The Genetics and Epigenetics of Fatigue

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Abstract: Fatigue is a common symptom and includes both physical and mental components. It can be associated with a variety of different syndromes and diseases, but in many cases is not associated with other comorbid conditions. Most humans have experienced acute fatigue in relation to different stressors. Acute fatigue typically decreases as the effect of the triggering factor is reduced and a normal homeostatic balance is restored. Fatigue that persists for 6 months or more is termed chronic fatigue. Chronic fatigue (CF) in combination with a minimum of 4 of 8 symptoms and the absence of diseases that could explain these symptoms, constitute the case definition for chronic fatigue syndrome. In spite of its prevalence, the biology of fatigue is relatively poorly understood and biological markers have not yet been identified. This literature search was performed in PubMed to identify research on the genetics and epigenetics of fatigue. Publications were included if fatigue was a major topic and the topic was combined with genetic and/or epigenetic measurements in adult humans. A total of 40 publications were identified. Although altered functioning in the hypothalamic-pituitary-adrenal axis, the serotonergic system, and associations with infectious agents have been identified, the search for genetic or epigenetic markers of fatigue, either in the context of CF or chronic fatigue syndrome (CFS) has been relatively unproductive or, in the case of epigenetics, nonexistent. Although several studies, both hypothesis-testing and hypothesis-generating, have been performed to search for biomarkers, they have mostly been underpowered, restricted by the heterogeneity of the phenotype, or limited by an unsystematic study design. To be able to confirm the hypothesis that risk for, or levels of, fatigue are influenced by the genetic or epigenetic background of an individual, studies need to be based on larger sample sizes with a more clearly defined phenotype. Studies need to focus not only on the influence of a single aspect such as single nucleotide polymorphisms (SNPs) or differential gene expression on disease risk or state, but also on the systems biology behind the disease in combination with information on environmental influences and validation of findings in functional studies.

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BACKGROUND

Fatigue is a common symptom whose descriptors include tiredness, weakness, lack of energy, and inability to concentrate. Fatigue is associated with a variety of syndromes and diseases, and may also be idiopathic. Fatigue can be conceptualized as a final common end point for psychological and biological processes. Fatigue is therefore both heterogeneous (occurring across different conditions) and multifactorial. This implies that more than 1 mechanism can play a role in its expression in an individual.

Most humans have experienced fatigue in relation to exercise, infection, exacerbations of systemic diseases, or in relation to psychological or social stressors. In fact, fatigue is so common that its prevalence ranks among the top 3 reported symptoms in most studies of symptom prevalence [1]. For the majority of individuals, fatigue will not persist at a high level, but gradually decreases as the triggering factor weakens and a normal balance is restored. This so-called acute fatigue is typically experienced by most people in relation to viral infections, or during, for example, radiation therapy. Acute fatigue has been associated with the inflammatory response, and pro-inflammatory cytokines are implicated as impor-

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tant mediators of the response, from the local immunologic insult to the entire organism, including the effect on immunologically competent cells and organ systems such as the central nervous system and the hypothalamic-pituitary-adrenal axis [HPA axis]).

However, in some people, fatigue persists because of continuing disease activity (eg, fatigue experienced by patients with advanced cancer) or as a "medically unexplained" symptom where there is no definite disease activity, including psychiatric disorders. This type, chronic fatigue (CF), is defined by convention as fatigue above a certain level that has lasted for 6 months or more [2]. Chronic fatigue syndrome (CFS) is characterized by CF and at least 4 of 8 other symptoms such as cognitive dysfunction or joint or persistent muscle pain, according to the U.S. Centers for Disease Control and Prevention (CDC) or Fukuda definition [3]. The CDC definition was launched primarily to standardize criteria and to enhance research on CFS. Although CF is relatively prevalent–for example, reported by 11% of the Norwegian population–CFS is found in less than 0.5% of the population [4,5].

CFS or CFS-like conditions had been observed for decades before the CDC definition was launched in 1994, but CFS has still not been accepted as a diagnostic entity within official diagnostic systems such as the International Classification of Diseases, 10th Revision. The labeling of the condition has varied throughout medical history, partly reflecting the dominant point of view of diseases at particular periods [6]. At present, different case definitions exist, but the CDC definition has been the criterion for case definition in the majority of publications since 1994.

Efforts to identify causes or mechanisms of CF or CFS have been relatively unsuccessful, and findings have often been difficult to replicate because of methodologic issues such as selection bias, absence of biological markers, and wide variations in the phenotype. Altered functioning in the HPA axis and the serotonergic system and associations with infectious agents have been demonstrated. CF and CFS must be regarded as complex conditions of probable multifactorial etiology and possibly involving multiple mechanisms [7]. In the clinic, efforts to detect any pathology by laboratory investigations have so far been futile. The present understanding of CFS reflects a diversity of etiologies by pointing to predisposing, triggering, and maintaining factors.

The rapid development of the field of molecular biology during recent decades has also influenced research on fatigue. This review is focused on genetics and epigenetics research conducted to understand the clinical phenotype of CF and CFS. Studies in the field of genetics aim to decipher the impact of variation in the DNA structure that can either be inherited, and are therefore found in the germline DNA (ie, inherited between individuals), or that arises during an individual's lifespan (ie, somatic mutations, inherited between cells). Core end points in the field of genetics are: 1) single nucleotide polymorphisms (SNPs), which are single base variations in the DNA structure which are found in 1% of the population or more and 2) copy number variations, which are segments of DNA that vary in copy number between genomes of different individuals ranging from one kilobase to several megabases in size [8]. The field of epigenetics studies the regulation of gene or chromosome function caused by mechanisms other than changes in the DNA and includes chromatin remodeling, histone modification, and DNA methylation and acetylation. Downstream products such as RNA transcripts and proteins are influenced by both the genetic and epigenetic background of an individual.

The introduction of modern molecular biology into research on fatigue can be regarded as part of the "natural spread" of the most recent research methods in medicine and biology into a diversity of fields. Still, knowledge is lacking concerning causality, pathophysiology, and diagnostics, including biological markers and eventual subgroupings, as well as the need for therapeutics, support the extension of molecular biology into research on fatigue and CFS [9]. Against this background, we have performed a literature review on genetic and epigenetic aspects of fatigue to determine the following: Which populations have been studied? What molecular end points have been explored and what are the main findings?

