TEMPLATE RICHIESTA ATTIVAZIONE TOPIC

Principal Investigator	Simona Lodato
Titolo del progetto	Preserving intestinal ecosystem and microbiota diversity: new strategy for prevention of Autism Spectrum Disorder
Curriculum	MEM standard
Area tematica (green/innovation) -> x info vedere in calce al template	Green OT7. Preservare e ripristinare gli ecosistemi e la biodiversità
Breve descrizione della riconducibilità del Progetto in funzione degli Obiettivi tematici indicati nel PNR	Autism spectrum disorder (ASD) is a heterogeneous group of severe neurodevelopmental disorders characterized by impaired communication, social skills and repetitive behaviors. Its incidence has dramatically increased in recent years, affecting 1 in 77 individuals in Italy, making ASD a major public health problem to face. This complex disorder, in fact, involves a high health, social and economic burden, as ASD is a lifelong condition that requires an early diagnosis, rehabilitation intervention, support for the families and training of health workers and educators assisting the patients. ASD has a multifactorial etiology, since both genetic and environmental factors have been demonstrated to contribute to its development. However, the main causes are not yet fully understood. Recent studies have shown a correlation between alterations of the intestinal microbiota and the onset of mental illness. Indeed, ASD patients present with an altered gut microbial composition and reduced diversity, with a consequent increase in gut permeability [1]; both factors having an influence on the development and functioning of the central nervous system. Chronic gastrointestinal (GI) symptoms are a common comorbidity in ASD patients and are correlated with autistic symptom severity. Interestingly, microbial-based interventions, including Fecal Microbial Transplantation (FMT) from healthy donors, improve significantly both GI and behavioral symptoms, although only temporarily [2,3]. These observations suggest that preserving the intestinal microbiota biodiversity may contribute to the prevention of ASD development and the amelioration of its manifestations. Exposure to maternal obesity *in utero* is an increasing risk of ASD onset in children. This data is of particular relevance considering the increasing worldwide prevalence of obesity in the last decades, due mainly to an increased intake of energy-dense foods high in fat and sugars. In mice, maternal high fat diet (HFD)-induced obesity causes behavioral deficits in the offspring, mediated by gut microbiota alterations [4], while supplementation with specific bacterial strains, including *L. reuterii,* improves the social deficits [5]. As a matter of fact, microbial dysbiosis and reduced microbial diversity is observed in both obese mice and humans. Several observations, including ours, show that HFD-induced gut dysbiosis

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causes intestinal barrier leakiness in female mice. It is, in particular, the disruption on the newly identified Gut Vascular Barrier (GVB) [6], located underneath the intestinal epithelium, that allows the dissemination of microbial derived products from the gut lumen to distal organs, including the brain. We have recently demonstrated that in pre-clinical models of NAFLD/NASH induced by high fat diet (HFD) feeding microbiota-driven GVB disruption is a triggering event occurring in the very early phase of disease onset. More interestingly, sealing of the GVB through pharmacological intervention is beneficial in both preventing and reverting liver steatosis and cirrhosis [7,8].

Gut microbiota affects also the permeability and tight junction formation of the vascular barriers located in the brain, namely the Blood Brain Barrier and Plexus Vascular Barrier (PVB), recently identified by our group (Carloni et al 2021). We demonstrated that gut microbiota dysbiosis (in experimental colitis) alters PVB permeability, secondary to GVB, suggesting a crosstalk between the two barriers and adding a new functional mechanism in the gut-brain axis regulation (Carloni et al 2021) [9].

The aim of this project directs to study the combination of gut microbiota changes and the disruption of vascular barriers in the intestine and brain as responsible events in ASD development. In particular, we will assess if maintenance of the intestinal ecosystem by preserving gut microbiota diversity and barrier functions can prevent ASD development during pregnancy, or influence brain functioning and ameliorate ASD symptoms in the adult life.

Aim 1. Characterization of gut microbiota-driven modulation of intestinal and brain vascular barriers in ASD mouse models

With this aim we will assess if gut microbiota changes in pregnant mothers affect intestinal and cerebral vascular barrier integrity, leading to the leakiness of bacterial products in the systemic circulation that reach the fetus and influence its brain development.

Modifications of gut microbiota will be analyzed in pregnant female mice with HFD-induced obesity and their offspring by metagenomic and metabolomic analyses. The integrity of gut and plexus vascular barriers will be analyzed by morphological analysis and functional assays, by assessing the diffusion of fluorescence-labelled dextran particles across the barriers.

Aim 2. Use of Rifaximin in preserving gut microbiota diversity and vascular barriers integrity

Rifaximin is a non-absorbable antibiotic with broad-spectrum activity and also with non-traditional antimicrobial effects. Beside its capacity of reducing bacterial virulence and translocation and anti-inflammatory properties, Rifaximin has the potential to induce a positive modulation of the gut microbiota. In fact, it favors the growth of bacteria beneficial to the host (such as *Bifidobacteria, Lactobacilli* and *Faecalibacterium prausnitzii*), without altering the overall gut microbiota composition [10]. Therefore, rifaximin can induce positive, namely "eubiotic" changes in the intestinal ecosystem, promising therapeutic advantages for several clinical conditions.

