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The Gut Microbiome and Alzheimer's Disease

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Abstract

Alzheimer's Disease's pathophysiology and pathogenesis are still poorly understood despite being one of the most researched neurocognitive diseases. This review examines a new area of research that explores the link between the gut microbiome and Alzheimer's Disease pathology. Potential treatments and therapeutic interventions stemming from microbiome research are discussed as well. Overall, while the research so far is promising, the literature in this area is still in its infancy, and there is much to be done to establish a causal role between microbiome dysbiosis and AD pathology. Moreover, assuming that microbiome research continues to support new models of AD, implications for the field are discussed.

The Gut Microbiome and Alzheimer's Disease

Alzheimer's Disease (AD) is a degenerative neurocognitive disorder characterized by progressive cognitive decline, affecting different areas such as memory, learning, executive functions, language, and other cognitive abilities (Alzheimer's Association, 2020). According to the Center for Disease Control and Prevention (CDC, 2020), approximately five million individuals in the United States currently suffer from AD, and as the population ages, the number is expected to triplicate to approximately 14 million people by 2060. While the death rate of other chronic conditions such as heart disease has decreased, AD's death rate is steadily increasing. Additionally, AD adds tremendous economic, psychological, physiological, and mental burdens to patients and their caregivers. Moreover, the cost of AD to the nation for 2020 is expected to be over $300 billion, with an anticipated increase to 1.1 trillion by 2050 (Alzheimer's Association, 2020; CDC, 2020).

Although AD is one of the most extensively researched disorders, its etiology and pathogenesis are still not fully understood. Research so far indicates that AD is characterized by extracellular accumulation of beta-amyloid protein (Aβ) plaques and intracellular neurofibrillary tangles of tau protein that lead to neuronal death, causing significant degeneration throughout the brain over time (Guo et al., 2020). Research also indicates that the interactions of several factors may be responsible for the onset of AD. Genetic research has identified over 23 genetic variants thought to be related to AD, particularly the APOE gene, which is considered to be directly involved in the production of the amyloid precursor protein that results in beta-amyloid protein plaques (van der Lee et al., 2018).

Additional genetic research points to the possibility of sex chromosomes being involved in AD pathology, since AD disproportionally affects more females than males (Pike, 2017). However, the research has not fully supported the involvement of sex chromosomes in AD pathology. Most research regarding sex differences focuses on the role of hormones. Some research indicates that estrogen may have neuroprotective qualities and plays a role in regulating synaptotoxicity, oxidative stress, and even neuroinflammation (Merlo, Spampinato, & Sortino,

2017). The literature also posited the possibility of females being at a higher risk of developing AD after menopause where there is a sharp decrease in estrogen and progesterone, while in males, testosterone is metabolized into estrogen, and the drop in testosterone with age tends to be much more gradual (Mielke, Vemuri, & Rocca, 2014).

Furthermore, some research also indicates that several environmental and sociocultural factors may influence AD's onset and severity. A literature review by Fratiglioni and Wang (2007) concluded that educational level, work complexity, leisure activities, and rich social networks were all associated with lower risks of dementia. Moreover, the literature has also shown that Black and Hispanic individuals have a higher risk of developing AD. Research regarding the differences between ethnicities has been inconclusive, with some research supporting higher genetic risk for Black and Hispanic individuals, while other research does not (Alzheimer's Association, 2020). Socioeconomic differences such as low socioeconomic status (SES), lower educational attainment and low quality of education, and greater exposure to adversity and discrimination, have also been studied and are thought to increase the prevalence of AD and other dementias in Black and Hispanic individuals (Alzheimer's Association, 2020). Nonetheless, the Alzheimer's Association (2020) also points out that research with representative samples of minority groups is scarce and in dire need to further understand these differences.

The Amyloid Cascade Hypothesis

The most accepted model of AD in the literature is called the Amyloid Cascade Hypothesis (ACH). First delineated in 1992 by Hardy and Higgins, the ACH suggests that AD results from an imbalance between the generation and clearance of amyloid-β protein. The Amyloid Precursor Protein (APP) is thought to be responsible for producing misfolded Aβ proteins that are insoluble and get deposited and accumulated in the brain. Aβ protein plaques are believed to be neurotoxic, leading to neurofibrillary tangles and, eventually, cell death, causing widespread neuronal loss (Hardy & Higgins, 1992).

Although a linear and simple model, the ACH has many drawbacks, as it leaves many questions unanswered. In a literature review by Herrup (2015), the evidence for and against the amyloid cascade hypothesis was reviewed. The ACH model's first criticism is that it assumes that amyloid is the driver and root cause of AD. However, as Herrup (2015) indicates, research with PET scans shows that not everyone with amyloid plaques in their brain develops cognitive impairment. Additionally, amyloid plaques are not exclusive to AD pathology, as they are also seen in other neurological conditions (Herrup, 2015).

On the other hand, according to the ACH model, removing amyloid from AD brains should stop the development of AD pathology (Herrup, 2015). In human anti-amyloid antibody therapy trials, few participants responded to this therapy. These participants showed a significant reduction in their amyloid burden. However, cognitive testing performance indicated that their cognitive impairment did not improve and continued to worsen, suggesting that AD pathology continued to progress regardless of amyloid status (Herrup, 2015).

Another critical point made by Herrup (2015) is that most AD research, including the ACH model, heavily relies on mice models of the disease. AD mice models are engineered to resemble human AD pathology by introducing human APP constructs into the mouse genome. These mice produce amyloid deposits in their brains and have behavioral and cognitive symptoms that resemble the human form of AD. However, Herrup (2015) indicates that these models recreate human familial AD (fAD), which is a type of AD with a clear genetic component and is responsible for the early-onset AD. Familial AD, however, accounts for a small percentage of AD cases.

Moreover, mice do not develop sporadic AD (sAD) naturally. In humans, sAD is responsible for the vast majority of AD cases. Therefore, although helpful, research with mice is not fully generalizable to humans (Herrup, 2015).

Thus, different techniques (immunization against Aβ peptides, strategies that enhance clearance of Aβ, and even anti-inflammatory treatments) appear effective at clearing Aβ plaque in mice brains, resulting in behavior and cognitive improvements, sometimes completely reversing AD pathology in mice (Herrup, 2015). However, trials of these methods with humans have not been as successful. As Herrup (2015) suggests, mice models more closely resemble fAD, supporting the ACH model, but do not resemble sAD, which affects most AD patients. Therefore, Herrup (2015) concludes that although amyloid is an essential factor of AD pathology, it does not seem to be the driver of AD's pathogenesis and suggests that future models redefine AD by looking beyond amyloid as the root cause of AD. Although the existing research is extensive and has made tremendous advances, it still does not provide an exact pathogenesis model and does not explain the differences in presentation of the disease from person to person. Unfortunately, this means that currently available treatments are merely able to manage some AD symptoms, but there is currently no known preventative treatment or cure for AD (Alzheimer's Association, 2020).

The Inflammatory Hypothesis of Alzheimer's Disease

As suggested by Herrup (2015), a new AD model examines inflammation as the driver of pathology, while the role of amyloid is seen as a factor in a complex chain of events. Inflammation has been linked to many disorders, either as a root cause or an exacerbating factor. Olsen and Singhrao (2015) summarize the inflammatory hypothesis and describe its two models: the intrinsic and extrinsic models (Olsen & Singhrao, 2015).