MATERIAL AND METHODS

A literature search was performed in the electronic database PubMed (www.ncbi.nlm.nih.gov/pubmed/). The following search term was used: "fatigue AND (gene OR gene expression OR SNP OR genetics OR epigenetics OR microRNA OR histone modification OR histone deacetylation OR DNA methylation OR protein expression OR HPA axis OR ubiquitin* OR mutation)." The search was limited to articles written in English, studies performed on samples collected from humans, and published between January 2000 and September 2009.

The titles of the resulting articles (n = 1794) were evaluated by 2 of the authors (H.L.-H. and K.V.R.) and selected if "fatigue" appeared to be a major topic combined with a genetic or epigenetic measurement. The search was thus not limited to CFS. The abstracts of the selected articles (n = 141) were subsequently read by 4 of the authors and the proposed inclusions/exclusions were then compared between all 4 and agreement was reached by consensus. Abstracts were included if the inclusion criteria were fulfilled, and the following exclusion criteria were not met: review articles, letters, protein or hormone measurements as a major topic without genetic measurements, non-adults (subjects age <18 years). The number of articles was thereby reduced from 141 to 58. Finally, the full set of articles (n = 58) were distributed randomly between the author pairs (H.E. + K.V.R., H.L.-H. + J.H.L.). Each author thus read half of the

articles. At this stage, 18 additional articles were excluded on the criteria stated previously and the search thus yielded 40 articles. The same pair of authors then extracted data from each article on publication year, clinical phenotype, methods of fatigue assessment, number of participants, main goal of the study, design, and biological end point. The findings of each author-pair were compared, discussed with the other author-pair if determined to be necessary, and then were combined into 3 tables.

RESULTS

Forty articles met the study criteria and are described in detail in Tables 1, 2, and 3. The articles were categorized by whether the study was mainly hypothesis driven (n = 15), hypothesis generating (n = 13), or if the aim of the study was to develop and test bioinformatic models without involving novel experimental studies (n = 12).

CFS was the clinical phenotype examined in the majority of the articles (n = 33). Fifteen of these articles were identified as based on data obtained in the "Wichita study" [10-21,33,44,45], which composed the majority of those categorized as bioinformatics mining studies (Table 3). The "Wichita study" is a collaborative study on fatigue in sufferers of CFS and of medically and psychiatrically unexplained fatigue identified from the general population of Wichita, Kansas [22].

Also included were articles exploring CF 1) in patients after verified infection (n = 2) [23,24], 2) during treatment for hepatitis C (n = 1) [25], 3) in multiple sclerosis (n = 1) [26], and 4) in hypothyroid individuals (n = 1) [27]. Further, 3 articles assessed fatigue in cancer survivors [28-30]. In the studies of CFS, definition of cases was mostly based on fulfilment of the CDC criteria (n = 32) [3]. Twenty-two studies reported that the CDC criteria were met without providing further details on data collection and sampling. Ten studies reported on assessments in addition to fulfilment of the CDC criteria, including different patient-reported outcomes and medical and/or psychological assessments. The majority of these studies were performed on the Wichita material. For those studies, a comprehensive presentation of all assessments has been presented in a separate article [31]. In the articles not researching CFS as the clinical phenotype, 1 study had used the CDC criteria for case definition [23] (Table 2). In the others, fatigue had been assessed by different patient-reported outcomes.

Case-control studies were the most frequently employed design (n = 29, including 1 twin study). The number of cases and controls in these studies ranged from 1 to 248 [32,33].

The biological systems considered in the hypothesis driven studies (Table 1) were the immune system (n = 9, primarily focusing on the innate immune system as opposed to the adaptive immune system), the HPA axis (n = 3), the serotonergic system (n = 4), and the thyroid system (n = 1) Two studies were counted both in the immune system and the serotonergic system section. In the hypothesis-driven studies, the authors investigated the association between a single or a handful of SNPs in a limited number of genes and the clinical phenotype. The only study with a more extended number of candidate genes is that of Smith et al, who investigated the influence of 77 SNPs in 14 genes involved in serotonin synthesis, signaling, metabolism, or transport [34]. Only in 3 of the hypothesis-driven studies were the genetic findings related to concurrent biochemical measurements of the respective gene product [27,33,35]. In 2 additional studies, a subset of the subjects had been previously examined with regards to the biochemical substrate of interest [29,36]. One study comparing mRNA expression of tumor necrosis factor (*TNF*), interferon (*IFN*)-γ, and interleukin (*IL*)*10* in blood cells from multiple sclerosis patients with and without fatigue measured serum catecholamine levels, but no measurements of the cytokines were described [26].

Several of the articles focusing on the immune system have investigated SNPs in the *TNF* gene, the *IL* genes, and the human leukocyte antigen (*HLA*) genes and reported positive associations with fatigue [25,26,28,29,35,37-39] (Table 1). For instance, an SNP in *IL17F* that antagonizes the proinflammatory effects was found at lower prevalence in CFS patients [39]. *IL6* and *IL1*β were studied in relation to fatigue during and after treatment of cancer and the results indicate that polymorphisms in *IL1B* may be a potential risk factor for persistent fatigue in breast cancer survivors [29].

IFNγ has been studied both at the genetic and the transcriptional level. One of the studies reported an association between a SNP in *IFN*γ located in intron 1 and CFS. A decreased level of the *IFN*γ low producers (homozygous for the variant A-allele at position +874) were found among CFS patients as compared with controls [37]. However, the study by Flachenecker et al, which focused on the association of cytokine transcription level with fatigue in multiple sclerosis patients, found no association with *IFN*γ expression [26].

Studies on the HLA complex reported an association between age of onset of CFS and the *HLA-DRB1**03 allele in combination with a promoter polymorphism in the *HTR2A* gene [35]. In another study, an increased frequency of the *HLA-DQA1**01 allele was found in CFS patients compared with controls [38]. Several of the gene expression studies showed that genes involved in the immune system were differentially expressed in peripheral blood cells of CFS patients and controls [10,40-43], as well as in chronically fatigued breast cancer survivors and controls [30] (Table 2).

Two of the 3 studies concentrating on the HPA axis showed that genetic variation in *NR3C1* is linked to CFS, although it should be noted that these articles are both based on the Wichita material [33,44,45].

