


HYPOTHESIS

Open Access



# The potential role of ocular and otolaryngological mucus proteins in myalgic encephalomyelitis/chronic fatigue syndrome

Kaylin Huitsing<sup>1†</sup>, Tara Tritsch<sup>1†</sup>, Francisco Javier Carrera Arias<sup>1,2</sup>, Fanny Collado<sup>2,3</sup>, Kristina K. Aenlle<sup>2,3,4</sup>, Lubov Nathason<sup>2,4</sup>, Mary Ann Fletcher<sup>2,3,4</sup>, Nancy G. Klimas<sup>2,3,4</sup> and Travis J. A. Craddock<sup>1,2,3,4,5,6\*</sup> 

## Abstract

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a debilitating illness associated with a constellation of other symptoms. While the most common symptom is unrelenting fatigue, many individuals also report suffering from rhinitis, dry eyes and a sore throat. Mucin proteins are responsible for contributing to the formation of mucosal membranes throughout the body. These mucosal pathways contribute to the body's defense mechanisms involving pathogenic onset. When compromised by pathogens the epithelium releases numerous cytokines and enters a prolonged state of inflammation to eradicate any particular infection. Based on genetic analysis, and computational theory and modeling we hypothesize that mucin protein dysfunction may contribute to ME/CFS symptoms due to the inability to form adequate mucosal layers throughout the body, especially in the ocular and otolaryngological pathways leading to low grade chronic inflammation and the exacerbation of symptoms.

**Keywords** Myalgic encephalomyelitis/chronic fatigue syndrome, Mucosal-immune system, Mucins, Inflammation, Computational modeling, Systems biology

<sup>†</sup>Kaylin Huitsing and Tara Tritsch are co-first authors and contributed equally to this work.

\*Correspondence:

Travis J. A. Craddock  
tcraddock@nova.edu

<sup>1</sup> Department of Psychology and Neuroscience, College of Psychology, Nova Southeastern University, 3300 S. University Drive, Fort Lauderdale, FL 33328-2004, USA

<sup>2</sup> Institute for Neuro-Immune Medicine, Nova Southeastern University, 3300 S. University Drive, Fort Lauderdale, FL 33328-2004, USA

<sup>3</sup> Miami Veterans Affairs Medical Center, 1201 NW 16th St, Miami, FL 33125-1624, USA

<sup>4</sup> Department of Clinical Immunology, Dr. Kiran C. Patel College of Osteopathic Medicine, Nova Southeastern University, 3300 S. University Drive, Fort Lauderdale, FL 33328-2004, USA

<sup>5</sup> Department of Computer Science, College of Engineering and Computing, Nova Southeastern University, 3300 S. University Drive, Fort Lauderdale, FL 33328-2004, USA

<sup>6</sup> Present Address: Center for Collaborative Research, Room 440, Nova Southeastern University, 3300 S. University Drive, Fort Lauderdale, FL 33328-2004, USA



**Introduction**

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a serious, long-term illness characterized by a persistent, unrelenting fatigue which is accompanied by a constellation of additional symptoms that affects many body systems. Meanwhile, the etiology of ME/CFS has yet to be fully elucidated. Among the additional symptoms tied to ME/CFS are rhinitis, sore throat, and dry eyes. Approximately, 75 to 80 percent of ME/CFS subjects appear to have an irritant rhinitis with increased mucin production (Naranch et al. 2002; Baraniuk et al. 1998), and there appears to be a relationship between ME/CFS and dry eye syndrome (Chen et al. 2018; Qanneta et al. 2014), with a previous clinical study demonstrating that sicca symptoms existed in about 70 percent of ME/CFS patients (Qanneta et al. 2014). Non-exudative pharyngitis with “crimson crescents” in the posterior pharynx is reported in upwards of 80 percent of ME/CFS patients (Cunha 1992). While other reports of related symptoms range widely (i.e. sore throat 19–84%, cervical lymphadenopathy 23–76%) it is clear that these symptoms are much more prevalent in ME/CFS as compared with healthy controls (Institute of Medicine 2015).

There are current data pointing to genetic connections of the symptoms above. A pilot study examining genome wide single nucleotide polymorphisms (SNPs) in 383 ME/CFS via the commercial company 23andMe showed that approximately 70–80% of ME/CFS subjects possess abnormal variants in genes encoding for airway, eye, and salivary mucin proteins (*MUC16*, *MUC19*, and *MUC22*) at 1.60 to 3.75 the reference population (see Table 1) (Perez et al. 2019). Many of these have the potential to generate dysfunctional mucin proteins. For example,

the Combined Annotation Dependent Depletion algorithm (CADD) (Kircher et al. 2014; Rentzsch et al. 2019) indicates a maximum score of 36 for *MUC19* SNP rs10784618 followed by 24.7 for *MUC19* SNP rs11564109 where scores above 20 indicate that a particular SNP is predicted to be among the one percent most deleterious substitutions, indicating that this variant is highly deleterious, and a potential disease mitigating variant.

Mucin-19 is a secreted gel forming mucin that has been detected in the submandibular gland, sublingual gland, respiratory tract, eye, and middle ear epithelium (Linden et al. 2008). The *MUC19* SNP rs10784618 is a nucleotide change of cytosine to adenine in chromosome 12 at position 40,834,955 (GRCh37-v1.4) (Kircher et al. 2014; Rentzsch et al. 2019). This change is a coding region of the gene resulting in a nonsense point mutation causing a premature stop codon at cysteine residue 1238 in the mucin-19 amino acid protein sequence (Kircher et al. 2014; Rentzsch et al. 2019). According to UniProt (The UniProt Consortium 2021) the mucin-19 protein (UniProtKB accession number Q7Z5P9) is typically 8384 amino acids long, this mutation can result in a severely truncated, incomplete, and dysfunctional protein product that is incapable of forming a proper protective barrier. The *MUC19* SNP rs11564109 variant is a missense coding sequence variant resulting in an amino acid change at position 1411 from cysteine to tyrosine (Kircher et al. 2014; Rentzsch et al. 2019). While the significance of this alteration is uncertain, it will affect overall protein conformation as cysteine plays an important role in gel mucin structure through the formation of disulfide bonds, whereas tyrosine cannot form such bonds (Meldrum et al. 2018). The remaining variants

**Table 1** Single Point Mutations in ME/CFS Mucin Proteins Compared to 1000 Genome Reference Population

Gene name	rsID	ME/CFS %	1000 Genome %	Ratio	Effect	Scaled CADD Score	Top % of deleterious changes	Mucin type	Location
<i>MUC16</i>	rs7245949	0.57	0.23	2.53	Missense T>I	12.17	6.09	Membrane bound	Airways, eye, reproductive organs, mesothelium
<i>MUC16</i>	rs1862462	0.50	0.25	2.02	Missense S>L	3.051	49.57		
<i>MUC16</i>	rs2547072	0.50	0.25	2.02	Missense T>I	0.635	86.41		
<i>MUC16</i>	rs1867691	0.50	0.22	2.30	Missense I>V	0.009	99.79	Gel forming	Airways, eye, middle ear, salivary glands
<i>MUC19</i>	rs10784618	0.77	0.48	1.60	Stop_gained	36	0.03		
<i>MUC19</i>	rs11564109	0.24	0.08	2.85	Missense C>Y	24.7	0.34		
<i>MUC19</i>	rs2588401	0.80	0.48	1.67	Missense A>T	7.453	18.01		
<i>MUC19</i>	rs10878538	0.26	0.11	2.33	5' UTR	4.114	38.82		
<i>MUC19</i>	rs1019709	0.82	0.40	2.05	Intron	1.241	75.17		
<i>MUC19</i>	rs2588402	0.80	0.48	1.67	Missense A>T	0.069	98.43	Membrane bound	Airways, eye, middle ear, salivary glands
<i>MUC22</i>	rs10947121	0.60	0.45	1.33	Missense L>P	5.542	27.95		
<i>MUC22</i>	rs3094672	0.75	0.20	3.67	Missense S>T	0.659	85.94		
<i>MUC22</i>	rs9262549	0.72	0.19	3.75	Missense S>T	0.27	93.98		

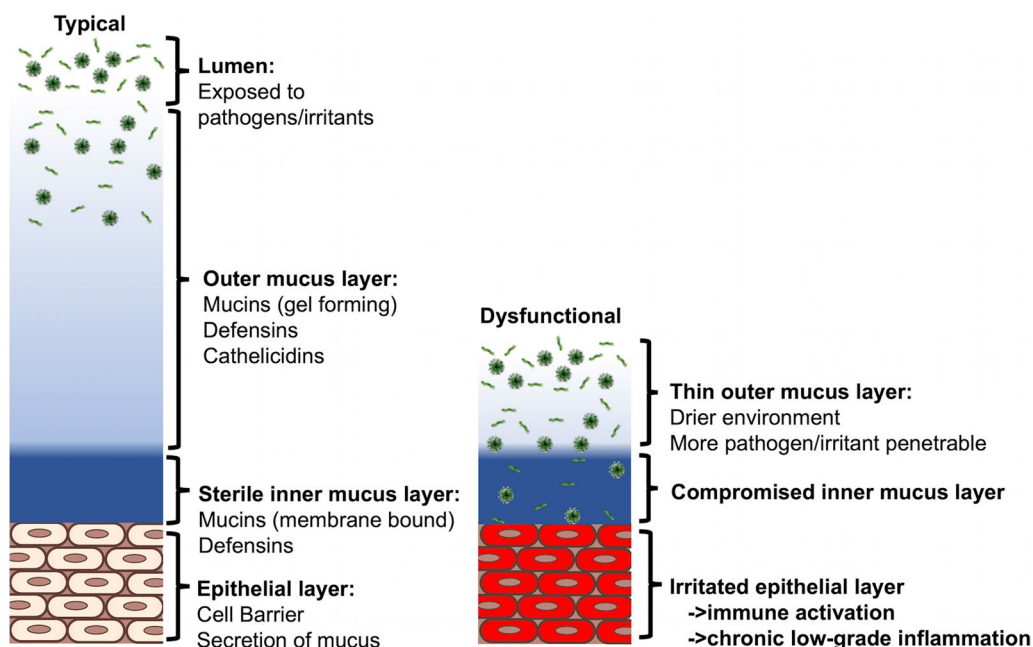
likewise result in amino acid changes (Kircher et al. 2014; Rentzsch et al. 2019), although the consequence of the substitutions is not clear as currently the three-dimensional structure of mucin-19 is not known.

