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A Systematic Review on the Microbiome, Pain, and Fatigue

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A Systematic Review on the Microbiome, Pain, and Fatigue

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B.S., Emory University, 2018

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Abstract

A Systematic Review on the Microbiome, Pain, and Fatigue

By Idiatou Diallo

Background

Both fatigue and pain are common public health problems that impose a great burden of disease in many individuals around the world, with limited standard treatment and prevention options. Research has shown that there is an overlap between the two conditions as significant associations have been found between the two. Thus, improved understanding of the common underlying mechanisms of both fatigue and pain is required to advance treatment and reduce prevalence. Interestingly, microbiome alterations have been implicated in the pathogenesis of both fatigue and pain. Therefore, reviewing microbiome research on fatigue and pain may help with effective novel treatments for both fatigue and pain.

Methods

We conducted a systematic review of the literature exploring the relationships between the microbiome, pain, and fatigue.

Results

Although there is a lack of comprehensive literature in this emerging area, current studies point to a variety of microbial taxa that are associated with both fatigue and pain.

Conclusion

Advancing microbiome research in both pain and fatigue will guide symptom research and provide alternate and novel opportunities for effective treatment for individuals suffering from both pain and fatigue.

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Introduction

A. Rationale

Fatigue, commonly defined as a “ feeling of tiredness, reduced energy level, muscle weakness, and cognitive impairments”[1, 2], is a frequent and costly public health problem that affects the mental, physical and social wellbeing of both children and adults. It has been associated with many health conditions such as diabetes, cancer, heart disease, sleep problems, depressive symptoms, and neurological conditions [3] along with significant occupational safety and performance implications [4-6]. Additionally, fatigue reportedly impacts between 6%-34% [1, 7-10] of the general population and is a common complaint of primary care patients [9, 10]. A common fatigue condition is chronic fatigue syndrome (CFS)/myalgic encephalomyelitis (ME) which is a long-term condition with complex dynamic symptoms (e.g. debilitating fatigue not due to physical activity for at least 6 months, post-exertional malaise, poor sleep, flu like symptoms, cognitive impairment, headache, and pain) without diagnostic indicators [11, 12]. It is estimated to affect around 0.2–0.4% of the world’s population [13] and approximately around 836,000 to 2.5 million Americans [14], although the condition is often underdiagnosed [15]. The subjective nature of the condition along with the frequent lack of medical explanations thus renders it a public health challenge [16].

Chronic pain, an important factor in determining a person’s quality of life [17], is also another emerging public health issue that remains generally unrecognized and underreported [18, 19]. Chronic pain impacts around 30%-50% of the world’s population [20]. There is an overlap between chronic fatigue and chronic pain, as significant associations have been found between the two conditions [21-24]. Many of the symptoms

essential in the diagnosis of CFS such as muscle pain, joint pain, sore throat, headaches, and painful lymph nodes, reflect pain [25]. Moreover, studies also show that fibromyalgia, a chronic multi-site pain condition that affects 3-6% of the world's population [26] is also strongly correlated with fatigue. The prevalence of fatigue ranges from 20%-70% among patients with fibromyalgia [27] with 35–70% of patients with CFS meeting the criteria for fibromyalgia [27]. The strong co-morbidity of chronic pain and chronic fatigue, suggests that they could be a single syndrome caused by overlapping mechanisms[28]. Therefore, understanding potential underlying mechanisms for the link between pain and fatigue could further elucidate the relationship and provide an alternative path for treatment for both conditions. Interestingly, alterations in the microbiome (the genetic material of the microbes that live inside and on the human body [29], have been implicated in the pathogenesis of chronic fatigue [30-33] as well as chronic pain[34, 35]. A prior study found that exercise challenge, its impact on pain and changes in the microbiome were largely linked to self-reported fatigue [36]. Furthermore, previous gene sequencing studies of stool bacteria have demonstrated an imbalance of intestinal bacterial populations in ME/CFS patients [30, 37, 38]. Additionally, altered plasma metabolites, some of which are products of the intestinal microbiome, have been identified in ME/CFS patients in contrast to healthy controls [39, 40], and there is evidence of an altered gut microbiome composition in pain patients in contrast to healthy controls [34, 41].