Four studies addressed the impact of genetic variation in the serotonergic system on CFS and treatment-induced fa-

Table 1. *Hypothesis-driven studies*

Study (Publication Year)	Clinical Phenotype	No. of Participants (Case/Control)	Fatigue Assessment	Molecular Biological End Point and Main Findings
Immune system				
Aouizerat et al (2009)	Cancer	185/103	Lee Fatigue scale	To test the hypothesis that an SNP in *TNF* (-308G>A, rs1800629) is associated with overall ratings of sleep disturbance and morning and evening fatigue. Individual's homozygote for the common allele was found to have a higher level of sleep disturbance ($P = .09$) and morning fatigue ($P = .02$).
Bull et al (2009)†	Hepatitis C, treatment-induced fatigue	98/0	FQ	To study the association between polymorphisms in the cytokine gene *IL6* (rs1800796) and the serotonin transporter gene (*5-HTT*) and treatment-induced fatigue and depression in patients with hepatitis C. The promoter SNP in IL-6, reducing the expression level of the gene, was found to be associated with fewer symptoms of depression ($P = .002$). The same was true but to a less extent for the variant giving a high expression level of the serotonin transporter gene ($P = .03$). Neither polymorphism was found to be associated with fatigue.
Carlo-Stella et al (2006)	CFS	80/140	CDC	By analyzing promoter SNPs in the cytokine genes *IL10, IL6,* and *TNF* as well as an SNP in intron 1 in *IFNγ* in CFS patients vs controls, the authors hope to confirm their hypothesis that CFS patients have a genetic predisposition to an inflammatory response. A significantly higher level of the *TNF*-857 T allele and a decreased level of the *IFNγ* low producers (homozygous for the variant A-allele) were found among patients as compared with controls ($P = .002$ and .04, respectively).
Collado-Hidalgo et al (2008)	Fatigue in BC survivors	33/14	SF36/MFSI	To investigate the role of cytokines in cancer-related fatigue by analyzing SNPs in the promoters of *IL1B* (-511) and *IL6* (-174). The results indicate that polymorphisms in IL1B may be a potential risk factor for persistent fatigue after cancer.
Flachenecker et al (2004)	MS	26 (MS-F)/11 MS (NF)	Krupp FSS	Cytokine mRNA expression was evaluated by RT-PCR for *TNF, IFNγ,* and *IL10* in peripheral blood cells. The aim was to assess the possible role of pro-inflammatory cytokines in MS patients, in particular with relation to level of fatigue. Median levels of TNF mRNA expression were found to be higher in MS patients with a high level of fatigue compared with patients with low levels of fatigue.
Metzger et al (2008)	CFS	89/56	CDC	To investigate the association between an SNP in *IL17F* (*rs763780,* T/C, His161Arg) and CFS. A significantly higher level of the CC and CT genotype was found in the controls vs the CFS patients ($P = .0018$), suggesting a protective effect. The Arg161 protein variant antagonizes the pro-inflammatory effect of the wild-type *IL17F*.
Ortega-Hernandez et al (2009)†	CFS	81/0	CDC	To determine the influence of SNPs in the serotonin pathway and HLA class II genes and autoantibodies on age at CFS onset and symptoms. Identifies, among others, an association with a promoter polymorphism in the *HTR2A* gene in combination with the *HLA-DRB103** allele for age at CFS onset in the third decade. In addition the *HTR2A* promoter SNP was found related to depressive symptoms.
Smith et al (2005)	CFS	40/102 (cadaveric controls)	CDC	To investigate the role of HLA class II antigens in CFS. An increased frequency of the HLA-DQA1*01 allele was found in patients with CFS ($P = .008$).
Sorensen et al (2009)	CFS	8/7	CDC	To test whether complement activation has a role in CFS the expression level of 9 genes in the classical and lectin pathways were evaluated with RT-PCR pre- and postexercise in patients with CFS and controls. A difference in the response of the lectin pathway between patients and controls were identified.
HPA-axis				
Rajeevan et al (2007)*‡	CFS	43/61 (IFS)+60 (NF)	First evaluation: CDC; second evaluation: MFSI/SF-36	To examine the association of 9 different polymorphisms in *NR3C1,* a major effector of the HPA-axis, with CFS and fatigue in CFS patients. Several SNPs were found associated with fatigue, and subjects homozygous for the major allele of all associated SNPs were at increased risk for CFS (OR ranging from 2.61 to 3.00).
Smith et al (2006)*	CFS	140/0	CDC	To investigate whether genetic differences in 11 genes related to the HPA axis function and mood-related neurotransmitter system were associated with CFS subtypes. Dividing the CFS patients into 5 subclasses, 3 subclasses could be distinguished by polymorphisms in genes related to HPA axis function or the neurotransmitter system (*POMC, NR3C1, MAOA, MAOB,* and *TPH2*).
Torpy et al (2004)	CFS	248/248	CDC	To test whether polymorphisms in the corticosteroid-binding globulin gene (*SERPINA6* (prev. CBG)) may act as a risk factor for CFS. No significant association was identified between polymorphisms in SERPINA6 and CFS. However, an increasing level of immunoreactive *SERPINA6* was found associated with increasing numbers of T-alleles for the common exon 3 polymorphism (c.825G/T, Ala224Ser).

Table 1. *Continued*

Study (Publication Year)	Clinical Phenotype	No. of Participants (Case/Control)	Fatigue Assessment	Molecular Biological End Point and Main Findings
Serotonergic system				
Bull et al (2009)†	Hepatitis C, treatment-induced fatigue	98/0	FQ	To study the association between polymorphisms in the cytokine gene *IL6* (rs1800796) and the serotonin transporter gene (*5-HTT*) and treatment-induced fatigue and depression in patients with hepatitis C. The promoter SNP in IL-6, reducing the expression level of the gene, was found to be associated with fewer symptoms of depression ($P = .002$). The same was true but to a lesser extent for the variant giving a high expression level of the serotonin transporter gene ($P = .03$). Neither polymorphism was found to be associated with fatigue.
Narita et al (2003)	CFS	78/50	CDC	To investigate the association between a promoter SNP in the serotonin transporter gene (*5-HTT*) coding for a protein involved in the HPA axis and mood-related neurotransmitter systems and subgroups of CFS patients. A significant increase in longer (L and XL) allelic variants was found in CFS patients compared with controls. The long variants are supposed to retain a higher transcriptional activity.
Ortega-Hernandez et al (2009)†	CFS	81/0	CDC	To determine the influence of SNPs in the serotonin pathway and HLA class II genes and autoantibodies on age at CFS onset and symptoms. Identifies among others an association with a promoter polymorphism in the *HTR2A* gene in combination with the *HLA-DRB103** allele for age at CFS onset in the third decade. In addition, the *HTR2A* promoter SNP was found related to depressive symptoms.
Smith et al (2008)*	CFS	40/55 (IFS) + 42 (NF)	CDC	77 polymorphisms in 14 genes related to serotonin synthesis, signaling, transport, and catabolism were studied. Three SNPs located in the *HTR2A* gene were found to be associated with CFS. The most compelling evidence was for rs6311 suggested to have increased promoter activity in functional studies.
Thyroid system				
van der Deure et al (2008)	Hypothyroidism	141/0	MFI-20	To study if polymorphisms in the *OATP1C1* gene in hypothyroid patients on Levaxin (LT4) therapy could influence well-being and neurocognitive functioning. Three polymorphisms were tested and an association with symptoms of fatigue and depression were identified for 2 of these (rs10770704 and rs10444412).

