thus could be used in trials where an RNA virus is suspected to be present. However, antiviral nucleotide analogues require active replication to be effective, and persistent RNA viruses may exhibit a very low replication rate[25].

With the observation that acute infection with enteroviruses often have serious consequences, including acute flaccid myelitis, various efforts to develop anti-enteroviral drugs are underway. When effective drugs are developed or existing drugs are repurposed for antiviral treatments, trials could investigate responses of individuals who had ME/CFS before 2020. Because the identity of the inciting virus was not determined, individuals who became ill in the numerous outbreaks of the mid-1980s[14,26] have never received anti-viral treatments analogous to those now being tested in Long COVID, such as Paxlovid, a drug that is not likely to affect viruses other than coronaviruses.

Many similarities in both symptoms and underlying pathophysiology in Long COVID and ME/CFS have been pointed out[27], and individuals with Long COVID who fulfill some ME/CFS diagnostic criteria are now being told they have ME/CFS, though there is a nomenclature issue. To prevent confusion, referring to "COVID-19-related ME/CFS" or "post-2020 ME/CFS" would be preferable to recognize that SARS-CoV-2 could not have incited cases that arose prior to 2020. While several comparisons of the two illnesses have been performed, there has been little attention to the possibility that a persistent virus could be a common feature of both illnesses. Treatment of individuals with pre-2020 ME/CFS with agents designed to specifically eliminate SARS-CoV-2 are unlikely to be beneficial, which is an important reason not to conflate the illnesses despite symptom similarities. While several similarities in molecular and biochemical abnormalities in Long COVID and pre-2020 ME/CFS have been detected[27], further comparisons between Long COVID and pre-2020 ME/CFS are critical to determine whether they can be distinguished despite many common symptoms. Currently, if someone's illness did not start immediately following a diagnosed case of COVID-19, it is not possible to know whether it was SARS-CoV-2 or some other agent that incited an illness fulfilling 2015 IOM Committee criteria.

Herpesviruses

A subset of people with ME/CFS (about 10%) report that the onset of their illness followed mononucleosis[28], caused by EBV, one of the nine types of human herpesviruses (HHVs). The precise role of HHVs in ME/CFS is not understood and requires substantial additional study. An issue is that normal recovery from mononucleosis can require a lengthy period[29,30], during which other types of infections or stresses could occur that are required for the development of ME/CFS. In that case, mononucleosis would be a predisposing condition, but not sufficient for development of ME/CFS. Furthermore, EBV is often reactivated following a variety of infections, psychological stress, and physical trauma[31], and EBV reactivation might sometimes be mistaken for an initial infection if tests are not interpreted properly. HHVs are not likely to be the cause of the numerous outbreaks of ME/CFS, as the epidemiology of those events does not fit with an HHV outbreak[29]. HHV6 was observed to be often reactivated in some of the victims of the Incline Village mid-1980s outbreak[32]. EBV and HHV6 reactivation has also been observed following COVID-19 but was not present in all individuals with Long COVID[33,34].

A longitudinal population study of US army personnel resulted in the observation that EBV seroconversion is a strong risk factor and likely to play a causal role in the development of multiple sclerosis (MS) [35], though whether EBV is both necessary and sufficient is not known. That study has sparked important inquiries into possible molecular mimicry with EBV protein sequences that could lead to the autoantibodies of MS[36]. A similar study should be performed to determine whether EBV is required before development of ME/CFS.

Longitudinal studies of ME/CFS following mononucleosis could be carried out to determine whether other infections are acquired as well as EBV in the fraction of individuals who are diagnosed with ME/CFS after an acute case of mononucleosis. One possible study design was used by Jason et al. [37], who collected blood from a large cohort of college students, of whom 5.3% developed mononucleosis, with 23% of them fulfilling ME/CFS diagnostic criteria 6 months later. The same authors showed that the number who still fulfill criteria for ME/CFS at 12 months and 24 months is progressively smaller[38]. Jason et al. also compared blood before and after mononucleosis and found that those who would later

develop ME/CFS exhibited differences in metabolite[39] and cytokine profiles[40] prior to their illness. Indeed, this study also revealed that individuals who had gastrointestinal (GI) symptoms before onset of mononucleosis and worse GI symptoms at onset were more likely to develop ME/CFS[40]. The preceding GI symptoms were unlikely to be due to EBV since they occurred before mononucleosis onset, but whether another virus may have been responsible is unknown. Serological studies of blood before and after mononucleosis leading to ME/CFS could provide some insights but would need to be interpreted with caution. Enteroviruses, for example, often cause asymptomatic infections and their frequent occurrence means that most individuals will exhibit antibodies from prior infections.

Initial infection with HHV6B causes roseola in children: by adulthood, ~95% of individuals are seropositive for both HHV6A and B[4]. However, as many as 1-2% of humans have inherited chromosomally integrated HHV-6[41]. An unanswered question is whether these individuals have an increased chance of developing ME/CFS, or whether HHV6 reactivation might not only predispose, but also perpetuate the illness in individuals, like those in Incline Village, for whom the inciting virus was neither EBV nor HHV6.

Another role that EBV or other HHVs might play in ME/CFS could be to prevent recovery, even when the inciting pathogen is not a herpesvirus. For example, the EBV protein deoxyuridine triphosphate nucleotidohydrolase (dUTPase) is thought to be neuroinflammatory and thus potentially involved in the neurological[42] or immunological[43] complications of ME/CFS. The role that EBV reactivation may play in Long COVID is also not yet clear[19]. Some published studies[44-46] as well as anecdotal reports suggest that certain individuals experienced significant improvement following anti-herpes viral drug treatments.

Human Endogenous Retroviruses (HERVs)

Endogenous retroviruses (ERVs) in the human genome resulted from integration of viral genes into the germ line of an infected host. These ERVs now form 5-8% of the human genome. ERVs are transcribed, often in a tissue-specific manner, and can influence global transcription and are critical to mammalian physiology. HERVs are named according to the particular tRNA that triggers reverse transcription; for example, HERV-K uses tRNA-lysine and HERV-W uses a tryptophan tRNA. HERV genes have remnants of their viral origins, with long terminal repeats (LTRs) and some have sequences homologous to capsid, polymerase, and envelope genes of the original exogenous retroviruses. Most ERVs are simply the LTRs, which can act as enhancers and promoters of human genes, depending on where they are inserted[47]. HERV expression is activated by environmental/lifestyle factors, and aging. In addition to forming some transcripts that are harmful, ERVs also have the potential to disrupt expression of human genes through insertional mutagenesis.

One possible way HERVs might be involved in ME/CFS is through expression of superantigens encoded by some HERVs. Superantigens bind non-specifically to MHC molecules and result in activation of B and T cells, thus also promoting cytokine release. For example, HERV-K18.1 is known to be activated by EBV[48]; thus, if EBV is activated or reactivated in ME/CFS, then harmful polyclonal T cells could play a role in pathophysiology. Not only EBV, but several other viruses are known to activate HERVs, including other herpesviruses such as HSV1 and HHV6[49], as well as influenza A[50], HIV[51], and Coxsackie B enterovirus[7]. The presence of epigenetic alterations is a hypothesis for altered gene expression in ME/CFS, and drugs that alter epigenetic marks are known to activate HERVs[6].

Expression of HERVs is also thought to be associated with several different autoimmune diseases[52]. For example, HERV-W is suspected as a factor in type-1 diabetes[53]. Expression of the HERV-W family is upregulated in PBMCs and brain tissue of individuals with MS[54] and may also factor into amyotrophic lateral sclerosis[55,56]. Autoimmune diseases are associated with detection of retroelement cDNA by the innate immune system[57]. Because of these disease associations, drugs that might prevent harmful HERV expression are under investigation[56]. The fact that autoimmunity is one hypothesis for the pathophysiology of ME/CFS makes it evident that there needs to be further inquiry into the possible role of HERVs.

So far, few studies have examined HERVs in ME/CFS. One study compared expression of the *env* gene of HERV K-18 in individuals with ME/CFS vs controls and did not detect any difference[49]. A subsequent study examined HERV K-18 expression more broadly with generic HERV-K primers and did detect greater transactivation in ME/CFS cases vs controls. When HERV-W genes were also globally examined, no difference in expression levels was detected between cases and controls[58]. Another report found that anti-HERV antibodies reacted to sections of gut biopsies in 8 of 12 individuals with ME/CFS but not in 8 controls[59]. In comparison of HERV-W envelope protein in plasma, no difference was found between 4 controls and people with pre-2020 ME/CFS, but elevated levels were seen in 12 individuals with Long COVID relative to controls and pre-2020 ME/CFS cases[60]. When total RNA of PBMCs was examined by qRT-PCR in 14 fibromyalgia cases, of which 7 also fulfilled ME/CFS criteria, elevated levels of several HERV classes were detected relative to 14 controls[61].

Conclusions

The possibility of persistent or reactivated chronic infections or transactivation of endogenous retroviruses is relevant to other webinar topics. What appear to be abnormalities in the immune system, in physiology, and in immunometabolism in people with ME/CFS could be natural responses to chronic or reactivated infections. All the following observations reported in ME/CFS are consistent with a response to a foreign antigen: altered immune cell metabolism, presence of inhibitor receptors on T cells characteristic of T cell exhaustion, expression of transcription factors that lead to T cell exhaustion, activation of classical monocytes for migration into tissue, inability of immune cells to respond to stimulation, and reduced cytotoxicity of natural killer cells. Indeed, one of the conclusions of the recently published intensive NIH intramural study was that a persistent antigen may be present in ME/CFS cases[62]. Likewise, the itaconate shunt could be operating as a result of an infection. Upregulation of expression of endogenous retroviruses could also be a factor in the elevated expression of cytokines and autoantibodies detected in some ME/CFS studies. Especially given the evidence of persistence of SARS-CoV-2 in at least some individuals with Long COVID, a syndrome with symptomatic overlap with ME/CFS, it will be essential to examine the viral origin(s) and the possible role that occult chronic infection or reactivated herpesviruses, or endogenous retroviruses may play in the pathophysiology of pre-2020 ME/CFS.

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Immune System

Introduction

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a multifaceted chronic disease characterized by extreme fatigue, post-exertional malaise (PEM), and a range of other symptoms. The exact cause of ME/CFS remains elusive, but the immune system is increasingly recognized as a key player in its pathology.

Background

The immune system's involvement in ME/CFS is suggested by several immunological abnormalities observed in individuals with ME/CFS:

Chronic Low-Grade Inflammation

Extensive research suggests altered cytokine levels and chronic inflammation in comparison to healthy controls may play a role in ME/CFS pathogenesis[1-8]. Montoya et al. [6] identified a cytokine signature associated with disease severity in people with ME/CFS, highlighting the strong immune system component of the disease. Furthermore, cytokine-cytokine correlations in plasma revealed a significant higher number of interactions in ME/CFS cases along with 13 inverse correlations that were mainly driven by the Interferon gamma-induced protein 10 (IP-10) [9]. However, systematic review found that cytokines' role as reliable biomarkers in ME/CFS remains inconclusive[10]. Further research is needed to elucidate the precise role of cytokines in ME/CFS and their potential as biomarkers or therapeutic targets. Establishing the causal relationships between cytokine levels, chronic inflammation, and the clinical manifestation of the disease is crucial.

Molecular Mimicry/Autoimmune Connections

Some pathogens can induce an immune response that cross-reacts with host tissues, leading to autoimmunity. There is some evidence linking autoantibodies and chronic symptoms in some people with ME/CFS[11-15]. However, this is not uniform across groups[12]. Further investigation is required to understand the specific link between autoimmunity and ME/CFS and the underlying mechanisms involved.

Immune Cell Dysfunction

Research indicates abnormalities in various immune cells, including natural killer (NK) cells, T cells, and B cells. Reduced NK cell activity and altered expression of NK cell activation markers have been extensively documented and correlated with the severity of ME/CFS[16-18]. Additionally, NK cells from people with ME/CFS demonstrate impaired calcium mobilization associated with transient receptor potential melastatin 3 (TRPM3) ion channels[17-19].

Studies have shown that T cell dysfunction is a prominent feature in ME/CFS, with alterations in various T cell subsets[4,20-22]. Abnormal distribution and dysfunction of CD4+ and CD8+ T cells have been implicated in the pathophysiology of ME/CFS [22,23]. Abnormalities in B cell populations have also been reported, with higher prevalence of naïve B cells in ME/CFS and fewer plasmablasts or switched

memory B cells[24,25]. These findings underscore the complex interplay of immune cell subsets in ME/CFS and the need for comprehensive research to understand the underlying immunological mechanisms.

Infection-associated Onset

Onset of ME/CFS following an infection could be the trigger for immune dysregulation. Several studies have explored the link between infectious triggers and the subsequent development of ME/CFS. For example, an association between parvovirus B19 infection and the onset of ME/CFS was observed 1-3 years post-infection[26]. There is considerable evidence for the involvement of enteroviruses in ME/CFS onset[27,28]. Some studies have characterized post-infectious ME/CFS as an inflammatory disorder triggered by an infectious pathogen, leading to an abnormal systemic immune response that persists beyond the clearance of the infection[29]. Others have indicated that ME/CFS onset involves a decisive infectious trigger that could lead to immunization against autoantigens involved in aerobic energy production and hormone receptors, resulting in post-exertional malaise and ME/CFS.

Not all individuals with ME/CFS have a clear preceding infectious episode. One explanation could be asymptomatic infections that lead to ME/CFS. Some researchers instead hypothesize that the heterogeneity of onset patterns indicates there could be multiple potential triggers other than infections. Indeed, it cannot be ruled out that the cumulative impact of various infections or repetitive infectious episodes may lead to ME/CFS through prolonged immune activation, potential establishment of persistent viral reservoirs, and the induction of epigenetic modifications, ultimately contributing to the observed heterogeneity in onset patterns. This theory underscores the complex interplay between multiple pathogens, host susceptibility factors, and their combined effects on immune and physiological systems in the development of ME/CFS.

Neuroimmune Interactions

The immune system and the nervous system are closely interconnected and may impact neurological symptoms such as pain[30]. This is supported by the fact that chronic immune activation can lead to dysregulation of the neuroendocrine system[31], affecting the release of hormones and neurotransmitters that play a crucial role in neurological and cognitive function, thus possibly contributing to the neurological and cognitive symptoms observed in ME/CFS. The precise mechanisms linking chronic immune activation to neuroendocrine dysfunction need further exploration. Establishing a direct causative relationship between immune activation and specific neurological symptoms is complex and requires more detailed investigation.

Role of Microbiome in Immune Dysregulation

The role of the microbiota in ME/CFS is a topic of growing interest, particularly in relation to its impact on the immune system. Several studies have investigated the potential links between the gut microbiome and ME/CFS, shedding light on the complex interactions between the microbiota and immune function[32-39] Perturbations in T cell subsets in people with ME/CFS suggest potential chronic infections or microbiome dysbiosis[4]. These findings underscore the complex interplay between the microbiome, immune dysfunction, and the development of ME/CFS.

Summary of Webinar and Closed Session Discussion
The objectives of this webinar were to (1) develop a clearer understanding of how the immune system is affected in individuals with ME/CFS; (2) determine the state of the science, current treatment limitations, unmet needs, and gaps in ME/CFS research; and (3) generate recommendations to improve the clinical research landscape of ME/CFS.

Clinical Immunology
Nancy Klimas (Nova Southeastern University) discussed how research in ME/CFS could be advanced through a multi-faceted approach. Key objectives included identifying and characterizing diagnostic immunological markers and mediators to facilitate targeted treatments for ME/CFS. There was an emphasis on increasing the robustness of data from published reports, as per Institute of Medicine recommendations, and on validating findings from smaller cohorts. Participants highlighted the importance of establishing robust biorepositories and data sharing platforms, as well as developing dedicated funding mechanisms for researchers. The role of the ME/CFS Clinician Coalition, supported by private donors, was acknowledged for its efforts to create evidence-based consensus guidelines, to prioritize and implement the clinical trials. The coalition has also been instrumental in addressing treatment prioritization issues, suggesting reliance on expert consensus treatment guidelines. The discussions underscored the critical need for research to reflect diverse demographics, including ethnicity, gender, age, and comorbid conditions, to ensure that findings are representative of the broader community. Lastly, the participants weighed the benefits of smaller, focused studies against larger, comprehensive ones and advocated for the creation of comprehensive biorepositories that might include additional biospecimens such as GI biopsies, tears, and saliva.

Autoimmunity
Carmen Scheibenbogen (Charité Hospital, Berlin) noted the potential of immune absorption studies to offer valuable insights despite being somewhat rudimentary. Participants were also curious about the specificity of these studies. The challenge lies in determining the effect of specific antibodies, which is not discernable with methods that remove all IgG.

Participants stressed the significance of validating and rigorously standardizing assays to detect autoantibodies, and that the presence of autoantibodies does not necessarily mean they are pathogenic; they might bind to an epitope without causing any discernible effect.

Moreover, participants speculated on the effectiveness of immune absorption as a treatment option, but concerns were raised regarding its regular use in clinical practice. Participants suggested exploring treatments that decrease antibody presence, which might be more applicable in the clinical setting.

Participants discussed international research efforts, emphasizing the importance of understanding global studies on ME/CFS. Participants expressed the need for better coordination across research endeavors in various countries, to ensure collective learning and progress. Many researchers are leveraging existing platforms such as clinicaltrials.gov to track ongoing studies; for example, the number of antiviral trials for ME/CFS are much more limited compared to those for Long COVID.

Immune Cell Types

Maureen Hanson (Cornell) emphasized the use of methods for analyzing individual immune cells or individual cell types to pinpoint the immune dysregulation in ME/CFS. Isolation of specific cell types allows identification of characteristics that might be hidden when PBMCs are analyzed together, as observed in a ME/CFS CD4+/CD8+ T cell analysis of cytokine associations and immunometabolism[22,40]. The first study of ME/CFS PBMCs with single-cell RNA sequencing (scRNAseq) has pinpointed alterations in classical monocytes and platelets[23], demonstrating that isolation of these cell types through flow sorting would allow greater depth of RNA sequencing and functional characterization. A promising report about Raman spectroscopy also illustrates the power of single cell analysis[41]. Spatial transcriptomic methods have not yet been applied to tissue in ME/CFS and have great potential to contribute to understanding disruptions in signaling and in tissue-resident immune cells. Unpublished flow cytometry studies, as well as scRNAseq, implicate T cell exhaustion in ME/CFS, which could be further investigated with comprehensive flow and mass cytometric panels and spatial transcriptomics.

Participants delved into the complexities of specific immune responses, particularly in relation to granulocytes, neutrophils, natural killer cells, and monocytes, and their potential as diagnostic markers. They noted that chronic immune activation can coexist with profound immunosuppression, drawing parallels with conditions like HIV infection. The discussion also covered potential therapeutic interventions, including antithrombotic therapy, and emphasized the importance of using biomarkers to guide clinical trials.

Immune Perturbations

In this discussion, Derya Unutmaz (Jackson Lab for Genomic Medicine, CT) focused on identifying and targeting specific metabolic and microbiota disruptions in individuals with ME/CFS to improve diagnosis and treatment. Key strategies include identifying actionable targets, measuring immune responses to interventions, conducting small-scale intervention trials, and developing sensitive biomarkers based on immune and metabolome data for more accurate diagnosis. In the project that was described, two groups of people with ME/CFS were analyzed, those with long-term (>10 years) and short term (<4 years) disease. The gut microbiome of long-term individuals was more similar to controls (with differences in low-abundance species and in heterogeneity). The abnormalities in the short-term cohort could result in potential increases in aberrant translocation of microbial metabolites that could affect host immune and metabolic processes. For example, one of the changes noted in short-term individuals was a reduction in potential immunomodulatory organisms (butyrate and tryptophan producers, e.g., *F. prausnitzii*), which could lead to long-term metabolic dysbiosis[38]. Thus, ME/CFS progression may begin with loss of beneficial microbes, particularly Short Chain Fatty Acid (SCFA) producers, resulting in downstream GI effects that are later reflected in plasma metabolite levels. This, in turn, may lead to more established metabolic and phenotypic changes in long-term ME/CFS individuals. Data linking metabolism to microbiome and then to immune system was also presented. Utilizing artificial intelligence (AI) the investigators were able to link microbiome and metabolomics data sets to clinical scores[38]. This approach may reveal actionable targets for therapeutic approaches to ME/CFS. AI-based approaches may also address the heterogeneity and complexity of the disease and identify better biomarkers for diagnostics.

For future directions, emphasis was placed on the need to understand the biological diversity among individuals with ME/CFS by designing studies on specific cohorts. Participants also highlighted the importance of generating ontology-based personalized treatments, acknowledging the diverse nature of the immune system, as evidenced by the unique immune profiles even in identical twins. They stressed the need for a robust set of biomarkers for clinical trials, noting the significance of systems biology in identifying changes in metabolites, microbiomes, and immune parameters. The discussion underlined the necessity of personalizing this information and the importance of designing trials that allow for the collection of pre- and post-intervention samples to better understand individual responses to treatment.

Gut-immune-metabolic Interplay

Armin Alaedini (Columbia) reviewed the growing evidence that ME/CFS is associated with immunologic and metabolic alterations [2,6,22,42,43]. Additionally, gastrointestinal symptoms of unknown etiology have been found to be common in individuals with ME/CFS [5,44,45]. However, the relationship between the immunologic, metabolic, and gastrointestinal abnormalities, and their relevance to core symptoms of ME/CFS, are poorly understood.

Recent studies have focused on some key areas: 1) Biomarkers: assessing the levels of specific biological markers indicative of intestinal damage and subsequent microbial translocation, which may trigger both systemic and localized immune activation, including neuroinflammation, in ME/CFS; 2) Impact of Exercise: investigating how exercise may affect the immune response in ME/CFS to shed light on the interplay between physical activity and immune function. 3) Metabolic Variances and Molecular Pathways: exploring metabolic differences in response to exercise, aiming to elucidate molecular pathways that offer deeper insights into the observed immune responses, particularly within the context of a compromised gut epithelium and microbial translocation. Relying on data from different cohorts of ME/CFS study participants, recent findings point to a state of downregulated innate immune response and upregulated antibody response in conjunction with increased intestinal damage in ME/CFS[46]. Additional data suggest that the observed immunologic dysfunction in ME/CFS may be mediated by alterations in glucose and citrate metabolism and an IL-10 immunoregulatory response. The findings provide insights into mechanistic pathways, biomarkers, and potential therapeutic targets, including in the context of exertion, with relevance to both intestinal and extra-intestinal symptoms in ME/CFS.

Recommendations for ME/CFS Clinical Trials

The participants emphasized the significant benefits of involving people with lived experience more actively in clinical trials, both as participants and in leadership roles, such as on advisory boards or chairing integration panels. This approach enhances trial design, aids in recruitment, and improves communication with the broader community. The concept of "citizen scientists," individuals with both lived experience and scientific expertise, was highlighted. The discussion also covered strategies for conducting effective clinical trials, including starting with smaller, exploratory, or Phase 2 trials before advancing to larger, randomized Phase 3 trials. NeuroNEXT was mentioned as a model for conducting studies on neurological diseases, collaborating with academia, private foundations, and industry. Questions were raised about the efficacy of stem cell treatments and the need for standardization in

their production. The importance of basic studies, such as those investigating the impact of butyrate-producing bacteria, was highlighted for their potential to inform future research and aid in the development of targeted treatments.

Conclusions

A comprehensive overview of the multifaceted approaches to ME/CFS research was performed, emphasizing the importance of identifying diagnostic markers, understanding immune and metabolic dysfunctions, and exploring gut-immune interactions. Key recommendations included more involvement of individuals with ME/CFS in clinical trial planning and leadership roles to enhance trial design and community engagement, with a trend towards greater involvement of individuals with ME/CFS in research. The discussions highlighted the need for robust biomarkers, the exploration of autoantibodies, and the understanding of immune cell functioning. The potential of precision therapies and ontology-based personalized treatments was underscored, considering the biological diversity among individuals with ME/CFS. Concerns about current diagnostic techniques, such as measuring neuroinflammation, and the efficacy of treatments like stem cells were discussed. The importance of small, exploratory trials as a precursor to larger studies was noted, alongside the need for international collaboration and better coordination in research. Finally, the working group emphasized the necessity of basic mechanistic studies to guide future research and the development of targeted treatments, marking a significant advancement in the roadmap of ME/CFS research.

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Nervous System

Introduction

The Nervous System group explored research priorities in six domains: cognition, dysautonomia, cerebrospinal fluid, neuroimaging, non-restorative sleep, and peripheral nervous system. In addition, needs specific to ME/CFS clinical trials were discussed. The goals of the discussion were to identify ME/CFS questions that need to be answered, list research priorities, prioritize studies that should be conducted immediately, suggest changes to ME/CFS clinical trials, highlight timely avenues for research, and propose paradigm-shifting ideas. Webinar speakers described issues concerning cognition (Gudrun Lange, Pain, and Fatigue Study Center), dysautonomia (Peter Rowe, Johns Hopkins), cerebral spinal fluid (Jonas Berquist, Uppsala University), neuroimaging (Jarred Younger, U. Alabama Birmingham), sleep (Janet Mullington (Harvard Medical School), the peripheral nervous system (Peter Novak, Brigham and Women's Hospital).

Background

Whether directly or indirectly, ME/CFS involves problems with the nervous system. Self-reported problems with concentration and memory are core components of ME/CFS diagnostic criteria, and research identifies objectively diminished performance in sustained attention, reaction time, and several other cognitive domains[1]. Widespread structural and functional abnormalities are found using several neuroimaging modalities[2]. There is no consensus on specific neuroimaging abnormalities that define ME/CFS[3]. The lack of consensus is likely due to the low number of confirmatory studies conducted and/or the presence of considerable disease heterogeneity with important subgroups.

Neuroinflammation has long been suspected in the pathophysiology of ME/CFS. There are several elements (cognitive disruption, fatigue, and altered mood) that ME/CFS shares with multiple sclerosis, long-term sequelae of stroke, Long COVID, traumatic brain injury, and neurodegenerative disorders[4]. Imaging studies also support a role of neuroinflammation in ME/CFS, particularly those using magnetic resonance spectroscopy (MRS) and positron emission tomography (PET) [4-7]. Larger studies, using the newest radioligands and magnetic resonance imaging (MRI) standards are needed.

Findings of ME/CFS abnormalities extend beyond the central nervous system and into the peripheral nervous system. Dysautonomia is of high concern, with several studies supporting irregularities in heart rate variability and orthostatic tolerance[8]. Small fiber neuropathy has been reported in a subgroup of individuals with ME/CFS[9,10].

It is unknown whether brain abnormalities are the cause or consequence of the core ME/CFS pathophysiological mechanism. It is also unknown if central abnormalities precede or follow peripheral nervous system problems. Also, treatments directly targeting the nervous system, such as vagus nerve stimulation and transcranial magnetic stimulation, are largely unexplored in ME/CFS.

Summary of Webinar and Closed Discussion

Cognition

Despite being a frequent complaint of ME/CFS sufferers, cognitive problems are understudied. The need for standardized and validated cognitive tests that reflect the clinical difficulties experienced by those with ME/CFS was stressed. Several specific recommendations for further research were identified.

First, psychometrically validated cognitive batteries specifically for ME/CFS are needed. For cognitive tests to be more widely adopted in the field, they must be easy for researchers to acquire, implement, and interpret. Critical questions are: 1) Are the cognitive tests employed in all ME/CFS studies reliable and valid? 2) Are the tools validated specifically in ME/CFS and do they capture the severity range of ME/CFS experience? 3) Are they able to be used longitudinally to track disease progress?

Second, some cognitive deficits in ME/CFS, such as delayed memory recognition, may become apparent only after a stress provocation. There are three implications of delayed cognitive deficits: 1) Studies using classic individual with disease/control contrasts without a stressor may miss important group differences. 2) Longitudinal designs (e.g., good-day/bad-day) should be considered, and these designs must use cognitive tasks validated for repeated use. 3) Standardized provocation tasks need to be validated and widely adopted. There is work undergoing to provide a passive exercise provocation approach that can be easily implemented by research teams.

