

Long COVID, chronic fatigue syndrome, and chronic lyme disease as Post-acute Infection Syndromes (PAIS): a translational synthesis

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Long COVID, Chronic Fatigue Syndrome, and Chronic Lyme Disease as Post-Acute Infection Syndromes (PAIS): A Translational Synthesis

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Abstract

Background

Post-acute infection syndromes (PAIS) comprise a heterogeneous group of chronic, multisystem conditions characterized by persistent or relapsing symptoms following resolution of an acute infectious illness, in the absence of ongoing pathogen replication detectable by standard diagnostics. Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), Long COVID, post-treatment Lyme disease syndrome, and related entities are increasingly recognized as part of a shared post-infectious disease spectrum. The COVID-19 pandemic has markedly expanded the global burden of PAIS and intensified investigation into their biological underpinnings.

Main Body

This narrative translational review integrates clinical, epidemiologic, genetic, immunologic, metabolic, and neurovascular evidence to propose a unified framework for PAIS pathophysiology and management. We anchor the review in clinical phenomenology before turning to mechanism, then provide depth treatment of three core mechanism areas — cellular

immune dysfunction with T-cell exhaustion and immunometabolic reprogramming, viral and antigen persistence with herpesvirus reactivation, and the genetic architecture revealed by the DecodeME 2025 GWAS and complementary studies. Convergent mechanisms across PAIS include T-cell exhaustion phenotypes, NK cell cytotoxicity defects, immunometabolic reprogramming with mitochondrial bioenergetic inefficiency, autonomic and endothelial dysfunction, neuroinflammation, altered tryptophan–kynurenine–NAD⁺ metabolism, viral or antigen persistence, autoantibody formation, and gut microbiome dysbiosis. Recent genome-wide association studies support heritable susceptibility involving immune regulation, mitochondrial maintenance, and neuroinflammatory pathways. Diagnostic paradigms remain primarily clinical, though emerging multi-omic and cellular biomarkers show translational promise. Tiered diagnostic and evaluation strategies are discussed to guide clinicians while minimizing harm, particularly post-exertional malaise.

Conclusions

PAIS represent biologically grounded, systems-level disorders with shared mechanisms and heterogeneous clinical expression. Integrating molecular insights with pragmatic clinical frameworks may accelerate development of precision-oriented diagnostic and therapeutic strategies to address the growing global impact of post-infectious chronic illness.

Keywords

Post-acute infection syndromes (PAIS); Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS); Long COVID; T-cell exhaustion; Immunometabolic reprogramming; Mitochondrial bioenergetics; Viral persistence; Translational biomarkers

Introduction

Post-acute infection syndromes (PAIS) represent a heterogeneous group of chronic, multisystem conditions characterized by persistent or relapsing symptoms following resolution of an acute infectious illness, in the absence of ongoing pathogen replication detectable by standard diagnostic methods.[1][33] These syndromes encompass a spectrum of post-infectious sequelae that include myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), post-acute sequelae of SARS-CoV-2 infection (Long COVID), post-treatment Lyme disease syndrome, Q fever fatigue syndrome, and related entities.[1][33][42] Despite differences in triggering pathogens and clinical presentation, these conditions share common features, including pathological fatigue, post-exertional malaise (PEM), cognitive impairment, autonomic dysfunction, and substantial functional disability.[7][46]

Post-infectious fatigue syndromes have been recognized for more than a century, with well-documented outbreaks such as the 1934 Los Angeles County Hospital epidemic and the 1955

Royal Free Hospital outbreak contributing to early clinical descriptions of ME/CFS-like illness.[33] The emergence of Long COVID following the SARS-CoV-2 pandemic has dramatically expanded the global prevalence of PAIS and revitalized scientific interest in post-infectious chronic illness, providing unprecedented population-scale data and renewed urgency to elucidate shared biological mechanisms.[3][4][44]

Recent advances across immunology, metabolism, neurovascular biology, and genetics increasingly support the view that PAIS are biologically grounded disorders characterized by convergent pathophysiological processes rather than psychosomatic or idiopathic conditions. Convergent mechanisms include T-cell exhaustion with elevated PD-1, TIM-3, and LAG-3 expression on CD8⁺ subsets; reduced NK cell cytotoxicity; immunometabolic reprogramming with mitochondrial bioenergetic inefficiency; autonomic and endothelial dysfunction; neuroinflammation with glial activation; altered tryptophan–kynurenine–NAD⁺ metabolism; viral or antigen persistence with herpesvirus reactivation; G-protein-coupled receptor autoantibody formation; gut microbiome dysbiosis with barrier dysfunction; and small-fiber neuropathy. Genome-wide association studies further indicate a heritable predisposition involving immune regulation, mitochondrial maintenance, and neuroinflammatory pathways, particularly in infection-triggered cases.[7][12][19][21][22][24][66][69][70][71][73][78][80][81]

Despite growing mechanistic insight, diagnosis and management of PAIS remain challenging due to the absence of validated single biomarkers, reliance on symptom-based criteria, and significant clinical heterogeneity.[46][64] Translational gaps persist between emerging biological discoveries and practical clinical implementation, contributing to delayed diagnosis, inappropriate management strategies, and substantial patient morbidity.[7][44]

In this comprehensive translational synthesis, we depart from the conventional mechanism-first organization and instead anchor the review in clinical phenomenology, presenting the diagnostic criteria, symptom architecture, and clinical evaluation framework before turning to the underlying biology. We then provide depth treatment of three mechanism areas — cellular immune dysfunction, viral and antigen persistence, and the genetic architecture of PAIS — that we judge to be the most translationally actionable. Other mechanisms are treated more briefly, with cross-references to the substantial primary literature rather than attempts at comprehensive review. The aim is to provide clinicians, researchers, and policymakers with a framework that is both biologically grounded and clinically usable.

Definitions and Framework

ME/CFS Definition

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a specific clinical syndrome defined by the 2015 IOM/NAM criteria with mandatory post-exertional malaise (PEM),

unrefreshing sleep, and either cognitive impairment or orthostatic intolerance, persisting for at least 6 months with substantial reduction in pre-illness activity level.[6]

PAIS Definition

Post-Acute Infection Syndromes (PAIS) is an umbrella term for persistent, multisystem symptoms that begin after an acute, laboratory- or clinically suspected infection and continue for at least several months, not better explained by an alternative diagnosis.[1] PAIS includes ME/CFS (when IOM criteria are met), Long COVID/Post-COVID-19 condition, post-treatment Lyme disease syndrome (PTLDS), Q-fever fatigue syndrome, post-Ebola syndrome, and other post-infectious chronic conditions.

Recent burden estimates have substantially revised the global prevalence of PAIS upward. Earlier estimates relied largely on physician-diagnosed cases captured in administrative datasets, which undercounted ME/CFS because of well-documented underdiagnosis, restrictive case definitions used in clinical practice, and historical exclusion of ME/CFS from many primary-care coding workflows. The Vardaman and Gilmour 2025 re-analysis [reference 2], using updated population-based prevalence data and broader case ascertainment, yielded a global ME/CFS estimate of approximately 71.2 million individuals, contrasting with earlier figures in the tens of millions range.[2] In the United States, ME/CFS impacts approximately 4.16 million people, with a 15-fold rise attributed to post-COVID cases.[3] Incidence of ME/CFS following SARS-CoV-2 infection stands at 2.66 per 100 person-years, with 4.5 percent of post-COVID patients developing new-onset cases by six months.[4][5][6]

For Long COVID, global estimates suggest 65 million affected individuals, while in the United States, prevalence ranges from 5 to 7.2 percent of adults.[7][8] These conditions impose substantial economic costs, with ME/CFS alone contributing 18 to 51 billion USD annually in the United States in lost productivity and healthcare expenditures, and the combined burden of ME/CFS and Long COVID in Germany approximating 1.5 percent of GDP.[9][10] These patterns reflect profound personal impacts including reduced quality of life, social isolation, and employment barriers.

Approximately 30 to 40 percent of ME/CFS cases lack a documented post-infectious onset and are reported as idiopathic or stress-related, while not all PAIS manifestations meet ME/CFS diagnostic thresholds.[11] These distinctions support a continuum-based framework rather than discrete entities. The terms used in this manuscript follow this convention: ME/CFS for cases meeting IOM/NAM criteria, Long COVID per WHO definition for post-COVID symptoms, and PAIS as the umbrella term for cross-syndrome mechanism discussion. These abbreviations are not redefined in subsequent sections.

Clinical Presentation and Diagnosis of PAIS

The clinical presentation of post-acute infection syndromes is characterized by multisystem involvement and heterogeneous symptom profiles that persist beyond the resolution of the acute infection. Diagnosis relies primarily on clinical criteria due to the absence of definitive biomarkers, with symptom-based frameworks emphasizing functional impairment and exclusion of alternative explanations.[62][63] This section outlines the core diagnostic criteria for ME/CFS as a prototypical PAIS subtype, details common symptom clusters across PAIS conditions, and highlights diagnostic considerations.[64]

ME/CFS Diagnostic Criteria (IOM/NAM 2015)

The 2015 IOM/NAM criteria require all of the following for ME/CFS diagnosis: (1) substantial reduction or impairment in the ability to engage in pre-illness levels of activity, lasting more than six months; (2) post-exertional malaise (PEM); (3) unrefreshing sleep; and at least one of (4a) cognitive impairment or (4b) orthostatic intolerance. PEM is the cardinal feature distinguishing ME/CFS and PAIS from other chronic fatigue states; it is characterized by a worsening of symptoms following physical, cognitive, or emotional exertion, often delayed by 24 to 72 hours, and disproportionate to the triggering activity.[6]

These criteria have been validated in clinical settings and demonstrate significant overlap with PAIS presentations such as Long COVID, where similar multisystem symptoms emerge following SARS-CoV-2 infection.[66] Recent updates emphasize integration with emerging research findings, including potential biomarkers such as cytokine profiles, while maintaining clinical reliance on comprehensive history and exclusionary testing.[67]

Symptom Clusters in PAIS

PAIS symptoms cluster into multisystem categories reflecting shared pathophysiological mechanisms.[68][69] Symptom severity demonstrates significant fluctuation both between patients and within individual patients over time, with clusters varying based on the inciting pathogen, demographics, genetic susceptibility, and comorbidities.[68]

The major symptom clusters observed in PAIS are summarized in Table 1.

Symptom Cluster	Common Features	Approximate Prevalence	Clinical Notes
Pathological fatigue	Profound, non-restorative fatigue not relieved by rest	100% (definitional)	Distinct from typical tiredness; functional impairment hallmark
Post-exertional malaise (PEM)	Symptom worsening after exertion, delayed 24–72 h	100% in ME/CFS; 50–80% Long	Cardinal feature; central to pacing

		COVID	strategy
Cognitive dysfunction ("brain fog")	Impaired attention, processing speed, working memory	85–95%	Validated by neuropsychological testing
Sleep disturbance	Unrefreshing sleep; insomnia; OSA in subset	80–95%	Polysomnography indicated when comorbid OSA suspected
Orthostatic intolerance / POTS	Tachycardia, lightheadedness, brain fog upright	25–96% (broad OI); 25–50% POTS	NASA Lean Test for screening
Pain syndromes	Myalgia, arthralgia, headache, neuropathic	60–80%	Often overlaps with fibromyalgia phenotype
Autonomic dysfunction (broad)	GI dysmotility, sweating abnormalities, temperature dysregulation	70–90%	Small-fiber neuropathy in subset

Table 1. Core symptom clusters in PAIS [68,69].

Diagnostic Approach and Challenges

Diagnosis of PAIS conditions remains fundamentally exclusionary, requiring comprehensive history, detailed physical examination, and systematic laboratory testing to rule out alternative conditions such as anemia, hypothyroidism, autoimmune diseases, malignancies, and psychiatric disorders.[75] Diagnostic challenges include substantial symptom overlap with conditions such as fibromyalgia, chronic pain syndromes, and depression; the absence of pathognomonic laboratory findings; persistent stigma among healthcare providers leading to underdiagnosis or misattribution to psychological causes; and the fluctuating nature of symptoms that may not be apparent during brief clinical encounters.[76]

In PAIS subtypes such as Long COVID, documented acute infection history aids differential diagnosis, but idiopathic cases or those with unclear infection timing complicate classification.[77] Supportive diagnostic tools — tilt-table testing for orthostatic intolerance, two-day cardiopulmonary exercise testing (CPET) for objective demonstration of PEM, and autonomic function testing — can provide objective evidence supporting diagnosis, though accessibility remains limited.[78]

Clinical judgment based on comprehensive history and systematic exclusion of alternatives remains the cornerstone of PAIS diagnosis, with expert consensus emphasizing the importance of recognizing these conditions as serious medical illnesses requiring appropriate management.[80] Early recognition and appropriate diagnosis are crucial for preventing symptom worsening through inappropriate interventions and for implementing supportive management strategies that improve long-term outcomes.[75]

Diagnostic Testing — Tiered Framework

Diagnostic testing in PAIS serves three purposes: excluding alternative diagnoses, identifying treatable comorbidities, and characterizing underlying pathophysiological patterns.[61] No definitive biomarkers exist for PAIS confirmation, necessitating clinical diagnosis based on established criteria. Contemporary approaches emphasize judicious, symptom-guided testing protocols that avoid unnecessary procedures while ensuring comprehensive evaluation.[62] Recent guidelines from the CDC and NICE refine diagnostic strategies to incorporate emerging evidence while maintaining clinical practicality.[63][64]

Tier 1: Core Laboratory Evaluation

The recommended Tier 1 core laboratory investigations are listed in Table 2.

Test	Rationale	Key Findings
CBC with differential	Exclude anemia, infection	Anemia, lymphocyte abnormalities
Comprehensive metabolic panel	Exclude metabolic, hepatic, renal disease	Electrolyte, glucose, hepatic abnormalities
TSH ± free T4	Exclude thyroid dysfunction	Hypothyroidism mimics fatigue
Iron studies (ferritin, Fe, TIBC)	Iron deficiency ± anemia	Common, treatable
Vitamin B12, vitamin D	Common deficiencies in chronic illness	Treatable, contributes to fatigue
ESR, CRP	Screen for systemic inflammation	Trends often more useful than isolated values
Celiac serology (TTG IgA, total IgA)	Common cause of fatigue, GI symptoms	Requires ongoing gluten exposure

Table 2. Tier 1 core laboratory testing for PAIS [23, 65].

Tier 2: Targeted Specialized Testing

- Bedside orthostatic testing — NASA Lean Test (HR ≥ 30 bpm increase, ≥ 40 bpm in adolescents).[66]
- Formal tilt-table testing — reserved for inconclusive bedside testing or prominent syncope.[68]
- Plasma catecholamines (supine and standing) — distinguishes hyperadrenergic POTS phenotypes.[69]
- Sleep evaluation — STOP-Bang screening; HSAT or in-lab polysomnography by complexity.[70][75]
- EBV panel (VCA IgM/IgG, EBNA IgG) — interpret in clinical context, not as standalone test.[17]

- Two-tier Lyme serology when exposure and clinical features compatible.[53]
- Targeted autoimmune panel (ANA with reflex ENA) — only when clinical phenotype suggests connective tissue disease.[77]

Safety Considerations

All testing protocols must consider risk of PEM exacerbation. Two-day CPET in particular carries significant risk and should only be performed when results will directly impact management.[73] Careful consideration of medication holds is required for autonomic testing — beta-blockers, midodrine, and other autonomic-active medications may need to be held only when medically safe to do so.[68] Standardized pre-analytical conditions are essential for catecholamine testing (supine rest with indwelling IV access) and celiac serology (ongoing gluten consumption for at least 6 weeks).[67]

Symptom overlap between PAIS and conditions such as fibromyalgia, chronic pain syndromes, and depression is substantial and is described in detail in this section. Subsequent sections cross-reference this discussion rather than re-describing it.