B. Objective

Despite these findings, most of the literature on the association between fatigue and pain has remained largely descriptive and does not fully delve into identifying potential

underlying mechanisms. Although a prior review examines the current evidence supporting microbiome alterations in ME/CFS individuals [31], to our knowledge, no published review has comprehensively examined the literature on the relationship between fatigue, pain, and the microbiome. Therefore, the goal of this review is to explore and further elucidate the microbiome differences in individuals with pain and fatigue. Advancing microbiome research in pain and fatigue will guide symptom research and provide alternate and novel opportunities for effective treatment for these individuals.

Methods

Search Strategy

A systematic search of published studies in PubMed, Embase, and the Web of Science from database inception through February 2020 was performed. Search terms included “fatigue,” pain”, “microbiome,” and permutations of these terms. Articles were also identified by reviewing the bibliographies of manuscripts.

Table 1: Search Queries Used in Each Database

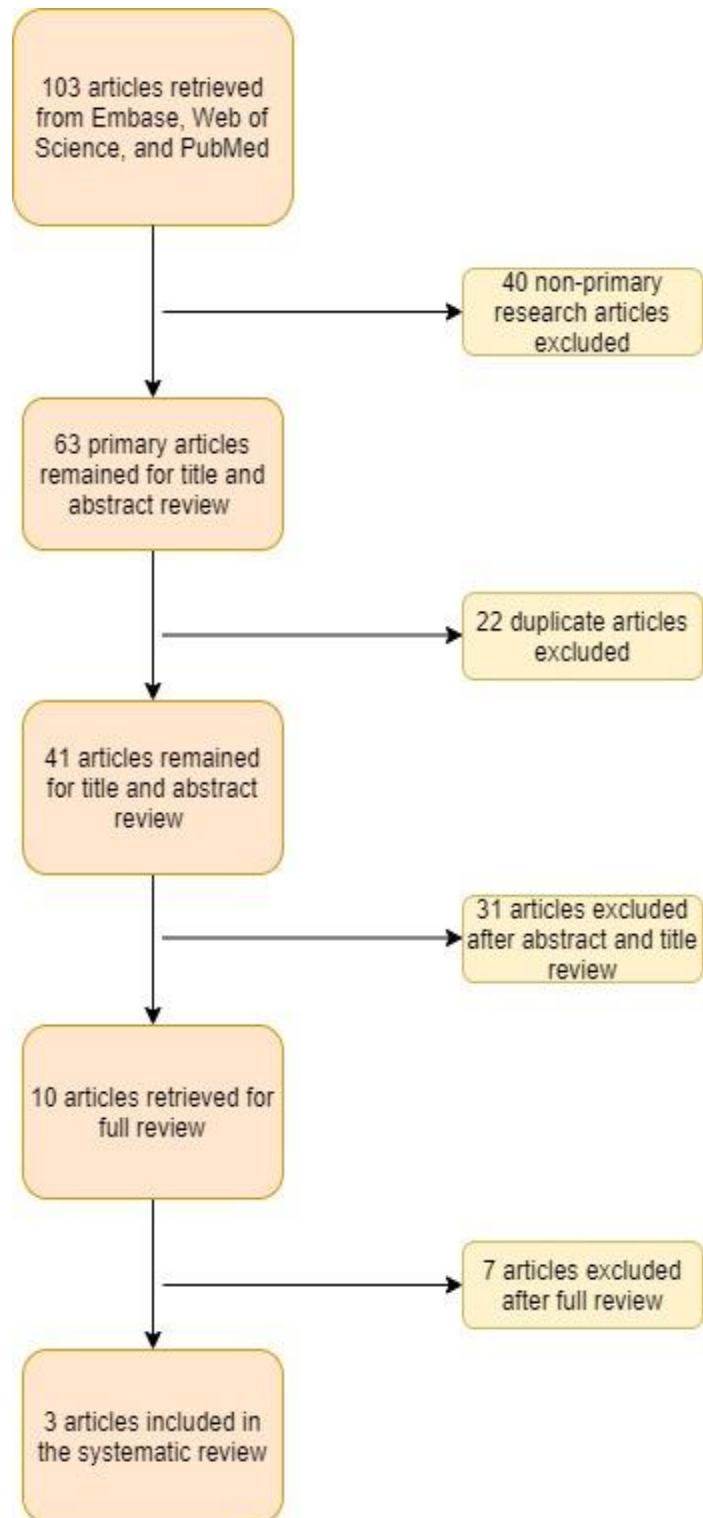
Database	Search Queries
Embase	'microbiome fatigue pain' OR (('microbiome'/exp OR microbiome) AND ('fatigue'/exp OR fatigue) AND ('pain'/exp OR pain))
PubMed	("microbiota"[MeSH Terms] OR "microbiota"[All Fields] OR "microbiome"[All Fields]) AND ("fatigue"[MeSH Terms] OR "fatigue"[All Fields]) AND ("pain"[MeSH Terms] OR "pain"[All Fields])
Web of Science	TOPIC: (microbiome pain fatigue) Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, ESCI, CCR-EXPANDED, IC Timespan=All years

