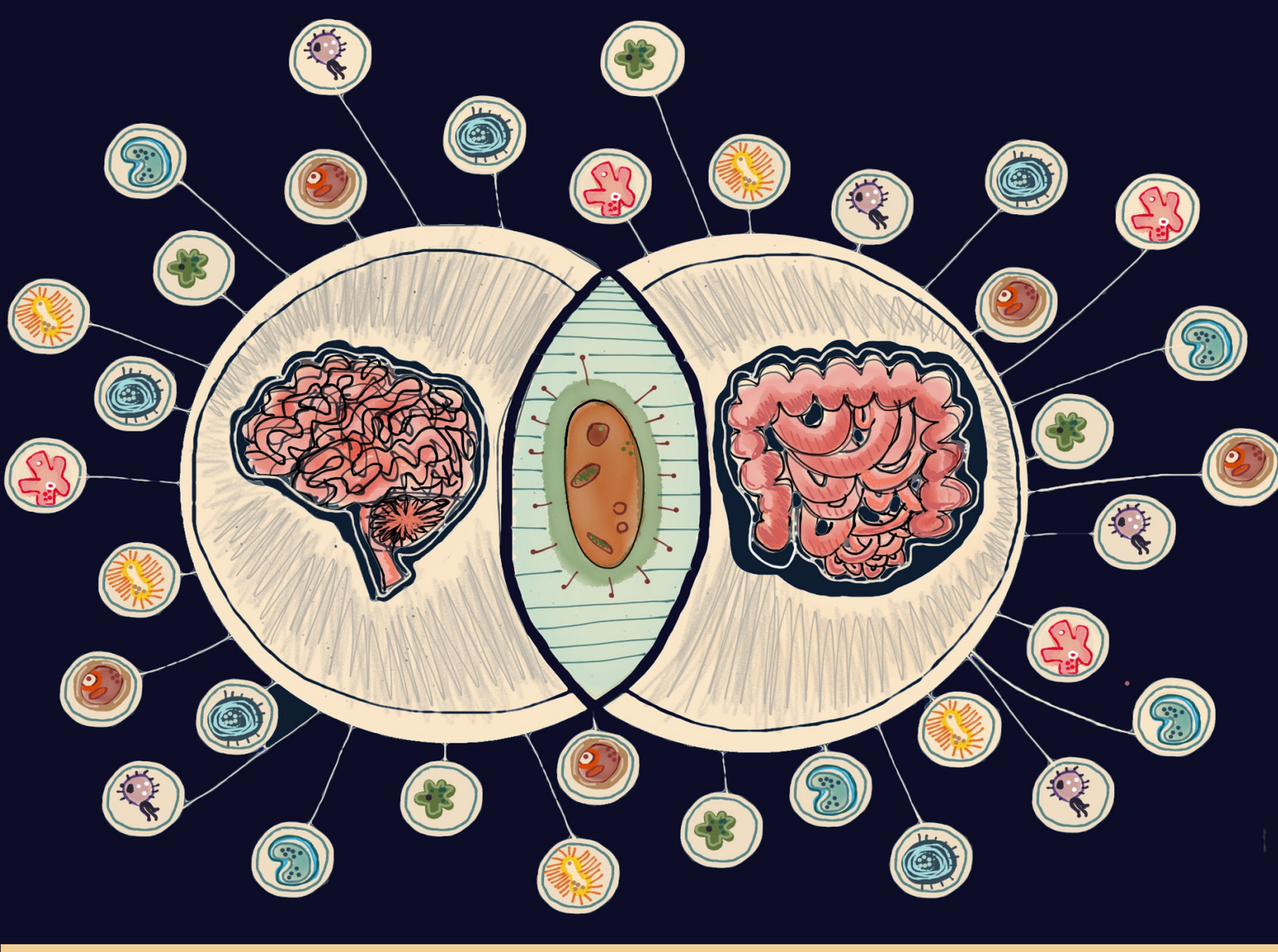

Ana Rita Cardoso Fernandes

The Pathogenesis of Parkinson Disease: The microbiota-gut-brain axis

Monografia realizada no âmbito da unidade Estágio Curricular do Mestrado Integrado em Ciências Farmacêuticas, orientada pelo Professor Doutor João António Nave Laranjinha e apresentada à Faculdade de Farmácia da Universidade de Coimbra

Julho 2016

· U C ·

Universidade de Coimbra

Ana Rita Cardoso Fernandes

The Pathogenesis of Parkinson Disease: The microbiota-gut-brain axis

Monografia realizada no âmbito da unidade Estágio Curricular do Mestrado Integrado em Ciências Farmacêuticas, orientada pelo Professor Doutor João António Nave Laranjinha e apresentada à Faculdade de Farmácia da Universidade de Coimbra

Julho 2016

· U C ·

UNIVERSIDADE DE COIMBRA

Cover picture adapted from:
Hair, kaitlyn, 2015. Me, Myself and Microbes [WWW Document]. Glas. Insight Into Sci. Technol. URL http://linkinghub.elsevier.com/retrieve/pii/S0165085 13002928 (accessed 6.28.16).

Declaração de Integridade

Eu, Ana Rita Cardoso Fernandes, estudante do Mestrado Integrado em Ciências Farmacêuticas, com o nº 2011155952, declaro assumir toda a responsabilidade pelo conteúdo da Monografia apresentado à Faculdade de Farmácia da Universidade de Coimbra, no âmbito da unidade de Estágio Curricular.

Mais declaro que este é um trabalho original e que toda e qualquer afirmação ou expressão, por mim utilizada, está referenciada na Bibliografia desta Monografia, segundo os critérios bibliográficos legalmente estabelecidos, salvaguardando sempre os Direitos de Autor, à exceção das minhas opiniões pessoais.

Coimbra, 30 de junho de 2016.

_____

Agradecimentos

Ao Professor Doutor João António Nave Laranjinha, agradeço a orientação, os sábios conselhos e todos os conhecimentos transmitidos durante o meu percurso académico.

À Dr.ª Bárbara Rocha, agradeço o diligente auxílio, a aprendizagem proporcionada e o exemplo que se tornou.

Aos meus pais, que tornaram possível esta concretização, pelo infindável apoio, pela minha educação e pelos valores que me incutiram. Ao meu impaciente irmão pela paciência e por me acompanhar todos estes anos.

Ao Thierry, pela constante presença, pela força e pela alegria.

À minha madrinha de curso, Rita Leão, pelo incansável altruísmo e pela prezada amizade.

À minha restante família e aos meus amigos e colegas que me acompanharam neste percurso, um sincero obrigada!