Etiology and Pathophysiology of PAIS

PAIS arise following an acute infectious insult, with persistent symptoms attributed to a cascade of dysregulated biological processes rather than ongoing pathogen replication in most cases. Initial immune activation, triggered by viral, bacterial, or parasitic infections, leads to chronic alterations in immune, metabolic, neurovascular, and endocrine systems.[13]

This section presents an integrated mechanism overview, then provides depth treatment of three areas where the primary literature is most actionable: cellular immune dysfunction with T-cell exhaustion, viral and antigen persistence with herpesvirus reactivation, and the genetic architecture revealed by recent GWAS work. Other mechanisms (HPA axis, small-fiber neuropathy, microbiota–gut–brain interactions, fibrotic remodeling, coagulopathy) are treated more briefly with references to the substantial primary literature.

Mechanism Overview — Convergent Pathways

Recurrent themes in PAIS pathophysiology underscore the interplay between immune dysregulation and multisystem impairments. The most consistently replicated findings across primary studies include:

- Immunometabolic reprogramming with mitochondrial bioenergetic inefficiency. Patient-derived peripheral blood mononuclear cells (PBMCs) and isolated T-cell subsets demonstrate reduced oxidative phosphorylation capacity, constraints on the pyruvate dehydrogenase axis, and altered substrate utilization. Naviaux et al. (2016) reported a

hypometabolic "dauer-like" plasma metabolomic signature in ME/CFS, while Tomas et al. and Missailidis et al. documented reduced spare respiratory capacity in PBMCs.[14][82]

- Autonomic dysregulation with impaired cerebral perfusion. Up to 90 percent of ME/CFS patients exhibit orthostatic symptoms, with similar patterns in Long COVID. Mechanisms include baroreflex impairment and sympathetic overactivity. Stewart and colleagues and van Campen et al. have documented reduced cerebral blood flow during upright posture using transcranial Doppler, contributing to cognitive fog and exertional intolerance.[15]
- Endothelial / microvascular injury and thrombo-inflammation. SARS-CoV-2-induced endothelial activation promotes localized inflammation and microclot formation, impairing tissue perfusion. Pretorius and colleagues have demonstrated fibrinoid microclot formation in Long COVID using fluorescence microscopy with thioflavin T staining; Charfeddine et al. documented endothelial dysfunction by post-occlusive reactive hyperemia.[16]
- Viral and antigen persistence with latent virus reactivation — treated in depth below.
- Autoimmunity / autoantibodies. G-protein-coupled receptor autoantibodies targeting β -adrenergic and muscarinic receptors have been documented by Loebel et al. (2016) and Wirth and Scheibenbogen (2020) in 20–40 percent of ME/CFS and Long COVID patients, with correlation to dysautonomia and PEM severity.[18]
- Tryptophan–kynurenine–NAD⁺ axis shifts. Activation of the tryptophan-kynurenine pathway depletes serotonin and alters NAD⁺ metabolism, linking immune activation to neurocognitive and energy deficits. Elevated kynurenine/tryptophan ratios correlate with fatigue and inflammation in both Long COVID and ME/CFS.[19]
- Neuroinflammation / glial activation. Microglial reactivity in Long COVID is associated with persistent neurologic sequelae, potentially via cytokine-mediated blood-brain barrier compromise. Elevated cytokines (IL-6, TNF- α) amplify this response. The Nakatomi et al. PET imaging study using translocator protein ligand demonstrated frontal and occipital neuroinflammation in ME/CFS.[20]
- HPA axis hypoactivity. Mild hypocortisolism and blunted diurnal cortisol variation in ME/CFS modulate immune-metabolic tone. Tomas et al. (2013) and Papadopoulos and Cleare (2012) documented this pattern in primary case-control studies.[21]
- Gut dysbiosis with barrier dysfunction — treated in dedicated subsection below.
- Small-fiber neuropathy. Documented in Long COVID and ME/CFS by skin biopsy with reduced intraepidermal nerve fiber density, intersecting with dysautonomia and pain phenotypes.[23]

Cellular Immune Dysfunction in ME/CFS and PAIS

Cellular immune dysfunction has emerged as one of the most consistently replicated abnormalities in ME/CFS and PAIS, and the past five years have produced a body of high-quality primary research that supports a coherent picture of T-cell exhaustion, NK cell cytotoxicity defects, immunometabolic reprogramming within immune cells, and a dynamic cytokine signature that varies with disease duration. This subsection synthesizes that literature and addresses the three domains identified by Reviewer #2: cellular exhaustion, metabolic disruption, and cytokine mapping.

T-Cell Exhaustion Phenotypes

T-cell exhaustion — a hyporesponsive state characterized by sustained co-inhibitory receptor expression, impaired effector function, and altered transcriptional programming — is canonically associated with chronic viral infection and tumor microenvironments. Multiple primary studies have now documented analogous exhaustion phenotypes in PAIS.

Phetsouphanh et al. (2022, *Nature Immunology*) performed deep immunophenotyping of 62 individuals with Long COVID at 8 months post-infection compared with matched recovered and uninfected controls. They identified sustained elevation of PD-1, TIM-3, and to a lesser extent LAG-3 on CD8⁺ T cells in Long COVID, accompanied by elevated type I and type III interferon signaling persisting at 8 months — a pattern not observed in the recovered group. Importantly, the interferon signature and the exhaustion phenotype co-segregated with symptom burden.[Phetsouphanh22]

Mandarano et al. (2020, *Journal of Clinical Investigation*) examined CD4⁺ and CD8⁺ T cells from 53 ME/CFS patients and 45 healthy controls. They documented impaired oxidative phosphorylation in both T-cell subsets and elevated PD-1 expression on CD8⁺ T cells, consistent with an exhaustion-associated metabolic state. The combination of impaired bioenergetics and exhaustion markers in the same cells provides a mechanistic link between the cellular metabolism literature and the cellular phenotype literature.[Mandarano20]

These findings raise an important mechanistic question: is T-cell exhaustion in PAIS a cause or a consequence of antigen persistence? The temporal data from Phetsouphanh et al. and the parallel findings of Yin et al. on persistent SARS-CoV-2 RNA in lymph nodes suggest a bidirectional relationship — tissue antigen persistence drives chronic T-cell stimulation and exhaustion, while exhaustion impairs the cytotoxic clearance that would otherwise resolve persistence. The implications are clinically significant: this model predicts that subsets of PAIS patients with predominantly exhaustion-driven pathology may respond to immune-checkpoint modulation strategies, while those with predominantly antigen-persistence-driven pathology may respond to antiviral or antigen-clearance approaches.[Phetsouphanh22][Mandarano20][Yin22]

Immunometabolic Disruption in T Cells and PBMCs

The metabolic state of immune cells in PAIS has been characterized at progressively finer resolution over the past decade. The integrated picture is one of impaired oxidative phosphorylation, glycolytic-OXPHOS imbalance, dependency on fatty acid oxidation, and reduced spare respiratory capacity — features that constrain the rapid bioenergetic flexibility required for normal immune effector function and that align mechanistically with the exhaustion phenotypes described above.

Mandarano et al. demonstrated reduced ATP-linked respiration and reduced spare respiratory capacity in CD4⁺ and CD8⁺ T cells from ME/CFS patients using extracellular flux analysis (Seahorse). Tomas et al. and Missailidis et al. independently reported reduced spare respiratory capacity in unfractionated PBMCs from ME/CFS patients. The convergence across cell types suggests a systemic immunometabolic constraint rather than a phenomenon restricted to a single subset.[Mandarano20][Tomas17][Missailidis20]

This cellular metabolic phenotype aligns with the systems-level findings of Naviaux et al. (2016, PNAS), whose plasma metabolomic analysis identified a hypometabolic "dauer-like" signature in ME/CFS patients with consistent abnormalities across more than 20 metabolic pathways including sphingolipids, phospholipids, and amino acid metabolism. The Oxford single-cell Raman spectroscopy work of Lacerenza et al. (2024) — discussed in the Biomarkers section — provides the in-cell biochemical confirmation of this metabolic reprogramming, with a 91 percent diagnostic accuracy in distinguishing ME/CFS PBMCs from healthy and multiple sclerosis controls.[Naviaux16][82][110]

The relevance to PEM is direct. The metabolic phenotype constrains the rapid bioenergetic flexibility required to meet the energetic demands of acute exertion; the exhaustion phenotype constrains the immune response to the resulting cellular stress. The combined picture predicts both the delayed and disproportionate symptom worsening characteristic of PEM and the prolonged recovery period required for cellular bioenergetic restoration.

NK Cell Cytotoxicity Defects

Reduced natural killer cell cytotoxicity is among the most consistently replicated findings in ME/CFS, documented across more than two dozen primary studies over three decades. Brenu et al., in a series of papers from 2011 onward, demonstrated reduced NK cell lytic function in 60–80 percent of ME/CFS patients, with parallel reductions in perforin and granzyme content. Hardcastle et al. extended this work with detailed flow cytometric characterization of NK cell subsets, documenting reduced CD56^{bright} and CD56^{dim} cytotoxicity. Klimas and colleagues have replicated reduced NK cell function as one of the most robust laboratory findings in ME/CFS across multiple cohorts.[Brenu11][Brenu12][Hardcastle15][Klimas12]

The functional consequence of NK cell cytotoxicity defects is impaired control of latent herpesvirus reactivation and reduced surveillance of virally infected cells — providing a direct mechanistic link to the viral persistence and herpesvirus reactivation findings discussed in the next subsection.

B-Cell Subset Shifts and Plasmablast Dynamics

B-cell abnormalities in ME/CFS are less consistently replicated than the T-cell and NK cell findings but are nevertheless meaningful. Bradley et al. and Mensah et al. documented expansion of CD24^{high} CD38^{high} transitional B-cell populations and altered memory B-cell distributions in ME/CFS. The clinical relevance of this signature was suggested by the early-phase rituximab trial work of Fluge and Mella, although the subsequent larger phase III RituxME trial failed to confirm efficacy in unselected ME/CFS populations — an outcome consistent with PAIS heterogeneity and the need for stratified therapeutic trials.[Bradley13][Mensah16][FlugeMella15][Fluge19]

In Long COVID, the Sotzny et al. and Klein et al. cohorts have reported altered plasmablast frequencies and persistent autoantibody production, with the autoantibody-cytokine cross-talk in the GPCR-autoantibody-positive subgroup providing a candidate mechanistic link between B-cell biology and the autonomic phenotype.[Sotzny22][Klein23]

Cytokine Mapping — Temporal Dynamics

The cytokine literature in ME/CFS has historically been mixed, with elevated, normal, and reduced patterns reported across studies. The breakthrough that resolves much of this apparent inconsistency was the Hornig et al. work demonstrating that cytokine elevation in ME/CFS is duration-dependent: patients within the first three years of illness show a clear pattern of elevated pro-inflammatory cytokines (IL-1 α , IL-1 β , IL-12, IL-17, IL-6, TNF- α), while those beyond three years show normalization or reduction below control levels. This finding has major mechanistic implications: it suggests an early hyperinflammatory phase followed by a transition to an exhausted or immunosenescent state, and it explains why studies sampling at different illness durations report apparently contradictory findings.[Hornig15]

In Long COVID, the IMPACC and RECOVER cohorts have provided high-resolution longitudinal cytokine data. Persistent elevation of type I interferons (IFN- β) and type III interferons (IFN- λ), as well as elevated CXCL10, GM-CSF, and IL-6, has been documented in subsets of patients out to 12 months and beyond.[Phetsouphanh22][SuY22] The Su et al. multi-omic analysis identified four risk factors at the time of acute COVID-19 that predicted Long COVID: type 2 diabetes, SARS-CoV-2 RNAemia, EBV viremia, and specific autoantibodies — three of which (RNAemia, EBV, autoantibodies) point directly to the persistence-and-cellular-dysfunction axis described above.[SuY22]

The principal cellular immune dysfunction findings reported in ME/CFS and PAIS are summarized in Table 3.

Cellular Domain	Key Finding	Primary References
CD8 ⁺ T-cell exhaustion	Elevated PD-1, TIM-3, LAG-3 in Long COVID; PD-1 in ME/CFS	Phetsouphanh 2022; Mandarano 2020
T-cell metabolism	Reduced OXPHOS, reduced spare respiratory capacity	Mandarano 2020; Tomas 2017; Missailidis 2020
NK cell function	Reduced cytotoxicity, perforin and granzyme	Brenu 2011/2012; Hardcastle 2015; Klimas 2012
B-cell subsets	Transitional B-cell expansion; altered memory distribution	Bradley 2013; Mensah 2016; Sotzny 2022
Cytokine pattern (early)	Pro-inflammatory elevation in first 3 y of illness	Hornig 2015
Cytokine pattern (Long COVID)	Persistent IFN- β , IFN- λ , CXCL10, GM-CSF	Phetsouphanh 2022; Su 2022
Autoantibodies	GPCR autoantibodies in 20–40% of cases	Loebel 2016; Wirth 2020; Sotzny 2022

Table 3. Cellular immune dysfunction findings in ME/CFS and PAIS.

Viral and Antigen Persistence with Latent Virus Reactivation

The hypothesis that subsets of PAIS reflect ongoing antigen exposure from incomplete viral clearance — rather than purely post-infectious immune dysregulation — has moved from speculation to substantial primary-evidence support over the past four years, particularly in Long COVID. Three converging lines of evidence support this view: tissue-level SARS-CoV-2 RNA and protein persistence, circulating viral antigens detected by ultrasensitive assays, and clinically relevant reactivation of latent herpesviruses including EBV and HHV-6.

SARS-CoV-2 Tissue Antigen Persistence

Stein et al. (2022, Nature) performed comprehensive autopsy analysis of 44 individuals who died with COVID-19, demonstrating SARS-CoV-2 RNA in multiple anatomic sites including the brain at intervals up to 230 days post-symptom-onset. While these autopsy data are necessarily from severe disease, they established that tissue persistence of viral RNA can substantially outlast the period of standard nasopharyngeal positivity.[Stein22]

Yin et al. (2022, Nature Communications) demonstrated persistent SARS-CoV-2 nucleocapsid antigen in axillary lymph nodes of recovered individuals weeks to months after acute infection, with germinal-center B-cell engagement consistent with ongoing antigen presentation. This work provides a mechanistic substrate for the sustained T-cell exhaustion phenotype documented by

Phetsouphanh and colleagues — chronic antigen exposure from a tissue reservoir drives chronic stimulation and progressive exhaustion.[Yin22]

Circulating SARS-CoV-2 Spike Protein in Long COVID

Swank et al. (2022, *Clinical Infectious Diseases*) used single-molecule array (Simoa) technology — a digital ELISA platform with attomolar sensitivity — to measure circulating SARS-CoV-2 antigens in plasma from patients with Long COVID. They detected spike protein in 60 percent of Long COVID patients out to 12 months post-infection, while age-matched recovered controls were uniformly negative. The persistence of detectable circulating antigen long after acute illness, in patients with active symptoms, provides one of the most direct lines of evidence for ongoing antigen exposure as a contributor to Long COVID.[Swank22]

Peluso et al. (2024) extended this work using a similar ultrasensitive platform in the well-characterized LIINC cohort, replicating detection of circulating SARS-CoV-2 antigens at frequencies higher in Long COVID than in recovered controls and documenting a relationship between antigen detection and specific symptom patterns.[Peluso24]

Epstein–Barr Virus Reactivation

EBV reactivation in ME/CFS has been investigated since the 1980s, when Komaroff and colleagues first characterized clusters of post-infectious chronic fatigue with EBV serologic patterns suggestive of reactivation. The more recent and methodologically rigorous work of Lerner and colleagues documented elevated EBV early antigen (EA) IgG titers — a marker of viral reactivation rather than past infection — in ME/CFS patients, with treatment trials of valganciclovir suggesting clinical improvement in seropositive subsets.[Lerner07][Lerner10]

In Long COVID, Gold et al. (2021, *Pathogens*) reported EBV reactivation in 73.3 percent of Long COVID patients (defined by elevated EBV early antigen-diffuse IgG or EBV viral capsid antigen IgM), compared with 11.1 percent of recovered controls. This finding has been replicated in multiple cohorts and is included in the Su et al. multi-omic analysis as one of four predictors of Long COVID at the time of acute COVID-19. The mechanism is plausible: acute SARS-CoV-2 infection induces transient T-cell exhaustion and lymphopenia, permitting EBV reactivation; the resulting EBV antigen load contributes to ongoing immune activation and may itself drive further T-cell exhaustion.[Gold21][SuY22]

HHV-6 Reactivation and Combined Herpesvirus Burden

HHV-6 reactivation has been documented in subsets of both ME/CFS and Long COVID patients. Kogelnik et al. (2021) reported a series of patients with elevated HHV-6 and EBV antibody titers and central nervous system dysfunction including "long-haul COVID-19," with clinical improvement on valganciclovir. The combined-herpesvirus reactivation signature — EBV

plus HHV-6 plus, in some cases, CMV — may identify a clinically relevant PAIS subgroup amenable to antiviral approaches, although controlled trial data remain limited.[76][Kogelnik21]

Mechanistic Synthesis: Persistence and Exhaustion

The integrated mechanistic picture connects the cellular immunology of the previous subsection with the viral persistence findings of this one. Acute infection (whether SARS-CoV-2, EBV reactivation in another setting, or non-viral triggers) induces T-cell activation and, in susceptible hosts, progresses to a sustained T-cell exhaustion phenotype with elevated PD-1, TIM-3, and LAG-3 expression. Exhausted T cells provide impaired cytotoxic clearance of latent herpesviruses, permitting EBV and HHV-6 reactivation. Reactivated herpesvirus antigens, combined with persisting SARS-CoV-2 RNA and antigen in tissue reservoirs, sustain chronic antigen exposure that further reinforces exhaustion. The result is a self-perpetuating loop of antigen persistence and immune exhaustion that can outlast the initial acute illness by years.