Article Selection

To be included in this literature review, each article had to fulfill inclusion criteria: (1) studies had to be conducted in humans, (2) written in English and were available as full text through institutional access (3) primary data articles, (4) and include individuals diagnosed with/or complaining of both fatigue and pain symptoms, and (5) include an evaluation of the participants' microbiome. Therefore, studies that did not assess pain, fatigue, and the microbiome were excluded. Studies that only included and assessed one or two of the key search terms (pain, fatigue, or microbiome) were excluded. Studies that solely assessed the metabolome and not the microbiome were also excluded.

Additionally, duplicate studies, conference abstracts, editorials, letters, and review articles were excluded. Thus, primary research articles that explored the microbiome in individuals with pain and fatigue were selected.

Overall, the literature search on fatigue, pain, and the microbiome identified 60 studies in Embase, 26 studies in PubMed, and 17 studies in the Web of Science Database giving a combined total of 103 studies. Of these, 63 were primary research articles and 40 were non-primary articles that were excluded. After 22 duplicates were removed, 41 primary research articles remained for title and abstract review. Of these, 10 articles were reviewed in full and 3 met the inclusion criteria. The flow diagram of the article search process and selection is shown in Figure 1.

Figure 1: Flow Diagram illustrating article review process

Review of Findings

There is a lack of comprehensive literature exploring the link between the microbiome, fatigue and pain, with only 3 studies identified that met the inclusion criteria. The table of evidence [table 2] below provides sample characteristics, methodologies and summary of findings on all reviewed articles. A detailed review on the 3 studies is discussed below.

Fatigue, Pain, Microbiome

A case study by Giloteaux et al. explored key variations in a pair of 34 year-old male Caucasian monozygotic twins who are discordant for ME/CFS [30]. The study was conducted by administering a two-day cardiopulmonary test (CPET) and by comparing the gut microbiome, fatigue and pain levels in two monozygotic twins. The gut microbiome was evaluated by collecting stool samples prior to the CPET and by using 16S rRNA sequencing. The ILL twin—which refers to the individual with ME/CFS—met the Fukuda definition for ME/CFS [42] and also complained of mild to moderate pain while the unaffected twin, referred to as WELL, was apparently healthy at the time of the evaluation. Both participants also completed the Chalder Fatigue Scale, the Bell Disability Scale, and the Medical Outcome Survey Short Form 36 (SF-36) for pain and fatigue assessments. ILL's scores were worse for all three scales. Specifically, ILL scored 10 on the Chalder Fatigue Scale while WELL scored 0 (the scale has a range of 0–11, with 0 equaling no fatigue). ILL also scored 40 on the Bell Disability Scale while WELL scored 100, which signified no impairments for WELL in contrast to ILL. Additionally, it is particularly important to note that the Physical (PCS) component summary score in the SF-36 form, which encompasses bodily pain, and physical functioning was significantly lower in the ILL twin (21.7) in contrast to the WELL twin

(55.6) and was also below the expected value (>50) in normal US populations. The gut microbiome analysis in both participants revealed sequences from the Bacteroidetes, Firmicutes, Verrucomicrobia, Actinobacteria, and Proteobacteria phyla. However, findings showed less microbial diversity in the ILL twin in contrast to the WELL twin. Additionally, the ILL twin had a greater abundance of Bacteroidetes (77%) and Firmicutes (74%) in contrast to the WELL twin who had a 19% abundance of Bacteroidetes and 17% abundance of Firmicutes. The ILL twin also had a higher abundance of bacteriophages such as of Siphoviridae and Myoviridae in contrast to the WELL twin. Interestingly, the WELL twin had a 5% abundance for Proteobacteria in contrast to a 1.5% abundance in the ILL twin. Lastly, the WELL twin had increased richness of Rikenellaceae, Ruminococcaceae, and Prevotellaceae at the family level when compared to the ILL twin, who also had decreased *Faecalibacterium* and *Bifidobacterium*. However, it is important to note that this is a case study focused on one pair of male monozygotic twins.

