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The Correlation between the Microbiome and Neurodegenerative Disorders such as Alzheimer Disease

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THE CORRELATION BETWEEN THE MICROBIOME AND NEURODEGENERATIVE DISORDERS SUCH AS ALZHEIMER'S DISEASE

Honors Thesis

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Introduction

Recent studies suggest that there are 1.3 times as many microbial cells as human cells in the body (Abbot, 2016). Another study claims that the combined genomes of this microbiota is 150 times that of the human genome (Zhuang & Shen, 2018). If you have ever had the feeling that you are not alone in an empty room you are most certainly correct. Your body is teeming with millions of organisms living in mutualistic symbiosis with you and surrounding microbes, but at times, this seemingly harmonious relationship can be disturbed, resulting in serious physical and psychological changes to the human body.

This thesis will explore many of the proven and possible ramifications of the oral and gut microbiota's metabolic systems. Primarily, literature regarding the current understanding of the human microbiome will be explored. This includes studies that review common human microbiome compositions and various contributors to differing microbiomes such as geographical location and diet. Next, there will be a review of specific methods utilized by microbes to influence the human body. Benefits of homeostasis of the human microbiome will be described. Contrastingly, there is abundant literature that supports the notion that disorders of the GI tract and neurodegenerative disorders of the brain actually stem from microbiome dysbiosis defined as any deviation from homeostatic concentrations of bacteria. The possible use of probiotics, prebiotics, and fecal transplants will be considered as treatment for microbiome dysbiosis and related disorders. Finally, I will turn to a review of neurodegenerative disorders, with a focus on Alzheimer's (AD) and its biological processes. Then I will expand on the dysbiotic microbial influence on the pathogenesis of AD. The conclusion of this paper will consist of possible holistic preventative measures for microbial dysbiosis and AD.

The Microbiome

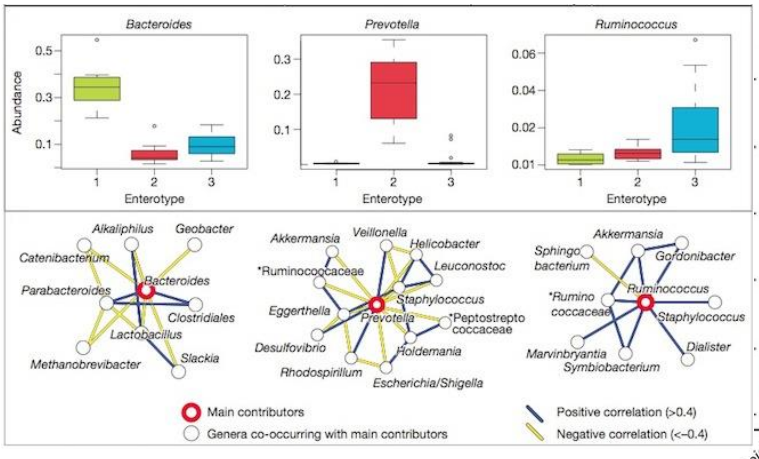
Introduction to the microbiome

In the past few years, scientists have begun to uncover the evolution and symbiosis of humans and microbes. Microbes include viral, bacterial, fungal, and archaeal organisms. The human microbiota is defined as the collection of roughly three pounds of microbial organisms found on and in the human body. The microbiome is the entirety of that microbial genomic code. The microbial proteome, specifically, is the microbial genes that are actually expressed and translated into proteins (Reid & Shannon, 2013).

What is considered a "normal" microbiome?

Only recently, scientists have begun to pay mind to the possible impact these microbes may have on animal health and disease. The study of metagenomics is made possible by novel sequencing techniques including high throughput sequencing such as Illumina. These novel techniques take a fraction of the time and effort to sequence large quantities of genetic data. This process usually involves a total extraction of genetic material from a sample (such as fecal or tooth plaque) followed by polymerase chain reaction (PCR) amplification of the 16S ribosomal RNA (or 16S rRNA) variable region. These amplified genes can then be sequenced through fluorescently labeled nucleotides that are analyzed and recorded using general electrophoresis and chromatography techniques. These data are then compared to other genomic sequences in rapidly growing bioinformatics databases (Alonso & Guarer, 2013).

The Human Microbiome Project was founded to orient future research by establishing a baseline or normal relative abundance of bacterial populations in the human microbiome. There are various distinct microbiomes in the human body that consist of characteristic bacterial communities such as the oral, gut, stomach, skin, and vaginal microbiome. Each microbial community can be further characterized by belonging to a different enterotype. Microbial enterotype is a classification of humans based on varying prominent bacterial concentrations. For instance, the three distinct enterotypes are based on a variation in concentration of one of three genera: *Bacteroides*, *Provetella*, and *Ruminoccocus*. Diagrams of these three enterotypes are shown in Figure 1 (Keim, 2011). This research is still in preliminary stages and far more research has to be done to provide supporting evidence of this form of gut enterotype classification.



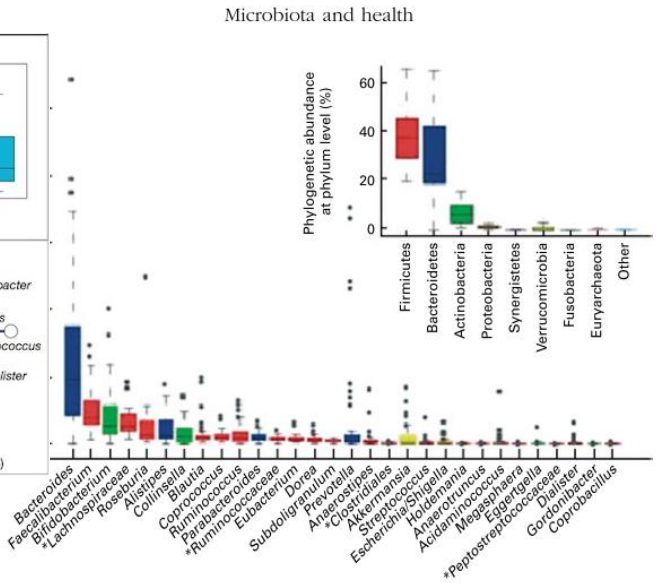


Figure 1

This figure shows the three most prevalent human gut microbiome enterotypes

Figure 2

Overall prevalence of various microbes in the human gut microbiome.

