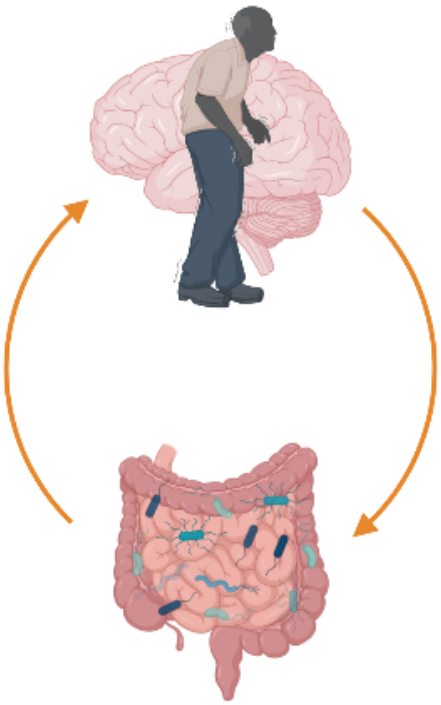
The Gut Microbiome: how it may affect the course of Parkinson's disease



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Abstract

Parkinson's disease (PD) is a growing problem as the mean age of the world's population increases and it has been the second most prevailing neurodegenerative disease in the world. Lately, the influence of the gut microbiome (GM) on PD has been studied. The gut-brain axis is a hot-topic not only in science but in general, because of the therapeutic potential through a combination of diet and faecal microbiota transplantation. In this paper the role of the GM is discussed firstly in gut diseases, but later on focusses on neurodegenerative diseases, in particular PD. It has been more evident that the composition of the gut microbiota is altered in patients suffering from irritable bowel syndrome or inflammatory bowel disease, but recently studies have shown that the GM is also disturbed in patients suffering from Alzheimer's disease (AD) and PD. The dysbiosis in the gut seem to affect the α-synuclein (α-syn) pathology in PD, and may result in short-chain fatty acids (SCFAs) disruption. Although, it is not yet clear if SCFAs have a positive or negative influence on the course of PD. The impact of heavy metals, in principal manganese, on PD is shortly discussed. α-Syn, SCFAs, and heavy metals appear to act on the neuroinflammatory system. In the end, the interplay between the GM and the main treatment of PD, levodopa, is reviewed. Though a lot is learned from recent findings, some studies revealed conflicting outcomes, ascertaining that more research is needed in the future before the GM could be used as a therapeutic target.

Key words: Parkinson's disease, gut microbiome, gut-brain axis, neurodegenerative disease

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Introduction

Age-related diseases are more common as we get older and also comprise neurodegenerative diseases. Parkinson's disease (PD) is the second most prevailing neurodegenerative disease and worldwide it affects around 1 to 2 persons per 1000 of the population. Due to the increase in age, it is probable that this number will be even doubled by 2030. In the early 1800's the disease was first described by James Parkinson, to whom the disease thanks its name. PD is mostly common among persons older than 65 years, but early-onset PD does exist. It appears that women are 1.5 times less likely to have PD than men. The mechanism behind PD is still not fully understood, though it has already been discovered that loss of or a distinctive degradation of the dopaminergic neurons in the substantia nigra pars compacta with dopamine deficiency within the striatum is an indication of PD. On top of that, the manifestation of intracellular eosinophilic inclusions, called Lewy bodies and Lewy neurites in the other neurons is often seen in PD patients too. Within these neurons the misfolded α-synuclein (α-syn) aggregates into insoluble particles which affects the entire nervous system, such as the sympathetic ganglia, salivary glands, adrenal medulla, vagus nerve, cutaneous nerves, sciatic nerve, and enteric nervous system (ENS). Symptoms often manifest clearly after two or more decades from the onset of pathological changes. The shortage of dopamine producing neurons results in impairment of motor control. About 60-70 percent of the neurons in the substantia nigra pars compacta are gone when the first symptoms of PD are present. The Lewy bodies impedes the normal function of neurons. The motor symptoms are notorious. Most people characterize PD with tremor and shuffling gait, but bradykinesia (slow movements), rigidity, and postural instability are common too. In addition, other observed motor symptoms are hypomimia (masked facial expression), blurred vision, impaired upward gaze, decreased eye blink rate, dystonia, stooped posture, difficulty turning in bed, scoliosis, kyphosis, inability to move or "freezing", palilalia (repetition in speech), and hypophonia (softer voice). (Beitz 2014; Caputi & Giron 2018) Besides the known motor symptoms, PD is also characterized by nonmotor symptoms such as gastrointestinal and urogenital dysfunction, neuropsychiatric issues, sleep, and pain/sensory disturbances. Especially gastrointestinal dysfunction, such as obstipation is affecting more than 80% of the PD patients. The problem with nonmotor symptoms is that they usually do not respond well enough to dopamine therapies, therefore causing difficulties maintaining quality of life. (Beitz 2014; Hegelmaier et al. 2020; Tan, Hor, Chong & Lim 2021) Even though, the whole mechanism behind PD is not yet known, it is acknowledged that PD is strongly influenced by environmental factors. (Hegelmaier et al. 2020) There is no cure for PD thus far, just some symptomatic treatments.