Similarly to the ACH, the intrinsic model assumes that the blood-brain barrier (BBB) is intact and that AD pathology is contained to factors within the brain (Olsen & Singhrao, 2015). According to this model, amyloid peptides lead to microglial activation as an immune response of the brain, causing neuroinflammation. Over time, neuroinflammation leads to neurotoxicity that then triggers the production of tau tangles and eventually results in neuronal death. In contrast with the ACH, the intrinsic model views inflammation from the prolonged overactivation of glial cells as the driver of pathology. Amyloid is seen as the activating factor but not responsible for pathogenesis on its own (Olsen & Singhrao, 2015).

On the other hand, the extrinsic model views AD pathology as a systemic issue rather than a purely neurological one (Olsen & Singhrao, 2015). According to the extrinsic model, bacteria or bacterial metabolites that cause systemic inflammation can affect different organs and can also weaken and seep through the BBB into the brain. Within the brain, these foreign contaminants activate a neuronal immune response by the glial cells. As with the intrinsic model, neuroinflammation then leads to neurotoxicity and, over time, results in neuronal death and widespread degeneration (Olsen & Singhrao, 2015).

The extrinsic model completely changes how AD has been studied and understood for decades. If bacteria or its metabolites are responsible for AD pathology, then the root cause may lay outside the brain. A promising new research area investigates the potential link between the gut microbiome and the brain as another possible factor in the etiology and pathogenesis of AD that has been overlooked until recently. The following sections will review the microbiome and will examine the existing literature regarding the link between the microbiome and AD pathology.

Microbiome

The term microbiome refers to a group or colony of microorganisms comprised of viruses, bacteria, archaea, eukaryotes, and other types of microbes that form an ecosystem within human's and other animal's bodies (Sochocka et al., 2019). These microorganisms live in different body sites and have different functions, living in commensal, symbiotic, and sometimes in parasitic relation to the human host. The largest of these colonies is the gut microbiome. The popular precognition was that the gut microbiome's function was to aid in the digestion of some foods, while its true function and relationship to the human host had been seriously underestimated (Sochocka et al., 2019). In 2007, the Human Microbiome Project (HMP) was established to sequence all microbial DNA that inhabits the human body and determine the role the microbiota plays, both in human health and disease (Blum, 2017).

Studies of the gut microbiome, in particular, have shown that changes or depletion of the microbiota (dysbiosis) have been linked to many gastrointestinal diseases, such as irritable bowel syndrome (IBS) and obesity (Sochocka et al., 2019). Dysbiosis has also been linked to other diseases such as allergies, cancers, metabolic disorders, and even neurological and psychological disorders, including anxiety, depression, Parkinson's disease, Alzheimer's Disease, ADHD, and Autism Spectrum Disorder. Although the gut microbiome tends to develop throughout childhood, it eventually reaches a period of relative stability throughout life. Nonetheless, certain factors and conditions are known to drastically impact microbial diversity, such as dietary changes, travel, antibiotics use, and chronic stress (Blum, 2017; Sochocka et al., 2019).

Gut Microbiome Development

Microbiome research has highly focused on the gut microbiome. Since the gut microbiome is composed of unique microbial life, the question as to how they colonize the human gut is central to understanding the microbiome and its relationship with the human body. Initially, it was assumed that babies were born microbe-free and acquired microbes after being exposed to them through the environment and contact with others. However, recent research shows evidence of a distinct microbiota in the vaginal canal and uterus, placenta, umbilical cord, and amniotic fluid. A similar microbiome to that of the mother has also been found in newborns' meconium. This suggests that microbial exchange may happen at or even before birth. Prenatal factors that have been shown to influence microbiome diversity include maternal health and nutrition, maternal substance and medication use, maternal stress, and term and mode of delivery. Some postnatal factors include breastfeeding and maternal nutrition, the use of formula versus breast milk, medications and antibiotics, skin contact, and nutrition, particularly during the first year of life. Although microbiome composition is somewhat stable, it also changes slightly throughout life (Principi & Esposito, 2016).

Gut Microbiome and Brain Functioning

The literature on microbiome functioning indicates that metabolites produced by the gut microbiome may play an essential role in the development and functioning of the brain (Mayer, Tillisch, & Gupta, 2015). Most of the research about the gut microbiota, brain development, and brain functioning comes from animal studies. As Mayer et al. (2015) indicated, most research comes from germ-free mice (GF) studies where mice are raised in entirely aseptic environments, which is achieved by removing the newborn mice by cesarean section and immediately transferring them to sterile environments. These studies have shown that GF mice, compared to normally raised mice, exhibit different feeding, social, and affective behaviors. These behaviors included anxiety

and depressive-like behaviors, aggression, and isolation. GF mice also displayed different metabolic functions and brain biochemistry, increased basal and HPA axis responses, memory impairments, and disrupted brain signaling systems. Moreover, in several of these research studies, the symptoms shown by GF mice were either mitigated, partially reversed, or fully reversed by providing prebiotics and specific strains of probiotics to the mice, suggesting possible therapeutic advantages of altering or influencing the gut microbiota (Mayer et al., 2015). Research with GF also suggests that the gut microbiome is involved in early brain development (Dinan & Cryan, 2017). Studies have shown that GF mice have compromised gene expressions that affect metabolic pathways such as the serotonergic system and the metabolism of steroid hormones. Moreover, GF mice have also shown lower expression of the brain-derived neurotrophic factor (BDNF), changes in the amygdala, and hypermyelination in the prefrontal cortical region (Dinan & Cryan, 2017). Although research with GF is promising, it is important to remember that these results do not necessarily translate to humans.

The Microbiota-Gut-Brain-Axis

How do the gut and microbiome influence the brain and vice versa? Meyer et al. (2015) delineated some of the pathways in which the brain and the gut microbiome interact and communicate. The Autonomic Nervous System (ANS) and its sympathetic and parasympathetic branches, as well as the hypothalamic-pituitary-adrenal (HPA) axis, play a large role in the function and regulation of intestinal activity (Dinan & Cryan, 2017). Thus, the gut microbiome is impacted by stress responses such as increased cortisol levels, adrenaline, and other stress response hormones. Moreover, ANS activity in the gastrointestinal tract has also been linked to immune responses (Mayer et al., 2015).

Communication between brain and gut microbiota is also known to be modulated and regulated by metabolites produced by the microbiome (Mayer et al., 2015; Dinan & Cryan, 2017). These metabolites include neurotransmitters such as serotonin, catecholamines, and GABA, which can bind to human receptors within the gut. Some of these metabolites also signal the brain by binding to and sipping into the endocrine and circulatory system or by signaling the brain directly through the vagus nerve and possibly through some spinal nerves. Moreover, evidence suggests that this is a bidirectional process with the brain communicating with the gut microbiota through neurotransmitters and hormones within the endocrine and cardiovascular system or through direct signaling via the vagus nerve. In recent literature, this bidirectional communication pathway has been termed the microbiota-gut-brain axis (Mayer et al., 2015; Dinan & Cryan, 2017). Communication between brain and gut microbiome and how it pertains to AD pathology will be discussed in greater detail in the following section.

The Gut Microbiome and Alzheimer's Disease

As previously stated, the hallmark of AD is the concentration of Aβ plaques and neurofibrillary tangles of tau protein found within the brains of AD patients. Though it is known that the Aβ plaques and tau protein tangles ultimately lead to widespread degeneration of the brain, the mystery lies in what triggers the production and accumulation of these proteins. While it had been previously hypothesized that AD was caused by different factors (i.e., genetics, hormones), research only partially supported these theories but never fully answered the riddle. The microbiota-gut-brain axis has recently received increased attention in the literature, linking dysbiosis to numerous diseases and disorders, including neurodevelopmental and neurocognitive disorders. Research with mice models of AD has observed that mice with AD-like symptoms have

significantly different strains of microbes than normal mice (He, Li, Sun, & Chen, 2020). It was also observed that many of the increased levels of specific strains of bacteria were known to be pro-inflammatory. Moreover, studies also suggest that increasing age was associated with decreased microbiota diversity. (He et al., 2020).