Nevertheless, a vast majority (more than 95%) of the gut microbes consist of *Firmicutes* and *Bacteroides* (Fig 2); (Alonso & Guarer, 2013). These two bacterial phyla are associated with digestion of carbohydrates, storing energy, and maintaining immune function.

Normal development of the human gut microbiome during one's life

The human microbiome varies within an individual's lifetime and is built in successive steps. In an infant, the gut is colonized by facultative anaerobes including *Streptococcus*, *Enterobacteriaceae*, and *Staphylococcus.* These microbes consume oxygen to create an anaerobic environment for strict anaerobes including *Clostridium, Bacteroides* and *Bifidobacteria*. After the first two years of life, the gut microbiome begins to increase in *Firmicutes* and resemble that of an adult microbiome. After a diverse adult microbiome is established, it is relatively stable and unchanging unless disrupted by a dramatic event (such as antibiotic regimens) (Oriach et al., 2016).

Gut Human Microbe Symbiosis and Homeostasis

Microbe host interaction

The gut microbiome interacts with the human host on a chemical signaling level and physical digestive level through metabolic intermediaries including cytokines, neurotransmitters, and short chain fatty acids (SCFA). The immune system also responds to microbial associated molecular patterns (MAMPs), which are molecules that are part of microbes including lipopolysaccharides (LPS) and peptidoglycan (PGS). The effect of these metabolites on the host can vary depending on the state of the host and the concentration of the metabolites (Minter et al., 2016).

Three principle microbial communication forms

1. **Blood**

Bacteria can communicate with the brain via blood circulation. This method is made possible by the breakdown of epithelial cell tight junctions in the gut, gums, and in the blood brain barrier. These tight junctions usually prevent the passage of water, bacteria and other metabolites across membranes. When these tight junctions are broken down, bacteria and bacterial metabolites (such as SCFA or cytokines) can more easily gain access to the blood and enter the brain. It is also important to note that because the mouth is closer to the brain, it is easier for bacteria to travel through the perforated gum lining to the brain relative to a leaky gut to the brain. Increased permeability of membranes in the body facilitate the influence of gut bacteria's communication to the brain leading to an inflammatory response of the central nervous system (CNS), which can lead to neural degeneration (Perez-Parado et al., 2017).

2. **Vagus nerve and the gut microbiome**

The vagus nerve stretches from the medulla oblongata in the brain stem to the gastrointestinal (GI) tract as an important modulator of the "brain-gut" axis. The nerve serves as a bidirectional chemical communication highway between the brain and the bacteria of the gut. Neurotransmitters produced by bacteria are able to access the brain using this "highway".

3. **Trigeminal and olfactory nerve and the oral microbiome**

The oral nasal cavity is innervated by the trigeminal nerve and the olfactory nerve that serve as direct highways for oral bacteria and associated metabolites to the brain. The bacteria start in the oral and nasal cavities and move through the olfactory nerve, the olfactory bulb, the perennial cortex (associated with facial recognition), and the hippocampus (associated with short

term memory). The Olfactory Hypothesis dictates that pathogens can enter the brain though the olfactory tract and increase production of amyloid plaques as well as neurofibrillary tangles. *Staphylococcus aureus* can travel to the olfactory bulb unimpeded regardless of immune mechanisms (Shoemark & Allen, 2014).

Microbial mechanisms in the human body

Gut microbes utilize these communication forms to perform a series of important jobs in the human body summarized in the following text: They facilitate immune function and promote the development of the immune system. By exposing the body to a variety of non-threatening microbes, the immune system tends not to overact to foreign bodies resulting in lower risk of autoimmune disease. A stable microbial community prevents the domination of the microbiota by another pathogenic bacterial strain (Sherwood, 2015). Microbes also aid in food digestion. For example obligate anaerobic gut microbes are responsible for metabolizing food substrate into energy, SCFA and other nutrients for humans; the human genome codes for fewer than 20 carbohydrate digestive enzymes. However, one strain of bacteria found in the human gut, *Bacteroides thetaiotamicron*, contains a genome encoding for 260 different carbohydrate digestive enzymes. Proof of the integral role of the microbiome on digestion can be seen in problems that arise in initial attempts to feed malnourished people. Starving people experience a depletion in gut microbial populations, as they provide no food or substrate for the microbes. If that starving person is given food, they will not gain substantial weight, nutrients or energy from that food until their gut microbiome has been reestablished (Reid & Greene, 2013). Recently, with the discovery of the influence of microbes on the brain, this topic is being thoroughly researched. Some SCFA are also integral in healthy acidification and regulation of the pH of the gut lumen to inhibit pathogenic invasion. *Firmicutes* produce the SCFA butyrate, which is

important in tissue maintenance because it provides nutrition and energy to the tissue. Butyrate is known to prevent leaky gut syndrome, which is caused by the breakdown of the epithelial cells in the cell wall that permits toxins, undigested food, and bacteria to leak into the bloodstream. Finally, SCFA treatment has been shown to ameliorate Irritable bowel disease (IBD) and colon cancer by preventing buildup of branched chain fatty acids (Alonso & Guarer, 2013).

Gut Microbiome Dysbiosis

What is dysbiosis?

Even though there is variance of microbial concentrations in the gut between individuals, the relative abundance of these bacterial species is generally constant throughout an individual's life. An imbalance of the microbes in one's gut can lead to a state of dysbiosis, which can dramatically increase the risk for a number of disorders. Dysbiosis can lead to a single organism dominating the microbiome or the drop in concentration of a single species whose functional role was integral to the maintenance of homeostasis in the human body. This change in bacterial relative abundance as well as the change in the bacterial metabolites present in the body can affect human metabolism on a physical and chemical level.

What causes dysbiosis?

Various environmental stressors can induce change in the relative abundance of microbes in the microbiome resulting in dysbiosis. These stressors include strong antibiotic regimens, access to clean water, general cleanliness, cesarean sections, reduced breastfeeding, access to animals or livestock, and dietary changes. Gut microbiome dysbiosis is most often caused by antibiotic treatment (especially broad spectrum treatments) that wipe out, indiscriminately, the

diverse population of the gut microbiota. These drugs are usually administered with intent to kill a single pathogenic bacteria, but result in the genocide of both "good" and "bad" bacterial species. This loss of diversity can accentuate dysbiosis instead of ameliorating it.