SNP = single nucleotide polymorphism; FQ = fatigue questionnaire; IL = interleukin; CDC = Centers for Disease Control and Prevention; CFS = chronic fatigue syndrome; SF36/MFSI = short form 36/multidimensional fatigue symptom inventory-short form; FSS = fatigue severity scale; RT-PCR = reverse transcription polymerase chain reaction; MS = multiple sclerosis; HLA = human leukocyte antigen; HPA = hypothalamic-pituitary-adrenal.

*Articles published on the Wichita data set.

†This article is listed both in the immune system section and the serotonergic system section.

‡Of the 9 SNPs included in this article, the results for 7 were already analyzed and published in Smith et al, 2006.

tigue [25,34,35,46]. The results indicate a role for *HTR2A* and *5-HTT* in CFS [34,46].

One article investigated the influence of the thyroid functioning on fatigue. This article identified 2 SNPs in the *ATP1C1* gene associated with symptoms of fatigue and depression [27]. However, a bioinformatics study based on the Wichita dataset suggests an immune-mediated loss of thyroid function as part of the pathogenesis of CFS (Table 3) [14].

The 13 hypothesis-generating studies compared the results from whole genome expression analysis using RNA isolated from whole blood or its leukocyte populations from patients (CFS in 10 articles, postinfectious fatigue in 2 articles, and CF in breast cancer survivors in 1) and controls (Table 2). The aim of these studies was to extract sets of differentially expressed genes between the compared groups, primarily using whole genome array and potentially to elucidate the affected pathways. To some degree, these studies support the importance of the genes and pathways investigated in the hypothesis driven studies, the immune system being one of the confirmed major biological systems. Also, 9 of the hypothesis-generating studies (eg, microarray studies using whole genome arrays) link gene expression differences related to the immune system with fatigue.

As can be expected from whole genome mRNA expression analysis, the lists of identified, differentially expressed genes are not necessarily overlapping. However, 15 of the 16 genes identified to be differentially expressed between CFS patients and controls in the article by Kaushik et al were also identified by Kerr et al [41,47]. The activity of genes involved in ion channel activity is recognized as different between CFS and control cases in several studies [11,24,42], indicating a possible malfunction in the ion channel system. Differentially expressed genes in the apoptotic system are another common finding from gene expression studies on CFS [11,41,48].

The 12 bioinformatical studies in Table 3 use various statistical approaches to explore primarily the Wichita data, either to look for differences between CFS and controls or to unravel the heterogeneity within the CFS samples. A search for differentially expressed genes between CFS and controls were performed in 2 of the articles and the resulting genes were found to be involved in pathways such as the endocrine system, connective tissue, oxidative stress, and ion transport, with immune function as the only common pathway identified in both studies [13,18]. Another study searching for differences in biological pathways identified yet another pathway; namely, the pathway of protein amino acid ADP-