INDEX

ABREVIATIONS/ACRONIMS:

ANS – Autonomic Nervous System

CNS – Central Nervous System

DMNV – Dorsal Motor Nucleus of the Vagus Nerve

ENS – Enteric Nervous System

GI – Gastrointestinal

LPS – Lipopolysaccharide

NMS – Non Motor Symptoms

PD – Parkinson's Disease

PIGD – Postural Instability and Gait Difficulty

SIBO – Small Intestine Bacterial Overgrowth

TLR - Toll-Like Receptor

ABSTRACT:

Parkinson's disease (PD), the second most prevalent neurodegenerative disorder in older adults, is mainly characterized by the loss of dopaminergic neurons in the *substantia nigra*, located in the midbrain. The neural loss in PD is usually linked to α-synuclein aggregation and accumulation in neural structures from the autonomic and the central nervous systems. Symptomatically, PD patients undergo motor features (tremor, bradykinesia, rigidity) but also a substantial number of non-motor symptoms (gastrointestinal impairment, sensorial dysfunctions, depression) that may precede the motor features by years. A markedly non-motor symptom among PD patients is constipation. Indeed, a brain-gut bidirectional communication ensuing the enteric nervous system (ENS) has been implied in the pathogenesis of PD.

In the recent years, the role of gut microbiota has been intensively discussed in the disease progression, largely motivated by accumulating evidences on the high prevalence of dysbiosis in PD patients. Therefore, a microbiota-gut-brain axis, encompassing reciprocal influence of microbiome and superior mental functions, has been recently advocated. The dysbiosis is thought to have an impact in the gut permeability leading to bacteria and endotoxins translocation which, in turn, may trigger α-synuclein accumulation and spreading through the ENS.

Environmental factors such as pesticides, herbicides, diet among others have been shown to exert an impact on the microbiota homeostasis and therefore in the disease progression, although further research is needed to ascertain the role of these factors in the etiology of the disease. Accordingly, manipulation of microbiota's composition with pre and probiotics and antibiotics, targeting certain bacterial species, was shown to reduce gut permeability and to improve the motor features in PD patients, respectively. Moreover, monoclonal antibodies and oligomers modulators, new therapeutics aiming the reduction of intra and extracellular α-synuclein, have been developed with positive preliminary results.

Thus, this work is aimed at discussing the microbiota-gut-brain axis as a novel approach to PD, emphasizing molecular mechanisms in connection with physiological processes and potential therapeutic strategies.

RESUMO:

A doença de Parkinson (PD) é a segunda perturbação neurodegenerativa mais prevalente na população acima de 65 anos. Caracteriza-se, essencialmente, pela perda de neurónios dopaminérgicos na *substantia nigra pars compacta*, localizada no mesencéfalo. É largamente aceite que a degeneração neuronal na PD surge a partir da agregação e acumulação de α-sinucleína em estruturas neuronais dos sistemas nervoso autonómico e central. Em termos de sintomatologia, os pacientes com PD experienciam sintomas motores (tremores, bradicinesia, rigidez), mas também um significativo número de sintomas não-motores (comprometimento gastrointestinal, disfunções sensoriais, depressão), que podem preceder os primeiros em vários anos. Um sintoma não-motor muito marcado em pacientes com PD é a obstipação. Consequentemente, uma comunicação bidirecional intestino-cérebro, envolvendo o sistema nervoso entérico, tem sido implicada na patogénese da PD.

Nos últimos anos, tem havido uma intensiva discussão na comunidade científica acerca do papel que o microbiota intestinal desempenhará na progressão da PD. Esta ideia tem sido suportada por evidências que demonstram uma elevada prevalência de disbiose em indivíduos com PD. Neste contexto, foi formulado o conceito "eixo microbiota-intestino-cérebro", que postula uma influência mútua entre o microbiota e as funções cerebrais. A disbiose tem um impacto na permeabilidade intestinal, o que possibilita a passagem de bactérias ou endotoxinas para além da lâmina própria, a designada translocação bacteriana. Por sua vez, este fenómeno poderá funcionar como um desencadeador da acumulação e transmissão da α-sinucleína através do sistema nervoso entérico.

Fatores ambientais como os pesticidas, herbicidas, dieta, entre outros, têm demonstrado ter um impacto significativo na constituição do microbiota e, portanto, na progressão da PD, apesar de ser necessária mais investigação neste âmbito. Por outro lado, a manipulação das estirpes bacterianas que constituem o microbiota com, por exemplo, pre e probióticos e com antibióticos de espectro para determinadas espécies bacterianas (*Helicobacter pylori*), demonstraram induzir uma redução da permeabilidade intestinal e uma melhoria nos sintomas motores, respetivamente. Adicionalmente, novas terapêuticas direcionadas para a redução da α-sinucleína intra e extracelular, de que são exemplo anticorpos monoclonais e oligómeros moduladores, têm sido desenvolvidas com resultados preliminares promissores.

Assim, este trabalho discute de modo crítico o eixo microbiota-intestino-cérebro como uma nova via envolvida na PD, relacionando mecanismos moleculares com processos fisiológicos e potências estratégias terapêuticas.

INTRODUCTION

The Parkinson's Disease

Parkinson's Disease (PD) is the second most common prevalent neurodegenerative disease affecting 1-2% of population above 65 years old (Valeria D. Felice et al., 2016). The essential symptoms for the clinical diagnosis are motor alterations such as bradykinesia, rigidity, rest tremor and postural instability. Notwithstanding, it has been realized that numerous non-motor symptoms (NMS) may be associated with PD that precede the onset of the motor symptoms by many years. These NMS encompass neuropsychiatric disorders (such as anxiety and depression), autonomic nervous system (ANS), including enteric nervous system (ENS) dysfunction, sleep disorders and sensory alterations (pain, hyposmia and taste impairment) (Fasano et al., 2015; Valeria D. Felice et al., 2016; Klingelhoefer and Reichmann, 2015). These may as well have a considerable or even greater impact than the motor symptoms in the patients quality of life (QoL), especially in the years before the installation of the disease. The main problems associated with the ANS and ENS dysfunction are gastrointestinal (GI) disorders, such as drooling, dental problems, constipation, impaired gastric emptying (gastroparesis), *Helicobacter pylori* infection and small intestinal bacterial overgrowth (SIBO) (Fasano et al., 2015; Valeria D. Felice et al., 2016). In particular, constipation was found to affect 80% of PD patients.

Biochemically, PD is characterized by the accumulation of α-synuclein in the brain, specifically in the *substantia nigra pars compacta.* The accumulation of α-synuclein in the form of Lewy bodies and Lewy neurites (Fasano et al., 2015), leads to selective degeneration of dopaminergic neurons, which then affects the signalling to other brain regions, such as the striatum (Klingelhoefer and Reichmann, 2015). Interestingly, a rostrocaudal gradient of phosphorylated α-synuclein in the ENS was identified in the gut at early stages of PD (Cersosimo et al., 2013).

In the framework of PD pathogenesis, an important mechanism that has been widely addressed is mitochondrial dysfunction. In fact, dysfunction of respiratory complex I, oxidative stress, inflammation and protein mishandling are considered hallmarks of PD. It has been suggested that α-synuclein aggregates inside neurons, impairing the mitochondrial activity of the complex 1. A vicious cycle then establishes as, in turn, mitochondrial dysfunction induces oxidative stress in the neuron, i.e. the concentration increase of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Moreover, a selective toxicity of the dopaminergic neurons was explained by the intrinsic sensitivity to complex I defects in the *substancia nigra* (Klingelhoefer and Reichmann, 2015).