Alzheimer's Disease

Introduction to AD

Dementia is a set of symptoms such as poor memory and inhibited learning/cognitive functions that results from a physical degenerative cause. AD is one of the most common causes of dementia. AD induces neural death in the brain especially in the cortex and hippocampus where higher cognitive functions occur and long term memories are stored. Symptoms of AD are progressive. Initially AD may not be detectable. Short term memory loss is the first detectable indicator of AD, followed by loss of motor skills and language, long-term memory loss, disorientation, and death (Shoemark & Allen, 2014).

Mechanisms and pathogenesis of AD

One of the major causes of AD is the formation of beta amyloid plaques that build up around neural cells in CNS. Amyloid precursor proteins (APP) are imbedded in the neural membrane and are believed to assist in neural growth and repair. In a normal brain, alpha and gamma secretase enzymes cleave APP's and the products are solubilized and recycled by cerebral fluid. However, in AD patients, beta and gamma secretase enzymes are used to cleave the APP's instead, which results in insoluble monomers called amyloid beta peptides. The buildup of these monomers form beta amyloid plaques (Cheignon et at., 2018). Amyloid protein denaturation and buildup spreads from neuron to neuron relatively quickly. This cascading

spread of insoluble misfolded proteins characteristic to AD has been labeled as prion-like (Pistollato et al., 2016). These plaques cause many pathogenic effects such as disrupting the transmission of neural signals if they form in the synaptic cleft of two neurons. The plaque can also stimulate immune responses that catalyze cascading inflammation. Often, if an immune response is stimulated, CNS glial cells such as microglia (macrophages) and astrocytes (that help form a blood brain barrier (BBB)) can become overactive. Microglia can be activated when their toll-like receptors (TLR) recognize MAMPS. When there is a dysbiotic abundance of bacteria exhibiting a specific MAMP, microglia become overactive and they phagocytize, not only bacteria/foreign particulates, but also components important to effective functioning of the nervous system, such as neural synaptic terminals as well as myelin sheaths (Minter et al., 2016). Myelin sheaths are protein coats that wrap around the axon of neural cells to insulate and increase the rate signal transmission by inducing salutatory signal transmission. Without myelin sheaths, signal transmission is inhibited dramatically (Pistollato et al., 2016). In AD sites of inflammation, astrocytes are over-stimulated and release proinflammatory cytokines including: tumor necrosis factor alfa (TNFα), interleukin-1 beta (IL1β), and apolipoprotien E (ApoE). The malfunction of these factors are involved in amyloid β peptides (Aβ) fibrillation and cerebral oxidation (Shoemark & Allen, 2014, & Vogt et al., 2017) which contribute to pathogenesis of AD.

Correlative evidence shows that buildup of beta amyloid plaque leads to the second major cause of AD: the buildup of protein Tau tangles within neural cells. Tau proteins are located on microtubules within the cytoskeletal matrix of the axon of a neural cell. Under normal conditions Tau proteins help maintain the stability of microtubules. Microtubules are used as cellular ‘highways’ to transport nutrients into the cell body and waste out of the cell body. However, in

AD patients, buildup of amyloid beta plaques stimulate a kinase enzyme (p38 Mitogen-activated protein kinases or MAPK) that phosphorylates Tau proteins, causing the Tau proteins to fall off the microtubule and accumulate in clumps of neurofibrillary tangles. As Tau proteins fall, the integrity of the microtubule degrades and they are broken down. This results in breakdown of the neural highway, a halt in transmission of nutrients to the cell body, and eventual neural death. (Cheignon, et at., 2018)

Oxidative stress is also a major contributor to the pathogenesis of Alzheimer's disease. Chemical reactions occurring can produce volatile oxygen species that can react with, and cause degradation of surrounding tissues. Specifically, incomplete reduction of oxygen in the electron transport chain can produce oxygen radicals that are known to break down lipids, proteins, and nucleic acids. Oxygen radical species include superoxide, peroxide, and hydroxide. Carbonylated proteins are known to be indicators of protein oxidation. A study performed by Hensley and his laboratory found a buildup of carbonylated proteins in the hippocampus and cortex. These regions are the main sites of Aβ plaque buildup and tissue degradation characteristic of AD (Hensley et al., 1995). Also, lipid peroxidation is hypothesized to contribute to asymmetry and permeability in phospholipid bilayers of synaptic regions of neuronal membranes. Finally, the lipid peroxidation product: 4-hydroxy-2-nonenal (4-HNE), is known to cause oxidation and degradation of Tau proteins (Liu et al., 2005).

Alzheimer's classifications

There are two principle classifications of AD. The first major form of AD is familial or early onset Alzheimer's which is caused mainly by genetic factors. Familial AD is dictated by dominant gene mutations in Presenilin (PS), which is one of the four core proteins that compose the gamma secretase complex (Shoemark & Allen, 2014). Malfunction of the gamma secretase

enzyme due to a PS mutation can lead to increase in production and aggregation amyloid beta plaque. An experiment shows that murine test subjects with PS knockout have a significant buildup of Aβ plaque as well as cortical atrophy. After nine months of PS inactivation there was 24% cortical neuron loss and 35% cortical volume loss. They also noted memory loss and loss in synaptic plasticity due to N-methyl-D-aspartate (NMDA) receptor inhibition. NMDA receptor proteins located on the synaptic cleft of neurons bind to glutamate to increase calcium influx and signal transduction in neurons. Finally, they found PS inhibited mice had a decrease in cyclic adenosine monophosphate (cAMP) response element activity. This cAMP molecule is used in signal propagation and signal cascades (Shen and Kelleher III, 2007). Additionally, trisomy of the 21st chromosome can increase the risk of early onset AD because the APP gene is located on this chromosome. With a higher production of APP these people have a higher chance of amyloid beta plaque buildup. (Shen & Kelleher, 2007).