Though, it was thought as common belief, Lederberg did not come up with microbiome as a term in 2001 and he also did not define a microbiota. When in the 1960s experiments on germ-free (GF) animals were done, the word microbiota was already used. In 1988, Whipps, Lewis, and Cooke came up with the definition of a microbiome in the way we use the term in science nowadays. It implies a "*convenient ecological framework*" consisting of a "*characteristic microbial community*" within its habitat, its physio-chemical properties, and its interactions with the environment. However, the term microbiome is also used to indicate a very small ecological niche of plant and animal life. Microbiome is not only being studied in medical science; microbiome research is very popular in agriculture, aquaculture, and horticulture too. Because of the development in engineering, microbiota is used to replace toxic chemicals in time, to create and to stimulate the use of sustainable resources, and to develop better ways to process our food. The human body consist of more than 100 trillion symbiotic microorganisms, including viruses, bacteria, fungi, and protozoa, and they all part of our microbiome. Now, the human microbiome is seen as an organ like the lungs or stomach. (Whipps, Lewis & Cooke 1988; Parker 2016; Prescott 2017; Berg et al. 2020)

The gut microbiome (GM)has become recognized as a metabolic organ, carrying approximately 150 times more genes than there are found in the entire human genome. Also, the microorganisms in the gut have been seen as very crucial for the maintenance of homeostasis lately, and it is even been considered as important as the liver. One of the main roles of the GM is that it has the potential to increase the energy extraction from our diet, increase the nutrient intake, and to change

the hunger signalling. The microorganisms living in the gut have a far more versatile metabolic gene pool than the human being, and they give their host unique and specific enzymes and provide a plethora of biochemical pathways. The GM is responsible for a large proportion of the metabolic processes in our body that helps the host with either xenobiotic processing or nutrient acquisition including the biosynthesis of vitamins and the metabolism of undigested carbohydrates. Besides the metabolic activity of the microbiome, the human microbiota is also a physical barrier, being one of the first “walls” of the immune system. Through competitive exclusion and antimicrobial substance production, the human microbiota protects its host against other pathogens. Therefore, the microbiome is important for immune system of the host and the development of the intestinal mucosa. (Wang et al. 2017)

Previous studies suggest that dysbiosis of the microbiome in the gut result in deterioration of cognitive and motor functions, which could lead to neurodegenerative diseases or worsening of the course of the disease. (Leo & Campos 2020; Arslanova et al. 2021) This deterioration is probably due to the relationship between the dysbiosis of the gut and neuroinflammation. This has been extensively studied in the last couple of years. Through neuroinflammation, the link between the dysbiosis and neurodegenerative diseases has been made as well. (Cerovic, Forloni & Balducci 2019; Li et al. 2019) Therefore, the main research question is what the relationship is between the GM and PD. In this review the focus will mainly lie on the effects of dysbiosis of the GM on PD, and how this results in the interaction between the GM neuroinflammatory response, and the course of the disease. At last, the influence of the GM on one of the treatments of PD, called levodopa, will also be discussed in this paper. Hopefully this review will help to have a better understanding on how the gut-brain axis affects the progression of PD and to learn if there are new possibilities for treatment.

1. Dysbiosis of the gut microbiome

The gut microbiome is a 'hot topic' not only in science, but as well in the general public. In particular the gut-brain axis is becoming an important aspect in the (bio)medical world. The increase in interest and knowledge in microbiomes has resulted in a new view on the use of good bacteria, nutrition and diet on health. As of late, the amount of research on the GM has skyrocketed, because there is growing evidence for the beneficial properties of a healthy microbiome and its use for novel medical treatments for an array of diseases. (Berg et al. 2020) Though, the subject is very popular now, in 1986 Linda R. Hegstrand and Roberta Jean Hine already published a study demonstrating the differences between GF and conventionally raised animals in their hypothalamic histamine levels due to differences in microbes. (Hegstrand & Hine 1968; Prescott 2017) Lately there has been increasingly more evidence that the microbiota is altered in patients suffering from neurodegenerative diseases, especially Alzheimer's disease (AD) patients and PD patients have a dysbiosis of the GM. (Hegelmaier et al. 2020; Ivakhniuk & Ivakhniuk 2021)