Table 2. *Hypothesis generating studies§*

Study (Publication Year)	Clinical Phenotype	# Participants (Case/Control)	Fatigue Assessment	Findings
Vernon et al (2002)	CFS	5/17	Holmes CFS criteria	Expression profiles of peripheral blood mononuclear cells were generated using the Atlas Human 1.2 Array II with 1176 genes. Eight genes were found differentially expressed in both an age-matched case-control analysis and when comparing all cases to all controls. Several of the differential expressed genes were involved in immunological functions and the results imply an immune dysfunction in the pathophysiology of CFS.
Powell et al (2003)	CFS	7/4	CDC	Expression profiling by differential-display PCR of lymphocytes identified 12 clones to be induced in CFS patients compared with controls. Four aligned to previously known genes (*MAD1L1, PIGK, MAIL,* and *CTSC*), 3 to predicted/hypothetical genes, and 4 to poorly characterized regions. The results indicate subtle changes in the immune system in CFS patients.
Whistler et al (2003)*‡	CFS	23/0	CDC	By grouping the CFS patients according to the onset of illness (gradually/sudden), 117 genes differentially expressed in peripheral blood mononuclear cells were identified when using the Atlas Human 3.81 oligonucleotide glass microarrays containing 3800 genes. The majority of these genes were involved in oxidative phosphorylation, glucose metabolism, purine and pyrimidine metabolism, and glycolysis.
Steinau et al (2004)	CFS	1/1	CDC	Differential display PCR of peripheral blood cells was used to assess expressional differences between 1 CFS patient and 1 healthy control. The analysis showed similar profile for the 2 samples, but a set of 10 genes involved in immune function was verified by RT-PCR.
Kaushik et al (2005)	CFS	25/25	CDC/FQ	Differential expression in peripheral blood mononuclear cells was assessed by a custom Nimblegen array containing probes for 9522 genes. Analysis of the microarray data revealed differential expression of 35 genes, of which 16 were confirmed by RT-PCR analysis. The profile generated based on this 16 genes indicate an activation of T cells and perturbation of neuronal and mitochondrial function in CFS patients as compared with healthy controls.
Whistler et al (2005)	CFS	5/5	CDC	A difference in baseline ion transport and ion channel activity that was exaggerated posttreatment was identified by comparing the expression level in mononuclear cells of CFS patients and healthy controls before and after exercise challenges using the Atlas Human 3.81 (3800 genes) oligonucleotide glass array. A difference in exercise-responsive genes between the 2 groups was also identified with complement activation being one of the processes differing only after challenge.
Whistler et al (2006)*	CFS	40/35 (IFS) +37 (NF)	First evaluation: CDC; second evaluation: MFI/SF-36	Identifies a list of 839 genes associated with fatigue through the analysis of gene expression levels in peripheral blood cells using the MGW 20K human array. Pathway analysis of the fatigue-associated genes implicated oxidative phosphorylation, gluconeogenesis, lipid metabolism, as well as multiple signaling pathways (mTOR, Wnt, MAPK, and Jak-STAT).
Cameron et al (2007)	Postinfective fatigue syndrome	7/8	SOFA	Comparing the expression level in peripheral blood cells using glass arrays (MWG Biotech) carrying 30K probes, 733 genes was identified to be differentially expressed between samples collected early during the illness and at the late (recovered) time point. Of these, 234 were found to be correlated to the severity of the fatigue symptom factor. Thirty-five genes, representing the signal transduction pathway, metal ion binding, and ion channel activity, were identified to have expression levels associated with the illness course of the disease.
Kerr et al (2008a)	CFS	Stage I: 25 vs 50 Stage II: 55 vs 75	CDC	A total of 182 genes were identified in stage I to be differentially expressed between CFS and healthy controls when analyzing the expression level in peripheral blood cells with Affymetrix Human Genome U133 Plus (47K) array. Eighty-eight of these genes were validated by RT-PCR, and among these there was an overrepresentation of the pathways immunity, inflammation, apoptosis, and neurological disease and function. Clustering of these genes revealed 7 CFS subtypes with distinct clinical differences.
Saiki et al (2008)	CFS	Stage I: 11 vs 11 Stage II: 18 vs 12	CDC	Identifies, using a custom microarray to analyze the of expression levels of 1467 stress-responsive genes in peripheral blood cells, 12 genes differentially expressed between CFS and healthy controls, of which 9 the results for genes were validated by RT-PCR. The identified genes pointed to an altered immune function and abnormal energy metabolism in the CFS patients.
Byrnes et al (2009)	CFS	44/44 (MZ twin study)	CDC	No differences in gene expression for any transcript were identified between CFS patients and their monozygotic co-twins when applying Affymetrix Human Genome U133 Plus (47K) array to analyze the expression level in peripheral blood cells.
Gow et al (2009)	Postinfectious CFS	Stage I: 8/7 Stage II: 10/10	CDC	Utilizing Human genome-wide Affymetrix GeneChip array (39K) to compare gene expression levels in peripheral blood mononuclear between cases and controls the authors find differential expression of genes linked to oxidative stress, apoptosis, and immune system.
Landmark-Høyvik et al (2009)	CF in BC survivors	49/88	FQ	The Illumina Human-6 expression bead chips (47K) were used to analyze the expression level in peripheral blood cells. The authors identify differentially expressed gene sets involved in the plasma and B-cell pathways between chronic fatigued and nonfatigued BC survivors.

FQ = fatigue questionnaire; CDC = Centers for Disease Control and Prevention; CFS = chronic fatigue syndrome; RT-PCR = reverse transcription polymerase chain reaction; NF = nonfatigued; IFS = idiopathic fatigue syndrome; CF = chronic fatigue; BC = breast cancer.

*Articles published on the Wichita data set.

†The aim of the studies is to identify differentially expressed genes between cases and controls or between specific subsets of cases.

‡This article refers the results from the longitudinal study on CFS in Wichita that laid the basis for the "Wichita study." Part of the samples is also included in the Wichita study.

Table 3. *Bioinformatical mining studies**

Study (Publication Year)	Clinical Phenotype	No. of Participants (Case/Control)	Fatigue Assessment	Findings
Lee et al (2009)†	CFS	46/19 (CFS-MDD) +36 (NF)	CDC	Using a 2-step model the causal relationship between SNPs in *NR3C1* and *COMT*, gene expression levels and disease was first established followed by a step to determine the significance of the genetic regulatory relationship. Multiple SNPs in NR3C1 were found to affect the risk of developing CFS with fatigue ± depression by interaction with the gene expression level, although this was not significant after FDR correction.
Presson et al (2008)†	CFS	Stage I: 87/0 Stage II: 39/0	CDC	By combining expression, SNP, and clinical data in an integrated approach a list of 20 candidate genes, in the endocrine, immune, and connective tissue pathways is identified to be causal drivers in a set of 299 genes correlating with CFS severity.
Aspler et al (2008)†	CFS	39/35 (IFS) + 37 (NF)	First evaluation: CDC; second evaluation: MFSI/SF-36	Constructs gene sets specific for different leukocyte subsets based on previously published data and analysis the Wichita expression dataset on this background. Identifies lower expression of the CD19+ B-cell subset in CFS patients indicating B-cell dysfunction.
Fuite et al (2008)†	CFS	39/37	CDC	Using network remodeling on the neuroendocrine and immune network, an indication of statistical significant differences between the networks of CFS and controls was identified. The results supports a possible immune-mediated loss of thyroid function in CFS exacerbated by blunted HPA axis responsiveness.
Bhattacharjee et al (2008)†	CFS	43/59 (IFS) + 58 (NF) (with available clinical data, additional samples included for the microbiological studies)	CDC	Bayesian hierarchical modeling and Markov Chain Monte Carlo computation was used to identify biomarker from microarray expression data, SELDI-TOF–based proteomics data, and genetic variation data taking into account clinical information first separately and then in an integrated manner. Based on the selection probability from SNP, expression, and proteomics analysis, gene regions or functional enrichments recognized by multiple sources were identified. Examples are the chromosome regions 5q31.3 and 17q21-23 in which the genes *NR3C1* and *ACE* resides.
Kerr et al (2008b)‡	CFS	55/75	CDC	Further bioinformatical analysis of the 7 distinct clinical phenotypes identified in Kerr et al (2008a). For each subtype, the differentially expressed genes (when compared with normal blood donors) were identified. The gene interactions, disease associations, and molecular and cellular function of the resulting gene sets were determined.
Emmert-Streib (2007)†	CFS	Subset of 227 individuals, actual number not given in article	CDC	Aiming to identify the biological processes rather than single genes affected by the chronic fatigue syndrome undirected dependency graphs were identified based on clinical data, the gene ontology database as well as gene expression data. The analysis indicates that the pathway of protein amino-acid ADP-ribosylation (linked to apoptosis, DNA repair, and disease response) is significantly changed by influence of the disease.
Goertzel et al (2006)†	CFS	43/58	CDC	By using machine learning algorithms (both support vector machines and simple enumerative search) to analyze genetic data the authors aim to determine if presence of CFS can be more accurately predicted by profiles generated from multiple SNPs (n = 28 in 9 genes) than would be expected by chance. Based on these 28 SNPs, CFS status could be predicted by 76% (CFS yes/no).
Fostel et al (2006)†	CFS	139 CFS	First evaluation: CDC; second evaluation: MFI/SF-36	Factor analysis using maximum likelihood estimation was used to identify biomarkers that distinguish subjects according to severity of fatigue and other symptom domains in subgroups. Four factors were found to constitute the optimal solution: fatigue, mood disturbance, concentration difficulties, and sleep/cognitive disturbance. No marker was identified able to separate all subpopulations of subjects, but a combination of 57 genes distinguished subjects along each factor dimension, although only significant for the extreme percentiles of CFS severity. The 57 candidates included, among other chemokine and androgen receptors, P450 cycle proteins and intracellular signaling components.
Fang et al (2006)†	CFS	12/11 (only included participants of the fatigue section of the study)	First evaluation: CDC; second evaluation: MFI	Comparing the 23 most and least fatigued as well as the 26 most and least depressed 188 and 164 fatigue- and depression-related genes, respectively, were identified. Twenty-four genes and 11 pathways common between the 2 subsets indicate a difference in eg immune response, apoptotic activity, and ion channel activity.
Broderick et al (2006)†	CFS	40/37 (IFS)+35(NF)	First evaluation: CDC; second evaluation: MFSI/SF-36	Principal component analysis and projection to latent structures identified 39 genes as principal contributors to the multiple dimensions that make up chronic fatigue syndrome. Seventeen of these 39 genes could be annotated and represented predominantly functions such as oxidative stress response, ion transport, and immune response.
Carmel et al (2006)†	CFS	111/0	CDC	Principal component and latent class analysis were used to classify the underlying gene expression profiles of unexplained chronic fatigue into 5 and 6 classes, respectively, fitting the concept of CFS being a heterogeneous disease. The 5 and 6 multiclass solutions could be discriminated by 32 and 26 genes, respectively, with 19 genes in common between them. Broadly, these genes were implicated in immune function, transcription, ubiquitination, and signal transduction.