Thus the maintenance of the redox balance is a crucial feature for maintaining homeostasis. Low levels of ROS are essential for the myriad of signalling pathways, such as gene transcription, protein kinase activation and phosphatase inhibition, among many others. Altered levels of these oxygen species, by disruption of the biological processes regulating the redox balance, may result in a persistent and unresolved inflammation, not only in the brain but also in the intestinal mucosa. The integrity of this barrier has a tremendous importance for exposure to exogenous noxious substances or even to the gut microbiota could lead to serious complications. Thereby the critical role of a rapid resealing of the epithelial layer (Aviello and Knaus, 2016).

The etiology of PD, a clinically heterogeneous disorder, is thought to be dominated by the influence of environmental factors (pesticides, herbicides, metals) over the genetic susceptibility. Moreover, the GI alterations in the early stages of PD suggest the involvement of gut signalling in the etiopathogenesis of the disease. Particularly, the bidirectional interaction between gut microbiota and the nervous system has been intensively studied in the recent years, regarding its influence in the development of PD (Bope and Kellerman, 2016; Scheperjans et al., 2015).

The synuclein spreading hypothesis

PD is a multicentre neurodegenerative disorder characterized by the accumulation and aggregation of α-synuclein in the *substantia nigra* (Mulak and Bonaz, 2015) and other brain regions. Nevertheless, there is growing evidence for abnormal α-synuclein accumulation outside the brain namely in the ENS neurons of the myenteric submucosal plexus of the GI tract (Fasano et al., 2015; Valeria D. Felice et al., 2016). The concentration gradient of phosphorylated α-synuclein (higher concentrations in the submandibular gland and lower in the colon, the enteric rostrocaudal gradient) follow the innervation pattern of the vagal nerve (Fasano et al., 2015) (Fig. 1). The parasympathetic fibres of the vagus nerve originate in the brainstem and innervate, among others, the abdominal viscera excluding the descending colon and rectum, thus controlling the motility and secretion of the great majority of the bowel. Likewise, phosphorylated α-synuclein is also detected in the olfactory bulb and correlates with PD (Fig. 1). Therefore, an α-synuclein trans-synaptic cell-to-cell transmission from the gut through the ANS to the substantia nigra has been proposed (Klingelhoefer and Reichmann, 2015). Another study suggests that α-synuclein removed from neurons, either are transported via endocytosis to neighbouring neurons or to neuronal precursors cells (Danzer et al., 2012).

This transmission have alternatively been explained as a "prion-like" mechanism where the misfolded α-synuclein propagates and accumulates augmenting the protein aggregates (Visanji et al., 2013). Accordingly, recent observations in experimental models reveal that misfolded protein can propagate from one neuron to another in a prion-like fashion led to the hypotheses of misfolded α-synuclein being itself a propagating agent (Derkinderen et al., 2014). Interestingly, given that both the olfactory and the GI systems are gateways to the external environment, these novel hypothesis support the pivotal contribution of environmental agents on the onset of the disease.

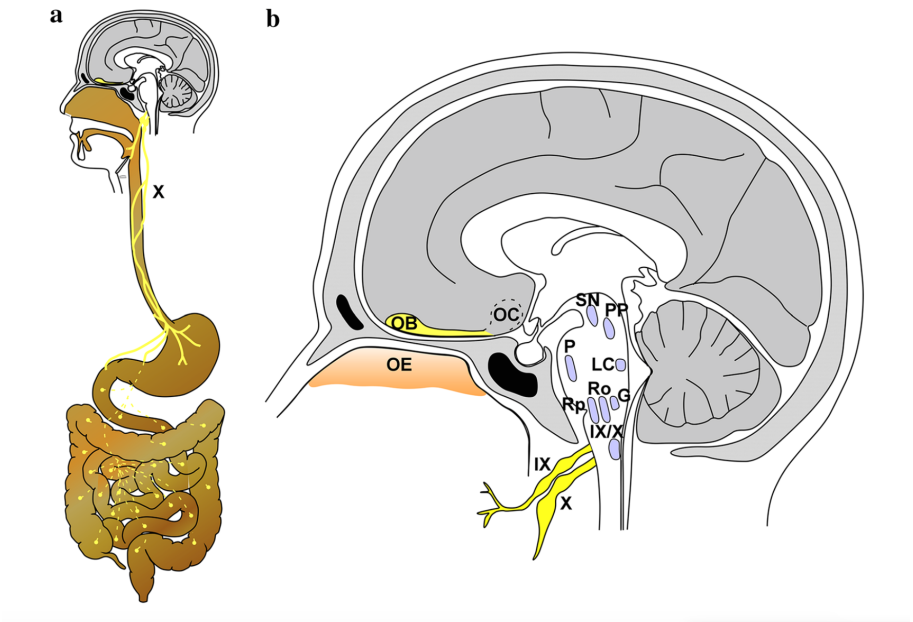

Figure 1: *Scheme showing the possible routes for α-synucleinopathy progression through the peripheral and central nervous systems (a) and the location of structures involved in α-synucleinopathy (b). G gigantocellular reticular nucleus, LC locus coeruleus, OB olfactory bulb, OC olfactory cortex, OE olfactory epithelium, P pontine nuclei, PP peduncle pontine nucleus, Ro nucleus raphe obscurus, Rp nucleus raphe pallidus, SN substantia nigra, IX glossopharyngeal nerve, IX/X glossopharyngeal/ vagal dorsal motor nucleus, X vagus nerve* (Ubeda-Bañon et al., 2014).

The spreading of the pathology from the gut, through the ENS, to the CNS has been supported by several experiments. The accumulation of inclusions similar to Lewy bodies in engrafted neuronal precursor cells and in grafted neurons in the PD patients who had received fetal mesencephalic transplants has been observed (Klingelhoefer and Reichmann, 2015). Also animal studies using mice that underwent an hemivagotomy, demonstrated less dopaminergic

cell death in the *substantia nigra* and less α-synuclein accumulation in the ipsilateral dorsal motor nucleus of the vagus nerve (DMNV) (Klingelhoefer and Reichmann, 2015; Pan-Montojo et al., 2012). Accordingly, an epidemiological study, where the results were adjusted for possible confounders, reported a lower risk of PD in patients who underwent truncal vagotomy (Svensson et al., 2015). In addition, the intact synaptic pathways turned out to be a requirement to the progression of the pathology given that disruption of nerve connections was found to cease the accumulation of α-synuclein in the ENS, the DMNV, the intermediolateral nucleus of the spinal cord and the *substantia nigra* (Klingelhoefer and Reichmann, 2015; Pan-Montojo et al., 2010).

The Braak's staging system

In order to distinct the different stages of PD, Braak and his colleagues developed the Braak's staging system, which was afterwards adjusted to subsequent evidence. In this staging system the onset of the pathology was asserted to begin in the olfactory bulb, the ENS, the intermediolateral nucleus (IML) of the spinal cord and the DMNV. In this regard it is important to emphasize the challenged validity of this system due to conflicting evidence, namely the diverse distribution of Lewy bodies through the body. Considering the neuropathological changes in PD follow a specific chronological and regional pattern, this staging system is consistent with the hypotheses that environmental factors may trigger the pathology and as well with spreading of the pathology from the ENS to the CNS (Klingelhoefer and Reichmann, 2015).