Literature reviews by Mancuso and Santangelo (2018) and Pellegrini, Antonioli, Colucci, Blandizzi, and Fornai (2018) explained how these pro-inflammatory bacteria in the gut might be directly responsible for the development and onset of AD. Firstly, the gut's relationship to the brain and other organ systems needs to be better understood, as the gut has often been referred to as "the second brain" or "the second immune system." As previously stated, it has been found that these bacteria also produce many metabolites, including neurotransmitters (Mancuso & Santangelo, 2018). Notably, the gastrointestinal system is under the control of the Enteric Nervous System (ENS), a subdivision of the ANS. Additionally, the gut is comprised of epithelial tissue, which aids in the absorption of nutrients, and together with a mucus layer, forms the gut barrier which isolates the gut and protects against pathogens from entering the system (Pellegrini et al., 2018).

Metabolites produced by the gut microbiome interact with the ENS and are also thought to affect the epithelium's protective mucus layer. In their review, Mancuso and Santangelo (2018) identified specific gut bacteria strains that had been shown to produce functional extracellular amyloid fibers within the gut. These amyloid fibers and other metabolites are known to disrupt the mucosal permeability, allowing pathogens and the bacterial-made amyloid fibers to seep through and possibly disseminate throughout the body, triggering an immune response. Over time, the spread of these pathogens leads to a chronic inflammatory response that further increases the permeability of the gut barrier and the BBB (Mancuso & Santangelo, 2018; Pellegrini et al., 2018; Sochocka et al., 2019).

Increased permeability of the BBB allows pathogens and bacterial-made amyloid fibers to enter and activate a cascade of events within the brain (Sochocka et al., 2019). Of importance, amyloid proteins, both bacterial and neuronal, are abundant in the human brain and can be absorbed and broken down by glial cells. However, Aβ proteins that are characteristic of AD are misfolded (Aβ40 and Aβ42 in specific), which cannot be broken down and eventually cluster together, forming plaques throughout the brain (Kowalski & Mulak, 2019). Kowalski and Mulak (2019) indicate there is evidence within the literature that Aβ40 and Aβ42 proteins then cross-seed to other types of amyloids, affecting the new proteins' shape and propagating the expression of misfolded proteins, leading to the spread and accumulation of plaques.

Overproduction of Aβ then activates microglia and astrocyte cells, which leads to chronic neuroinflammation (Kowalski & Mulak, 2019). These inflammatory responses lead to over-reactive glial cells, which, over time, become impaired in their ability to clear amyloid plaques, causing neuroinflammation and widespread glial cell death, increasing neurotoxicity, leading to overall neuronal death and degeneration of the brain. Likewise, neuroinflammation and increased neurotoxicity also cause tau proteins within the neurons to misfold. In normal conditions, tau proteins help modulate the stability of axonal microtubules. In AD, misfolded tau proteins accumulate, creating tubules that cause neurons to collapse. Misfolded tubules have also been observed to spread from neuron to neuron, affecting normal tau proteins to fold abnormally (Kowalski & Mulak, 2019). This research supports the extrinsic model of the inflammatory hypothesis of AD. A continuation is a review of the existing research with humans linking microbiome bacteria and their metabolites to AD pathology.

Human Research of Microbiome and Alzheimer's Disease

Although research with humans is limited for obvious ethical reasons, some studies have explored the link between dysbiosis and AD. In a study by Vogt et al. (2017), the bacterial taxonomic composition of fecal samples of AD patients was compared to healthy controls (HC) to examine differences in gut microbiome between groups. A bacterial 16S rRNA gene sequencing on DNA was performed on fecal samples from a total of 50 participants (AD=25; HC=25) who were matched by age and sex. Differences in microbiota composition were analyzed at different phylogenetic levels between groups (Vogt et al., 2017).

At the Phylum level, both groups had similar microbiota compositions, with Firmicutes accounting for 78% of all microbial DNA in the samples, followed by Bacteroidetes making up 15% of the samples, and Actinobacteria and Verrucomicrobia making up approximately 2.6% each of the DNA found in the samples (Vogt et al., 2017). Nonetheless, differences were noted at the Family and Genus levels, with some strains found to be increased, while other strains were reduced in the AD group compared to the HC group. Table 1 specifies the strains that were noted to differ for the AD group. Of importance, some of the increased strains in the AD group (i.e., Bacteroides) are known to be pro-inflammatory bacteria. These bacteria were identified as gram-negative bacteria, meaning that they have an outer protective layer that can diffuse into the gut, which causes irritation. Likewise, some of the bacteria decreased in the AD group (i.e., Bifidobacterium, SMB53, Dialister) are known to be anti-inflammatory strains (Vogt et al., 2017).

Table 1

Strains Found to Differ Between Control Group and AD Participants.

Phylogenetic level	Increased Strains	Decreased Strains
Family	Bacteroidaceae Rikenellaceae Gemellaceae	Ruminococcaceae Bifidobacteriaceae Clostridiaceae Mogibacteriaceae Turicibacteraceae Peptostreptococcaceae
Genus	Blautia Bacteroides Alistipes Phascolarctobacterium Bilophila Gemella	Bifidobacterium SMB53 Dialister Clostridium Turicibacter Adlercreutzia cc115

Note. Adapted from "Gut Microbiome Alterations in Alzheimer's Disease," by Vogt, N. M., Kerby, R. L., Dill-McFarland, K. A., Harding, S. J., Merluzzi, A. P., Johnson, S. C., ... & Bendlin, B. B, 2017, *Scientific reports*, *7*(1), 1-11.

Vogt et al. (2017) then conducted a second part of the study to explore if differences in bacteria at the Genus level correlated with known biomarkers of AD. Cerebrospinal Fluid (CSF) from lumbar punctures was collected from a total of 40 participants (AD=9 and HC=31; 21 of the

HC participants were not part of the original sample study). Biomarkers from the CSF included Aβ42/Aβ40 (amyloid burden), phosphorylated tau (p-tau; a marker of neurofibrillary tangles), the ratio of p-tau/Aβ42 (a marker of the level of AD pathology), and chitinase-3-like protein (YKL-40; a marker of astroglial/microglial activation). The analysis revealed notable trends between the microbial DNA from fecal samples and CSF biomarkers (Vogt et al., 2017).

Positive correlation between the abundant bacteria in AD patients and levels of p-tau and p-tau/Aβ42 in CSF were found (Vogt et al., 2017). These correlations were particularly strong for the Bacteroides and Blautia strains. In other words, the higher the levels of these strains, the higher the CSF biomarkers of AD pathology. Similarly, a negative correlation was reported between less abundant bacteria in AD patients and increased levels of YKL-40, with the correlation being particularly strong for the SMB53 and Dialister strains. Thus, the higher the levels of these strains, the lower the levels of YKL-40 in the CSF and vice versa (Vogt et al., 2017).

This is one of the first studies investigating the relationship between gut microbiota and AD pathology. Vogt et al. (2017) found differences in microbiota composition between AD participants and healthy controls at different phylogenetic levels. Additionally, they explored how these differences in bacterial composition were related to known CSF biomarkers, with clear correlations between the abundance (or depletion) of certain strains and the levels of biomarkers in CSF (Vogt et al., 2017). Nonetheless, while these results evidence a link between gut microbiome composition and AD pathology, it does not show a causal relationship between one and the other. Future research needs to investigate further these differences and their significance with larger, more diverse samples.

