Project 10

Name/title of the PhD course	**Pharmaceutical Sciences**
Name of the PhD coordinator	Prof. Rosaria Meli
Name/Title of the PhD project	***High fat diet-shaped intestinal microbiota and risk for Parkinson's disease: pharmacological control by prebiotics***
Department of reference	Department of Pharmacy, University of Naples "Federico II (http://www.farmacia.unina.it/)
Working conditions, research team, infrastructures, equipment	Team of researchers affiliated with the Department: -Prof. Francesca Lembo, Associate Professor of Microbiology and Clinical Microbiology -Prof. Giuseppina Mattace Raso, Associate Professor of Pharmacology -Dr. Lorena Coretti and Claudio Pirozzi, Researchers -Dr. Chiara Annunziata, Postdoctoral Researcher -Dr. Federica Comella, Luigia Turco and Nicola Opallo, Ph.D. Main projects the team works with: Study of functional connections between intestinal microbiota and specific host pathophysiological conditions through the means of animal models. The projects the teamwork is involved in (see below) provide for close cooperation and exchange of expertise between Pharmacology and Microbiology groups of the Department: a) Microbiota-gut-brain-axis: -Study of microbiota signatures in neurodevelopmental disorders in particular Autism Spectrum Disorders (ASD). -Deciphering the role of microbiota in the pathogenesis of neurological and neurodegenerative disorders, epilepsy and Parkinson's disease, respectively. -Pharmacological and nutritional control of dysbiosis related to CNS disorders such as mood disorders and neurodegenerative diseases. b) Microbiota-gut-heart-axis: Microbiota profiles associated with heart failure in a murine model of Transverse Aortic Constriction Analysis of microbiota composition during aging in a murine model of mitochondrial impairment and endothelial dysfunction. c) Identification of molecular targets and pharmacological/nutraceutical and microbiome-based strategies for the therapy or prevention of neurodegenerative diseases and neuropsychic development (i. e. Parkinson's disease, ASD) d) The pharmacological modulation of hepatic, vascular, and renal alterations, secondary to hormonal and metabolic dysfunctions.
Scientific context	Parkinson's disease (PD) is a neurodegenerative disorder with mostly unknown etiology, associated to cognitive and motor dysfunction. It is characterized by two main detrimental processes: a progressive reduction of dopaminergic neurons in the substantia nigra (SN) pars compacta (SNpc), and an intraneuronal accumulation of Lewy bodies, containing misfolded α-synuclein (α-Syn). The dopaminergic reconstitution is the principal therapeutic option in PD patients; today the current gold-standard therapy remains the combination of levodopa and carbidopa, even if it often becomes less effective over time and is not devoid of side effects. Only 10% of the PD cases originate from genetic defects while environmental factors appear to play a role in most cases. Among these, interest has been growing in the influence of food and nutrients on the development of PD, as nutrition is a potentially modifiable factor (Boulos *et al.*, 2019). Unhealthy diets, including those high in fat, can impact gut microbiota homeostasis and the overall energy balance leading to obesity and low-grade inflammation, called "metainflammation". All those alterations converge into several common detrimental mechanisms linking obesity to the development of central nervous system disorders. In this regard, many studies have demonstrated that obese individuals have an augmented risk for developing neurodegenerative disorders, including PD. Moreover, modification of the gut microbiota profiles has been shown to be involved in the pathogenesis of PD. The potential role of the microbiota-gut-brain axis in the pathogenesis and severity of PD has been identified: disturbed gut microbiota could lead to gut barrier integrity disruption, local and systemic inflammation, affecting blood-brain barrier (BBB), leading to neurodegeneration. Together with microbiota composition analysis, the researcher reported that the microbes present in the intestines of individuals with PD are more able to degrade the mucin and glycans of the host and to contribute to the deficiency of a type of vitamin B (folate), as well as to the increased blood levels of the amino acid cysteine with respect to the intestinal bacteria of control individuals. Taken together results suggest a shift to an inflammatory state associated with reduced anti-inflammatory activity by host intestinal metabolites (Dorines *et al.*, Cell Reports, 2021). Intriguingly, germ-free mice overexpressing α-Syn (GF Thy1-α-Syn mice) did not show typical features of PD, including α-synucleinopathy, impaired movement, and microgliosis. Notably, their recolonization by fecal transplant of PD patients revealed a higher score in PD patterns (Sampson *et al.*, Cell, 2016). Western diet is among the greatest risk factors for PD development, whereas quite recently the Mediterranean diet has been associated with lower rates of developing parkinsonism, and with slower progression of parkinsonian signs (Agarwal *et al.*, J Nutr Health Aging, 2018; Jackson *et al.*, Front Neurol., 2019). Because the diet is one of the principal modulators of gut microbiota, high fat diet could induce the overgrowth of potentially pathogenic bacteria with a reduction of the intestinal microbiota eubiotic state, affecting PD development and progression. Currently, the relationship between fat overnutrition, dysbiosis, metainflammation, and PD pathogenesis remains overlooked even though evidence supports the idea that targeting the

	gut-brain axis in PD through the modulation of gut microbiota composition could be one of the main novel and encouraging strategies to limit PD pathogenic mechanisms and prevent the progression of the disease. In particular, the proposed project is focused to clarify the role of both diet and gut microbiota composition as risk factors in the pathophysiology of PD and testing the effects of "targeted" nutraceutical compounds supplementation on PD symptoms upon high-fat diet.