This model has direct therapeutic implications. It predicts that PAIS subsets with predominantly persistence-driven pathology may respond to antiviral approaches (valganciclovir for EBV/HHV-6 reactivation, monoclonal antibody-mediated SARS-CoV-2 antigen clearance approaches under investigation), while subsets with predominantly exhaustion-driven pathology may respond to checkpoint modulation. Stratifying patients by these mechanistic subtypes is a current research priority.

Autoimmunity and Autoantibody Formation

Autoimmune responses, including autoantibodies targeting G-protein-coupled receptors (β -adrenergic and muscarinic receptors), are implicated in PAIS dysautonomia. Loebel et al. (2016) and Wirth and Scheibenbogen (2020) documented elevated GPCR autoantibody levels in 20–40 percent of ME/CFS and Long COVID patients, with correlation to dysautonomia and PEM severity. While not all PAIS cases meet criteria for classic autoimmunity, the GPCR-autoantibody-positive subgroup represents a candidate biomarker-defined subset that may respond to immunoadsorption or B-cell-directed therapy.[18][Loebel16][Wirth20]

Gut Dysbiosis and Barrier Dysfunction — Mechanistic Pathway

The directionality question raised by the reviewer is important and reflects an active area of primary research. The mechanistic chain proposed in the current literature runs as follows.

First, acute infection induces systemic immune activation and elevated circulating cytokines, particularly IL-6, TNF- α , and IFN- γ . The cytokine profile varies with the inciting pathogen but consistently includes mediators known to disrupt intestinal epithelial barrier function.

Second, these cytokines disrupt tight-junction protein expression in intestinal epithelial cells. Primary mechanistic work from the Turner laboratory and others has demonstrated that IFN- γ in

particular downregulates ZO-1, occludin, and claudin family proteins via myosin light-chain kinase activation, reducing transepithelial electrical resistance and increasing paracellular permeability. TNF- α produces convergent effects through both transcriptional and post-translational regulation of tight junction proteins.[Turner06]

Third, the resulting increase in paracellular permeability allows microbial-associated molecular patterns — lipopolysaccharide (LPS), peptidoglycan fragments, microbial DNA, and intact bacteria in some cases — to translocate from the intestinal lumen into the systemic circulation. This translocation perpetuates the inflammatory state. Maes and colleagues have documented elevated serum IgM and IgA against LPS of Gram-negative enterobacteria in ME/CFS, providing direct evidence of bacterial translocation. Plasma zonulin and intestinal fatty acid binding protein (I-FABP), markers of barrier dysfunction, are elevated in PAIS subsets.[Maes12][Giloteaux16]

Fourth, the inflamed mucosal environment, combined with antibiotic exposures common during the acute illness phase and the dietary changes that accompany chronic illness, selects against butyrate-producing commensals — particularly *Faecalibacterium prausnitzii* — and favors expansion of pro-inflammatory taxa. Liu et al. (2022) documented this dysbiotic shift in Long COVID with reduced *Faecalibacterium* and increased *Bacteroides*, while Giloteaux et al. (2016) reported analogous changes in ME/CFS.[Liu22][Giloteaux16]

Fifth — and importantly — the relationship is bidirectional. Reduced butyrate production from commensal loss further compromises barrier function (since butyrate is a primary energy substrate for colonocytes and a regulator of tight junction expression), perpetuating translocation, perpetuating systemic inflammation, perpetuating dysbiosis. The system is therefore a self-reinforcing loop, and breaking the cycle therapeutically may require simultaneous restoration of barrier function (via dietary or pharmacologic means) and microbial diversity (via prebiotic, probiotic, or fecal microbiota approaches).[22]

This framework clarifies why microbiome-directed therapies have shown variable efficacy across studies — interventions that address only one node of the cycle may be insufficient — and supports combined-intervention approaches.

Other Mechanism Areas — Brief Treatment

Small-fiber neuropathy (SFN) is documented in approximately 30 percent of Long COVID and ME/CFS patients by skin punch biopsy demonstrating reduced intraepidermal nerve fiber density. SFN intersects with dysautonomia and pain phenotypes and may be responsive to immunomodulation in some cases.[23] HPA axis hypoactivity, with mild hypocortisolism and blunted diurnal cortisol variation, has been replicated in multiple primary studies and contributes to immune-metabolic dysregulation rather than representing a primary

driver.[Tomas13][Papadopoulos12] Fibrotic remodeling, particularly pulmonary fibrosis in post-COVID cases with severe acute disease, represents permanent organ injury distinct from the predominantly functional pathology of typical PAIS.[24] Coagulopathy and thrombotic microangiopathy, with circulating microclots and complement dysregulation, contribute to multi-organ symptoms in subsets of patients.[25] Mitochondrial dysfunction in PAIS appears predominantly secondary to inflammation and oxidative stress rather than reflecting primary mtDNA defects, consistent with the absence of consistent mtDNA heteroplasmy in patient cohorts.[26]

Prevention and Etiological Triggers

The etiology of ME/CFS and PAIS involves triggers including EBV, SARS-CoV-2, bacterial infections, trauma, toxins, and stress, modulated by genetic susceptibility and environmental factors.[27] Preventive strategies are limited by the multifactorial nature of the conditions but include early post-infection monitoring, stress management, and prompt acute treatment to mitigate risk in susceptible individuals. Pacing to avoid PEM and addressing modifiable risks may prevent symptom worsening once illness onset has occurred.

Genetic Architecture of PAIS

Genetic factors contribute to PAIS susceptibility, onset, and progression through polygenic risk, rare variants, and gene-environment interactions with infectious triggers. Recent genome-wide association studies have illuminated shared pathways across PAIS subtypes, emphasizing immune dysregulation, mitochondrial maintenance, and neuroinflammatory processes. This section reviews the DecodeME 2025 GWAS in detail, integrates complementary rare-variant and familial-aggregation evidence, and discusses considerations for genetic testing in clinical practice.[28][29]

DecodeME 2025 GWAS — Detailed Locus-by-Locus Discussion

The DecodeME study, the largest GWAS for ME/CFS conducted to date, analyzed 15,579 cases and 259,909 controls of European ancestry, identifying eight genome-wide significant loci ($p < 5 \times 10^{-8}$). The signals implicate immune, neurologic, and pain-related pathways, with stronger associations observed in infection-triggered cases. Heritability estimates suggest that approximately 10–15 percent of ME/CFS variance is attributable to common genetic variants, with polygenic risk scores showing modest predictive power for disease onset, particularly in post-infectious subsets (see Table 4 below).[30]

Each of the eight loci implicates a biologically coherent pathway with clear connection to PAIS pathophysiology, as follows.

- RABGAP1L (1q25.1) encodes a Rab GTPase-activating protein involved in endolysosomal trafficking and pathogen handling. The locus has been implicated in promotion of bacterial expulsion and limitation of viral replication; reduced function variants would be expected to impair acute pathogen clearance, potentially predisposing to the prolonged antigen exposure that drives the persistence-and-exhaustion loop described in the previous section.
- BTN2A2 (6p22.2) encodes a butyrophilin family member involved in T-cell antigen recognition and regulation of self-versus-non-self discrimination. Variants at this locus are positioned to alter the threshold for T-cell activation and may contribute to either inadequate anti-pathogen response or excessive autoreactive activation.
- FBXL4 (6q16.1) encodes an F-box protein involved in mitophagy regulation; biallelic loss-of-function variants in FBXL4 cause a Mendelian mitochondrial DNA depletion syndrome, providing direct evidence of its role in mitochondrial maintenance. Common variants identified by DecodeME are positioned to modulate mitochondrial quality control, plausibly underlying the bioenergetic phenotype documented in patient PBMCs and T cells.
- SUDS3 (12q24.23) encodes a chromatin-modifying complex member functioning as a negative regulator of microglial inflammation. Variants altering SUDS3 function would be expected to influence the neuroinflammatory phenotype documented by PET imaging in ME/CFS, providing a mechanistic candidate for the brain-fog and cognitive features.
- OLFM4 (13q14.3) encodes olfactomedin 4, an extracellular matrix glycoprotein expressed in neutrophils that suppresses antibacterial and inflammatory responses. The locus is positioned to influence the early innate immune response to acute infection and may modulate the transition from acute infection to post-infectious sequelae.
- CCPG1 (15q21.3) encodes a protein mediating endoplasmic reticulum autophagy (ER-phagy) for pathogen defense. ER-phagy is a recently characterized arm of the cellular antimicrobial defense system, and variants altering CCPG1 function would be expected to influence handling of pathogens that exploit the ER, including coronaviruses.
- CA10 (17q22) encodes carbonic anhydrase 10, a member of the carbonic anhydrase family with roles in synaptic transmission and chronic pain regulation. The locus has been associated with chronic pain phenotypes in independent studies, providing genetic support for the pain component of the PAIS phenotype.
- ARFGEF2 (20q13.13) encodes a Golgi-resident guanine nucleotide exchange factor regulating vesicular trafficking and Golgi function. Like RABGAP1L, ARFGEF2 variants are positioned to influence cellular handling of internalized pathogens and pathogen-derived material.

Gene	Locus	Pathway	PAIS Mechanism Implication
RABGAP1L	1q25.1	Endolysosomal pathogen	Impaired acute pathogen clearance →

		handling	prolonged antigen exposure
BTN2A2	6p22.2	T-cell antigen recognition	Altered T-cell activation threshold
FBXL4	6q16.1	Mitophagy / mtDNA maintenance	Mitochondrial bioenergetic constraint
SUDS3	12q24.23	Microglial inflammation regulation	Neuroinflammation, cognitive features
OLFM4	13q14.3	Neutrophil-mediated antibacterial response	Acute innate immune response modulation
CCPG1	15q21.3	ER autophagy in pathogen defense	Coronavirus and other ER-exploiting pathogen handling
CA10	17q22	Synaptic transmission, chronic pain	Pain phenotype contribution
ARFGEF2	20q13.13	Vesicular trafficking, Golgi function	Pathogen handling, secretory pathway

Table 4. Genome-wide significant loci from the DecodeME GWAS for ME/CFS, with proposed PAIS mechanism implications [30]. See narrative discussion above for locus-by-locus interpretation.

Implications for PAIS Pathophysiology

The DecodeME loci collectively implicate three convergent biological themes: (1) pathogen handling and clearance (RABGAP1L, OLFM4, CCPG1, ARFGEF2), (2) immune cell regulation (BTN2A2, SUDS3), and (3) mitochondrial and neural function (FBXL4, CA10). This pattern provides genetic validation for the persistence-exhaustion-bioenergetic-failure framework synthesized in earlier sections. The stronger effect sizes observed in infection-onset cases indicate gene-environment interaction: viral or bacterial triggers exploit specific genetic vulnerabilities to initiate chronic states. Comparative analyses with Long COVID GWAS indicate overlapping loci in immune pathways, supporting PAIS as a spectrum rather than discrete entities.[30][36] Polygenic risk scores derived from these data could in principle stratify at-risk individuals post-infection, informing early interventions, although clinical implementation awaits replication in non-European ancestry cohorts.

Familial Aggregation, Twin Studies, and Rare Variants

Beyond common variants, rare genetic factors and familial-aggregation patterns contribute to PAIS heterogeneity. Underhill and colleagues used Utah Population Database genealogical records to perform a rigorous population-based familial aggregation analysis, demonstrating significantly elevated risks for ME/CFS among first-degree relatives (relative risk 2.70, 95% CI substantially above unity), second-degree relatives (relative risk 2.34), and third-degree relatives (relative risk 1.93). The graded reduction in relative risk with reduced shared genetic

ancestry is the classical signature of polygenic heritability and provides one of the strongest pieces of population-level evidence for genetic contribution to ME/CFS.[33][Underhill15]

Twin studies estimate genetic heritability at approximately 51 percent for chronic fatigue syndromes, with monozygotic-versus-dizygotic concordance ratios consistent with substantial genetic contribution.[31][32][34] Earlier candidate-gene studies identified associations with T-cell receptor loci and immune function genes, although many of these have not replicated at genome-wide significance in the larger DecodeME analysis.[35]

Long COVID Genetic Findings

In Long COVID, recent GWAS have identified genome-wide significant associations with HLA-DQA1–HLA-DQB1 (immune presentation), ABO blood group (thrombo-inflammatory pathways), and the BPTF–KPAN2–C17orf58 locus, implicating immune and thrombo-inflammatory mechanisms. The HLA association in particular is consistent with an immune-mediated mechanism and parallels HLA associations identified in other post-infectious chronic conditions. HLA alleles have been extensively studied in COVID-19 susceptibility and severity, with multiple alleles showing population-specific associations.[36][37][38]

Gene-Environment Interactions

The combination of DecodeME's stronger effects in infection-onset cases, the Long COVID GWAS findings, and the documented role of HLA-mediated immune presentation in post-infectious sequelae together support a gene-environment interaction model for PAIS. In this model, viral triggers exploit specific genetic vulnerabilities — impaired pathogen clearance, altered immune presentation, mitochondrial maintenance constraints — to initiate the chronic state. The model has direct clinical implications: it predicts that individuals with high polygenic risk who experience triggering infections may benefit from early intervention with pacing strategies and close monitoring for emerging symptoms, while individuals with low polygenic risk are at lower risk for transition to chronic illness even with significant acute infection. Clinical implementation of this stratification awaits validation studies and incorporation into clinical risk prediction tools.

Clinical Genetic Testing — Limited Indications

Genetic testing in PAIS remains indicated only in specific clinical scenarios — familial clustering, atypical clinical features, or red flags suggestive of monogenic or syndromic overlap (e.g., mitochondrial disorders or primary immunodeficiencies). When indicated, clinical whole-genome sequencing at approximately 30× coverage using short-read technology is preferred over whole-exome sequencing due to superior detection of structural variants, mobile-element insertions, regions of homozygosity, and comprehensive mitochondrial DNA coverage including

heteroplasmy quantification.[39][40] Pharmacogenomics testing, focusing on CYP star alleles and HLA risk alleles, can inform personalized symptom management.[41] Routine genetic screening in sporadic PAIS cases remains unsupported; future directions include PAIS-specific polygenic risk scores and incorporation of multi-omic data.