The second and most common form of Alzheimer's is sporadic or late onset. This form of AD is caused by mainly environmental factors and a few hereditary factors. With respect to the latter genetic links, the e4 allele of apolipoprotein E can increase risk of late onset AD. Normal allelic expression (not type e4) of apolipoprotein E results in the breakdown of amyloid beta. However, the e4 allelic version of this protein does break down amyloid beta tissue as well (Cheignon, et at., 2018). Environmental risk factors for CNS aggravation and AD pathogenesis include increased age (typically, people above the age of 60), diet high in fats and sugar and low in fiber, microbiome composition (as will be the focus in this paper) as well as other factors. Because sporadic AD is more common and is more likely to be affected by the environment, namely gut and oral microbial communities, this form will be the focus of this study and

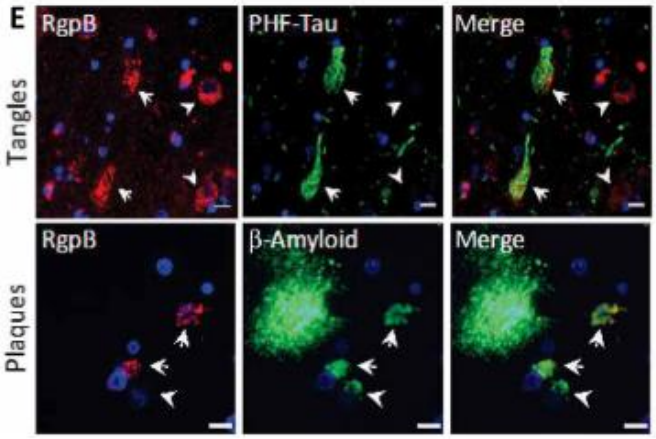
hereafter. Any discussion of AD is referring to the sporadic form unless otherwise noted (Cheignon, et at., 2018).

Effect of Oral Microbiome on AD

Bacteria found in the oral microbiome have been known to contribute to AD pathogenesis. The bacteria of the oral microbiome are located closer to the brain than the gut and therefore have a shorter distance to travel to affect the brain. These bacteria and their metabolites can pass from frequently aggravated gums into the blood stream and through the blood brain barrier. Various types of oral bacteria are correlated with ageing such as anaerobic bacteria. Some anaerobic strains of bacteria produce tumor necrosis factor alpha **(**TNFα) proinflammatory cytokine that has been known to contribute to the progression of AD. A major limitation of the study of the oral microbiota is that many strains of bacteria in the mouth are difficult to culture in laboratory or entirely uncultivable. This is mainly due two main factors: The first being there are many unknown obligatory symbiotic relationships between specific strains of bacteria that would have to be accounted for to grow that strain in culture. Metabolites such as growth factors (GF) provided by one species of bacteria may permit a second species to grow (Strandwitz et al., 2019). The second factor is that it is difficult to grow anaerobic bacteria in culture (Shoemark & Allen, 2014).

Dominy et al. (2019) found that AD murine models had a much higher concentration of *Porphyromas gingivalis*, an anaerobic bacterium known to cause periodontitis. In AD mice there were increased concentrations of *P.gingivalis*, which produces neurotoxic, proteolytic gingipian enzymes, which are needed for the survival of the bacteria and pathogenic effect on the host.

There are three types of gingipains produced by *P. gingivalis* including: lysine-gingipain (Kgp), arginine-gingipain A (RgpA), and arginine-gingipain B (RgpB). High concentrations of gingipains Kgp and RgpB directly correlated to build up of Tau tangles and increase in concentration of ubiquitin. Ubiquitin is an enzyme that is used in the production of APP's and correlates to a higher concentration of Aβ plaque deposition. Figure 3 superimposes a stain of cerebral RgpB buildup, a stain of Tau tangles, and a stain of Aβ plaque. It is clear that the protein aggregations directly correlate with the gingipain depositions. Figure 4 shows an increase in concentration of RgpB, Kgp, Tao, and ubiquitin load in AD patients compared to controls.