Mucin-16 and mucin-22 are both membrane-bound mucins that are present on epithelial cells and serve as receptors and sensors to mediate signal transduction. Mucin-16, known as ovarian tumor marker CA125 due to its overexpression in ovarian and endometrial cancer (Felder et al. 2014), is present in a number of normal tissues, but mainly ocular surface epithelia such as the cornea, conjunctiva, lacrimal gland, accessory lacrimal glands, efferent tear ducts and also nose, uvula and larynx (Kutta et al. 2008). Mucin-16 can restrict or facilitate microbial invasion at the apical surface of the epithelium (Chatterjee et al. 2021). For example, it has been shown that there is greater binding of *Staphylococcus aureus* to in vitro-cultured corneal cells when mucin-16 is depleted (Blalock et al. 2007). Mucin-22 is a relatively novel membrane-bound mucin with previously unknown pathophysiological roles (Hijikata et al. 2011; Fini et al. 2020). Recent work indicates that mucin-22 appears to play an important protective role against severe coronavirus disease (COVID)-19 infection, with certain variants offering improved protection (Castelli et al. 2022). These variants in *MUC22* however did not include those observed in Table 1. Another variant in *MUC22* not listed in Table 1 appears to be associated with the risk of

childhood asthma (Chen et al. 2017). Thus, while the role of ME/CFS associated variants in *MUC22* are unknown, evidence suggests they may affect the respiratory tract and response to environmental pollutants or pathogens (Castelli et al. 2022; Chen et al. 2017). The variants in Table 1 for both *MUC16* and *MUC22* result in amino acid changes in the extracellular region of the membrane bound protein. The highly glycosylated extracellular mucin domains form a tight mesh structure that protects cells by bind pathogens to inhibit invasion (Putten and Strijbis 2017). It is known that serine and threonine repeats in this region are the sites of glycosylation (Brown et al. 2013), thus if these variants produce functional changes in these mucin proteins, it would therefore be in the ability of these mucins to interact with and bind pathogens.

**The hypothesis**

The role of these mucus proteins (mucin-16, mucin-19 and mucin-22) in the ear, nose, throat, respiratory tract, and eye is to protect and prevent infection (Linden et al. 2008; Dhanisha et al. 2018). Exposure to the environment risks exposure to viral and bacterial pathogens (Fig. 1). The first layer of protection from these pathogens is the outer mucus layer formed of gel like mucins (such as mucin-19), and anti-bacterial peptides such as defensins and cathelicidins (Linden et al. 2008; Dhanisha et al. 2018; Repentigny et al. 2015). This outer mucus layer

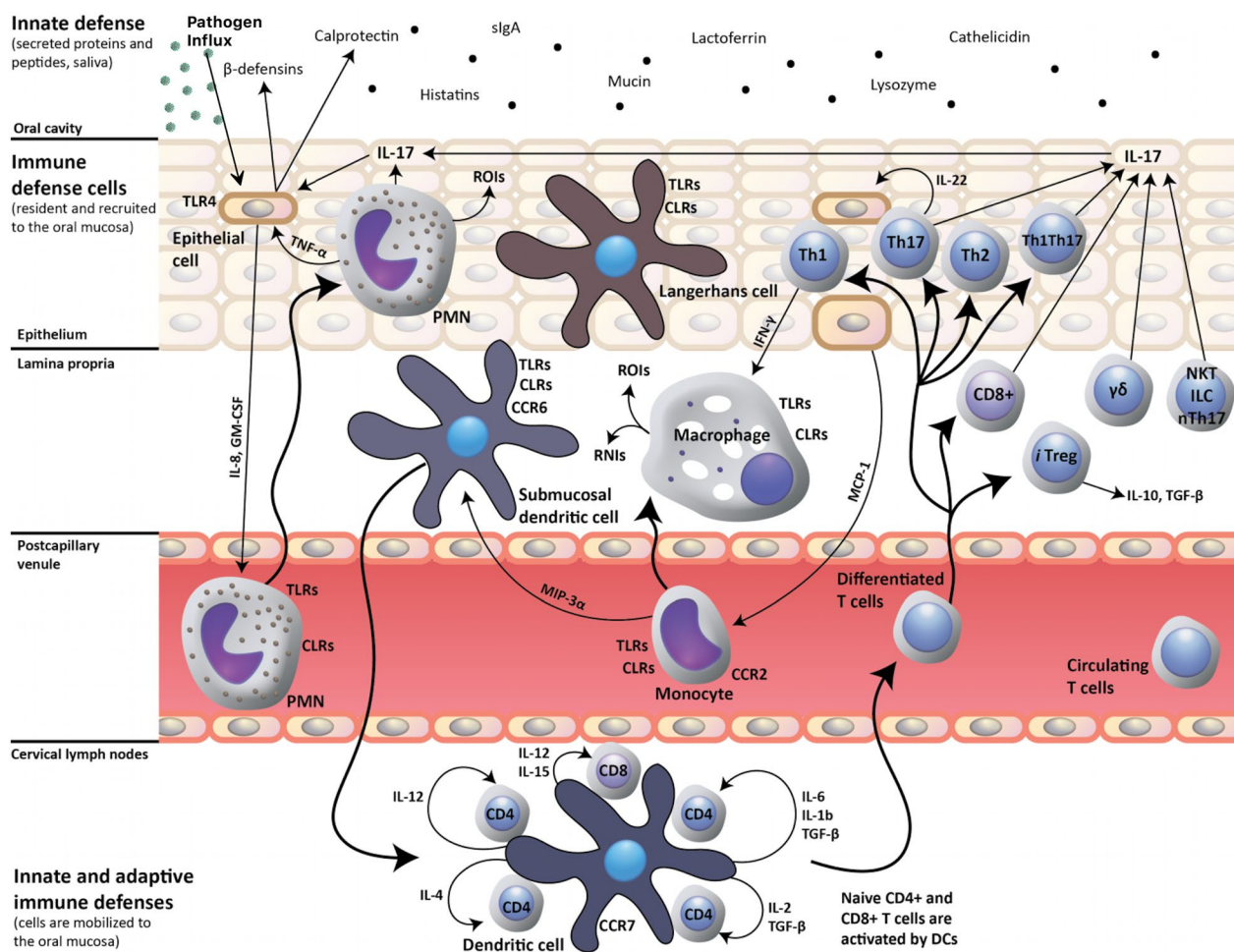


**Fig. 1** The mucosal protective barrier. (Left) Pathogens present in the lumen are prevented from reaching the epithelium via a first layer of gel-like mucins and antibacterial peptides, and a second layer of membrane bound mucins (Linden et al. 2008; Dhanisha et al. 2018). (Right) Dysfunctional outer and inner mucin proteins lead to a compromised inner mucus layer and irritated epithelium (Linden et al. 2008; Dhanisha et al. 2018)

serves to protect the second inner mucus layer adhered to the epithelium, keeping it sterile to avoid irritating the epithelial layer (Fig. 1, left). Dysfunctional changes that decrease the outer gel layer of mucus (i.e., through non-functional mucin-19), coupled with an inner mucus layer incapable of binding pathogens (i.e., dysfunctional mucin-16 and mucin-22) would result in a compromised mucus layer leading to a chronically irritated epithelial layer (Fig. 1, right).

When pathogens manage to cross the mucus layer the response by the immune system from resident and recruited immune defense cells containing T-cells, dendritic cells, and macrophages, and further from the

immune system within the blood comprising of peripheral blood mononuclear cells (Fig. 2) would result in a chronic low-grade inflammation (Repentigny et al. 2015; Zhang et al. 2023; Song et al. 2020). Expanding on the roles of the mucosa and cytokines within the ocular and otolaryngological environment, there is speculation as to the resulting deficits in the mucin-16, mucin-19 and mucin-22 proteins. While preliminary studies have shown that numerous individuals have variants in these proteins (1000 Genomes Project Consortium (2015), it is hypothesized that these variants cause dysfunction in the mucosal protective barrier. A dysfunctional mucosal barrier will result in a compromised barrier between



**Fig. 2** Host response to oral pathogen influx. A protective host response to infection is dependent on dendritic cell-mediated induction of Th17 cell-mediated adaptive immunity, which, by the production of interleukin (IL)-17 upregulates the innate expression of mucosal antimicrobial peptides ( $\beta$ -defensins, calprotectin) by epithelial cells. IL17 also up-regulates IL8 and granulocyte–macrophage colony-stimulating factor (GM-CSF) production by epithelial cells, which in turn trigger recruitment of polymorphonuclear neutrophils (PMN) to the oral mucosa. Innate-like cell populations, including  $\gamma\delta$  T-cells, Natural Killer T cells (NKT), innate lymphoid cells (ILC) and natural Th17 cells (nTh17), also produce IL17 and may participate in the mucosal host response. CLR, C-type lectin receptors; RNI, reactive nitrogen intermediates; ROI, reactive oxygen intermediates; and TLR, toll-like receptors. Image adapted from (Repentigny et al. 2015) under the Creative Commons Attributions License 4.0 International (CC BY 4.0)