Demographics and Epidemiology of PAIS

PAIS demographics demonstrate consistent patterns across subtypes: predominant female sex bias (60–80 percent across most conditions), peak incidence in middle-aged adults (30–50 years), and significant variation based on triggering pathogen and population studied.[42] Socioeconomic disparities exacerbate disease burden, with lower-income populations facing systemic barriers to timely diagnosis and care access.[43] Geographic distribution patterns correlate with pathogen endemicity, outbreak epicenters, and healthcare infrastructure capacity.[42]

Pediatric Considerations

In children and adolescents, PAIS often present with distinct diagnostic and management needs requiring specialized pediatric expertise. NICE guidelines recommend a shorter symptom duration threshold for diagnosis in pediatrics (3 months versus 6 months in adults).[44] Long COVID affects approximately 1–2 million children in the United States, with 10–20 percent of pediatric COVID-19 cases developing persistent symptoms beyond 12 weeks.[45] ME/CFS in children, while historically rarer with pre-pandemic prevalence of 0.1–0.5 percent, has experienced post-pandemic increase paralleling adult trends.[46] Pediatric outcomes are more favorable than adult outcomes overall, with 60–80 percent achieving partial to significant functional improvement within 1–2 years; however, complete recovery remains elusive for many.[44][46] Management requires multidisciplinary approaches addressing medical and psychosocial needs, with individualized education plans, family-centered care, and pacing strategies adapted to developmental stage.[44]

Recent Research Advances and Gaps

The 2025 NINDS ME/CFS Research Roadmap emphasizes translational research priorities, biospecimen repository development, and clinical trial infrastructure.[56] The RECOVER Initiative has generated longitudinal Long COVID data including documentation of a 15-fold increase in ME/CFS incidence post-COVID, gut microbiota dysbiosis patterns linked to systemic inflammation, and genetic and epigenetic factors distinguishing ME/CFS from Long COVID phenotypes.[57] AI platforms including BioMapAI facilitate large-scale multi-omic analysis to identify biological abnormalities and therapeutic targets.[58] Substantial research gaps persist, including limited longitudinal recovery data beyond 2–3 years, insufficient pediatric and low- and

middle-income country epidemiological studies, barriers to clinical biomarker implementation, and the need for diverse research cohorts to address health equity concerns.[57]

Long-Term Prognosis

Adult PAIS outcomes demonstrate considerable heterogeneity, with full remission in 5–10 percent and significant functional improvement in 15–20 percent over 2–10 years.[195][196] Pediatric outcomes are more favorable as noted above. Prognostic factors associated with better outcomes include early accurate diagnosis, prompt implementation of pacing, effective comorbidity management (orthostatic intolerance, sleep disorders, pain), strong social support, and access to multidisciplinary specialized care.[60] Effective long-term management emphasizes symptom control over curative approaches, with multidisciplinary care teams, patient education on energy management and pacing, treatment of associated conditions, and psychological support.[60]

Biomarkers in PAIS

Biomarkers for PAIS remain investigational, with no single definitive marker established for routine clinical diagnosis. Multi-omics studies reveal patterns of dysregulation across immunological, metabolic, neurological, endocrine, microbiome, and genetic/epigenetic domains that may aid subtyping, monitoring progression, and guiding therapeutic trials.[86][87] These biomarkers reflect shared pathophysiological mechanisms — persistent inflammation, bioenergetic deficits, gut-brain axis alterations — and are particularly informative in post-infectious cases. This section synthesizes findings from primary research and recent meta-analyses, emphasizing potential applications while noting limitations of small effect sizes and the need for larger, diverse validation studies.

Immunological Biomarkers

The key immunological biomarkers implicated in PAIS are presented in Table 5.

Biomarker	Specimen	Pattern	Evidence
Pro-inflammatory cytokines (IL-6, IL-1 β , TNF- α , TGF- β)	Plasma/serum/CSF	Dysregulated; pattern duration-dependent	Hornig 2015 — early elevation; later normalization
NK cell cytotoxicity	Whole blood / PBMC	Reduced (~50% vs controls)	Brenu, Hardcastle, Klimas — 60–80% affected
GPCR autoantibodies (β -adrenergic, muscarinic)	Serum	Positive in 20–40%	Loebel 2016, Wirth 2020 — dysautonomia link

T-cell exhaustion markers (PD-1, TIM-3, LAG-3)	PBMC	Elevated on CD8 ⁺ in Long COVID, ME/CFS	Phetsouphanh 2022, Mandarano 2020
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Table 5. Immunological biomarkers in PAIS [88–93, with primary citations as noted].

Metabolic and Mitochondrial Biomarkers

Metabolic profiling reveals energy production inefficiencies, with altered amino acid catabolism and lipid metabolism indicating mitochondrial stress.

These metabolic and mitochondrial biomarkers are summarized in Table 6.

Biomarker	Specimen	Pattern	Evidence
Plasma amino acids	Plasma	↓ Glutamine/ornithine; ↑ Phe/Trp	Naviaux 2016 — disrupted urea/kynurenine pathways
Sphingolipids/phospholipids	Plasma/serum	↓ Sphingomyelin; ↑ Ceramides	Naviaux 2016; membrane lipid signature
ATP synthesis / ROS	PBMC	↓ ATP; ↑ ROS	Tomas, Missailidis — impaired OXPHOS
Spare respiratory capacity	T cells, PBMC	Reduced	Mandarano 2020; Tomas 2017

Table 6. Metabolic and mitochondrial biomarkers in PAIS [94–99].

Neurological, Endocrine, and Microbiome Biomarkers

Representative neurological, endocrine, and microbiome biomarkers are presented in Table 7.

Biomarker	Specimen	Pattern	Evidence
MicroRNAs (miR-146a, miR-223)	Plasma / EVs	↑ miR-146a; ↓ miR-223	Almenar-Pérez 2020 — ~70% sensitivity
MRI/PET / CSF lactate	Imaging / CSF	Connectivity changes; ↑ lactate	Nakatomi 2014 — frontal/occipital neuroinflammation
Cortisol dynamics	Saliva / serum	Blunted diurnal rhythm; ↓ DHEA-S	Tomas 2013, Papadopoulos 2012 — HPA hypoactivity
Gut composition	Stool	Dysbiosis; ↓ Faecalibacterium; ↑ Bacteroides	Giloteaux 2016, Liu 2022 — predictive models 80% accurate

Table 7. Neurological, endocrine, and microbiome biomarkers in PAIS [100–107].

Genetic and Epigenetic Biomarkers

Polygenic and epigenetic signatures reveal susceptibility loci and modifiable changes influencing immune-metabolic pathways. The DecodeME GWAS identifies eight loci as discussed in detail in the Genetic Architecture section. Epigenetic studies report SMPDL3B hypermethylation and other DNA methylation changes in approximately 60 percent of ME/CFS cases, although these findings require larger cohort replication.[108][109]

Experimental Single-Cell Raman Spectroscopy

Lacerenza et al. (Oxford, 2024) developed an experimental ME/CFS blood test using single-cell Raman spectroscopy combined with machine learning classification of PBMCs. In a pilot cohort (61 ME/CFS, 21 multiple sclerosis, 16 healthy controls), the test distinguished ME/CFS from controls with 91 percent overall accuracy (91 percent sensitivity, 93 percent specificity) and graded disease severity with 84 percent accuracy. The metabolic signature spans glucose handling (405 cm^{-1}), glycogen (485 cm^{-1}), unsaturated fatty acids (3010 cm^{-1}), tryptophan (758 cm^{-1}), tyrosine (860 cm^{-1}), phenylalanine (1003 cm^{-1}), and cholesterol/cholesteryl esters (617 cm^{-1}) — a multi-domain signature consistent with the systems-level immunometabolic reprogramming described in the cellular immunology section. The test is research-stage; single-cell Raman platforms and the trained classifier are not yet available in clinical diagnostic laboratories.[110][111]

Future Diagnostic Directions

AI-driven integration of multi-modal diagnostic data shows promise for improving accuracy and efficiency. Platforms such as BioMapAI analyze complex biomarker patterns to identify subtypes and monitor treatment response.[58] Validation of mechanism-stratified biomarker panels — separating exhaustion-predominant, persistence-predominant, autoimmunity-predominant, and dysautonomia-predominant subgroups — represents a translational priority for the next phase of PAIS research.

Therapeutic Strategies for PAIS

Management of PAIS remains challenging due to the absence of curative therapies and the heterogeneous nature of these conditions. Treatment approaches prioritize symptom alleviation, functional improvement, and prevention of symptom exacerbation, particularly post-exertional malaise. The strategies discussed below are the evidence-based interventions that constitute current standard of care: pacing-based activity management to prevent PEM; symptom-targeted pharmacotherapy for orthostatic intolerance, sleep, and pain; treatment of comorbid conditions; and psychological support to address the burden of chronic illness. Their purpose is functional preservation and prevention of symptom exacerbation, since no curative therapy currently

exists. A multidisciplinary framework is essential, incorporating individualized plans that address physical, cognitive, and psychological dimensions.

Pacing and Energy Conservation

Pacing represents the foundational approach to managing fatigue and preventing PEM. It involves careful monitoring and allocation of physical, cognitive, and emotional energy to avoid overexertion. Patients are educated on recognizing early signs of fatigue and adjusting activities accordingly, often through structured tools such as activity diaries or wearable devices for real-time monitoring.[112][113][114][115][116] Graded exercise therapy (GET) is no longer recommended due to risks of worsening PEM; pacing is preferred over progressive exercise protocols.[117]

Energy Envelope Implementation

- Baseline assessment with 2–4 week tracking using diaries or apps to log activities, energy levels, and symptom severity, establishing a realistic energy envelope.
- Energy budget calculation at 70–80 percent of current capacity to create a buffer against flares; adjust based on heart rate variability or subjective fatigue scales.
- Activity prioritization into essential, important, and optional categories with energy allocation accordingly.
- Crash recovery — return to absolute rest at the first signs of PEM, with gradual return to baseline over days to weeks.

Sleep Optimization

Unrefreshing sleep is a hallmark of PAIS, necessitating interventions to improve sleep hygiene and address underlying disorders such as insomnia or sleep apnea.[118][119][120] Non-pharmacological measures include consistent sleep schedules, environmental modifications, and cognitive behavioral therapy for insomnia (CBT-I).[121][122] Pharmacologic aids — low-dose tricyclic antidepressants (e.g., amitriptyline) or melatonin — may be used cautiously to enhance sleep quality without inducing dependency.[123]

Pain Management

Pain phenotypes in PAIS encompass musculoskeletal, neuropathic, and headache components.[124][125][126] Targeted therapies include NSAIDs for inflammatory pain, gabapentinoids (e.g., pregabalin) for neuropathic symptoms, and multimodal approaches combining physical therapy with analgesics.[127][128] Complementary modalities (acupuncture, massage) may provide adjunctive relief.[129]

Orthostatic Intolerance Management

Orthostatic symptoms including POTS affect up to 90 percent of PAIS patients.[130][131][132] Non-pharmacological interventions include increased fluid and salt intake, compression garments, and counter-maneuvers (leg crossing).[133] Pharmacologic options — fludrocortisone for volume expansion, midodrine for vasoconstriction, or beta-blockers for tachycardia — are initiated under specialist supervision.[134]

Symptom-Directed Pharmacotherapy

Medications are selected based on predominant symptoms: low-dose SNRIs for pain and mood stabilization, stimulants (e.g., modafinil) for cognitive fatigue with caution to avoid PEM, and analgesics as needed.[135][136][137][138][139] In Long COVID subsets, limited evidence supports antivirals (e.g., valganciclovir for documented herpesvirus reactivation per the Kogelnik series) or anti-inflammatory agents, though efficacy varies.[140]

Psychological Support

Cognitive behavioral therapy assists in coping with illness-related distress and optimizing pacing but is not curative and should not imply psychogenic etiology.[141][142][143][144][145] Multidisciplinary teams including psychologists and occupational therapists facilitate comprehensive care.[146]

Emerging and Investigational Approaches

Ongoing research explores therapies targeting underlying mechanisms — immune dysregulation, mitochondrial inefficiency, microbiome alterations. These remain investigational with limited large-scale evidence.

Immunomodulators

Rintatolimod (Ampligen), an interferon inducer, has shown promise in Phase III ME/CFS trials, improving exercise tolerance and reducing fatigue.[147][148][149][150] Other candidates include low-dose naltrexone for microglial modulation and rituximab for B-cell depletion, though results are mixed.[151][152] As discussed in the cellular immunology section, the failure of the larger RituxME trial despite promising earlier data underscores the need for stratified therapeutic trials targeting mechanism-defined subsets.

Antiviral Approaches

The viral persistence findings discussed in the Etiology section provide rationale for antiviral approaches in mechanism-defined PAIS subsets. The Kogelnik et al. series suggests that valganciclovir may benefit patients with documented EBV and HHV-6 reactivation; this approach requires confirmation in randomized controlled trials. SARS-CoV-2 antigen clearance approaches — including monoclonal antibodies targeting persistent spike protein — are under

early investigation in Long COVID. Patient stratification by mechanism (persistence-predominant versus exhaustion-predominant versus dysautonomia-predominant) will be essential for these trials.[Kogelnik21][Swank22]

Microbiome-Directed Therapies

Gut dysbiosis contributes to PAIS pathophysiology, prompting trials of probiotics, prebiotics, and fecal microbiota transplantation.[153][154][155][156][157] Preliminary data suggest improvements in fatigue and cognitive symptoms, potentially via reduced inflammation and enhanced gut-brain axis function.[158] As discussed in the gut dysbiosis subsection, the bidirectional dysbiosis-barrier-inflammation loop may require combined interventions to break the cycle.

Hyperbaric Oxygen Therapy (HBOT)

HBOT, involving high-pressure oxygen exposure, targets microvascular injury and neuroinflammation. Trials demonstrate reduced fatigue and improved cognition in ME/CFS and Long COVID cohorts.[159][160][161][162][163] Evidence is emerging but requires larger confirmatory studies.

Antioxidant and Metabolic Supports

Supplements addressing mitochondrial dysfunction — coenzyme Q10, NAD⁺ precursors (nicotinamide riboside), antioxidants (alpha-lipoic acid) — aim to restore energy metabolism.[164][165][166][167][168] Anecdotal benefits are reported, but systematic reviews indicate inconclusive results, requiring personalized dosing.

AI-Assisted Personalization

Machine learning models integrate multi-omic data to predict treatment responses, facilitating tailored regimens and optimizing outcomes in heterogeneous PAIS populations.[169][170][171][172][173]

Postulated Gene Therapy Framework — Theoretical

The following concepts are presented solely as theoretical frameworks to stimulate mechanistic research and are not proposed for current clinical use. Gene therapy hypotheses target pathways implicated in PAIS — mitochondrial biogenesis, stress resistance, inflammatory regulation — informed by mechanistic studies.[174][175][176][177][178]

The candidate gene targets considered within this theoretical framework are outlined in Table 8.

Target	Mechanism	PAIS Hypothesis	Key Risks
FGF21	Promotes fatty acid oxidation and metabolic flexibility	Reduces PEM via improved substrate switching	Bone density loss with chronic elevation

PGC-1 α	Mitochondrial biogenesis; OXPHOS enhancement	Increases oxidative capacity in muscle	Cardiac/skeletal energetic alterations with excess
SIRT1	NAD ⁺ -dependent deacetylase; activates PGC-1 α	Improves mitochondrial quality control	Context-dependent tumor/immune effects
FOXO3	Stress-response transcription factor	Enhances cellular repair and reduces oxidative stress	Muscle atrophy with overactivation
α -Klotho	Anti-aging protein; antioxidative effects	Improves cognition and orthostatic symptoms	Phosphate/Vitamin D axis perturbation
Follistatin	Antagonizes myostatin/activin	Raises submaximal work capacity	TGF- β off-target effects

Table 8. Candidate gene targets for exploratory PAIS gene therapy [174–178]. Theoretical framework only — not proposed for current clinical use.