	Furthermore, it also has the potential to regulate brain functions, as suggested by its beneficial activity in hepatic encephalitis patients [11,12]. We propose to test if Rifaximin treatment of HFD-induced obese pregnant mice protects the offspring from ASD development as well as administration to ASD offspring improves its gut microbial ecosystem and integrity of vascular barriers, with consequent amelioration of behavioral defects.
Referenze	1 Vuong, H.E. and Hsiao, E.Y. (2017) Emerging Roles for the Gut Microbiome in Autism Spectrum Disorder. *Biol. Psychiatry* 81, 411–423 2 Kang, D.-W. *et al.* (2017) Microbiota Transfer Therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. *Microbiome* 5, 10 3 Kang, D.-W. *et al.* (2019) Long-term benefit of Microbiota Transfer Therapy on autism symptoms and gut microbiota. *Sci. Rep.* 9, 5821 4 Buffington, S.A. *et al.* (2016) Microbial Reconstitution Reverses Maternal Diet-Induced Social and Synaptic Deficits in Offspring. *Cell* 165, 1762–1775 5 Sgritta, M. *et al.* (2019) Mechanisms Underlying Microbial-Mediated Changes in Social Behavior in Mouse Models of Autism Spectrum Disorder. *Neuron* 101, 246-259.e6 6 Spadoni, I. *et al.* (2015) A gut-vascular barrier controls the systemic dissemination of bacteria. *Science* 350, 830–4 7 Sorribas, M. *et al.* (2019) FXR modulates the gut-vascular barrier by regulating the entry sites for bacterial translocation in experimental cirrhosis. *J. Hepatol.* 71, 1126–1140 8 Mouries, J. *et al.* (2019) Microbiota-driven gut vascular barrier disruption is a prerequisite for non-alcoholic steatohepatitis development. *J. Hepatol.* 71, 1216–1228 9 Brescia, P. and Rescigno, M. (2021) The gut vascular barrier: a new player in the gut–liver–brain axis. *Trends Mol. Med.* 27, 844–855 10 Ponziani, F.R. *et al.* (2017) Eubiotic properties of rifaximin: Disruption of the traditional concepts in gut microbiota modulation. *World J. Gastroenterol.* 23, 4491–4499 11 Patel, V. *et al.* (2021) Rifaximin reduces gut-derived inflammation and mucin degradation in cirrhosis and encephalopathy: RIFSYS randomised controlled trial. *J. Hepatol.* DOI: 10.1016/j.jhep.2021.09.010 12 Bass, N.M. *et al.* (2010) Rifaximin treatment in hepatic encephalopathy. *N. Engl. J. Med.* 362, 1071–81

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Pagina 16 del PNR https://www.mur.gov.it/sites/default/files/2021-01/Pnr2021-27.pdf
Il Green Deal europeo è stato presentato l'11 dicembre 201931 come primo atto della nuova Commissione e quale parte integrante di una strategia europea per attuare l'Agenda 2030 delle Nazioni Unite, dichiarando le sfide ambientali e climatiche come il compito che definisce la nostra generazione. Nell'ambito del Green Deal la Commissione Europea riorienta il processo di coordinamento macroeconomico del semestre europeo per integrarvi gli Obiettivi di sviluppo

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sostenibile delle Nazioni Unite (SDG), al fine di porre la sostenibilità e il benessere dei cittadini al centro della politica economica. Il Green Deal europeo prevede un piano d'azione volto a ripristinare la biodiversità, a ridurre l'inquinamento e a promuovere l'uso efficiente delle risorse passando a un'economia pulita e circolare. Esso si articola in otto Obiettivi Tematici (OT): OT1. Rendere più ambiziosi gli obiettivi dell'UE in materia di clima per il 2030 e il 2050; OT2. Garantire l'approvvigionamento di energia pulita, economica e sicura; OT3. Mobilitare l'industria per un'economia pulita e circolare; OT4. Costruire e ristrutturare in modo efficiente sotto il profilo energetico e delle risorse; OT5. Accelerare la transizione verso una mobilità sostenibile e intelligente; OT6. "Dal produttore al consumatore": un sistema alimentare equo, sano e rispettoso dell'ambiente; OT7. Preservare e ripristinare gli ecosistemi e la biodiversità; OT8. Obiettivo "inquinamento zero2 per un ambiente privo di sostanze tossiche. Tutti i sei grandi ambiti di ricerca e innovazione trattati nel PNR stabiliscono connessioni con i suddetti obiettivi del Green Deal europeo. Alcuni lo fanno in maniera meno esplicita, altri in maniera più esplicita evidenziando il contributo ai target qualitativi o, laddove presenti, quantitativi. A titolo di esempio si riporta di seguito una sintesi delle connessioni più rappresentative tra i grandi ambiti di ricerca e innovazione del PNR 2021-27 e il Green Deal europeo.

1. **Salute.** Gli obiettivi del PNR sono focalizzati a migliorare la capacità diagnostica, lo sviluppo di tecnologie sanitarie efficaci e innovative, incluse le tecnologie digitali, assieme allo sviluppo di nuovi approcci diagnostici e terapeutici e l'identificazione dei fattori di rischio per infertilità. Tali finalità richiamano alcuni degli obiettivi del GD, di cui OT8 e OT3 sono i più evidenti.

Tematiche generali area Salute: pagina 35
Nel SNSI: https://www.agenziacoesione.gov.it/wp-content/uploads/2019/06/Strategia_Nazionale_di_Specializzazione_Intelligente_Italia.pdf
le aree di specializzazione regionale: Salute (pagina 80)
Aree tematiche nazionali: Salute, Alimentazione, qualità della vita (pag. 93)
Traiettorie tecnologiche di sviluppo a priorità nazionale: Salute (pag.100)