A similar study by Zhuang et al. (2018) attempted to replicate these findings. Bacterial 16S rRNA gene sequencing was performed on fecal samples from 86 participants (AD=43; normal controls [NC]=43) who were also matched in sex and age. Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria were the dominant bacteria. Their analysis at different phylogenetic levels also found differences between AD and control groups beginning at the phylum level, with a mild decrease in the abundance of Bacteroidetes and Actinobacteria observed among AD patients. Differences were also noted between the HC group and the AD group at the Class, Order, and Family levels, with some strains being increased and some strains being decreased (Zhuang et al., 2018). Refer to Table 2 for detailed strains found to differ in the AD group.

Table 2

Strains Found to Differ Between Normal Control Group and AD Group.

Phylogenetic Level	Increased Strains	Decreased Strains
Phylum		Bacteroidetes
		Actinobacteria
Class	Actinobacteria	Negativicutes
	Bacilli	Bacteroidia
Order	Lactobacillales	Bacteroidales
		Selenomonadales
Family	Ruminococcaceae	Lanchnospiraceae
	Enterococcaceae	Bacteroidaceae
	Lactobacillaceae	Veillonellaceae

Note. Adapted from "Gut Microbiota is Altered in Patients with Alzheimer's Disease," by Zhuang, Z. Q., Shen, L. L., Li, W. W., Fu, X., Zeng, F., Gui, L., ... & Zheng, P, 2018, *Journal of Alzheimer's disease*, *63*(4), 1337-1346.

Although Zhuang et al. (2018) had a larger sample, their results only show a relation between the gut microbiome and AD pathology, but its implications remain unclear. Additionally, their analysis did not include a comparison to other biomarkers (i.e., CSF or blood plasma), limiting the conclusions drawn from their results. Moreover, different bacteria at different levels were found between the study by Zhuang et al. (2018) and the study by Vogt et al. (2017). This difference may be due to differences in participants' geographical location and diets (Vogt's study was done in Wisconsin while Zhuang's study was done in China). Another factor might be ethnic/racial differences between the samples, but unfortunately, neither researcher specified their sample's racial breakdown. While race and ethnicity might or might not be an influential factor, the inclusion of diverse populations in future research is crucial for generalizing conclusions across populations.

Another study by Cattaneo et al. (2017) sought to explore an association between brain amyloidosis, gut microbiome strains, and inflammatory markers. Blood and stool samples were collected from a total of 83 participants (Healthy Controls [HC]=10; Cognitively Impaired Amyloid positive [Amy+] = 40; Cognitively Impaired Amyloid Negative [Amy-]=33). Participants underwent a comprehensive clinical assessment to establish their level of cognitive functioning and amyloid status. Clinical assessment included a physical and a neurological examination. Neuropsychological examinations included assessments for the domains of global cognition, memory, language, visuospatial/constructional, executive functioning, non-verbal reasoning, and upper limb apraxia. Lastly, participants also had an amyloid positron-emission tomography (PET) scan done to detect the participants' amyloid status (Cattaneo et al., 2017).

Microbial DNA from stool samples was analyzed for specific bacteria known to be inflammatory and abundant in other neurological conditions (Escherichia/Shigella and P.

aeruginosa; Cattaneo et al., 2017). Anti-inflammatory bacteria known to be depleted in individuals with other neurological disorders were also analyzed (E. rectale, E. hallii, F. prausnitzii, and B. fragilis). Likewise, blood samples underwent gene expression analyses for the presence of pro-inflammatory cytokines related to peripheral inflammatory state (CXCL2, CXCL10, IL-1b, IL-6, IL-18, IL-8, NLRP3, TNF-a), as well as known anti-inflammatory cytokines (IL-4, IL-10, and IL-13). Their analyses indicated that compared to the other conditions, Amy+ participants had a significantly higher abundance of the pro-inflammatory strain Escherichia/Shigella and higher levels of pro-inflammatory cytokines IL-6, CXCL2, NLRP3, and IL-1b (Cattaneo et al., 2017).

Additionally, the abundance of Escherichia/Shigella strains had a positive correlation with pro-inflammatory cytokines IL-1b, CXCL2, NLRP3 (Cattaneo et al., 2017). In other words, the higher the abundance of these pro-inflammatory bacteria in stool samples, the higher the levels of pro-inflammatory cytokines in blood samples, indicating a possible relationship between gut microbiome dysbiosis and peripheral inflammation. Moreover, anti-inflammatory strain E. rectale negatively correlated with pro-inflammatory cytokines IL-1b, CXCL2, and NLRP3. Thus, the higher the abundance of this anti-inflammatory strain in stool samples, the lower the inflammatory cytokines in stool samples (Cattaneo et al., 2017).

All in all, Cattaneo et al. (2017) demonstrated a relationship between the gut microbiome and peripheral inflammation. Their study had robust diagnostic and exclusion criteria, including PET scan, neuropsychological evaluation, and complete clinical assessment. Nonetheless, Cattaneo et al. (2017)'s study had some significant limitations. Firstly, the researchers did not collect information or control for differences in participants' dietary habits, which is a known factor to impact microbiome composition. Furthermore, although this is one of the first studies that examined the relationship between the gut microbiome and peripheral inflammation, this study had a small sample size and did not report on patients' race or ethnicity. Future research will benefit from the inclusion of larger, more diverse samples (Cattaneo et al., 2017).

Zhao, Cong, and Lukiw (2017a) conducted a series of studies to explore the role of strains of gram-negative bacteria as possible pro-inflammatory bacteria and their potential impact on the brain. Zhao et al (2017a) explained that gram-negative bacteria have an outer layer made up of Lipopolysaccharide (LPS), which serves as a protective barrier from the environment. Under conditions of dysbiosis, gram-negative bacteria secrete LPS into the environment, irritating the GI tract epithelia, and inducing a pro-inflammatory immune response. The peripheral inflammation response then leads to a weakened gut barrier and BBB, as was previously mentioned in the Inflammatory Hypothesis of AD section. Therefore, bacteria and their metabolites (including bacterial-made amyloid and other neurotoxic molecules) roam in the peripheral system and CNS, creating a cascade of consequences. *Bacteroides fragilis* (*B. fragilis*) and *Escherichia coli* (*E. coli)* are two gram-negative bacteria that are highly abundant in the human gut and are known to produce highly inflammatory and neurotoxic metabolites (Zhao, Cong, & Lukiw, 2017a).

To examine whether bacterial LPS is, in fact, able to seep into the CNS, Zhao and colleagues (2017a) did an immunohistochemical analysis of human brain tissue samples of the temporal lobe neocortex (HC=6; AD=6) and hippocampus (HC=2; AD=4). Samples were matched by age and gender, and no significant differences were noted between groups. Their results indicate that LPS was on average twice as abundant in all samples of temporal neocortex tissue of AD brains. Likewise, LPS was found to be three times as abundant in three of the four samples of hippocampus tissue of AD donors, with one advanced AD case having a 26-fold increase in LPS. This is the first study ever to report the presence of bacterial LPS in human brains with AD. Researchers conclude these findings to support the Inflammatory Hypothesis of AD, indicating

that bacterial LPS can leak through the gut barrier and BBB and is found at disproportionally higher rates in brains with AD pathology (Zhao et al., 2017a).

A follow-up study by Zhao, Cong, Jaber, and Lukiw (2017b) sought to replicate the findings of the previous study by doing an immunohistochemical analysis on human brain tissue of the temporal lobe neocortex (HC= 4; AD=4) and hippocampus (HC=3; AD=3). Additionally, researchers assessed for localized accumulation of LPS in the tissue samples by using DAPI staining. Their results indicated LPS was seven times more abundant in the neocortex of the temporal lobe of AD samples and 21 times more abundant in the hippocampus of AD samples. Additionally, researchers found that 75% of LPS in AD samples was clustered around neuronal cell nuclei. Researchers concluded that LPS might play a role in the neuronal degeneration observed in AD brains (Zhao, Cong, Jaber, & Lukiw 2017b).