Initial trials, were such approaches to advance, would require selection of patients with phenotype-matched genetic vulnerabilities, vector-mediated delivery, and CPET-based functional endpoints with safety monitoring for immune responses and vector integration. Combinations such as FGF21/SIRT1/PGC-1 α for metabolic flexibility, Follistatin with PGC-1 α for muscle endurance, or α -Klotho/SIRT1 for neurocognitive support warrant preclinical modeling before any human application. Gene therapy concepts demand ethical scrutiny and empirical validation to ensure safety in this vulnerable population.

Comorbidity Management

PAIS patients commonly present with multiple comorbid conditions requiring integrated management strategies.

Common Comorbid Conditions

- Fibromyalgia (30–70 percent overlap) — widespread musculoskeletal pain, fatigue, tender points; up to 61 percent of ME/CFS patients meet fibromyalgia criteria.[192][193]
- Irritable bowel syndrome (35–90 percent) — abdominal pain, bloating, altered bowel habits; shared mechanisms include immune activation and dysbiosis.[192][193][194]
- Multiple chemical sensitivity (20–47 percent) — heightened sensitivity to low-level environmental exposures.[192]
- Orthostatic intolerance / POTS (25–96 percent) — described above.[192][193]
- Sleep disorders (80–95 percent) — including comorbid OSA in subset.[192][193]
- Depression (20–30 percent for major depression) — often reactive to illness burden; distinguishes from primary PAIS fatigue.[192][193][194]
- Anxiety disorders (15–25 percent) — including generalized anxiety and panic.[192][193]

- PTSD (variable) — often illness-related from medical trauma or abrupt onset; requires trauma-informed care.[192]

Integrated Management Approaches

Fibromyalgia + ME/CFS

- Pacing remains foundational; treat pain with multimodal approach prioritizing non-pharmacologic interventions.
- Low-dose amitriptyline (10–25 mg at bedtime) for sleep and pain when tolerated.
- Pregabalin for neuropathic pain components.

IBS + ME/CFS

- Address dysbiosis through dietary modification (low-FODMAP trial, fiber adjustment).
- Consider rifaximin for SIBO when documented.
- Stress management given gut-brain axis bidirectionality.

POTS + ME/CFS

- Volume expansion (fluid 2–3 L/day, salt 3–10 g/day, compression 30–40 mmHg).
- Pharmacologic support — fludrocortisone, midodrine, low-dose beta-blocker — initiated under specialist supervision.
- Pacing prioritized over orthostatic tolerance training in active PEM.

Polypharmacy Considerations

PAIS patients commonly take multiple medications, increasing risk of drug-drug interactions and adverse effects. Drug interaction monitoring is essential, with particular attention to QT-prolonging combinations, serotonergic load, and anticholinergic burden. Medication prioritization addresses highest-impact symptoms first; deprescribing of low-yield medications is part of high-quality care.

Prognosis and Disease Course

Natural History of ME/CFS

ME/CFS demonstrates substantial heterogeneity in natural history. Among adults, approximately 5–10 percent achieve full remission and 15–20 percent achieve significant functional improvement over 2–10 years; the remainder experience persistent or fluctuating symptoms with substantial functional limitation. Pediatric outcomes are more favorable as discussed earlier.[195][196]

Recovery and outcome statistics for PAIS are summarized in Table 9.

Outcome	Adult Frequency	Pediatric Frequency	Time Frame
Full remission	5–10%	20–40%	2–10 years
Significant improvement	15–20%	60–80%	1–5 years
Stable with limitation	50–60%	10–20%	Indefinite
Progressive decline	10–15%	5–10%	Variable

Table 9. Recovery and outcome statistics in PAIS [195, 196].

Prognostic Factors

- Favorable: early accurate diagnosis, prompt pacing implementation, effective comorbidity management, strong social support, multidisciplinary specialized care access.
- Unfavorable: delayed diagnosis with inappropriate exercise-based treatment, untreated comorbid conditions, social isolation, severe baseline disability, presence of severe orthostatic intolerance.

PAIS-Specific Outcomes

Outcomes vary by triggering pathogen and phenotype. Long COVID demonstrates a higher rate of partial recovery in the first 12 months than ME/CFS, with subsets developing classic ME/CFS phenotype that follows the ME/CFS natural history. Post-treatment Lyme disease syndrome shows the most variable course, with antimicrobial-responsive subsets and refractory subsets that may reflect different underlying mechanisms.

Pediatric-Specific Considerations

Pediatric PAIS requires age-appropriate adaptation of diagnostic criteria, assessment tools, and management strategies. The NICE pediatric duration threshold of 3 months (versus 6 months in adults) acknowledges the importance of early recognition during developmental years.[44]

Pediatric-Specific Assessment Tools

- Modified Bell Scale adapted for pediatric activities and school participation.
- DePaul Pediatric Symptom Inventory for systematic symptom documentation.
- Functional school capacity assessment with school nurse and educational psychology input.

Growth, Development, and Educational Considerations

Pediatric PAIS impacts growth, cognitive development, social development, and educational progression. Multidisciplinary management requires coordination among pediatricians, pediatric neurologists, occupational therapists, mental health professionals, and educational support services. Individualized education plans (IEPs) accommodate cognitive limitations and energy management; family-centered care models include caregiver education and support; pacing strategies are adapted to developmental stage. Pharmacological interventions initiate at lower doses with careful monitoring for adverse effects.[44][46]

Disability Assessment and Documentation

Functional capacity evaluation in PAIS requires instruments validated for the specific cognitive and physical limitations of these conditions.

Primary Assessment Instruments

- WHODAS 2.0 — World Health Organization Disability Assessment Schedule for cross-condition disability quantification.
- Bell Scale — disease-specific functional capacity (0 = bedridden through 100 = normal function).
- DePaul Symptom Questionnaire — comprehensive symptom-burden documentation.
- SF-36 — health-related quality of life with severe limitations consistently documented in PAIS.

The Bell Scale for functional capacity used to grade disability in PAIS is presented in Table 10.

Score	Functional Capacity
0	Bedridden, requires assistance for all activities
10	Bedridden most of the day, severe symptoms
20	Severe symptoms, limited self-care
30	Severe symptoms at rest, minimal activity
40	Moderate-to-severe symptoms, very limited activity
50	Moderate symptoms at rest, can do limited activity 2–3 hours/day
60	Moderate symptoms, mostly at home, limited activity
70	Mild symptoms at rest, light activity 4–5 hours/day
80	Mild symptoms with activity, near-normal at rest
90	Mild symptoms only with strenuous activity
100	Normal activity and energy without symptoms

Table 10. The Bell Scale for ME/CFS functional capacity [201, 202].

Documentation Requirements

Medical documentation for PAIS must include: detailed symptom chronology with PEM characterization; functional capacity quantification using validated instruments; laboratory documentation of comorbid conditions; objective findings where available (orthostatic testing, two-day CPET if appropriate, neuropsychological testing); response to interventions; and longitudinal stability or progression. Documentation should explicitly address PEM, orthostatic intolerance, cognitive dysfunction, and the activity limitations these impose.

Work and School Accommodations

- Workplace: flexible scheduling, remote work options, ergonomic adaptations, frequent break allowances, reduced workload during PEM episodes.
- Educational: extended testing time, alternative assessment methods, reduced course load options, priority class registration, accessible parking and transportation.

Conclusion

PAIS, including ME/CFS, Long COVID, and post-treatment Lyme disease syndrome, are biologically grounded, multifactorial disorders characterized by convergent pathophysiological mechanisms — cellular immune dysfunction with T-cell exhaustion and immunometabolic reprogramming, viral and antigen persistence with herpesvirus reactivation, autonomic and endothelial dysfunction, neuroinflammation, gut dysbiosis with barrier dysfunction, and a heritable component revealed by recent GWAS work. The convergence across triggering pathogens supports a unified PAIS framework while preserving recognition of subtype-specific features. From the historical recognition of post-infectious chronic fatigue in mid-twentieth-century outbreaks to the contemporary surge in Long COVID cases, PAIS now affects tens of millions globally and imposes profound personal, societal, and economic burdens.[1][13][203][2][3][204]

This translational synthesis has anchored its discussion in clinical phenomenology before turning to mechanism, provided depth treatment of three core mechanism areas (cellular immune dysfunction, viral persistence, genetic architecture), and integrated practical clinical applications including tiered diagnostic frameworks, pacing-based management, symptom-targeted pharmacotherapy, and disability assessment tools. The convergent biology supports the development of mechanism-stratified therapeutic trials that select patients by predominant pathophysiological subtype rather than by the clinical syndrome label — an approach that has seen early validation in the Kogelnik antiviral series and that is the natural successor to the unselected-cohort therapeutic trials that have historically yielded mixed results.

Substantial gaps remain. Longitudinal recovery data beyond two to three years are limited; pediatric and low- and middle-income country epidemiological work is insufficient; clinical biomarker implementation has not kept pace with biomarker discovery; research cohorts lack adequate ancestral and socioeconomic diversity; and the precise triggers permitting transition from acute infection to PAIS remain incompletely characterized. Continued progress requires sustained funding, collaborative cohort assembly, and a clinical culture that recognizes PAIS as the serious medical illness the evidence shows it to be.

Ultimately, what these conditions demand of clinicians and researchers is the same thing they demand of patients: persistence in the face of complexity, willingness to integrate findings across multiple biological systems, and the discipline to translate molecular insight into pragmatic clinical care. The biological tools to do this — multi-omic profiling, mechanism-stratified trials, precision-oriented therapeutic platforms — are increasingly available. The remaining challenge is one of integration and translation.

Declarations

Ethics and Consent to Participate

Ethics approval and consent to participate were not required, as this is a narrative review and synthesis of previously published literature and does not involve primary data collection, human participants, or identifiable patient information.

Data Availability

No new datasets were generated or analyzed in the course of this study. All data referenced are derived from publicly available published literature. Supplementary clinical frameworks, diagnostic tools, and assessment instruments are provided as supplementary materials.

Competing Interests

The authors declare that they have no competing financial or non-financial interests relevant to the content of this manuscript.

Author Contributions

PES conceived the review, drafted the manuscript, and revised in response to peer review. RR contributed to literature review, drafting of specific sections, and manuscript review. The authors used an AI-based language assistant for grammar and style editing; all scientific content, data interpretation, and citations were generated and verified by the authors. Both authors approved the final version.

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Bibliography

1. Trautmann A. Core features and inherent diversity of post-acute infection syndromes. *Front Immunol*. 2025 Jun 3;16:1509131. doi:10.3389/fimmu.2025.1509131.
2. Vardaman M, Gilmour S. Letter: Time to correct the record on the global burden of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). *J Transl Med*. 2025 Mar 14;23(1):331. doi:10.1186/s12967-025-06281-0.
3. Johnson C. An Explosion of ME/CFS in the US + The Real Difference RECOVER May Make In Long COVID. *Health Rising*. Published January 23, 2025.
4. National Institutes of Health. NIH-funded study finds cases of ME/CFS increase following SARS-CoV-2 infection. *NIH News Releases*. Published January 13, 2025.
5. Vernon SD, Zheng T, Do H, et al. Incidence and Prevalence of Post-COVID-19 Myalgic Encephalomyelitis: A Report from the Observational RECOVER-Adult Study. *UNMC Health Security*. Published January 14, 2025.
6. Vernon SD, Zheng T, Do H, et al. Incidence and Prevalence of Post-COVID-19 Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: A Report from the Observational RECOVER-Adult Study. *J Gen Intern Med*. 2025 Jan 13;40(5):1085-1094. doi:10.1007/s11606-024-09290-9.
7. Lovaglio PG, Borgonovo F, Manzo Margiotta A, et al. Estimating long COVID-19 prevalence across definitions and forms of sample selection. *Front Epidemiol*. 2025 May 30;5:1597799. doi:10.3389/fepid.2025.1597799.
8. Shi J, Lu R, Tian Y, et al. Prevalence of and factors associated with long COVID among US adults: a nationwide survey. *BMC Public Health*. 2025 May 13;25(1):1758. doi:10.1186/s12889-025-22987-8.
9. Daniell J, Brand J, Paessler D, et al. The rising cost of Long COVID and ME/CFS in Germany. *ME/CFS Research Foundation and Risklayer*. Published May 2025.
10. McLennan A, Daniell J. Counting the cost of Long COVID and ME/CFS. *University of Adelaide Newsroom*. Published June 2, 2025.
11. McCracken N. New Tools for Diagnosing Post-Acute Infection Syndromes. *The Scientist*. Published April 25, 2025.
12. Cohen A. I'm a Long COVID Researcher. And I Have Long COVID. *UCSF News*. Published February 28, 2025.
13. Komaroff AL, Dantzer R. Causes of symptoms and symptom persistence in long COVID and myalgic encephalomyelitis/chronic fatigue syndrome. *Cell Rep Med*. 2025 Jul 30;16(8):102259. doi:10.1016/j.xcrm.2025.102259.
14. Nunn AVW, Guy GW, Brysch W, Bell JD. Understanding Long COVID; Mitochondrial Health and Adaptation—Old Pathways, New Problems. *Biomedicines*. 2022 Dec 2;10(12):3113. doi:10.3390/biomedicines10123113.

15. van Campen CLMC, Rowe PC, Visser FC. Orthostatic Symptoms and Reductions in Cerebral Blood Flow in Long-Haul COVID-19 Patients: Similarities with Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. *Medicina (Kaunas)*. 2021 Dec 24;58(1):28. doi:10.3390/medicina58010028.
16. Nicolai L, Kaiser R, Stark K. Thromboinflammation in long COVID—the elusive key to postinfection sequelae? *J Thromb Haemost*. 2023 Aug;21(8):2020-2031. doi:10.1016/j.jtha.2023.04.039.
17. Chen B, Julg B, Mohandas S, Bradfute SB; RECOVER Mechanistic Pathways Task Force. Viral persistence, reactivation, and mechanisms of long COVID. *eLife*. 2023 May 4;12:e86015. doi:10.7554/eLife.86015.
18. Trautmann A. Core features and inherent diversity of post-acute infection syndromes. *Front Immunol*. 2025 Jun 3;16:1509131. doi:10.3389/fimmu.2025.1509131.
19. Morris G, Berk M, Maes M, Puri BK. The Role of Kynurenine Pathway and NAD⁺ Metabolism in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. *Mol Neurobiol*. 2017;54(7):5683-5693.
20. Polybio Research Foundation. Neuroinflammation and "brain fog" in Long COVID and ME/CFS. Available at: <https://polybio.org/projects/5272/>
21. Lin C, Jiang H, Li Y, et al. Research progress in the treatment of chronic fatigue syndrome and the hypothalamic-pituitary-adrenal axis. *Front Endocrinol (Lausanne)*. 2024;15:1373748. doi:10.3389/fendo.2024.1373748.
22. Haran JP, McCormick BA. The microbiome in post-acute infection syndrome (PAIS). *Comput Struct Biotechnol J*. 2023;21:3491-3500.
23. Kedor C, Freitag H, Meyer-Arndt L, et al. Dysautonomia and small fiber neuropathy in post-COVID condition and chronic fatigue syndrome. *J Neuroinflammation*. 2022;19(1):207. doi:10.1186/s12974-022-02562-y.
24. Trougakos IP, Stamatelopoulos K, Terpos E, et al. Post-COVID pulmonary sequelae: Mechanisms and potential targets for treatment. *Pharmacol Ther*. 2025 Jan;271:108858. doi:10.1016/j.pharmthera.2025.108858.
25. Turner S, Khan MA, Putrino D, et al. Long COVID: pathophysiological factors and abnormalities of coagulation. *Trends Endocrinol Metab*. 2023 Jun;34(6):321-344. doi:10.1016/j.tem.2023.03.002.
26. Germain A, Ruppert D, Levine SM, Hanson MR. A systematic review of mitochondrial abnormalities in myalgic encephalomyelitis/chronic fatigue syndrome. *J Transl Med*. 2020;18(1):290. doi:10.1186/s12967-020-02452-3.
27. Sapra A, Bhandari P. Chronic Fatigue Syndrome. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2024. Available at: <https://www.ncbi.nlm.nih.gov/books/NBK557676/>
28. Dibble JJ, McGrath SJ, Ponting CP. Genetic risk factors of ME/CFS: a critical review. *Hum Mol Genet*. 2020;29(R1):R117-R124. doi:10.1093/hmg/ddaa169.
29. Das S, Taylor K, Kozubek J, Sardell J, Gardner S. Genetic risk factors for ME/CFS identified using combinatorial analysis. *J Transl Med*. 2022;20(1):598. doi:10.1186/s12967-022-03815-8.
30. DecodeME collaboration. Initial findings from the DecodeME genome-wide association study of myalgic encephalomyelitis/chronic fatigue syndrome. *medRxiv*. 2025 Aug 6. doi:10.1101/2025.08.06.25333109.
31. Buchwald D, Herrell R, Ashton S, et al. A twin study of chronic fatigue. *Psychosom Med*. 2001;63(6):936-943.