1.2 The influence of the gut microbiome on other diseases

Reasonably, the GM has an influence on the health of the gut. Therefore, a plethora of studies have been done on the effect of GM on the gastrointestinal tract, though there is growing evidence that the liver is also affected by the GM. Dysbiosis could induce liver damage due to toxins produced by the microbiome. Also, the production of ammonia, ethanol, and acetaldehyde may have an effect on the liver function. Patients suffering from liver cirrhosis showed a higher number of species of Fusobacteria and Proteobacteria, and a lower number of Bacteroidetes in the gut, and some studies found that there is a possibility that these changes could arise from the oral microbiome, which was significantly different from healthy controls. (Wang et al. 2017)

Faecal microbiota transplantation is one of the used therapies at the moment for gastrointestinal tract disorders. Irritable bowel syndrome (IBS) patients differ in their microbiome composition. Normally, IBS is tackled by the use of diets such as low FODMAP (fermentable oligo-, di-, monosaccharides, and polyols) or NICE (National Institute for Health and Care Excellence). However, some patients do not seem to respond well on these diets. 50-70% of IBS patients respond to low FODMAP-diet, while the diet is hard to maintain and expensive. In the long run it could even cause dietary deficiency. NICE on the other hand is simpler and therefore easier to maintain, it only asks patients to reduce the intake of sugary foods and drinks, food containing high amounts of fat, beans, cabbage, onions, and to replace wheat products with spelt. In addition, it is recommended to take psyllium husk fibres. It appeared that patients that do not respond well to the diets, have a more disturbed GM. For these patients, faecal microbiota transplant could be the solution, but more research is needed to learn how the GM affects the course of the disease. (El-Salhy, Hatlebakk & Hausken 2019)

Obesity is a growing problem all over the world with the number of obese being tripled since 1975. Unfortunately, the existing drug therapies are still not successful and therefore new insights are needed. Although it is not yet proved that an altered microbiome has a role in obesity or food addiction, the intestinal hormones ghrelin, GLP1 and peptide YY are influenced by short-chain fatty acids (SCFAs). The level of ghrelin, which is a hunger hormone, is higher when there is a disturbance in the production of SCFAs, and the level of satiety hormones GLP1 and peptide YY are lower. The disturbance of SCFAs could be a result of dysbiosis of the GM. (Gupta, Osadchiy & Mayer 2020)

Worldwide 47 million people are affected by AD and is still the most spread form of dementia. 15-20 years before clinical symptoms are shown, the pathogenesis has already started with AD and in these years the brain's function decreases due to neuronal cell death, synaptic alterations, vessel damage, and chronic neuroinflammation. Hyperphosphorylated tau and amyloid-β (Aβ) plaques result in brain lesions and are the main characteristics of AD. Although it was long thought that Aβ was the main culprit for AD, failed anti-Aβ trials have shown otherwise. Lately it has been considered that the influence of the gut-brain axis on neuroinflammation is a more attractive target than the Aβ oligomers. A few studies have found that the microbial diversity was lower in faecal samples of AD

patients compared to the healthy controls. (Cerovic, Forloni & Balducci 2019; Ivakhniuk & Ivakhniuk 2021) Mouse models showed how broad-spectrum antibiotics or GF conditions in AD mice could lower the number of Aβ plaques and that introduction of the GM from AD mice in GF mice increased the Aβ pathology. (Cerovic, Forloni & Balducci 2019) Also the effect of diet on AD has been studied. It appeared that high-fat diets, especially saturated fats, and diets high in simple sugars are linked to a higher risk for AD. A Mediterranean diet is associated with a lower risk for cognitive disorders. Mediterranean diets consist of high-fibre products, ω-3 fatty acids, and antioxidants which are known to be beneficial for the health. (Leo & Campos 2020) There have been some studies done with diet interventions, like caloric restriction, among others. The purpose of caloric restriction is to lower the daily caloric intake and the diet appeared to have neuroprotective and age-delaying potential. Moreover, fasting could change GM composition promoting anti-inflammatory microbes. (Cerovic, Forloni & Balducci 2019; Fontana et al. 2021) Whether caloric restriction or any other dietary intervention is safe to implement for the elderly suffering from AD has yet to be investigated.

As mentioned, the GM affects not only the health of the gut, but also the rest of the body including the brain. Several studies have shown that the GM of patients suffering from disease of the abdomen, but also neurodegenerative diseases has been altered. However, most studies are done on animal models and for the translation to possible therapy for human diseases more (clinical) studies are needed, especially long-term studies.