Braak and his colleagues suggested that the disease would start in the gut and spread to the CNS via the vagus nerve and the spinal cord, thus establishing the gut-brain axis as a central pathway in the disease. Several evidences subsequently corroborated this hypotheses, namely the finding of Lewy bodies (comprised of mainly α-synuclein and ubiquitin) in post-mortem cases of early PD and the recent study demonstrating that α-synuclein injected in the gut wall of rats migrated to the brain in the vagus nerve at a rate estimated to be 5-10 mm per day (Ghaisas et al., 2016; Holmqvist et al., 2014). This may implicates a start of pathology in the most distal terminals of the vagus nerve, far away from the central nervous system (Vizcarra et al., 2015).

In this work we will discuss the role of microbiota and other environmental factors in PD through the microbiota-gut-brain axis.

The microbiota

The human microbiota, known for many years as the microflora, is now perceived as the superorgan of the human body, outnumbering the eukaryote cells by a factor of 10. These microbes (bacteria, viruses, archaea) cover all body surfaces but 10% of which inhabit the human gut, where 10^{12} bacterial cells per gram are found in the colon. Although the microbiota comprises several phyla, 90% of the bacteria belong to the *Bacteroidetes* and the *Firmicutes*. The composition of microbiota is highly variable between individuals and even within an individual, certain physiological and pathological conditions may modify the bacterial profile. Nevertheless, a functional redundancy within certain microbial groups allowing the microbiota's proper function has been suggested (Cassani et al., 2015; Sekirov et al., 2010). Gut microbiota contributes to the human homeostasis by preventing colonization by pathogens, synthesising molecules such as immunomodulatory short-chain fatty acids (SCFA), vitamins (folate and thiamine) and neurotransmitters such as serotonin (5-HT) and γ-aminobutyric acid (GABA). These microbes also selectively allow the absorption of certain substances (vitamins, medication, toxic compounds), harvest energy from otherwise ingestible nutrients and modulate local and systemic immune-inflammatory responses (Cassani et al., 2015; Ghaisas et al., 2016; Scheperjans et al., 2015). The influence of the intestinal microbiota in the nervous system, through what has been referred has the gut-brain axis, has been highlighted with implications in neurodegenerative diseases like PD, Alzheimer's Disease (AD) and Amyotrophic Lateral Sclerosis (ALS), among many other multiorganic diseases (Autism, Diabetes Mellitus, Multiple Sclerosis) (Fang, 2015; Ghaisas et al., 2016).

THE MICROBIOTA-GUT-BRAIN AXIS: A NOVEL CONCEPT IN PD

The motor symptoms in PD are likely to appear only after the degeneration of over 80% of the dopaminergic neurons in the *substantia nigra* and the dysfunction of the nigrostriatal dopaminergic pathway (Klingelhoefer and Reichmann, 2015). This reveals a considerable time interval between the onset of the disease and the diagnosis.

Constipation is the most common GI symptom in PD, reported in 80-90% of the patients and it has also been reported to develop as far back as 15.3 years before the motor features. Moreover, impaired gastric emptying (gastroparesis) has a prevalence in 70%-100% (Fasano et al., 2015). This clinical evidence is in line with early disturbances in gut homeostasis.

Recent research has highlighted an important gut feature in PD: *the intestinal microbiota, also referred as the second brain*. Evidence is now accumulating that supports central role of

intestinal microbiota not only in the gut homeostasis but also in the regulation of a myriad of physiological processes contributing to human health. In particular, it has been recognized the microbiota-gut-brain axis, a bidirectional communication between the CNS and the gastrointestinal tract involving neural pathways but also immune and endocrine mechanisms. Microbiota modulates digestive processes (motility, secretion), immune function, perception and emotional response to visceral stimuli (Valeria D. Felice et al., 2016), influencing brain activity, levels of neurotransmitters receptors and neurotrophic factors (Scheperjans et al., 2015).

Several symptoms associated with microbiota-gut-brain axis have been likewise related to some PD symptoms, mainly the early GI involvement. Moreover, the evidence that environmental factors influence both gut bacteria and PD support the role of dysbiosis (altered gut microbiota profile) in PD. Accordingly, Scheperjans *et al,* by studying the composition of fecal microbiome from 72 PD patients using high throughput pyrosequencing showed a reduction of 77.6% of the Prevotellaceae abundance in comparison with control subjects. *Prevotella* is the main contributor of a gut microbiome enterotype (a suggested microbiota stratification) (Scheperjans et al., 2015). This enterotype is associated with higher levels of the neuroactive SCFA (produced from soluble fibres) and high biosynthesis capacity of thiamine and folate (Arumugam et al., 2011). They similarly found a positive correlation between the Enterobacteriaceae abundance and the postural instability and gait difficulty (PIGD) phenotype, which are the non-tremor dominant patients. This PD phenotype tend to have a worse prognosis and show more severe α-synucleinopathy in the colonic ENS (Scheperjans et al., 2015). The relevance of this work becomes evident when considering it establishes a connection between the gut microbiota and the motor phenotype of PD (Wood, 2014). In this study the researchers also show higher levels of *Lactobacillaceae*, *Verrucomicrobiaceae*, *Bradyrhizobiaceae* and *Clostridiales Incertae Sedis IV*. The deregulation of the Prevotella and Lactobacillus has been associated with impaired ghrelin secretion in PD patients (Scheperjans et al., 2015). This incretin, which abnormal function might be implicated with gastroparesis (Fasano et al., 2015), has a regulatory function of the nigrostriatal dopamine pathway, which may imply a protective role in PD. Furthermore, Lactobacillaceae modulate activity of ENS neurons and vagal afferents, thereby, this bacterial family may have an impact in the α-synuclein secretion (Scheperjans et al., 2015).

Remarkably, Cakmak highlighted the effect of decreased Prevotella abundance in PD, showing that it may be associated with the decrease of hydrogen sulphide, a gaseous neurotransmitter secreted by certain bacteria of this family and that plays a protective role on dopaminergic neurons in rat models (Cakmak, 2015).

On the other hand, Cassani *et al.* by measuring the concentration of urinary indican (indoxyl sulphate), a marker of intestinal dysbiosis, in PD and control patients observed significantly higher indican urinary concentrations in the PD patients than in the control group. These results were consistent with those observed in *de novo* patients, suggesting that the detection does not depend of the duration of the disease. The indican is a metabolite originated from the bacterial metabolism of tryptophan in the gut. Therefore, conditions that interfere with the bacterial balance, such as SIBO, malabsorption or constipation, will lead to intraluminal increase of amino acids, including tryptophan, that is in turn converted into indican (Cassani et al., 2015). In sum, both studies support the role of gut microbiota in PD.

One of the deleterious consequences related to changes in the gut microbial profile is an increase of gut epithelial permeability that leads to local and systemic inflammation, likely due to translocation of bacteria or bacterial antigens and endotoxins (Valeria D. Felice et al., 2016; Hyland et al., 2014), that is, the passage of viable resident bacteria from the GI tract to normal sterile tissues (Potgieter et al., 2015). The translocation of such substances has been hypothesised to be an environmental factor that triggers α-synuclein accumulation and aggregation in the colon of PD patients (Scheperjans et al., 2015). In turn, the α-synucleinopathy may, as discussed above (*see The spreading hypothesis*), spread via the vagal nerve up to the DMNV, ultimately contributing to the neurodegenerative process.