Third, there is a critical need for remote cognitive tests. Internet-based cognitive tests would significantly increase the number and diversity of individuals with ME/CFS able to contribute to research. Home-based cognitive tests would also substantially decrease the burden for all participants. It is also likely that home-based tests more truly represent typical daily functioning. This is because the effort of getting to a laboratory can be taxing and even lead to a Post-Exertional Malaise (PEM) flare, affecting the cognitive battery scores.

Dysautonomia

There was consensus that more dysautonomia research is needed. While there are important connections between ME/CFS and dysautonomia, the causality of that relationship is still unclear. It is unknown, for example, whether dysautonomia precedes or follows ME/CFS, and if both conditions are caused by a third factor, such as mast cell activation syndrome, hypermobility syndrome, or a pathogen. A greater understanding of the sequence of events will allow more effective targeting for optimal treatment.

One reason for low productivity in this area is that common research paradigms and tools are accessible only to a few experts in the field, often involving medical doctors and special equipment such as the tilt table. It was therefore stressed that valid measures of orthostatic intolerance and autonomic function be created that can be used by the general research community. The simpler NASA lean test has identified some ME/CFS cases with orthostatic intolerance[11].

It was noted that most research on dysautonomia issues in ME/CFS has focused on POTS, even though the incidence of POTS in individuals with ME/CFS varies widely in studies. Other forms of orthostatic intolerances should be investigated more closely in future research.

It was also pointed out that improving sympathovagal balance may lead to significant improvement in ME/CFS. Therefore, treatments that target circulatory dysfunction, hyperhidrosis, and other autonomic issues need greater attention in ME/CFS.

Cerebrospinal fluid (CSF)

CSF studies are rarely conducted in ME/CFS, but they have unique ability to interrogate the central nervous system (CNS). Neuropeptides and other proteins that may be important in ME/CFS, such as orexin/hypocretin, cannot be assessed with any other method. The peripheral blood supply does not provide a useful proxy for the brain environment in many cases. Neuroimaging scans cannot measure most neurochemicals or pro-inflammatory agents that may be dysregulated in the central nervous system. Analytes of interest that can be estimated through neuroimaging techniques, such as lactate, can also be more accurately quantified through direct CSF sampling. Some critical CNS questions can therefore only be answered by using lumbar punctures. It was noted that CSF studies have classically been performed more outside of the United States.

The discussants agreed that while it is true that there are potential side effects of lumbar puncture (the most common being headaches), modern techniques have substantially reduced those risks. These practices include replacing CSF volume immediately after a draw, using atraumatic noncutting needles, and employing fluoroscopy techniques for accurate needle placement.

Because of the difficulty in acquiring CSF samples for ME/CFS studies, clinicians and researchers using this technique are requested to biobank CSF samples for use by other research groups. CSF samples can be stored by BioSEND.

Neuroimaging

The discussion began by noting that there are very few published papers on ME/CFS neuroimaging, even though those few papers have been influential in the field. Fortunately, several groups have more recently begun this type of work and replicated earlier reports on brain abnormalities in ME/CFS. These include both magnetic resonance imaging (MRI) studies examining important analytes with spectroscopy, and positron emission tomography (PET) studies of microglia-mediated brain inflammation.

A call was made to explore blood-based correlates of neuroimaging findings whenever possible. While most neuroimaging approaches are non-invasive and low burden, the widescale adoption of ME/CFS neuroimaging may be prohibitively expensive. Also, several of the scans employed in ME/CFS studies are experimental and not typically available in hospitals and clinics. While it is possible to export these scans to other locations for clinical use, cheaper and simpler assessment techniques are desirable.

Two additional points were raised during the neuroimaging discussion. One point was that some elements of pathology can be examined only with autopsy studies. Microstructure abnormalities and chemical imbalances can typically not be assessed with noninvasive imaging. Donated brain tissue is maintained by the NIH NeuroBioBank. There are multiple centers throughout the U.S. that can coordinate accepting donations, with good geographic coverage. Registration for the program is managed by the Brain Donor Project , which can also coordinate whole-body donations. At present, the number of brains of ME/CFS cases available in biobanks is quite small.

A second point was raised that the glymphatic system has not been well studied or incorporated into neuroimaging protocols. Because the glymphatic system is important in clearing waste lactate and other chemicals from the brain, impaired function may affect sleep, cognition, and other aspects of ME/CFS.

Sleep

Non-restorative sleep is a hallmark of ME/CFS, though little is known about it. Readily available at-home measures can capture some variables (such as heart rate, breathing rate, and basic electrocardiograph data), but usually exclude critical measures such as EEG. The discussants prioritized possible approaches to improve sleep measures in ME/CFS and close research gaps.

The intriguing possibility was raised that EEG could serve as the foundation of the first ME/CFS biomarker or signature. Several members stressed that earlier attempts to characterize different conditions using EEG should be revisited. A 2011 work[12] showed a very good separation between ME/CFS, healthy control, and depression comparison groups. With large enough samples, and several disease comparison groups, it may be possible to describe a unique ME/CFS signature. A particular avenue of interest is using machine learning and artificial intelligence tools to extract critical signals from EEG data. It is suspected these approaches could raise accuracy even above the 90% rate reported with earlier approaches.

Several other important questions were raised that may be answered with EEG. First, what is the relationship between abnormal sleep and abnormal daytime fatigue? Second, how does brain activity change as ME/CFS severity increases or decreases? Third, does sleep quality precede or follow improvements in ME/CFS? Fourth, how does sleep change during PEM flares? Fifth, how do ME/CFS treatments impact sleep quality?

Two medications (sodium oxybate and histamine H3 receptor antagonist/inverse agonists) were mentioned as potential ME/CFS treatments. These medications are used in the treatment of narcolepsy and may provide benefits to individuals with ME/CFS. Both treatments are too expensive for most individuals to use off-label, and insurance coverage can be hard to obtain. Both treatments need further research (efficacy and safety) before recommending widespread use. Sodium oxybate was not approved by the FDA for fibromyalgia treatment, and preliminary trials in ME/CFS were terminated early by the industry sponsor. It is noted, however, that the drug is FDA-approved for cataplexy in adults with narcolepsy, and excessive daytime sleepiness.

Peripheral Nervous System

An interesting discussion revolved around the true relationship between dysautonomia, small fiber neuropathy, and ME/CFS. Dysautonomia and small fiber neuropathy are contemporarily considered to be two distinct conditions. The former is typically associated with orthostatic intolerance and gastrointestinal issues, while the latter is associated with painful burning sensations. Recent clinical observations suggest that the distinction may not be as clear as originally conceptualized, with perhaps 90% of individuals with dysautonomia showing evidence of small fiber neuropathy. Small fiber neuropathy should be tested even when individuals do not show a circumscribed classic burning sensation.

More research is also needed to understand causal pathways between these conditions. We do not know if dysautonomia or small fiber neuropathy lead to the other, or if both are caused by a third factor. The instigating event may be a toxic exposure, genetic abnormality, viral assault, or autoimmune condition, among many other possibilities. There is a need to better understand the "inflammatory reflex" by which uncontrolled pro-inflammatory activity can influence neural circuits in a way that could cause or exacerbate autonomic issues.

Overarching Suggestions for Clinical Trials

First, the number of ME/CFS trials needs to be drastically increased. There are very few properly powered, randomized controlled trials (RCTs). Some attention is needed to understand why relatively few ME/CFS clinical trial applications are submitted to NIH, and whether there are real or perceived obstacles, or whether there is simply a small number of ME/CFS investigators with appropriate experience in designing and conducting large trials. It was noted that many in the scientific community have the impression that NIH does not fund ME/CFS clinical trials, or only funds trials that incorporate a validated objective physiological mechanism. Researchers should know that NIH invites ME/CFS clinical trials and has open mechanisms to support those studies. Some confusion may be due to NIH using a special emphasis panel for ME/CFS applications, except clinical trials, which are reviewed by a separate review panel and use other funding mechanisms. It was also noted that clear guidance on best practices in ME/CFS clinical trials could widen the pool of interested investigators to young scientists and researchers in other fields, especially given the influx of researchers into the Long COVID field.

Second, the need for more at-home trials was reiterated. While decentralized clinical trials are becoming more popular, there are very few true mobile clinical trials that enable all participation to be done at home. There are a significant number of ME/CFS participants who are homebound or located too far from clinical trial sites to participate. To capture the diversity of ME/CFS participants (including the entire range of ME/CFS severity), mobile clinical trial designs should be better utilized whenever possible.

Third, it was again stressed that the effort required to attend a laboratory site may be sufficient to provoke a PEM state. This disease exacerbation due to study participation can be harmful to the participants and adversely affect study data. Every effort must therefore be made to reduce the study burden, whether conducted at a clinical location or at home.

Several other important points were briefly raised by the discussants. 1) Biomarkers and treatments for other diseases (such as neurodegenerative and rheumatologic disorders) should be investigated to see

if they provide clues of ME/CFS pathophysiology. 2) While fibromyalgia and ME/CFS have overlapping elements, these conditions should be handled separately given the special requirements needed for managing chronic pain. More work is needed to fully understand the relationship between these two disorders. 3) There is a need for common outcomes, data collection procedures, inclusive/exclusion approach, and data storage and sample repositories. Harmonization of procedures allows more direct comparisons to be made between studies and can reduce the time to make important advances. 4) Clinical trials need to be large enough to provide confidence in results, provide generalizable results, and accommodate exploration of subgroups. 5) It is possible that monotherapies are not sufficient to adequately manage the complexity and heterogeneity of ME/CFS. 6) It can sometimes take over a year to meet regulatory requirements and launch a clinical trial. Efforts to streamline processes and fast-track approvals would increase the success of clinical trials. 7) Available resources such as NeuroNEXT (Network for Excellence in Neuroscience Clinical Trials), CDER (Center for Drug Evaluation and Research), and the NINDS ME/CFS CDE (Common Data Elements) initiative should be used.

Conclusions

Current evidence suggests that the nervous system has a critical role in ME/CFS that is poorly specified. In all domains discussed, the need for more research was clearly identified. The relative paucity of studies in these areas may be related to misperceptions about funding mechanisms. For future clinical trials in ME/CFS, particular attention should be given towards reducing participant burden, biobanking of samples, harmonization of procedures, considering subtypes, comparisons to other disease groups, and increasing statistical power. Nervous system studies should specifically leverage neuroimaging scans (MRI, EEG, PET), cognitive batteries, and lumbar punctures, when feasible. Accessible dysautonomia testing, remote cognitive testing sensitive to ME/CFS, and peripheral correlates of neuroimaging results need to be further developed, and their development should be prioritized.

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Circulation

Introduction

The Circulation webinar aimed to (1) develop a clearer understanding of circulation and the vasculature in individuals with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS); (2) determine the state of the science, current treatment limitations, unmet needs, and gaps in ME/CFS research; and (3) generate potential recommendations to improve the clinical research landscape of ME/CFS. What is known about endotheliitis in ME/CFS and Long COVID was explained by Jane Mitchell (Imperial College London) and Resia Pretorius *(*Stellenbosch University, South Africa). Issues surrounding hypovolemia and cerebral blood flow were discussed by Frans Visser and Linda Van Campen (Stichting Cardio Zorg, The Netherlands). Abnormalities in red blood cells were described by Jiandi Wan (U. California, Davis). David Systrom, Jr (Brigham and Women's Hospital, Harvard Medical School) informed the group about vascular dysregulation and its manifestation during exercise. This report summarizes what is known about systemic circulatory abnormalities in ME/CFS and Long COVID, knowledge gaps, and recommendations for future ME/CFS clinical trials.

Background and Summary of Webinar and Closed Session

Endotheliitis

Endotheliitis is an immune-inflammatory response of endothelial cells to infectious pathogen invasion that results in endothelial dysfunction, a central factor in cardiovascular disease. Endothelial cells, which form a thin internal lining throughout the vasculature, play a key role in vasoactivity through three hormonal pathways: (1) eNOS (vasodilator); (2) PGI2 (vasodilator); and (3) ET-1 (vasoconstrictor). ME/CFS and individuals with Long COVID display endotheliitis, including reduced flow-mediated dilation, increased ET-1 release, and altered endothelial biomarkers[1–4].

Individuals with ME/CFS experience inappropriate constriction in some areas of the body and inappropriate dilation in others. In septic shock, the inflammatory form of NO (iNOS) is produced in an unregulated manner and results in decreased vasoconstriction. Individuals with ME/CFS may be overproducing iNOS in areas with decreased constriction and overproducing eNOS in areas experiencing excess constriction.

The cytokine storm induced by SARS-CoV-2 is believed to be a mechanism leading to endothelial dysfunction in individuals with COVID-19, primarily through reducing eNOS release and increasing ET-1 release.

The observed dysregulation that exists in individuals with ME/CFS and Long COVID, where inappropriate vasoconstriction and dilation occur, reflects the complexity of endothelial dysfunction underlying the conditions and warrants additional research to uncover the underlying mechanisms. The full landscape of cytokines and endothelial biomarkers must be established to understand the resulting endothelial dysfunction and stratify individuals with ME/CFS and Long COVID. Research is needed to understand the mechanisms underlying endothelial dysfunction in ME/CFS and their similarity to those

underlying endotheliitis that results from COVID-19. Clinical trials using EndoPAT, the only FDA-approved point-of-care measure of endothelial function, are recommended as an outcome variable[5]. The EndoPAT augmentation index is less variable than the reactive hyperemia index and thus could be a potential outcome measure of endothelial function in a heterogeneous population. In addition, the measurement of plasma endothelin-1 (ET-1), prostacyclin, and metabolites of nitric oxide can be used as treatment targets for clinical trials. Treatment trials can use drugs that increase vasodilation (e.g., arginine, prostacyclin, treprostinil) or that decrease vasoconstriction (e.g., ambrisentan) with EndoPAT measures serving as a clinical outcome. Selective NO inhibitors could be used to further investigate the roles of iNOs and eNOS in ME/CFS.

Thrombotic Endotheliitis and Microclots in Long COVID and Related Conditions Like ME/CFS

Damaged endothelial cells and platelet hyperactivation are key players in Long COVID, and likely ME/CFS[6–9]. As many as 43-45% of individuals with Long COVID fulfill diagnostic criteria for ME/CFS[10–12]. Direct inflammatory molecule binding to fibrinogen, as well as the presence of SARS-CoV-2 spike protein S1, can induce new clot formation and hyperactivation of platelets.[13] These anomalous amyloid deposits, found in both ME/CFS and Long COVID, are especially resistant to fibrinolysis and have been coined "microclots"[6,7]**.** The presence of microclots is not the only focus; their contents, activity, and biochemical characteristics are also important. Platelet behavior and microclots can be characterized according to grading systems. Proteomic plasma analysis indicated microclots were present in acute and Long COVID in higher numbers than individuals with diabetes or healthy controls[6]. This analysis identified highly expressed inflammatory proteins, including α(2)-antiplasmin (α2AP) (which can inhibit fibrinolysis), several fibrinogen chains, and Serum Amyloid A (SAA).[6] 80% of individuals with Long COVID in a study testing anticoagulant treatment reported symptom improvement and no presence of clots following treatment[14].

There is a need to expand novel blood-borne biomarkers (e.g., endothelial debris markers, biofilm presence) that allow researchers to assess microclots across diseases. In addition, a cost-effective and scalable diagnostic test should be developed that can identify microclots and larger-scale studies are needed to confirm the microclot findings in ME/CFS[7] and Long COVID. Additional recommendations include 1) analyzing self-reported survey data from thousands of individuals on symptom improvement for medications that are known to have anti-coagulant and platelet effects; 2) launching a randomized clinical trial with anti-coagulant and platelet drugs alone and in combination vs. placebo, with outcomes to include microclot quantification, validated questionnaires, wearable devices, cardiopulmonary exercise testing, blood flow, e.g., EndoPAT.

Hypovolemia/Blood Volume

Currently, a major, effective first line treatment for orthostatic intolerance is directed at increasing blood volume by increasing salt intake. Blood volume, plasma volume, and red blood cell mass are often decreased in individuals with ME/CFS, and reduced blood volume has been correlated with exercise intolerance[15–19]. The root causes of hypovolemia are heterogeneous (e.g., autonomic nervous system dysfunction, changes to vascular tone or musculature), and treatments will differ based on the underlying mechanism. Measuring blood volume can be informative for diagnostic and therapeutic purposes but is rarely done because of the high temporal and financial burden of current measurement techniques and their accompanying radiation exposure. ME/CFS researchers must normalize blood

volume measurements in any studies of orthostatic intolerance (OI) or hypovolemia[19]. Some individuals with ME/CFS who have OI and decreased exercise capacity exhibit decreased blood and plasma volume when compared to individuals with ME/CFS without those symptoms[20]. More research is needed on the relationship between blood and plasma volume and OI. The link between clinical worsening of OI to hypovolemia is poorly understood. Future recommendations include longitudinal studies to understand the relationship of OI to hypovolemia and interventional trials of PO fluid and electrolyte loading vs. IV crystalloid treatment.

Orthostatic Intolerance: The Role of Cerebral Blood Flow and Circulation

Orthostatic intolerance is required in the IOM Diagnostic Criteria for ME/CFS and has been reported as one of the most impairing aspects of the disease[21–23]**.** OI symptoms differ across individuals, but some signs are consistent. Signs include POTS, orthostatic hypotension, syncope, and blue extremities and limbs[24,25]. Symptoms of OI are caused by a reduction of blood flow to the brain and activation of the stress system. Reduced blood flow can be caused by gravity, reduced blood volume, and decreased O_2 extraction[22,26]. Reduced venous return of blood to the heart in individuals with ME/CFS is seen twice as often as in healthy controls. Reduced O_2 uptake may point to mitochondrial complications[26].

A clear relationship exists between cerebral blood flow and several OI symptoms. Reduction in cerebral blood flow can reduce the individual's pain threshold and increase memory and concentration problems. Cerebral blood flow abnormalities are observed in individuals with ME/CFS when sitting and standing[25]. OI and cerebral blood flow abnormalities can be improved by compression stockings[16]**.** Most individuals with ME/CFS (90 percent) exhibit disturbed cerebral autoregulation[27]. Relationships between reductions in blood volume and reductions in red blood cell (RBC) velocity/microclots are unknown. In addition, relationships between OI-induced hypocapnia and cerebral vasoconstriction are based on small single-center studies[28]. There is also a need to understand the pathogenesis of orthostatic and exercise hyperventilation and hypocapnia. Future recommendations include 1) further explore the correlation between reductions in blood volume and reductions in red blood cell (RBC) velocity/microclots. 2) investigate relationships between OI-induced hypocapnia and cerebral vasoconstriction in well-powered studies of ME/CFS and Long COVID. 3) Randomized clinical trials of calcium channel-blockers, e.g., amlodipine in improving orthostatic-related cerebral vasoconstriction and OI. RCT of mitigators of hyperventilation, e.g., sertraline, HIF-1 blocker.

Impaired Oxygen Sensitivity of Red Blood Cells in ME/CFS

Deoxygenation of normal RBC hemoglobin leads to the export of vasodilator and antiadhesive S-nitrosothiols (SNOs) and adenosine triphosphate (ATP) in parallel with oxygen transport in the respiratory cycle. Together, these mediated responses to shear stress and oxygen offloading promote the efficient flow of blood cells and in turn, optimize oxygen delivery. RBC oxygen sensitivity is impaired in ME/CFS and Long COVID. Whether impaired RBC deformability in ME/CFS contributes to impaired oxygen delivery/utilization to and by end organs such as the CNS and exercising limb skeletal muscle is still unknown. In addition, whether impaired RBC metabolism in response to local hypoxia contributes to systemic vascular dysregulation in ME/CFS is still poorly understood. Future recommendations include 1) developing an ME/CFS diagnostic test using PO_2-mediated RBC capillary velocity, 2) using longitudinal monitoring of RBC capillary velocity to track an individual's response to treatment (e.g., medication, surgical intervention), 3) investigating the effects of circulating cytokines on RBC capillary

flow in ME/CFS, and 4) screening drugs that can improve RBC deformability and sensitivity to PO_2 changes (e.g., salmeterol, xanomeline).

Neurovascular Dysregulation During Exercise

In ME/CFS, there is evidence of systemic vascular dysregulation, including preload failure, that overlaps with POTS and orthostatic hypotension, and peripheral left to right shunting[29–31]. Systemic vascular dysregulation has been linked to a high prevalence of small fiber neuropathy in ME/CFS[30]. A randomized controlled trial assessed pyridostigmine treatment in ME/CFS and observed an increase in peak exercise cardiac output through increased vasoconstriction. This suggests that exercise intolerance is linked to neurovascular dysregulation because pyridostigmine, a neuroactive drug, mitigates vascular dysregulation and decreases symptoms in some individuals with ME/CFS[32]. However, little is known about the contribution of the circulatory dysfunction of dysautonomia, hypovolemia, endotheliitis, microclots, and RBC abnormalities to symptoms in ME/CFS. There is a need to identify circulatory pathophysiologic subsets in ME/CFS to enrich clinical trials and ultimately, direct therapy. Future recommendations include 1) determining the cause of systemic vascular dysregulation in ME/CFS with practical, easily administered testing in the field, 2) with respect to neurovascular dysregulation, determining the utility of POTS- drugs repurposed for the treatment of preload failure and peripheral left to right shunting. A Harvard/Open Medicine Foundation "LIFT" Life Improvement trial will measure three questionnaires, collect blood for omics analyses, and treat individuals with ME/CFS with pyridostigmine and low-dose naltrexone (LDN).

Conclusions

With a focused goal on identifying research priorities concerning circulation issues in ME/CFS, the insights gained in this webinar from individuals with lived experiences, discussions on endotheliitis, microclots, endothelial damage, blood volume abnormalities, and neurovascular dysregulation reflect the complex nature of ME/CFS pathophysiology and the urgency of understanding circulatory disturbances in this condition. The report highlights some of the circulatory challenges faced by individuals with ME/CFS, potential avenues for therapeutic interventions, and the need for methodologies to stratify individuals with ME/CFS to provide effective targeted treatment. The research priorities discussed range from identifying biomarkers and uncovering the pathophysiologic mechanisms of the disease to launching randomized clinical trials of repurposed drugs. Collaborative research efforts hold promise for advancing the diagnostic accuracy of this condition and exploring therapeutic measures to better understand this complex condition and improve the quality of life of individuals with ME/CFS.

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Metabolism

Introduction

There are overarching questions regarding the metabolic factors influencing the onset, susceptibility, and severity of ME/CFS. Can understanding these factors pave the way for innovative treatments or prevention strategies?

Background

Recent advancements in metabolomics offer potential for discovering biomarkers in complex chronic diseases like ME/CFS, which involve disruptions in multiple body systems[1,2]. The term "metabolomics" refers to the study of the metabolites that are produced by the biochemical reactions in living organisms which can be measured in biological fluids, cells, or tissues. Through sophisticated large-scale methodologies, metabolomics provides unique insights into diseases like ME/CFS based on their metabolite signatures. As a complement to other "*omic*" approaches (e.g., genomics and proteomics), metabolomics is closer to the phenotypes influenced by various environmental factors affecting disease pathophysiology. Unlike genomics, which explores inherited human genome changes and gene interactions, metabolomics offers a real-time perspective on current cellular modifications resulting from gene interactions with stressors since disease onset. Various specimens, such as plasma, serum, urine, peripheral blood mononuclear cells (PBMCs) and specific immune cells (e.g., NK cells, T and B lymphocytes), have been analyzed to track metabolite changes in individuals with ME/CFS compared to healthy controls, employing NMR and mass spectrometry as popular analytical methods. Additionally, studies have explored fecal microbiota and metabolites in ME/CFS, considering the gut microbiota's influence on host metabolism, given reported gut issues in ME/CFS.

ME/CFS is associated with disruptions in energy metabolism, amino acid utilization (including branch chain AAs and tryptophan), and disturbances in lipid metabolism[3,4]. Germain et al. conducted three separate studies[5-7] suggesting impairments in lipids, steroids, and redox metabolic pathways. Nagy-Szakal et al. confirmed lipid metabolism abnormalities, highlighting elevated ceramide levels in individuals with ME/CFS with Irritable Bowel Syndrome (IBS) [9]. Their study proposed a predictive model based on fecal metagenomics and plasma metabolomics. Hoel et al. created a metabolic phenotypes map through metabolomics and lipidomics analysis, indicating impaired lipid metabolism in people with ME/CFS[10]. Individuals with ME/CFS were categorized into three metabotypes, revealing potential compensatory metabolic adaptations due to exertion-induced tissue hypoxia. Che et al.'s metabolomics study using plasma samples identified impaired peroxisomal metabolism and tricarboxylic acid (TCA) cycle dysfunction [11]. While these findings correlated with common ME/CFS symptoms, a reproducible list of disease-specific metabolites as a signature or for assessing symptom severity is lacking. Challenges, including variable recruitment, sample types, sizes, and disease heterogeneity, may contribute to this inconsistency. In biomarker discovery, including metabolomics, monitoring molecule/metabolite changes before and after post-exertional malaise (PEM) induction may be crucial. A standard provocation maneuver allows tracking alterations in response to exercise, providing insights into this cardinal symptom and minimizing confounders by using each individual as their own control.

McGregor et al. explored metabolite profiles in individuals with ME/CFS with self-reported PEM scores, linking hypermetabolism, glycolysis, and acetylation deregulation to PEM symptoms[12]. However, limitations included a small sample size, a limited number of identified metabolites, and PEM evaluations relying on self-reported scores over 7 days and 12 months, rather than a standardized PEM-inducing exercise challenge. To address these limitations, Germain et al. conducted a recent, comprehensive metabolomics study. They assessed metabolite changes in plasma samples collected before and after two cardiopulmonary exercise tests (CPETs) with a 24-hour recovery interval, aiming to induce PEM[6]. Significant differences in metabolites and pathways between individuals with ME/CFS and controls during the recovery period were observed, with distinct patterns in females versus males. This provided evidence for disruptions in lipid and energy metabolism in the context of PEM. Several pathways shared disrupted glutamate metabolism, even though plasma glutamate levels did not significantly differ between individuals with ME/CFS and controls. This aligns with glutamate's critical role in various metabolic processes and brain functionality, both impacted in ME/CFS. The same CPET strategy was used to analyze metabolites in urine in a small sample of study participants before exercise and 24 hours later, providing the intriguing finding that exercise affected urine metabolites far more in sedentary controls than in individuals with ME/CFS[13]. As an easily obtained biofluid, additional studies of urine with larger cohort sizes and following an exercise challenge, may be informative.

The most recent Germain et al. study[6] identified dysregulation in butyrate metabolism, consistent with the previously reported lower levels of butyrate-producing bacteria in the gut of individuals with ME/CFS[14]. Recent microbiome research by Guo et al. supports the connection between gut microbiota and the observed metabolic profile in ME/CFS, emphasizing the inverse association of butyrate-producing microbes with fatigue severity. Butyrate, a short-chain fatty acid produced by specific gut microbiota such as *Faecalibacterium prausnitzii* and *Eubacterium rectale*, plays a crucial role in maintaining gut health by meeting energy and immunological needs [15]. Considering these findings, incorporating gut microbiome profiling into metabolomics research in ME/CFS becomes important for a more comprehensive understanding of pathological mechanisms.

Summary of Webinar and Closed Session Discussion

The objectives of the Metabolomics Webinar were to (1) gain a clearer understanding of metabolic impacts on individuals with ME/CFS, (2) assess the current state of science, treatment limitations, research gaps, and unmet needs in ME/CFS, and (3) formulate potential recommendations to enhance the clinical research landscape for ME/CFS.