SNP = single nucleotide polymorphism; FDR = false discovery rate; CDC = Centers for Disease Control and Prevention; SF36/MFSI = short form 36/multidimensional fatigue symptom inventory-short form; HPA = hypothalamic-pituitary-adrenal; SELDI-TOF = surface-enhanced laser desorption/ionization time-of-flight; CFS = chronic fatigue syndrome.

*The criteria used to separate the studies in Table 2 and Table 3 was whether or not the study included new experiments in the wet-lab. A majority of the studies are using the Wichita data set to test or develop bioinformatical/statistical models to further increase our knowledge of CFS.

†Articles published on the Wichita data set. For the samples included, the analysis of gene expression levels in peripheral blood cells were performed using the MGW 20K human array.

‡Uses the experimental results from Kerr et al (2008a) where the expression level in peripheral blood cells was analyzed with Affymetrix Human Genome U133 Plus (47K) array (see Table 2).

ribosylation as different between CFS and controls [16]. An integrated analysis of proteomics data, gene expression, and SNP data identified several chromosomal regions as potential marker regions of CFS; for instance, 17q21-23, where the *ACE* gene resides, and 5q31.3, which includes the *NR3C1* gene [15]. Multiple SNPs in the *NR3C1* gene were found to be associated with the risk of developing CFS in a parallel study, confirming that this gene might play a role in the development of CFS [21]. Several of the articles have looked into the heterogeneity of CFS by analyzing differences in gene expression within the CFS samples [17,19,20]. One study showed that gene expression could distinguish the CFS subjects according to the onset of the illness (gradual or sudden) [20]. Another study subdivided the CFS subjects into 5 or 6 classes based on the gene expression, where the separating genes were involved in immune function, transcription, ubiquitination, and signal transduction [19]. A search for biomarkers that could distinguish CFS subjects by fatigue severity and accompanying symptoms identified chemokine and androgen receptor genes, genes coding for P450 cycle proteins, as well as genes involved in intracellular signaling [17].

No direct studies on the epigenetic contribution to fatigue were identified.

DISCUSSION

A main finding of this review is that there is a great heterogeneity within genetic studies of fatigue in terms of sample sizes, sample descriptions, and findings. The phenotype, as difficult as it is to define, is further subjected to enlarged variation by the inclusion of 8 articles on fatigued populations suffering from conditions other than CFS. As for CFS, the complexity is more striking than the uniformity in terms of affected biological systems and genetic aberrations, although some systems such as the immune system and the HPA axis were implicated in more than 1 study. Two studies in mice that were excluded (only human studies were reviewed) strengthen the hypothesis that immune dysfunction is associated with fatigue [49,50]. Mice with the *RAG2* gene knocked out, resulting in absent T and B cells, displayed behaviors interpreted as sustained fatigue [49]. *IL10*-deficient mice showed aggravated level of fatigue and motor deficits after peripheral immune stimulation, indicating a protective effect of *IL10* [49]. Still, in our view, the findings from the reviewed studies cannot be regarded as conclusive because of a lack of confirmatory findings, the limited number of studies, and the relatively small number of subjects studied so far.

The sample sizes are evaluated as relatively small because the number of cases varied between 1 and 248. The largest sample sets were found in those studies grouped as hypothesis driven (Table 1; median 79, range 8-248), whereas the smallest sample sets were found among the hypothesis-generating studies (Table 2; median 18, range 1-55). In 33 studies, the phenotype was CFS and in most of these studies the description of the phenotype was restricted to stating that the samples were collected from patients who fulfilled the CDC criteria. One might question whether this is a sufficient description, because information on how this conclusion was reached was mostly lacking and other descriptors such as onset and duration of condition, comorbidities, and other clinical variables were not described. This might imply that the studies are performed on heterogeneous materials, thus reducing the chance of consistent findings. For the so-called Wichita material, the sampling of data was extensive and has been reported in a separate article [31]. Still, for these and other studies using the same dataset in separate articles, the referral to the original article containing information about the samples and procedures used to collect them was often lacking.