Project Research plan	This project will be organized in two phases. The first phase of the study will investigate the role of high-fat diet (HFD) as a potential PD risk factor, likely able to negatively impact the general health status of control mice and/or worsen the motor symptoms of PD, through the alteration of gut microbiota composition and function. In the second phase the effects of CA and PUFA-ω3 on reshaping of microbiota profiles, PD symptoms, and central and peripheral parameters will be tested.
Research and Training Innovative aspects	This project will explore a knowledge gap between the involvement of diet and gut microbiota in the onset and progression of PD. Specifically will be elucidated if malnutrition in terms of high-fat diet and nutraceutical intervention could impact on PD symptoms and progression by means of gut microbiota modulation. Through a multidisciplinary approach that involves behavioral, molecular and high-throughput NGS techniques, the main goal of this project will be the clarification of the role of gut microbiota in PD and the identification of possible effective new prebiotic treatment to feed and select bacteria able to positively impact on PD phenotype. This study may open new perspectives for the development of innovative and tailored therapeutic strategies willing to improve the quality of life of PD patients.
Inter-Multidisciplinary aspects	The project will use multi-disciplinary experimental methods to fulfill the proposed aims, by integrating data from behavior, molecular biology, high-throughput Next Generation Sequencing (NGS) and statistical approaches. The proposed aims (Research objectives, RO) will be addressed as follows: -RO1: Effect of High fat Diet on Parkinson's disease progression: the effect of diet intake will be assessed firstly by behavioral tests. The PhD student will be guided to acquire the knowhow of Parkinson's model and associated tests, use of stereotaxic surgery in mice and animal handling. The effects of high fat diet will be evaluated on metabolic parameters (i.e. the monitoring of body weight gain, fat accumulation, insulin-resistance, and serum parameters) and the severity of PD phenotype (behavioral tests). -RO2: Effect of High fat Diet on gut microbiota and intestinal homeostasis in PD mice: to this aim the PhD student will perform molecular biology and high-throughput NGS approaches techniques. Data analysis of microbiota sequencing will be performed at APC Microbiome Center (APC), under the supervision of Prof Cryan, worldwide recognized expert in the field of the microbiota-gut-brain axis. Intestinal homeostasis will be evaluated by gut permeability assay, tight junction, and cytokine gene transcription. -RO3: Identification of PD-associated bacteria: correlation among behavioral, brain, gastrointestinal and microbiota data to identify specific microbial species driving possible phenotypic changes induced by PD and diet treatment (acquisition of statistical skills). - RO4: Effect of prebiotic administration on promoting the growth of bacteria reduced in high fat diet PD mice. The efficacy of prebiotic supplementation in the diet will be studied on 1) gut microbiota as read out of treatment in order to assess the reduction of potential pathogenic bacteria and growth of eubacteria; 2) locomotor activity and cognitive skills (behavioral tests), gut inflammation (cytokine and immune response-related gene transcription, inflammatory enzyme expression), gut permeability (tight junction gene transcription and histological analysis). To complete this aim and to increase insights in prebiotics features, the PhD student will be hosted by the Neilos s.r.l. to assist the formulation and production of several prebiotics.