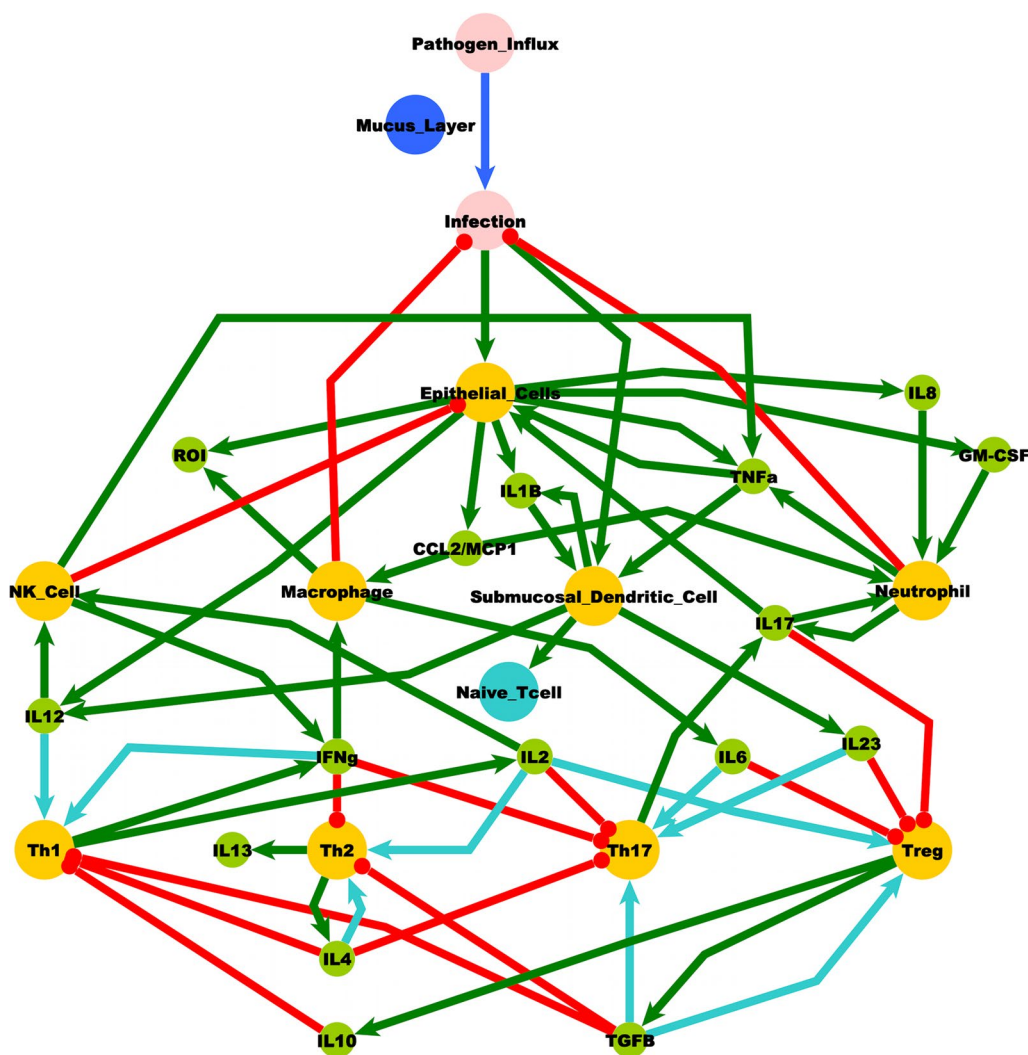
epithelial cells and the environment. Smaller or weaker barriers permit pathogen access and infiltration leading to a persistent low-grade inflammation in which cytokines will be consistently released contributing to continuous sickness behaviors like those seen with ME/CFS.

### Evaluation of the hypothesis

#### Discrete ternary logic analysis of regulatory network

Our previous work (Craddock et al. 2014, 2015, 2018; Fritsch et al. 2014) suggests that the complexity of the mucosal-immune signaling system can allow for multiple regulatory modes beyond what is typically considered

typical health. To provide a theoretical framework for our hypothesis here we compile molecular and cellular signaling information from various studies and reviews in the literature (Repentigny et al. 2015; Ohradanova-Repic et al. 2023; Romero et al. 2004; Kato 2015; Barnes et al. 2022; Mettelman et al. 2022; Sato and Kiyono 2012; Davis et al. 2017; Costello et al. 2015; Laulajainen-Hongisto et al. 2020) to create a logically consistent, theoretical model of a general ocular and otolaryngological mucosal-innate immune signaling system to explore the role of mucus protection in the homeostatic regulation of the innate immune system and the perpetuation of chronic low-grade inflammation (see Fig. 3). Logic



**Fig. 3** A literature-based logic model of mucosal-immune system interactions. Nodes: Yellow, distinct immune cell types; Green, immune signaling molecules; Red, environmental pathogens. Edges: Red, inhibition; Green, stimulation. Conditionals: Cyan, Naive T Cell dependent stimulation; Blue, Mucus layer dependent stimulation. GM-CSF—granulocyte–macrophage colony-stimulating factor; IFNg—interferon  $\gamma$ ; IL—interleukin; ROI—reactive oxygen intermediary; NK—natural killer cells; CCL2/MCP1—chemokine ligand 2/monocyte chemoattractant protein 1; TGFB—transforming growth factor  $\beta$ ; Th—T helper cell; TNFa—tumor necrosis factor  $\alpha$ ; Treg—regulator cell

rules are applied to this connectivity diagram to predict the system's homeostatic behavior. Using a similar approach reported in (Craddock et al. 2015; Craddock et al. 2014; Thomas 1991; Thomas et al. 1995; Mendoza and Xenarios 2006) the mucosal-immune system in Fig. 3 was captured as a logic connectivity model consisting of interconnected nodes with three discrete states:  $-1$  (suppressed),  $0$  (normal) and  $+1$  (increased). In brief, the state of the system at a point in time was described by an assignment of discrete states to all nodes. The state that each node in the system transitions to in the next time step was determined from a set of balanced ternary logic statements [see (Craddock et al. 2015)], the node's current state, and the defined interactions (i.e. activate or inhibit) of the neighboring input nodes. The logic is such that an increase in activators raises the node value, while a decrease in inhibitors decreases the value. In cases where both activators and inhibitors were increased, the node value remained unchanged. While the number of activators and/or inhibitors for a given variable may remain static, they may also be allowed to change based on predefined conditions, such as the state of one or more variables as described in Arias et al. (2021). In the system described in Fig. 3 conditional edges are dependent on the state of Naïve T Cells. The system is updated asynchronously (allowing only one variable to change at a time), such that for each current state there are potentially several subsequent states towards which it may evolve. The number of states, and the values they can be assigned, determine the total number of states available to the model system. By analyzing all possible states of the system, a temporal sequence of states was discerned. Steady states were defined as those states for which the current system state did not evolve in time. The steady states of the mucosal-immune system are given in Table 2.

Beyond normal homeostatic regulation, our model predicts alternate self-perpetuating conditions consistent with chronic inflammation. Three stable states are shown in Table 1 with SS0 corresponding to a typical healthy state, while both SS1 and SS2 present with a stable altered Th1 immune profile. As such, these simulations of pathogen influx with deficient mucus protection were shown to be theoretically capable of forcing the system to a state of immune activation supporting a potential role for the mucosal-immune signaling system's own homeostatic drive in perpetuating chronic low-grade inflammation.

#### Comparison to ME/CFS cytokine panels

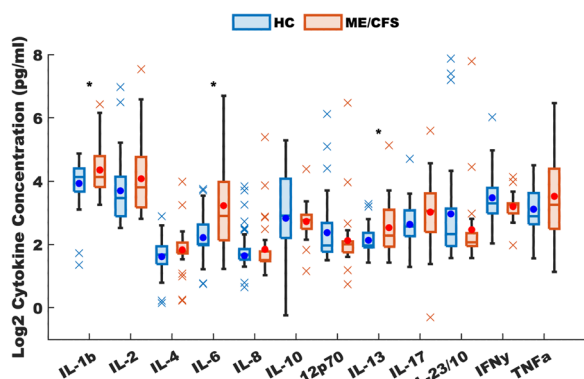
To determine the applicability of this model to ME/CFS in specific, we compared our model predicted homeostatic stable states to cytokine signaling profiles in blood of female subjects with ME/CFS. Clinical data obtained