1.2 The dysbiosis of the gut microbiome in Parkinson's disease

A previous study by Keshavarzian et al. has shown that the composition of the bacteria in the mucosa of PD patients differ from healthy controls. In healthy controls the mucosal-associated microbial communities were richer in bacteria from the family Coprobacillaceae (Firmicutes, class Erysipelotrichi) and genera *Dorea* (Firmicutes, class Clostridia) than in PD patients. In addition, they found a decrease of the anti-inflammatory *Faecalibacterium* (Firmicutes, class Clostridia) in the mucosa of the PD patients. Furthermore, in the mucosa of the PD subjects there was a significant increase in proinflammatory bacteria such as from the family Oxalobacteraceae (Protobacteria, class Betaproteobacteria) and genus *Ralstonia* (Proteobacteria, class Betaproteobacteria, family Oxalobacteraceae). The bacteria from the genus *Faecalibacterium* (Firmicutes, class Clostridia) were the only butyrate-producing bacteria that was significantly less abundant in mucosal PD than in healthy controls. After analysing the faecal samples, it was discovered that at the phylum level the bacteria from Bacteroidetes, Proteobacteria, and Verrucomicrobia are more present in samples of PD patients than in those of the healthy controls, while the amount of Firmicutes bacteria was higher in healthy controls. In PD faecal samples *Akkermansia* (Verrucomicrobia, class Verrucomicrobiae), *Oscillospira* (Firmicutes, class Clostridia), and *Basteroides* (Bacteroidetes, class Bacteroidia), bacteria that may be seen as proinflammatory, were at the genus level significantly more abundant. Whereas the putative anti-inflammatory bacteria *Blautia*, *Coprococcus*, and *Roseburia* (Firmicutes, class Clostridia) were less abundant in PD faecal samples. These bacteria are SCFAs producers. Overall, the GM of PD patients shifts to a more proinflammatory condition in comparison to the healthy controls. (Keshavarzian et al. 2015)

SCFAs are small organic monocarboxylic acids and play an important role in the intestines to support the body health and gastrointestinal function. Acetate, propionate, and butyrate are most common metabolites produced by the GM through the anaerobic fermentation of indigestible polysaccharides like dietary fibre and resistant starch. These metabolites are known for their energy supply, their role in neurotrophic factors, and the regulation of T regulator cells. SCFAs reduces the risks of colorectal cancer as well. There is still not much known about the signalling by SCFAs, but that SCFAs bind to G protein-coupled receptors (GPCRs) is already acknowledged. The activation of these receptors results in various effects depending on which cell they are expressed. In the immune tissue of the gut, the peripheral nervous system and the central nervous system (CNS), the interaction between SCFAs and histones has been discovered. Histone deacetylase activity is being inhibited by the SCFAs throughout several cell populations, resulting in the acetylation of lysine

residues in nucleosomal histones. So even though just a small part of the colon-derived SCFAs enters the systemic circulation, they still have a lot of influence on different organs and systems. Butyrate is a metabolite which is capable of inducing T-regulatory cells to differentiate and therefore affecting the immune system through controlling inflammation. Disruption of the metabolites ends in quite a few pathological abnormalities such as inflammatory bowel disease (IBD) and obesity. (Silva, Bernardi & Frozza 2020)

Because of the dysbiosis in the gut of PD patients, there have been (clinical) studies that tested the effects of rectal enemas or faecal microbiota transplantation. This treatment is used for patients with IBD and in the past some studies have suggested that there are some similarities between the GM of PD patients and IBD patients. Bowel cleansing is a well-established treatment and a well-tolerated intervention. (Hegelmaier et al. 2020) In a study performed on rats, the scientists concluded that the negative effects of metal neurotoxicity might have been alleviated by faecal microbiota transplant from the control groups. They have found a significantly downregulated expression of Tau, an important marker for AD, after faecal microbiota transplantation in comparison with the group without treatment. Amyloid-β and Tau aggregates are able to act as molecular patterns that induce oxidative stress, produce ROS, and trigger toll like receptors (TLRs) and NLRP3 (pyrin domain-containing protein 3) inflammasomes, a member of the nucleotide-binding and oligomerisation domain-like receptors (NLRs). All this activity results in microglial production of inflammatory cytokines. In addition to Tau, they found less expression of inflammatory factors meaning that the faecal microbiota transplantation could inhibit the immune response initiated by metal toxicity. (Wang et al. 2020)