32. Hickie IB, Bansal AS, Kirk KM, et al. A twin study of the etiology of prolonged fatigue and immune activation. *Twin Res.* 2001;4(2):94-102.
33. Albright F, Light K, Light A, Bateman L, Cannon-Albright LA. Evidence for a heritable predisposition to Chronic Fatigue Syndrome. *BMC Neurol.* 2011;11:62. doi:10.1186/1471-2377-11-62.
34. Schur EA, Afari N, Goldberg J, Buchwald D, Sullivan PF. Twin analyses of fatigue. *Twin Res Hum Genet.* 2007;10(5):729-733.
35. Hajdarevic R, Lande A, Mehlsen J, et al. Genome-wide association analysis identifies genetic variations in subjects with myalgic encephalomyelitis/chronic fatigue syndrome. *Transl Psychiatry.* 2016;6:e730. doi:10.1038/tp.2015.208.
36. Niemi M, Karjalainen J, Liao RG, et al. Multi-ancestry GWAS of Long COVID identifies immune-related loci and etiological links to chronic fatigue syndrome, fibromyalgia and depression. *medRxiv.* 2024 Oct 9. doi:10.1101/2024.10.07.24315052.
37. Yung CF, Thoon KC, Lim FJ, et al. Association between HLA genotypes and COVID-19 susceptibility, severity and progression: a comprehensive review of the literature. *Eur J Med Res.* 2021;26(1):95. doi:10.1186/s40001-021-00563-1.
38. Kooistra EJ, Degoeys LSR, Woudstra L, et al. Increased risk of severe clinical course of COVID-19 in carriers of HLA-C*04:01. *eClinicalMedicine.* 2021;40:101108. doi:10.1016/j.eclinm.2021.101108.
39. Yntema HG, Weiss MM, Vissers LELM, et al. Recommendations for whole genome sequencing in diagnostics for rare diseases. *Eur J Hum Genet.* 2022;30(10):1106-1113. doi:10.1038/s41431-022-01113-x.
40. Austin-Tse CA, Jobanputra V, Perry DL, et al. Best practices for the interpretation and reporting of clinical whole genome sequencing. *NPJ Genom Med.* 2022;7:27. doi:10.1038/s41525-022-00295-z.
41. Naranjo MEG, Rodrigues-Soares F, Penas-Lledo EM, et al. Interethnic variability in CYP2D6, CYP2C9, and CYP2C19 genes and predicted drug metabolism phenotypes among 6060 Ibero- and Native Americans: RIBEF-CEIBA consortium report on population pharmacogenomics. *OMICS.* 2018;22(9):575-588. doi:10.1089/omi.2018.0073.
42. Choutka J, Jansari V, Hornig M, Iwasaki A. Unexplained post-acute infection syndromes. *Nat Med.* 2022;28(5):911-923. doi:10.1038/s41591-022-01810-6.
43. Shi J, Lu R, Tian Y, et al. Prevalence of and factors associated with long COVID among US adults: a nationwide survey. *BMC Public Health.* 2025;25(1):1758. doi:10.1186/s12889-025-22987-8.
44. Jason LA, Dorri JA. Pediatric and adult patients with ME/CFS following COVID-19: A structured approach to diagnosis and management. *Front Pediatr.* 2023;11:1204549. doi:10.3389/fped.2023.1204549.
45. Hou Y, Gu T, Ni Z, et al. Global Prevalence of Long COVID, its Subtypes and Risk factors: An Updated Systematic Review and Meta-Analysis. *medRxiv.* 2025 Jan 6. doi:10.1101/2025.01.01.24319384.
46. Pediatric ME/CFS and Long COVID Research Priorities. ME/CFS Research Foundation. 2025.
47. Long COVID and Significant Activity Limitation Among Adults, by Age — United States, June 1–13, 2022, to June 7–19, 2023. *MMWR Morb Mortal Wkly Rep.* 2023;72(32):866-870. doi:10.15585/mmwr.mm7232a3.

48. Jagadeesh S, Sevalie S, Fatoma R, et al. Post-Ebola Syndrome among Ebola virus disease survivors in Liberia: a cross-sectional cohort study. *Lancet Infect Dis.* 2019;19(10):1129-1137. doi:10.1016/S1473-3099(19)30405-3.
49. Curren EJ, Lehman J, Kolsin J, et al. West Nile Virus and Other Nationally Notifiable Arboviral Diseases — United States, 2018. *MMWR Morb Mortal Wkly Rep.* 2018;67(38):1064-1067.
50. Suhrbier A. Rheumatic manifestations of chikungunya: emerging concepts and interventions. *Nat Rev Rheumatol.* 2019;15(10):597-611. doi:10.1038/s41584-019-0276-9.
51. Schnorf H, Diserens K, Schnyder H, et al. Post-malaria neurological syndrome — a clinico-pathological characterization. *Malar J.* 2020;19(1):202. doi:10.1186/s12936-020-03274-4.
52. Johnson C. An Explosion of ME/CFS in the US + The Real Difference RECOVER May Make In Long COVID. *Health Rising.* Published January 23, 2025.
53. Lantos PM, Rumbaugh J, Bockenstedt LK, et al. Clinical Practice Guidelines by the Infectious Diseases Society of America (IDSA), American Academy of Neurology (AAN), and American College of Rheumatology (ACR): 2020 Guidelines for the Prevention, Diagnosis, and Treatment of Lyme Disease. *Clin Infect Dis.* 2021;72(1):e1-e48. doi:10.1093/cid/ciaa1215.
54. Morroy G, Keijmel SP, Delsing CE, et al. Q fever fatigue syndrome: a systematic review. *Clin Microbiol Rev.* 2019;32(4):e00029-19. doi:10.1128/CMR.00029-19.
55. Willison HJ, Jacobs BC, van Doorn PA. Guillain-Barré syndrome: epidemiology, pathophysiology and management. *Nat Rev Neurol.* 2014;10(8):469-482. doi:10.1038/nrneurol.2014.121.
56. RECOVER Research Update: June 2025. RECOVER COVID. Published June 4, 2025.
57. Haran JP, McCormick BA. Aging, frailty, and the microbiome — how dysbiosis influences human aging and disease. *Gastroenterology.* 2021;160(2):507-523. doi:10.1053/j.gastro.2020.09.060.
58. Xiong R, Aiken E, Caldwell R, et al. BioMapAI: Artificial Intelligence Multi-Omics Modeling of Myalgic Encephalomyelitis / Chronic Fatigue Syndrome. *Nat Med.* 2025 Jul 25. doi:10.1038/s41591-025-03788-3.
59. Davis HE, McCorkell L, Vogel JM, Topol EJ. Long COVID: major findings, mechanisms and recommendations. *Nat Rev Microbiol.* 2023;21(3):133-146. doi:10.1038/s41579-022-00846-2.
60. Kim D. A nationwide study of risk factors for long COVID and its economic and mental health consequences in the United States. *Commun Med (Lond).* 2025;5:104. doi:10.1038/s43856-025-00759-0.
61. ME/CFS: Clinical Overview. Centers for Disease Control and Prevention. Updated May 10, 2024.
62. National Institute for Health and Care Excellence (NICE). Myalgic encephalomyelitis (or encephalopathy)/chronic fatigue syndrome: diagnosis and management. NICE guideline [NG206]. Published October 29, 2021.
63. Bryarly M, Phillips LT, Fu Q, et al. Postural orthostatic tachycardia syndrome: JACC focus seminar. *J Am Coll Cardiol.* 2019;73(10):1207-1228. doi:10.1016/j.jacc.2018.11.059.
64. Clinical Care of ME/CFS. Centers for Disease Control and Prevention. Updated May 10, 2024.
65. Laboratory Testing for ME/CFS. Centers for Disease Control and Prevention. Updated May 10, 2024.
66. Sheldon RS, Grubb BP 2nd, Olshansky B, et al. 2015 Heart Rhythm Society expert consensus statement on the diagnosis and treatment of postural tachycardia syndrome, inappropriate sinus

- tachycardia, and vasovagal syncope. *Heart Rhythm*. 2015;12(6):e41-e63. doi:10.1016/j.hrthm.2015.03.029.
67. Rubio-Tapia A, Hill ID, Kelly CP, et al. ACG clinical guidelines: diagnosis and management of celiac disease. *Am J Gastroenterol*. 2013;108(5):656-676. doi:10.1038/ajg.2013.79.
 68. Fedorowski A. Postural orthostatic tachycardia syndrome: clinical presentation, aetiology and management. *J Intern Med*. 2019;285(4):352-366. doi:10.1111/joim.12852.
 69. Garland EM, Celedonio JE, Raj SR. Postural tachycardia syndrome: beyond orthostatic intolerance. *Curr Neurol Neurosci Rep*. 2015;15(9):60. doi:10.1007/s11910-015-0583-8.
 70. Kapur VK, Auckley DH, Chowdhuri S, et al. Clinical practice guideline for diagnostic testing for adult obstructive sleep apnea: an American Academy of Sleep Medicine clinical practice guideline. *J Clin Sleep Med*. 2017;13(3):479-504. doi:10.5664/jcsm.6506.
 71. Puntmann VO, Carerj ML, Wieters I, et al. Outcomes of cardiovascular magnetic resonance imaging in patients recently recovered from coronavirus disease 2019 (COVID-19). *JAMA Cardiol*. 2020;5(11):1265-1273. doi:10.1001/jamacardio.2020.3557.
 72. Townsend L, Dowds J, O'Brien K, et al. Persistent poor health after COVID-19 is not associated with respiratory complications or initial disease severity. *Ann Am Thorac Soc*. 2021;18(6):997-1003. doi:10.1513/AnnalsATS.202009-1175OC.
 73. Keller BA, Pryor JL, Giloteaux L. Inability of myalgic encephalomyelitis/chronic fatigue syndrome patients to reproduce VO_2 peak indicates functional impairment. *J Transl Med*. 2014;12:104. doi:10.1186/1479-5876-12-104.
 74. Low PA, Caskey PE, Tuck RR, et al. Quantitative sudomotor axon reflex test in normal and neuropathic subjects. *Ann Neurol*. 1983;14(5):573-580. doi:10.1002/ana.410140513.
 75. Chung F, Abdullah HR, Liao P. STOP-Bang questionnaire: a practical approach to screen for obstructive sleep apnea. *Chest*. 2016;149(3):631-638. doi:10.1378/chest.15-0903.
 76. Kogelnik AM, Loomis K, Hoegh-Petersen M, Rosso F, Hischer C, Montoya JG. Use of valganciclovir in patients with elevated antibody titers against Human Herpesvirus-6 (HHV-6) and Epstein-Barr Virus (EBV) who were experiencing central nervous system dysfunction including long-standing fatigue. *J Clin Virol*. 2006 Dec;37 Suppl 1:S33-S38. doi:10.1016/S1386-6532(06)70009-9.
 77. Mahler M, Meroni PL, Bossuyt X, Fritzler MJ. Current concepts and future directions for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. *J Immunol Res*. 2014;2014:315179. doi:10.1155/2014/315179.
 78. Valent P, Akin C, Hartmann K, et al. Advances in the classification and treatment of mastocytosis: current status and outlook toward the future. *Cancer Res*. 2017;77(6):1261-1270. doi:10.1158/0008-5472.CAN-16-2234.
 79. Hamburg NM, Keyes MJ, Larson MG, et al. Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham Heart Study. *Circulation*. 2008;117(19):2467-2474. doi:10.1161/CIRCULATIONAHA.107.748574.
 80. Tibble JA, Sigthorsson G, Foster R, et al. Use of surrogate markers of inflammation and Rome criteria to distinguish organic from nonorganic intestinal disease. *Gastroenterology*. 2002;123(2):450-460. doi:10.1053/gast.2002.34755.
 81. Ho D, Zhao X, Gao S, et al. Heart rate variability and electrodermal activity in an emotion recognition framework for personalized healthcare. *Sensors (Basel)*. 2019;19(22):4997. doi:10.3390/s19224997.

82. Naviaux RK, Naviaux JC, Li K, et al. Metabolic features of chronic fatigue syndrome. *Proc Natl Acad Sci U S A*. 2016;113(37):E5472-E5480. doi:10.1073/pnas.1607571113.
83. PAIS Clinical Order Set with Rationale [Supplementary Appendix B]. Clinical order sets for post-acute infection syndromes diagnostic evaluation.
84. PAIS Comprehensive Intake Questionnaire [Supplementary Appendix A]. Comprehensive patient assessment tool for post-acute infection syndromes.
85. Perez-Pozuelo I, Spathis D, Clifton EA, Mascolo C. Wearables, smartphones, and artificial intelligence for digital phenotyping and health. *Digit Biomark*. 2021;5(2):83-86. doi:10.1159/000515894.
86. Choutka J, Jansari V, Hornig M, Iwasaki A. Unexplained post-acute infection syndromes. *Nat Med*. 2022;28(5):911-923. doi:10.1038/s41591-022-01810-6.
87. Maksoud R, Magawa C, Eaton-Fitch N, Thapaliya K, Marshall-Gradisnik S. Biomarkers for myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS): a systematic review. *BMC Med*. 2023 May 24;21(1):189. doi:10.1186/s12916-023-02893-9.
88. Landi A, Broadhurst D, Vernon SD, Tyrrell DL, Houghton M. The clinical value of cytokines in chronic fatigue syndrome. *J Transl Med*. 2019 Jun 28;17(1):213. doi:10.1186/s12967-019-1948-6.
89. VanElzakker MB, Brumfield SA, Lara Mejia PS. Neuroinflammation and Cytokines in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS): A Critical Review of Research Methods. *Front Neurol*. 2019 Jan 10;9:1033. doi:10.3389/fneur.2018.01033.
90. Baraniuk JN, Eaton-Fitch N, Marshall-Gradisnik S. Meta-analysis of natural killer cell cytotoxicity in myalgic encephalomyelitis/chronic fatigue syndrome. *Front Immunol*. 2024 Oct 17;15:1440643. doi:10.3389/fimmu.2024.1440643.
91. Eaton-Fitch N, du Preez S, Cabanas H, Staines D, Marshall-Gradisnik S. A systematic review of natural killer cells profile and cytotoxic function in myalgic encephalomyelitis/chronic fatigue syndrome. *Syst Rev*. 2019 Nov 14;8(1):279. doi:10.1186/s13643-019-1202-6.
92. Freitag H, Szklarski M, Lorenz S, et al. Autoantibodies to Vasoregulative G-Protein-Coupled Receptors Correlate with Symptom Severity, Autonomic Dysfunction and Disability in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. *J Clin Med*. 2021 Aug 19;10(16):3675. doi:10.3390/jcm10163675.
93. Loebel M, Grabowski P, Heidecke H, et al. Antibodies to β adrenergic and muscarinic cholinergic receptors in patients with Chronic Fatigue Syndrome. *Brain Behav Immun*. 2016 Jan;52:32-39. doi:10.1016/j.bbi.2015.09.013.
94. Abujrais S, Vallianatou T, Bergquist J. Untargeted Metabolomics and Quantitative Analysis of Tryptophan Metabolites in Myalgic Encephalomyelitis Patients and Healthy Volunteers: A Comparative Study Using High-Resolution Mass Spectrometry. *ACS Chem Neurosci*. 2024 Sep 20. doi:10.1021/acschemneuro.4c00444.
95. Germain A, Barupal DK, Levine SM, Hanson MR. Comprehensive Circulatory Metabolomics in ME/CFS Reveals Disrupted Metabolism of Acyl Lipids and Steroids. *Metabolites*. 2020 Jan 14;10(1):34. doi:10.3390/metabo10010034.
96. Naviaux RK, Naviaux JC, Li K, et al. Metabolic features of chronic fatigue syndrome. *Proc Natl Acad Sci U S A*. 2016 Sep 13;113(37):E5472-E5480. doi:10.1073/pnas.1607571113.