Nagy et al. explored the degree to which the gastrointestinal microbiome and peripheral inflammation are associated in ME/CFS patients [43]. The study was comprised of 50 ME/CFS patients and 50 healthy matched controls that were dominantly Caucasian, and had a mean age of 51.1 and 51.3, respectively. Participants completed medical history reports, symptom rating scales, the Short Form 36 Health Survey (SF-36)—which measures bodily pain—the Multidimensional Fatigue Inventory (MFI), which assesses fatigue, and provided fecal and blood samples. The gut microbiome was assessed via metagenomic sequencing. Irritable bowel syndrome (IBS) co-morbidity was also assessed and data analysis was conducted based on the diagnostic groups ME/CFS, ME/CFS +

IBS, ME/CFS without IBS, and controls. With regard to the gut microbiome, there were several differences at the family level. Specifically, results showed a decreased richness of Lachnospiraceae and Porphyromonadaceae and an increased abundance of Clostridiaceae in the ME/CFS cohort (with and without IBS) in contrast to the controls. Findings also displayed differences at the genus level, with the ME/CFS cohort showing decreased abundance of Dorea, Faecalibacterium, Coprococcus, Roseburia, and Odoribacter and increased richness of Clostridium and Coprobacillus in contrast to controls. In general, the findings displayed a decrease in 9 bacterial species in the ME/CFS cohort in contrast to the controls (Faecalibacterium prausnitzii, Faecalibacterium cf., Roseburia inulinivorans, Dorea longicatena, Dorea formicigenerans, Coprococcus catus, Odoribacter splanchnicus, Ruminococcus obeum, and Parabacteroides merdae) and an increase in 3 bacterial species (Clostridium asparagiforme, Clostridium symbiosum, and Coprobacillus bacterium). Additionally, ME/CFS patients with IBS had distinct taxa from the ME/CFS patients without IBS. Specifically, there were higher levels of unclassified Alistipes and reduced concentrations of Faecalibacterium in ME/CFS subjects with IBS. On the other hand, there were increased concentrations of unclassified Bacteroides and decreased levels of Bacteroides vulgatus in ME/CFS subjects without IBS. Interestingly, in the ME/CFS patients with IBS, decreased concentrations of *Coprococcus comes* was correlated with worse fatigue scores and decreased abundance of *Faecalibacterium* was linked to worse pain scores. Additionally, pain and fatigue scores were more severe in ME/CFS +IBS patients with high BMI in contrast to other groups. This is interesting as findings show a reduced richness of Faecalibacterium species in ME/CFS +IBS patients with high BMI in contrast

to other study groups. Overall, pain, and fatigue levels were correlated with the richness of distinct bacterial taxa and metabolic pathways in the ME/CFS cohort. Additionally, this is a cross-sectional study with a small sample size for the evaluation of the microbiome and pain in different subgroups of patients with ME/CFS.

Minerbi and colleagues investigated the differences in the gut microbiome between 77 women with Fibromyalgia (FM) and 79 healthy controls [44]. The FM patients were dominantly Caucasian and had a mean age of 46 ± 8 . All participants were interviewed by a pain physician, had stool samples collected and analyzed via 16S rRNA sequencing, and participants also completed several questionnaires including: the FM Survey Diagnostic Criteria and Severity Scale and the FM Impact questionnaires which included assessments of pain and fatigue; the Physical Activity self-Administered Questionnaire, and a Sleep Scale from the Medical Outcomes Study. The gut microbiome predominant phyla across all participants consisted of Bacteroidetes (48%), Firmicutes (40%), Proteobacteria (4%), and Actinobacteria (2%). Several operational taxonomic units (OTUs)—also known as clusters of organisms—were found to be differentially abundant (DA). Specifically, there were 53 OTUs higher in FM and 18 OTUs greater in unrelated controls. The study also found a significant association between the abundance of several taxa and FM along with disease severity measures such as pain intensity, widespread pain index, and fatigue. Specifically, pain intensity and widespread pain index were positively correlated with the abundance of unclassified Clostradiales, Bacteroides, Clostridium, Lachnospirillum, Ruminococcaceae, along with Eisenbergiella Massilensis in the FM group in contrast to the control group. Pain intensity was also positively associated with Blautia Hydrogenotrophica. Furthermore, fatigue was positively linked to the abundance