Hegelmaier et al. concluded that a combination of a vegetarian diet including a high dose of anti-inflammatory acting SCFAs, and bowel cleansing have a positive effect on the GM and clinical course in PD. To measure the differences in motor symptoms in PD patients a well-known clinical evaluation scale was used. The Unified Parkinson Disease Ratings Scale (UPDRS) has different levels from UPDRS I-IV, wherein UPDRS-III evaluates the motor symptoms of people suffering from PD. This scale tests speech, rest tremor, facial expression, rigor, action or postural tremor of the hands, finger dexterity, agility of the legs, (rapidly changing) hand movements, getting up from the chair, posture, gait, postural stability and movement of the body. UPDRS-I evaluate behaviour, mood, and mentation, within the UPDRS-II a self-assessment about daily life experience is comprised, and UPDRS-IV is a self-assessment about the complications of therapy. After therapy, the data of the clinical study showed that the motor function improved and the UPDRS-III decreased significantly. However, there were no significant differences before and after the vegetarian diet intervention only, but as they have explained themselves, it is probably due to the short period of the intervention. Whereas the outcome of an enema is directly visible, the outcome of a change in diet probably will take some time. Yet it is still promising to look into this matter in the future. The daily dose of levodopa decreased as well after the combined therapy, but the interaction between levodopa and the GM interaction will be reviewed later. (Hegelmaier et al. 2020)

GM dysbiosis seems to have a big attribution in several diseases. In PD, patients tend to have a more proinflammatory GM compared to healthy controls and that these changes lead to a lower production of SCFAs. Because of this, the functionality of the gut and the overall health decreases. Studies have shown that the use of a faecal microbiota transplant together with a healthy diet is capable to improve the symptoms of PD. Patients experienced less motor dysfunction and though not all interventions showed significant results, it is a promising start.

2. The relation between the gut microbiome, neuroinflammation, and Parkinson's disease

Dysbiosis of the GM has already been linked to neuroinflammation by triggering the misfolding aggregation of α-syn in the ENS and then spreading to the brain. Due to the increased permeability of the intestinal walls or also called a "leaky gut", it is easier for pathogens to enter the body resulting in intestinal inflammation. In PD patients this phenomenon has been observed in preclinical and clinical studies. Though, a lot remain unclear about the leaky gut and its influence on the course of neurodegenerative diseases such as AD and PD. The increase of α-syn deposition in the gastrointestinal tract has been linked to bacterial endotoxin exposure. While exposure to a low number of bacterial endotoxins, or lipopolysaccharides (LPS) in early life could improve the immune system to fight against systemic diseases, dysregulation of the immune structure initiated by LPS are the cause of inflammatory responses in local and systemic tissues, which in turn might result in α-syn pathology (Caputi & Giron 2018; Tan et al. 2021)

2.1 α-Synuclein

In PD α-syn pathology affects the motor function of the patient. In a study by Sampson et al. the importance of the GM was discovered. Using GF and antibiotic treated specific pathogen free (SPF) mice they mimic respectively the absence of GM and gut dysbiosis. First, they have tested if the Thy1-αSyn (alpha-synuclein overexpressing: ASO) mouse strain really displays lesser motor functions than the wild type (WT) mice. They have learned that indeed the SPF-ASO mice performed worse than the SPF-WT mice in motor function tasks, but that the GF-ASO and GF-WT do not differ so much in the results. For this study they measured the motor function by means of beam traversal, pole descent, nasal adhesive removal, and hindlimb clasping reflexes. Hereafter, the α-syn aggregation of GF and SPF mice is determined in different brain sections using Western blots of brain extracts. In GF-ASO mice they found significantly less α-syn aggregates than in SPF-ASO mice, though there are regional differences in the brain. In the frontal cortex, caudal putamen, and inferior midbrain where the substantia nigra is located, the α-syn aggregation in GF-ASO is lower than in SPF-ASO, whereas in the cerebellum almost the identical amount of α-syn aggregation has been detected. Nevertheless, deriving from these results, it might be possible to conclude that the GM is indeed needed for α-syn pathology. (Sampson et al. 2016)

The activation of microglia is an observed phenomenon in PD. α-Syn is known for activating microglia, though degenerating dopaminergic neurons also trigger this activation. Microglia phagocytose α-syn through TLRs, TLR-2 and TLR-4 in specific, which then leads to the production of reactive oxygen species (ROS). (Sampson et al 2016; Baizabal-Carvallo & Alonso-Juarez 2020) Oxidative stress is caused by a disproportion of ROS and is known to be harmful for the human health. Several neurological diseases are linked to oxidative stress, including AD and PD. (Pizzino et al. 2017) When microglia are activated, an inflammatory process within the substantia nigra is put into operation whereby dopaminergic neurons are lost and possibly promoting a loop of degenerating dopaminergic neurons and activation of microglia, (Sampson et al 2016; Baizabal-Carvallo & Alonso-Juarez 2020) TLRs are a part of a range of pattern recognition receptors, which are important for the innate immune system. As the first "defence system" of the human body, the innate immune system is crucial for recognising an infection in the early stages. (Caputi & Giron 2018) This might be the reason that a leaky gut is problematic in the course of PD as it impedes the function of the first defence wall of the gut.