Metabolism-Immunology Interplay

Shuzhao Li (Jackson Laboratory) emphasized integrating system immunology data with existing ME/CFS cohort data and suggested exploring the mapping of flow cytometry data onto single-cell transcriptomic data. Such data is not abundant; only one single cell RNAseq study has been performed and published in ME/CFS so far[16]. Utilizing biofluid metabolomics for biomarker identification faces challenges, particularly with plasma or serum. Some molecules exhibit variable concentrations, while others (e.g., glucose) are homeostatically regulated. Recognizing the less variable nature of glucose levels in blood compared to muscle tissues, researchers should adopt a kinetic approach to modeling data, as suggested by participants referencing Joshua Rabinowitz's work on isotope tracing in mice[17]. The discussion included the potential use of metabolomics to stratify ME/CFS cohorts into subgroups

and explore endophenotypes for understanding and predicting individual responses. Dynamic metabolomic analyses are crucial for capturing cell functional output in response to stimuli, aligning with concerns about the polypharmacy effect in metabolomic data due to comorbidities and medications in individuals with ME/CFS. Participants recommended investigating medication impact on illness and symptoms. The "Anna Karenina principle" was suggested to assess the metabolic profile similarity between healthy controls and those with ME/CFS, aiding in identifying molecular signatures. The "Anna Karenina principle" is a concept borrowed from Leo Tolstoy's novel "Anna Karenina." In simple terms, it suggests that successful outcomes result from many factors working together harmoniously, but failure can arise from just one factor going wrong.

Consequences of altered metabolism

Jessica Maya (Cornell) highlighted the importance of analyzing individual types of immune cells, given that cell types normally differ in immunometabolism. Also, observing their responses pre- and post-stimulation may uncover their behavior under immune challenges. Altered CD8 T cell metabolism is suggestive of T cell exhaustion [18,19]. Survey data and correlation analyses between individual-reported medication use, symptom severity, and molecular data were considered valuable to identify influences on molecular changes. Single-cell transcriptomics can be used to probe metabolism by comparing expression of genes involved in metabolic pathways[16]. Several participants cautioned against oversimplifying immune cell metabolism, emphasizing the uniqueness of different immune cell metabolic programs and the necessity to consider tissue-specific metabolism. Proposals were made to leverage tissue biopsies for a comprehensive study of diverse cell types in different tissues, acknowledging the differences between immune cells in tissue and the circulatory system. Discussion encompassed the challenges of translating *in vitro* findings to *in vivo* studies, especially when targeting specific immune cells in treatments. The consensus was that while *in vitro* studies provide foundational knowledge, robust *in vivo* applications are considerably more complex. Participants suggested using established *in vivo* models (e.g., mice) from other diseases. Technical challenges in cell-based analyses were also raised, including loss of cell viability in ME/CFS samples compared to controls, necessitating rapid processing and robust sample collection for accurate experimental results.

Single-Cell Raman Technologies

Jiabao Xu (Univ. Glasgow, UK) stressed the significance of single-cell technologies in studying heterogeneous PBMC subpopulations in individuals with ME/CFS, recognizing potential differences within these subpopulations. Raman spectroscopy, a label-free analytical method, offers detailed chemical and structural insights into molecules and their interactions within a sample by measuring variations in scattered light under different conditions. Single-cell Raman spectroscopy (SCRS) provides real-time information about the macromolecules within cells, presenting their actual status through Raman spectra. In an initial pilot study, the '*fingerprint*' of ME/CFS in PBMCs and a cell model lacking mitochondrial DNA was compared to healthy controls. Higher phenylalanine-associated Raman bands in ME/CFS or model cells, compared to healthy ones, led to the suggestion of phenylalanine as a potential diagnostic biomarker for ME/CFS[20, 21]. Analyzing specific subpopulations, particularly CD4 and CD8 T cells, could yield more precise metabolic data. The consensus was on the necessity of combining immune profiling with cellular analysis to uncover ME/CFS mechanisms and potentially develop targeted treatments. Regarding iPSCs generated from PBMCs, participants discussed their potential use in creating muscle cell and neuron models for ME/CFS research. While some favored this approach,

others expressed reservations. Reprogramming PBMCs to generate iPSCs might significantly alter cells, potentially erasing ME/CFS-specific characteristics, including crucial DNA methylation patterns. The absence of a clearly defined genetic component in ME/CFS further complicates iPSC use, unlike in genetic epilepsies with known gene defects. To address these concerns, participants suggested alternative approaches like cellular phenomics, for a more accurate representation of ME/CFS-affected tissues.

Computational Perspectives

Wenzhong Xiao (Harvard Medical School) acknowledged challenges in achieving reproducible metabolic analysis results from blood samples, expressing the hope that overcoming these issues could yield short-term benefits for individuals with ME/CFS. However, there are limited data from crucial areas like muscle tissue or the CNS. Therefore, to obtain more robust and reliable data, there is a need to expand research into other prospective tissues affected by ME/CFS. Additionally, computational sciences and AI have the potential to revolutionize the application of metabolomics in decoding ME/CFS by efficiently processing vast and complex datasets, uncovering intricate metabolic patterns that may be indicative of the disease. The advanced analytical capabilities of AI can identify subtle relationships within metabolomic data, enabling the discovery of precise biomarkers and providing a more comprehensive understanding of the metabolic alterations associated with ME/CFS

Microbial Metabolism

Brent Williams (Columbia) highlighted the potential insights from fecal metabolomics in understanding ME/CFS, referencing ongoing work on fecal transplants for clinical trials led by Simon Carding's team in the UK. Concerns were raised about the limited publication of data from these trials, mainly presented at conferences. Participants urged consideration of geographical variations in the microbiome, cautioning against generalizing European studies to those done in the U.S. due to distinct microbiome profiles and metabolic outputs. *In vivo* models, specifically fecal microbiota transplants into mice, could be used to study ME/CFS phenotypes, metabolic, and immune cell profiling. Participants also discussed a study using ^{13}C-labeled dietary fiber to trace metabolites in feces[22], illustrating the impact of microbiome-derived metabolites on phenotypes. Mention was made of another study on bacterium-deficient microbiomes showing direct influences on physical responses. Participants highlighted clinical and experimental studies in epilepsy, where fecal transplants controlled seizures[23] and induced brain epigenetic changes. Technical aspects of microbiome studies were discussed, comparing the limitations of 16S ribosomal RNA sequencing with more detailed shotgun metagenomics. Emphasis was placed on the critical need for accurate species-level data. Participants also suggested revisiting existing *omics* data with advanced statistical methods for deeper insights.

Metabolic characterization of biofluids

Christopher Armstrong (University of Melbourne, Australia) stressed the importance of selecting appropriate control groups for ME/CFS studies, particularly emphasizing the inclusion of sedentary controls to mitigate the confounding effects of exercise on immune function. They shared recruitment strategies, such as using questionnaires to ensure genuinely sedentary controls, while recognizing challenges in finding perfectly matched controls due to the unique activity limitations of people with ME/CFS. Acknowledging deconditioning as a significant variable, some participants noted that inherent differences may exist in the disease itself, regardless of activity levels. Conducting research requiring

individuals with ME/CFS to stop medications was deemed challenging. The group discussed the value of fasted metabolomics samples to control dietary influences, with differing opinions on the potential benefits of postprandial samples. They considered a standardized glucose tolerance test and highlighted the stability of longitudinal measures when fasting is observed.

Recommendations for ME/CFS Clinical Trials

Participants highlighted the potential of genetic modeling in ME/CFS research, exemplified by initiatives like the Jackson Laboratory's project aiming to create genetic models for mutations in every human gene. While this ambitious project offers new tools, ensuring these models accurately represent the intricate and multifaceted nature of ME/CFS, a condition lacking a single genetic cause, remains a challenge. The success of these models depends on their ability to capture the disease's complexity and provide clinically relevant insights. Challenges related to individuals taking numerous supplements and medications were discussed, emphasizing the potential confounding effects on data and the role they might play in the chronic nature of the illness. Some participants suggested starting clinical trials with a "*clean slate*," although acknowledging the difficulty for individuals discontinuing medications. The logistical and financial challenges of controlling diet in studies were considered. Future experiments in metabolomics or exposomics must be meticulously planned, considering covariance, and ensuring sufficient statistical power for meaningful results.

Conclusions

Metabolic exploration in ME/CFS promises insights for addressing this intricate condition. Research priorities advocate a collaborative, multi-disciplinary approach with a focus on metabolism-immunology intersections. Examining immune cell subsets, testing metabolic drugs, and understanding ME/CFS extracellular impacts are vital aspects. Cutting-edge technologies like single-cell Raman spectroscopy and AI-driven diagnostics show potential. Microbial metabolism emphasizes fecal metabolomics, *in vitro*/*in vivo* models, and interventions hold promise. Clinical trial recommendations stress data repositories and acknowledgment of metabolomic diversity. In conclusion, the outlined priorities offer a metabolic roadmap for ME/CFS, enabling collaboration and strategic approaches to unravel complexities for targeted treatments and improved outcomes.

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Physiology

Introduction

ME/CFS is a chronic disease that affects multiple systems. Symptoms can be quite variable in nature and severity, but there are several that are commonly associated with the disease: post-exertional malaise (PEM), brain fog, non-refreshing sleep, fatigue, orthostatic intolerance, and muscle/joint pain. Mechanisms underlying ME/CFS pathologies are not known, but the nature of the symptoms suggests neurological, immune, and energy systems dysfunctions. The Physiology webinar explored ME/CFS pathologies at the level of cell and systems physiology.

Summary of Webinar and Closed Session

PET Imaging for Whole Body Immune Responses

Because ME/CFS is a multisystem chronic disease, technology enabling imaging of active physiological processes throughout the body would be a significant advance. MRI enables structural imaging and fMRI enables imaging of changes in metabolism, but neither make it possible to image cellular/biochemical processes underlying physiological processes. Michele James (Stanford) described advances in positron emission tomography (PET imaging) using a growing toolbox of molecular/cellular markers that make it possible to locate, quantify, and compare active physiological processes throughout the body. An early version of this methodology used uptake of Fluorodeoxyglucose (FDG) to image metabolic activity in body tissues. Metabolic suppression in the forebrain and the brainstem was observed in individuals with ME/CFS. While this approach is getting closer to functional measurements, it is not specific as to cellular mechanisms. Accordingly, researchers are developing and using marker compounds that label specific cell types; for example, immune cells involved in inflammation or glial cells involved in supporting neuronal processes[1]. These methods, which have been previously applied mostly to regional studies, such as the brain, can be used in whole body scans to image the global distribution of markers related to processes of interest such as neuroimmune activity that may be involved in multisystem components of ME/CFS. The potential value of PET methods for early disease detection, disease staging, and monitoring of therapeutic efficacy was emphasized. Continued development and validation of PET tracers/biomarkers should be high priority research as well as the development of AI/ML methods for analysis of the massive amounts of data that can be obtained by these new imaging approaches. Use of promising PET tracers should be incorporated into phase 1 and 2 clinical trials whenever possible.

The Cell Danger Response (CDR) and ME/CFS

Robert Naviaux (UC San Diego) introduced the CDR concept by summarizing the events of the pathogenetic process/responses triggered by trauma or infection and the subsequent healing process/responses that he terms salugenesis[2,3]. Importantly, both processes are energetically demanding. The CDR involves the release of ATP by the damaged or infected cell to generate a pool of extracellular ATP (eATP) that signals an injury or disease process is occurring and triggers salugenic responses. While these responses are energy demanding, they occur at the same time metabolism is suppressed. The hypothesis is that the suppression of metabolism is at the root of fatigue; normally fatigue, along with inflammatory processes that generate pain, are adaptive responses to facilitate

salugenic processes. Postulating the development of a hypersensitivity to the purinergic signaling by eATP makes the connection of ME/CFS and other chronic illness such as Long COVID[4]. There is a strong need for deeper research on the CDR and purinergic signaling, including the mechanism of hypersensitivity, and possible antipurinergic therapies in chronic diseases that involve fatigue and inflammatory processes.

The Itaconate Shunt and ME/CFS

Robert Phair's (Integrative Informatics, Inc.) central thesis is that the central cause of ME/CFS is the inability of cells to produce ATP. The itaconate shunt is the hypothetical mechanism that converts cells from an energy-producing to an impaired energy-producing condition. Moreover, although ME/CFS is a multisystem disease, it is a mosaic or cell autonomous disease. Not every cell is a sick cell. The symptoms of the disease depend on which cells are sick, and the severity of the disease depends on the proportion of cells that are sick. By comparing whole body metabolic rates and cellular metabolic rates, Phair estimates that in a group of individuals with ME/CFS that have been studied, the proportion of sick cells ranged from 9 to 45%. Research approaches that use sampling of cells must recognize that not all the cells in the sample are sick cells thus introducing variability in the results. The itaconate shunt is a shifting of carbon from the TCA cycle to a pathway that returns carbon to pyruvate and acetyl CoA without going through the TCA cycle. The critical component of this shunt is the enzyme Cis-Aconitate Dehydrogenase (CAD). When that enzyme is expressed in sick cells and imported into the mitochondria, it shifts cis-aconitate from conversion to isocitrate to itaconate instead. Itaconate does not enter the TCA cycle but is converted to itaconyl-CoA that goes through several more reactions to form pyruvate. The proposal is that this futile cycle has the adaptive function of reducing resources from invading pathological organisms. In support of that hypothesis, it is known that interferon alpha can turn on the itaconate shunt. In response to an infection, the innate immune system expresses interferon alpha, which binds to its receptors and activates the JAK-STAT signaling pathway. Among the hundreds of genes turned on by that pathway, ACOG1 codes for CAD, which turns on the itaconate shunt. Another action of the JAK-STAT signaling is increased expression of interferon alpha, thus creating a positive feedback loop. Normally there are signals that turn off that positive feedback loop, thus the hypothesis arises that failure of those mechanisms result in chronic activation of the itaconate shunt. There are several relevant pharmacological approaches that should be investigated. If the hypothesis is correct, strategies could include inhibitors of the JAK-STAT pathways, blocking of the interferon alpha receptor, and blocking of CAD, perhaps by bisphosphonates.

Overlapping Mechanisms in Individuals in the Intensive Care Unit (ICU) and Those with ME/CFS

Dominic Stanculescu has surveyed clinical information on several chronic critical illnesses to search for possible common underlying mechanisms[5-8]. Similarities between such illness and with ME/CFS were found in several areas: 1) in vascular systems – hypoperfusion and endotheliopathy, 2) in the gastrointestinal system, injury to intestinal integrity, 3) in the central endocrine systems, suppression of pulsatile pituitary releases, and 4) in peripheral endocrine system, low thyroid hormone function. In normal recovery energy is shifted to essential organs and repair, whereas in chronic illnesses, the recovery processes are not initiated. High priority should be given to studies of pulsatile pituitary release of ACTH and GH in individuals with ME/CFS, studies of the thyroid function, studies of GI hormones, and elements that perpetuate hypometabolism and inflammation in chronic critical illnesses and in ME/CFS.

Refreshing Sleep

Since a defining characteristic of ME/CFS is non-refreshing sleep, it is important to ask what refreshing sleep is. There is not a clear answer to that question[9]. Rebecca Robbins (Harvard Medical School) recently led a consensus report of leading sleep experts on what is refreshing sleep (Robbins et al. 2021). Gold standards involve in-clinic polysomnographic recordings overnight, which cannot replicate someone's normal sleep environment. As a result, there is growing interest in using wearable devices for recording sleep characteristics in naturalistic environments[10]. One interesting result is that people with insomnia typically have few obvious differences in quantitative measures of sleep, yet they have striking differences in qualitative evaluations of their sleep. The consensus report defines restorative sleep as that which leads to improved subjective alertness, cognitive function, mood, energy and/or will being relative to the immediate pre-sleep period. Applying a survey tool REST-Q to a large, nationally representative sample of US adults they found that 33% had low sleep satisfaction, 39% some satisfaction, and 28% highly satisfied. High research priorities for ME/CFS populations would be to explore the subcomponents of restorative/refreshing sleep such as daytime grogginess, apply the REST-Q survey in individuals with ME/CFS, and explore correlations between sleep satisfaction measures with quality of life and other dimensions of care in individuals with ME/CFS.

Non-refreshing Sleep

Maiken Nedergaard described the recent hypothesis of sleep function – removal of wastes from the brain parenchyma[11]. She explained how cerebrospinal fluid (CSF) moves from the ventricles, where it is produced, to the subarachnoid space surrounding the brain. Where arteries penetrate the brain, CSF flows through the perivascular space between the arterial endothelium and the membranes of the astrocyte that constitute the blood/brain barrier. This movement of CSF is propelled by the pulsations in the arteries. Remarkable imaging results show that this flow of CSF through the extracellular space of the brain occurs almost exclusively during sleep. The mechanism for that exclusivity is reduction in resistance to flow due to shrinkage of cell volumes and expansion of the extracellular space. Current work reveals tight coupling between the slow waves of the EEG, and the occurrence of reciprocal movements of CSF in the parenchyma and the fourth ventricle[12]. Furthermore, the slow waves are coupled to the firing of cells in the locus coeruleus (LC). Since the neurotransmitter of the LC is nor-epinephrine (NE), a controller of vasomotion. The refreshing quality of sleep likely depends on healthy glymphatic flow, and that in turn depends on the robustness of the slow wave activity driven by the firing pattern of the LC neurons[13]. She therefore proposes that critical research to advance these interesting insights into the refreshing function of sleep requires continued work on the glymphatic system and brain waste removal and how that is influenced by NE oscillations and quality of NREM sleep. Normative clinical studies of individual variation in constitutional sleep need and quality of NE oscillations might be informative as well. Future work on individuals with ME/CFS should include electroencephalographic characterizations of their non-rapid eye movement (NREM) sleep and slow wave activity (SWA). Investigations of therapies that have influences on the NE oscillations might be of interest plus their effects on SWA, sigma power (related to cognitive function), and cognitive function as well.

Metabolism and ME/CFS

Karl Tronstad (U. Bergen, Norway) pointed out that ME/CFS is a disease that impairs energy metabolism, yet there is no clear explanation for the energetic effects of the disease. ME/CFS involves dysregulation in the immune system and perhaps resetting or re-adjusting the immune system might prove beneficial to individuals with ME/CFS. Comparisons with normal energetic challenges were made to gain insight into possible causes of the ME/CFS condition of lack of energy and post-exertional malaise (PEM). The normal responses of individuals engaged in exhaustive endurance exercise include inhibition of oxidative phosphorylation and elevation of glycolysis, inhibition of pyruvate dehydrogenase, lowered anaerobic threshold, increased lactate production, and expression of hypoxia inducible factor (HIF) [14]. These are largely responses to exercise also seen in ME/CFS, but they do not resolve rapidly. A possible mechanism was recently described in a study of endoplasmic reticulum stress that was shown to cause the expression and release of a protein WASF3 that disrupts mitochondrial function[15]. Normal muscle cells exposed to serum from people with ME/CFS exhibit signs of energy metabolism stress[16]. To identify possible factors and differences between normal energy phenotypes and individuals with ME/CFS, a large metabolomics study resulted in distinguishing differences between subsets of patents, comprised largely of three metabolic phenotypes[17]. One group resembled patterns seen in endurance athletes and fasting, another group appeared to reflect dyslipidemia, and one group was lesser but overlapping patterns with the other two groups. The energetic state of individuals with ME/CFS reflect normal adaptations to energy stress, but they do not resolve, possibly due to immune system dysfunctions.

ME/CFS and Long COVID: BH4 and Nitric Oxide (NO)

Ronald Davis (Stanford) observed that the drug Abilify was shown to mitigate ME/CFS symptoms in a large percentage of ME/CFS test subjects and that the long-term treatment correlated with increased dopamine levels. The formation of dopamine from phenylalanine and tyrosine depends on the enzyme BH4. In individuals with ME/CFS, the formation of dopamine is low, and the levels of phenylalanine are high, suggesting low BH4 activity. BH4 is also required for citrulline + NO production from arginine, and in individuals with ME/CFS, the arginine/citrulline ratio is high, suggesting low BH4 activity. BH4 is difficult to measure directly, as it is degraded rapidly in blood samples. Fe, Mn, and Cu are low in individuals with ME/CFS, and their uptake by the metal transporter DMT1 requires NO, which in turn depends on BH4.

Several intriguing observations merit further study. Crashes in ME/CFS (temporary exacerbations of symptoms) activate the innate immune system and repeated activations may damage the ability to recover. Another issue to investigate is the overlap or mistakes in distinguishing between MS and ME/CFS. About half of the individuals with ME/CFS studied carry antibodies that break down myelin basic protein[18]. Given that both diseases are suspected to be autoimmune, it would be worthwhile to determine whether there are other commonalities such as effects of infection by EBV or other herpesviruses.

Extracellular Vesicles

Dr. Ludovic Giloteaux reviewed current knowledge of extracellular vesicles (ECVs), which carry signals around the body and are in all body fluids. They contain a wide variety of molecules, including cytokines and RNAs, and have been shown to function in coagulation, suppression of immune cells, and to facilitate angiogenesis, and probably many other phenomena. Studies have comparing ECVs of ME/CFS and healthy individuals and in response to exercise. ECVs have been found to be more abundant in individuals with ME/CFS than controls[19-22]. Analysis of miRNAs in ECVs in ME/CFS suggested dysregulation of neuroimmune pathways[19]. The cytokine content of EVs differed between ME/CFS cases and controls[23]. Cargos of ECVs from both individuals with ME/CFS and controls began to change with onset of exercise and are different at the end. Alterations in the proteome of ECVs due to exercise are correlated with symptom severity[23]. There is much to be learned by further research in this relatively new area that may be involved in many cell-cell signalling relationships as well as immune system controls and cell responses to stressors.

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Less Studied Pathologies

Introduction

ME/CFS has several less studied pathologies that can significantly impact disease severity, clinical presentation, and quality of life. These include connective tissue disorders, spinal and mechanical disorders, mast cell activation disorders, gastrointestinal conditions, neuroendocrine dysfunction, and reproductive health conditions. Notably, the Roadmap webinar was the first event of its kind discussing in-depth some of these topics in ME/CFS, such as connective tissue, spinal, and reproductive conditions, signifying important progress in the field.

Background and Summary of Webinar and Closed Discussion

Connective Tissue Disorders (CTDs)

Connective tissue (CT) is the most abundant and diverse type of tissue in the body[1]. CT binds, supports, and protects organs, and includes vascular endothelium, lymph, fascia, meninges, bone, cartilage, tendons, and ligaments. CT is dynamic and consists of collagen (over 25 different types), proteoglycans, glycoproteins (e.g., fibronectin, laminin), and elastin, meaning that CT abnormalities can impact many parts of the body and many bodily functions. (For example, type IV collagen is an abundant component of the brain/blood barrier.)

CTDs are heterogeneous: There are over 200 types, and they can be hereditary such as Ehlers-Danlos Syndrome (EDS) or acquired such as autoimmune CTDs[1]. Dr. Ilene Ruhoy (Mt. Sinai) discussed how the body is a cohesive network of tissues, the physiology of CT, and collagen abnormalities found in hEDS. CTDs can drive diffuse symptoms depending on the body part affected and can lead to spinal and mechanical conditions.

CT damage and disorders, especially CTDs presenting with joint hypermobility, are an emerging issue in ME/CFS. They have no treatments and urgently require further research. Key questions include: What mechanisms contribute to CTDs? Do subsets of individuals with ME/CFS develop acquired CTDs? If so, what may be the roles of chronic inflammation, immune dysregulation, metabolic and mitochondrial dysfunction, hypoperfusion, and environmental exposures and infection in connective tissue damage? Why might some people be more susceptible to CTDs than others? Do people with pre-existing or hereditary/genetic hypermobility have increased risk for developing ME/CFS? In these individuals, does their hypermobility/CTDs become more symptomatic or more severe after developing ME/CFS?

Beth Pollack (MIT) overviewed the literature on CTDs in ME/CFS and in commonly comorbid illnesses. Studies found that 12%-19% of people with ME/CFS may have hypermobile Ehlers-Danlos Syndrome (hEDS), and 50%-81% of individuals with ME/CFS may be hypermobile.[2–4] This is significantly higher than general population prevalence. It is estimated that only 0.02%-0.2% of the population may have EDS, and 3.4% may have symptomatic hypermobility (joint hypermobility with widespread pain)[5–7].

There is a need to study across a group of illnesses comorbid with ME/CFS to learn more about contributing factors to CTDs and the roles of CTDs in these illnesses. There are high rates of comorbidity between ME/CFS and POTS, fibromyalgia, mast cell activation syndrome (MCAS), Long COVID, and dysautonomia — all of which have elevated rates of hEDS and hypermobility (except for Long COVID, where EHR data and case studies suggest a connection and research is in progress)[3,8–12]. Symptoms of CTDs overlap with all these illnesses and include myalgias, fatigue, GI issues, headache, neurological and gynecological symptoms and more[13–19].

Research establishing biomarkers, therapeutic targets, and treatments is a top priority. CTDs impact the body in many ways beyond joint hypermobility, and there is an acute need for improved detection and diagnosis of different types of CTDs. Additionally, there is need for more comprehensive screening for joint hypermobility that goes beyond the Beighton scale and includes other commonly affected joints, such as in the neck. Screening for CTDs using existing tools is recommended in ME/CFS research and may inform phenotyping research.

Spinal and Mechanical Conditions

The spine is a series of joints, supported by ligaments and connective tissue that stabilizes and runs throughout the spine. Damage to these ligaments and other tissue can cause spinal and other mechanical disorders. Dr. Ruhoy's discussed spinal conditions seen in ME/CFS, hEDS, and POTS, based on literature and experience in her neurosurgical clinic. These conditions include: craniocervical instability (CCI), atlantoaxial instability (AAI), tethered cord syndrome (TCS), chiari malformation, Eagle's syndrome, various nerve, and vascular compression syndromes (e.g., thoracic outlet syndrome, jugular vein compression), spinal stenosis, degenerative disc disease, as well as cerebrospinal fluid (CSF) leaks, and idiopathic intracranial hypertension and hypotension.

CCI and AAI, instabilities of the upper cervical spine, impact the craniocervical junction (CCJ) - a small, highly vascularized, and innervated area serving as a "superhighway" to the brain. CCI can compress the brain stem, cervical nerve roots, basilar artery, jugular vein, as well as restrict blood flow and CSF into and out of the brain. Through compression of the brainstem, vagus nerve and other cranial nerves, it can affect autonomic function, and can cause symptoms including dysphasia, headaches, sleep issues, neuromuscular dysfunction, vertigo, syncope, and pain[20–22]. Many of these symptoms, including those related to dysautonomia, overlap with ME/CFS symptoms.

Pollack's talk covered the prevalence of spinal conditions in the literature of ME/CFS and associated complex chronic illnesses. This included an MRI study of 205 individuals with ME/CFS (but without healthy controls) showing 80% have disc bulging or hernias in the cervical spine; 83% have signs of intracranial hypertension; 64% have obstructions in C1–T2; over half have spinal cord compressions at C5–C6, and 28% at C6–C7.[4] Additionally, 26.5% (n=913) of individuals with ME/CFS have severe spine problems according to an EHR study of ME/CFS clinics[23]. Case studies report overall symptom improvements, including orthostatic symptoms, in individuals with severe ME/CFS post spinal stenosis surgery[24].

TCS is common in EDS: 40% of individuals with EDS (n=223) at EDS clinics have it, and it frequently co-occurs with CCI.[25] TCS, where the spinal cord is attached to spinal tissue, can be congenital or acquired.

Research on mechanisms in acquired TCS is needed. TCS can cause cord stretch injury via torsion on the tethered cord pulling on the brainstem, which can result in symptoms and overlapping pathologies also seen in ME/CFS, such as cerebral hypoperfusion, dysautonomia, and neuroinflammation.[26,22,27] Klinge et al found inflammatory cell invasion (mast cells, leukocytes, microglia) in the filum of individuals undergoing hEDS surgical TCS vs. non-hEDS TCS individuals, suggesting a possible inflammatory basis for TCS in hEDS that requires further research[26]. She also found a key difference in the tissue between hEDS and non-hEDS TCS filum: hEDS filum was half as elastic, increasing risk for cord stretch injury and its related neurological symptoms[26].

A top priority for research is studying mechanisms of spinal disorders and developing therapeutics to prevent and treat them non-invasively. There is also a need for earlier diagnosis for spinal and mechanical issues and increased access to clinical care. Julie Rehmeyer pointed out that thousands of individuals with ME/CFS with spinal issues connect in online groups and frequently report experiencing significant diagnostic delays due to too few neurosurgeons who specialize in these issues relative to the need by individuals with ME/CFS.