Lipopolysaccharide (LPS), a major component of Gram-negative bacteria wall, modulates GI motility and when it surpasses its physiological effects, it may increase gut permeability. LPS has been associated with pro-inflammatory reactions through the LPS/Toll-Like Receptor/Nuclear Factor-Kappa B (NF-κ B) pathway and with the production of inflammatory cytokines upon LPS absorption in the gut. Indeed, LPS is used as a toxin-induced model of PD (Fang, 2015). LPS binding protein (LBP), a pro-inflammatory marker, was shown to be increased in PD patients (Valeria D. Felice et al., 2016) implicating high exposure to LPS. High-fat diets have also been shown to induce gut microbiota alterations, increasing the number of LPS-containing bacteria (Francino, 2016). This is an example of one environmental factor influencing the microbiota composition and ultimately affecting the development of PD. It is noteworthy that α-synuclein itself exhibits pro-inflammatory effects. Extracellular α-synuclein was shown to release cytokines and activates microglia. Whereas, neuron-derived α-synuclein led to nuclear fragmentation and caspase 3 activation of the affected cells (Klingelhoefer and Reichmann, 2015).

In a different context, but still regarding GI mucosal physiology, *Prevotellaceae* has been shown to promote mucin synthesis. The decrease of mucin due to the low levels of *Prevotella* may weaken even more the gut wall, increasing as well its permeability (Vizcarra et al., 2015).

Still in connection with gut barrier function, Clairembault *et al.* demonstrated morphological changes in the gut, namely the down regulation of the tight junction component – occludin – in PD patients (Clairembault et al., 2015). Moreover, a decreased tight junction ZO-1 in duodenum and in distal colon was identified in MPTP mouse models (Fang, 2015).

The number of bacteria in the small intestine is tightly controlled by several intrinsic mechanisms, such as the gastric acid, which destroys a considerable number of bacteria, and ileocaecal valve, among others (Fasano et al., 2015; Grace et al., 2013). Diseases related with impaired GI motility, such as Parkinson's and Diabetes Mellitus, predispose for abnormal translocation of bacteria to the small intestine mucosa (Derkinderen et al., 2014) (Fig. 2). PD patients with SIBO have more severe motor fluctuations than those without, likely because SIBO may disrupt the small intestinal barrier leading to immune activation or impaired absorption of antiparkinsonian medication (Fasano et al., 2015).

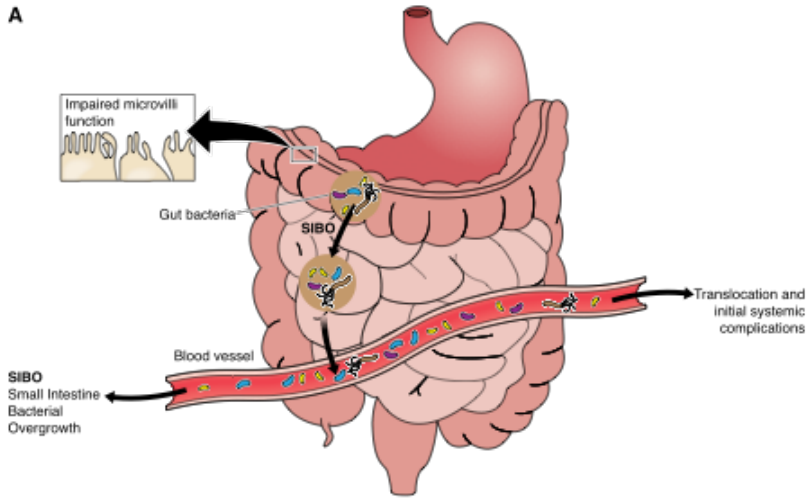

***Figure 2**: Gut microbiota translocation from the colon to the small intestine, causing small intestine bacterial overgrowth (SIBO). SIBO facilitates the entrance of some of the displaced microbiota into the bloodstream by breaching the endothelial barrier. (Adapted from:* (Sekirov et al., 2010)*).*

The microbiota has been suggested as a primer of the innate immune system through a mechanism referred to as molecular mimicry (MM). MM is explained by similarities of nucleotide sequence and/or protein configuration among certain microbial and human proteins. These may cross-seed between each other, leading to an altered response of the immune system (Friedland, 2015), which may be either reduced or enhanced. Hence, MM would have an influence the health and disease of an individual. In Parkinson's, the cross-seed would happen between an exogenous protein from amyloid-containing bacteria and the endogenous amyloid (α-synuclein in the PD case). The immune response to the "hybrid"

amyloid structure would be enhanced relatively to the endogenous amyloid through TLR-mediated pathways (Friedland, 2015). Besides the cross-seeded misfolding elicited by bacterial proteins in PD, Friedland also proposed that these proteins induce inflammation and oxidative stress, thereby causing cellular toxicity in the neural structures (Friedland, 2015).

OTHER ENVIRONMENTAL FACTORS

Extensive evidence indicates an inverse relation between coffee drinkers and cigarette smokers and the PD incidence. Several explanations for this relationship have been pointed out, among them the premorbid personality trait related with coffee-drinking and smoking dislike or the neuroprotective role of caffeine and nicotine in the neural structures. Derkinderen et al proposed another hypothesis to explain this evidence namely the mitigation of the intestinal inflammation due to alterations in the gut microbiota following the consumption of coffee and cigarettes. This reduction in inflammation would result in a decreased misfolding of α-synuclein in the ENS, minimizing its propagation to the CNS (Derkinderen et al., 2014). Indeed, not only coffee but also other caffeine-containing beverages such as black tea and Chinese and Japanese tea have shown this relation with PD prevalence. (Mulak and Bonaz, 2015). Coffee and tobacco were also found to increase bacteria that counteract certain forms of chronic GI infection, which is the case of *H. pylori*. Moreover, coffee has been shown to increase *Bifidobacterium* in the gut, with ensuing anti-inflammatory properties (Derkinderen et al., 2014).

As already discussed, diet plays also an important role in PD pathogenesis. Accordingly, it has been reported a higher prevalence of PD among individuals consuming dairy products (Cassani et al., 2015). The modulation of the microbial composition leading to a more efficient uptake of energy from nutrients has been advocated. In line with this notion, the consumption of a Mediterranean diet is recommended in early PD (Barichella et al., 2009).

Chemical substances such as pesticides and fungicides as paraquat, rotenone and maneb as well as heavy metals such as iron, lead, mercury, cadmium, arsenic and manganese have also been shown to increase the risk of PD. Likewise, factors as living in rural areas, farming and drinking well water have as well been pointed as risk factors for PD. For these environmental factors, the relation with the microbiota has not been determined (Ghaisas et al., 2016).