**Table 2** Stable behaviors of the mucosal-immune system

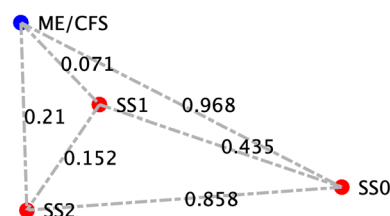
Nodes	SS0	SS1	SS2
Submucosal dendritic cell	0	1	1
Infection	0	0	0
Epithelial cell	0	0	-1
IL1 $\beta$	0	1	1
TNF $\alpha$	0	1	0
IL12	0	1	1
IL8	0	0	-1
CCL2/MCP1	0	0	-1
GM-CSF	0	0	-1
Naive Tcell	0	1	1
Th1	0	1	1
Th2	0	0	-1
Th17	0	0	-1
Treg	0	0	-1
IFN $\gamma$	0	1	1
IL2	0	1	1
IL6	0	1	1
TGF $\beta$	0	0	-1
IL4	0	0	-1
IL10	0	0	-1
IL23	0	1	1
IL17	0	1	0
IL13	0	0	-1
Neutrophil	0	1	0
ROI	0	1	1
Macrophage	0	1	1
NK cell	0	1	1

as part of a larger on-going study investigating changes in cytokines in ME/CFS was used as a basis for comparison with the predicted resting states [see (Morris et al. 2019)]. A total of 65 female subjects (29 with ME/CFS, 36 healthy controls), and 53 male subjects (25 with ME/CFS, 28 healthy controls) were selected without exclusion for ethnicity from the patient population within the Institute for Neuroimmune Medicine at Nova Southeastern University (NSU) in Fort Lauderdale, Florida, directed by Nancy Klimas, M.D. All subjects signed an informed consent approved by the Institutional Review Board (IRB) of NSU, Fort Lauderdale, Florida. Included subjects presented with acute onset and with an illness duration of at least 4 years. ME/CFS was diagnosed according to current research case definitions (Fukuda et al. 1994; Carruthers et al. 2003): fatigue of greater than 6 months duration and at least four of eight symptoms including exercise-induced relapse, myalgia, arthralgia, headache of a new and different type, nonrestorative sleep, cognitive complaints, sore throat, and tender lymph nodes. All ME/CFS study subjects presented with a 36-short

form health survey (SF-36) summary physical composite score below the 50th percentile, based on population norms. Healthy controls were self-defined as sedentary (no regular exercise program, sedentary employment). Plasma concentrations of interleukin (IL) 1 $\beta$ , IL2, IL4, IL6, IL8, IL10, IL12p70, IL13, IL17, IL23, IFN $\gamma$  and TNF $\alpha$  were measured via Q-Plex multiplex ELISA (Quansys Biosciences, Logan, Utah) from blood obtained at rest. A meta-analysis was used to calculate the significance of similarity between the inflammatory profiles of subjects with ME/CFS, and the equilibrium states predicted by the logic model. To do this the cytokine profiles were compared to each model-predicted steady-state behavior of the mucosal-immune system through the application of Brown's theoretical approximation (Brown 1975) of Fisher's statistics, as conducted in our previous work (Craddock et al. 2014, 2015, 2018; Fritsch et al. 2014; Arias et al. 2021; Rice et al. 2014). This method was chosen as it provides a meta-analysis technique to combine non-independent probabilities and obtain an overall significance measure P based on a set of *p*-values obtained from independent *t*-tests. The aggregate value P ranges between 0 and 1, with 0 indicating complete overlap and 1 being the farthest distance from a stable state. This method is applicable as the model elements do not express independently, as evidenced by the connectivity of the mucosal-immune interaction model (Fig. 3). The above-mentioned cytokine data were compared against the model predicted states based on the 12 measured variables. To visualize the comparison of the measured states with the model-predicted stable states the multi-dimensional co-expression profiles (Figs. 4 and 6)

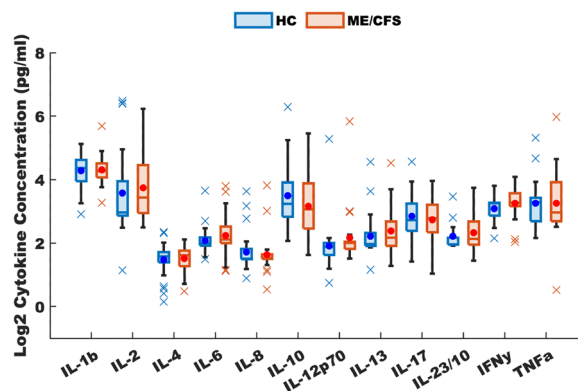


**Fig. 4** Log<sub>2</sub> normalized plasma cytokine concentrations at rest for female ME/CFS subjects compared to healthy sedentary controls. Horizontal line indicates the median, solid dot indicates the overall mean, box shows the lower and upper quartiles, x indicates outliers, and whiskers indicate the minimum and maximum values that are not outliers. \**p* < 0.05 for two-tailed heteroscedastic *t*-test. Note: IL23 concentration is reduced by a factor of 10 to fit on the graph scale



**Fig. 5** Projections of the comparison between the model predicted stable states and female ME/CFS cytokine profiles. SS0 indicates typical health, SS1 is a stable state with an increased Th1 immune profile, and SS2 is a stable state with an increased Th1 and decreased Th2, Th17 and Treg immune profile. Distances between points are measures of dissimilarity as determined by an aggregate P-value based on Brown's methods for combining multiple non-independent statistical tests

were projected into a two-dimensional space using multidimensional scaling as done previously (Arias et al. 2021) (see Figs. 5 and 7). Here, the dissimilarity matrix defined by the aggregate P value is scaled such that the 2D Euclidean distances between points approximate the corresponding dissimilarities. This is performed using the function *mdscale* in MATLAB to minimize Kruskal's stress criterion normalized by the sum of squares of the dissimilarities. After comparing the stable states in Table 2 with the female cytokine profiles of ME/CFS patients (Fig. 4), we found that SS1 was the most closely aligned with the ME/CFS profile (Fig. 5). The SS1 state is characterized as an inflammatory state by increased pro-inflammatory cytokines (IL1 $\beta$ , IL2, IL6, IL12, IL17, IL23, IFN $\gamma$ , TNF $\alpha$ ), activation of the innate immune cells (Neutrophil, Macrophage, NK Cell, Submucosal Dendritic Cell) and a shift towards Th1 immunity. The next nearest



**Fig. 6** Log<sub>2</sub> normalized plasma cytokine concentrations at rest for male ME/CFS subjects compared to healthy sedentary controls. Horizontal line indicates the median, solid dot indicates the overall mean, box shows the lower and upper quartiles, x indicates outliers, and whiskers indicate the minimum and maximum values that are not outliers. \**p* < 0.05 for two-tailed heteroscedastic *t*-test, no significant differences found. Note: IL23 concentration is reduced by a factor of 10 to fit on the graph scale

state was the SS2 state. The SS2 state is characterized as a mixed inflammatory state by an increase in some pro-inflammatory cytokines (IL1 $\beta$ , IL2, IL6, IL12, IFN $\gamma$ ), a decrease in other pro-inflammatory cytokines (IL8, IL23, TNF $\alpha$ ), a decrease in anti-inflammatory cytokines (IL4, IL10, TGF $\beta$ ), activation of some of the innate immune cells (Macrophage, NK Cell, Submucosal Dendritic Cell), an upward shift towards Th1 and downward shift away from Th2, Th17, and Treg immunity. Female ME/CFS cytokine profiles were furthest from the typically healthy state SS0. This is consistent with our hypothesis that ME/CFS presents with a low-grade inflammatory profile. However, when comparing the male cytokine profile of ME/CFS subjects (Fig. 6), it was found to align near equidistant from health (SS0) and the SS1 state, with the SS1 being slightly more favorable (Fig. 7), but unlike females it was found to be the closest to the SS2 mixed inflammatory state. The difference between male and female cytokine profiles is consistent with previous work suggesting a sex difference in males with ME/CFS (Jeffrey et al. 2019; Nkiliza et al. 2021; Friedberg et al. 2023; Lim and Son 2021; Cheema et al. 2020; Pollack et al. 2023).

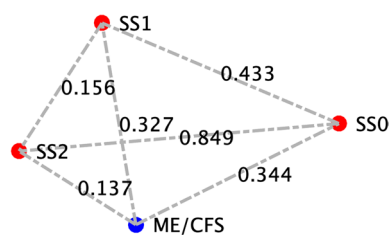
### Consequences of the hypothesis and discussion

The ocular and otolaryngological mucus layers normally act to protect the epithelial tissue from irritants, microorganisms and pathogens entering the body (Linden et al. 2008; Dhanisha et al. 2018). Changes in the mucus layer lining can often be symptoms of illness such as diabetes (Negrato and Tarzia 2010), human immunodeficiency virus (HIV) (Heron and Elahi 2017), vitamin deficiency (Philipone et al. 2017) or even neurodegenerative illnesses such as Alzheimer's and Parkinson's diseases (Aufret et al. 2021; Paraskevaïdi et al. 2020). Here we have presented a hypothesis that the symptoms observed in the chronic illness of ME/CFS may, in part, be associated

with a compromised ocular and otolaryngological mucus layer leading to increased likelihood of irritation of the epithelial layer in these regions resulting in a chronic low-grade inflammation. This is consistent with findings indicating a preponderance of rhinosinusitis symptoms in subjects with unexplained chronic fatigue and bodily pain (Chester 2003).

The alignment of several immune markers modeled here with experimental data from men and women with ME/CFS supports at least partial involvement of the body's own homeostatic drive in facilitating the perpetuation of this condition and a chronic dysregulated immune system. When considering these alignments, however, it is important to remember that the hypothesis outlined above does not state that ME/CFS results solely from inflammation. Instead, it is proposed that homeostatic drive might be a significant contributor to the persistence of illness mechanisms, which may be exacerbated by a compromised mucosal protective barrier. These naturally occurring alternate immune regulatory regimes, once entered, provide an alternate stable homeostasis resistant to change, that can support many chronic pathological processes (Craddock et al. 2014; Fritsch et al. 2014), including ME/CFS symptoms related to inflammation (Komaroff 2017). This alignment is also not expected to remain static (Fritsch et al. 2014). Under typically healthy conditions small perturbations of the immune system result in a regulatory response that will fluctuate in time around its normal homeostatic state (Fritsch et al. 2014). Likewise, an illness in the vicinity of an alternate stable state will also fluctuate, albeit differently from health (Fritsch et al. 2014). This is consistent with literature findings of mixed and variable inflammation profiles in ME/CFS (VanElzakker et al. 2019; Blundell et al. 2015; Strawbridge et al. 2019). It is this overall altered inflammation regulation which can correlate with symptoms in ME/CFS (Komaroff 2017), even though static cytokine profiles themselves are transient and variable.