Mast Cell Activation Disorders (MCAD)

MCAD, which include MCAS, are characterized by disordered and pathological mast cell activation. MCAS has heterogeneous symptoms that significantly overlap with ME/CFS symptomatology including fatigue, pain, allergies to food and environmental contaminants, and gastrointestinal, dermatological, neurocognitive, and cardiovascular issues.[28] There is circumstantial evidence, but limited research on the prevalence of MCAS and ME/CFS. In a study of 160 individuals with ME/CFS, 7% had a MCAS diagnosis[29]**.**

Mast cells are found throughout the body and tend to cluster at sites that interface with the external environment, and nerves and blood vessels. Mast cells degranulate and can release hundreds of mast mediators, chemicals that perform a variety of functions, and can impact many organ systems. Mast cells in part are immune sentinels that can recruit and activate various immune cells to address various pathogens, impact blood flow, or mediate tissue remodeling. Critically, some mast cell mediators, such as tryptase and histamine, can directly damage collagen[30,31]. They can affect factors impacting collagen synthesis, such as fibroblasts[32]. MCAD and chronic inflammation arise when mast cells chronically degranulate not only to threat, but to a variety of allergens or benign triggers. More research is needed on how MCA may be mechanistically implicated in CTDs and other ME/CFS pathologies. Dr. Anne Maitland (Metrodora Institute and Mt. Sinai) reviewed the pathophysiology of MCAD and how it may intersect with ME/CFS. Studying the role of dysfunctional barrier immunity in triggering MCS is critical, including tissue and epithelial barrier damage.

Gastrointestinal (GI) Conditions

GI dysfunction is common in ME/CFS as well as in overlapping chronic illnesses such POTS, MCAS, fibromyalgia and EDS[33–36]. Individuals with ME/CFS commonly report gastrointestinal symptoms, such as constipation and diarrhea, and 38-98% have irritable bowel syndrome[37]. Research in ME/CFS has found GI pathologies including GI barrier impairment; reduced microbial diversity, and dysbiosis.[38–40] Notably, research has found differences in microbial signatures of people who have had ME/CFS for less

than or more than ten years, and correlations between certain microbes and some ME/CFS symptoms, including fatigue and dysautonomia[40,41].

Dr. Laura Pace (U. Utah) discussed in part how the gastrointestinal tract interfaces with the nervous, immune, and endocrine systems. Microbes in the gut secrete substances that can influence nervous system, endocrine, and immune function, and inflammation[42,43]. She also discussed how intestinal barrier permeability may contribute to systemic inflammation and sustained immune dysfunction[44–46]. Key areas of ME/CFS research include how microbial dysbiosis, intestinal permeability, and dysautonomia may contribute GI symptoms; and conversely, how dysbiosis and intestinal barrier dysfunction may be implicated in immune, endocrine, and neurological dysfunction in ME/CFS.

Neuroendocrine Dysfunction

About 75% of individuals with ME/CFS are biologically female. Premenopausal women have an elevated risk for both ME/CFS and Long COVID, suggesting an important role for sex hormones in disease onset and pathophysiology[19]. The recently published NIH intramural study highlighted sex differences in ME/CFS[47]. There is great need to explore the crosstalk between hormones and ME/CFS symptomatology, and how hormones may impact immune, cardiovascular, metabolic, mitochondrial, and neurological dysfunction in ME/CFS.

As Dr. Roumiana Boneva (CDC) and Dr. Elizabeth Unger (CDC) summarized in their talks, reduced levels of gonadal/sex hormones may contribute to ME/CFS symptoms through several known pathomechanisms: impaired sleep, loss of neuroprotective effects of progesterone and estradiol, increase in pro-inflammatory cytokines, up-regulation of inflammation, possible increased pain perception due to loss of estradiol's and progesterone's effects on anti-nociceptive pathways, and reduction of estrogen- and progesterone-facilitated healing and repair mechanisms[48–53].
Natalie Thomas (U. Melbourne) summarized evidence for alterations in adrenal stress (cortisol), gonadal sex (estrogen, progesterone), thyroid (T3/T4), and renal (aldosterone), and neuroendocrine systems in ME/CFS, all of which have sex differences[54]. Broadly, in ME/CFS, there appears to be a downregulation of steroid hormone levels and an increase of upstream dysregulation of the hypothalamus and pituitary in individuals with ME/CFS, as evidenced by anti-hypothalamus antibodies (AHA) and anti-pituitary antibodies (APA)[54]. Cortisol has many physiological roles including in increasing energy utilization, moderating inflammatory and autonomic responses, and alterations in cortisol levels are important to continue to study in ME/CFS.

Endocrine dysfunction has broad physiological effects and may be implicated in both the heterogeneity and fluctuating symptomatology in ME/CFS. Estradiol deficiency has been found in ME/CFS, and progesterone deficiency has also been found in the luteal phase in premenopausal women with ME/CFS[54]. Both hormones play mediating roles in immune and mitochondrial functions, and neuroprotection — all systems that are dysfunctional in ME/CFS[54]. For example, a study of healthy people, conducted across a period of two menstrual cycles, found that increased in serum estradiol was associated with a decrease in C-reactive protein (CRP), a measure of inflammation, while increased serum luteal progesterone was associated with increased CRP, suggesting that these endogenous hormones may have pro- and anti-inflammatory effects[55].

There are significant knowledge gaps regarding hormones in ME/CFS. Examination of hormones in ME/CFS has often been secondary in nature, such as a part of proteomics research, and is not yet a primary focus of ME/CFS research. Research has largely been cross-sectional and measured one hormone at a time, rather than studying hormonal fluctuations longitudinally and how a network of hormones may interact. The menstrual cycle, sex differences, and diurnal fluctuations in hormones contribute to research complexities and data variability. Research should focus on whether hormonal medications are possible therapeutics for ME/CFS, especially sex hormones because of their ability to modulate immunity and other bodily systems.

Reproductive Health Conditions

Reproductive conditions and reproductive health related symptoms are relatively common in females with ME/CFS. Much of the literature consists of epidemiological or survey-based studies, often with small cohorts. There is urgent need for research on underlying mechanisms and treatments, and how reproductive health conditions and the menstrual cycle may be implicated in ME/CFS pathophysiology and symptomatology. The reproductive impacts of ME/CFS in males has not been studied, however erectile dysfunction and reduced sperm count has been found in Long COVID, suggesting the need for further research in ME/CFS[56].

Females with ME/CFS report increased rates of polycystic ovarian syndrome; uterine fibroids, pelvic pain, gynecological surgery, hysterectomy, endometriosis, and early menopause compared to controls[19,57–59]. Females with ME/CFS disproportionately report irregular menstrual cycles, amenorrhea, dysmenorrhea, excessive menstrual bleeding, and bleeding between periods.[19,59.]

Half to two thirds of females with ME/CFS report increased illness symptoms before menses; therefore, the menstrual cycle should be considered when conducting symptom surveys[19]. A longitudinal case/control study found that early onset menopause is a risk factor for ME/CFS and menopause exacerbated symptoms in 38% of perimenopausal and postmenopausal women with ME/CFS.[19] Pregnancy is reported as a trigger of both ME/CFS and POTS, accounting for 3-10% of cases and can also improve, maintain, or worsen symptoms[19,60]. A retrospective, observational, case control study found that women who had been pregnant in the previous year were over 31 times more likely to develop ME/CFS, highlighting the need to study mechanistically how pregnancy and other reproductive events increase risk for developing ME/CFS[19].

In EHR and survey studies of individuals with ME/CFS, 20.1-36% reported endometriosis, which is 2-3.5 times greater than general population prevalence[19,23]. Emelia von Saltza discussed the great need for developing earlier and less invasive diagnostics and treatments for endometriosis. Treatment options are limited: both diagnosis and treatment involve surgery, and average diagnostic delay for endometriosis ranges from 6.7-10.4 years[61,62].

There is emerging research possibly implicating infection (pathogenic bacteria) in the etiopathogenesis of endometriosis. A 2023 study found Fusobacterium in endometrial tissue of 64% of 79 women with endometriosis and just 7% of healthy controls[63]. In mouse models of endometriosis, mice inoculated with Fusobacteria had increased and larger endometrial lesions; antibiotic treatment reduced fusobacterium and the number and size of endometrial lesions in mice[63]. Further murine and human

subjects research on infection, immunity, and endometriosis in infection-associated illnesses is warranted.

Conclusions and Cross-cutting Themes

Including screening for these less studied topics in research studies on ME/CFS is an essential and cost-effective next step to advance research. Scientists may not be aware of best practices to screen for these pathologies, and standardized instruments and protocols for appropriate screening should be shared within the field.

There is urgent need for deep phenotyping research within ME/CFS and across this group of associated illnesses, focusing on these less studied pathologies. Tissue-based and multi-omic research is a top priority for most of these topics. To enable this research, the field could establish a tissue bank (like the NINDS-supported NeuroBioBank) for ME/CFS neurosurgical tissue, biopsies, endometrial surgical tissue, GI surgical tissue and more.

There is also a need for comprehensive research that looks across traditional silos in research and clinical care. This approach, like systems biology, would examine the interconnections, bidirectional relationships, and altered homeostasis between these less studied pathologies and other pathologies in ME/CFS. Similarly, associated illnesses (e.g., dysautonomia, Long COVID, EDS, MCAD, chronic Lyme disease, fibromyalgia, and Sjogren's syndrome) share many of these less studied pathologies. Clinical studies and therapeutic trials with multiple comparator illness cohorts are important directions for research.

Specialty clinics are already using off-label therapeutics for some of these less studied pathologies. A list of potential therapeutics that have anecdotal off-label clinical efficacy should be developed so that they can be repurposed for treatment or prioritized for clinical trials. Case studies about successful treatments of these less studied pathologies should be published to support advancing towards therapeutics research.

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Genomics and Genetic Susceptibility

Introduction

The overarching questions articulated by the research and people with lived experience communities are: what are the genetic and epigenetic factors that contribute to the development of ME/CFS, affect susceptibility for it and/or its severity? Could this knowledge lead to new treatments, diagnostic tools, or prevention approaches? In this section, priorities are presented for research to address these questions.

Background

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a long-term, multisystem illness. The highly debilitating and unpredictable manifestations of ME/CFS make it a family disease—impacting the function, financials, and well-being of the immediate and extended family. Moreover, the prevalence of ME/CFS in blood relatives who do not live together is higher than in the general community[1,2], suggesting that genetic factors contribute to ME/CFS risk. However, ME/CFS diagnoses do not follow a predictable Mendelian pattern[3], implying that in most cases there is not one genetic variant that increases ME/CFS risk, but rather several affecting more than one gene. Analysis of a large U.S. health insurance dataset to assess the genetic and environmental contributions in twin and sibling pairs found evidence for heritability of risk for ME/CFS[4]. A classic twin study was conducted using 146 female-female twin pairs, providing evidence supporting familial aggregation, suggesting that genes may play a role in the etiology[5]. Therefore, it is likely that ME/CFS is a multifactorial disorder, with multiple genetic factors contributing to the clinical presentation. For instance, an infection might trigger ME/CFS and contribute to its etiology, as a diagnosis often follows acute infection from viral and non-viral pathogens[6].

A susceptible genetic profile might lead to a physiology that predisposes an individual at risk to develop an aberrant immune/inflammatory response when triggered by an infection. The risk might even be increased by prior exposure to infections, physical trauma, or other environmental factors. The current leading hypothesis is that the varied genetic contributions exert a relatively small individual effect, but that their combination disrupts a physiological pathway or process leading to a pathological condition and a clinical manifestation.

Identifying such factors requires the study of large populations and of well-characterized cohorts. Unfortunately, adequately robust studies have not been done in ME/CFS, and the field has not benefitted from advances in methods and tools of genetic research that have improved our understanding of many other complex diseases. The lack of such research represents a major deficiency, as genetic studies can objectively identify genes, molecules and pathways that are involved in ME/CFS, that can in turn be targeted therapeutically. Genetically supported targets double the success rate in clinical development[7], thus a key objective for genetic and epigenetic studies is to identify targets for therapeutic interventions.

Summary of Webinar and Closed Session

Genome-Wide-Association Studies (GWAS) in ME/CFS

The evidence suggests that while genetic factors likely contribute to ME/CFS risk, they have a relatively small individual effect. A genome-wide association study (GWAS) is a common method for discovering genetic causes of disease, particularly when its etiology is unknown. This *hypothesis-free* approach is useful for discovering new pathophysiological mechanisms as it is not influenced by pre-existing biological assumptions. Chris Ponting (University of Edinburgh) emphasized the importance of large cohort size (and substantially more controls than cases) to adequately power GWAS to find DNA variants of small effect. While studies of hundreds of cases may be sufficient to identify common variants with very strong effects (as in monogenic diseases), other disease areas have demonstrated that sample sizes of tens of thousands to 100,000 cases are required to discover variants with weak effects. It is therefore not surprising that the few GWAS conducted with small ME/CFS sample sizes did not find reliable risk loci[8,9,10]. The largest GWAS published to date examined a total of 2,532 individuals with ME/CFS for the genome-wide analyses and 460 individuals with ME/CFS for a targeted analysis. ME/CFS risk loci displaying genome-wide significance were not identified[11]. As of November 2023, Dr. Ponting and his colleagues completed enrollment of 21,632 out of the planned 25,000 UK participants in the DecodeME study—the largest ever ME/CFS study, with GWAS as its primary objective. Funding of £3.2M for the study was provided jointly by the Medical Research Council and the National Institute for Health Research in the UK. Participants are adults ≥ 16 years with all levels of disease severity. Saliva samples for DNA analysis are collected from home, as well as data from a paper or online questionnaire. The study is co-produced with the community and deidentified individual-level data will be shared for further research analyses. The first results are forecasted for the summer of 2024, and are expected to identify five associations that will pinpoint chromosomal locations containing DNA variants that change the activity of genes, leading to susceptibility to ME/CFS. Results from the study could also enable detection of genetic signals shared with other diseases or traits, and aid in stratification of ME/CFS subtypes. To complement the DecodeME study, Dr. Ponting is advocating for establishing a large and representative USA cohort, with 50,000 ME/CFS participants, advancing the field to have datasets closer in magnitude to other disease areas.

Genetic datasets of large populations can also be interrogated to identify genetic and molecular interactions. Steven Gardner (Precision Life, Inc.) shared the results of GWAS and a combinatorial analysis of genetic risk factors for ME/CFS and Long COVID using UK Biobank data, in a case–control design with 1000 cycles of fully random permutation[12]. Results were augmented by a series of replication and cohort comparison experiments, including use of disjoint Verbal Interview CFS, post-viral fatigue syndrome and fibromyalgia cohorts also derived from UK Biobank, and comparison of results for overlap and reproducibility. No genome-wide significant variants were identified in over 500,000 markers tested. This sample size was underpowered to detect weak effects. However, combinatorial analysis revealed 199 SNPs mapping to 14 genes that were significantly associated with 91% of the cases in the ME/CFS population. Many of the genes identified are known to be linked to the key cellular mechanisms hypothesized to underpin ME/CFS, including vulnerabilities to stress and/or

infection, mitochondrial dysfunction, sleep disturbance and autoimmunity. Three of the critical SNPs were replicated in the post-viral fatigue syndrome cohort and two SNPs replicated in the fibromyalgia cohort. In a more recent study, they compared two subpopulations of individuals with Long COVID from Sano Genetics' Long COVID GOLD study cohort (https://sanogenetics.com/study/gold/), focusing on people with severe or fatigue dominant phenotypes[13]. A comparative analysis identified overlap in the genes associated with fatigue-dominant Long COVID and ME/CFS, including genes involved in circadian rhythm regulation, and insulin regulation. Overall, 39 SNPs associated with Long COVID in this study can be linked to 9 genes identified in the combinatorial analysis of individuals with ME/CFS from the UK Biobank. Importantly, among the 73 genes associated with Long COVID, 42 are potentially tractable for drug discovery approaches, with 13 of these already targeted by drugs in clinical development, potentially leading to drug repurposing for use in treating Long COVID and/or ME/CFS.

The caveats are that these are still small data sets, with largely self-reported diagnoses and a potentially high rate of misdiagnosis. There is limited ancestral diversity, other potential biases of age and activity level, as well as very limited information on disease onset relapses and recovery. Additional independent, larger datasets will further improve the disease insights and validate potential treatment options, as well as the development of diagnostic algorithms using low-density genotype arrays so individuals with ME/CFS can be tested to determine their risk of the disease for a relatively low cost.

GWAS in Long COVID

The GWAS findings common to ME/CFS and Long COVID suggest the potential for discoveries in Long COVID to contribute to the understanding of ME/CFS. Vilma Lammi (University of Helsinki, Finland) described GWAS for Long COVID and the challenges of designing such a study. An international collaboration ultimately included up to 6,000 Long COVID cases and over a million population controls from 24 studies across 16 countries in the COVID-19 Host Genetics Initiative. The team identified a genome-wide significant variant in the *FOXP4* locus, that has been previously associated with COVID-19 severity, lung function, and lung cancer. They have replicated their results in six independent cohorts[14]. While this gene may be more specific to longer-term outcomes of SARS-CoV-2 infection, the study shows the potential of GWAS to be constructed based on discrete datasets across borders and still yield meaningful results. Other studies that leverage genetic databases were described by Anniina Tervi (University of Helsinki) who shared results of a genetic correlation analyses for ME/CFS and some common comorbidities using summary level statistics from different European ancestry population cohorts (e.g., FinnGen). The preliminary results indicate that there might be a shared genetic component for ME/CFS with Raynaud's syndrome, hEDS, insomnia, asthma and Sjogren disease.

Taken together, it is clear that: 1) the analysis of large genetic datasets has a high potential to contribute to understanding ME/CFS; 2) the advances in genetic methods and tools have not been utilized sufficiently in ME/CFS; and 3) there is an urgent need to share data and create access to genetic information on a large scale. During the closed session, Stella Aslibekyan (23andMe) shared that their database has more than 13 million participants and that more than 100,000 individuals self-reported as having ME/CFS. They presented results of a GWAS in 50,000 self-reported cases of Long COVID and identified that the immune-related loci remained genome-wide significant in the multi-ancestry analysis. Furthermore, they found strong genetic correlations for Long COVID with ME/CFS, fibromyalgia, chronic pain, and dysautonomia[15]. Thus, large datasets with ME/CFS genetic information

already exist around the world that can aggregate the necessary cohort size to make significant discoveries. This realization led to a strong desire expressed in the closed-session discussion to create a consortium for sharing data for analysis. While sharing individual level data is not possible in some instances (depending on participants' consent), summary statistics are sufficient for GWAS (but not for combinatorial analysis). Most agreed that a rapid analysis of existing and soon to be released data (DecodeME, All of US) is an important step in determining further investments. The discussion also highlighted the need for merging Whole Genome Sequencing information with detailed phenotypical and multi-omic data, although the mechanism to amass such datasets was a matter of debate.

Family Studies and Case Reports

Fereshteh Jahaniani (Stanford) illustrated the potential power of such an approach, applied to family-based studies involving multiple cases of ME/CFS and case/control association studies, to identify rare variants that contribute to ME/CFS. An unpublished study identified rare deleterious variants that were part of pathways implicated in dysregulation in energy metabolism and coagulation cascades in a family that had members with hypermobile spectrum disorder, Ehlers-Danlos syndrome, and ME/CFS. An extraordinary example of how the identification of a single variant in a single case may shed a light on the pathophysiology of ME/CFS was recently presented at the NIH ME/CFS conference by Paul Hwang (NIH). A rare genetic mutation leading to overexpression of Wiskott-Aldrich Syndrome Protein Family Member 3 (WASF3) was found in a 38-y-old woman suffering from long-standing fatigue and exercise intolerance. The mutation can disrupt mitochondrial respiratory super complex formation. Expanding on these findings, skeletal muscle biopsy samples obtained from a small cohort of individuals with ME/CFS showed increased WASF3 protein levels and aberrant endoplasmic reticulum (ER) stress activation relative to non-sedentary controls[16].

The research approaches of studying large population genetics and interrogation of deeply genotyped / phenotyped cases have their distinct advantages, operational challenges, and cost-benefit calculations. Therefore, the panel's consensus recommendation was to pursue both approaches in parallel, while cross-referencing results.

Epigenetics

The integration of epigenomic research in ME/CFS was a topic of considerable discussion. Alain Moreau (Université de Montréal, Canada) described the possible contribution of epigenetics, the inherited or acquired modifications that affect gene expression but not gene structure (e.g., base-pair sequence), including the methylome, histone modification and small non-coding RNAs. Infection by herpesviruses has been reported to impact the expression of microRNAs (miRNAs), short non-coding RNA sequences which have been suggested to be epigenetic factors modulating ME/CFS pathogenic mechanisms. Several microRNAs (miRNAs) have been found recently to be differentially expressed in ME/CFS cases and showed that some of these miRNAs were significantly reduced in individuals with fibromyalgia compared to both individuals with ME/CFS and healthy controls[17]. MicroRNAs could be used to stratify individuals with ME/CFS in different subgroups associated with specific symptoms (e.g., dysautonomia, brain fog and cognitive dysfunctions) that are exacerbated by post-exertional malaise (PEM) as well as using such stratification to predict therapeutic response among different subgroups of individuals with ME/CFS. MicroRNAs have been suggested as potential biomarkers that could be used for diagnostic purposes, although differences exist by race and ethnicity. There appears to be a shift in the immune

inflammatory response from transient to chronic in ME/CFS, as reflected in DNA methylome changes, and DNA methylation changes can be transmitted from an affected parent to an offspring. DNA methylation alterations occur in ME/CFS, similar to microRNA signatures. Thus, methylation patterns may also be useful as biomarkers for clinical testing.

Conclusions

The evidence suggests that while genetic factors likely contribute to ME/CFS risk, they have a relatively small individual effect. Recent studies indicate, however, that combinatorial analysis evaluating the predictive value of multiple loci present in the same individuals may be much more powerful to illuminate the genes, cells and processes that causally modulate disease risk for the whole population. Importantly, genetically-supported targets double the success rate in clinical development, so it is critically important to identify specific genes or physiological pathways that can be targets for therapeutic interventions. Genetic factors underlying ME/CFS susceptibility can be studied by analyses of common variation within large populations (i.e., genome-wide association studies: GWAS) and of rarer variation within smaller, enriched cohorts (e.g., whole genome sequencing of case-control, families, and severely affected individuals). These research methods have their distinct advantages and cost-benefit calculations, so it is recommended that both approaches be pursued in parallel, cross-referencing results. Genetic information lends itself easily to digitalization and decentralized research, so data sharing is of the outmost importance. Technological advances in analysis and interpretation of genomic data—machine learning and artificial intelligence, in particular—enable widened scope, increased speed, and reduced cost of such studies in ways that were not previously possible, including through public-private partnerships. The inherent advantages of genetic research in ME/CFS-overcoming biases of diagnoses, hypothesis-free approach for unknown / multiple etiologies, and ease of data harmonization and referencing – argue that this strategy must be deployed now. While a singular genetic factor in ME/CFS is unlikely to exist, we must recognize that advancements, even if relevant to only a subset of individuals with ME/CFS, are significant. Thus, there is an impetus to embark on ME/CFS genetic and epigenetic research with renewed vigor and increased investments at this time.

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ME/CFS Research Priorities

Chronic Infection

Chronic Infections (other than Herpesviruses)

1. Employ modern methodologies to assess prevalence of chronic infections by enteroviruses or other RNA viruses in ME/CFS cases that arose before 2020, assaying tissue samples as well as biofluids.
2. Determine the prevalence of SARS-CoV-2 chronic infection in individuals exhibiting Long COVID that fulfill IOM or international criteria for ME/CFS.
3. Determine whether molecular markers differentiate pre-2020 ME/CFS cases from Long COVID cases.
4. Perform serological studies of individuals with ME/CFS to determine if prior viral exposures differ from control subjects.
5. Investigate whether acute enteroviral infections result in ME/CFS, even without becoming chronic.
6. If chronic RNA virus infections exist in ME/CFS, determine whether viruses persist due to immune evasion or niche microenvironment and assess downstream consequences.
7. Develop vaccines and drug treatments for both acute and chronic enteroviral (EV) infections in association with other diseases, including type 1 diabetes, cardiomyopathy, acute flaccid myelitis, and ME/CFS.
8. Conduct clinical trials assessing anti-viral (entero-, SARS CoV-2) drugs on ME/CFS and Long COVID symptoms and disease severity.

Herpesviruses

1. Assess whether new primary infections with herpesviruses, especially EBV and HHV6A-B, trigger ME/CFS onset, with or without involvement of other viruses, using longitudinal serum sampling.
2. Determine through longitudinal studies the incidence and frequency of EBV and HHV-6A/B reactivation in individuals with ME/CFS and whether reactivation levels correlate with symptom type or severity.
3. Conduct clinical trials to assess impact of anti-herpesvirus treatments on symptoms, symptom flares and disease severity, and on biomarkers for herpesvirus reactivation.

Human Endogenous Retroviruses (HERVs)

1. Evaluate levels of expression of HERVs in immune and non-immune cells from individuals with ME/CFS and compare them to both healthy control groups and comparison groups with other fatiguing illnesses.
2. Investigate correlations of HERV expression with symptomology and disease severity.
3. Increase mechanistic studies of HERV expression and where and how HERV expression affects immune function (including response to residual RNA viruses).
4. Conduct anti-viral therapy-based clinical trials if/when positive correlations of HERV expression with disease are identified.

Overarching

1. Develop biorepositories—serum/plasma, leukocytes, saliva, nasopharynx swabs, feces, biopsies, autopsies—from ME/CFS individuals for the assessment of multiple infectious agents (viruses, bacteria, fungi, protozoa).
2. Mine large longitudinal population biorepositories to identify and analyze blood samples collected before and after individuals developed ME/CFS, analogous to studies identifying the association of multiple sclerosis with prior EBV infection.
3. Identify sub-groups of ME/CFS individuals based on physiological and molecular measures, by considering, for example, individuals with/without chronic RNA virus infection, absence/presence of reactivated HHVs, and illness for less/more than three years.
4. Include carefully matched healthy controls and, if feasible, disease comparison cohorts with other chronic fatiguing illnesses in studies of ME/CFS.
5. Use combinatorial data from multiple sources to increase the precision and accuracy of identifying ME/CFS disease signatures.
6. Fund replication studies to verify important findings.

Immune System

Clinical Immunology

1. Identify and characterize diagnostic immunological markers and mediators of ME/CFS. Increase the power of observations from published reports (per Institute of Medicine recommendations).
2. Expand robust sample biorepositories (https://searchmecfs.org) and data sharing platforms (https://mapmecfs.org)
3. Engage in consensus meetings with health care providers and expert advocates by leveraging the ME/CFS Clinician Coalition.
4. Prioritize studies that focus on differences in gender, age, stage of illness, and comorbid conditions; and ensure research has representative demographics.

Autoimmunity

1. Elucidate the function and signaling of G protein-coupled receptor (GPCR) autoantibodies in ME/CFS.
2. Identify and characterize other autoantibodies associated with ME/CFS.
3. Investigate the role of B cell and B cell receptor signaling contributing to ME/CFS.
4. Establish a reliable, consistent animal model for serum transfer assays.
5. Conduct clinical studies targeting either B cells or aged adipose B (AAB) cells.

Immune Cell-Types

1. Investigate the signaling pathways responsible for driving dysregulation.
2. Determine whether immune cells in ME/CFS are “exhausted” and identify the mechanisms underlying such exhaustion.

3. Assess whether granulocytes function normally in individuals with ME/CFS.
4. Clarify whether immune cells are functioning abnormally within tissues and organs.
5. Explore how monocytes and platelets are contributing to immune abnormalities and symptoms in ME/CFS.

Immune Perturbations

1. Identify actionable metabolic or microbiota targets that are disrupted in individuals with ME/CFS.
2. Measure immune outputs during interventions that target these disruptions to rapidly assess their biological impact.
3. Conduct small intervention trials based on available targets to evaluate changes in immune response, metabolic pathways, and microbiome.
4. Develop sensitive and specific biomarkers based on immune and metabolome data for accurate ME/CFS diagnosis.
5. Design research studies on specific cohorts to determine the biological diversity of individuals with ME/CFS.
6. Generate ontology-based personalized treatments for ME/CFS.