The role of TLRs in the course of PD has to be investigated more, but recently more evidence has surfaced that TLR-2 and TLR-4 have a double agenda. Through activation in microglia, they induce neurotoxicity, but also phagocyte misfolded α-syn causing the opposite of neurotoxicity, namely neuroprotection. During their differentiation macrophages derived from human hematopoietic stem and progenitor cells were challenged with a TLR2 agonist. Afterwards these macrophages were inoculated in irradiated mice and subsequently showed a lenient phenotype. The release of inflammatory mediators and ROS was as a result lowered. When TLR-4 is lost a few things seem to happen. When a TLR-4 knock-out mouse model is used, it resulted in reduced effect of the pesticide

called rotenone which is widely used in animal models of PD. Rotenone affects the intestinal barrier integrity, colonic α-syn deposition, dopaminergic cell loss, microglial activation, and motor function impairment. However, TLR-4 depletion also increases dopaminergic neurodegeneration in the substantia nigra pars compacta, because extracellular α-syn cannot be recognized and therefore is not phagocytosed by microglia. (Caputi & Giron 2018; Tan et al. 2021) Evidently more research is needed to learn when the activation of microglia by TLRs is detrimental for the human health. Recently scientists have learned that SCFAs are also involved in the activation of microglia. Butyrate seems to increase TLR-dependent responses by inducing their expression, but the underlying mechanism behind it is still to be discovered. (Caputi & Giron 2018)

Motor dysfunction in PD is mainly affected by misfolded α-syn aggregation. Disrupted microbiota could be the cause of this. Neuroinflammation is then induced by α-syn through microglia activation, which causes the build-up of ROS. Degeneration of dopaminergic neurons could also induce this process, however neuroinflammation is also the culprit of neuronal degeneration resulting in a loop of processes. Nevertheless, the exact underlying mechanism of α-syn in PD and if it could be a therapeutic target needs to be investigated more in the future.

2.2 Short-chain fatty acids

As mentioned before, SCFAs execute various roles within the human body and researchers have learnt that SCFAs are probably important in the microbiota-gut-brain axis. It is suggested that the integrity of the blood-brain barrier (BBB) is maintained by SCFAs, which is associated with regulated passage of nutrients and molecules from the circulation to the brain. This is necessary for the brain development and the preservation of CNS homeostasis. This is supported by a study that used GF mice to investigate the effect on the BBB. It was found that there were less tight junction proteins, resulting in an increased permeability of the BBB. (Silva, Bernardi & Frozza 2020) Besides the protection of the BBB, SCFAs help with neuronal survival, inflammatory cascades, and endocrine signalling as well. (Tan et al. 2021) Butyrate seem to be capable of lowering the inflammatory reaction by decreasing microglial activation and pro-inflammatory cytokines secretion, and induces functional and morphological changes in the microglia towards a homeostatic state and inhibits LPS induced pro-inflammatory alterations. (Silva, Bernardi & Frozza 2020) These microbial metabolites (butyrate, acetate, and propionate) are essential for intestinal barrier function, regulation of intestinal motility, and immunological processes in the body and therefore it is highly probable that they are also important to tackle the leaky gut syndrome. The clinical study of Hegelmaier led to the conclusion that dietary interventions with SCFAs could indeed improve the condition of PD patients, but how and via which pathways exactly has not been elucidated yet. (Hegelmaier et al., 2020)

Although, there is evidence of the neuroprotective function of SCFAs, there are also counter statements saying that SCFAs contribute to motor dysfunction in PD by activating microglia. SCFA-fed animals given the anti-inflammatory compound minocycline showed less microglia activity, α-syn aggregation, and improved their motor function. Sampson and his team concluded that the adverse symptoms were the result of SCFAs. (Sampson et al., 2017) Another study showed that under homeostatic conditions microglia in SPF mice matured and functioned normally while the microglia in GF mice were scrubbed. After oral application of SCFAs to GF mice, the microglia were driven to maturation, meaning that the SCFAs are likely to initiate microglia activation. (Erny et al. 2015) In the end there is definitely more research needed to understand the role of SCFAs in PD, but it is highly possible that they play a prominent role in course of the disease.