In order to assess the possible role of LPS in AD pathology, Zhao, Jaber, and Lukiw (2017c) replicated the previous studies by analyzing temporal neocortex tissue (HC=12; AD=15). Similar to the previous study, an Immunohistochemical analysis and DAPI staining were conducted with the tissue samples. Additionally, a run-on gene transcription on human neuronal-glial (HNG) cells in co-culture incubated with LPS (2.5 weeks) was used to assess the capacity of production of messenger RNA (mRNA). An mRNA molecule is an RNA strand that is complementary to a DNA strand of a gene. It is created in the nucleus of cells and exits into the cytoplasm, where a ribosome "reads" it and makes specific proteins. Like the previous study, Zhao et al., (2017c) found approximately 90% of LPS clustered around nuclei in AD brain tissue. Run-on transcription analyses revealed a significantly reduced output of mRNA. Zhao et al. (2017c) conclude that LPS may contribute to the degeneration of brain cells by blocking mRNA from exiting the cells' nuclei, which represses transcriptional activity, disrupting the read-out of genetic information (Zhao, Jaber, & Lukiw, 2017c).

The studies by Zhao et al. (2017a, 2017b, 2017c) are one of the first studies to explore the possible role the gut microbiome plays in AD pathology. Their results show gram-negative bacteria LPS in human brain samples, which disproportionally accumulate in brains with AD compared to control samples. Moreover, LPS was found to cluster around cell nuclei, and run-on transcription demonstrated reduced mRNA output in the samples. These results may allude to how LPS contributes to neuronal degeneration in AD brains and opens the door for new research to investigate further the link between bacterial metabolites and AD pathology (Zhao et al., 2017a; Zhao et al., 2017b; Zhao et al., 2017c). Nonetheless, to be able to generalize results, future research should be cognizant of having larger, diverse samples and detailing participants' full demographic data.

On a similar note, an earlier study by Riviere, Riviere, and Smith (2002) sought to explore the role of oral bacteria and the onset of AD. According to Riviere et al. (2002), early literature shows a possible relationship between oral health and AD pathology. Thus, to determine whether *Borrelia burgdorferi* or any of seven species of oral *Treponema* could be detected in the human brain, Riviere et al. (2002) conducted a series of studies comparing tissue samples from donors who had AD and from non-AD controls. For the first study, frontal lobe cortexes from 34 donors (AD=16; HC=18) underwent molecular and immunological testing for the presence of *Borrelia burgdorferi* or *Treponema*. Researchers indicated the immunological method assessed for the presence of species-specific antigens in the tissue samples, while the molecular testing procedure tested for the presence of actual bacterial RNA (Riviere, Riviere, & Smith, 2002).

Using both the molecular and the immunological techniques, B. burgdorferi was found to be present in both groups (AD=5/16; HC=1/18; Riviere et al., 2002). Similarly, six of the seven

types of *Treponema* species (were found in both groups using the molecular techniques (AD= 14/16; HC=4/18). The immunological technique also found *Treponema* to be present in both groups. However, this method was more sensitive than the molecular as it detected antigens for three additional donor samples (AD=14/16; HC=7/18). To assess if *Treponema* was able to reach the CNS through the trigeminal brain, the second study examined frozen trigeminal ganglia from five donors (AD=3; Control= 2), using the molecular method. Results revealed that all five samples contained different *Treponema* species, with some donors having multiple species (Riviere et al., 2002).

To further assess the presence of *Treponema* in AD brains compared to healthy controls, the third study examined tissue from trigeminal ganglia, pons, and hippocampus of four donors (AD=1; Control=3) using the immunological method (Riviere et al., 2002). Antigens for Treponema were found in the trigeminal ganglia, pons, and hippocampus of the AD donor and the trigeminal ganglia and pons of the three control donors. Lastly, to explore the possibility of differences in Treponema prevalence in live individuals, the fourth study collected saliva from 33 live participants (AD=17; Control= 16). Molecular testing was done after collection and repeated after the saliva was cultivated for seven days to amplify for viable *Treponema*. The analysis did not reveal a significant difference between groups, either pre-or post-cultivation. Thus, the presence of *Treponema* in live patients does not necessarily relate to AD pathology (Riviere et al., 2002).

While Treponema and B. burgdorferi were present in the frontal lobe cortex of both groups, the AD group had significantly higher rates and higher diversity of Treponema species compared to healthy controls (Riviere et al., 2002). Moreover, Treponema was also present in other brain structures (trigeminal ganglia, pons, and hippocampus) of donors from both groups. This is evidence that oral bacteria are able to invade the CNS. Furthermore, Treponema was present in trigeminal ganglia samples, suggesting that oral bacteria may reach the CNS through the trigeminal nerve (Riviere et al., 2002). Nonetheless, while these results evidence that oral bacteria can be present in the CNS, the role it plays in AD pathology is unclear.

Overall, the research studies discussed above add essential value to the existing AD literature. While these are some of the first studies to explore the relationship between microbiome and AD pathology, it is clear that there is a long way before meaningful results solve the mystery that is AD. Importantly, future research needs to have larger sample sizes and to diversify the samples. This goes beyond demographic characteristics of age and sex (which all studies reported). Researchers need to recruit racially and ethnically diverse samples purposely to be able to generalize results reliably. This is of particular importance as the existing literature has disproportionally studied White individuals, leaving important racial characteristics unexplored. For instance, it is known that Black and Hispanic individuals are disproportionally more likely to develop AD pathology. However, genetic or sociocultural factors are largely unexplored. For instance, differences in diets or geographic locations of residence (including factors such as access to clean water or balanced meals) can significantly impact the gut microbiome.

Furthermore, while microbiome research is promising, there is also ample criticism. As mentioned throughout this review, most researchers have relied on 16s rRNA sequencing to assess for bacterial DNA in their samples. According to Hanage (2014), however, this method is better at distinguishing bacteria at higher taxonomic levels but loses accuracy at lower, more specific, taxonomic levels. Therefore, while 16s rRNA sequencing shows differences between AD groups and controls, it is not specific enough to reveal significant differences between groups. Thus, other methods need to be explored for research to produce meaningful results (Hanage, 2014).

Another vital factor delineated by Hanage (2014) is that all of the research so far only shows correlations but is far from showing causation. Consequently, interpretations from these studies must be taken with a grain of salt. Likewise, while the research shows correlations, there is no clear-cut evidence (yet) that supports a specific model of AD pathogenesis. Moreover, while research studies tend to be highly controlled (i.e., excluding participants with comorbid disorders), the generalizability of these results is questionable in real-life cases. Furthermore, while this research attempts to solve AD's mystery from an entirely new perspective, Hanage (2014) questions how much of it is hype. Lastly, an essential factor to consider is the bidirectionally of the gut-microbiota-brain-axis. Thus, it is important to question if gut dysbiosis is responsible for disease states or if dysbiosis results from disease states (Hanage, 2014). Unfortunately, there are currently far more questions than answers when it comes to AD.

Implications of Microbiome Research and Alzheimer's Disease

While the literature regarding microbiome and AD is still in its infancy, there are several potential implications for the future. Firstly, if research supports that the microbiome has a causal role in AD pathology (as in the extrinsic model of the inflammatory hypothesis of AD), the way AD is studied, understood, and conceptualized can change tremendously. AD could go from being considered a purely neurological disorder to a systemic disorder. Hence, in practice, this would push for a network medicine approach with a team of medical professionals that could include immunologists, gastroenterologists, endocrinologists, neurologists, neuropsychologists, and even dietitians and nutritionists. Moreover, microbiome research could potentially open the door for treatments aimed at preventing or even reversing the effects of AD.