Gut-Immune-Metabolic Interplay

1. Explore the connection between altered immune, metabolic, and gut functions and their relevance to ME/CFS symptoms.
2. Investigate the role of neuroinflammation in ME/CFS, especially its relationship with systemic and gastrointestinal inflammation.
3. Develop biomarkers to stratify study participants with ME/CFS based on disease etiology and underlying mechanisms.
4. Elucidate the distinct mechanisms underlying the gut-immune-metabolic interplay as well as the biomarker profiles of individuals with ME/CFS.
5. Design precision therapies tailored to specific subsets of individuals with ME/CFS.

Nervous System

Cognition

1. Develop and validate objective tools for remote cognitive assessment in ME/CFS.
2. Produce cognitive batteries specifically for use in ME/CFS studies, with tests easily administered, completed, and scores so that cognitive assessments can be integrated into more research and clinical protocols.

Dysautonomia

1. Explore the impact of orthostatic stress in ME/CFS pathology, symptoms, and disease severity.
2. Investigate circulatory dysfunction in ME/CFS neural, immune, and autonomic symptoms.
3. Determine relation between orthostatic intolerance and mast cell activation syndrome in

ME/CFS.

Neuroinflammation

1. Investigate cerebrospinal fluid to reveal ME/CFS immunological and neuroinflammatory abnormalities.
2. Test role of neuroinflammation in ME/CFS using pharmaceuticals that cross the blood-brain-barrier and modulate inflammatory processes.

Neuroimaging

1. Better understand the effects that travel, preparation, and instrumentation have on neuroimaging results.
2. Conduct neuroimaging scans [magnetic resonance imaging (MRI), positron emission tomography (PET)] and electroencephalogram (EEG) in study participant-comparison designs to uncover brain abnormalities specific to ME/CFS.
3. When feasible, incorporate neuroimaging scans in clinical trials to identify central nervous system changes associated with improved symptom severity.

Disordered Sleep

1. Develop and validate biomarkers for non-restorative sleep.
2. Leverage machine learning to clarify EEG correlates of non-restorative sleep in ME/CFS.
3. Identify ways to subgroup ME/CFS participants by individual sleep patterns.
4. Understand the causes of sleep phase reversal in ME/CFS.
5. Determine if small fiber neuropathy drives ME/CFS, orthostatic intolerance, cerebral hypoperfusion, and postural orthostatic tachycardia syndrome.
6. Examine characteristics of orthostatic intolerance with and without ME/CFS.

Circulation

Endotheliitis/opathy

1. Establish the full landscape of cytokines and endothelial biomarkers to understand the resulting endothelial dysfunction and stratify individuals with ME/CFS.
2. Determine pathophysiologic mechanisms underlying endothelial dysfunction in ME/CFS and their similarity to those that underly endotheliitis resulting from COVID-19.
3. Determine utility of scalable functional biomarkers of systemic endothelial dysfunction, e.g., EndoPAT.
4. Launch randomized clinical trials of repurposed drugs that enhance vasodilation, e.g., vasodilation (e.g., arginine, prostacyclin, treprostinil) or decrease pathologic vasoconstriction, e.g., ambrisentan).

Thrombotic Endotheliitis and Microclots

1. Identify additional novel, practical, clinically useful biomarkers that allow researchers and clinicians to assess microclots e.g., endothelial debris markers, biofilm presence, 2AP (an

inhibitor of fibrinolysis).
2. Expand imaging flow cytometry and microscopy testing more widely in pathology labs to determine the presence and role of microclots.
3. Determine if anticoagulant/antiplatelet therapy improves microcirculatory blood flow, endothelial function, and symptoms.

Hypovolemia/Blood Volume

1. Further develop minimally invasive, non-radioactive techniques for assessing blood volume for diagnostic and therapeutic purposes in ME/CFS.
2. Emphasize that research studies in ME/CFS must normalize blood volume measurements in any studies of orthostatic intolerance (OI) or hypovolemia.
3. Determine the relationship between blood and plasma volume and OI.
4. Investigate the role of disease course in hypovolemia, potentially through longitudinal studies and as the disease becomes more severe.

Orthostatic Intolerance: The Role of Cerebral Blood Flow and Circulation

1. Identify the signs of POTS, orthostatic hypotension, syncope and blue extremities and limbs and how they differ across individuals with ME/CFS.
2. Identify the underlying mechanisms of disturbed cerebral autoregulation.
3. Determine the role of orthostatic hyperventilation and hypocapnia in provoking cerebral vasoconstriction, lightheadedness, and decreased cognition.
4. Determine whether drugs with relative specificity for blocking of cerebral vasoconstriction, e.g., amlodipine, improve upright cerebral blood flow and symptoms by transcranial Doppler, functional MRI, and tests of cognitive function.

Impaired Oxygen Sensitivity of Red Blood Cells from ME/CFS

1. Develop and validate an ME/CFS diagnostic test using PO_2 -mediated RBC capillary velocity.
2. Use longitudinal monitoring of RBC capillary velocity to track individual response to treatment (e.g., medication, surgical intervention) in people with ME/CFS.
3. Investigate the effects of circulating cytokines on RBC capillary flow in ME/CFS.
4. Screen drugs that can improve RBC deformability and sensitivity to PO_2 changes (e.g., salmeterol, xanomeline).
5. Evaluate the efficacy of hyperbaric and supplemental oxygen therapies in reducing ME/CFS symptom severity.

Neurovascular Dysregulation During Exercise

1. Identify and differentiate circulatory pathophysiologic subtypes of ME/CFS, including dysautonomia, hypovolemia, endotheliitis, microclots, and RBC abnormalities.
2. Investigate the role of mitochondrial dysfunction in reduced O_2 uptake and in symptomatology of ME/CFS.
3. Identify and validate organic and scalable biomarkers, which can eventually be incorporated into randomized clinical trials (RCTs).

4. Facilitate placebo-controlled clinical trials with repurposed drugs directed at the underlying circulatory pathophysiology, e.g., pyridostigmine, low dose naltrexone.

Lymphatic System

1. Determine whether mechanisms underlying vascular endothelial dysfunction are also impacting lymph vessel endothelium.
2. Expand mechanistic research to lymphatic system pathologies, their prevalence, and roles in ME/CFS pathophysiology, including lymph vessel damage, lymph stagnation, lymph node swelling, glymphatic dysfunction, and lymphedema.

Metabolism

Metabolism-Immunology Interplay

1. Prioritize research into state-of-the-art systems immunology including detailed transcriptomic data, bulk and single cell-RNA-seq, and immune receptor repertoires.
2. Integrate systems immunology data with existing ME/CFS cohort data.
3. Focus on biochemical hypotheses in well-planned exposomics and metabolomics.
4. Generate advanced biochemical modeling to simulate and predict phenotypes and endophenotypes of individuals with ME/CFS.
5. Leverage ex vivo cell models and preclinical models to develop therapeutics.
6. Expand the existing ME/CFS biorepository to include biospecimens from clinical studies and trials.

Consequences of Altered Metabolism

1. Understand the altered metabolic states/reprogramming in all specific immune cell subsets of ME/CFS before and after stimulation.
2. Investigate the consequences of metabolic abnormalities (e.g., exhaustion) in specific immune cell populations including T cells.
3. Test drugs in vitro that modify metabolic pathways in specific immune cell subsets and determine if it is it possible to test in vivo.
4. Unravel the effect of ME/CFS extracellular environment on immune cell populations.
5. Examine immune cells in different tissue types for altered metabolic states.

Single-Cell Raman Technologies

1. Implement advanced machine learning into single-cell Raman spectroscopy to achieve greater than 90% sensitivity and specificity.
2. Leverage peripheral blood mononuclear cells (PBMCs) to diagnose ME/CFS.
3. Elucidate further the differences in using frozen versus fixed samples for analysis.
4. Identify new health care technologies for early diagnosis and prevention.
5. Incorporate complex data and machine learning approaches for enhanced health care solutions.

Computational Perspectives

1. Understand tissue metabolism in ME/CFS particularly in muscles and in the central nervous system (CNS).
2. Integrate plasma metabolomics with profiles of other diseases to infer the disease mechanism.
3. Incorporate plasma metabolomics with tissue data in ME/CFS to determine disease mechanisms and potential treatments.
4. Identify metabolomic biomarkers for disease diagnosis.
5. Elucidate predictive biomarkers for targeted treatments.

Microbial Metabolism

1. Address the lack of fecal metabolomics studies in ME/CFS.
2. Explore prospective studies and leverage existing well-powered, data-rich studies.
3. Focus on longitudinal single-omics or multi-omics datasets.
4. Develop and employ in vitro and in vivo models.
5. Investigate mechanisms and phenotypes linked to microbiome and metabolomic disturbances.
6. Assess the efficacy of metabolite, probiotic, prebiotic, or symbiotic interventions.
7. Focus on microbiome and metabolome data mining.

Metabolic Characterization of Biofluids

1. Conduct large-scale diagnostic biomarker projects and their validation.
2. Identify objective metabolite markers for disease improvement.
3. Characterize metabolic changes in response to external stressors.
4. Establish connections between metabolome to physiological measures and function.
5. Implement tracking of symptoms of ME/CFS for enhanced clustering and delivering personalized medicine.
6. Evaluate the impact of “deconditioning” and ensure ii accounted for i ies

Physiology

PET Imaging for Whole Body Immune Responses

1. Explore the potential of PET tracers and MRI techniques to detect subtle and nuanced inflammatory responses.
2. Streamline assessment of advanced 3T or 7T MRI techniques for early detection, disease staging, and monitoring the efficacy of therapies.
3. Implement whole-body imaging studies to assess the systemic impact of ME/CFS.
4. Conduct studies to understand potential sex differences in the immune response associated with ME/CFS.
5. Validate PET tracers/biomarkers in human tissues, cells, and potentially mouse models.
6. Apply spatial transcriptomics and proteomics techniques to clinical samples to map the molecular landscape of ME/CFS at a cellular level.
7. Develop new image analysis methods using AI/ML to enhance the detection and understanding of ME/CFS.
8. Leverage most promising PET tracers in Phase 1 and Phase 2 clinical trials.

Cell Danger Response: Extracellular ATP (eATP)

1. Conduct comprehensive studies to elucidate how eATP signaling contributes to fatigue and pain, particularly in DOMS and chronic fatigue syndromes.
2. Investigate eATP signaling mechanisms for immobility to prevent further injury and limit transmission of infections.
3. Explore the role of eATP in initiating the healing cycle (i.e., salugenesis).
4. Investigate potential biomarkers and diagnostic criteria for eATP hypersensitivity to facilitate early diagnosis and targeted treatment.
5. Study how alterations in eATP signaling might affect recovery processes and contribute to the persistence of symptoms.
6. Launch a double-blind, placebo-controlled, RCT to test the safety and efficacy of antipurinergic therapy for ME/CFS.
7. Conduct RCTs for Long-COVID and post-Lyme disease to assess the efficacy of APT in these syndromes as well.
8. Implement longitudinal studies to track the changes in eATP signaling over time in individuals with ME/CFS.

Itaconate Shunt in ME/CFS

1. Develop and refine technologies to detect and isolate sick cells in individuals with ME/CFS at a high resolution.
2. Generate multiplexing techniques to simultaneously analyze multiple cellular markers and pathways involved in ME/CFS.
3. Create ultra-sensitive assays, such as single-molecule ELISA, to detect extremely low concentrations of biomarkers and mechanistic disease signatures in the blood, potentially in the aM - fM range.
4. Apply zero-inflated Poisson statistics-aware technology to accurately interpret

data where only a small fraction of cells are affected.

5. Investigate the contribution of intravascular sources to ME/CFS pathology, considering how circulating factors may affect cellular health.
6. Explore the interaction between blood components and sick cells to understand systemic effects.
7. Conduct studies to determine the predisposing factors (genetic, environmental, and immunological) for ME/CFS.
8. Leverage AI/ML to analyze complex datasets and identify patterns and markers indicative of ME/CFS

Overlapping Mechanisms in Individuals in the ICU and People with ME/CFS

1. Further explore the overlaps in mechanisms of illnesses induced by physical, infectious, and/or emotional stressors, including ME/CFS, chronic critical illness, post-intensive care syndrome (PICS), cancer-related fatigue, post-viral fatigue, PACS, Long-COVID, heat stroke, and fibromyalgia.
2. Assess the pulsatile pituitary secretions in ME/CFS and their relationship to the severity of illness and physiological alterations in ME/CFS. Specifically, assess (i) the relationship between pulsatile ACTH secretions and the integrity/function of the adrenal glands, and (ii) the relationship between pulsatile GH secretions and the balance between catabolic and anabolic activities. (Central mechanisms in chronic critical illness).
3. Investigate the HPT axis function in ME/CFS (including thyroid hormone function at tissue level) and relationship to the severity of illness, hypometabolic state, immune system activity and specific organ/tissue symptoms. (Central mechanisms in chronic critical illness).
4. Explore the role of interlinkages between inflammation, intestinal injury, pituitary suppression, low thyroid hormone function, endothelial function, and mitochondrial function in the persistence of illness in ME/CFS. (As documented in chronic critical illness).
5. Study the potential applicability of chronic critical illness treatment trials to develop new therapeutic avenues for ME/CFS.

Refreshing Sleep

1. Conduct comprehensive studies exploring various subcomponents of restorative and refreshing sleep (e.g., grogginess, sleep inertia, and depth of sleep) in people with ME/CFS.
2. Leverage REST-Q to assess the prevalence and characteristics of restorative sleep in individuals with ME/CFS.
3. Assess how improvements or impairments in restorative sleep impact daily functioning, mental health, and physical health in these individuals.
4. Investigate how restorative sleep influences other dimensions of care in ME/CFS, including pain management, cognitive function, and emotional well-being.
5. Explore potential interventions that could enhance the restorative quality of sleep in people with ME/CFS and evaluate their efficacy.

6. Implement longitudinal studies to track changes in restorative sleep patterns over time in individuals with ME/CFS and their impact on disease progression and symptom severity.
7. Use wearable sleep trackers and other technological tools for continuous and precise monitoring of sleep patterns.
8. Combine sleep quality assessments with other biological parameters, such as hormone levels, inflammatory markers, and immune system function, to understand the complex interactions in ME/CFS.

Nonrestorative Sleep

1. Explore the relationship between norepinephrine (NE) oscillation patterns and memory performance, particularly the role of sigma power and sleep spindles in memory consolidation during NREM sleep.
2. Assess the impact of chronic non-restorative sleep on long-term cognitive function and neurological diseases (e.g., onset of Alzheimer's Disease).
3. Evaluate the effectiveness of current sleep aids on NE oscillation patterns and develop new therapeutic strategies that could promote optimal NE oscillation densities during NREM sleep.
4. Explore non-pharmacological interventions (e.g., cognitive-behavioral therapy) for insomnia to increase NE oscillations.
5. Assess how NE oscillations affect glymphatic function and potential buildup of neurotoxic waste products.
6. Examine the impact of acute and chronic stress on NE oscillation patterns and the resulting quality of sleep.
7. Investigate the possible roles of impairment of deep, slow-wave sleep and glymphatic system clearance in producing non-restorative sleep and other ME/CFS symptoms (brain fog, post-exertional malaise, hypothalamic dysfunctions).
8. Identify individual differences in NE oscillation patterns and their implications for personalized sleep medicine.
9. Explore genetic, environmental, and lifestyle factors that influence NE oscillation patterns and restorative sleep.

Metabolism and ME/CFS

1. Investigate repurposing existing drugs for the treatment of ME/CFS based on pathophysiological insights.
2. Clarify mechanisms underlying ME/CFS pathophysiology, focusing on the immune system, metabolism, and neurology.
3. Utilize high-throughput technologies to deeply characterize the immune system in people with ME/CFS, including single-cell analyses and cytokine profiling.
4. Conduct larger, well-controlled studies with harmonized methodologies to ensure the robustness and reproducibility of findings.
5. Establish comprehensive databases to collect data on ME/CFS, including clinical, biochemical, and genetic data.
6. Investigate the role of the immune system in different stages of ME/CFS.

7. Identify and characterize subgroups of individuals with ME/CFS to tailor treatments and understand variability in disease progression and response to therapy.
8. Utilize machine learning and data mining technologies to detect patterns that may not be evident through traditional analysis.

ME/CFS and Long-COVID: BH4 and Nitric Oxide

1. Develop and validate methods to measure BH4 in blood.
2. Determine if ME/CFS is a cell autonomous disease where more cells involved indicates an individual with more severe disease.
3. Determine if and how energy depletion from exertion causes crashes and may activate innate immunity and restart the disease.

Extracellular Vesicles (EVs)

1. Investigate the cellular origins of EVs released post-exercise and map their distribution and target tissues in the body.
2. Explore the mechanisms of EV uptake and the subsequent signaling pathways activated in recipient cells.
3. Analyze how EV content from biofluids (CSF, urine, muscle interstitial fluid) correlates with symptomatology in ME/CFS.
4. Perform comprehensive studies to correlate changes in plasma proteomics with EV proteomics post-exercise in individuals with ME/CFS.
5. Determine specific proteins that are preferentially packaged into EVs and their implications for disease state and severity.
6. Implement sex-stratified analyses to determine if there are differences in EV response and disease manifestation between male and female people with ME/CFS.
7. Explore whether sex-specific factors influence the EV proteome and the associated symptom severity.

Less Studied Pathologies

Connective Tissue Disorders (CTDs) in ME/CFS

1. Define the biological mechanisms and biomarkers, especially of acquired or exacerbated CTDs after development of ME/CFS. Research 1) Immune and inflammatory mechanisms
   2) Possible metabolic and mitochondrial mechanisms 3) Genetics, and roles of germline vs. somatic mutations 4) Possible roles for perfusion and hypoxia 5) infections (bacterial, viral, fungal).
2. Develop and validate diagnostic and predictive tools for comprehensive whole-body evaluation of CTDs and expand and improve upon hypermobile joint based CTD evaluation.
3. Identify how CTDs may impact ME/CFS pathophysiology, symptomatology, risk, onset, trajectories, and severity.
4. Identify why CTDs are over-represented in ME/CFS. Determine prevalence of pre-

existing and hereditary CTDs vs. newly acquired in ME/CFS, and whether pre-existing or genetic CTDs increase the risk for ME/CFS.

Spinal and Mechanical Disorders in ME/CFS

1. Identify therapeutic targets and develop non-invasive treatments (e.g., anti-inflammatory and immunomodulating therapies, regenerative tissue technologies for ME/CFS-related spinal disorders).
2. Study the prevention and prediction of the development of spinal disorders, and how they may contribute to ME/CFS pathophysiology symptomatology, especially: upper cervical instabilities, chiari malformation, and tethered cord syndrome.
3. Utilize histology, high resolution imaging, and in-depth analysis of a variety of types of neurosurgical tissue to identify phenotypes and tissue-based biomarkers/mechanisms of connective tissue damage underlying spinal disorders.
4. Conduct multi-omic, immune, and pathogen profiling of neurosurgical tissue and fluids to develop hypotheses for causes of the disorders.
5. Validate accessible low/no risk assessments in research and clinical practice to screen for spinal disorders in ME/CFS research to study prevalence and identify biomarkers.
6. Include MRI studies of spinal issues in ME/CFS and incorporate an examination of spinal and mechanical issues into already planned MRI studies.
7. Analyze neurosurgical outcomes and develop tools to predict them. Understand risk factors and conduct research to prevent the need for additional neurosurgeries.

Mast Cell Activation Disorders (MCAD), Including Mast Cell Activation Syndrome (MCAS) in ME/CFS

1. Develop and validate standardized testing and screening tools and identify objective biomarkers for MCAD.
2. Establish mechanistic endotypes and genotypes to advance MCAD translational research.
3. Investigate the crosstalk between epithelial barrier impairments and barrier immunity, MCAD, and autonomic nervous system dysfunction.
4. Study risk factors and possible triggers of MCAD, including infection and environmental exposures (mold, chemicals).
5. Determine how mast cell mediators contribute to or drive collagen and connective tissue breakdown.
6. Develop new treatments to block MCA and associated mediators.
7. Identify the role of MCA in ME/CFS symptomatology, phenotypes, severity, and pathophysiology. Study what improves and how individuals with ME/CFS benefit from existing MCA treatments.

Gastrointestinal Dysfunction in ME/CFS

1. Study intestinal barrier function, neurogastrointestinal function, dysmotility, and dysbiosis.
2. Identify how immune, neurological, and connective tissue dysfunction contribute to GI issues in ME/CFS, and how GI issues may contribute to them.

3. Perform in-depth imaging studies of the GI tract and intestinal barrier. Utilize latest technologies (e.g., 3D tissue level mapping, next gen intestinal biopsies during routine endoscopy, confocal laser endomicroscopy).
4. Conduct GI-tissue based microbiota analysis, going beyond stool, using longitudinal and spatial analysis.
5. Conduct multi-omic and deep immune profiling of the GI tract: tissue-based research, mass cytometry, single cell profiling, complement pathways, cytokines, and more.
6. Develop treatments and utilize drug repurposing to treat GI dysfunction, GI tract infections, and dysbiosis (e.g., SIBO).

Neuroendocrine Dysfunction in ME/CFS

1. Identify how symptoms and pathologies of ME/CFS are moderated by steroid levels/sex hormones and steroid network relationships.
2. Identify the broad effects of hormones on diverse physiological systems (including immune, metabolic, mitochondrial, neurological, cardiovascular, and renal).
3. Conduct studies and clinical trials to identify whether different types of hormonal medications, steroids, thyroid medications, and gender-affirming hormone therapies exacerbate or mitigate ME/CFS pathologies and symptoms.
4. Comprehensively screen for, diagnose, and manage endocrine disorders in ME/CFS.
5. Identify the roles of hormonal sex differences in sex dimorphisms in ME/CFS, as well as female/premenopausal elevated risk for ME/CFS. Examine risk of ME/CFS in transgender individuals.

Reproductive Health and Menstrual Science in ME/CFS

1. Expand research on the prevalence of menstrual and reproductive health symptoms and conditions in ME/CFS, and their roles in ME/CFS symptoms, severity, risk, pathophysiology, and illness trajectories.
2. Collect gynecologic, menstrual, and reproductive medical history systematically in ME/CFS research.
3. Find out why menstruating women report ME/CFS symptom exacerbation prior to menses to identify therapeutic treatments.
4. Determine how reproductive events/phases (e.g., menstrual cycle, pregnancy, menopause, puberty) affect ME/CFS.
5. Investigate elevated rates of endometriosis in ME/CFS, including risk, etiology, and mechanisms.
6. Study reproductive tract pathologies by analyzing/profiling biological samples: vaginal swabs, menstrual effluence, tissue. Examine dysbiosis, infection, and immunity.
7. Study the impact of ME/CFS on male fertility and sexual function.
8. Develop non-invasive early diagnostics and therapeutics for reproductive health conditions in ME/CFS.

Genomics/Genetic Susceptibility

Genetic Risk Factors

1. Establish a comprehensive, large, and representative cohort in the United States to conduct robust genetic studies.
2. Investigate common deoxyribonucleic acid (DNA) variants associated with ME/CFS to understand their role in the disease's development.
3. Analyze and compare genetic data between females and males to identify potential sex- specific genetic factors.
4. Compare genetics between individuals who report different types of onset and suspected disease triggers.
5. Conduct whole genome sequencing, especially in the most severely affected individuals with ME/CFS to identify rare DNA variants that may play a significant role in the condition.
6. Explore the possibility of gene mutations or rare genetic variants that may be contributing to ME/CFS.

Combinatorial Analysis of Genetic Risk Factors from United Kingdom (UK) Biobank

1. Expand and compare the results of multiples studies that identified ME/CFS risk associated single nucleotide polymorphism (SNP) combinations.
2. Compare SNPs identified in ME/CFS that were also identified in severe and/or fatigued Long COVID cohorts.
3. Develop improved genotypic differential triage and diagnostic tools that can accurately identify individuals with ME/CFS and Long COVID.
4. Investigate the underlying mechanisms associated with the identified SNPs in ME/CFS genes.
5. Understand how these genetic variations may contribute to the development and progression of ME/CFS and Long COVID.
6. Evaluate opportunities for drug repurposing by identifying existing medications that may have therapeutic potential for treating ME/CFS and Long COVID.

Genetic Susceptibility in Long COVID and ME/CFS

1. Investigate the prevalence and impact of variants in the *FOXP4* gene in non-European populations; understanding how these variants may contribute to the development and severity of ME/CFS in different ethnic groups.
2. Elucidate how *FOXP4* gene variants may be linked to respiratory symptoms in individuals with ME/CFS.
3. Determine if FOXP4 gene variants are associated with the risk and severity of Long COVID.
4. Examine the shared genetic components between ME/CFS and other conditions like Raynaud's syndrome, hypermobile Ehlers-Danlos syndrome, and asthma.
5. Utilize population-level data to conduct hypothesis-free screens for genetic variants associated with both Long COVID and ME/CFS.
6. Perform in-depth variant-to-function analyses to elucidate the functional

consequences of specific genetic variants associated with the diseases.

Case-Control and Family Studies

1. Investigate the burden of rare potentially causative genetic variants in ME/CFS.
2. Prioritize and study top candidate genes identified through burden tests, with a focus on their roles in regulating mitochondrial function, cell signaling, T-cell receptor activity, and inflammation.
3. Utilize machine learning frameworks to analyze genetic data in an unbiased manner, potentially uncovering hidden disease genes and disease-associated pathways.
4. Apply AI/ML techniques to predict ME/CFS disease risk and clinical outcomes, contributing to personalized medicine approaches.
5. Combine family and population-based studies to gain comprehensive insights into the genetic architecture of ME/CFS.
6. Develop methods to distinguish between causal, contributing, and co-segregating genetic variants, as well as platform-specific artifacts in genetic analyses.

Contributions of Epigenomics

1. Identify circulating microRNAs associated with specific ME/CFS symptoms, including PEM, dysautonomia, cognitive dysfunctions, immune cell dysfunctions, and autoantibodies production.
2. Investigate the biological mechanisms underlying changes in microRNA expression in response to PEM induction in individuals with ME/CFS.
3. Identify the genes targeted by validated pathogenic microRNAs causing ME/CFS; are these effects systemic or tissue/cell-specific?
4. Explore the contribution of lncRNAs in ME/CFS pathogenesis.
5. Investigate the potential interactions of lncRNAs with microRNAs on ME/CFS; develop cellular and animal models for mechanistic and drug screening studies.
6. Investigate the links between post-infection syndromes and DNA methylation alterations in ME/CFS onset.
7. Understand the epigenetic factors involved in the development of the condition and their potential relationship with microRNA dysregulation

APPENDICES

Appendix 1: Nomination Form for PWLE ME/CFS
Appendix 2: Key Takeaways from Interviews with PWLE ME/CFS
Appendix 3: Webinar Agendas
Appendix 4: Research Priorities from the Community
Appendix 5: Rosters

A. ME/CFS Research Roadmap Working Group of Council and PWLE ME/CFS
B. Webinar Planning Groups
C. NINDS & RLA Staff
D. Trans-NIH ME/CFS Working Group

Appendix 6: NIH Funding for ME/CFS Research: FY2008-FY2022

Appendix 1: Nomination Form for PWLE ME/CFS

Self-Nomination for ME/CFS Research Roadmap Working Group

OMB#: 0925-0766 Expiration date: 04/2023

Public reporting burden for this collection of information is estimated to average 20 minutes per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to: NIH, Project Clearance Branch, 6705 Rockledge Drive, MSC 7974, Bethesda, MD 20892-7974, ATTN: PRA (0925-0766). Do not return the completed form to this address.