The microglia can also be triggered with the assistance of astrocytes via secreting the calcium-binding protein S100β. This way astrocytes have an influence on PD as well. S100β is overexpressed in the substantia nigra and its level is increased in the cerebrospinal fluid in patients with PD. Therefore, S100β has been introduced as a marker of PD progression. S100β binds to the receptor for advanced glycation end products on neuron and glial cells and these receptors may have a role as a ligand for α-syn. (Baizabal-Carvallo & Alonso-Juarez 2020)

For the production of SCFAs, a healthy GM is essential. SCFAs have a beneficial role in human health. Not only do they contribute to energy supply, but they have a neuroprotective function as well. The integrity of the BBB is maintained by SCFAs and they help with neuronal survival. Despite of the positive characteristics of SCFAs, some studies have shown the negative features of SCFAs on the disease course of PD, because they activate microglia and in turn worsen motor function in mouse models. Thus, how SCFAs affect PD has to be elucidated more, but the importance of the role of SCFAs is almost certain. (Westfall et al. 2017)

2.3 Manganism

The GM might be altered by heavy metals too and apparently the production of key metabolites can be disrupted as well. Manganese (Mn) is one of the heavy metals that is an important trace element whose function under normal physiological conditions is to act as a cofactor for a myriad of enzymes. Though an overload of cellular Mn accumulation could be neurotoxic to a person when overexposed chronically, which may lead to manganism or PD-like symptoms. An overload is characterised by neurological deficit, neuroinflammation, tremor, dystonia, spasticity, and bradykinesia. Recently, chronic overexposure of Mn is considered as one of the environmental factors that could cause PD, therefore neurotoxicity induced by Mn has been a point of interest in science. (Wang et al. 2020)

Several studies have shown that disturbed Mn levels causes motor dysfunctions in animals. Besides motor function, scientists have discovered that the cognitive and behavioural functions were also altered by abnormal Mn levels. According to a study by Wang et al. Mn exposure led to a clear degeneration and ultimately also necrosis of the pyramidal cell layer in the hippocampus. They hypothesised that overexposure of Mn triggers an inflammatory response, and they indeed found elevated (pro-)inflammatory cytokines and a significant higher number of T-cells in peripheral blood of Mn-treated rats, which will most likely activate the immune system and generate an inflammatory response in the host in the end. It is thought that the metal toxicity is emerged from an Imbalance of redox cycling, and pro-inflammatory cytokines are able to excite ROS. The same study showed that the Mn-treated rats had significantly less antioxidants than the control groups. Peripheral (pro-)inflammatory cytokines can probably pass through the BBB and induce central inflammatory responses. Thus, Mn exposure results in upregulated transcriptions associated with inflammation, neurodegeneration, and activation of microglia. This was supported by the data from Wang's research. (Wang et al. 2020)

Just like TLRs, NLRs are important for the innate immune system. We know most of the NLRP3 inflammasome and it consists of the adapter protein apoptosis-associated speck-like protein (ASC) and procaspase-1. In the case of Mn-exposure NLRP3 is triggered by TLR-4, which has activated the nuclear factor kappa B (NF-κB) signalling pathway. Through NF-κB the transcription of pro-IL-1β and pro-IL-18 is upregulated. The second step is forming a complex of the NLRP3, ASC, and procaspase-1. This transforms procaspase-1 into caspase-1, and will mature IL-1β and IL-18. When active, IL-1β is able to induce continuous oxidative stress or pro-inflammatory environment in the brain, leading to increased neuroinflammation. Rats overexposed to Mn showed increased levels of NLRP3 and IL-1β. They also showed raised caspase-1 cleavage. Altogether, these processes are evidence of NLRP3 inflammasome activation. Initiating the NLRP3 inflammasome mechanism by Mn/TLR-4 might result in ROS production, further activation of NLRP3, neuroinflammation, and neurodegeneration through active IL-1β. (Shao et al. 2015; Wang et al. 2020)

Although not much is known yet about manganism in other neurodegenerative diseases, it is interesting to investigate the effect of heavy metals on (neurodegenerative) diseases. Until now, there seems to be a strong correlation between aluminium and AD besides manganism and PD. Other metals showed inconsistent results in different studies. (Cicero et al. 2017)

3. How the gut microbiome affects the drug therapy with Levodopa

Levodopa (L-3,4-dihydroxyphenylalanine or L-DOPA) is a drug treatment often used to treat PD patients with. Usually, levodopa will be combined with an aromatic amino acid decarboxylase inhibitor such as carbidopa. Unfortunately, the dosage of levodopa and/or decarboxylase inhibitor varies significantly among PD patients to provide the brain with enough amounts of dopamine. As a result, this treatment seems to be ineffective in a portion of PD patients. The efficacy of levodopa also decreases over time which causes the necessity to use higher and more frequent dosages. Researchers have discovered that the bacteria in the gut have a metabolizing effect on levodopa. *Lactobacillus* and *Enterococcus* especially have tyrosine decarboxylase properties that are capable to decarboxylate levodopa and explain the need for higher dosage in some PD patients. (van Kessel et al. 2019) A small group of PD patients that have a heightened level of *Helicobacter pylori* struggle with the treatment as well due to the possible binding of *H. pylori* to levodopa, making the drug less effective. (van Kessel et al. 2019; van Kessel & EL Aidy 2019) However, the results differ between studies which indicate that the exact mechanism behind the effect of *H. pylori* should be studied further.