A large proportion (15 of 40 [37.5%]) of the included studies was based on a limited number of cases from the Wichita study (n = 55). Of 33 studies on CFS, 15 used the Wichita material. The other 17 (one set of material published twice) [41,48] studies on CFS included in total 786 subjects, inferring that 841 subjects fulfilling the CDC criteria have been studied so far. Fifteen articles based on the same material of 55 subjects raise a methodological question: how many analyses can be performed on the same dataset without the risk of false-positive rates exceeding an acceptable level? Although high-throughput genomic analyses have the potential to unravel the complex biology behind fatigue, the methods are subject to potential problems that result from the number of candidate biomarkers or predictors (eg, gene transcripts, SNPs) being significantly greater than the number of cases. This problem further increases by using the same dataset to test multiple different bioinformatical or statistical models, with the results being evaluated independently of each other. In high-throughput molecular analysis, a model cannot be judged exclusively by its fit to the data used to develop it because the number of variables is much larger than the number of samples; this increases the risk of false discoveries. This raises the issue of what kind of validation should be required. Internal validation is appropriate for the initial studies in which a genomic profile is developed. Such studies are often based on specimens from 1 institution, and the microarray assay is conducted in 1 laboratory. Consequently, the data might not reflect the full range of variability in prognostic influences and tissue-handling procedures. Ideally, external validation on an independent dataset should be conducted. The number of articles published on a dataset derived from the same material is therefore of concern, because the chances of reporting false-positive results thus increases and consistency between the results are less valuable considering the restricted context of 1 dataset.

Our search did not identify any studies analyzing the direct impact of epigenetic variation such as methylation level or degree of histone modification on the risk or level of

CF or CFS. It is true that the gene expression level is influenced not only by environmental influences and the genetic background of an individual, but also the epigenetic state. As such, the gene expression studies can be viewed as pseudo-epigenetic, although there is still a limited potential to decipher an epigenetic contribution.

The reviewed studies cannot be regarded as exhaustive because the literature search was restricted to 1 database. A hand-search was not performed and the combination of search terms was not fully validated. Although standard methodology was applied in the extraction of data, we cannot rule out the possibility that we have missed some relevant studies.

CONCLUSION

To confirm the hypothesis that risk and level of fatigue are influenced by the genetic or epigenetic background of an individual, future studies can draw on the experience from cancer research. The trend in this research area is to perform intergroup studies with sufficient sample sizes focusing not only on the influence of a single aspect such as SNPs or differential gene expression on disease risk or state, but also on the biology behind the disease in combination with information on environmental influences. Validation of the findings in molecular studies to explore the mechanism for the association is also needed. This shift, although challenging, is, in our opinion, needed in the research field of fatigue to further expand our knowledge of a symptom with substantial costs, both for those affected and the greater society.

REFERENCES

1. Wessely S. Chronic fatigue: symptom and syndrome. Ann Intern Med 2001;134:838-843.
2. Wessely S, Hotopf M, Sharpe M. Chronic Fatigue and its Syndromes. Oxford, UK: Oxford University Press; 1998.
3. Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. The chronic fatigue syndrome: A comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. Ann Intern Med 1994;121:953-959.
4. Loge JH, Ekeberg O, Kaasa S. Fatigue in the general Norwegian population: normative data and associations. J Psychosom Res 1998;45:53-65.
5. Prins JB, van der Meer JW, Bleijenberg G. Chronic fatigue syndrome. Lancet 2006;367:346-355.
6. Wessely S. Chronic fatigue syndrome: A 20th century illness? Scand J Work Environ Health 1997;23(Suppl 3):17-34.
7. Ryan JL, Carroll JK, Ryan EP, Mustian KM, Fiscella K, Morrow GR. Mechanisms of cancer-related fatigue. Oncologist 2007;12(Suppl 1): 22-34.
8. Cook EH, Jr., Scherer SW. Copy-number variations associated with neuropsychiatric conditions. Nature 2008;455:919-923.
9. Vernon SD, Whistler T, Aslakson E, Rajeevan M, Reeves WC. Challenges for molecular profiling of chronic fatigue syndrome. Pharmacogenomics 2006;7:211-218.
10. Aspler AL, Bolshin C, Vernon SD, Broderick G. Evidence of inflammatory immune signaling in chronic fatigue syndrome: A pilot study of gene expression in peripheral blood. Behav Brain Funct 2008;4:44.
11. Fang H, Xie Q, Boneva R, Fostel J, Perkins R, Tong W. Gene expression profile exploration of a large dataset on chronic fatigue syndrome. Pharmacogenomics 2006;7:429-440.
12. Whistler T, Taylor R, Craddock RC, Broderick G, Klimas N, Unger ER. Gene expression correlates of unexplained fatigue. Pharmacogenomics 2006;7:395-405.
13. Presson AP, Sobel EM, Papp JC, et al. Integrated weighted gene co-expression network analysis with an application to chronic fatigue syndrome. BMC Syst Biol 2008;2:95.
14. Fuite J, Vernon SD, Broderick G. Neuroendocrine and immune network re-modeling in chronic fatigue syndrome: An exploratory analysis. Genomics 2008;92:393-399.
15. Bhattacharjee M, Botting CH, Sillanpaa MJ. Bayesian biomarker identification based on marker-expression proteomics data. Genomics 2008;92:384-392.
16. Emmert-Streib F. The chronic fatigue syndrome: A comparative pathway analysis. J Comput Biol 2007;14:961-972.
17. Fostel J, Boneva R, Lloyd A. Exploration of the gene expression correlates of chronic unexplained fatigue using factor analysis. Pharmacogenomics 2006;7:441-454.
18. Broderick G, Craddock RC, Whistler T, Taylor R, Klimas N, Unger ER. Identifying illness parameters in fatiguing syndromes using classical projection methods. Pharmacogenomics 2006;7:407-419.
19. Carmel L, Efroni S, White PD, Aslakson E, Vollmer-Conna U, Rajeevan MS. Gene expression profile of empirically delineated classes of unexplained chronic fatigue. Pharmacogenomics 2006;7:375-386.
20. Whistler T, Unger ER, Nisenbaum R, Vernon SD. Integration of gene expression, clinical, and epidemiologic data to characterize chronic fatigue syndrome. J Transl Med 2003;1:10.
21. Lee E, Cho S, Kim K, Park T. An integrated approach to infer causal associations among gene expression, genotype variation, and disease. Genomics 2009;94:269-277.
22. Hampton T. Researchers find genetic clues to chronic fatigue syndrome. JAMA 2006;295:2466-2467.
23. Gow JW, Hagan S, Herzyk P, Cannon C, Behan PO, Chaudhuri A. A gene signature for post-infectious chronic fatigue syndrome. BMC Med Genom 2009;2:38.
24. Cameron B, Galbraith S, Zhang Y, et al. Gene expression correlates of postinfective fatigue syndrome after infectious mononucleosis. J Infect Dis 2007;196:56-66.
25. Bull SJ, Huezo-Diaz P, Binder EB, et al. Functional polymorphisms in the interleukin-6 and serotonin transporter genes, and depression and fatigue induced by interferon-alpha and ribavirin treatment. Mol Psychiatry 2009;14:1145.
26. Flachenecker P, Bihler I, Weber F, Gottschalk M, Toyka KV, Rieckmann P. Cytokine mRNA expression in patients with multiple sclerosis and fatigue. Mult Scler 2004;10:165-169.
27. van der Deure WM, Appelhof BC, Peeters RP, et al. Polymorphisms in the brain-specific thyroid hormone transporter OATP1C1 are associated with fatigue and depression in hypothyroid patients. Clin Endocrinol (Oxf) 2008;69:804-811.
28. Aouizerat BE, Dodd M, Lee K, et al. Preliminary evidence of a genetic association between tumor necrosis factor alpha and the severity of sleep disturbance and morning fatigue. Biol Res Nurs 2009;11:27-41.
29. Collado-Hidalgo A, Bower JE, Ganz PA, Irwin MR, Cole SW. Cytokine gene polymorphisms and fatigue in breast cancer survivors: early findings. Brain Behav Immun 2008;22:1197-1200.
30. Landmark-Hoyvik H, Reinertsen KV, Loge JH, Fossa SD, Borresen-Dale AL, Dumeaux V. Alterations of gene expression in blood cells associated with chronic fatigue in breast cancer survivors. Pharmacogenom J 2009;9:333-340.
31. Vernon SD, Reeves WC. The challenge of integrating disparate high-content data: Epidemiological, clinical and laboratory data collected during an in-hospital study of chronic fatigue syndrome. Pharmacogenomics 2006;7:345-354.