Beyond the potential exacerbation of immune activation caused by a compromised mucus layer it is difficult to discuss the consequences of these mucin variants without knowing if they are over/under expressed in ME/CFS. The GWAS SNP analysis presented here only exposes the presences of these variants in the illness cohort. However, past studies have shown immune, and brain functional consequences associated with the mucin SNPs found in ME/CFS as presented in Table 1. For the *MUC16* SNP rs7245949 a study of the association with tumor mutation burden has shown it is associated with lower expression in markers of T-cell responses (Wang et al. 2020). The remaining three *MUC16* SNPs (rs1862462, rs1867691, rs2547072) have all been shown to be significantly associated with changes in grey matter



**Fig. 7** Projections of the comparison between the model predicted stable states and male ME/CFS cytokine profiles. SS0 indicates typical health, SS1 is a stable state with an increased Th1 immune profile, and SS2 is a stable state with an increased Th1 and decreased Th2, Th17 and Treg immune profile. Distances between points are measures of dissimilarity as determined by an aggregate P-value based on Brown's methods for combining multiple non-independent statistical tests



volumes in ten brain regions in a study of genetic biomarkers for Alzheimer's Disease (Zeng et al. 2021). Two of the *MUC19* SNPs (rs2588401 and rs2588402) have been associated with the chronic inflammatory disorder asthma (Karunas et al. 2015a, b; Levchenko et al. 2018), while *MUC19* SNP rs2588401 shows an association with acute lymphocytic leukemia, a cancer of immature lymphocytes, a type of white blood cell involved in the body's immune system (Alcântara et al. 2022). The *MUC22* SNP rs10947121 has been associated with ankylosing spondylitis, a type of arthritis that causes inflammation in the joints and ligaments of the spine, as well as peripheral joints like the knees, ankles, and hips (Chen et al. 2016). Finally, an association between *MUC22* SNP rs3094672 and the autoimmune disorder multiple sclerosis has been shown, albeit with a protective role (Dankowski et al. 2015). To the best of our knowledge, beyond these handful of studies, the functional consequences of the mucin variants identified here in ME/CFS have not been investigated.

The association of these variants with inflammatory disorders may point to a role in ME/CFS. Many studies of ME/CFS have found evidence of abnormal T cell populations (Mandarano et al. 2020; Rivas et al. 2018; Brenu et al. 2016; Fletcher et al. 2000), and reduced natural killer (NK) cell function (Barker et al. 1994; Whiteside and Friberg 1998; Fletcher et al. 2002; Brenu et al. 2012, 2014; Klimas et al. 1990). Studies indicate NK cell function correlates with illness severity (Rivas et al. 2018; Ojo-Amaize et al. 1994; Strayer et al. 2015), however the reason for this reduced function is unknown. As T and NK cells need to form immune synapses with their target which involve very close cell–cell contact (Bromley et al. 2001) the presence of an adhesive or anti-adhesive molecule, like mucins, on the surface of cells may have significant consequences for cell interactions. As discussed above, *MUC16* SNPs associated with ME/CFS are associated with T cell and lymphocyte dysfunction. Additionally, mucin-19 is suggested to specifically alter CD4+ T cell response (McBride et al. 2020). It's hypothesized that this dysfunction is due to the large and heavily glycosylated mucin-16 preventing the establishment of a robust immunologic synapse between T cells and major histocompatibility complex presentation of antigens on the cell surface (Wang et al. 2020). This is supported by flow cytometry results which suggest significant deficits in the expression of receptors and adhesion molecules on subsets of CD8+ T cells in ME/CFS patients may contribute to disease pathogenesis (Brenu et al. 2016). Moreover, mucin-16, either detached or adhered to the cell surface, has been shown to directly inhibit the natural cytotoxicity mechanism of NK cells (Gubbels et al. 2010). NK cell activation triggered by inflammatory mediators,

cytokines, and chemokines, including IL-2 and IL-12 following recognition of stressed, and infected cells leads NK cells to lyse target cells and secrete IFN $\gamma$  and TNF $\alpha$  (Hardcastle et al. 2015). The reduced ability of NK cells to recognize and clear infected and stressed epithelial cells coupled with the proposed increased propensity of the epithelium to be irritated and infected in ME/CFS due to dysfunction in the mucus layer is expected further exacerbate this problem leading to an increase in associated symptoms with a decrease in NK cell function consistent with literature.

While studies of the direct functional consequences of ME/CFS identified mucin SNPs are lacking, the consequences of a dysfunctional mucus barrier also have additional relevance for ME/CFS and its symptomatology. For example, chemical sensitivities are recognized as a common symptom of ME/CFS (Carruthers et al. 2003, 2011) with multiple chemical sensitivity being a common comorbidity in the illness (Carruthers et al. 2003; Reid 1999). Triggers include pesticides, perfume and petrochemicals, and natural irritants like mold and wood-fire smoke, and can lead to symptoms of headache, migraine, cognitive impairment, dizziness, fatigue, nausea, vomiting, cardiac abnormalities, skin rashes, asthma, and anaphylaxis (Damiani et al. 2021) all of which are common symptoms of ME/CFS. A dysfunctional ocular and otolaryngological mucus layers would lead to a sensitive epithelium that may be irritated due to environmental exposures (i.e. chemical or biological) leading to “flares” of symptoms as the immune system is further triggered. This is consistent with the “kindling” theory of ME/CFS (Jason et al. 2009). ME/CFS has also been associated with exposure to infectious agents, and there have been multiple reported “outbreaks” of illness (Monro and Puri 2018). Various bacteria, including members of the gut microbiome, and viruses such as human parvovirus B19, enteroviruses, as well as the herpesviruses Epstein–Barr virus (EBV), human herpesvirus-6 types A and B (HHV-6), and human cytomegalovirus (HCMV), have been implicated as possible etiological pathogens of ME/CFS (Ariza 2021; Cox et al. 2022). The symptom similarities between Long COVID (post-acute sequela of SARS-COV-2 infection) and ME/CFS also suggest that COVID-19 may play a similar role in disease onset (Komaroff and Lipkin 2023). These pathogens are all found in, and can be transmitted by, saliva or respiratory droplets. A compromised otolaryngological mucus layer would allow for increased risk of initial infection. Furthermore, the herpes viruses (i.e. EBV, HHV-6, and HCMV) remain latent within the body within salivary glands and epithelial cells and occasionally are reactivated (Grinde 2013). Potential triggers of this reactivation include environmental irritants leading to inflammation (Stoeger and Adler 2019;

Williams et al. 2019). As such, increased irritation in these regions due to a dysfunctional mucus protective barrier would lead to a greater incidence of viral reactivation and its associated symptoms.

Finally, similar to our findings presented here, many studies indicate a sex difference in ME/CFS in symptom presentation, onset and triggers (Friedberg et al. 2023; Thomas et al. 2022), immune measures (Jeffrey et al. 2019; Cheema et al. 2020; Smylie et al. 2013), metabolism (Nkiliza et al. 2021; Thomas et al. 2022), and gut microbiota (Wallis et al. 2017, 2018) with the female sex at greater risk for developing the illness and suffering endocrine events over the illness course (Pollack et al. 2023; Thomas et al. 2022). Females with ME/CFS report greater irregularities in their menstrual cycles, with menopause and pregnancy affecting their symptomatology (Pollack et al. 2023). Endometriosis, a disease in which tissue similar to the lining of the uterus grows outside the uterus, is a comorbid condition in women with ME/CFS, and is associated with chronic pelvic pain, earlier menopause, hysterectomy, and more ME/CFS-related symptoms (Wirth and Löhn 2023). While the mucins identified here in ME/CFS cluster in the ocular and otolaryngological areas, mucin-16 is also present in the endometrium with its overexpression in ovarian and endometrial cancer denoting it as a known ovarian tumor marker (Felder et al. 2014). Serum levels of mucin-16 are increased in women with endometriosis, however no changes have been found in the expression of mucin-16 in the endometrium of women with endometriosis (Liu et al. 2020). In ovarian cancer, mucin-16 is reported to be an immunosuppressive factor by acting on the surface of NK cells, B cells, monocytes, and neutrophils leading to an inflammatory and immunosuppressive phenotype (Wu et al. 2023). As such increased levels of mucin-16 in serum would additionally add to an altered immune profile in ME/CFS. As it has also been shown that dexamethasone, as a synthetic steroid similar to the stress hormone cortisol, upregulates the expression of mucin-16, this provides an additional link between mucin-16 expression, stress, and immune dysregulation in ME/CFS (Seo et al. 2007; Karlan et al. 1988). Should the variants in *MUC16* noted here result in a dysfunctional mucus that further contributes to these changes in inflammation seen in ME/CFS it may explain the overall sex differences and additional symptoms observed in females. Further investigation however is needed.

Overall, we have presented genetic evidence suggesting a dysfunctional mucus barrier in most individuals with ME/CFS. This dysfunction, in conjunction with environmental exposures to chemical and biological triggers, potential latent viral infection, altered T and decreased NK cell function are expected to contribute to the overall

triggering of symptoms in ME/CFS. Future work investigating the role of the ocular and otolaryngological mucus layer are ultimately needed to confirm this hypothesis.