Antibiotics are known to induce significant alterations in the microbiota that can persist for months or years. These compounds have shown to change gene expression, protein activity and overall metabolism of the gut microbiota, in addition to taxa replacement within the bacterial community. Following antibiotics exposure, increased susceptibility towards

intestinal infections and a shift in SCFA production have been observed. These changes have also been detected during gut dysregulation of PD patients. The effectiveness of both innate and adaptive immune responses is as well disturbed. One example of this indirect alteration caused by antibiotics is the different repertoire of microbial-associated molecular patterns (MAMP) observed in the receptors of the immune epithelial cells (Francino, 2016).

THERAPEUTICS AND BIOMARKERS/FUTURE DIRECTIONS

Diagnosis

The reported accuracy of a clinical PD diagnosis is 26% to 92%, improving with the disease duration and the responsiveness to medication (Adler et al., 2014; Scheperjans et al., 2015). With this in mind, it became critical to develop more accurate diagnostic tools. Hence, several biomarkers have been studied. Scheperjans *et al* proposed the *Prevotella* quantification in the fecal microbiome not as a PD positive biomarker because of its low specificity for PD, but as an exclusion biomarker seeing that a person with a high abundance of Prevotellaceae was very unlikely to have PD. It was also suggested a combined quantification of the Prevotella and other 4 bacterial families and the employment of the Wexner total score as a clinical measure of constipation, that showed a specificity of 90.3% (Scheperjans et al., 2015).

On the other hand, colonic α-synuclein has been reported not to have high specificity for the diagnosis of PD. This evidence together with the lower α-synuclein quantity in the inferior GI tract neglects colonic α-synuclein as biomarker (Fasano et al., 2015). Moreover, it has been shown that synuclein accumulates in ENS neurons with aging without any association with PD (Mulak and Bonaz, 2015). The rostrocaudal α-synuclein distribution gives, thereby, the submandibular gland a potential location to measure the referred protein. This hypotheses is supported by a post-mortem study where α-synuclein was found in the submandibular glands of every PD patients and in any of the controls (Beach et al., 2013; Fasano et al., 2015).

The altered gut microbiota in PD has implied a reduction SCFA-producing bacteria and considering that other intestinal diseases show a loss of butyrate-producing bacteria with increase of opportunistic pathogens, butyrate might have a potential as a biomarker for intestinal health and ultimately for PD (Ghaisas et al., 2016).

Therapeutics and disease management:

Regarding the therapeutic approaches available to PD patients, the medication is mainly to treat the symptoms as the pathology has not been fully understood. However, some

research has been developed and two approaches been proposed, namely the reduction of intracellular and/or extracellular α-synuclein levels and preservation of the mitochondrial activity.

Oligomers modulators provide a new advance on the prevention of protein aggregation and may become a disease-modifying therapy for PD. Anle138b is an oligomer modulator which blocks the formation of pathological protein aggregates by targeting structure-dependent epitopes. This oligomer has inhibited α-synuclein accumulation and subsequent neuronal degeneration in different mouse models, with no apparent toxicity (Klingelhoefer and Reichmann, 2015; Wagner et al., 2013)

Monoclonal antibodies against extracellular α-synuclein have been also studied with some positive preliminary results (for instance, the reduction of Lewy bodies and neurites formation) (Klingelhoefer and Reichmann, 2015).

In order to restore the mitochondrial activity impaired by the excess of α-synuclein, some compounds have been proposed such as polyphenols due to its capacity to modulate mitochondrial activity and mitochondrial cell death (Klingelhoefer and Reichmann, 2015).

The use of antibiotics to treat infections, like H. pylori and SIBO, have been as well considered for improvement of some PD symptoms. In the eradication studies performed, the obliteration of these conditions in PD patients improved their symptomatology with less motor fluctuations (Fasano et al., 2015; Valeria D. Felice et al., 2016).

A supplementary dietetic approach may be considered in order to re-establish the ideal proportions of bacteria in the gut microbiota. One example is the prebiotics, which are compounds metabolized only by the bacteria, thereby favouring specific changes in the activity and composition of the gut microbiome leading to the improvement of the host's health. Other example may be the probiotics, which are microbes administered to the host to confer health benefits (Ghaisas et al., 2016). The simultaneous use of both may as well be considered. In fact, treatment with both pre and probiotic has been shown to improve intestinal permeability as well as systemic inflammation (Valeria D Felice et al., 2016; Kelly et al., 2015) (see Fig. 3). Microbiome transplantation is also an hypotheses with preliminary reports forwarded (Mulak and Bonaz, 2015). Considering the decreased abundance of *Prevotella*, supplementation in SCFA and vitamins (folate and thiamine) may have a therapeutic potential (Fasano et al., 2015) as their decrease may lead to reduced production of essential vitamins and impaired gut hormones secretion (Ghaisas et al., 2016). The diet *per se* should also be highlighted as an approach taking into account the many benefits of a healthy nourishment in the gut health. For instance, it was shown that the ingestion of green-leafy vegetables provides nitrate anion which

in turn was suggested to ensure epithelial integrity and mucus production during dysbiosis (Rocha et al., 2014).

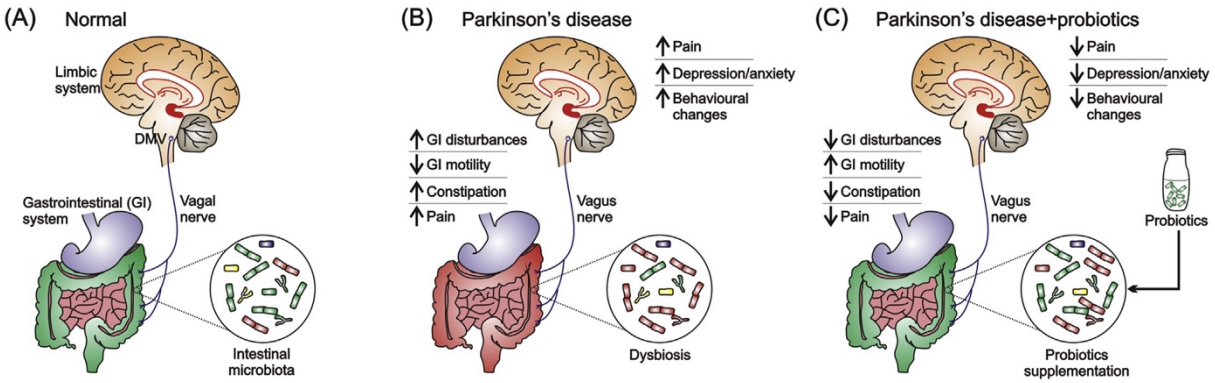

Figure 3: *The brain-gut axis in health and disease, with relevance to Parkinson's disease. (A) The healthy bi-directional communication between the brain and the gut, highlighting the involvement of the vagus nerve. (B) The brain-gut axis and non-motor symptoms of Parkinson's disease including both central and GI dysfunction. (C) The manipulation of the gut microbiota through the use of probiotics and potential alleviation of non-motor symptoms of Parkinson's disease. SN: substantia nigra; DMV: dorsal motor nucleus of the vagus* (Valeria D Felice et al., 2016).