The National Institutes of Health (NIH) is leading an effort to develop a Research Roadmap for myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). Towards this end, the National Institute of Neurological Disorders and Stroke (NINDS) has formed the **ME/CFS Research Roadmap Working Group of the National Advisory Neurological Disorders and Stroke (NANDS) Council**. This Working Group will include ME/CFS basic and clinical experts from the research community, leaders of ME/CFS non-profit advocacy and research organizations, and people who are living with ME/CFS, have a family history of ME/CFS, are caregivers/care partners of people living with ME/CFS, or identify as ME/CFS patient advocates.

To identify members of the public who are living with ME/CFS, have a family history of ME/CFS, are caregivers/care partners or advocates for those living with ME/CFS to participate in the Working Group, NINDS is asking for interested individuals to self-nominate. Nominees should be comfortable expressing their individual view(s) on a panel that includes clinician scientists, researchers, and subject matter experts.

Please answer the questions below to nominate yourself as a potential participant in this Working Group. All information shared via this form is done entirely voluntarily. Please send any questions to MECFSResearchRoadmap@ninds.nih.gov.

The deadline to submit responses is **February 9, 2023 at 5:00 pm ET**.

1. I am self-nominating to serve on the ME/CFS Research Roadmap Working Group of NANDS Council. *

- ◯ Yes
- ◯ No

2. Please share how you identify: *

- ☐ Person living with ME/CFS
- ☐ Person with a family history of ME/CFS
- ☐ Caregiver/care partner for person(s) living with ME/CFS
- ☐ Patient advocate
- ☐ None of the above

3. Please provide your first and last name. *

4. Please provide your preferred email address to receive correspondence. *

5. Please provide your preferred phone number for communications. *

6. Please confirm you meet the following Qualifications of Eligibility (check all that apply): *

- [ ] Experience with ME/CFS, e.g., as a person with living with ME/CFS, person with a family history of ME/CFS; a family member or a caregiver/care partner of a person with ME/CFS; or a patient advocate
- [ ] Have some knowledge of current research on ME/CFS
- [ ] Available and able to participate in up to seven or eight four-hour virtual workshops (videoconference or phone) over the next 10-12 months. These workshops will include frequent breaks. In addition, applicants can request accommodations in the application form.
- [ ] Have a reasonable command of the English language
- [ ] Able to clearly and succinctly articulate your views through oral and written communications (use of proxy permitted)
- [ ] Have a reasonable level of comfort with email and in navigating the internet (downloading and uploading files, filling forms etc.), with assistance, if needed
- [ ] Over the age of 18
- [ ] Willing to have your name publicly posted on the roster for the ME/CFS Research Roadmap Working Group

7. Please share the reason(s) for your interest in participating in this research roadmap development process. *

8. Please indicate which ME/CFS advocacy groups, organizations, or foundations you are affiliated with, if any. *

9. Please provide a **brief** description of any relevant experience; for example, experience with the disease, experience seeking out or participating in clinical research, or experience interacting with the broader ME/CFS community. Please be as specific as possible relating to involvement and/or interactions (ex. advisory, consultancy, participatory) with regard to ME/CFS research efforts. *

10. Please send one resume (in Microsoft Word format) to MECFSResearchRoadmap@ninds.nih.gov with the email subject line: Self-Nomination for ME/CFS RRWG – [YOUR FULL NAME]. *

- ◯ I will email my resume ASAP

11. Please include any accommodation requests, including use of a communication proxy, here. *

12. If I am not selected for this Working Group, I would be interested in future engagement opportunities. *

- ○ Yes, you can retain my data and connect with me regarding participation in future engagement opportunities
- ○ No, please do not retain my data and do not contact me about future engagement opportunities.

Appendix 2: Key Takeaways from Interviews with PWLE ME/CFS

"NINDS can help better include PWLE by seeking, understanding, respecting, and validating our often years and many decades of personal experiences with the disease. We have been through a lot of struggles and have much to contribute with our years of experience. Make us feel that our opinion and perspective and suggestions all matter and are important, that we are helping to make a difference to make progress in this field and research. Seek our input and value the importance of our contribution." – PWLE ME/CFS subject matter expert (SME) on the ME/CFS Research Roadmap team.

Mid-way through the ME/CFS Research Roadmap development process the people with lived experience serving as subject matter experts (PWLE SMEs) on the Working Group of Council and webinar planning subgroups were asked to provide feedback on our efforts to include them in the process. This is a summary of their written feedback and main takeaways from small group interviews. Most of the comments focused on topics to include in the roadmap itself and how to ensure that PWLE feedback was incorporated. Here we focus on feedback related to engagement of PWLE in the process. We also discussed how we addressed concerns about the process and implemented ideas from PWLE. We are extremely grateful for the participation of the PWLE SMEs, and all that we have learned from them, not only about ME/CFS but also about how to best include PWLE of neurological disorders and research advocates.

Importance of Including PWLE ME/CFS in the Process

All PWLE surveyed and interviewed expressed the importance of including PWLE in this process as subject matter experts who can provide unique insights and identify the most critical research directions, and potentially encourage the most impactful strategies that may lead to treatments for the most pressing symptoms. Most also identified additional benefits to including PWLE. *"More than anything, people living with ME/CFS need HOPE, whether they have lived with it for decades, years or have just found out they have this condition. As every day goes by, they need to know that efforts are truly being made to find the best answers for the causes and treatments of this often-misunderstood disease."*

Several individuals also felt that they were honored to be included and they appreciated the experience for many reasons, primarily they learned a great deal from the process, and they are now equipped to work in a similar capacity in the future. *"This has been an interesting process, it was a very good experience for me, and helped me feel more involved, and like I can take more action now. I joined the process more for what I could learn than what I thought I could bring. I have learned a lot from PWLE and from researchers."*

Many shared that hearing from PWLE often provides scientists and policy makers with a greater understanding of what people with ME/CFS are dealing with both from the condition and current medical care. *"There is often a vast gap of empathy and communications that exists*

between individuals with ME/CFS and Healthcare Providers. So, it's likely that the gap between PWLE and Scientific Researchers is even more formidable."

Further, some individuals highlighted the importance of exposing more PWLE to scientific considerations and processes that go into which treatments to pursue. Many PWLE and advocates that are not interacting with scientists may advocate strongly for something that science suggests is not a good option, with more interaction between scientists and PWLE both can understand each other and build more trust. Trust allows for greater productivity. Some PWLE MECFS shared that because of their participation on this working group and a better understanding of how to effectively work with scientists and policy experts they have been contacted by researchers for more collaborative opportunities and they have felt empowered to engage more actively with their own primary care team.

Impact of Involvement of ME/CFS PWLE Those surveyed felt that there were examples where the PWLE participants tangibly impacted the development of the priorities. *"Going in, I thought that including PWLE was a pro forma exercise and that our perspectives would be sidelined. This wasn't the case."* Specific examples of this impact included items such as: the PWLE comment that "if sick people were consulted, we'd probably be willing to accept more risk in trials than researchers expect" a point that was repeated by researchers in later discussions and the point from a PWLE that "the speakers be urged to orient discussions toward developing treatments" which was incorporated into instructions to the speakers.

It's difficult to capture all the specific examples where inclusion of PWLE ME/CFS as subject matter experts shaped the research priorities. Nevertheless, most PWLE reported that they felt that their inclusion allowed for a greater understanding by both sides of the vastness of areas to consider in developing a Research Roadmap for ME/CFS.

Orientation to the Working Groups and Clarity of the Process

NINDS staff learned a great deal from the PWLE MECFS planning committee members about how to optimally include PWLE in committees to define research priorities not only for MECFS but for disorders in general. Here we describe some of the lesson's learned from both successful processes and areas for improvement from the PWLE MECFS member perspective. Many PWLE SMEs were pleased with the process, "I've been amazed at how well the Nervous System Committee functioned, getting the webinar up and running in record time. I appreciate being able to listen in and then to contribute…". There were many positive comments about the process and PWLE SME inclusion and ideas for improvement.

Balancing Information Load with Procedural Clarity

The most prominent theme for improvement was around clarity of the process and roles. The organizing team strove to balance providing details and clarity and avoiding information overload (for PWLE SMEs and other SMEs).

"As time went on participation got easier as we started to orient to the process, but it would be good to have a clearer idea of the process and what to expect when we joined the group." "In

my planning group the PWLE felt unsure about how to participate and contribute" One great suggestion from a PWLE SME is to "provide more orientation to the process but make it optional. A recording would make it easier for people to participate. Keep in mind that for PWLE sitting on committees like this is a new experience."

Additional orientation and role definition would benefit all the committee members as scientists don't frequently work with people with different technical expertise. This engagement is a teachable skill. "The webinar experience has overall been positive— but sometimes it seemed unclear in which capacity lived experience members re supposed to engage. Researchers also didn't always seem confident in how to respond or engage with the lived experience aspect. Ask PWLE for their opinion and perspective and listen and apply, as in my experience it does not appear to be recognized."

"It would be nice if there was more transparency into the process - it's hard to understand what is going on". The weekly update email (a suggestion from one PWLE) was extremely appreciated by not just the PWLE SMEs but all the committee members and NINDS staff. Another area for improved clarity that all committee members needed was around how to use Box and other applications.

"It's hard to keep up" – When PWLE MECFS were invited to participate in this process we acknowledged that it would be highly technical and that as Lived Experience Subject Matter Experts they wouldn't be able to follow all topics covered (which is expected when adding any subject matter expert to a diverse group of technical experts). In the future it would be beneficial to continually repeat this as it's not fully appreciated. The chairs of the groups worked to find a balance between making the science accessible to the community and those observing and what one PWLE MECFS SME phrased the "importance that we are not just regurgitating past information."

Reiterating the Value of the PWLE SME Inclusion

The PWLE SMEs that we interviewed appreciated that we wanted their feedback about how we were doing at our efforts to include them in the process.

Some PWLE SMEs shared that more clarity is needed on why some comments and ideas are integrated while others are left out. Scientists have been trained to think and move through a problem in a specific way, and sometimes forget that the process isn't universal, so it is not clear to the whole group the reasoning behind why some ideas are pursued and others are not. It would enhance participation to provide assistance with explaining the decision making during these conversations. "If what is suggested by PWLE is not an ideal research priority for example, then share that with them and why, or else it is presumed our input didn't matter or count, which defeats the purpose of being involved as a PWLE, and the goal at least for me is to contribute, make a difference and expedite progress towards effective treatments and ultimately a cure."

Some PWLE SME felt like their participation was an afterthought or not as highly valued as the other SMEs. One suggested solution for this would be to conversations should start with PWLE not with researchers - ME charities in the UK have a list of priorities that started with PWLE.

Who to Include and How to Include PWLE

It was important from the beginning for committees to include multiple PWLE SMEs for diversity of perspective and in the words of one PWLE SME "include more PWLE that you think you'll need, because some promising appointees will drop out or not contribute, whether due to their health or their timidity."

One goal that organizers struggled with fully accomplishing was to include insight from PWLE that are considered "very severe". The severely impacted have important insights that are often left out because of the logistical difficulty around their inclusion. This leads to a lack of focus by scientists on the research that is needed to address the needs of this population. PWLE SMEs interviewed urged us to "Recognize that the very severe group of individuals with ME/CFS who are being left out of this research for PWLE and need to be included in some way, whether that is their caregiver assisting in their communication to advocate their needs and what they view as important, so they are included. Many severe are unable to do electronics and/or communicate but very little, so create platforms that are flexible and adaptable for involvement. Speak and communicate slowly and simply so that ALL levels of severity can process the information given, especially the highly technical information." This is not only important for this specific process but "clinicians have more exposure and tend to work with individuals with less severe disease" so increasing engagement will broaden the perspective of the scientists leading research.

Another aspect that is always difficult to balance is who within the community can/should speak for the community. Some PWLE appreciated when PWLE SMEs who were also researchers were active within the group, but it was also noted that often there is an overrepresentation of people with advanced degrees. It's easier and faster to incorporate those PWLE SMEs into the highly technical conversations than to include PWLE SMEs that do not have medical backgrounds but as one PWLE SME noted. "It's clear from online discussions that less-educated people feel mystified and/or alienated by the biomedical research process. If their voices aren't included somehow, this will probably continue - and researchers won't understand their lived experience, which may be different." Attempts were made to include people with different comfort and familiarity levels into the deliberations at the levels they were able to participate. Less intense options like participating in the IdeaScale or webinar Q&A were promoted to collect ideas and thoughts from PWLE MECFS that did not have the broader PWLE perspective to speak for the MECFS community. There was also a suggestion that using live voting during the webinars would provide another option for participation.
A longer-term solution is continually working to build more comfort from the whole community, giving all PWLE options to have more exposure to the science. One PWLE SME shared "I was also fortunate to virtually attend the recent "NIH NIAID Advancing ME/CFS Research: Identifying Targets for Intervention and Learning from Long COVID", which offered more live interaction among both physical and virtual attendees. Many comments were made

and positive things that were presented and/or learned, in a strong atmosphere sharing and partnering in what is to come in ME/CFS Research." These types of experiences help to build the understanding between PWLE MECFS and scientists that can allow for more productive partnerships.

Balancing Urgency with Diligence and Simplicity with Specificity

Balancing time and intensity of this effort was a contestant challenge throughout this process. The organizers battled with the need to work swiftly to spur progress while also brining along all stakeholders. This meant balancing the need to cover all relevant topics, research, and SMEs with the need to limit the time and intensity of the work for PWLE MECFS whose condition limits their ability to participate. While this compromised both, there was a continual effort throughout to find the best balance and this should continue to be a top priority not only with this community but with other neurological disorder PWLE SMEs.

Furthermore because of this battle with time, the speakers time was limited and PWLE wanted more time to share their experiences, though one PWLE noted that "many PWLE are looking for more of a support group feel" and this could potentially be addressed in the future with a separate venue for PWLE to collect their thoughts as a community. One suggestion from a PWLE member was to collect in advance the It would be nice if PWLE could each put together a 1-page background on their experience to give the researchers. Another suggested "working more actively with non-profit advocacy groups to collect and summarize comments from PWLE."

Another timing that required balance included the need to hold conversations during working hours when many working PWLE were not able to participate. This was alleviated by providing the recordings after each webinar and collecting comments and questions via IdeaScale and email. Some PWLE MECFS SMEs shared that they would have liked to be there in person but most expressed that in person meeting would be impossible for most PWLE MECFS.

While I got to speak my spiel for a few minutes, for me these presentations mostly told people what everyone already knew. Almost everything novel was N=1. In contrast, online discussion forums include the experiences of many more people, and it would be productive for researchers to *systematically* consult this rich resource - then report on this publicly in meetings.

Appendix 3: Webinar Agendas

Eight Webinars were held between August 25, 2023, and January 11, 2024. They are not listed in the order of occurrence, but rather in the order in which the webinar reports are ordered in the overall report.

Chronic Infection Webinar
November 30, 2023
Webinar Chair: Maureen Hanson, PhD; Cornell University
Webinar Moderator: Vicky Whittemore, PhD; NIH/NINDS

Agenda

Introduction
Vicky Whittemore, PhD; NIH/NINDS

Lived Experience
David Holcomb

Chronic Infections in Long COVID
Michael Peluso, MD, MHS; University of California, San Francisco

Chronic Infection in ME/CFS (Other than Herpesviruses)
Maureen Hanson, PhD; Cornell University

Infection/ Reactivation of Herpesviruses and ME/CFS
Anthony Komaroff, MD; Harvard

Endogenous Retroviruses and ME/CFS
Simon Carding, PhD; The Quadrum Institute, University of East Anglia

Panel Discussion
Vicky Whittemore, PhD; NIH/NINDS

Immunology Webinar
October 19, 2023
Webinar Chair: Derya Unutmaz, MD; Jackson Laboratories
Webinar Moderator: Vicky Whittemore, PhD; NIH/NINDS

Agenda

Introduction
Vicky Whittemore, PhD; NIH/NINDS

Clinical Immunology of ME/CFS
Nancy Klimas, MD; Nova Southeastern University

Evidence for Autoimmunity in ME/CFS
Carmen Scheibenbogen, MD; Charité-Universitätsmedizin Berlin

Immune Cell-type Approaches to Identify Mechanisms of ME/CFS
Maureen Hanson, PhD; Cornell University

Predictive and Mechanistic Insights into Immune Perturbations During ME/CFS
Derya Unutmaz, MD; Jackson Laboratories

Gut-Immune-Metabolic Interplay in ME/CFS
Armin Alaedini, PhD; Columbia University

Lived Experience
Angela Termini

Lived Experience
Tracy Duvall

Discussion: Research Priorities - what is clinically translatable?
Moderator: Vicky Whittemore, PhD; NIH/NINDS

Closing Remarks
Vicky Whittemore, PhD; NIH/NINDS

Nervous System Webinar
August 25, 2023
Webinar Chair: Jarred Younger, PhD; University of Alabama, Birmingham
Webinar Moderator: Vicky Whittemore, PhD; NIH/NINDS

Agenda

Introduction
Vicky Whittemore, PhD; NIH/NINDS

Lived Experience
Trisha Fisher

Cognition

Gudrun Lange, PhD; Pain & Fatigue Study Center, Mt. Sinai

Dysautonomia
Peter Rowe, MD; Johns Hopkins Medicine

Cerebral Spinal Fluid Studies
Jonas Bergquist, MD; PhD; Uppsala University

Neuroimaging
Jarred Younger, PhD; University of Alabama, Birmingham

Sleep
Janet Mullington, PhD; Harvard Medical School

Peripheral Nervous System
Peter Novak, MD, PhD; Brigham and Women's Hospital

Panel Discussion
Moderator: Vicky Whittemore, PhD; NIH/NINDS

Closing Remarks
Jarred Younger, PhD; University of Alabama, Birmingham & Vicky Whittemore, PhD; NIH/NINDS

Circulation Webinar
January 11, 2024
Webinar Chair: David Systrom, MD; Brigham & Women's Hospital Harvard
Webinar Moderator: Vicky Whittemore, PhD; NIH/NINDS

Agenda

Introduction
Vicky Whittemore, PhD; NIH/NINDS

Lived Experience
Gwynn Dujardin

Endotheliitis
Jane Mitchell, PhD; Imperial College London

Microclots
Resia Pretorius, PhD; Stellenbosch University

Hypovolemia

Frans Visser, MD; Stichting Cardiozorg

Cerebral Blood Flow
Linda Van Campen, MD; Stichting Cardiozorg

Red Blood Cell Abnormalities
Jiandi Wan, PhD; University of California, Davis

Neurovascular Dysregulation
David Systrom, Jr, MD; Brigham & Women's Hospital Harvard

Panel Discussion
Moderator: Vicky Whittemore, PhD; NIH/NINDS

Metabolism Webinar
October 26, 2023
Webinar Chair: Alain Moreau, PhD; University de Montreal
Webinar Moderator: Vicky Whittemore, PhD; NIH/NINDS

Agenda

Introduction
Vicky Whittemore PhD

Lived Perspective of an Individual with ME/CFS – Progress Made via Self-education, Determination, Self-tracking, and Analysis
Chris Wikman

The Interplay Between Metabolism and Immunology in ME/CFS
Shuzhao Li, PhD; The Jackson Laboratory

Investigations and Consequences of Altered Metabolism in ME/CFS Immune Cells
Jessica Maya, PhD; Cornell University

Single-Cell Raman Technologies for Diagnosis and Investigation of ME/CFS Immune Cells
Jiabao Xu, PhD; University of Glasgow

Studying Metabolomics in ME/CFS - A Computational Perspective
Wenzhong Xiao, PhD; Massachusetts General Hospital Harvard

Microbial Metabolism in ME/CFS Pathogenesis: The State of Supporting Evidence and Prevailing Knowledge Gaps
Brent Williams, PhD; Columbia University

Metabolic Characterization of Biofluids in ME/CFS
Chris Armstrong, PhD; The University of Melbourne

Closing Remarks
Vicky Whittemore, PhD; NIH/NINDS

Physiology Webinar
December 8, 2023
Webinar Chair: H. Craig Heller, PhD; Stanford University
Webinar Moderator: Vicky Whittemore, PhD; NIH/NINDS

Agenda

Introduction
Vicky Whittemore, PhD; NIH/NINDS

Illuminating Whole Body Immune Responses in ME/CFS Using PET
Michele James, PhD; Stanford University

The Cell Danger Response
Robert Naviaux, MD, PhD; University of California, San Diego

Update on the Itaconate Shunt Hypothesis
Rob Phair, PhD; Integrative Informatics

Lived Experience: Searching for Mechanisms of ME/CFS in Other Conditions
Dominic Stanculescu; Independent Researcher, Belgium

Consensus report on what is refreshing sleep?
Rebecca Robbins, PhD; Harvard Medical School

Non-refreshing Sleep
Maiken Nedergaard, MD, DMSc; University of Rochester and University of Copenhagen

Metabolism and ME/CFS
Karl Tronstad, PhD; University of Bergen

BH4: A Potential Culprit
Ron Davis, PhD; Stanford University

Extracellular Vesicles
Ludovic Giloteaux, PhD; Cornell University

Panel Discussion
Moderator: Vicky Whittemore, PhD; NIH/NINDS

Less Studied Pathologies in ME/CFS
January 5, 2024
Webinar Chair: Beth Pollack, MIT
Webinar Moderator: Vicky Whittemore, PhD; NIH/NINDS

Agenda

Introductions
Vicky Whittemore, PhD; NIH/NINDS & Beth Pollack; MIT

Research Overview of Pathologies and Co-morbidities
Beth Pollack; MIT

Connective Tissue Disorders and Cranio-cervical Instability in ME/CFS
Ilene Ruhoy, MD, PhD; Mount Sinai South Nassau

Mast Cell Activation Disorders
Anne Maitland, MD, PhD; Mount Sinai

Lived Experience
Julie Rehmeyer

Gastrointestinal Issues in ME/CFS
Laura Pace, MD, PhD; University of Utah

Introduction to Reproductive Health Conditions in ME/CFS
Beth Pollack; MIT

Lived Experience
Emelia von Saltza

Reproductive Health Conditions in ME/CFS
Roumiana Boneva, MD, PhD; CDC & Elizabeth Unger, MD, PhD; CDC

Panel Discussion
Moderator: Vicky Whittemore, PhD; NIH/NINDS

Closing Remarks
Beth Pollack, MIT & Vicky Whittemore, PhD; NIH/NINDS

Genomics/Genetic Susceptibility Webinar
November 1, 2023
Webinar Chair: Oved Amitay, Solve ME/CFS Initiative
Webinar Moderator: Vicky Whittemore, PhD; NIH/NINDS

Agenda

Introductions
Vicky Whittemore, PhD; NIH/NINDS & Oved Amitay, Solve ME/CFS Initiative

Lived Experience
Hayla Sluss, University of Massachusetts

Session 1: Large Datasets and Genome Wide Association Studies (GWAS)

Genetic Risk Factors of ME/CFS: A Critical Review (GWAS)
Chris Ponting, DPhil; University of Edinburgh

Combinatorial analysis of Genetic Risk Factors for ME/CFS, UK Biobank
Steve Gardner, PhD; PrecisionLife

Genetic Susceptibility in Long COVID and ME/CFS: Long COVID Host Genetics Initiative (International Network)
Hanna Ollila, PhD; Stanford University/Harvard University
Vilma Lammi, PhD; Institute for Molecular Medicine Finland, University of Helsinki
Anniin Maria Tervi; Institute for Molecular Medicine Finland, University of Helsinki

Session 2: Enriched Cohorts and Epigenetics

Characterizing the Genetic Basis of ME/CFS through Case-Control and Family Studies
Fereshteh Jahaniani, PharmD, PhD; Stanford University
Varuna Chander, PhD; Stanford University

Contribution of Epigenomics to ME/CFS Pathogenesis: Past, Present and Future
Alain Moreau, PhD; Université de Montréal

Summary and Closing Remarks
Kristina Allen-Brady, PhD, MSPT, MPT; University of Utah

Appendix 4: Research Priorities from the Community

The research priorities listed below were submitted via the crowdsourcing tool, IdeaScale, or via email.

Chronic Infection Research Priorities

- Expand research using tissue studies of the GI-tract including immunoassays and imaging to investigate locations of immune activity (B-cells, macrophages, CD-4, CD-8, T-regs, etc.) in the tissue microenvironment.
- Expand on research using EBV and HHV-6 tissue studies of the upper GI tract including immunoassay and imaging to investigate locations of immune activity (B-cells, macrophages, CD-4, CD-8, T-regs, etc.) in the microenvironment.
- Investigate the role of EBV reactivation in ME/CFS.
- Identify where the pathogen reservoirs are located and identify treatments to eliminate them.
- Determine the types of tissues infected by Candida, its relationship to the gluten antigen, and its role in chronic immune activation, leaky gut, and microbiome dysfunction.
- Identify specific HERVs that are important and correlate the most common ones to physiological states or processes in different subtypes of individuals with ME/CFS.
- Determine why ERVs are expressed in some individuals, but not others and why HERV activation in immune dysfunction is non-uniform.
- Identify the history of infection for individuals with ME/CFS and how the type and number of past infections impacts current immune functioning.
- Investigate polyclonal hypergammaglobulinemia and hypogammaglobulinemia to determine their cause, compare their pathophysiology and impact on immune system functioning, and identify appropriate treatments.
- Define adaptive immune responses (B and T cells) in individuals with ME/CFS, screen them against epitope libraries from viruses and other pathogens to look for homology between target pathogen epitopes and potential autoantigens to identify persistent viral antigens that may be promoting inflammatory responses contributing to disease symptoms.
- Consider a clinical trial of peptide Thymosin Alpha 1 as treatment to improve pathological levels of fatigue.
- Targeted clinical trials should be prioritized, particularly by drug repurposing.
- A survey of what treatments have shown benefit for people with ME/CFS would help to determine which clinical trials to prioritize to support studies that will improve their functional capacity.

- Determine the role viruses continue to play in the perpetuation of the illness.
- Apply recent research findings that residual SARS-CoV2 virus and viral fragments are active in Long-COVID and live in viral reservoirs in tissues in the body to determine if this is also occurring in ME/CFS.
- Determine if other pathogens, including Bartonella and Parvovirus B19, are playing a role in the pathophysiology of ME/CFS.
- Utilize data and artificial intelligence from a large cohort of individuals with ME/CFS to gain insights from journaling food intake, supplement trials, stress and ME/CFS symptoms to correlate symptom flares and remissions to specific types of foods (and alcohol intake) and discover which supplements/treatments are beneficial.
- Developing experimental subtype definitions beyond established ME/CFS criteria to allow more closely defined cohorts with distinct laboratory detected traits, which would reduce heterogeneity and improve signal to noise in results and reproduction attempts.
- Develop studies that tie findings of immune dysregulation and persist antigen back to the disease symptoms.
- Investigate the gut-brain axis and the role of the vagus nerve in ME/CFS.
- Learn from the research and clinical trials for Long COVID to determine what might be the best treatments for ME/CFS.
- Develop targeted clinical trials of anti-fungals, anti-virals, anti-bacterial in well characterized cohorts of individuals with ME/CFS.
- Investigate the role of fungi, yeast, bacteria, toxins, and endotoxins in ME/CFS pathophysiology and develop targeted treatments.
- Determine the role that various stressors acting alone or together with an infectious agent, are causing ME/CFS.
- Develop studies that look across chronic post-infectious diseases to determine the common pathways, or how they differ depending on the infectious agent.
- Study families where more two or more individuals in the family have ME/CFS to determine the potential trigger, genetic susceptibility, etc.
- Investigate the differences between individuals who had COVID, who developed Long COVID and those with Long COVID who also meet the diagnostic criteria for ME/CFS and compare these groups to individuals who had ME/CFS before 2020.

Immune System Research Priorities

- Investigate vaccine intolerance in a subset of individuals with ME/CFS – identify the cause, develop preventive measures and treatments.
- Investigate Inborn Errors of Immunity (Primary Immune Deficiencies), a known comorbidity in a subset of individuals with ME/CFS (COVID, IgA Deficiency, etc.), treat the condition when possible and study its impact on functioning and quality of life.
- Investigate the Interferon Genetic Signature in individuals with ME/CFS.