#### Abbreviations

CADD	Combined annotation dependent depletion algorithm
CCL2/MCP1	Chemokine ligand 2/monocyte chemoattractant protein 1
CLRs	C-type lectin receptors
COVID	Corona virus disease
EBV	Epstein–Barr virus
GM-CSF	Granulocyte–macrophage colony-stimulating factor
HCMV	Human cytomegalovirus
HHV6	Human herpesvirus-6 types A and B
IL	Interleukin
ILC	Innate lymphoid cells
ME/CFS	Myalgic encephalomyelitis/chronic fatigue syndrome
NK	Natural killer
NKT	Natural killer T
NSU	Nova Southeastern University
nTh17	Natural Th17 cells
PMN	Polymorphonuclear neutrophils
RNIs	Reactive nitrogen intermediates
ROIs	Reactive oxygen intermediates
SF-36	36-Item short form health survey
SNP	Single nucleotide polymorphisms
SS	Steady state
TGFB	Transforming growth factor $\beta$
Th	T helper cell
TLRs	Toll-like receptors
TNFA	Tumor necrosis factor $\alpha$
Treg	T regulator cell

#### Acknowledgements

The authors would like to thank Mr. Zachary Barnes for data collection, organization, and quality control. The authors would also like to thank Ms. Beth Gilbert and Mr. Tatum Hedrick for proofreading.

#### Author contributions

Conceptualization, TJAC; Literature Review, KH and TT; mathematical analysis, FJCA and TJAC; Patient recruitment and assessment, FC; cytokine analysis, KA; resources, MAF, NGK and TJAC; writing—original draft preparation, KH, TT and TJAC; writing—review and editing, KH, TT, FJCA, FC, KA, NGK and TJAC; funding acquisition, MAF, NGK, and TJAC. All authors have read and agreed to the published version of the manuscript.

#### Funding

This research was supported by National Institutes of Health awards R01AI065723 (Fletcher PI), R01NS090200 (Fletcher PI), and R01AR057853 (Klimas PI) for recruitment and assessment of ME/CFS subjects, and Department of Defense Congressionally Directed Medical Research Program awards W81XWH-16-1-0632 (Craddock PI), W81XWH-16-1-0552 (Craddock PI), and W81XWH-15-1-0582 (Craddock PI) for the design of the logic model analysis. The funders had no role in the design of the study, in the collection, analysis, and interpretation of data, or in the writing of the manuscript. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the funding agency.

#### Availability of data and materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

#### Declarations

##### Ethics approval and consent to participate

This study was conducted according to the guidelines of the Declaration of Helsinki. The study protocol was approved by the Institutional Review Boards of the Miami Veteran Affairs Human Research Protections Program under

protocol #4987.76 and #4987.81 and Nova Southeastern University under protocol 06021413F. All subjects were recruited from the Miami Veterans Administration Medical Center and gave written informed consent.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

Received: 14 July 2023 Accepted: 5 December 2023

Published online: 03 January 2024

#### References

- 1000 Genomes Project Consortium. A global reference for human genetic variation. *Nature*. 2015;526(7571):68.
- Alcântara ALd, Pastana LF, Gellen LPA, Vieira GM, Dobbin EAF, Silva TA, Pereira EEB, Rodrigues JCG, Guerreiro JF, Fernandes MR. Mucin (MUC) family influence on acute lymphoblastic leukemia in cancer and non-cancer native American Populations from the Brazilian Amazon. *J Personal Med*. 2022;12(12):2053.
- Arias FJC, Aenlle K, Abreu M, Holschbach MA, Michalovic LT, Kelly KA, Klimas N, O'Callaghan JP, Craddock TJA. Modeling neuroimmune interactions in human subjects and animal models to predict subtype-specific multidrug treatments for gulf war illness. *Int J Mol Sci*. 2021;22(16):8546.
- Ariza ME. Myalgic encephalomyelitis/chronic fatigue syndrome: the human herpesviruses are back! *Biomolecules*. 2021;11(2):185.
- Auffret M, Meuric V, Boyer E, Bonnaure-Mallet M, Vérin M. Oral health disorders in Parkinson's disease: more than meets the eye. *J Parkinsons Dis*. 2021;11(4):1507–35.
- Baraniuk JN, Clauw DJ, Gaumont E. Rhinitis symptoms in chronic fatigue syndrome. *Ann Allergy Asthma Immunol*. 1998;81(4):359–65.
- Barker E, Fujimura SF, Fadem MB, Landay AL, Levy JA. Immunologic abnormalities associated with chronic fatigue syndrome. *Clin Infect Dis*. 1994;18(1):S136–41.
- Barnes MV, Openshaw PJ, Thwaites RS. Mucosal immune responses to respiratory syncytial virus. *Cells*. 2022;11(7):1153.
- Blalock TD, Spurr-Michaud SJ, Tisdale AS, Heimer SR, Gilmore MS, Ramesh V, Gipson IK. Functions of MUC16 in corneal epithelial cells. *Invest Ophthalmol vis Sci*. 2007;48(10):4509–18.
- Blundell S, Ray K, Buckland M, White P. Chronic fatigue syndrome and circulating cytokines: a systematic review. *Brain Behav Immun*. 2015;50:186–95.
- Brenu EW, Van Driel ML, Staines DR, Ashton KJ, Hardcastle SL, Keane J, Tajouri L, Peterson D, Ramos SB, Marshall-Gradisnik SM. Longitudinal investigation of natural killer cells and cytokines in chronic fatigue syndrome/myalgic encephalomyelitis. *J Transl Med*. 2012;10:1–11.
- Brenu EW, Huth TK, Hardcastle SL, Fuller K, Kaur M, Johnston S, Ramos SB, Staines DR, Marshall-Gradisnik SM. Role of adaptive and innate immune cells in chronic fatigue syndrome/myalgic encephalomyelitis. *Int Immunol*. 2014;26(4):233–42.
- Brenu EW, Broadley S, Nguyen T, Johnston S, Ramos S, Staines D, Marshall-Gradisnik S. A preliminary comparative assessment of the role of CD8+ T cells in chronic fatigue syndrome/myalgic encephalomyelitis and multiple sclerosis. *J Immunol Res*. 2016;2016:9064529.
- Bromley SK, Burack WR, Johnson KG, Somersalo K, Sims TN, Sumen C, Davis MM, Shaw AS, Allen PM, Dustin ML. The immunological synapse. *Annu Rev Immunol*. 2001;19(1):375–96.
- Brown MB. 400: A method for combining non-independent, one-sided tests of significance. *Biometrics*. 1975;31:987–92.
- Brown R, Hollingsworth MA. Mucin family of glycoproteins. In: Brown R, Hollingsworth MA, editors. *Encyclopedia of biological chemistry*. 2nd ed. Amsterdam: Elsevier; 2013. p. 200–4.
- Carruthers BM, Jain AK, De Meirleir KL, Peterson DL, Klimas NG, Lerner AM, Basted AC, Flor-Henry P, Joshi P, Powles AP. Myalgic encephalomyelitis/chronic fatigue syndrome: clinical working case definition, diagnostic and treatment protocols. *J Chronic Fatigue Syndr*. 2003;11(1):7–115.
- Carruthers BM, van de Sande MI, De Meirleir KL, Klimas NG, Broderick G, Mitchell T, Staines D, Powles AP, Speight N, Vallings R. Myalgic encephalomyelitis: international consensus criteria. *J Intern Med*. 2011;270(4):327–38.
- Castelli EC, de Castro MV, Naslavsky MS, Scliar MO, Silva NS, Pereira RN, Ciriaco VA, Castro CF, Mendes-Junior CT, Silveira ED. MUC22, HLA-A, and HLA-DOB variants and COVID-19 in resilient super-agers from Brazil. *Front Immunol*. 2022;13: 975918.
- Chatterjee M, Huang LZ, Mykytyn AZ, Wang C, Lamers MM, Westendorp B, Wubbolts RW, van Putten JP, Bosch B-J, Haagsmans BL. Glycosylated extracellular mucin domains protect against SARS-CoV-2 infection at the respiratory surface. *bioRxiv*. 2021;11: e02374.
- Cheema AK, Sarria L, Bekheit M, Collado F, Almenar-Pérez E, Martín-Martínez E, Alegre J, Castro-Marrero J, Fletcher MA, Klimas NG. Unravelling myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS): Gender-specific changes in the microRNA expression profiling in ME/CFS. *J Cell Mol Med*. 2020;24(10):5865–77.
- Chen S, Li B, Peng X, Zhuo X, Han C, Liu J, Li G, Lei C, Yang Z, Wu Z. Association of variants in MUC22 with ankylosing spondylitis in the young Chinese Guangxi Zhuang population. *Int J Clin Exp Med*. 2016;9(9):18270–80.
- Chen J, Zhang J, Hu H, Xue M, Jin Y. Polymorphisms of TGFBI, TLE4 and MUC22 are associated with childhood asthma in Chinese population. *Allergol Immunopathol*. 2017;45(5):432–8.
- Chen C-S, Cheng H-M, Chen H-J, Tsai S-Y, Kao C-H, Lin H-J, Wan L, Yang T-Y. Dry eye syndrome and the subsequent risk of chronic fatigue syndrome—a prospective population-based study in Taiwan. *Oncotarget*. 2018;9(55):30694.
- Chester AC. Symptoms of rhinosinusitis in patients with unexplained chronic fatigue or bodily pain: a pilot study. *Arch Intern Med*. 2003;163(15):1832–6.
- Costello M-E, Robinson PC, Benham H, Brown MA. The intestinal microbiome in human disease and how it relates to arthritis and spondyloarthritis. *Best Pract Res Clin Rheumatol*. 2015;29(2):202–12.
- Cox BS, Alharshawi K, Mena-Palomo I, Lafuse WP, Ariza ME. EBV/HHV-6A dUTPases contribute to myalgic encephalomyelitis/chronic fatigue syndrome pathophysiology by enhancing TFH cell differentiation and extrafollicular activities. *JCI Insight*. 2022;7(11): e158193.
- Craddock TJA, Fritsch P, Rice MA Jr, Del Rosario RM, Miller DB, Fletcher MA, Klimas NG, Broderick G. A role for homeostatic drive in the perpetuation of complex chronic illness: Gulf War Illness and chronic fatigue syndrome. *PLoS ONE*. 2014;9(1): e84839.
- Craddock TJA, Del Rosario RR, Rice M, Zysman JP, Fletcher MA, Klimas NG, Broderick G. Achieving remission in gulf war illness: a simulation-based approach to treatment design. *PLoS ONE*. 2015;10(7): e0132774.
- Craddock TJA, Michalovic L, Kelly KA, Rice M Jr, Miller D, Klimas N, Morris M, O'Callaghan J, Broderick G. A logic model of neuronal-glial interaction suggests altered homeostatic regulation in the perpetuation of neuroinflammation. *Front Cell Neurosci*. 2018;12:336.
- Cunha BA. Crimson crescents—a possible association with the chronic fatigue syndrome. *Ann Intern Med*. 1992;116(4):347.
- Damiani G, Alessandrini M, Caccamo D, Cormano A, Guzzi G, Mazzatenta A, Micarelli A, Migliore A, Piroli A, Bianca M. Italian expert consensus on clinical and therapeutic management of multiple chemical sensitivity (mcs). *Int J Environ Res Public Health*. 2021;18(21):11294.
- Dankowski T, Buck D, Andlauer TF, Antony G, Bayas A, Bechmann L, Berthele A, Bettecken T, Chan A, Franke A. Successful replication of GWAS hits for multiple sclerosis in 10,000 Germans using the exome array. *Genet Epidemiol*. 2015;39(8):601–8.
- Davis MM, Tato CM, Furman D. Systems immunology: just getting started. *Nat Immunol*. 2017;18(7):725–32.
- De Repentigny L, Goupil M, Jolicoeur P. Oropharyngeal candidiasis in HIV infection: analysis of impaired mucosal immune response to *Candida albicans* in mice expressing the HIV-1 transgene. *Pathogens*. 2015;4(2):406–21.
- Dhanisha SS, Guruvayoorappan C, Drishya S, Abeeesh P. Mucins: structural diversity, biosynthesis, its role in pathogenesis and as possible therapeutic targets. *Crit Rev Oncol Hematol*. 2018;122:98–122.
- Felder M, Kapur A, Gonzalez-Bosquet J, Horibata S, Heintz J, Albrecht R, Fass L, Kaur J, Hu K, Shojaei H. MUC16 (CA125): tumor biomarker to cancer therapy, a work in progress. *Mol Cancer*. 2014;13(1):1–15.
- Fini ME, Jeong S, Gong H, Martinez-Carrasco R, Laver NM, Hijikata M, Keicho N, Argüeso P. Membrane-associated mucins of the ocular surface: New