of unclassified Clostradiales, Bacteroides, Lachnoclostridium, Ruminococcaceae, Clostridium along with Eisenbergiella Massilensis, Intestinimonas Butyriciproducens, and Clostridium Scidens in the FM group in contrast to the control group. Nevertheless, it is important to note that this is a cross-sectional study conducted in a small sample of women.

Discussion

Summary of Evidence

Overall, the gut microbiome findings across the three studies show key common variations predominantly in the Bacteroidetes and Firmicutes phyla among individuals with both pain and fatigue in contrast to controls. Findings also show common variations across studies at the family level, with alterations in the abundance of Ruminococcaceae, Lachnospiraceae, and Clostridiaceae among participants with pain and fatigue in contrast to controls. There were also several similar findings at the genus level across the studies, with variations in the abundance of Clostridium, Lachnoclostridium, Bacteroides, and Faecalibacterium in individuals with pain and fatigue compared to controls.

Specifically, Giloteaux et al. [30] found that the ILL twin had a greater richness of Bacteroidetes and Firmicutes in contrast to the WELL twin. Conversely, the WELL twin had increased richness of Ruminococcaceae, when compared to the ILL twin, who instead had a decreased abundance of Faecalibacterium. Additionally, although this study did not directly assess the correlation between pain scores, fatigue scores, and the microbiome, the results do illustrate that the ILL twin—who scored worse on the pain

and fatigue related questionnaires—also has a decreased bacterial diversity in contrast to the WELL twin. This is pertinent as decreased bacterial diversity has been correlated with many human diseases and conditions [45]. These findings are supported by studies which found that individuals with chronic pelvic pain [34] and individuals with fatigue [46] have a significantly decreased gut microbiome diversity in contrast to controls.

Moreover, Nagy et al. [43] found a decreased abundance of Lachnospiraceae and an increased richness of Clostridiaceae in the ME/CFS cohort (with and without IBS) when compared to the controls. Findings also displayed decreased richness of *Faecalibacterium*, and increased abundance of *Clostridium* in contrast to controls.

Moreover, there was an increased abundance of unclassified *Bacteroides* in ME/CFS subjects without IBS. Interestingly, in the ME/CFS patients with IBS, decreased richness of *Coprococcus comes* was linked to worse fatigue scores and decreased concentrations of *Faecalibacterium* was associated with worse pain scores. Additionally, pain and fatigue scores were more severe in ME/CFS +IBS patients with high BMI in contrast to other groups. There was also a decreased abundance of *Faecalibacterium* species in ME/CFS +IBS patients with high BMI in contrast to other groups. However, further work on clarifying the link between *Faecalibacterium*, pain and fatigue is needed to explain these findings, as a recent study suggests that *Faecalibacterium* is more abundant in fatigued individuals in contrast to controls [46]. Lastly, Minerbi et al.[44] found that pain intensity, widespread pain index, and fatigue were correlated positively and significantly with the abundance of unclassified Clostradiales, *Bacteroides*, *Clostridium*, *Lachnoclostridium*, *Ruminococcaceae*. These results corroborate findings from Giloteaux et al. as the ILL twin had worse pain and fatigue scores and also had a greater

richness of Bacteroidetes (the phylum of Bacteroides as seen in Minerbi et al.) and Firmicutes (the phylum of Clostradiales, Clostridium, Lachnoclostridium, and Ruminococcaceae).

Limitations

This review has several limitations that must be acknowledged. First, it is pertinent to note that this review is limited to a descriptive approach. This is partly due to the 3 reviewed articles slightly differing in their purpose and methodologies, and due to the articles reporting on the relative rather than on the absolute abundance of different subgroups (e.g. genera), which is gold standard and assesses the absolute number of microorganisms to identify taxonomic changes in lieu of informing abundance in a relative manner[47]. The studies were also small with sample sizes ranging from 2 to 156 and were likely underpowered.