Secondment opportunities	As part of this research, the Ph.D. student will carry out a secondment at: 1) **University College Cork, APC**, Microbiome Center (Cork, Ireland) under the supervision of Prof. John F. Cryan; https://apc.ucc.ie. Length of the foreseen secondment: 4-6 months 2) **Neilos s.r**.l. (Naples, Italy) under the supervision of Dr. A. Bagnulo; http://www.neilos2015.com. Length of the foreseen secondment: 4 months.
Main Supervisors: Prof. Francesca Lembo (https://www.docenti.unina.it/francesca.lembo)	
Brief CV	Academic positions. 2001-2006: University Researcher in Microbiology and Clinical Microbiology (SSD MED07), Faculty of Pharmacy, University of Naples Federico II.2006 to date: Associate Professor in Microbiology and Clinical Microbiology, Department of Pharmacy, University of Naples Federico II. 2020: National Scientific Habilitation to Full Professor in Microbiology and Clinical Microbiology achieved in the ASN 2018/20 session. Prof. Francesca Lembo has been co-tutor of several Ph.D. students in Translational Medicine and Molecular Medicine and Medical Biotechnology Ph.D. programs at the University of Studies of Campania Luigi Vanvitelli and University of Naples Federico II. She supervised Ph.D. students in their experimental training and in the elaboration of their Ph.D. theses.
Publications	Professor Francesca Lembo has authored 47 publications in peer-review journals. H-index 24 (SCOPUS) 1) Boccella N, Paolillo R, Coretti L, D'Apice S, Lama A, Giugliano G, Schiattarella GG, Cuomo M, d'Aquino ICavaliere G, Paciello O, Mollica MP, Mattace Raso G, Esposito G, Lembo F, Perrino C. Transverse aortic constriction induces gut barrier alterations, microbiota remodeling and systemic inflammation. Sci Rep. 2021 Apr 1;11(1):7404. 2) Citraro R, Lembo F, De Caro C, Tallarico M, Coretti L, Iannone LF, Leo A, Palumbo D, Cuomo M, Buommino E, Nesci V, Marascio N, Iannone M, Quirino A, Russo R, Calignano A, Constanti A, Russo E, De Sarro G. First evidence of altered microbiota and intestinal damage and their link to absence epilepsy in a genetic animal model, the WAG/Rij rat. Epilepsia. 2021 Feb;62(2):529-541. 3) Coretti L, Paparo L, Riccio MP, Amato F, Cuomo M, Natale A, Borrelli L, Corrado G, Comegna M, Buommino E, Castaldo G, Bravaccio C, Chiariotti L, Berni Canani R, Lembo F. Gut Microbiota Features in Young Children With Autism Spectrum Disorders. Front Microbiol. 2018. 19;9:3146. Erratum in: Front Microbiol. 2019 May 03;10: 920.

	4) Cristiano C, Pirozzi C, Coretti L, Cavaliere G, Lama A, Russo R, Lembo F, Mollica MP, Meli R, Calignano A, Mattace Raso G. Palmitoylethanolamide counteracts autistic-like behaviours in BTBR T+tf/J mice: Contribution of central and peripheral mechanisms. Brain Behav Immun. 2018. 74:166-175. 5) Coretti L, Cristiano C, Florio E, Scala G, Lama A, Keller S, Cuomo M, Russo R, Pero R, Paciello, Mattace Raso G, Meli R, Cocozza S, Calignano A, Chiariotti L, Lembo F. Sex-related alterations of gut microbiota composition in the BTBR mouse model of autism spectrum disorder. Sci Rep. 2017. 28;7: 45356.
Projects participation	Funded projects participation: 1)Researcher participating in the Project admitted to STAR (Territorial Support for Research Activities) funding 2016. Title: "Integrate epigenetics, metabolomics and intestinal microbiota profiles to obtain a personalized medicine in case of heart failure" (Project number: 16-CSP-UNINA-055). 2) Researcher participating in the Project PRIN (Projects of Major National Interest) funding 2015. Title: "Biological and pharmacological HDAC inhibitors in a genetic model of epilepsy and in experimental pain models: role of the microbiome and SCFAs" (Prot. 2015XSZ9A2_001). 3) Researcher participating in the Project PRIN (Projects of Major National Interest) funding 2020. Title: Glymphatic system: a new player in the gut-brain axis. Natural resources to maintain homeostasis.