Presently, the available treatments for AD deal with some of the symptoms but provide no actual cure for the disease. Moreover, AD is diagnosed in life based on the symptoms observed/reported and changes in cognition. However, this is a provisional diagnosis, and confirmed AD pathology is only done postmortem after autopsy. Thus, misdiagnosing or underdiagnosing is a serious problem in the field today. If microbiome research can potentially identify specific strains responsible for AD pathology, fecal samples could be used to look for biomarkers. Importantly, since gut dysbiosis has been linked to several diseases, fecal sample screeners could become standard practice and allow for the early detection of AD and many other disorders and could also aid in the rule-out process.

In AD, early detection could significantly change an individual's prospects, especially if treatments are developed to slow or reverse the effects of AD. It is known that AD pathology is present for several years before symptoms are apparent. Thus, early detection could potentially save millions of dollars to the nation and could provide financial relief to individual families who care for family members affected by AD, as well as increase the quality of life for the patient.

Early Research for Potential Treatments of Alzheimer's Disease

Assuming that the microbiome has a causal role in the pathology of AD, it could be presumed that influencing the gut microbiome would impact AD pathology. Akbari et al. (2016) conducted a double-blind research study to assess if probiotic supplementation would improve cognitive and metabolic functioning in AD patients. Participants were matched for disease severity, gender, BMI, and age. The criteria from the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association (NINDS-ADRDA) were used to establish AD diagnosis. As part of the exclusion criteria, participants could not have other diagnoses of metabolic disorders or chronic infections, and no

participants taking probiotic supplementation in the last six weeks were allowed to participate. A total of 60 AD patients (probiotic=30; Control=30) participated in this study (Akbari et al., 2016).

For the intervention, the probiotic group received probiotic supplementation for 12 weeks (Akbari et al., 2016). Participants were supplied with 200 ml/day probiotic milk containing a solution with the following strains: Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum, and Lactobacillus fermentum. The control group received the same amount of milk daily without a probiotic solution. While there are no specific known strains that aid AD pathology yet, the strains selected were chosen based on literature suggesting they have anti-inflammatory and health properties on humans. Moreover, the Mini-Mental Status Examination was administered pre-and post-intervention as a measure of global cognition. Additionally, blood samples were collected after 12 hour fasting periods pre-and post-intervention as well. Blood samples were assessed for biomarkers of oxidative stress (total antioxidant capacity [TAC], Total glutathione [GSH], Malondialdehyde [MDA], and Plasma nitric oxide [NO]), inflammation (High sensitivity C-reactive protein [hs-CRP]), and Metabolic functioning (fasting plasma glucose [FPG], Triglyceride [TG], Cholesterol [LDL, and HDL], Insulin resistance [HOMA-IR], B-Cell function [HOMA-B], and Quantitative insulin check [QUICK]; Akbari et al., 2016).

Comparison of pre-and post-intervention assessments revealed significant differences between groups (Akbari et al., 2016). MMSE scores were observed to improve in the Probiotic group (+27.90% ± 8.07) after 12-week probiotic supplementation, while they had a slight decrease in the Control group (−5.03% ± 3.00, $p<0.001$). Additionally, the probiotic group had decreased levels of HOMA-IR (+28.84% ± 13.34) compared to Controls (+76.95% ± 24.60, $p = 0.002$). The Control group also had higher levels of HOMA-B (+75.62% ± 23.18) compared to the Prebiotic group (+3.45% ± 10.91), while the QUICKI levels increased in the probiotic group (−1.83% ± 1.26) compared to the Control group (−4.66% ± 1.70). Furthermore, Triglyceride levels were observed to be decreased in the probiotic group (−20.29% ± 4.49mg/dl) compared to the Control group (−0.16% ± 5.24mg/dl; Akbari et al., 2016).

Overall, HOMA-IR, HOMA-B, and QUICKI are markers of insulin resistance and insulin sensitivity (Akbari et al., 2016). These results suggest that the Probiotic group had improved metabolic function after probiotic supplementation (Akbari et al., 2016). Nonetheless, there were no statistically significant differences between groups for FPG, LDL, and HDL biomarkers. The biomarker for inflammation (hs-CRP) was also found to differ with a statistically significant decrease for the Probiotic group (−17.61% ± 3.70) compared to the control group (+45.26% ± 3.50 μg/ml). For the biomarkers of oxidative stress, only MDA was found to have a significant difference between groups, with a significant decrease in the Probiotic group (−22.01% ± 4.84 μmol/l) compared to the Control group (+2.67% ± 3.86 μmol/l). There were no statistically significant differences for the other markers of oxidative stress (TAC, GSH, and NO; Akbari et al., 2016).

The study by Akbari et al. (2016) was one of the first to explore the possibility of probiotic supplementation as a treatment option for AD. After 12 weeks of probiotic supplementation, results showed improved performance in a cognitive measure, better metabolic functioning, and decreased inflammatory biomarkers for the Probiotic group compared to the Control group. Nonetheless, there are some significant limitations to consider. For instance, the MMSE was the only measure used to assess global cognitive functioning. This measure is a screener for cognitive functioning but does not encompass the depth of the many cognitive domains impacted by AD. Thus, future research will benefit from utilizing more ample neuropsychological batteries to better

assess the changes in cognition and specific cognitive domains impacted by such interventions. Future research should also analyze fecal samples pre-and post-intervention to assess the actual changes observed in participants' gut microbiome. Furthermore, considering the newness of such intervention, future research should include follow-up assessments (6-12 months or longer) to evaluate if results are still present months or years after the intervention or if supplementation needs to be repeated.

A similar study by Ton et al. (2020) also examined if probiotic supplementation impacted the metabolic and cognitive performance of patients with AD. A total of 13 participants diagnosed with AD were included in this study. Researchers followed diagnostic criteria set by the Alzheimer's Association and National Institute of Aging. Moreover, they had extensive exclusion criteria, including no neurological or psychiatric comorbidities, no metabolic or autoimmune disorders, no irritable bowel syndrome, and no current use of immunosuppressant medication or antibiotics. Participants were supplemented for 90 days with 2mL per kilogram of weight of fermented milk with Kefir Grains with the following strains of bacteria: *Acetobacter aceti, Acetobacter sp., Lactobacillus delbrueckii delbrueckii, Lactobacillus fermentum, Lactobacillus fructivorans, Enterococcus faecium, Leuconostoc spp., Lactobacillus kefiranofaciens, Candida famata, and Candida krusei.* According to the researchers, Kefir grains have been shown to have pro-health effects on several conditions, including gastric ulcers, cardiac and vascular dysfunction, and atherosclerosis (Ton et al., 2020).

Blood samples were also collected pre-and post-intervention (Ton et al., 2020). Samples were assessed for the presence of oxidative stress biomarkers (Quantification of Reactive oxidative species [ROS], Superoxide anion [O2-], hydrogen peroxide [H2O2], peroxynitrite/hydroxyl radical [ONOO- /OH-], nitric oxide [NO], and Advance Oxidation Protein Product [AOPP]), and biomarkers of inflammation (Pro-inflammatory: IL-6, IL-8, IL-, 1b, IL-12p70, and TNF-α; Anti-inflammatory: IL-10). Additionally, a cognitive battery was administered pre-and post-intervention as well. Cognitive domains included Global cognition (MMSE), Memory (Immediate and delayed; Recall board with ten concrete objects.), Visuospatial and Abstraction abilities (Similarities test and Cookie theft test [part of the Boston diagnostic aphasia examination]), Executive and language function (Boston Naming Test and Verbal fluency test), Constructive abilities (Clock drawing test), and Attention (Trail Making Test; Ton et al., 2020).