- genes, new protein functions and new biological roles in human and mouse. *Prog Retin Eye Res.* 2020;75:100777.
- Fletcher MA, Maher K, Patarca-Montero R, Klimas N. Comparative analysis of lymphocytes in lymph nodes and peripheral blood of patients with chronic fatigue syndrome. *J Chronic Fatigue Syndr.* 2000;7(3):65–75.
- Fletcher MA, Maher KJ, Klimas NG. Natural killer cell function in chronic fatigue syndrome. *Clin Appl Immunol Rev.* 2002;2(2):129–39.
- Friedberg F, Adamowicz JL, Bruckenthal P, Milazzo M, Ramjan S, Zhang X, Yang J. Sex differences in post-exercise fatigue and function in myalgic encephalomyelitis/chronic fatigue syndrome. *Sci Rep.* 2023;13(1):5442.
- Fritsch P, Craddock TJA, del Rosario RM, Rice MA, Smylie A, Folcik VA, de Vries G, Fletcher MA, Klimas NG, Broderick G. Succumbing to the laws of attraction: exploring the sometimes pathogenic versatility of discrete immune logic. *Syst Biomed.* 2014;1(3):179–94.
- Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. The chronic fatigue syndrome: a comprehensive approach to its definition and study. *Ann Intern Med.* 1994;121(12):953–9.
- Grinde B. Herpesviruses: latency and reactivation—viral strategies and host response. *J Oral Microbiol.* 2013;5(1):22766.
- Gubbels JA, Felder M, Horibata S, Belisle JA, Kapur A, Holden H, Petrie S, Migneault M, Rancourt C, Connor JP. MUC16 provides immune protection by inhibiting synapse formation between NK and ovarian tumor cells. *Mol Cancer.* 2010;9:1–14.
- Hardcastle SL, Brenu EW, Johnston S, Nguyen T, Huth T, Ramos S, Staines D, Marshall-Gradisnik S. Serum immune proteins in moderate and severe chronic fatigue syndrome/myalgic encephalomyelitis patients. *Int J Med Sci.* 2015;12(10):764.
- Heron SE, Elahi S. HIV infection and compromised mucosal immunity: oral manifestations and systemic inflammation. *Front Immunol.* 2017;8:241.
- Hijikata M, Matsushita I, Tanaka G, Tsuchiya T, Ito H, Tokunaga K, Ohashi J, Homma S, Kobashi Y, Taguchi Y. Molecular cloning of two novel mucin-like genes in the disease-susceptibility locus for diffuse panbronchiolitis. *Hum Genet.* 2011;129:117–28.
- Institute of Medicine. Beyond myalgic encephalomyelitis/chronic fatigue syndrome: redefining an illness. Washington, DC: The National Academies Press; 2015. p. 304.
- Jason L, Porter N, Herrington J, Sorenson M, Kubow S. Kindling and oxidative stress as contributors to myalgic encephalomyelitis/chronic fatigue syndrome. *J Behav Neurosci Res.* 2009;7(2):1.
- Jeffrey MG, Nathanson L, Aenlle K, Barnes ZM, Baig M, Broderick G, Klimas NG, Fletcher MA, Craddock TJ. Treatment avenues in myalgic encephalomyelitis/chronic fatigue syndrome: a split-gender pharmacogenomic study of gene-expression modules. *Clin Ther.* 2019;41(5):815–35 e6.
- Karlan BY, Amin W, Casper SE, Littlefield BA. Hormonal regulation of CA125 tumor marker expression in human ovarian carcinoma cells: inhibition by glucocorticoids. *Can Res.* 1988;48(12):3502–6.
- Karunas A, Yunusbaev B, Fedorova YY, Gimalova G, Khusnutdinova E. Association of MUC19 gene polymorphic variants with asthma in Russians based on genome-wide study results. *Russ J Genet.* 2015a;51:1135–43.
- Karunas A, Yunusbaev B, Fedorova YY, Gimalova G, Khusnutdinova E. Association of polymorphic variants of gene MUC19 with asthma in Russians according to the results of a genome-wide study. *Genetika.* 2015b;51(11):1315–24.
- Kato A. Immunopathology of chronic rhinosinusitis. *Allergol Int.* 2015;64(2):121–30.
- Kircher M, Witten DM, Jain P, O’roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. *Nat Genet.* 2014;46(3):310.
- Klimas NG, Salvato FR, Morgan R, Fletcher MA. Immunologic abnormalities in chronic fatigue syndrome. *J Clin Microbiol.* 1990;28(6):1403–10.
- Komaroff AL. Inflammation correlates with symptoms in chronic fatigue syndrome. *Proc Natl Acad Sci.* 2017;114(34):8914–6.
- Komaroff AL, Lipkin WI. ME/CFS and Long COVID share similar symptoms and biological abnormalities: road map to the literature. *Front Med.* 2023;10:1187163.
- Kutta H, Willer A, Steven P, Bräuer L, Tsokos M, Paulsen F. Distribution of mucins and antimicrobial substances lysozyme and lactoferrin in the laryngeal subglottic region. *J Anesth.* 2008;213(4):473–81.
- Laulajainen-Hongisto A, Toppila-Salmi SK, Luukkainen A, Kern R. Airway epithelial dynamics in allergy and related chronic inflammatory airway diseases. *Front Cell Dev Biol.* 2020;8:204.
- Levchenko A, Piskunov V, Konoplya N, Bushueva O, Raspopov A, Mezentseva O, Polonikov A. Genetic aspects of chronic rhinosinusitis. *Russ J Genet.* 2018;54:910–8.
- Lim E-J, Son C-G. Prevalence of chronic fatigue syndrome (CFS) in Korea and Japan: a meta-analysis. *J Clin Med.* 2021;10(15):3204.
- Linden S, Sutton P, Karlsson N, Korolik V, McGuckin M. Mucins in the mucosal barrier to infection. *Mucosal Immunol.* 2008;1(3):183–97.
- Liu L, Wang Y, Chen X, Tian Y, Li TC, Zhao L, Chen Q, Wei M, Zhang S. Evidence from three cohort studies on the expression of MUC16 around the time of implantation suggests it is an inhibitor of implantation. *J Assist Reprod Genet.* 2020;37:1105–15.
- Mandarano AH, Maya J, Giloteaux L, Peterson DL, Maynard M, Gottschalk CG, Hanson MR. Myalgic encephalomyelitis/chronic fatigue syndrome patients exhibit altered T cell metabolism and cytokine associations. *J Clin Investig.* 2020;130(3):1491–505.
- McBride K, Banos-Lara Mdr, Cheemarla NR, Guerrero-Plata A. Human metapneumovirus induces mucin 19 which contributes to viral pathogenesis. *Pathogens.* 2020;9(9):726.
- Meldrum OW, Yakubov GE, Bonilla MR, Deshmukh O, McGuckin MA, Gidley MJ. Mucin gel assembly is controlled by a collective action of non-mucin proteins, disulfide bridges, Ca<sup>2+</sup>-mediated links, and hydrogen bonding. *Sci Rep.* 2018;8(1):5802.
- Mendoza L, Xenarios I. A method for the generation of standardized qualitative dynamical systems of regulatory networks. *Theor Biol Med Model.* 2006;3(1):13.
- Mettelman RC, Allen EK, Thomas PG. Mucosal immune responses to infection and vaccination in the respiratory tract. *Immunity.* 2022;55(5):749–80.
- Monro JA, Puri BK. A molecular neurobiological approach to understanding the aetiology of chronic fatigue syndrome (myalgic encephalomyelitis or systemic exertion intolerance disease) with treatment implications. *Mol Neurobiol.* 2018;55:7377–88.
- Morris MC, Cooney KE, Sedghamiz H, Abreu M, Collado F, Balbin EG, Craddock TJ, Klimas NG, Broderick G, Fletcher MA. Leveraging prior knowledge of endocrine immune regulation in the therapeutically relevant phenotyping of women with chronic fatigue syndrome. *Clin Ther.* 2019;41(4):656–74 e4.
- Naranch K, Repka-Ramirez S, Park Y-J, Velarde A, Finnegan R, Murray J, Pheiffer A, Hwang E, Clauw D, Baraniuk J. Differences in baseline nasal secretions between chronic fatigue syndrome (CFS) and control subjects. *J Chronic Fatigue Syndr.* 2002;10(1):3–15.
- Negrato CA, Tarzia O. Buccal alterations in diabetes mellitus. *Diabetol Metab Syndr.* 2010;2(1):1–11.
- Nkiliza A, Parks M, Cseresznye A, Oberlin S, Evans JE, Darcey T, Aenlle K, Niedospial D, Mullan M, Crawford F. Sex-specific plasma lipid profiles of ME/CFS patients and their association with pain, fatigue, and cognitive symptoms. *J Transl Med.* 2021;19(1):1–15.
- Ohradanova-Repic A, Pražnicová R, Gebetsberger L, Moskalets T, Skrabana R, Cehlar O, Tajti G, Stockinger H, Leksa V. Time to kill and time to heal: the multifaceted role of Lactoferrin and Lactoferricin in host defense. *Pharmaceutics.* 2023;15(4):1056.
- Ojo-Amaize EA, Conley EJ, Peter JB. Decreased natural killer cell activity is associated with severity of chronic fatigue immune dysfunction syndrome. *Clin Infect Dis.* 1994;18(1):S157–9.
- Paraskevaïdi M, Allsop D, Karim S, Martin FL, Crean S. Diagnostic biomarkers for Alzheimer’s disease using non-invasive specimens. *J Clin Med.* 2020;9(6):1673.
- Perez M, Jaundoo R, Hilton K, Del Alamo A, Gemayel K, Klimas NG, Craddock TJ, Nathanson L. Genetic predisposition for immune system, hormone, and metabolic dysfunction in myalgic encephalomyelitis/chronic fatigue syndrome: a pilot study. *Front Pediatr.* 2019;7:206.
- Philipone E, Yoon AJ. Mucosal manifestations of nutritional deficiencies. In: Philipone E, Yoon AJ, editors. *Oral pathology in the pediatric patient: a clinical guide to the diagnosis and treatment of mucosal lesions.* Cham: Springer; 2017. p. 21–3.
- Pollack B, von Saltza E, McCorkell L, Santos L, Hultman A, Cohen AK, Soares L. Female reproductive health impacts of Long COVID and associated illnesses including ME/CFS, POTS, and connective tissue disorders: a literature review. *Front Rehab Sci.* 2023;4:1122673.
- Qanneta R, Fontova R, Pàmies A. Etiology of sicca syndrome in a consecutive series of 199 patients with chronic fatigue syndrome. *Reumatol Clin (eng Ed).* 2014;10(4):269–70.