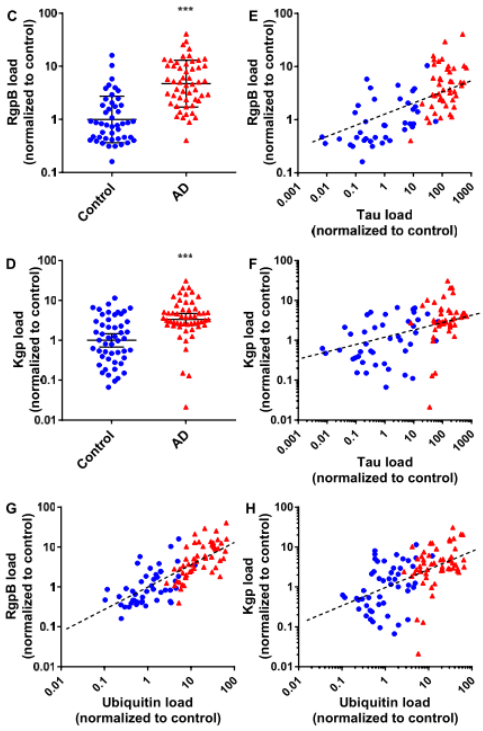


Figure 3

Cerebral RgpB build up causes Aβ plaque aggregation

Figure 4

C & D: show gingipain build up increased in Alzheimer mice

E & F: show tau load increase in AD mice

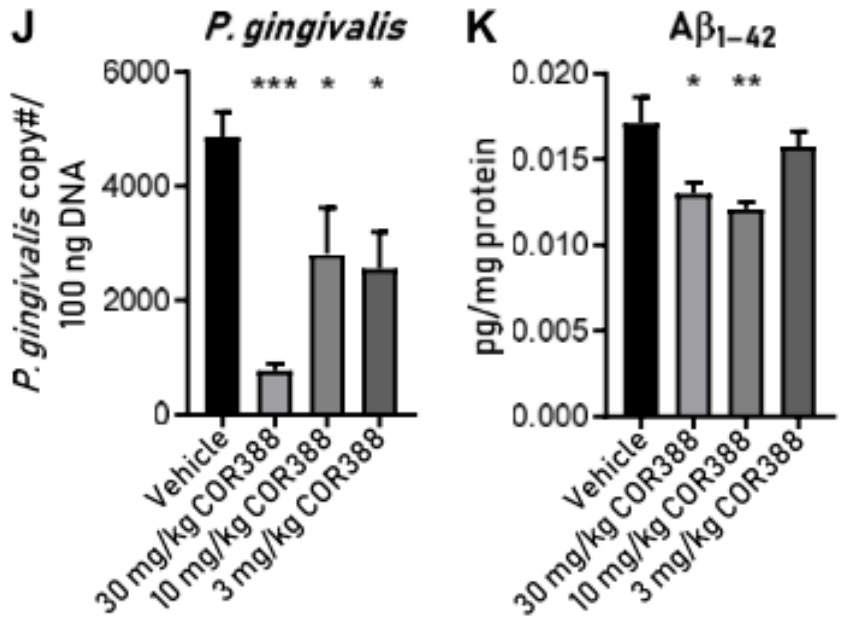
G & H: show ubiquitin build up (indicating Aβ plaque aggregation)

These results led to a follow up experiment where a short peptide narrow spectrum antibiotic called COR388 was designed to block Kgp proteolytic activity. Inhibition of Kgp led to a dramatic decrease in *P. gingivalis* in the oral microbiota and a correlative decrease in Aβ protein. The Diagram in Figure 5 shows a decrease of bacteria and Aβ protein build up with an increase in COR388 antibiotic (Dominy et al., 2019).

Figure 5

J: Higher concentrations of COR388 antibiotic lead to lower concentrations of *P.gingivalis*

K: Higher concentrations of COR388 antibiotic lead to lower concentrations of Aβ protein buildup



Effect of the Gut and Stomach Microbiome on AD

The human gut microbiota alone is composed of over 10^4 organisms including over 2000 bacterial species. Metabolic activities of gut microbes release exudates that are used as signaling molecules by the host body and can influence various aspects of homeostasis (Pistollato et al., 2016).

Some scholars argue that amyloid proteins produced by bacteria are analogous to those found in the brain that lead to Aβ plaque buildup. Amyloid proteins are used by bacteria to form biofilms (often with cytotoxic effect to the host) and facilitate bacterial adhesion, aggregation, colonization, and tissue invasion (Pistollato et al., 2016). Additionally, a study performed by Asti and Gioglio, 2014 found that buildup of amyloid A proteins in senile plaques of AD patients lead to subsequent upregulation of various cytokines or signaling molecules including interleukins (IL) and tumor necrosis factors (TNF), specifically: IL-6, TNF- alpha, IL-12, p19 and IL-10. If over expressed, these cytokines stimulate inflammation. It is likely that they play a role in AD pathogenesis (Asti & Gioglio, 2014). Aggregate amyloid proteins have been known to cause reactive oxygen species accumulation as well as microglial inactivation. Amyloid aggregation can activate nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) signaling, which results in a signaling cascade that impairs microglia phagocytosis of Aβ plaque. *Escherichia coli*, *Salmonella enterica*, *Salmonella typhimurium*, *Bacillus subtilis*, *Mycobacterium tuberculosis*, and *Staphylococcus aureu* are all known to produce amyloid protein. In fact, abnormally high concentrations of *E.coli* have been correlated to the formation and aggregation of Aβ fibrils and AD pathogenesis (Pistollato et al., 2016).

Notably, the presence of LPS metabolites in normal quantities are used to control homeostatic mechanisms, inflammation, permeability, and sepsis. However, a buildup of LPS can result in activation of signaling pathways that cause neurodegeneration and inflammation in the brain. *In vitro* studies show that a large concentration of LPS added to bacterial amyloid proteins can result in protein polymerization/aggregation that could contribute to oxidative stress of the brain. A buildup of LPS metabolites can also exacerbate gut leakiness and increase levels of proinflammatory cytokines such as interleukin (IL)17A and IL-22. These two cytokines have been associated with AD in past studies.

Studies show that both *Lactobacillus* and *Bifidobacterium* break down glutamate to produce *gamma*-Aminobutyric acid (GABA), an inhibitory neurotransmitter. Abnormal concentrations of these two types of bacteria could lead to abnormal concentrations of GABA which can lead to poor mental health including AD and anxiety. Other SCFA present in disproportional amounts can be a threat and they are integral in glycolysis and energy derivation in the brain as well as immune response and other homeostatic mechanisms (Pistollato et al., 2016).

A study was performed by Minter et al. 2016 in Stanford University on the effect of gut microbial communities on microglial activity and AD pathogenesis. This study focused on male murine models that were subjected to rounds of antibiotic treatment (ABX mice) compared to a control group (vehicle). When subject to rounds of antibiotic treatment their gut microbial population changed in relative species diversity but not overall quantity of microorganisms. Change in dominant bacterial strains changed the overall gene expression, metabolite production, cytokine and chemokine production. In the host for example, CCL11 is a bacterial metabolite that can function as a cytokine to cross the blood brain barrier and lead to microglial activation

and phagocytosis. CCL11 was found to be elevated in ABX mice. In conclusion, they found that ABX mice actually had altered microglial morphology and microglia that were generally more efficient. In the same study study, the ABX mice with increased microglial activity had a decrease in Aβ plaque and tested better for long term memory (Minter et al., 2016). It should be noted that an increase of microglial activity and efficiency could be a preventative measure for AD because this phagocytic enzyme can consume Aβ plaque. However, an overstimulation of microglia can be a causative agent of AD and memory loss as microglia consume nonpathogenic particulates such as neural synapses (neurite degeneration) or myelin sheaths. Finally, a major limitation of this study is that these scientists confirmed a correlation between a change in the gut microbiome composition, microglial activity, and AD pathogenesis but they were *not* able to identify which particular species of bacteria or bacterial gene was causing the inhibition of microglial activity. In a later interview when Sangram S. Sisodia, one of the scientists working on these experiments, was asked about a possible next step to better understand how the gut microbiome effects microglial activity and protein buildup, he said a series of bacterial gene knockout experiments could be performed to determine which gene was contributing to microglial inhibition (Minter et al., 2016).