Results indicated an overall improvement in cognitive performance on all domains assessed (Ton et al., 2020). Refer to Table 3 for a summary of the percentage increase in scores of cognitive assessments pre- and post-supplementation. Moreover, some pro-inflammatory cytokines were decreased post supplementation (TNF-α, IL-8, and IL12p70). There was, however, no difference in other pro-inflammatory (IL-1b and IL-6) or anti-inflammatory cytokines (IL-10). Additionally, there was a decrease in most oxidative stress biomarkers (O2-, H2O2, ONOO- /OH-, AOPP), except for NO, which had an increase (Ton et al., 2020).

Table 3

Percentage of Increase in Cognitive Performance of Assessments Pre- and Post- Probiotic Supplementation.

Domains	Assessments	% increase pre- and post-supplementation ($p<0.05$)
Global Cognitive Functioning	MMSE	28%
Memory	Immediate Memory	~66%
	Late Memory	~62%
Visual-Spatial and Abstraction Abilities	Similarities	~2-fold
	Cookie Theft picture	~2-fold
Executive and Language Functions	Boston Naming Test	~30%
	Verbal Fluency Test	~25%
Constructive Abilities	Clock Drawing test	~46%
Attentative Function	Trail Making Test	40%

Note. Adapted from "Oxidative Stress and Dementia in Alzheimer's Patients: Effects of Synbiotic Supplementation," by Ton, A. M. M., Campagnaro, B. P., Alves, G. A., Aires, R., Côco, L. Z., Arpini, C. M., ... & Vasquez, E. C., 2020, *Oxidative Medicine and Cellular Longevity*, *2020*, NA-NA.

The study by Ton et al. (2020) demonstrated that probiotic supplementation influenced cognitive functioning and inflammatory and oxidative stress biomarkers in patients with AD. Nonetheless, there are some limitations to consider. This study was not a controlled trial and did not have a control group for comparison. Moreover, the small sample size and lack of information on patients' demographics make it difficult to generalize results. Furthermore, while the researchers stated following guidelines for neuropsychological testing appropriate for a Brazilian population (where the study was conducted), it is unclear how the cognitive domains were established and how each domain's assessments were chosen.

Lastly, a case study by Hazan (2020) details the case of an 82-year-old patient who presented with a recurrent Clostridium difficile infection (CDI), which was causing symptoms of diarrhea, abdominal pain, and fever. The patient had been through multiple antibiotics courses, yet symptoms continued to recur, and stool samples continued to test positive for CDI. Additionally, the patient had been diagnosed with AD, showing a progressive cognitive decline for the past five years. Hazan (2020) reported that the patient's most recent MMSE score was a 20/30, which is

considered to be in the moderately impaired range. Moreover, previous neuropsychological testing revealed significant deficits in memory, learning (nonverbal), attention, and response inhibition (executive functioning). Lastly, the patient's wife reported behavioral changes such as no longer enjoying socializing, flattened affect, confusion, memory loss, and that he required assistance for activities of daily living such as taking his medication, preparing food, and bathing (Hazan, 2020).

The patient underwent a Fecal Matter Transplant (FMT) procedure primarily for the treatment of the recurrent CDI (Hazan, 2020). A single 300 mL FMT infusion was performed from the patient's 85-year-old wife, who presented cognitively intact and was healthy. According to Hazan (2020), FMT has shown promising effects in mice models of AD, and results indicate significant improvement in cognitive functioning. Moreover, FMT has also been studied in humans for other disorders, including gastrointestinal disorders and even neurodegenerative and neurodevelopmental disorders (Hazan, 2020).

Immediately after surgery, the patient and his wife reported a complete absence of CDI symptoms. Two months post-surgery, a stool test was negative for the presence of CDI. At a two-month follow-up visit, the patient was re-administered an MMSE and scored a 26/30, which is considered to be within the normal cognitive functioning range. Moreover, the patient's wife reported improvement in his affect and felt like he had also improved mentally. At a four-month follow-up visit, the patient and wife continued to report memory improvement and indicated he was now able to remember important dates and better recall past experiences. Lastly, at a six-month follow-up, the patient was re-administered the MMSE and scored 29/30, which is within the normal cognitive functioning range. The patient self-reported improved mood and was described by the researcher as "more interactive, and showed more expressive affect" (Hazan, 2020, p. 3).

The results from this case study by Hazan (2020) show tremendous improvement in cognitive functioning in a patient diagnosed with AD after an FMT. Importantly, cognitive performance continued to improve at two, four-, and six-months follow-ups. While this is the first study to document the effects of FMT on AD, it is necessary to conduct a controlled clinical trial to better study FMT's effects on cognition on a representative sample of the population. Furthermore, a significant limitation to consider is that Hazan (2020) only used one measure to assess cognitive functioning. The researcher mentioned a previous neuropsychological battery administered to the patient; however, it was not specified how long before the FMT was the battery administered, or what assessments were included in the battery. While the patient's MMSE performance drastically improved from the moderately impaired range to the normal cognitive functioning range, future research needs to assess different cognitive domains to better understand how FMT impacts performance in different cognitive domains. Lastly, a fecal analysis for specific bacteria strains pre-and post-FMT would have been adequate to understand how the patient's microbiome was impacted after the FMT in terms of strains present and diversity of microbes.

Discussion

AD is one of the most common neurodegenerative disorders worldwide. It creates a tremendous psychological, emotional, and financial burden to patients and their families. Research thus far is extensive, particularly examining genetics, hormones, and even environmental and sociocultural factors (Alzheimer's Association, 2020; CDC, 2020; van der Lee et al., 2018; Pike, 2017; Merlo et al., 2017; Mielke et al., 2014; Fratiglioni & Wang, 2007). Nonetheless, AD's pathogenesis is still an elusive mystery, as the most accepted model of AD (the ACH; Hardy & Higgins, 1992) is not fully supported by research. A new shift in the literature attempts to study

AD differently by assuming that inflammation is the driver of pathology (Inflammatory Hypothesis) rather than the amyloid protein plaques themselves (Herrup, 2015; Olsen & Singhrao, 2015).

An exciting new area of research is the possible role that gut bacteria play in AD pathogenesis. The microbiome has been severely underestimated and understudied, as it was considered that its only function was to aid in digestion. Nonetheless, research shows that the microbiota may play a role in human development (Blum, 2017; Sochocka et al., 2019; Principi & Esposito, 2016; Mayer et al., 2015; Dinan & Cryan, 2017). The literature also supports a link between gut dysbiosis and many disorders, including psychiatric, neurologic, and metabolic conditions (Blum, 2017; Sochocka et al., 2019). Unfortunately, most research (for AD and microbiome) heavily relies on mice models due to ethical implications. Consequently, it is difficult to generalize those results to humans (Hanage, 2014). The present review focused on research studies with human subjects to explore the possible link between the gut microbiome, dysbiosis, and AD pathology. Considering the novelty of this area of research, the existing literature is limited.

Research studies comparing microbiota from fecal samples from AD patients versus healthy controls found differences in strains between groups at different taxonomic levels, with some strains being increased while other strains were decreased in the AD groups (Vogt et al., 2017; Zhuang et al., 2018, Cattaneo et al., 2017). Notably, some of the increased strains in AD groups were known to be inflammatory bacteria, while some of the decreased strains were anti-inflammatory bacteria.

Additionally, one study found a positive correlation between increased pro-inflammatory strains and higher pro-inflammatory cytokines and a negative correlation between a higher abundance of anti-inflammatory strains and lower levels of pro-inflammatory cytokines in blood samples (Cattaneo et al., 2017).