- Reid HD. Multiple chemical sensitivity: a 1999 consensus. *Arch Environ Health*. 1999;54(3):147–9.
- Rentzsch P, Witten D, Cooper GM, Shendure J, Kircher M. CADD: predicting the deleteriousness of variants throughout the human genome. *Nucleic Acids Res*. 2019;47(D1):D886–94.
- Rice MA Jr, Craddock TJA, Folcik VA, del Rosario RM, Barnes ZM, Klimas NG, Fletcher MA, Zysman J, Broderick G. Gulf War Illness: Is there lasting damage to the endocrine-immune circuitry? *Syst Biomed*. 2014;2(4):80–9.
- Rivas JL, Palencia T, Fernández G, García M. Association of T and NK cell phenotype with the diagnosis of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). *Front Immunol*. 2018. <https://doi.org/10.3389/fimmu.2018.01028>.
- Romero P, Wagg J, Green ML, Kaiser D, Krummenacker M, Karp PD. Computational prediction of human metabolic pathways from the complete human genome. *Genome Biol*. 2004;6(1):R2.
- Sato S, Kiyono H. The mucosal immune system of the respiratory tract. *Curr Opin Virol*. 2012;2(3):225–32.
- Seo KY, Chung S-H, Lee JH, Park MY, Kim EK. Regulation of membrane-associated mucins in the human corneal epithelial cells by dexamethasone. *Cornea*. 2007;26(6):709–14.
- Smylie AL, Broderick G, Fernandes H, Razdan S, Barnes Z, Collado F, Sol C, Fletcher MA, Klimas N. A comparison of sex-specific immune signatures in Gulf War illness and chronic fatigue syndrome. *BMC Immunol*. 2013;14(1):29.
- Song D, Cahn D, Duncan GA. Mucin biopolymers and their barrier function at airway surfaces. *Langmuir*. 2020;36(43):12773–83.
- Stoeger T, Adler H. “Novel” triggers of herpesvirus reactivation and their potential health relevance. *Front Microbiol*. 2019;9:3207.
- Strawbridge R, Sartor M-L, Scott F, Cleare AJ. Inflammatory proteins are altered in chronic fatigue syndrome—a systematic review and meta-analysis. *Neurosci Biobehav Rev*. 2019;107:69–83.
- Strayer D, Scott V, Carter W. Low NK cell activity in chronic fatigue syndrome (CFS) and relationship to symptom severity. *J Clin Cell Immunol*. 2015;6(348):2.
- The UniProt Consortium. UniProt: the universal protein knowledgebase in 2021. *Nucleic Acids Res*. 2021;49(D1):D480–9.
- Thomas R. Regulatory networks seen as asynchronous automata: a logical description. *J Theor Biol*. 1991;153(1):1–23.
- Thomas R, Thieffry D, Kaufman M. Dynamical behaviour of biological regulatory networks—I. Biological role of feedback loops and practical use of the concept of the loop-characteristic state. *Bull Math Biol*. 1995;57(2):247–76.
- Thomas N, Gurchich C, Huang K, Gooley PR, Armstrong CW. The underlying sex differences in neuroendocrine adaptations relevant to myalgic encephalomyelitis chronic fatigue syndrome. *Front Neuroendocrinol*. 2022;66: 100995.
- van Putten JP, Strijbis K. Transmembrane mucins: signaling receptors at the intersection of inflammation and cancer. *J Innate Immun*. 2017;9(3):281–99.
- 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;9:1033.
- Wallis A, Butt H, Ball M, Lewis DP, Bruck D. Support for the microgenderome invites enquiry into sex differences. *Gut Microbes*. 2017;8(1):46–52.
- Wallis A, Ball M, Butt H, Lewis DP, McKechnie S, Paull P, Jaa-Kwee A, Bruck D. Open-label pilot for treatment targeting gut dysbiosis in myalgic encephalomyelitis/chronic fatigue syndrome: neuropsychological symptoms and sex comparisons. *J Transl Med*. 2018;16:1–16.
- Wang X, Yu X, Krauthammer M, Hugo W, Duan C, Kanetsky PA, Teer JK, Thompson ZJ, Kalos D, Tsai KY. The association of MUC16 mutation with tumor mutation burden and its prognostic implications in cutaneous melanoma. *Cancer Epidemiol Biomark Prev*. 2020;29(9):1792–9.
- Whiteside TL, Friberg D. Natural killer cells and natural killer cell activity in chronic fatigue syndrome. *Am J Med*. 1998;105(3):275–34S.
- Williams M, Cox B, Alharshawi K, Lafuse W, Ariza M. Epstein–Barr virus dUT-Pase contributes to neuroinflammation in a cohort of patients with encephalomyelitis/chronic fatigue syndrome. *Clin Ther*. 2019;41:848–63.
- Wirth KJ, Löhn M. Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and comorbidities: linked by vascular pathomechanisms and vasoactive mediators? *Medicina*. 2023;59(5):978.
- Wu Y, Liu Q, Xie Y, Zhu J, Zhang S, Ge Y, Guo J, Luo N, Huang W, Xu R. MUC16 stimulates neutrophils to an inflammatory and immunosuppressive phenotype in ovarian cancer. *J Ovarian Res*. 2023;16(1):181.
- Zeng A, Rong H, Pan D, Jia L, Zhang Y, Zhao F, Peng S. Alzheimer’s Disease Neuroimaging Initiative (ADNI). Discovery of genetic biomarkers for Alzheimer’s disease using adaptive convolutional neural networks ensemble and genome-wide association studies. *Interdiscip Sci Comput Life Sci*. 2021;13(4):787–800.
- Zhang R, Zhang L, Li P, Pang K, Liu H, Tian L. Epithelial barrier in the nasal mucosa, related risk factors and diseases. *Int Arch Allergy Immunol*. 2023;184(5):481–501.

## Publisher’s Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more [biomedcentral.com/submissions](https://biomedcentral.com/submissions)