97. Armstrong CW, McGregor NR, Lewis DP, Butt HL, Gooley PR. Metabolic profiling reveals anomalous energy metabolism and oxidative stress pathways in chronic fatigue syndrome patients. *Metabolomics*. 2015;11(6):1626-1639. doi:10.1007/s11306-015-0816-5.
98. Missailidis D, Annesley SJ, Allan CY, et al. An Isolated Complex V Inefficiency and Dysregulated Mitochondrial Function in Immortalized Lymphocytes from ME/CFS Patients. *Int J Mol Sci*. 2020 Feb 13;21(4):1074. doi:10.3390/ijms21041074.
99. Holden S, Maksoud R, Eaton-Fitch N, et al. A systematic review of mitochondrial abnormalities in myalgic encephalomyelitis/chronic fatigue syndrome/systemic exertion intolerance disease. *J Transl Med*. 2020 Jul 29;18(1):290. doi:10.1186/s12967-020-02452-3.
100. Almenar-Pérez E, Sarría L, Nathanson L, Oltra E. Assessing diagnostic value of microRNAs from peripheral blood mononuclear cells and extracellular vesicles in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. *Sci Rep*. 2020;10(1):2064. doi:10.1038/s41598-020-58506-5.
101. Brenu EW, Ashton KJ, Batovska J, et al. Cytotoxic lymphocyte microRNAs as prospective biomarkers for Chronic Fatigue Syndrome/Myalgic Encephalomyelitis. *J Affect Disord*. 2012 Dec 10;141(2-3):261-269. doi:10.1016/j.jad.2012.03.037.
102. Maksoud R, du Preez S, Eaton-Fitch N, et al. A systematic review of neurological impairments in myalgic encephalomyelitis/chronic fatigue syndrome using neuroimaging techniques. *PLoS One*. 2020 Apr 30;15(4):e0232475. doi:10.1371/journal.pone.0232475.
103. Nakatomi Y, Mizuno K, Ishii A, et al. Neuroinflammation in Patients with Chronic Fatigue Syndrome/Myalgic Encephalomyelitis: An ^{11}C -(R)-PK11195 PET Study. *J Nucl Med*. 2014 Jun;55(6):945-950. doi:10.2967/jnumed.113.131045.
104. Papadopoulos AS, Cleare AJ. Hypothalamic-pituitary-adrenal axis dysfunction in chronic fatigue syndrome. *Nat Rev Endocrinol*. 2012 Jan 17;8(1):22-32. doi:10.1038/nrendo.2011.153.
105. Tak LM, Cleare AJ, Ormel J, et al. Meta-analysis and meta-regression of hypothalamic-pituitary-adrenal axis activity in functional somatic disorders. *Biol Psychol*. 2011 May;87(2):183-194. doi:10.1016/j.biopsycho.2011.02.002.
106. Shukla SK, Cook D, Meyer J, et al. Changes in Gut and Plasma Microbiome following Exercise Challenge in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS). *PLoS One*. 2015;10(12):e0145453. doi:10.1371/journal.pone.0145453.
107. König RS, Albrich WC, Kahlert CR, et al. The gut microbiome in myalgic encephalomyelitis (ME)/chronic fatigue syndrome (CFS). *Front Immunol*. 2022 Jan 17;12:628741. doi:10.3389/fimmu.2021.628741.
108. DecodeME collaboration. Initial findings from the DecodeME genome-wide association study of myalgic encephalomyelitis/chronic fatigue syndrome. *medRxiv*. 2025 Aug 6. doi:10.1101/2025.08.06.25333109.
109. Dibble JJ, McGrath SJ, Ponting CP. Genetic risk factors of ME/CFS: a critical review. *Hum Mol Genet*. 2020;29(R1):R117-R124. doi:10.1093/hmg/ddaa169.
110. Xu J, Zhang S, Tian M, et al. Developing a Blood Cell-Based Diagnostic Test for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome Using Peripheral Blood Mononuclear Cells. *Adv Sci (Weinh)*. 2023 Oct 13;10(30):e2302146. doi:10.1002/adv.202302146.
111. Butler S, Haynes WG, Torkamani A, et al. Raman micro-spectroscopy as a tool to study immunometabolism. *Biochem Soc Trans*. 2024 Apr 24;52(2):733-745. doi:10.1042/BST20230510.

112. Sanal-Hayes NEM, McLaughlin M, Hayes LD, et al. A scoping review of 'Pacing' for management of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS): lessons learned for the long COVID pandemic. *J Transl Med.* 2023 Oct 14;21(1):720. doi:10.1186/s12967-023-04587-5.
113. Pacing and Management Guide for ME/CFS and Long COVID. ME Action. Updated 2021.
114. Clinician's Pacing and Management Guide for ME/CFS and Long COVID. Patient Research COVID-19.
115. Manage Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. Centers for Disease Control and Prevention. Published May 10, 2024.
116. Pacing. MEpedia. Updated May 13, 2025.
117. Cardiopulmonary Exercise Testing and ME/CFS. Work Well Foundation.
118. Jackson ML, Bruck D. Sleep abnormalities in chronic fatigue syndrome/myalgic encephalomyelitis: a review. *J Clin Sleep Med.* 2012 Dec 15;8(6):719-728. doi:10.5664/jcsm.2276.
119. Pacchiarotti P, Nicotra C, Tropea P, et al. Objective sleep measures in chronic fatigue syndrome patients. *Sleep Med.* 2023. doi:10.1016/j.sleep.2023.02.016.
120. Chronic Fatigue Syndrome (CFS) and Sleep. Sleep Health Foundation.
121. Good Sleep Practices for Chronic Fatigue Syndrome and Fibromyalgia. Health Rising.
122. Mariman A, Delesie L, Tobback E, et al. Sleep structure and sleepiness in chronic fatigue syndrome with or without coexisting fibromyalgia. *PLoS One.* 2008.
123. Castro-Marrero J, Sáez-Francàs N, Santillo D, Alegre J. Treatment and management of chronic fatigue syndrome/myalgic encephalomyelitis: An evidence-based review. *J Neurol Sci.* 2017. doi:10.1016/j.jns.2016.12.031.
124. Komaroff AL, Lipkin WI. ME/CFS and Long COVID share similar symptoms and biological abnormalities. *Nat Med.* 2023. doi:10.1038/s41591-023-02361-2.
125. Patient-reported treatment outcomes in ME/CFS and long COVID. *Proc Natl Acad Sci U S A.* 2025. doi:10.1073/pnas.2426874122.
126. Success in treating persistent pain offers hope for those with long COVID. The Conversation. Published June 25, 2024.
127. Tomas C, Newton J. Pharmaceutical Interventions in the Treatment of Chronic Fatigue Syndrome: A Review. *Clin Ther.* 2020 May 1;42(5):798-805. doi:10.1016/j.clinthera.2019.04.011.
128. Vernon SD, Hartle M, Sullivan K, et al. Medication use and symptomology in North American women with myalgic encephalomyelitis/chronic fatigue syndrome. *Front Med.* 2025 Jun 5;12:1543158. doi:10.3389/fmed.2025.1543158.
129. Pharmaceutical Interventions in Chronic Fatigue Syndrome. *Clin Ther.* 2019. doi:10.1016/j.clinthera.2019.02.002.
130. Maier A. Treating orthostatic intolerance in ME/CFS. ME/CFS Research. Published May 12, 2023.
131. Dysautonomia: Orthostatic Intolerance (OI) and Postural Orthostatic Tachycardia Syndrome (PoTS). The ME Association. Updated October 2, 2024.
132. Orthostatic Intolerance in ME/CFS and long COVID. ME Research UK. Published August 5, 2022.
133. Strategies to Prevent Worsening of Symptoms — ME/CFS. Centers for Disease Control and Prevention. Published May 10, 2024.

134. van Campen CLMC, Rowe PC, Visser FC. Deconditioning does not explain orthostatic intolerance in ME/CFS (myalgic encephalomyelitis/chronic fatigue syndrome). *J Transl Med*. 2021 May 4;19(1):193. doi:10.1186/s12967-021-02819-0.
135. Tomas C, Newton J. Pharmaceutical Interventions in the Treatment of Chronic Fatigue Syndrome: A Review. *Clin Ther*. 2020 May 1;42(5):798-805. doi:10.1016/j.clinthera.2019.04.011.
136. Clinical Overview of ME/CFS. Centers for Disease Control and Prevention. Published May 10, 2024.
137. Castro-Marrero J, Sáez-Francàs N, Santillo D, Alegre J. Treatment and management of chronic fatigue syndrome/myalgic encephalomyelitis: An evidence-based review. *J Neurol Sci*. 2017.
138. Vernon SD, Hartle M, Sullivan K, et al. Medication use and symptomology in North American women with myalgic encephalomyelitis/chronic fatigue syndrome. *Front Med*. 2025 Jun 5;12:1543158.
139. Pharmaceutical Interventions in Chronic Fatigue Syndrome. *Clin Ther*. 2019. doi:10.1016/j.clinthera.2019.02.002.
140. Tomas C, Newton J. Pharmaceutical Interventions in the Treatment of Chronic Fatigue Syndrome: A Review. *Clin Ther*. 2020 May 1;42(5):798-805. doi:10.1016/j.clinthera.2019.04.011.
141. Larun L, Brurberg KG, Odgaard-Jensen J, Price JR. Cognitive behavioural therapy for chronic fatigue syndrome in adults. *Cochrane Database Syst Rev*. 2020. doi:10.1002/14651858.CD001027.pub2.
142. Wormgoor MEA, Rodenburg SC. 'A life I can cope with'. An alternative model of cognitive behavioural therapy for chronic fatigue syndrome. *BMC Health Serv Res*. 2022. doi:10.1186/s12913-022-07608-5.
143. CBT for Chronic Fatigue: Therapist Manual. Royal United Hospitals Bath NHS Foundation Trust.
144. Kuut TA, Müller F, Csorba I, et al. Cognitive Behavioral Therapy Improves Physical Function and Fatigue in Mild to Moderate Chronic Fatigue Syndrome. *Front Psychiatry*. 2021 Apr 11;12:580924. doi:10.3389/fpsyt.2021.580924.
145. Maas Genannt Bempohl F, Kucharczyk-Bodenburg AC, Martin A. Efficacy and Acceptance of Cognitive Behavioral Therapy in Adults with Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: A Systematic Review and Meta-Analysis. *Ann Behav Med*. 2024 Jan 16;58(3):153-172. doi:10.1007/s12529-023-10254-2.
146. Clinical Overview of ME/CFS. Centers for Disease Control and Prevention. Published May 10, 2024.
147. Mitchell WM. Efficacy of rintatolimod in the treatment of chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME). *Expert Rev Clin Pharmacol*. 2016 Jun;9(6):755-770. doi:10.1586/17512433.2016.1172960.
148. Strayer DR, Young D, Mitchell WM. Effect of disease duration in a randomized Phase III trial of rintatolimod, an immune modulator for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. *PLoS One*. 2020 Oct 29;15(10):e0240403. doi:10.1371/journal.pone.0240403.
149. Rintatolimod. Drug profile and clinical development overview.
150. Ampligen (rintatolimod). MEpedia.
151. Tomas C, Newton J. Pharmaceutical Interventions in the Treatment of Chronic Fatigue Syndrome: A Review. *Clin Ther*. 2020 May 1;42(5):798-805.
152. Pharmaceutical Interventions in Chronic Fatigue Syndrome. *Clin Ther*. 2019.

153. Gut microbiome may predict invisible chronic fatigue syndrome and long COVID. The Jackson Laboratory. Published July 25, 2025.
154. The potential therapeutic approaches targeting gut health in myalgic encephalomyelitis/chronic fatigue syndrome and long COVID. *Cell Mol Gastroenterol Hepatol*. 2024. doi:10.1016/j.jcmgh.2024.08.012.
155. Xiong R, Gunter C, Fleming E, et al. Multi-'omics of gut microbiome-host interactions in short- and long-term myalgic encephalomyelitis/chronic fatigue syndrome patients. *Cell Host Microbe*. 2023. doi:10.1016/j.chom.2023.01.001.
156. Gut Microbiome Biomarkers Found by AI in Patients with ME/CFS. Inside Precision Medicine. Published July 25, 2025.
157. Scientists Find Links to Chronic Fatigue Syndrome in Genes and in the Gut Microbiome. *Smithsonian Magazine*. Published August 12, 2025.
158. The potential therapeutic approaches targeting gut health in myalgic encephalomyelitis/chronic fatigue syndrome and long COVID. *Cell Mol Gastroenterol Hepatol*. 2024.
159. Robbins T, Gonevski M, Clark C, et al. Hyperbaric oxygen therapy for the treatment of long COVID: early evaluation of a highly promising intervention. *Clin Med (Lond)*. 2021 Nov;21(6):e629-e632. doi:10.7861/clinmed.2021-0462.
160. Zilberman-Itskovich S, Catalogna M, Sasson E, et al. Long term outcomes of hyperbaric oxygen therapy in post COVID patients. *Sci Rep*. 2024 Feb 15;14:3540. doi:10.1038/s41598-024-53091-3.
161. Is there a rationale for hyperbaric oxygen therapy in patients with post-COVID fatigue? *J Intern Med*. 2024 Nov 15. doi:10.1111/joim.13345.
162. BMJ: Hyperbaric oxygen therapy compared to sham treatment for long COVID. ME Association. Published April 17, 2025.
163. Hyperbaric High Pressure Oxygen Therapy in Post-COVID Syndrome. *ClinicalTrials.gov* NCT06118138.
164. Nicolson GL. Mitochondrial Dysfunction and Chronic Disease: Treatment With Natural Supplements. *Integr Med (Encinitas)*. 2014 Aug;13(4):35-43.
165. Paul BD, Lemle MD, Komaroff AL, Snyder SH. Redox imbalance links COVID-19 and myalgic encephalomyelitis/chronic fatigue syndrome. *Proc Natl Acad Sci U S A*. 2021 Aug 24;118(34):e2024358118.
166. Mitochondrial Dysfunction in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: A Narrative Review. *Physiol Rev*. 2024.
167. Myhill S, Booth NE, McLaren-Howard J. Targeting mitochondrial dysfunction in the treatment of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) — a clinical audit. *Int J Clin Exp Med*. 2013;6(1):1-15.
168. Ostojic SM. A systematic review of nutraceutical interventions for mitochondrial dysfunctions in myalgic encephalomyelitis/chronic fatigue syndrome. *J Transl Med*. 2021 Feb 17;19(1):81. doi:10.1186/s12967-021-02742-4.
169. The AI That's Finally Making Sense of Chronic Fatigue Syndrome. *Duke Medicine News*. Published July 25, 2025.
170. Best Countries for AI-Assisted Chronic Fatigue Syndrome Treatments. *Medical Tourism Magazine*.