Another series of studies sought to explore the role LPS from gram-negative bacteria (known to be pro-inflammatory) played in AD pathology (Zhao et al., 2017a; Zhao et al., 2017b; Zhao et al., 2017c). Samples of human brains were analyzed for the presence of LPS from healthy and AD donors (Zhao et al., 2017a). In a follow-up study, the samples were stained to assess how LPS localized within the brain (Zhao et al., 2017b). In a final third study, run-on gene transcription was conducted in order to assess if LPS had an impact on the production of mRNA (Zhao et al., 2017c). Results indicated that LPS was present at disproportionately higher rates on brain tissue samples of AD donors (Zhao et al., 2017a). Moreover, LPS was primarily found to cluster around cell nuclei, and run-on transcription revealed a significantly reduced mRNA output (Zhao et al., 2017a; Zhao et al., 2017b; Zhao et al., 2017c). These studies are the first to evidence the presence of LPS within the CNS. Additionally, the researchers demonstrated how LPS might contribute to neuronal degeneration by disrupting transcriptional activity (Zhao et al., 2017a; Zhao et al., 2017b; Zhao et al., 2017c).

Similarly, an earlier study by Riviere et al. (2002) analyzed brain tissue samples from healthy and AD donors to evaluate whether oral bacteria could invade the CNS in a series of studies. The samples were analyzed for the presence of *Borrelia burgdorferi* and *Treponema* by using immunological and molecular techniques. Results revealed the presence of both bacteria in the tissue samples from healthy and AD donors, with AD donors having higher instances of the bacteria (*Treponema* in particular) and higher diversity (several species per sample compared to control groups). Their analysis also found the presence of *Treponema* in trigeminal nerve tissue samples. Although the bacteria were found in both groups, Riviere et al. (2002)

evidenced that oral bacteria can, in fact, travel to the CNS. Moreover, their analysis suggests that oral bacteria may reach the CNS through the trigeminal nerve. Nonetheless, their studies did not explore the role, if any, that oral bacteria have in the pathology of AD (Riviere et al., 2002).

While these studies provide a foundation for microbiome research and AD, they have considerable limitations, including small, homogeneous samples. Additionally, the literature thus far shows correlations, but it is a long way from finding a causal link between microbiota and AD pathology. Therefore, research concerning therapeutic interventions that directly impact gut microbiota to reduce or reverse AD pathology is scant. Nonetheless, some pioneering studies attempt to analyze the impact of altering the gut microbiome and the effects on the symptoms of AD, including cognitive deficits.

Akbari et al. (2016) conducted one of the first studies investigating the impact of probiotic supplementation on AD patients. The participants completed the MMSE to assess for cognitive changes and provided blood samples pre-and post-intervention, which were analyzed for biomarkers of oxidative stress and metabolic functioning. After 12 weeks of supplementation, MMSE scores had significantly improved from pre-intervention in the probiotic group, while the control group's performance slightly worsened. Additionally, the prebiotic group also had decreased levels of some metabolic (HOMA-IR), inflammation (hs-CRP), and oxidative stress (MDA) biomarkers (Akbari et al., 2016). While this study shows improvement after probiotic supplementations in patients with AD, one of their main drawbacks was the lack of cognitive assessments to analyze the intervention's impact on different cognitive domains.

A similar study by Ton et al. (2020) also examined if probiotic supplementation with fermented milk from Kefir grains would reduce AD symptoms after 90 days of supplementation. This study also collected blood samples pre-and post-intervention and analyzed the samples for inflammation and oxidative stress biomarkers. Additionally, participants completed a full neuropsychological battery to explore the impact of probiotics on different cognitive domains (global cognition, memory, visuospatial and abstraction abilities, executive and language function, constructive abilities, and attention). After a 90-day supplementation, patients had significant improvement in performance in all domains assessed. Moreover, some pro-inflammatory cytokines (IL-1b and IL-6) and oxidative stress biomarkers (O2-, H2O2, ONOO- /OH-, AOPP) also had significantly decreased (Ton et al., 2020). While this study better explored the impact of supplementation on different cognitive domains, their sample was very small, and their study did not include a comparison group.

Lastly, a case study by Hazan (2020) studied the effects of FMT on an 82-year-old patient with a Clostridium difficile infection (CDI) and AD. At a follow-up visit after the procedure, the patient's CDI had entirely resolved. At a two month follow up, the patient's MMSE score had improved from the moderately impaired range (20/30) to the normal cognitive functioning range (26/30). At a four-month follow-up, the patient and his wife continued to report improvement in cognition and mood, and at a six month follow up, the patient's MMSE score showed continued improvement (29/30). Researchers concluded that FMT had a beneficial impact on the patient's AD symptoms (both cognitive and emotional) and resolved the ongoing CDI the patient initially presented with. While this case study showed improvement in the patient's cognitive performance over time (follow-ups at two, four, and six months), the researchers only used one measure (MMSE) to assess cognitive changes, significantly limiting the conclusions that could be drawn. Moreover, while this is a very promising start, future studies with much more robust and diverse samples are needed to examine the effects of FMT on AD pathology.

Limitations

The current literature review had some significant limitations to consider. Firstly, due to the limited research available, the theoretical literature far exceeds the research conducted to establish causality of pathology or possible therapeutic interventions. Thus, the conclusions drawn from this review are limited in terms of actual clinical applicability. Additionally, while there is existent international research that might have added substantial value to this review, there were studies with no translation available to be included. Moreover, the theoretical literature far exceeds the research conducted to establish causality of pathology or possible therapeutic utility. Thus, the majority of conclusions reached from these studies are based on the assumption that the theoretical portions are correct and should, therefore, be interpreted with a slight degree of healthy skepticism. Additionally, the lack of demographic data of participants available in the existent research makes it difficult to generalize to all individuals who are affected by AD. Lastly, due to the lack of cognitive assessment and follow-up measures in the existing studies, conclusions regarding the actual therapeutic potential to treat AD's cognitive symptoms remain limited.

Future Directions

While the research thus far is promising, it is scant and needs to address important limitations. Primarily, future research needs to actively strive to change the current status quo. As previously discussed, the existing literature is not mindful of recruiting diverse samples or providing and delineating the participants' demographic data beyond sex and age. Therefore, large scale studies are needed to have truly representative samples of the population. International collaboration is also necessary to compare results from different countries and explore racial, cultural, and social influences. While AD has been considered a purely neurological disorder for decades, it is impossible to ignore the environmental and sociocultural factors that may influence pathology. Specific to microbiome research, factors such as diet or access to potable water may have a tremendous impact on gut health.

As discussed throughout this paper, the notion of AD as a purely neurological condition is proving to be an outdated one. Therefore, future research can tremendously benefit from collaboration from different research specialties, which may be highly beneficial to understand the complexity that is AD. Additionally, considering the different specialties involved in this type of research and the need for international collaboration, researchers need to be mindful of using internationally recognized diagnostic criteria and thoroughly delineate diagnostic processes and assessments. Moreover, future research needs to include cognitive assessment data to be able to explore the impact gut health has on cognition and the potential effects of therapeutic interventions. Lastly, longitudinal and cross-sectional studies need to be prioritized to establish the long-term changes of the gut microbiome, the long-term effects on AD pathology, and the potential therapeutic utility of such interventions.

All in all, the work in this area of research is just beginning. Researchers need not only to collaborate internationally, but interprofessional collaboration is essential as well. It is clear that a crucial factor is time; for the advancement of research and technological strategies to refine research methods. Moreover, future research needs to emphasize causal relationships and move beyond correlational relations to explore the therapeutic potential of manipulating the gut microbiome and the underlying mechanisms of such interventions.

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