Additionally, all 3 studies were conducted in populations that were predominantly Caucasian and female which reduces the generalizability of the findings (refer to Table 3). However, it is important to note that women are more likely to report fatigue [48], more likely to be diagnosed with ME/CFS [49], and are more likely to report chronic pain including FM [50] in contrast to men.

Moreover, the 3 studies evaluated the microbiome by conducting fecal analysis.

However, while Giloteaux et al. and Minerbi et al. utilized 16S rRNA sequencing which provides the taxonomic composition of a microbial sample by sequencing the gene encoding of the small subunit of ribosomal RNA (16S) [51, 52], Nagy et al. analyzed stool samples via metagenomic sequencing which sequences all given genomic information encoded by a microbial sample [51, 52]. Thus, it is important to note that we

cannot directly compare the metagenomic findings to the 16S rRNA sequencing results which only targets 16S rRNA genes. Moreover, using fecal samples for analyzing the microbiome is a limitation as stool samples are more representative of the microbiome populating specific luminal areas in contrast to mucosal segments, and is thus not a good representative of the diversity of the microbiome as a whole [53-55]. Therefore, paired mucosal and luminal/fecal samples should be considered. Furthermore, although 16S rRNA sequencing aids in determining the taxonomic composition of fecal microbial samples, according to research [51], taxonomy remains inadequate in identifying microbial function.

While Minerbi et al. directly elucidated associations between distinct taxa and pain and fatigue symptoms, Giloteaux et al. did not assess the link between distinct taxa and pain and fatigue scores. On the other hand, while Nagy et al. show the association between the ME/CFS +IBS group, pain/fatigue scores, the abundance of distinct taxa, and does also state that pain, and fatigue levels were linked with the richness of distinct bacterial taxa, these distinct species were not elucidated in the study.

Future Areas of Interest

A future area of interest includes the association between metabolites, the gut microbiome, pain and fatigue. This is an important next step as there is a strong association and interplay between the human microbiome and host metabolism [56]. A study by Malatji et al. [57] showed that three metabolites (Hippuric acid, lactic acid, 2-Hydroxyisobutyric acid) observed in the urine samples of an FM patient group suggest alterations in the gut metabolome, with the most discriminatory variable between the FM group and controls being the 2-Hydroxyisobutyric acid. This is pertinent as 2-Hydroxyisobutyric acid has

been previously associated with faecalibacterium prausnitzii [58]. The findings also showed increased levels of three metabolite markers (taurine, creatine and succinic acid) which were important for the distinction between FM patients and controls and were significant markers of pain and fatigue in FM. Interestingly, prior animal studies suggest that creatine is correlated with bacteroides[59]. Moreover, succinate, the anion of succinic acid, is a gut microbiota-derived metabolite that mediates intestinal homeostasis and energy metabolism [60]. On the other hand, while the association between taurine and the microbiome remains unclear, some studies suggest a beneficial effect [61]. In the Minerbi et al. article, invariable alterations in the richness of butyrate metabolism-related bacteria were detected. Specifically, *F. Prausnitzii* and *B. Uniformis* were decreased in FM patients, whereas *Intestinimonas Butyriciproducens*, *Flavonifractor Plautii*, *Butyricoccus Desmolans*, *Eisenbergiella Tayi*, and *Eisenbergiella Massiliensis* were increased in FM patients. These alterations had metabolic effects such that serum levels of butyric acid in FM patients were particularly higher in contrast to unrelated controls, whereas levels of propionic and isobutyric acid were lower when compared to controls. The link between the metabolome, microbiome, pain, and fatigue is thus another area of potential interest that is more comprehensive and may provide improved alternate and novel opportunities for effective treatment of both chronic fatigue and pain.

Overall, the limited existing human studies on the microbiome in individuals with chronic pain and fatigue report abundance-specific findings of distinct taxa. However, the sample sizes are relatively small ranging from 2 to 156, and no consensus has emerged regarding which bacterial taxa are most relevant to pain and fatigue. Pain and fatigue-

related microbial differences in contrast to controls remains unclear. Additionally, future studies should employ larger sample sizes, should utilize a functional system of classification such as that explained by Connor et al. [51], should evaluate the presence of related metabolites, and control for other demographic or biological factors that may be pertinent covariates, such as BMI as seen in Nagy et al.[43]. Furthermore, evaluating patients with pain and fatigue who are unmedicated and not undergoing mindfulness interventions at the time of the microbiome assessment may also aid in elucidating the possible confounders associated with pain relievers, or other medications that alleviate pain or fatigue symptoms.