A study was performed by Vogt et al. 2017 on fecal samples of 50 human patients, 25 of whom had AD. They found an overall decrease in biodiversity, an increase in *Bacteroides* and a decrease in *Fimicutes* and *Bifidobacterium.* Biodiversity was measured in operational taxanomic units (OTU) or bacterial types. In AD patients there was an increase in 14/83 OTU and the rest decreased. This led to a significant overall loss in biodiversity and richness in the gut microbiomes of AD patients. There was a defined increase *Bacteroides* and *Blautia* among other

bacteria. Additionally, there was a significant loss of *Firmicutes* and *Bifidobacterium* (Vogt et al., 2017).

This combination of change in bacterial relative abundance is a microbial recipe for disaster. The *Bacteroides* Gram-negative cell wall has an outer LPS (also known as lipoprotein A) layer that stimulates the immune system to release of proinflammatory cytokines. Therefore, an increase in this bacteria results in systemic inflammation. *Firmicutes* exudate butyrate is important in nourishment and maintenance of the BBB. *Bifidobacterium* has anti-inflammatory effects and decreases membrane permeability. Studies show that an increase in *Bifidobacterium* in mice results in decreased bacterial translocation through permeable membranes. *Bifidobacterium* is found in many probiotics. In summary, if *Bacteroides* has increased access to the CNS through the blood stream after a breakdown of the gut membrane and BBB (facilitated by the decrease of *Bifidobacterium* and Firmicutes), *Bacteroides* LPS has a more direct access to the brain and can cause cerebral inflammation (Vogt et al., 2017).

Brain tissue surrounding plaque buildup of AD patients was found to have abnormal presence of LPS. Studies have shown that *in vitro* cultures, LPS stimulates fibrillogenesis in amyloid beta peptide. Additionally, *in vivo* studies in mice show that injections of LPS increased amyloid beta plaque and Tau aggregation. Postmortem studies show that human AD brain tissue contained Gram-negative *E. coli* and LPS aggregation in the brain along with AD plaque deposition. An increase in permeability allows Gram-negative bacteria and LPS more direct access to the brain (Vogt et al., 2017).

This article also mentions that type 2 diabetes is known to be comorbid with AD. Both of these disorders are characterized by a decrease in the richness of *Firmicutes* and an increase in the richness of *Bacteroides* and therefore display similar states of gut dysbiosis. It is also worth

noting that insulin resistance, a hallmark of diabetes, which causes a decrease of glucose cerebral metabolism is also correlated to an increase in amyloid plaque deposition.

Another study performed by Zhuang & Shen (2018) showed a clear difference in the composition of gut microbiomes of patients with AD compared to controls. They noted that AD patients had a high concentration of *Ruminococcus* bacteria in comparison to controls. High concentrations of *Ruminococcus* generally correlate to low concentrations of N-acetyl aspartate, which is an indicator of neuronal health. This study also mentions the possible contributions of *Helicobacter pylori*, a stomach bacterium, to an increase in Aβ plaque deposition in AD patients (Zhuang & Shen, 2018).

Environmental Risk Factors for Alzheimer's Disease Pathogenesis

A major limitation in this thesis investigation is the fact that there are multiple contributing independent variables that play a role in the pathogenesis of sporadic AD. Specifically, the evolving human diet and age are two factors that play integral roles in AD prevalence and development.

Aging: Increase in AD prevalence as human lifespan increases

As people age, their risk of developing AD is greatly increased. Concurrently, as people age, there is a notable change in many physiological factors and microbial concentrations. There have been dramatic increases in life expectancy over the course of human history, particularly in the last hundred years. Even more recently, in 2010 global life expectancy has reached sixty eight years, which indicates a twenty-one year increase just since 1995. This steep increase in

life expectancy is attributed to controlling the spread of communicable diseases as well as a developed understanding of medicine and its application (Wayne, 2019). Due to the fact that AD diagnosis and pathogenesis occurs later in life, AD appears to be a recent health issue among humans because of a relatively recent increase of the average human lifespan. Based on current ascending trends, the number of people with AD is projected to more than double by 2050 from 5.8 million to 13.8 million (Kondro, 2010). Many scholars have questioned the change in prevalence of AD over time and some attribute it to a change in the composition of the microbiome of the human population. It is difficult to separate the aging process from AD development to determine the precise causal agent for AD. The main factors associated with both aging and AD are increased microbial dysbiosis, increased total bacterial load, a weakening immune system, and a decrease in tissue integrity of the BBB (Shoemark & Allen, 2014).

Aging is indirectly correlated to the immune system's ability to function. Microglia, for instance become less effective in clearing plaque and waste buildup as age increases. This makes the body more susceptible to microbial dysbiosis as there are fewer checks and balances in the body against a rapid increase in relative abundance of pathogenic bacteria (Minter et al., 2016). In the mouth, there is also a decrease in lysozyme containing saliva production, which permits the growth of bacteria (Shoemark & Allen, 2014).

In the gut microbiome of the elderly, there is a marked change in microbial concentrations including an increase in Gram-negative *Bacteroidetes* and decrease in *Firmicutes* and *Bifidobacteria* (Pistollato et al., 2016).

Also, in the oral microbiome, AD patients have a noteworthy increase in the concentration of anaerobic bacterial species due to an increase in pocket depth and the use of dental prostheses. This overall increase in anaerobic bacteria and overall bacterial load results in

a stimulation of inflammatory pathways by evoking the increased release of TNFα and IL1β. These are immune system triggers released by glial cells upon pathogenic bacterial encounters. Increased concentrations of TNFα can also contribute to breakdown of the BBB (Shoemark & Allen, 2014).

To reiterate, a major complicating aspect of my research paper is the fact that aging and microbial compositional shifts occur concurrently. Therefore, it is difficult to know if the bacterial change is a causal factor of AD or simply occurs independently at the same time.