A radically different approach involves nitric oxide (•NO) which, at physiological levels, have function such as control of the gastric mucosal blood flow, gut motility and barrier integrity (Aviello and Knaus, 2016). However, abnormal high levels of •NO lead to alteration of the gut motility, vascular tone, blood supply, mucosal barrier function and immune response (Savidge, 2014). Enteric glia has been pointed as an important regulatory •NO source via production of reactive S-nitrosothiol intermediates, exerting protective effects. S-nitrosylation is an easily reversible post-translational modification of a protein or peptide with a cysteine (Cys) residue induced by •NO (Savidge, 2014).

Recent advances in •NO therapeutics are the identification of possible targets that are aberrantly S-nitrosylated and clinically responsive to the therapeutic reversion. The goal is to selectively control the nitrosylation state of the affected proteins (Savidge, 2014).

Among others, Parkinson's is one of the diseases where this type of aberrant regulatory protein was detected. With this in mind, preclinical studies have shown to exert positive feedback on the targeted S-nitrosothiol therapy in the CNS, conferring an evident improvement of the intestinal barrier dysfunction (Savidge, 2014). Medicines targeting aberrant S-nitrosothiol proteins are expected to surpass the limitations of the pharmacological NO donors, thanks to a new deliver approach. Improved knowledge on the druggable targets and in this action mechanism in vivo would give new insights to this approach (Savidge, 2014).

CONCLUSION

In this work it is highlighted a novel pathway that might exert a critical impact in PD, the bidirectional communication between the microbiota, the gut and the central nervous structures (the microbiota-gut-brain axis). Along this axis, a myriad of interactions might be possible but, as discussed, inflammation, with its subsequent increase in the gut permeability, is at the crossroads in the axis. Environment factors are also considered important players in the modulation of the functionality of microbiota-gut-brain axis in PD.

Finally, the enteric glia may confer a wide range of protective functions through NO derived signals and the therapeutics arising from this rational may reveal new targets in disorders associated with GI inflammation and permeability (Savidge, 2014), as is the case of PD.

It is largely accepted that, a better diagnosis accuracy is required to begin the PD treatment as earlier as possible. In this regard, the microbiota has provided some conceivable biomarkers such as, the *Prevotella* and *Enterobacteria* quantifications in fecal samples of the subjects. Certain therapeutics have been studied as an intervention in the pathology, particularly those with potential as disease-modifying molecules that may alter the natural history of the disease. Manipulation of the gut microbiota composition and function may have an impact on the QoL of PD patients, specially in the reduction of motor fluctuations and non-motor symptoms such as pain, depression and constipation (Valeria D. Felice et al., 2016).

In spite of all these novel advances, it is clear that further research should be performed towards an enhanced knowledge of the pathophysiology, as well as towards the causal relationships between the gut microbiota and the pathogenesis of PD, in order to explore this relationship for improved diagnosis and therapeutics.

REFERENCES:

- Adler, C.H., Beach, T.G., Hentz, J.G., Shill, H.A., Caviness, J.N., Driver-Dunckley, E., Sabbagh, M.N., Sue, L.I., Jacobson, S.A., Belden, C.M., Dugger, B.N., 2014. Low clinical diagnostic accuracy of early vs advanced Parkinson disease: clinicopathologic study. Neurology **83**, 406–12.
- Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D.R., Fernandes, G.R., Tap, J., Bruls, T., Batto, J.-M., Bertalan, M., Borruel, N., Casellas, F., Fernandez, L., Gautier, L., Hansen, T., Hattori, M., Hayashi, T., Kleerebezem, M., Kurokawa, K., Leclerc, M., Levenez, F., Manichanh, C., Nielsen, H.B., Nielsen, T., Pons, N., Poulain, J., Qin, J., Sicheritz-Ponten, T., Tims, S., Torrents, D., Ugarte, E., Zoetendal, E.G., Wang, J., Guarner, F., Pedersen, O., de Vos, W.M., Brunak, S., Doré, J., Antolín, M., Artiguenave, F., Blottiere, H.M., Almeida, M., Brechot, C., Cara, C., Chervaux, C., Cultrone, A., Delorme, C., Denariaz, G., Dervyn, R., Foerstner, K.U., Friss, C., van de Guchte, M., Guedon, E., Haimet, F., Huber, W., van Hylckama-Vlieg, J., Jamet, A., Juste, C., Kaci, G., Knol, J., Lakhdari, O., Layec, S., Le Roux, K., Maguin, E., Mérieux, A., Melo Minardi, R., M'rini, C., Muller, J., Oozeer, R., Parkhill, J., Renault, P., Rescigno, M., Sanchez, N., Sunagawa, S., Torrejon, A., Turner, K., Vandemeulebrouck, G., Varela, E., Winogradsky, Y., Zeller, G., Weissenbach, J., Ehrlich, S.D., Bork, P., 2011. Enterotypes of the human gut microbiome. Nature **473**, 174–180.
- Aviello, G., Knaus, U., 2016. ROS in gastrointestinal inflammation: Rescue Or Sabotage? Br. J. Pharmacol. **16**, 1-10.
- Barichella, M., Cereda, E., Pezzoli, G., 2009. Major nutritional issues in the management of Parkinson's disease. Mov. Disord. **24**, 1881–1892.
- Beach, T.G., Adler, C.H., Dugger, B.N., Serrano, G., Hidalgo, J., Henry-Watson, J., Shill, H.A., Sue, L.I., Sabbagh, M.N., Akiyama, H., 2013. Submandibular gland biopsy for the diagnosis of Parkinson disease. J. Neuropathol. Exp. Neurol. **72**, 130–6.
- Bope, E.T., Kellerman, R.D., 2016. The Nervous System, in: Conn's Current Therapy 2016. Elsevier Inc., pp. 683–686.
- Cakmak, Y.O., 2015. Provotella-derived hydrogen sulfide, constipation, and neuroprotection in Parkinson's disease. Mov. Disord. **30**, 1151.
- Cassani, E., Barichella, M., Cancello, R., Cavanna, F., Iorio, L., Cereda, E., Bolliri, C., Zampella Maria, P., Bianchi, F., Cestaro, B., Pezzoli, G., 2015. Increased urinary indoxyl sulfate (indican): New insights into gut dysbiosis in Parkinson's disease. Park. Relat. Disord. **21**, 389–393.