32. Steinau M, Unger ER, Vernon SD, Jones JF, Rajeevan MS. Differential-display PCR of peripheral blood for biomarker discovery in chronic fatigue syndrome. J Mol Med 2004;82:750-755.

33. Torpy DJ, Bachmann AW, Gartside M, et al. Association between chronic fatigue syndrome and the corticosteroid-binding globulin gene ALA SER224 polymorphism. Endocr Res 2004;30:417-429.

34. Smith AK, Dimulescu I, Falkenberg VR, et al. Genetic evaluation of the serotonergic system in chronic fatigue syndrome. Psychoneuroendocrinology 2008;33:188-197.

35. Ortega-Hernandez OD, Cuccia M, Bozzini S, et al. Autoantibodies, polymorphisms in the serotonin pathway, and human leukocyte antigen class II alleles in chronic fatigue syndrome: Are they associated with age at onset and specific symptoms? Ann N Y Acad Sci 2009;1173:589-599.

36. Sorensen B, Jones JF, Vernon SD, Rajeevan MS. Transcriptional control of complement activation in an exercise model of chronic fatigue syndrome. Mol Med 2009;15:34-42.

37. Carlo-Stella N, Badulli C, Silvestri De, , et al. A first study of cytokine genomic polymorphisms in CFS: Positive association of TNF-857 and IFNgamma 874 rare alleles. Clin Exp Rheumatol 2006;24:179-182.

38. Smith J, Fritz EL, Kerr JR, Cleare AJ, Wessely S, Mattey DL. Association of chronic fatigue syndrome with human leucocyte antigen class II alleles. J Clin Pathol 2005;58:860-863.

39. Metzger K, Fremont M, Roelant C, De MK. Lower frequency of IL-17F sequence variant (His161Arg) in chronic fatigue syndrome patients. Biochem Biophys Res Commun 2008;376:231-233.

40. Saiki T, Kawai T, Morita K, et al. Identification of marker genes for differential diagnosis of chronic fatigue syndrome. Mol Med 2008;14:599-607.

41. Kerr JR, Petty R, Burke B, et al. Gene expression subtypes in patients with chronic fatigue syndrome/myalgic encephalomyelitis. J Infect Dis 2008;197:1171-1184.

42. Whistler T, Jones JF, Unger ER, Vernon SD. Exercise responsive genes measured in peripheral blood of women with chronic fatigue syndrome and matched control subjects. BMC Physiol 2005;5:5.

43. Vernon SD, Unger ER, Dimulescu IM, Rajeevan M, Reeves WC. Utility of the blood for gene expression profiling and biomarker discovery in chronic fatigue syndrome. Dis Markers 2002;18:193-199.

44. Rajeevan MS, Smith AK, Dimulescu I, et al. Glucocorticoid receptor polymorphisms and haplotypes associated with chronic fatigue syndrome. Genes Brain Behav 2007;6:167-176.

45. Smith AK, White PD, Aslakson E, Vollmer-Conna U, Rajeevan MS. Polymorphisms in genes regulating the HPA axis associated with empirically delineated classes of unexplained chronic fatigue. Pharmacogenomics 2006;7:387-394.

46. Narita M, Nishigami N, Narita N, et al. Association between serotonin transporter gene polymorphism and chronic fatigue syndrome. Biochem Biophys Res Commun 2003;311:264-266.

47. Kaushik N, Fear D, Richards SC, et al. Gene expression in peripheral blood mononuclear cells from patients with chronic fatigue syndrome. J Clin Pathol 2005;58:826-832.

48. Kerr JR, Burke B, Petty R, et al. Seven genomic subtypes of chronic fatigue syndrome/myalgic encephalomyelitis: A detailed analysis of gene networks and clinical phenotypes. J Clin Pathol 2008;61:730-739.

49. Golumbek PT, Keeling RM, Connolly AM. RAG2 gene knockout in mice causes fatigue. Muscle Nerve 2007;36:471-476.

50. Krzyszton CP, Sparkman NL, Grant RW, et al. Exacerbated fatigue and motor deficits in interleukin-10-deficient mice after peripheral immune stimulation. Am J Physiol Regul Integr Comp Physiol 2008;295:R1109-R1114.