Human bacterial coevolution

The contributions of *H. pylori* in the stomach microbiome to AD pathogenesis is currently being investigated. Scientists have hypothesized that *H. pylori* may contribute to Aβ plaque production as well as Tau phosphorylation, resulting in a progression of AD (Zhuang & Shen, 2018). However, *H. pylori* has only recently been incorporated as a part of the human stomach microbiome during the transition from a hunter-gatherer lifestyle to a more sedentary agricultural lifestyle during the Neolithic age (generally within the past 10,000 years, with independent origins of agriculture worldwide). For instance, in central Africa nearly 4,500 years ago, *H. pylori* strains were introduced to rainforest hunter-gatherer communities by agricultural communities (Patin et al., 2013). Humans have lived as hunter gatherers/foragers for the vast majority of human evolutionary history of nearly two million years (e.g., genus *Homo)* with a relatively diverse, "broad spectrum" dietary regime that stands in contrast to today's "Western" diet (Warinner, 2015). While it is difficult to determine the prevalence of AD that far in the past of human evolutionary history (though there are tantalizing hints that the selection for genes tied to brain metabolic development in the origin of anatomically modern *H. sapiens* species 50-200 thousand years ago may be associated with AD), it is still interesting to see the way in which the

human microbiome has developed over time, with implications on susceptibility to AD (Rogers, 2015).

Diet: Increase of AD prevalence as human diet changes

Diet has been linked to an alarmingly rapid rate of decrease in microbial diversity. (Davenport et al., 2017). There are two principle diet types: Western and Non-Western-style diets, that result in two very distinct gut microbiomes (Oriach et al., 2016). It should be taken into account that these two diet types are not specific to geographical location but rather to diet composition. Foods in Non-Western diets could include things like sweet potato, wheat, barley, lentils, berries, vegetables (Lipski, 2010). The Non-Western diet is considered a more traditional and natural diet that resembles the hunter gatherer diet. The Non-Western diet is generally composed of low fat, low sugar, and high fiber foods. Alternatively, foods included in the Western diet include things like cheese, butter, high-fructose corn syrup, and foods with increased sodium concentrations (Cordain, 2005). The Western diet focuses more on processed contemporary foods and is composed of high fat, high sugar, high meat, and low fiber foods (Oriach et al., 2016). The westernization of diets has come about with a transition from a hunter gatherer to sedentary lifestyle (Warinner, 2015).

1. **The Western-style diet**

Experiments have shown that the altered gut microbiota due to the Western diet causes increased inflammation and induced dementia (Oriach et al., 2016). On the other hand, there are some strains of bacteria ingested that are labeled "psychobiotics" that correlate to improved mental health. However, very little is known about the specific mechanisms of the microbial metabolites and their interactions with the brain (Oriach et al., 2016, & Serkov et al., 2018*). In vivo* murine studies showed that high fat, high sugar diets (emulating Western diet) resulted in a

dysbiotic change in the microbiome and a consequential decrease in memory (memory was tested using the Morris water maze test where the mouse must find a platform within a circular pool of water that helps it escape the pool) (Oriach et al., 2016). The results of this study are summarized in Table 1 that follows. (Note that good performance on the Morris Mouse Maze test means good spatial short term memory)

Table 1

Morris Mouse Maze Studies on Diet and Behavior

Diet	Intervention Length	Microbial Changes	Behavioral changes
High fat	8 weeks	↑*[Firmicutes & Ruminococcaceae]* ↓ [bacterial diversity]	↑anxiety ↓memory (Morris water maze test)
High sucrose diet	2-5 weeks	↑*[Clostridiales, Lactobacillus, & Lactococcus]* ↓ *[Baceriodites]*	↓memory (morris water maze test)
Meat containing diet	3 months	↑[bacterial diversity]	↑long term memory ↓anxiety like behavior
Western style diet	21 days`	↑*[Firmicutes]* ↑*[Spirochaetes]*	↓SCFA

Another study dictated that high fat diets specifically resulted in a decrease in *Bifidobacterium*. When *Bifidobacterium* is present in moderate quantities, it has anti-inflammatory effects and decreases membrane permeability (Serkov et al., 2018). Yet another

study showed that Western diets resulted in a disproportionally large increase in the concentration of *Firmicutes* to *Bacteriodetes* ratio. *Firmimcutes* and *Bacteroidetes* are two of the most prevalent bacteria already present in the human gut microbiome. If *Firmicutes*/ *Baceriodetes* ratio increases, there is a reduction of the total diversity of the microbiome which leads to dysbiosis as well as increased membrane permeability. Researchers speculate that the rise in concentration of *Bacteriodetes* occurs because *Bacteriodetes* metabolize the increased amounts of amino acids derived from meats found in western diets (Pistollato et al., 2016).

2. The Non-Western diet

The Non-Western diet results in an increase in *Proteobacteria* (Davenport et al., 2017) and *Provecella* (Pistollato et al., 2016). *Provecella* break down plant material found in higher quantities in the Non-Western diet. Non-Western diets rich in fiber encourage the growth of a diversity of "good microbes" such as *Bifidoboacterium* in the gut. Fiber is considered a major prebiotic or substrate that encourages bacterial growth that is beneficial to the host. Murine models show that mice with higher fiber diets had improved overall mental health (Oriach et al., 2016).

Holistic Preventative Measures for AD

A common theme in my research paper is that a single metabolite or bacterial strain present in dysbiotic proportions can contribute to neurodegeneration and other serious impairments. This leads to the conclusion that is counterintuitive to the traditional clinical approach of searching for a single pathogenic culprit for a disorder. Instead of targeting the presence of a specific "bad bacterium" for a disease state, the problem seems to be the absence of

"good bacterium". This absence and decrease in interspecies bacterial competition provides the ecological space for the overgrowth of other types of bacteria. This results in a disproportionate concentration of bacterial metabolic exudates that likely contribute to the pathogenesis of AD.

Therefore, some of the best holistic preventative measures that can be taken to lower risk for AD are those that establish and maintain a stable and diverse gut and oral microbiome. The establishment of a healthy diverse gut microbiome in the first two years of life is important in a generally more resilient immune system and improved health overall (Reid and Greene, 2013).

Diet

Homeostatic microbial concentrations can be enforced by dietary intake that resembles a more traditional, Non-Western diet. Clinical data shows that patients are less likely to develop AD if they have a higher intake of whole grains, fiber, fruit, vegetables, legumes, fish, and low fat dairy. Many of these foods act as prebiotics. A prebiotic is a substance that encourages the growth of certain beneficial strains of bacteria. Foods that should be avoided include those that are high in fat and refined sugar. Patients who follow this type of diet have a significant reduction in Aβ plaque aggregation and higher cerebral metabolic rates (indicating increased cortical brain function) and decreased risk of AD development (Pistollato et al., 2016). There are also some herbs and teas that have neuroprotective properties (Pistollato et al., 2016).