- Cersosimo, M.G., Raina, G.B., Pecci, C., Pellene, A., Calandra, C.R., Gutierrez, C., Micheli, F.E., Benarroch, E.E., 2013. Gastrointestinal manifestations in Parkinson's disease: Prevalence and occurrence before motor symptoms. J. Neurol. **260**, 1332–1338.
- Clairembault, T., Leclair-Visonneau, L., Coron, E., Bourreille, A., Le Dily, S., Vavasseur, F., Heymann, M.-F., Neunlist, M., Derkinderen, P., 2015. Structural alterations of the intestinal epithelial barrier in Parkinson's disease. Acta Neuropathol. Commun. **3**, 12.
- Danzer, K.M., Kranich, L.R., Ruf, W.P., Cagsal-Getkin, O., Winslow, A.R., Zhu, L., Vanderburg, C.R., McLean, P.J., 2012. Exosomal cell-to-cell transmission of alpha synuclein oligomers. Mol. Neurodegener. **7**, 42.
- Derkinderen, P., Shannon, K.M., Brundin, P., 2014. Gut feelings about smoking and coffee in Parkinson's disease. Mov. Disord. **29**, 976–979.
- Fang, X., 2015. Potential role of gut microbiota and tissue barriers in Parkinson's disease and amyotrophic lateral sclerosis. Int J Neurosci **126**, 771–776.
- Fasano, A., Visanji, N.P., Liu, L.W.C., Lang, A.E., Pfeiffer, R.F., 2015. Gastrointestinal dysfunction in Parkinson's disease. Lancet Neurol. **14**, 625–639.
- Felice, V.D., Quigley, E.M., Sullivan, A.M., O'Keeffe, G.W., O'Mahony, S.M., 2016. Microbiota-gut-brain signalling in Parkinson's disease: Implications for non-motor symptoms. Parkinsonism Relat. Disord. **27**, 1–8.
- Francino, M.P., 2016. Antibiotics and the human gut microbiome: Dysbioses and accumulation of resistances. Front. Microbiol. **6**, 1–11.
- Friedland, R.P., 2015. Mechanisms of Molecular Mimicry Involving the Microbiota in Neurodegeneration. J. Alzheimer's Dis. **45**, 349–362.
- Ghaisas, S., Maher, J., Kanthasamy, A., 2016. Gut microbiome in health and disease: Linking the microbiome-gut-brain axis and environmental factors in the pathogenesis of systemic and neurodegenerative diseases. Pharmacol. Ther. **158**, 52–62.
- Grace, E., Shaw, C., Whelan, K., Andreyev, H.J.N., 2013. Review article: Small intestinal bacterial overgrowth - Prevalence, clinical features, current and developing diagnostic tests, and treatment. Aliment. Pharmacol. Ther. **38**, 674–688.
- Holmqvist, S., Chutna, O., Bousset, L., Aldrin-Kirk, P., Li, W., Björklund, T., Wang, Z.-Y., Roybon, L., Melki, R., Li, J.-Y., 2014. Direct evidence of Parkinson pathology spread from the gastrointestinal tract to the brain in rats. Acta Neuropathol. **128**, 805–20.
- Hyland, N.P., Quigley, E.M.M., Brint, E., 2014. Microbiota-host interactions in irritable bowel syndrome: epithelial barrier, immune regulation and brain-gut interactions. World

J. Gastroenterol. **20**, 8859–66.

- Kelly, J.R., Kennedy, P.J., Cryan, J.F., Dinan, T.G., Clarke, G., Hyland, N.P., 2015. Breaking down the barriers: the gut microbiome, intestinal permeability and stress-related psychiatric disorders. Front. Cell. Neurosci. **9**, 392.
- Klingelhoefer, L., Reichmann, H., 2015. Pathogenesis of Parkinson disease-the gut-brain axis and environmental factors. Nat. Rev. Neurol. **11**, 625–36.
- Mulak, A., Bonaz, B., 2015. Brain-gut-microbiota axis in Parkinson's disease. World J. Gastroenterol. **21**, 10609–10620.
- Pan-Montojo, F., Anichtchik, O., Dening, Y., Knels, L., Pursche, S., Jung, R., Jackson, S., Gille, G., Spillantini, M.G., Reichmann, H., Funk, R.H.W., 2010. Progression of Parkinson's Disease Pathology Is Reproduced by Intragastric Administration of Rotenone in Mice. PLoS One **5**, 1-9.
- Pan-Montojo, F., Schwarz, M., Winkler, C., Arnhold, M., O'Sullivan, G.A., Pal, A., Said, J., Marsico, G., Verbavatz, J.-M., Rodrigo-Angulo, M., Gille, G., Funk, R.H.W., Reichmann, H., 2012. Environmental toxins trigger PD-like progression via increased alpha-synuclein release from enteric neurons in mice. Sci. Rep. **2**, 898.
- Potgieter, M., Bester, J., Kell, D.B., Pretorius, E., 2015. The dormant blood microbiome in chronic, inflammatory diseases. FEMS Microbiol. Rev. **39**, 567–591.
- Rocha, B., Correia, M., Barbosa, R., Laranjinha, J., 2014. A dietary-driven redox modulation of gut microbiome-host interactions: the rescue of epithelial barrier and mucus production during dysbiosis by dietary nitrate. Free Radic. Biol. Med. **75**, S36–S37.
- Savidge, T.C., 2014. Importance of NO and its related compounds in enteric nervous system regulation of gut homeostasis and disease susceptibility. Curr. Opin. Pharmacol. **19**, 54–60.
- Scheperjans, F., Aho, V., Pereira, P.A.B., Koskinen, K., Paulin, L., Pekkonen, E., Haapaniemi, E., Kaakkola, S., Eerola-Rautio, J., Pohja, M., Kinnunen, E., Murros, K., Auvinen, P., 2015. Gut microbiota are related to Parkinson's disease and clinical phenotype. Mov. Disord. **30**, 350–358.
- Sekirov, I., Russell, S., Antunes, L., 2010. Gut microbiota in health and disease. Physiol. Rev. **90**, 859–904.
- Svensson, E., Horváth-Puhó, E., Thomsen, R.W., Djurhuus, J.C., Pedersen, L., Borghammer, P., Sørensen, H.T., 2015. Vagotomy and subsequent risk of Parkinson's disease. Ann. Neurol. **78**, 522–529.

- Ubeda-Bañon, I., Saiz-Sanchez, D., De La Rosa-Prieto, C., Martinez-Marcos, A., 2014. α-Synuclein in the olfactory system in Parkinson's disease: Role of neural connections on spreading pathology. Brain Struct. Funct. **219**, 1513–1526.
- Visanji, N.P., Brooks, P.L., Hazrati, L.-N., Lang, A.E., 2013. The prion hypothesis in Parkinson's disease: Braak to the future. Acta Neuropathol. Commun. **1**, 2.
- Vizcarra, J.A., Wilson-Perez, H.E., Espay, A.J., 2015. The power in numbers: Gut microbiota in Parkinson's disease. Mov. Disord. **30**, 296–298.
- Wagner, J., Ryazanov, S., Leonov, A., Levin, J., Shi, S., Schmidt, F., Prix, C., Pan-Montojo, F., Bertsch, U., Mitteregger-Kretzschmar, G., Geissen, M., Eiden, M., Leidel, F., Hirschberger, T., Deeg, A.A., Krauth, J.J., Zinth, W., Tavan, P., Pilger, J., Zweckstetter, M., Frank, T., Bähr, M., Weishaupt, J.H., Uhr, M., Urlaub, H., Teichmann, U., Samwer, M., Bötzel, K., Groschup, M., Kretzschmar, H., Griesinger, C., Giese, A., 2013. Anle138b: a novel oligomer modulator for disease-modifying therapy of neurodegenerative diseases such as prion and Parkinson's disease. Acta Neuropathol. **125**, 795–813.
- Wood, H., 2014. Parkinson disease: Gut reactions—can changes in the intestinal microbiome provide new insights into Parkinson disease? Nat. Rev. Neurol. **11**, 66.