- Investigate an Immune Activation Test that gauges the system's antibody load, the aggregate number of antibodies from infection exposures, autoimmune processes, epitope spreading, and other factors that activate the immune system in real-time and cause it to consume the body's resources.
- Investigate the role of autoantibodies and if eliminating autoantibodies lowers the total antibody load and potentially preventing downstream immune cell exhaustion, neurologic inflammation, metabolic dysfunction, and tissue destruction from the chronic state of immune activation an infinite loop driven by a constant supply of autoantibodies.
- Investigate the impact of normal and abnormal aging of immune system functioning in individuals with ME/CFS across the life span.
- Identify the target antigens recognized by 'exhausted' T cells to clarify drivers of this exhaustion, and understanding if the immune system is responding to foreign or self-antigens (or both) to guide the use of existing immune-modulating therapies in clinical trials.
- Design clinical trials that target dysfunctional CD8 T-cells with severe deficiencies in production of IFNg and TNFa.
- Potential treatment options are already widely researched so this is an area where targeted clinical trials should be prioritized, particularly by repurposing FDA approved drugs.
- Investigate how the innate immune system gets rewired in ME/CFS and how this can be treated and prevented.
- Identify the groups of cells activated by mast cells and confirm their correlation with ME/CFS viral and bacterial infections.
- Study hyperbaric oxygen therapy (HBOT) and its anti-inflammatory effects in MECFS.
- Utilize Artificial Intelligence (A/I) to find existing drugs that could help MECFS stratify the individuals by immunologic subsets.
- Investigate the role multiple viral infections of macrophages in ME/CFS.
- Investigate the crosstalk between the circadian clock and macrophages (which may explain the night-time condition improvement in ME/CFS).
- Investigate how recovery from exertion may be immune-mediated.
- Study T-regs at different times in disease (PEM after physical exertion and baseline).
- Investigate the interaction of the sensory nervous system and immune cells in MECFS.
- Determine if there is a cohort of individuals with ME/CFS that meet the biomarkers for anti-inflammatory drugs and assess their effectiveness in ME/CFS.
- Design precision therapies tailored to specific subsets of individuals with ME/CFS.
- Utilize urine as a biospecimen to investigate the role of mast cell activation (not just blood).
- Investigate the occurrence of autoimmune disorders in family members of individuals with ME/CFS and determine if there is a shared underlying pathology.
- Investigate how multiple hits with different pathogens impacts the immune system, and the role of reactivation of viruses in ME/CFS.

- Initiate studies to determine the role and impact of different living environments on the immune system and overall health of individuals with ME/CFS.
- Identify why some individuals with ME/CFS sustain recurrent infections and others do not to understand how this relates to latent infection being periodically reactivated and what that activation looks like symptomatically.
- Identify yet unknown viruses (but also perhaps bacteria and fungi) that may linger and lay dormant but also easily activated leading to chronic infections. And further to know whether chronic infections are the same predator being constantly reactivated, or different pathogens.
- Continue to explore the role of TRPM in ME/CFS and ensure that all TRPM research utilizes stratification by strictest criteria (ICC), gender, type of onset, pre/post menopause, severity of illness, etc.
- Determine if there is increased complement activation as well as high expression of STING, cGas, and IFN-α in individuals with ME/CFS as has been demonstrated in Long COVID and determine if these pathways should be enhanced or inhibited and what is causing them to be highly expressed.
- Investigate chronic antigen exposure, i.e., if there is an antigen we need to decipher if it is one or many and be able to do this at a personalized level for each individual with ME/CFS. However, we need to know if it's the antigen itself causing ME or if the antigen is a bioproduct of some other process in the body gone.
- Consider a clinical trial of immunotherapy/CAR-T cell therapy for ME/CFS.
- Develop collaborations with biotech to investigate treatments using immunometabolism drugs that are either approved or in their pipeline.
- Determine if helminthic therapy would be effective to treat symptoms of ME/CFS.
- Investigate those individuals with ME/CFS whose health significantly improved after a specific COVID vaccine to understand this response and who may benefit from this type of treatment.
- Initiate rigorous studies of integrative medicine approaches to evaluate the efficacy and specific mechanisms that may help with development of more effective therapies.
- There is a significant need for a test for EBV reactivation that is widely available.
- There is a significant need for validated biomarker(s), lab tests and animal models for ME/CFS to advance research.
- Determine the efficacy and impact of the use of specific diets on the immune system and overall health of individuals with ME/CFS.

Nervous System Research Priorities

- Investigate the connection between intracranial hypertension (without papilledema) and cerebrospinal fluid leaks and how this process could account for the physical and cognitive dysfunction in ME/CFS.
- Study periodic paralysis and symptoms resulting in no diagnosis or Functional Neurological Disorder diagnosis in severely ill individuals with ME/CFS.

- Investigate paresthesia's, temporary paralyses of body and limbs, breathing paralysis, numbness, and tingling, fasciculations, gait walk and sometimes falls, because of lack of control and sudden weakness of the legs.
- Identify if antigen-specific immune responses are driving neuroinflammation, or if it is more of a non-specific innate immune response and connecting neuroinflammation defined by brain imaging to peripheral immune responses.
- Investigate the relationship between autonomic dysfunction and ME/CFS symptoms, such as the significant increase in heart rate upon standing and tilting observed in individuals with ME/CFS to unravel the complexities of this condition.
- Investigate the role of structural issues and compression syndromes of the brainstem, vagus nerve, accessory nerve, and blockage of venous outflow at the level of the upper cervical vertebrae in ME/CFS.
- Identify atypical presentation of structural conditions using CT myelogram or other novel imaging techniques.
- Investigate the role that dysfunctional neuronal signaling plays in causing POTS in individuals with ME/CFS and develop targeted treatments.
- Identify changes in brain metabolism and the root cause of these changes.
- Investigate the cause of extreme and debilitating extreme light and sound sensitivity in ME/CFS and develop targeted treatments.
- Run a contest to collect ideas for the underlying pathologies that might explain all the symptoms of PEM.
- Investigate small fiber neuropathy and determine if it is a causative mechanism of ME/CFS and Long COVID.
- Investigate causes of PEM and include clinical trials to ameliorate this debilitating aspect of ME/CFS.
- Investigate the role of low spectral power in the nervous system and how it may contribute to the understanding of symptoms in ME/CFS.
- Investigate the role of disordered sleep and inability to sleep in individuals with ME/CFS and how sleep patterns change over time and length of illness.
- Identify treatments for insomnia and unrefreshing sleep for individuals with ME/CFS.
- Investigate the common symptom of "wired and tired" – the imbalance with the parasympathetic nervous system - to determine the cause and identify treatments.
- Investigate activation of microglia cells in the brain in ME/CFS.
- Identify the specific pathology or diagnostic in ME/CFS that will lead to successful clinical trials.
- Expand the use of neuroimmune imaging in ME/CFS to explore CNS and PNS involvement in ME/CFS.
- Design studies that monitor the nervous system 24-72 hours after exertion to identify the changes that result in PEM.

- Perform a longitudinal observational study of individuals with POTS with small fiber neuropathy and alpha synuclein.
- Investigate the prevalence and cause(s) of reduced brain blood flow velocity.
- Investigate the prevalence of dementia and Alzheimer's Disease in individuals with ME/CFS.
- Investigate if dysfunction of inhibitory neurons and what neural circuits cause an over excited state and subsequent neurotoxicity that is the basis for the myriad of symptoms associated with PEM - light and sound intolerance, dizziness, tinnitus, nausea, headache, orthostatic intolerance, exhaustion, and pain.
- Investigate if dysfunction in how astrocytes regulate glutamate toxicity plays a role in ME/CFS.
- Identify the cause of fluctuation in cognitive function in efforts to identify patterns associated with more functional and less functional days.
- Research studies need to consider the energy depletion incurred to participate in the study and include ways to assess it.
- Find or develop tests/instruments that can be used several times during a study, for example before and after exertion, on a good day and on a bad day, at the beginning of a session and when energy declines.
- Investigate the association between POTS or orthostatic intolerance and mast cells that is seen in some, but not all individuals with ME/CFS.
- Investigate if blood brain barrier (BBB) permeability changes following exertion in individuals with ME/CFS and what causes changes in BBB permeability to develop targeted treatments.
- Investigate if neuron fusion occurs in ME/CFS (as has been shown for Long COVID) and if it is the cause of the electrical arcs in the brain experienced by individuals with ME/CFS.
- Investigate if IRG1 is upregulated in neurons in the brain in individuals with ME/CFS.
- Determine if there is a link between CCL11 and brain fog in ME/CFS as has been shown for Long COVID.
- Determine if there is evidence of immune cell infiltration in the CNS and the presence of abnormally behaving cells such as NKG2D+CD8+ T cells that may cause the symptoms of ME/CFS.
- Investigate the bidirectional relationship of the gut and the brain in ME/CFS, as well as any dysregulations in the enteric nervous system and the vagus nerve.
- Further elucidate the role of the HPA-axis and the Kyneurine pathway in ME/CFS.
- Investigate the role of dysfunctional afferent nerve signaling in hypersensitivity to any exogenous and endogenous stimuli which may be a key feature of ME/CSF pathophysiology.
- Further investigate the role of reduced CSF catecholamines in ME/CFS and the role this plays in orthostatic intolerance (via impaired neurovascular sympathetic tone), cognitive impairment (reduced arousal), and sleep cycle regulation via neuroimmune interactions.

- Investigate the role the ventral brain (the upstream ‘gateway hub’ from the body to the dorsal or upper brain) plays in ME/CFS.
- Initiate rigorous clinical studies to determine if “brain retraining “is a beneficial intervention for individuals with ME/CFS.
- Investigate if a damaged/missing circulating protein could result in a synapse type exhibiting vague, semi repeatable malfunction which seemed to evade a controlled experiment and if each common synapse type would likely have a discernible syndrome associated with destruction of the circulating protein which carried its neurotransmitter.
- Design studies to differentiate between signs and symptoms of ME/CFS that may seem indicative of an "oversensitive" or "hyperactive" nervous system but could instead be related to other conditions such as autonomic dysfunction or small fiber neuropathy so as not to misinterpret these symptoms as solely due to central sensitization.
- Investigate the triggers and causes of PEM including neuroinflammation and its effects on the autonomic nervous system, potential reduction in cerebral blood flow, and the significance of reduced catecholamines in CSF so that targeted treatments can be developed.
- Investigate if there are similarities in the pathophysiology of ME/CFS and migraines that explain the overlapping symptoms of sensitivity and pain.
- Identify the cause of extreme muscle pain experienced by some individuals with ME/CFS so that targeted treatments can be developed.
- Empower investigators to think outside the box and identify the novel disease mechanism that is underlying the cause of ME/CFS (and potentially other diseases such as Long COVID).
- Leverage data being collected on wearables by individuals with ME/CFS to better understand the subtypes of disease and the symptoms experienced by individuals with the disease.
- Empower a cross discipline team to do a "whiteboard exercise" to explore brain/brainstem inflammation as the possible root cause of the major known symptoms (PEM) of ME/CFS.
- Investigate whether PEM is a protective or immune system state triggered by an intrusion into the brain or brainstem by infections that attack the CNS and have crossed the blood brain barrier.
- Investigate whether there are markers of neuroinflammation and/or viruses in the cerebrospinal fluid of individuals with ME/CFS.
- Investigate dorsal root ganglionitis and (viral) infections/inflammation in or around the Vagus Nerve from autopsies from individuals with ME/CFS and test drugs or other options in trials to target these infections/inflammations in the spinal cord/dorsal roots/vagus nerve.

- Design studies utilizing cognitive tests to include studies done at baseline, immediately after physical exertion like a bicycle test, and during PEM (perhaps the day after the physical exertion), after an orthostatic stress like a tilt table test.
- Use longer tests for up to an hour to test for mental fatigue and perhaps PEM after mental exertion, not small duration tests (5-10 minutes).
- Determine if administering a physical exertion after a long duration cognitive task makes it more difficult to complete the task and if it hastens PEM.
- Design a robust, well powered qEEG study, with analysis of different brain areas to investigate theta waves and investigate the deep anatomical origin from which they generate, which could provide clues pointing to the pathophysiological mechanism.
- Determine if neuromodulatory treatments would benefit individuals with ME/CFS.
- Include individuals with ME/CFS who had a gradual onset of the disease, in future studies and clinical trials.
- Determine if intranasal delivery of IgG or a similar 'medicine' can reduce neuroinflammation in the brain enough to cause significant symptom relief for individuals with ME/CFS.
- Include individuals with well-controlled co-morbidities (such as anxiety or OCD) in neuro-imaging studies or trials.
- Investigate whether ME/CFS is an ion channel disease (channelopathy).
- Research studies of Eastern medicine should be explored for symptom mitigation such as acupuncture reflexology, and Thai massage.
- Initiate basic and clinical research on the effects of glymphatic clearance on the core symptomology of ME/CFS: fatigue, brain fog, chronic insomnia, PEM, POTS/OI. Disruptions of glymphatic clearance, autonomic functions and cerebral hypoperfusions could potentially form self-sustaining vicious cycles.
- Perform a large, rigorous PET imaging study in individuals who have PEM compared to when they do not to conclusively determine if neuroinflammation is occurring in ME/CFS and its role in PEM.
- Determine if blocking or destruction of the neuroglobin, but only when its oxygen binding site is free, on a very large number of neurons is a possible cause for PEM/PESE.
- Utilize the appropriate cognitive tests to assess individuals with ME/CFS, and consider their current health status, educational level, socioeconomic status, etc.
- Clinical trials of medications being used for orthostatic intolerance and dysautonomia to test their efficacy that would lead to better access to these medications.
- Develop a data repository where individuals with ME/CFS can upload their labs, imaging studies, etc. for use in research.
- Investigate neurotransmitter (potentially glutamate and serotonin) dysfunction, likely a downstream effect of mastic cell/immune system, epithelial/vascular and/or structural issues in MECFS to develop treatments for neurotransmitter homeostasis issues that could prove to greatly increase quality of life, save neurons from death and disease, and

may address symptoms including severe overstimulation, inability to process sounds, lights, movement, or other sensory stimulation, myoclonus, hypereflexia, insomnia, vestibular dysfunction, nausea, pain, terror attacks, autonomic dysfunction.
- Investigate how the vagus nerve modulates the sickness response due to local and specific infectious agents.
- Expand robust sample biorepositories of postmortem nervous system tissue for research purposes.
- Utilize animal models to address research issues that are not possible to address in human subjects.

Circulation Research Priorities

- Investigate the cause, treatment efficacy and prevention of hypovolemia, and include testing of all individuals with ME/CFS in clinical workups.
- Develop a diagnostic test for red blood cell deformity and carry out clinical trials to determine the impact on red blood cells with treatments such as anti-infective drugs, vitamin B12, etc.
- Identify the role of micro clotting in ME/CFS and identify treatments and preventative strategies.
- Examine the interrelationships of the affected systems in ME/CFS. We need a more integrated, comprehensive approach to treatment that addresses the complex interactions between systems.
- Investigate the decoupling between fluid status and blood pressure in various physiologic states like POTS in individuals with ME/CFS and identify effective treatments.
- Investigate the use of an external sensor to measure PA pressure to detect fluid depleted states in ME/CFS and determine if the measures correlate with the invasive methods used in CPETs.
- Explore the potential of clinical trials with normal saline infusion for those early in their disease course.
- Implement extracranial Doppler cerebral blood flow measurement as a standard procedure to diagnose ME/CFS in addition to other diagnostic criteria and make it a standardized test that is included in guides for clinicians.
- Prioritize research on the role of mitochondrial dysfunction in reduced O_2 uptake and in symptomatology of ME/CFS.
- Utilize wearable to study objective outcome measures of blood flow to the head in MECFS research and determine how it relates to or may be affected by orthostatics, particularly in nervous system and circulation research.
- Carry out studies of blood cells to identify problems with shape and deformability on a large population of individuals with ME/CFS which may help identify subtypes or different presentations of disease.
- Study reduced brain blood flow velocity in individuals with ME/CFS with and without POTS and investigate treatments for orthostatic intolerance in both groups.

- Investigate if neurological symptoms such as pain, tingling, restless legs are related to circulatory issues in ME/CFS.
- Identify the role of circulatory issues, neurological issues, MCAS, and structural issues in causing headaches and identify effective treatments.
- Determine the cause of transient or permanent swollen lymph nodes and identify effective treatments.
- Investigate the presence, cause and significance of vast inflammation or other potential issues in the bone marrow of individuals with ME/CFS and identify effective treatment to resolve the inflammation.
- Investigate endothelial cell dysfunction and its relationship to bone marrow inflammation.

Metabolism Research Priorities

- Identify the role of the ambient concentration of CO2 in air or the room in ME/CFS and utilize these measures in metabolic studies.
- PEM is one of the core symptoms of ME/CFS and needs to be accounted for in the inclusion criteria for any metabolic research studies conducted.
- There is a critical need to examine the interrelationships of the affected systems in ME/CFS.
- Develop therapeutics targeting the inhibition of WASF3, ER stress pathways, and mitochondrial dysfunction, and their effect on PEM in individuals with ME/CFS, measured with improvement in 2-day CPET.
- Identify if calcium channelopathies are involved in the endoplasmic reticulum stress response pathway that causes the overexpression of WASF3 and mitochondrial dysfunction in ME/CFS.
- Investigate impairment in glycolysis in individuals with ME/CFS and identify treatment targets.
- Investigate whether the itaconate shunt hypothesis is impacting individuals with ME/CFS.
- Evaluate the Kynurenine pathway in individuals with MECFS of all severities and length of disease.
- Explore glucocorticoid resistance at the level of the receptor or other targets in the pathway, not just cortisol levels.
- Investigate the role of xenobiotics and how diets, medication, environmental exposures are involved in disease onset and to understanding the cause(s) of post-exertional malaise or PEM, which could be triggered by various activities or exposures.
- Combine systematic approaches involving metabolomics, systematic immune profiling, transcriptomics, and other omics approaches, with comprehensive symptomatology

collections and time-series clinical measurements (e.g., blood sugars) to investigate the nature of the post-exertional malaise.

- Utilize Single-Cell Raman technologies to Identify metabolomic biomarkers for disease diagnosis.
- Investigate tissue metabolism in muscles and in the Central Nervous System (CNS) to identify metabolomic biomarkers for disease diagnosis.
- Identify objective metabolite markers that can be used to measure disease improvement.
- Implement tracking of symptoms of ME/CFS for enhanced clustering and delivering personalized medicine.
- Expand existing research to explore the theory that people with ME/CFS are in a state of Dauer or a hypometabolic state.
- Investigate whether PEM could be caused by two or more distinct phenomena: 1) a systemic inflammatory event, such as might occur with ischemia-reperfusion or mast cell activation, that causes widespread vascular damage, lowering individuals' baselines substantially and triggering an extended period of fatigue, pain, and nausea; 2) PEM as a result of the damage caused by an inflammatory event.
- Utilize qualitative and mixed methods research to investigate whether and to what extent a relapse-recovery pattern exists in ME/CFS and Long COVID.
- Conduct longitudinal research on how people's experiences of post-exertional malaise change over time and length of disease.
- Explore if and how vascular healing is slowed in ME/CFS by connective tissue disorders.
- Investigate sulfur metabolism in ME/CFS and how disturbance in sulfur metabolism in ME/CFS may impact DHEA-S and catecholamine levels, levels of homocysteine in cerebrospinal fluid, and sensitivity to medications (sulfation affects drug detoxification).
- Expand studies of fecal metabolomics in ME/CFS.
- Investigate the connection between brain metabolism and visual impairment in ME/CFS. Along with cognitive impairments, disabling vision problems are common in ME, worsening in PEM episodes.
- Continue and expand research on mitochondrial function and the role of the immune system on mitochondrial function in ME/CFS.
- Explore the role of melatonin and its role in sleep disorders in ME/CFS, as well as its impact on other symptoms associated with the disease.
- Carry out clinical trials with compounds that enhance aerobic oxidation in mammals.
- Investigate UPR/endoplasmic reticulum stress as a mechanism for translating a lot of stresses into PEM.
- Investigate individuals with ME/CFS who have endometriosis as an ME/CFS subtype with increased insulin sensitivity to gain insights into the pathophysiology of the disease.
- Investigate the role of hypoglycemia in the occurrence of POTS in individuals with ME/CFS.

- Make available a list of known metabolic differences in individuals with ME/CFS and currently available lab tests that are generally different to assist individuals with ME/CFS in obtaining the appropriate lab tests.

Physiology Research Priorities

- Address the challenges of the multisystem heterogeneous symptoms and population with whole body research.
- Utilize existing databases at VA, All of US, etc. for additional data to uncover information about the course of illness, variety of comorbidities, and to develop personalized treatments.
- Develop large longitudinal studies of individuals with ME/CFS across all age groups, i.e., children, adults, and seniors and across all illness severity groups, i.e., mild, moderate, and severe stratified by gender comparing groups to each other and to healthy matched controls to inform precision and personalized diagnoses and treatments.
- Include individuals with ME/CFS in the Integrated Personal Omics Profiling Studies at Stanford University to address challenges of heterogenous presentation.
- Develop an ME/CFS Symptoms Data Base to manage the challenge of ME/CFS's multitude of symptoms of varying severity.
- Develop an ME/CFS Decision-Making Support System for clinicians to address the challenge of ME/CFS's multisystem heterogenous presentation.
- Investigate thyroid dysregulation in ME/CFS comorbidity to understand its relationship to inflammation, dysregulation of the HPA axis, pain, carpal tunnel, tissue destruction and possible surgery.
- Develop educational videos for new researchers to the ME/CFS field, clinicians, individuals with ME/CFS, families, caregivers, and advocates, university professors, medical and biomedical students, and new researchers to the field its purpose to describe current ME/CFS research, ME/CFS's multisystem heterogenous presentation, history, epidemiology including outbreaks, symptoms, treatments, research priorities, etc.
- Investigators should carefully consider inclusion criteria for their ME/CFS research studies. Individuals with ME/CFS who have experienced physiological triggers (e.g., spinal injury, head trauma) or environmental factors may potentially fall into a different subtype from infection-associated forms of the disease.
- Investigate the high overlap between ME/CFS and connective tissue diseases and the potential subtyping opportunities.
- Develop consensus for use of case definitions in all ME/CFS research, i.e., the ICC and IOM definitions (not Fukuda).
- Initiate physiological research on PEM.
- Investigate the prolonged prodromal period, i.e., a long period between full-blown ME and the most probable infectious trigger experienced by at least 1/3 of individuals with ME/CFS and identify methods to identify and study individuals who are “pre-ME/CFS”.
- Investigate immune network analysis of CSF in individuals with ME/CFS with atypical and classical presentations.

- Investigate how eATP signaling contributes to fatigue and pain, particularly in delayed onset muscle soreness (DOMS) and chronic fatigue syndromes.
- Investigate the overlaps in mechanisms of illnesses induced by physical, infectious, and/or emotional stressors, including ME/CFS, chronic critical illness, post-intensive care syndrome, (PICS), cancer-related fatigue, post- viral fatigue, PACS, long-COVID, heat stroke, and fibromyalgia.
- Emphasize the importance of PEM in ME/CFS across all research priorities as it is the hallmark symptom of ME/CFS.
- Assess the pulsatile pituitary secretions in ME/CFS and their relationship to the severity of illness and physiological alterations in ME/CFS.
- Investigate the HPT axis function in ME/CFS (including thyroid hormone function at tissue level) and relationship to the severity of illness, hypometabolic state, immune system activity and specific organ/tissue symptoms.
- Explore the role of interlinkages between inflammation, intestinal injury, pituitary suppression, low thyroid hormone function, endothelial function, and mitochondrial function in the persistence of illness in ME/CFS.
- Assess how norepinephrine oscillations affect glymphatic function and potential buildup of neurotoxic waste products.
- Examine the impact of acute and chronic stress on norepinephrine oscillation patterns and the resulting quality of sleep.
- Perform comprehensive studies to correlate changes in plasma proteomics with EV proteomics post-exercise in individuals with ME/CFS.
- Prioritize research into the correlation between EV protein level changes post- exercise and specific ME/CFS symptoms like myalgia, arthralgia, fatigue, and post-exertional malaise (PEM).
- Utilize PET scanning to further investigate body-wide problems or dysregulation within bone marrow.
- Adopt the Canadian name of PENE, post-exertional neuroimmune exhaustion instead of post-exertional malaise or fatigue.
- Further characterize what happens during a crash to the neuroimmune, cardiac, biochemical, mito-cellular etc. systems during a crash.
- Investigate the role of Mg in forestalling/mitigating a crash.
- Identify and characterize individuals with ME who have or are at risk for hyponatremia - those that are beyond thirsty all the time - to see if this symptom is related to the disease and if so, how, and why.
- Identify what causes decline in function even when an individual is staying within their energy envelope, taking the prescribed medications, and doing all the right things.
- Investigate endocrine statuses- both pituitary and adrenal gland - to understand physiological processes, and difference between men and women with ME/CFS.
- Investigate the experience of post-exertional neuroimmune exhaustion as compared to those who have other conditions with PEM to identify if they are the same experience with different labels or two different energy production issues.

- Perform long term continuous (daily for a few years) self-bio sampling (fingerstick blood, saliva, urine, etc.) to gain insights into biochemical changes precipitating PEM development.
- Investigate the heterogeneity of presentations of symptoms between individuals with ME/CFS and utilize PET imaging (as well as other imaging) might to look at different ME/CFS subsets to see if differences in imaging in different systems might explain some of the differing symptoms.
- Prioritize a clinical trial of antipurinergic therapy (APT) in ME/CFS.
- Investigate semaglutides for ME/CFS to address metabolic issues with insulin, kidneys, cholesterol, and liver function.
- Investigate common nutritional deficiencies associated with ME/CFS (including but not limited to vitamin C, vitamin B complex, sodium, magnesium, zinc, folic acid…) or any that might help with mitochondrial support.
- Provide more information on how to help with symptoms now while researchers are unraveling this complex disease.
- Replicate the recent findings published in Nature for MECFS study leg pain (quads) and identify targeted treatments.
- Investigate how the physiology of exertion and recovery may be disrupted in ME. The goal of such research would be to uncover more central control mechanisms that could be creating the ON-OFF switch for the changes seen at the cellular level.

Less Studied Pathologies Research Priorities

- Institute a more holistic approach to research on ME/CFS that also includes consideration of environmental exposures.
- Investigate the overlap with connective tissue diseases and the impact of viruses on connective tissue diseases which significantly impact quality of life for subsets of individuals with ME/CFS. Research into Mast Cell Activation Syndrome holds the potential to deliver treatment options for people with ME/CFS.
- Investigate the laxity of connective tissue that results in “leaky gut” in ME/CFS and Ehlers Danlos to determine if connective tissue breakdown/dysfunction is present, and the role it may also play in blood brain barrier (BBB) dysfunction and vascular laxity.
- Further explore the genetic and symptom overlap between ME/CFS and Ehlers Danlos, and specifically include those who have the mild form of Ehlers Danlos.
- Study the high urine output (as much as 9 liters/day) that is experienced by some individuals with ME/CFS.
- Perform rigorous clinical studies treat different groups of people with ME/CFS (with pain/without pain) with full body cold laser therapy to determine its efficacy to treat both pain and PEM.
- Investigate the prevalence of structural issues in ME/CFS and whether any of these issues precede or pre-dispose individuals to the disease and/or trigger other/worsening structural issues.