Plant phenolics and iron

Another remedial approach is an increased intake of antioxidants including plant derived foods that contain phenol groups including green tea, dates, virgin olive oil, and red wine. These substances have been correlated with lower plaque and Tau aggregations as well as less cerebral inflammation. Phytochemicals and other plant components such as tea phenolics have been

known to hinder the proliferation of *Clostridium difficile*, and *Clostridium perfringens* (the increased concentration of these types of bacteria has been known to cause a disease state of the gut). Iron containing red meat is an oxidant and can worsen cerebral oxidation. Abnormally high intake of iron containing red meat can result in the formation of fibrin fibers that cause red blood cell to clot. These blood clots can then prevent oxygen from accessing the CNS. Therefore, low amounts of red meat consumed could be a preventative measure for AD pathogenesis (Pistollato et al., 2016).

Prebiotics and probiotics

In addition to prebiotics and various phenolics, a balanced intake of probiotics in foods such as kefir and kambucha, or other foods that are rich in "good" bacteria (such as *Lactobacillus* and *Bifidobacterium*) help maintain a healthy gut microbiome. For instance, the implementation of probiotic and prebiotic regimens in the elderly have resulted in an increase in B*ifidobacterium* and decrease in inflammatory cytokines including IL-5, IL-6, IL-1b, IL-8, and TNF-a (Pistollato et al., 2016).

Fecal microbiota transplants

An interesting possible method to remedy a dysbiotic gut microbiome is fecal microbiota transplants (FMT). FMT donations from healthy donors with a diverse gut microbiome are being used in hospitals today to treat *C. difficile* infections (CDI) by reestablishing a diverse gut microbiome (Austin et al., 2019). There are also promising studies that show that FMT in mice can dramatically reduce the prevalence of Parkinson's Disease (PD) (Yang et al., 2018). While the effects of the microbiome and FMT in PD have been more thoroughly studied than in AD,

these two neurodegenerative disease states have analogues mechanisms. With more testing it is highly plausible that FMT could be used in a similar manner to treat AD.

Oral hygiene

Additionally, with regard to the oral microbiome, maintaining good oral hygiene could decrease risk for AD. Good oral hygiene prevents the stratification of bacteria in the mouth that provide anaerobic niches leading to increased populations of anaerobic bacteria. Specifically, avoiding the overgrowth of anaerobic bacteria *P. gingivalis* has been shown to decrease the buildup of Aβ plaque in the brain (Shoemark & Allen, 2014).

Conclusion

In conclusion, gut and oral microbiome does indeed play a role in the development of AD. The healthiest microbiome has a more varied relative abundance of a diverse array of bacteria. Deviation from a symbiotic state of bacterial abundance can have serious local and systemic ramifications. The gut microbiome is established at a young age and should remain relatively constant after the age of two. Microbes have an important role in systemic signaling and digestion in the human body and are hugely beneficial to the host under homeostatic conditions. Microbes can communicate with the brain through bacterial translocation or the transfer of their metabolites and cytokines throughout the blood stream. This form of communication is more prevalent when there is an increased permeability of membranes. Additionally, microbes can communicate to the brain by stimulating the transmission of neurotransmitters through the nervous system to the brain.

Late onset AD can be brought about by genetic allelic expression of apoe4 and by environmental factors such as dysbiosis of the microbiome. AD is characterized by an overactive immune response caused by a buildup of Aβ plaque and Tau protein in the brain. Microbial dysbiosis can contribute to the pathogenesis of AD. AD patients often have gut microbiome dysbiosis caused by an increase in Gram-negative *Bacteroides* and a decrease in *Firmicutes* and *Bifidobacterium. Bacteroides* induce the production of LPS that invoke cerebral inflammation and Aβ aggregation. *Firmicutes* and *Bifidobacterium* help prevent membrane permeability. *Firmicutes* specifically produce SCFA butyrate that helps nourish the epithelial membrane of the gut. There is also an overall decrease in bacterial biodiversity in AD patients. The oral microbiome is characterized by an increase in anaerobic bacteria such as *P. gingivalis,* producing gingipain enzymes that buildup in exactly the same locations as Aβ plaque and Tau tangles.

The composition of the gut and oral microbiomes has changed over the course of human history and within a single life span. The human microbiome is sensitive to a multitude of environmental variables. Diet is one of the most prominent influences on the composition of the microbiome. Non-Western or traditional diets characteristic of hunter-gatherer populations have lower amounts of sugar and fat and higher amounts of fiber. Experiments show that these diet types have a stabilizing effect on the gut microbiome with an increase in *Bifidobacterium.* We see the opposite effect in those that consume a western type diet with more fats and sugars and less fiber. This results in a decrease in *Bifidobacterium* and an increase in the *Firmicutes* and *Bacteroides* ratio resulting in an overall decrease in bacterial diversity, and an increase in membrane permeability and inflammation. AD was also shown to be comorbid with Diabetes 2, both of which have a characteristically similar state of dysbiosis. Furthermore, the inhibition of glucose metabolism as a result of diabetes may contribute to the pathogenesis of AD. Aging also

contributes heavily to AD pathogenesis. There is a similar dysbiotic shift in older people that resembles that of AD patients, specifically an increase in Gram-negative bacteria in the gut and an increase of anaerobic bacteria in the mouth.

Finally, holistic methods of AD prevention are mainly based on the attempt to maintain a stable, diverse microbiota. These methods include the following measures: moderate consumption of probiotics, prebiotics, and plant derived phenolics. There should also be a limited consumption of red meats. This generally reflects a Non-Western diet type. Research shows that perhaps FMT could be used to reestablish a functional gut microbiome and lessen the pathogenic causative agents and effects of AD. It is clear that there is more research to be done regarding the mechanistic effect of the microbiome and neurodegenerative disorders such as AD, as there are countless contributing factors in the equation. However, it is interesting to see a clear correlation between two systems that were once thought to be entirely isolated. Hopefully, further research will lead to a better understanding of this novel intrinsic ecosystem and perhaps contribute to AD therapies or preventative measures.

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