Conclusion

Due to the complexity and subjective nature of both chronic pain and fatigue, diagnosing and treating the symptoms and related conditions remain a public health challenge.

According to the CDC, current treatment for those who suffer from pain and fatigue is currently limited to pain-relievers, such as aspirin and acetaminophen, or to counseling, which might involve mindfulness techniques that aid in learning new ways to deal with pain [62]. Further research on the association between the microbiome, particularly the Bacteroidetes and Firmicutes phyla, along with pain and fatigue might help in providing alternate and novel opportunities for effective treatment for individuals suffering from pain and fatigue. Since research has mainly focused on females and Caucasians, it is also pertinent to explore these associations in males and different ethnic groups as current findings might not reflect microbiome patterns prevalent in males and various ethnic groups suffering from pain and fatigue.

Table 2: Fatigue, Pain, Microbiome

<i>Title (publication type)</i>	<i>Authors</i>	<i>Subjects</i>	<i>Pain/fatigue Measures</i>	<i>Key Findings</i>
A Pair of Identical Twins Discordant for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome Differ in Physiological Parameters and Gut Microbiome Composition (case study)	Gilateaux et al [30]	Twins: one with chronic fatigue syndrome (ME/CFS), the other a control	Fatigue syndrome (ME/CFS) Self-Reported Pain; and Medical Outcome Survey Short Form 36 (physical component-bodily pain)	ILL twin had worse pain and fatigue scores and complaints. Reduced microbial diversity in ILL twin in contrast to WELL twin. ILL twin had a greater abundance of Bacteroidetes and Firmicutes in contrast to the WELL twin WELL twin had a higher abundance of Proteobacteria, Rikenellaceae, Ruminococcaceae, and Prevotellaceae ILL had reduced abundance of Faecalibacterium and Bifidobacterium and an increased richness of Siphoviridae and Myoviridae
Fecal metagenomic profiles in subgroups of patients with myalgic encephalomyelitis/chronic fatigue syndrome (cross sectional)	Nagy et al [43]	50 ME/CFS patients and 50 matched healthy controls	Fatigue syndrome (ME/CFS) The 1994 CDC Fukuda; The SF-36-bodily pain	At the Family level: decreased richness of Lachnospiraceae and Porphyromonadaceae and an increased abundance of Clostridiaceae in the ME/CFS cohort in contrast to the controls At the Genus Level: decreased abundance of Dorea, Faecalibacterium, Coprococcus, Roseburia, and Odoribacter and increased richness of Clostridium and Coprobacillus in the ME/CFS cohort in contrast to the controls In ME/CFS + IBS participants, decreased concentrations of <i>Coprococcus comes</i> was linked to worse fatigue scores and decreased abundance of Faecalibacterium was linked to worse pain scores
Altered microbiome composition in individuals with fibromyalgia (cross-sectional)	Minerbi et al [44]	77 patients with Fibromyalgia (FM) and 79 controls	Fatigue: the FM Survey Diagnostic Criteria and Severity Scale questionnaire, and the FM Impact Questionnaire Pain: the FM Survey Diagnostic Criteria and Severity Scale questionnaire, and the FM Impact Questionnaire	Pain intensity, widespread pain index, and fatigue were positively associated with the abundance of unclassified Clostradiales, Bacteroides, Clostridium, Lachnoclostridium, Ruminococcaceae.

Table 3: Demographics of Study Participants

Study Name	Gilateaux et al		Nagy et al		Minerbi et al	
	Cases N=1	Controls N=1	Cases N=50	Controls N=50	Cases N=77	Controls N=79
Sex						
Female	0	0	41	41	77	N/P *
Male	1	1	9	9	0	N/P
Race						
Caucasian	1	1	49	48	N/P **	N/P
Asian	0	0	1	1	N/P	N/P
Other	0	0	0	1	N/P	N/P
Mean Age	34.3	34.3	51.1	51.3	46 ± 8	N/P

N/P: not provided

N/P*: not provided /Majority female

N/P**: not provided/ Predominantly Caucasian

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