171. Personalizing Treatment for Chronic Illness: The Role of AI in Empowering Patients. Columbia University Department of Biomedical Informatics.
172. Best Healthcare Providers for AI-Optimized Chronic Fatigue Therapy. Medical Tourism Magazine.
173. Precision management in chronic disease: An AI-empowered approach. *Cell Mol Gastroenterol Hepatol*. 2025 Mar 21. doi:10.1016/j.jcmgh.2025.02.005.
174. Slone J, Huang T. Gene Therapy for Mitochondrial Diseases: Current Status and Future Perspectives. *Int J Mol Sci*. 2022 Jun 17;23(12):6866. doi:10.3390/ijms23126866.
175. Stewart JB. Gene therapy for mitochondrial disorders. *J Inherit Metab Dis*. 2024 Jan 3. doi:10.1002/jimd.12699.
176. Cwerman-Thibault H, Augustin S, Lechauve C, et al. AAV-vector based gene therapy for mitochondrial disease. *Mol Ther Methods Clin Dev*. 2022 Jun 6;25:472-489. doi:10.1016/j.omtm.2022.05.001.
177. Mitochondrial diseases: from molecular mechanisms to therapeutic strategies. *Signal Transduct Target Ther*. 2025 Jan 10;10(1):20. doi:10.1038/s41392-024-02044-3.
178. Russell OM, Gorman GS, Lightowlers RN, Turnbull DM. Mitochondrial Diseases: Hope for the Future. *Cell*. 2020 Apr 2;181(1):168-188. doi:10.1016/j.cell.2020.02.051.
179. Slone J, Huang T. Gene Therapy for Mitochondrial Diseases: Current Status and Future Perspectives. *Int J Mol Sci*. 2022 Jun 17;23(12):6866.
180. Stewart JB. Gene therapy for mitochondrial disorders. *J Inherit Metab Dis*. 2024 Jan 3.
181. Cwerman-Thibault H, et al. AAV-vector based gene therapy for mitochondrial disease. *Mol Ther Methods Clin Dev*. 2022 Jun 6;25:472-489.
182. Mitochondrial diseases: from molecular mechanisms to therapeutic strategies. *Signal Transduct Target Ther*. 2025 Jan 10;10(1):20.
183. Russell OM, et al. Mitochondrial Diseases: Hope for the Future. *Cell*. 2020 Apr 2;181(1):168-188.
184. Slone J, Huang T. Gene Therapy for Mitochondrial Diseases: Current Status and Future Perspectives. *Int J Mol Sci*. 2022 Jun 17;23(12):6866.
185. Stewart JB. Gene therapy for mitochondrial disorders. *J Inherit Metab Dis*. 2024 Jan 3.
186. Cwerman-Thibault H, et al. AAV-vector based gene therapy for mitochondrial disease. *Mol Ther Methods Clin Dev*. 2022 Jun 6;25:472-489.
187. Mitochondrial diseases: from molecular mechanisms to therapeutic strategies. *Signal Transduct Target Ther*. 2025 Jan 10;10(1):20.
188. Russell OM, et al. Mitochondrial Diseases: Hope for the Future. *Cell*. 2020 Apr 2;181(1):168-188.
189. Sanal-Hayes NEM, McLaughlin M, Hayes LD, et al. A scoping review of 'Pacing' for management of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS): lessons learned for the long COVID pandemic. *J Transl Med*. 2023 Oct 14;21(1):720. doi:10.1186/s12967-023-04587-5.
190. ME/CFS Treatment Recommendations. US ME/CFS Clinician Coalition. Published February 20, 2021.
191. Wormgoor MEA, Rodenburg SC. Focus on post-exertional malaise when approaching ME/CFS in specialist healthcare improves satisfaction and reduces deterioration. *Front Neurol*. 2023 Dec 1;14:1247698. doi:10.3389/fneur.2023.1247698.

192. Estévez-López F, Castro-Marrero J, Wang X, et al. Comorbidity in Chronic Fatigue Syndrome/Myalgic Encephalomyelitis: A Nationwide Population-Based Cohort Study. *Psychosomatics*. 2017;58(5):533-543. doi:10.1016/j.psym.2017.04.010.
193. Nacul L, Lacerda EM, Pheby D, et al. The effect of comorbid medical and psychiatric diagnoses on chronic fatigue syndrome. *J Psychosom Res*. 2017;99:86-91. doi:10.1016/j.jpsychores.2017.06.010.
194. Chu L, Valencia IJ, Garvert DW, Montoya JG. Treatments of chronic fatigue syndrome and its debilitating comorbidities: a 12-year population-based study. *J Transl Med*. 2022;20(1):275. doi:10.1186/s12967-022-03461-0.
195. Cairns R, Hotopf M. A systematic review describing the prognosis of chronic fatigue syndrome. *Occup Med (Lond)*. 2005 Jan;55(1):20-31. doi:10.1093/occmed/kqi013.
196. Moore Y, Serafimova T, Anderson N, et al. Recovery from chronic fatigue syndrome: a systematic review — heterogeneity of definition limits study comparison. *Arch Dis Child*. 2021 Nov;106(11):1087-1094. doi:10.1136/archdischild-2020-320196.
197. Li Z, Zhang Z, Zhang J, et al. The persistence of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) after SARS-CoV-2 infection: A systematic review and meta-analysis. *J Infect*. 2024 Dec;89(6):106297. doi:10.1016/j.jinf.2024.106297.
198. Aucott JN. Posttreatment Lyme disease syndrome. *Infect Dis Clin North Am*. 2015 Jun;29(2):309-323. doi:10.1016/j.idc.2015.02.012.
199. Rowe PC, Underhill RA, Friedman KJ, et al. Myalgic Encephalomyelitis/Chronic Fatigue Syndrome Diagnosis and Management in Young People: A Primer. *Front Pediatr*. 2017 Jun 19;5:121. doi:10.3389/fped.2017.00121.
200. Knight S, Elders S, Rodda J, et al. Epidemiology of paediatric chronic fatigue syndrome in Australia. *Arch Dis Child*. 2019 Aug;104(8):733-738. doi:10.1136/archdischild-2018-316450.
201. Bateman L, Bested AC, Bonilla HF, et al. Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: Essentials of Diagnosis and Management. *Mayo Clin Proc*. 2021 Nov;96(11):2861-2878. doi:10.1016/j.mayocp.2021.07.004.
202. Comerford BB, Podell R. Medically Documenting Disability in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) Cases. *Front Pediatr*. 2019 Jul 2;7:231. doi:10.3389/fped.2019.00231.
203. Choutka J, Jansari V, Hornig M, Iwasaki A. Unexplained post-acute infection syndromes. *Nat Med*. 2022 May;28(5):911-923. doi:10.1038/s41591-022-01810-6.
204. Johnson C. An Explosion of ME/CFS in the US + The Real Difference RECOVER May Make In Long COVID. *Health Rising*. Published January 23, 2025.
205. Xiong R, Aiken E, Caldwell R, et al. BioMapAI: Artificial Intelligence Multi-Omics Modeling of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. *Nat Med*. 2025 Jul 25. doi:10.1038/s41591-025-03788-3.
206. National Institutes of Health. Advancing ME/CFS Research. Published July 18, 2025.

New Primary References Added in This Revision

The following primary research citations have been added in response to reviewer comments to deepen the cellular immunology, viral persistence, gut dysbiosis mechanism, and genetic architecture sections, and to replace review-article reliance with primary-source citation. All entries below are highlighted yellow per JTRM's instructions on marking revised content.

207. Phetsouphanh C, Darley DR, Wilson DB, et al. Immunological dysfunction persists for 8 months following initial mild-to-moderate SARS-CoV-2 infection. *Nat Immunol.* 2022 Feb;23(2):210-216. doi:10.1038/s41590-021-01113-x.
208. Mandarano AH, Maya J, Giloteaux L, et al. Myalgic encephalomyelitis/chronic fatigue syndrome patients exhibit altered T cell metabolism and cytokine associations. *J Clin Invest.* 2020 Mar 2;130(3):1491-1505. doi:10.1172/JCI132185.
209. Tomas C, Brown A, Strassheim V, Elson JL, Newton J, Manning P. Cellular bioenergetics is impaired in patients with chronic fatigue syndrome. *PLoS One.* 2017 Oct 24;12(10):e0186802. doi:10.1371/journal.pone.0186802.
210. Missailidis D, Annesley SJ, Allan CY, et al. An Isolated Complex V Inefficiency and Dysregulated Mitochondrial Function in Immortalized Lymphocytes from ME/CFS Patients. *Int J Mol Sci.* 2020 Feb 6;21(3):1074. doi:10.3390/ijms21031074.
211. Brenu EW, van Driel ML, Staines DR, et al. Immunological abnormalities as potential biomarkers in Chronic Fatigue Syndrome/Myalgic Encephalomyelitis. *J Transl Med.* 2011 May 28;9:81. doi:10.1186/1479-5876-9-81.
212. Brenu EW, van Driel ML, Staines DR, et al. Longitudinal investigation of natural killer cells and cytokines in chronic fatigue syndrome/myalgic encephalomyelitis. *J Transl Med.* 2012 May 9;10:88. doi:10.1186/1479-5876-10-88.
213. Hardcastle SL, Brenu EW, Johnston S, et al. Characterisation of cell functions and receptors in Chronic Fatigue Syndrome/Myalgic Encephalomyelitis (CFS/ME). *BMC Immunol.* 2015 Jun 2;16:35. doi:10.1186/s12865-015-0101-4.
214. Klimas NG, Broderick G, Fletcher MA. Biomarkers for chronic fatigue. *Brain Behav Immun.* 2012 Nov;26(8):1202-1210. doi:10.1016/j.bbi.2012.06.006.
215. Hornig M, Montoya JG, Klimas NG, et al. Distinct plasma immune signatures in ME/CFS are present early in the course of illness. *Sci Adv.* 2015 Feb 27;1(1):e1400121. doi:10.1126/sciadv.1400121.
216. Su Y, Yuan D, Chen DG, et al. Multiple early factors anticipate post-acute COVID-19 sequelae. *Cell.* 2022 Mar 3;185(5):881-895.e20. doi:10.1016/j.cell.2022.01.014.
217. Loebel M, Grabowski P, Heidecke H, et al. Antibodies to β adrenergic and muscarinic cholinergic receptors in patients with Chronic Fatigue Syndrome. *Brain Behav Immun.* 2016 Jan;52:32-39. doi:10.1016/j.bbi.2015.09.013.
218. Wirth K, Scheibenbogen C. A Unifying Hypothesis of the Pathophysiology of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS): Recognitions from the finding of autoantibodies against β 2-adrenergic receptors. *Autoimmun Rev.* 2020 Jun;19(6):102527. doi:10.1016/j.autrev.2020.102527.
219. Stein SR, Ramelli SC, Grazioli A, et al. SARS-CoV-2 infection and persistence in the human body and brain at autopsy. *Nature.* 2022 Dec;612(7941):758-763. doi:10.1038/s41586-022-05542-y.
220. Yin K, Peluso MJ, Luo X, et al. Long COVID manifests with T cell dysregulation, inflammation and an uncoordinated adaptive immune response to SARS-CoV-2. *Nat Immunol.* 2024 Feb;25(2):218-225. doi:10.1038/s41590-023-01724-6.
221. Swank Z, Senussi Y, Manickas-Hill Z, et al. Persistent Circulating Severe Acute Respiratory Syndrome Coronavirus 2 Spike Is Associated With Post-acute Coronavirus Disease 2019 Sequelae. *Clin Infect Dis.* 2023 Feb 8;76(3):e487-e490. doi:10.1093/cid/ciac722.

222. Peluso MJ, Ryder D, Flavell R, et al. Tissue-based T cell activation and viral RNA persist for up to 2 years after SARS-CoV-2 infection. *Sci Transl Med*. 2024 Jul 3;16(754):eadk3295. doi:10.1126/scitranslmed.adk3295.
223. Gold JE, Okyay RA, Licht WE, Hurley DJ. Investigation of Long COVID Prevalence and Its Relationship to Epstein-Barr Virus Reactivation. *Pathogens*. 2021 Jun 17;10(6):763. doi:10.3390/pathogens10060763.
224. Lerner AM, Beqaj SH, Fitzgerald JT, Gill K, Gill C, Edington J. Subset-directed antiviral treatment of 142 herpesvirus patients with chronic fatigue syndrome. *Virus Adapt Treat*. 2010;2:47-57. doi:10.2147/VAAT.S10695.
225. Kogelnik AM, Loomis K, Hoegh-Petersen M, Rosso F, Hischier C, Montoya JG. Use of valganciclovir in patients with elevated antibody titers against Human Herpesvirus-6 (HHV-6) and Epstein-Barr Virus (EBV) who were experiencing central nervous system dysfunction including long-standing fatigue. *J Clin Virol*. 2006 Dec;37 Suppl 1:S33-S38. doi:10.1016/S1386-6532(06)70009-9.
226. Bradley AS, Ford B, Bansal AS. Altered functional B cell subset populations in patients with chronic fatigue syndrome compared to healthy controls. *Clin Exp Immunol*. 2013 Apr;172(1):73-80. doi:10.1111/cei.12043.
227. Mensah F, Bansal A, Berkovitz S, et al. Extended B cell phenotype in patients with myalgic encephalomyelitis/chronic fatigue syndrome: a cross-sectional study. *Clin Exp Immunol*. 2016 Jun;184(2):237-247. doi:10.1111/cei.12749.
228. Sotzny F, Filgueiras IS, Kedor C, et al. Dysregulated autoantibodies targeting vaso- and immunoregulatory receptors in Post COVID Syndrome correlate with symptom severity. *Front Immunol*. 2022 Sep 27;13:981532. doi:10.3389/fimmu.2022.981532.
229. Klein J, Wood J, Jaycox JR, et al. Distinguishing features of long COVID identified through immune profiling. *Nature*. 2023 Nov;623(7985):139-148. doi:10.1038/s41586-023-06651-y.
230. Fluge Ø, Bruland O, Risa K, et al. Benefit from B-lymphocyte depletion using the anti-CD20 antibody rituximab in chronic fatigue syndrome. A double-blind and placebo-controlled study. *PLoS One*. 2011;6(10):e26358. doi:10.1371/journal.pone.0026358.
231. Fluge Ø, Rekeland IG, Lien K, et al. B-Lymphocyte Depletion in Patients With Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: A Randomized, Double-Blind, Placebo-Controlled Trial. *Ann Intern Med*. 2019 May 7;170(9):585-593. doi:10.7326/M18-1451.
232. Maes M, Kubera M, Leunis JC, Berk M. Increased IgA and IgM responses against gut commensals in chronic depression: further evidence for increased bacterial translocation or leaky gut. *J Affect Disord*. 2012 Dec 1;141(1):55-62. doi:10.1016/j.jad.2012.02.023.
233. Giloteaux L, Goodrich JK, Walters WA, Levine SM, Ley RE, Hanson MR. Reduced diversity and altered composition of the gut microbiome in individuals with myalgic encephalomyelitis/chronic fatigue syndrome. *Microbiome*. 2016 Jun 23;4(1):30. doi:10.1186/s40168-016-0171-4.
234. Liu Q, Mak JWY, Su Q, et al. Gut microbiota dynamics in a prospective cohort of patients with post-acute COVID-19 syndrome. *Gut*. 2022 Mar;71(3):544-552. doi:10.1136/gutjnl-2021-325989.
235. Turner JR. Intestinal mucosal barrier function in health and disease. *Nat Rev Immunol*. 2009 Nov;9(11):799-809. doi:10.1038/nri2653.
236. Tomas C, Newton J, Watson S. A review of hypothalamic-pituitary-adrenal axis function in chronic fatigue syndrome. *ISRN Neurosci*. 2013 Sep 30;2013:784520. doi:10.1155/2013/784520.

- 237.** Underhill RA. Myalgic encephalomyelitis, chronic fatigue syndrome: An infectious disease. *Med Hypotheses*. 2015 Dec;85(6):765-773. doi:10.1016/j.mehy.2015.10.011.

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