- Investigate the highly overlapping comorbidities, including CTDs, also commonly present with idiopathic intracranial hypertension (IIH) without papilledema and are found to have internal jugular vein compression or stenosis, Eagle's syndrome, cerebral venous congestion (e.g., venous sinus stenosis), and other systemic venous compression syndromes (Nutcracker, May-Thurner, MALS, etc.). Intracranial pressure dynamics, cerebral venous outflow disorders, and systemic venous compression syndromes in people with ME/CFS with and without CTDs and identify treatment options.
- improve methods of screening for connective tissue disorders beyond Beighton scores in people with ME/CFS.
- There is a significant need for mechanistic research on connective tissue disease, spinal/mechanical conditions, and male and female reproductive health in ME/CFS.
- Investigate ear issues in individuals with ME/CFS including feelings of fullness, pain, tinnitus, and hearing issues (other than processing) to determine the cause and potential treatments.
- Really exciting to see these co-morbidities of ME/CFS being brought to the attention of the ME/CFS research community! While there is a ton of remaining research on all these conditions, some of these co-morbidities have treatment options that can make a difference for people with ME/CFS right now, if they could access the right medical professionals.
- Integrate studies of the co-morbidities (less studied pathologies) into ME/CFS research to promote diagnosis and treatment of these co-morbidities in individuals with ME/CFS.
- Define the biological mechanisms and biomarkers, especially of acquired or exacerbated CTDs after development of ME/CFS.
- Identify possible genetic vs. somatic mutations, metabolic and mitochondrial mechanisms, and the role of infections (bacterial, viral, fungal) in the less studied pathologies of ME/CFS.
- Investigate elevated rates of endometriosis in ME/CFS, including risk, etiology, mechanisms and treatment and prevention options.
- Examine and compare these pathologies across co-occurring illnesses to understand why some also commonly occur in comorbid conditions (e.g., dysautonomia, Ehlers Danlos Syndrome, MCAD, Long COVID, chronic Lyme disease, fibromyalgia, Sjogren's syndrome).
- Determine the incidence of inflammatory bowel disease, Crohn’s, and Ulcerative Colitis in individuals with ME/CFS, determine if ME/CFS symptoms get worse during IBD flares, and identify and test treatment options.
- Include individuals over the age of 65 years in research studies on ME/CFS.
- investigate the cause of chronic tendon injury and muscle weakness and pain and burning in the feet from walking, identify and test treatments to lessen or eliminate the symptoms in individuals with ME/CFS.
- Initiate rigorous microbiome altering trials to determine the impact on the overall health of individuals with ME/CFS.
- Perform rigorous studies in individuals who are severely affected with ME/CFS to identify treatments to improve their symptoms and overall health.

- Investigate the improvements/exacerbations of symptoms seen in women with ME/CFS during pregnancies.
- Explore the use of provoked urinalysis to discover heavy elements from long past exposures,
  investigate dental amalgams as a source of mercury exposure, and test for evidence of human parasites in individuals with ME/CFS.
- Develop effective treatments and reliable biomarkers for testing, specifically for Mast Cell Activation Syndrome and GI dysfunction.

Genomics and Genetic Susceptibility Research Priorities

- Perform pharmacogenetics and nutrigenomics research on individuals with ME/CFS to guide treatment development and clinical care.
- Do family studies with families who have 2 or more individuals with ME/CFS.
- Utilize genomics-informed research for the development of both diagnostics and treatments.
- Perform genome-wide association studies in a large, diverse, representative US cohort of individuals with ME/CFS to identify SNPs involved in the disease's development.
- Perform whole genome sequencing and identification of genes and SNPs that can be compared in cohorts of individuals with severe and mild/moderate ME/CFS, people with Long Covid with and without ME/CFS, individuals with ME/CFS with and without infectious onset, and those with and without connective tissue diseases, and stratify the analyses by sex to identify sex-specific genetic factors.
- Perform genetic analyses that include calcium and potassium channelopathies, mitochondrial DNA mutations; variants associated with hypermobility or partial EDS/CTD phenotypes, Long Covid-related variants that have been found including variants associated with TLR4 receptor-mediated inflammation, circadian rhythm regulator variants (e.g., the CLOCK gene, which also causes mitochondrial dysfunction & pain), and variants associated with insulin regulation.
- Investigate if there are specific genomic markers and/or gene variants that predispose an individual to develop ME/CFS.
- Include individuals who are very severely ill and homebound or bedbound in research studies.
- Work collaboratively to share genomic and genetic data to accelerate the research.
- Examine the shared genetic components between ME/CFS and other conditions like Raynaud’s syndrome, hypermobile Ehlers-Danlos syndrome, and asthma.
- Prioritize the identification of pathogenic circulating microRNAs associated with specific ME/CFS symptoms, including post exertional malaise (PEM), dysautonomia, cognitive dysfunctions, immune cell dysfunctions, and autoantibodies production.
- Understand the epigenetic factors involved in the development of the condition and the potential relationship with microRNA dysregulation.

- Perform research on the mechanical inner workings of immunometabolomics and attempt to do system coupling between the pathophysiological mechanisms at play between immunological signaling, metabolic homeostasis/signaling and histone code.
- Establish a database where individuals with ME/CFS who have genetic sequencing data can share their data so that it can be used for research.
- Expand research on WASF3 to determine its role in ME/CFS and identify treatment targets.

Overarching Research Priorities

- Form more significant partnerships with PWLE ME/CFS in all aspects of research which would benefit the process.
- This disease is complex. A gentle reminder to take a step back when you need to.
- Use ICC, CCC, SEID and not Fukuda case definitions in research.
- Adopt standardized research methods across studies.
- Include severe/very severe individuals with ME/CFS regardless of age or length of illness in research studies.
- Drop 'effort preference' research.
- Fund nanoneedle research.
- Cut the red tape so individuals with ME/CFS get treatment options as quickly as possible.
- Push information to the medical community as hard as possible when you find it.
- Research/educate best practices for pacing, including the role of aggressive rest and publish best practices for pacing.
- Identify clear biological markers (biomarkers) that are safe and accessible to individuals with ME/CFS.
- Need deeper probing into PENE when individuals with ME/CFS raise "fatigue" as a concern to their doctors?
  (support push for fatigue screening protocols).
- Identify best practice protocols for supporting care from home as one progresses from severe to very severe including appropriate hospital protocols for the very severe.
- Evidence from clinical trials to support insurance payment for helpful treatments - e.g., Occupational Therapy by practitioner who know pacing for PENE.
- Education of Social Security & insurance doctors, family members, caregivers, and other practitioners about ME/CFS.
- Recognition of and help for suicide ideation after stressful events/encounters.
- Include neurodivergence in all studies (autism spectrum disorder, ADHD, PMDD and PTSD).
- Investigate neuroimmune disease related to allergies which are more common in people with Autism and ADHD (and ME/CFS).
- Research studies should be inclusive of all individuals and not just focus on Caucasians and from all socioeconomic groups.

- Encourage an integrative approach across research centers that can significantly accelerate advancements. By fostering an environment that promotes the sharing of data and biospecimens, we can enhance the efficiency and scope of the research endeavors.
- Recognize the diversity among individuals with ME/CFS is crucial for advancing our understanding and treatment strategies.
- Adopt standardized research methodologies, including and caregiver inputs.
- Prioritize studies that include pediatric populations that can provide crucial insights into the disease's development and long-term implications.
- Investigate the interconnectedness of ME/CFS with conditions such as connective tissue disorders and MCAS.
- Investigate ME/CFS from various inducers by broadening the scope of research to include a variety of ME/CFS triggers beyond infections to unveil critical insights into immune dysfunctions and potential therapeutic avenues and including the impact of physical trauma and environmental factors on disease onset and progression.
- Investigate the gender differences seen in ME/CFS.
- Address disparities in healthcare access and integrating ME/CFS into medical curricula can enhance disease recognition and management, promoting health equity.
- Capitalize on findings from related research areas, such as neurology and immunology to provide novel approaches to understanding and treating ME/CFS.
- Development and utilize appropriate animal models to facilitate a systemic understanding of ME/CFS pathophysiology and potential treatment strategies.
- Ensure diversity among researchers and study participants to fostering an inclusive and comprehensive research environment.
- Address and mitigate unconscious biases in grant review processes is vital for supporting researchers from diverse backgrounds and circumstances.
- Move away from basic/exploratory research on ME/CFS and focus on testing a variety of treatments for ME/CFS.
- Examine the reason that most people with ME/CFS reach tolerance when they take dopamine stabilizer drugs.
- All research priority areas need more funding!!
- We need studies aimed at characterizing PEM.
- Research into the cognitive symptoms associated with ME/CFS as well as post-exertional malaise.
- Research needs to be based on proper characterization of cohorts.
- Lack of sufficient data collection at the medical practitioner level.
- Direct resources towards investigations which could greatly improve questionnaires, surveys, and pre-printed forms to give doctors the tools for asking the right questions and identifying comorbidities. This will enable information to be accumulated in a way that can better differentiate ME from ME/CFS and help to illuminate ME/CFS in some people with Long Covid.

- Research studies need to include this group in all studies (as well as those over age 65) whenever possible. Some of the answers we are looking for may be found in those that have survived this debilitating disease for years/decades.
- Investigate the impact of ME/CFS of brain circuits and sensitivity to psychoactive substances.
- Investigate the role of inflammation in the brain and how it alters synaptic transmission and promotes hyperexcitability and neuronal damage.
- Investigate the role of trauma and emotional stress in ME/CFS along with a genetic predisposition plus viral infection – how do all of these come together to "cause" or make ME/CFS symptoms worse?
- Support treatment trials in the next 5 years designed specifically to identify and characterize responders.
- Develop a plan to include ME/CFS arms in Long COVID treatment trials.
- Convene working groups of experts to prepare a more comprehensive analysis of treatment trial options.
- Expand the use of real-world data (RWD) and digital twins to improve precision subtyping.
- Validate surrogate clinical trial endpoints that can minimize potential harms/burdens to individuals with ME/CFS during study participation.
- Promote decentralized trial designs to extend the access to clinical studies to people with ME/CFS who are house-bound/bed-ridden.
- Rank drug repurposing candidates based on biology-informed probabilities of success.
- Explore the discovery of digital biomarkers, especially based on medical imaging results.
- Encourage meaningful engagement of individuals with ME/CFS in all stages of the research process.
- Add engagement requirements to studies on ME/CFS.
- Carefully characterize study participants for research on ME/CFS and consider rule outs and comorbidities during the sample selection process.
- Study the cognitive side of PESE/PEM.
- Prioritize research to identify and validate biomarkers to improve both clinical care and research.
- Provide support for an ME/CFS clinical treatment trials network.
- Include a cohesive, cross-cutting, integrated PEM-focused research strategy and priorities to fully elucidate the symptom of PEM (across triggers and disease severity) and its underlying pathology, to identify and validate the biological and individual reported measures needed to identify, measure, and monitor PEM, and to recommend trials on methods to manage, treat, and eventually prevent PEM.
- Concentrate on the top 10+ priorities already ranked in the JLA consultation.; see: https://www.jla.nihr.ac.uk/priority-setting-partnerships/ME-CFS/top-10-priorities.htm
- Support longitudinal studies into the effect of pacing in ME and Long COVID.
- Support research on the skeletal muscle proteome.
- Investigate the correlations between ME/CFS and cancer.

- Support research by physical and occupational therapist to develop safe and effective treatments/therapies for individuals with ME/CFS.
- Research the mental health burden of medical gaslighting and abuse on people with ME/CFS.
- Need clarification of the ME/CFS construct. Primary case definitions and diagnostic criteria for Myalgic Encephalomyelitis (ME) differ from those for chronic fatigue syndrome (CFS). Many have argued for the retirement of CFS as a diagnostic category. However, as ME/CFS continues to be used, it should be identified and investigated as a spectrum rather than as one entity. Survey instruments and NIH-supported studies need to be designed to better elucidate cohorts and identify their location on the spectrum.
- Initiate a Symptom Mapping Project. Facilitation of a project devoted specifically to the detailed mapping of symptoms is urgently needed. The Symptom Mapping Project should have foci distinct from efforts to identify and describe underpinning mechanisms and pathologies.
- Investigate the natural history of the illness. The course of the disease must be far better explored than it has been to date. In the case of ME/CFS, particularly in view of emerging evidence from Long COVID, natural history necessarily includes reasonably believed initiating events and exposures (e.g., infection, toxic exposure, traumatic accident, etc.).
- Survey instrument development. New survey instruments are needed to refine the ME/CFS construct, comprehensively elaborate the symptom complex, and understand how it becomes triggered and progresses. Current publicly available instruments are substantially wanting on all these fronts.
- Establishes knowledge requirements for applicants for and recipients of funding for ME/CFS research, based on the body of knowledge amassed for this initiative and evolving over time with new findings.
- Utilize tools/wearables for persons with ME/CFS to self-monitor and collect data on health measures.
- Conduct epidemiology studies to characterizes ME/CFS to identify the various symptoms of the disease and identify subgroups.
- Research mental health burden of medical gaslighting and abuse on individuals with ME/CFS.
- Identify the underlying immune, metabolic, or other reasons that individuals with ME/CFS don't interact with food/weight loss treatments the way other people do generally and if there is a way to figure out if these odd interactions are pointing to broken pathways or other possible treatable options.
- There is a need for immediate emergency funding for the ME/CFS Research Roadmap May 2024. All the research presented and discussed in the webinars are worthy of funding.
- Identify genetic changes in immune regulation in ME/CFS.
- Instead of provoking PEM through exercise, researchers should attempt to reduce or eliminate symptoms through some intervention.

- Focus clinical trials on sensory overload and its causes: photophobia, hyperacusis, intolerance to multiple simultaneous stimuli, such as crowds, individuals talking at the same time, etc., or stimuli in quick succession, such as images, lights, movements, etc. and take these limitations into consideration during medical and clinical trial proceedings.
- Progressively prioritize the vast number of questions is to 1) organize findings and questions by major hypotheses about what maintains the disease (of course, some findings will fit in multiple hypotheses), 2) identify ways to falsify these hypotheses, and 3) do so. Once a hypothesis has been posited, the findings and questions associated only with it should be discarded.
- Integrate the research priorities across topic areas and generate a smaller list of key priorities.
- Individuals with ME/CFS have found cures for themselves. Other people with ME/CFS have found medication treatments that are life changing for the better, even if they did not restore 100% functionality. We need to catalog these success stories.
- Identify a liaison with ARPA-H to promote research on ME/CFS.
- Future trials should include cohorts of individuals with ME/CFS who have overlapping conditions.
- Ensure that all individuals included in research on ME/CFS have PEM.
- Investigate whether people with ME/CFS with and without depression differ on various measures.
- Perform autopsy studies to study viral persistence.
- Going forward much greater attention should be paid to recruiting and examining many more men with ME/CFS. It is perfectly possible that the test results from men will illuminate new information and directions to explore than what has been gleaned so far.
- Investigate if ME/CFS is driven by a simple change in population amongst the brain microbiome.
- Utilize new brain imaging tools to elucidate the cause(s) of ME/CFS.
- For a very complex, heterogeneous disease we need to go deeper and look at the effects on all tissues/organs.
- Cohorts in studies of ME/CFS should represent the heterogeneity of the disease, as well as in trials that focus on specific dysfunctions/dysregulation of systems.
- PEM is a hallmark of ME and as such warrants extensive and careful study and research on PEM should be a priority.
- Ensure that young people with ME/CFS are appropriately studied to find ways of treating and curing the disease so they can be fully engaged in their lives.
- Stakeholders should be fully involved at all stages of ME research to include this expertise and ensure that research is meaningful and appropriate for people with ME/CFS and ensure that wording does not allow for misinterpretation.
- Conduct clinical trials that match to the symptoms of individuals with ME/CFS.
- Use AI to draw further conclusions/identify patterns from research studies on ME/CFS.
- Include individuals with ME/CFS on every funded study to be sure the study uses effective designs that meet individuals where they are at in terms of needs.

- Investigate the central sensitization hypothesis in ME/CFS.
- To properly study any disease state, both environmental and genetic factors must be investigated.
- Study the exposome of individuals with ME/CFS to identify environmental factors that influence ME/CFS to develop a diagnostic biomarker and find new therapeutics.
- Investigate why symptoms vary predictably with the phase of the menstrual cycle and why those changes are not the same for everyone.
- Focus on treatments (perhaps cross referenced across all the areas) to investigate 1) new drugs 2) ideas for repurposing old drugs or 3) current or smaller trials that are so far indicating success but need validation.
- Focus on researching the chemical, medication, and supplement sensitivities that many of us with ME/CFS experience.
- Pay attention to study design issues and utilize diagnostic criteria that includes PEM, includes individuals with severe ME/CFS, Increased use of diseased control groups, analysis of data and results by separating cohorts into groups based on sex, age, length of illness, severity, and common comorbidities.
- Partner with private sector companies who have new technologies which could be leveraged for research on ME/CFS.
- Write a comprehensive review paper collating and discussing current biological findings of ME/CFS and related conditions.
- Investigate cognitive exertion that can cause profound and sudden PEM and is a hallmark of ME/CFS yet is extremely under-researched.
- Investigate if the cause of ME/CFS after an infection/virus or if you got it more by stress/lifestyle issues results in different symptoms and response to treatments.
- Study individuals who recovered from ME/CFS.
- Encourage researchers to submit and share their "big idea" behind ME/CFS.
- Encourage the creation and validation of protocols to assess PEM that are more accessible, less expensive, less risky, and more likely to be tolerated by a spectrum of individuals with ME/CFS.
- Conduct studies of pacing in ME/CFS to reduce/eliminate symptoms of the disease.
- Investigate the role of the blood brain barrier, changes in ME/CFS, and the role of intracranial hypertension.
- Focus research on system coupling between the pathophysiological mechanisms at play between immunological signaling, metabolic homeostasis/signaling and histone code.
- There needs to be a balance between clinical studies (clinical trials), clinical tests, surveys (e.g., a national NIH-administered Disease Registry like those for cancer and MS) and basic science (e.g., human and animal studies on neuroimmune interactions, fatigue, sleep disruption, immune system interventions). Inclusion of basic science research is necessary for longer-term understanding of ME/CFS proximal and distal causes and persistence and eventual discovery of cures.
- Prioritize faster over slower research studies through support for smaller, pilot studies with more rapid review and funding timelines than larger, slower projects.

- Concentrate research on what changes are occurring when individuals with ME/CFS have respite from their symptoms.
- Encourage theory-building -- although there may be a variety of precipitating triggers (distal events), there well may be a "final common path" of similar responses (proximal events of neural, immune, and circulatory responses) that constitute the persistent disease state and its characteristic symptomology.
- A coordinated questionnaire may need to be developed as a research instrument to unify findings, and this tool would need to be carefully designed to collect every bit of information that could be relevant. I would recommend that many individuals with ME/CFS be involved in its construction.
- Researchers need to be more specific and transparent in their reporting of the specific symptoms and characteristics of the individuals included in their studies, the type of fatigue experienced by the study participants, etc.
- Support researchers who are working under the assumption that ME/CFS is a biological disease.
- Seeking out survivors of that initial cohort in Lake Tahoe and proactively fill in the missing facts from that outbreak.
- Awareness of the definition of "fatigue" in ME/CFS research to understand (a) the diversity of experience, (b) how bad it can get, and (c) that it's not "fatigue" in the conventional sense, it's an energy disorder we can't explain within our current theoretical framework.
- Identify “where is the problem not?". Find a situation where the problem doesn't occur. Find the places (other than the learned behavior cases which we know how to retrain) where an intervention sent physical ME/CFS into remission.
- Investigate the lifecycle to this illness from being initiated by an event to manifesting as illness that does not recover.
- Utilize better precision in distinguishing Post Viral Fatigue Syndrome from ME/CFS, especially in the new Long COVID era. Apart from the distinct symptomatology, the two conditions differ significantly in recovery rates.
- Develop better diagnostics to define which individuals are hurt from activity (recovery impairment) and who may benefit from well-monitored activity, even in the presence of fatigue.
- Investigate the various factors that likely contribute to the difficulty to work in ME/CFS: fatigue, PEM, orthostatic intolerance, the unpredictable fluctuating nature of the illness which makes it difficult to meet the expectations of employers who look for consistency and reliability, executive dysfunction, slowed processing speed and others.
- Investigate the co-morbidity of ADHD with ME/CFS.
- Researchers should collaborate with people with ME/CFS and study the true psychosocial issues in ME/CFS, especially in children, adolescents, and young adults as they are most likely the most vulnerable.
- Coordinate research efforts on post-infectious diseases.
- Support researching, training, and improving care coordination and service provision for those with MECFS. This would include case management and social work services for

those who are inpatient and outpatient, as well as training for primary care management.

- Focus research on the physiology of exertion - how is the dynamic interplay of body systems orchestrated for exercise in healthy subjects and how/why does it go wrong in people with ME/CFS?
- Develop a coordinated strategy to investigate repurposing drugs to treat ME/CFS.
- Build consensus around clinical trial endpoints that are acceptable to regulators.
- Support a data-driven hypothesis generation strategy that includes comprehensive "-omics" testing (particularly focusing on severe ME/CFS cases that employ unbiased metabolomics and metagenomics approaches), and utilize the data gathered from these tests to cluster individuals with ME/CFS into distinct phenotypes to guide the formulation of targeted hypotheses.
- Incorporate the use of novel technologies in ME/CFS research and in particular advancements in technological research on the brain that could lead researchers in discovering aspects that have remained invisible until now.
- Explore the use of gene therapy (CRISPR) to treat individuals with ME/CFS.
- Develop an international consensus on how to define severity of disease in ME/CFS.
- Focus the research on clinical treatment trials and not observational studies with a focus on treatment so dysfunction of the autonomic nervous system the immune system, and the Itaconate pathway.
- Support a comprehensive facility where up to 50 concurrent studies could be run and would provide the research staff with a unique opportunity to look at the data in the aggregate / make comparisons / draw hypotheses/ and draw conclusions.
- Identify the prevalence of post-concussion syndrome among people who go on to develop ME/CFS.
- Test treatments for cognitive dysfunction in ME/CFS.
- Prioritize the development of studies that allow remote participation, and from as many states as possible and that include the sickest individuals.
- Develop and validate a diagnostic biomarker(s) for ME/CFS.
- Investigate environmental factors that propagate the illness (even for those with viral triggers) and relies upon the initial destruction of the epithelial barrier, and subsequent exposure to insoluble nanoparticles such as titanium dioxide, which clog lymphatics and can be mechanically damaging without being toxic.
- Pay attention to the spectrum or staging of this disease which may lead to more focused, perhaps preventative, and even better managed care.
- Investigate "pathogenic" biofilms in any/all mucosal tissues (which prevent immune surveillance) containing one or more acetaldehyde producing species, allowing a slippery slope of microbiome dysbiosis which is exacerbated by deficiency of Bifidobacterium spp. and lactobacillus spp. that leads to a cascade of events and the symptoms of ME/CFS.
- Investigate the role of superantigens produced by Staphylococcus aureus, Streptococcus pyrogens and SARS - CoV-2 as the cause of CFS and Long Covid.

Appendix 5: Rosters

A. ME/CFS Research Roadmap Working Group of Council Roster

Co-Chair: Lucinda Bateman, MD; Bateman Horne Center, Salt Lake City, UT
Co-Chair: Maureen Hanson, PhD; Cornell University, Ithaca, New York

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Simon Carding, Ph.D.; Norwich Medical School, University of East Anglia, Norwich, UK
H Craig Heller, PhD; Stanford University, Stanford, CA
David Holcomb; San Diego, CA
Leonard Jason, PhD; DePaul University, Chicago, IL
Cort Johnson; Health Rising, Henderson, NV
Chloe Jones; University of Alabama, Birmingham, Birmingham, AL
Laurie Jones; #MEAction, Santa Monica, CA
Nancy Klimas, MD; Nova Southeastern University, Ft. Lauderdale, FL
Anthony Komaroff, MD; Harvard Medical School, Boston, MA
Gudrun Lange, PhD; Mt. Sinai, New York, NY
Susan Levine, MD; Private Practice, New York, NY
W Ian Lipkin, MD; Columbia University, New York, NY
Alain Moreau, PhD; University of Montreal, Montreal, Canada
Benjamin Natelson, MD; Mt. Sinai, New York, NY
Beth Pollak; Massachusetts Institute of Technology, Cambridge, MA
Chris Ponting, PhD; The University of Edinburgh, Edinburgh, Scotland
Richard Simpson; Invest in ME Research, Hampshire, UK
David Systrom, MD; Brigham and Women's Hospital, Harvard University, Boston, MA
Linda Tannenbaum; Open Medicine Foundation, Agoura Hills, CA
Elizabeth Unger, MD, PhD; Center for Disease Control and Prevention, Atlanta, GA
Derya Unutmaz, MD; The Jackson Laboratories, Farmington, CT
Sumeeta Varma, MD, MSCI; New York, NY
Chris Wikman; Germantown, MD
Jarred Younger, PhD; University of Alabama, Birmingham, Birmingham, AL
Executive Secretary: Vicky Whittemore, PhD; National Institute of Health/National Institute of Neurological Disorders and Stroke, Bethesda, MD

People with Lived Experience ME/CFS (PWLE MECFS)

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Peter Cariani
Gwynn Dujardin
Tracy Duvall
Lisa Engel
Tess Falor
Kenneth Friedman
Thomas Giersch
Nancy Harkness
Michael Hermus
David Holcomb
James Holcomb
Chloe Jones
Ikuko Kato
David Kim
Roshan Kumar
Derek Simmonds
Hayla Sluss
Lorraine Steefel
Angela Termini
Elizabeth Weaver
Chris Wikman

B. ME/CFS Research Roadmap Webinar Planning Groups

Chronic Infection
Chair: Maureen Hanson
Simon Carding
Kenneth Friedman
David Holcomb
Ikuko Kato
Nancy Klimas
Anthony Komaroff
Elizabeth Unger

Immune System
Chair: Derya Unutmaz
Tracy Duvall
David Kim
Nancy Klimas
Roshan Kumar
Susan Levine
Alain Moreau
Angela Termini
Sumeeta Varma

Nervous System
Chair: Jarred Younger
Lisa Engel
H. Craig Heller
Leonard Jason
Chloe Jones
Laurie Jones/Jaime Seltzer
Gudrun Lange
Benjamin Natelson
Lorraine Steefel

Circulation
Chair: David Systrom
Lucinda Bateman
Peter Cariani
Gwynn Dujardin
Benjamin Natelson
Beth Pollack
Elizabeth Weaver
Jarred Younger

Metabolism
Chair: Alain Moreau
Tess Falor
Thomas Gierach
W. Ian Lipkin
Derek Simmonds
Derya Unutmaz
Chris Wikman

Physiology
Chair: H. Craig Heller
Maureen Hanson
Nancy Harkness
Richard Simpson
David Systrom

Less Studied Pathologies
Chair: Beth Pollack
Lucinda Bateman
Maureen Hanson
Michael Hermus
Cort Johnson
Nancy Klimas

Genomics & Genetic Susceptibility
Chair: Oved Omitay
Miriam Boyer
James Holcomb
Anthony Komaroff
Alain Moreau
Chris Ponting
Hayla Sluss
Linda Tannenbaum

C. The NIH and Contractor Teams for the ME/CFS Research Roadmap

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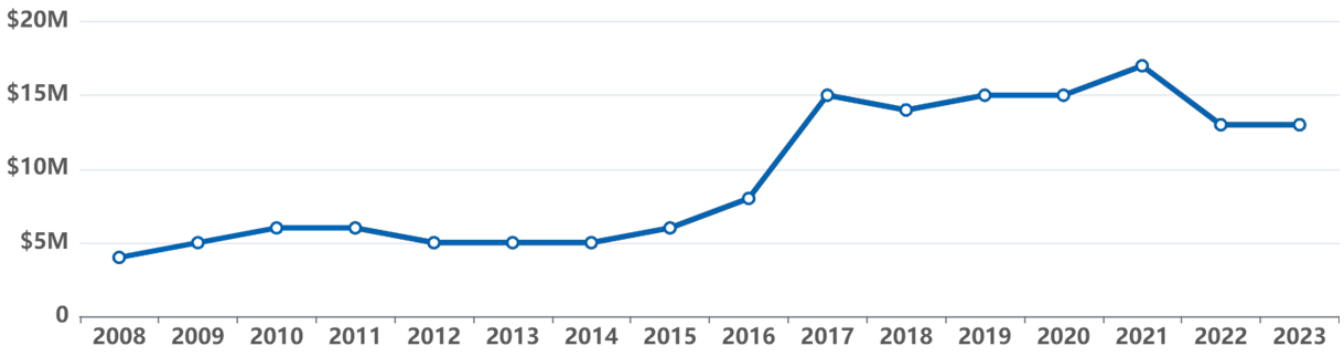
Appendix 6: NIH ME/CFS Research Funding: Fiscal Year 2008 – 2023



Data from https://report.nih.gov/funding/categorical-spending#/