Most PD patients experience gut motility problems and although they are treated with levodopa, it still seems that this problem is not fully resolved. Around 10% of the levodopa is not absorbed by the intestines and a study by van Kessel et al. showed that *Clostridium sporogenes* could deaminate the leftover levodopa in the distal gastrointestinal tract to 3-(3,4-dihydroxyphenyl)-propionic acid (DHPPA) which reduces the gut motility ex vivo. The mechanisms behind the inhibitory function of DHPPA on gut motility is yet to be discovered, it is thought that DHPPA hinders the acetylcholine-induced muscle contractions in the gut. (van Kessel et al. 2020)

4. Conclusion & Discussion

Logically, the GM is disturbed in patients suffering from chronic disorders of the gut like IBD or celiac disease, but recent discoveries show that the GM is also altered in patients suffering from neurodegenerative diseases (Hirschberg et al. 2019). Normally as people become of age the GM changes, but the GM of PD patients still has a more proinflammatory tendency than their age-matched healthy controls. The dysbiosis of the gut may affect the α-syn pathology in PD. Motor function impairment is due to a shortage of dopamine in the brain and is caused by degeneration of the dopaminergic neurons. Microglia activation occurs because of the degeneration. However, α-Syn seem to activate microglia as well. Due to neuroinflammation the dopaminergic neurons will further degenerate. (Sampson et al. 2016)

When opportunistic bacteria overrule the good bacteria in the gut, the production of SCFAs will decrease. Anaerobic fermentation of dietary fiber and resistant starch by bacteria produce these metabolites and they are known to play a role in energy supply and neuroprotection, (Westfall et al. 2017) though the conclusions differ between papers as some say that SCFAs initiate microglia activation and therewith neuroinflammation (Sampson et al. 2016)

To overcome the dysbiosis of the GM, the use of probiotics is proposed as a therapeutic strategy. Probiotics are the good bacteria that have a positive influence on the host when given in an adequate quantity. (Hirschberg et al. 2019) If probiotics could be used to treat PD needs further investigation as there are still gaps in the current data. Probiotics do not work well for patients who have Crohn's disease. (Hirschberg et al. 2019; Tan et al. 2021) As mentioned before, faecal microbiota transplantation showed promising results in PD patients and could be a potential therapy, but long-term studies need to be done before it could be implemented. (Hegelmaier et al. 2020)

Heavy metals like lead and mercury are harmful for human health, but in what way (heavy) metals increases the risk of developing neurodegenerative diseases is still not known. (Giacoppo et al. 2014). Manganism seem to influence motor function in animal models and give PD-like symptoms. An overload of Mn could also initiate neuroinflammation via TLRs and NLRs. Despite, the possible significant correlation between Mn neurotoxicity and PD, several meta-studies found that the increased risk of PD could not be linked to Mn. (Cicero et al. 2017)

Currently the treatment for PD is levodopa often combined with an aromatic amino acid decarboxylase inhibitor. Unfortunately, not all PD patients respond well on the treatment and the efficacy of levodopa decreases over time. It raised the question if GM also interacts with the drug and if via acting on GM the use of levodopa could be made more beneficial for the patient to prevent higher dosage with the negative consequences of side-effects. Apparently, PD patients' dysbiosis of the gut could result in a poor function of levodopa because of the metabolism of levodopa by *Lactobacillus* and *Enterococcus*. Besides, *C. sporogenes* could worsen the gut-motility function via DHPPA. (van Kessel et al. 2019; van Kessel et al. 2020) It has been proposed to use dopamine antagonists directly on the gut because it seemed that dopamine or dopamine agonists in the gut lowers the stomach motility. The absorption of levodopa is dependent on the gut-motility. However, the results did not show an improved motility of the gut nor prevented the inhibitory function of dopamine, and usually dopamine agonists are given together with levodopa. Thus, before current treatments will be adjusted, more research on the effect of dopamine on gut-motility is needed. (van Kessel & El-Aidy 2019)

Overall, it can be concluded that the gut-brain axis has a relevant role in the course of PD. The exact underlying mechanisms are yet to be discovered, but more needs to be explored as there are gaps and contradictions in current literature before the gut-brain axis could be used as a therapeutic target for PD.

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