EXPLORING THE ROLE OF MICROBIOTA-GUT-BRAIN AXIS MODULATION IN AUTISM SPECTRUM DISORDER USING THE BTBR MOUSE MODEL

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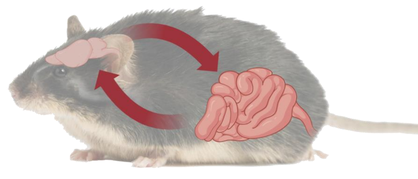
INTRODUCTION

Autism Spectrum Disorder (ASD) is a complex neurodevelopmental condition characterized by **impaired social interactions** and **repetitive behaviours**. Recent research highlights the **role of the microbiota-gut-brain axis in ASD**, where a leaky gut and low-grade intestinal inflammation can trigger a proinflammatory state within the brain. In this context, the **BTBR T+tf/J (BTBR) mouse model of ASD** provides an invaluable platform for investigating potential interventions and dissecting the involvement of the microbiota-gut-brain axis.



We aimed to **characterise the gut microbiota of BTBR mice** and investigate how two neuroprotective and anti-inflammatory treatments, namely **Palmitoylethanolamide (PEA)**, a PPAR-α agonist, and a formulation containing **dimethylglycine combined with B-vitamins (DMG-vitB)**, which reduces the oxidative stress, can affect ASD-like symptoms, inflammatory markers, gut permeability, and the composition of the gut microbiota.

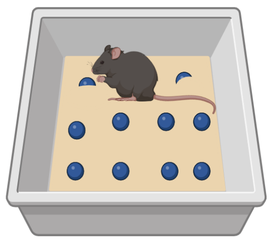
METHODS

Animals:

Prosocial strain: **C57Bl/6J** mice

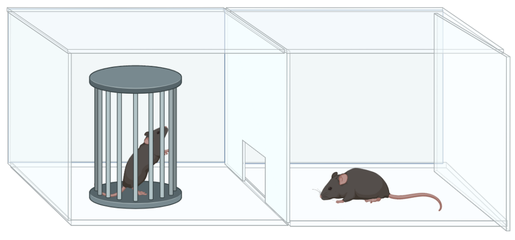
ASD model: **BTBR** T + tf/J mice

Behavioural tests



Marble burying

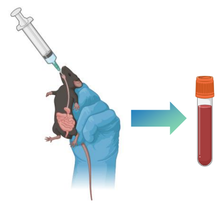
Self-grooming



Social approach

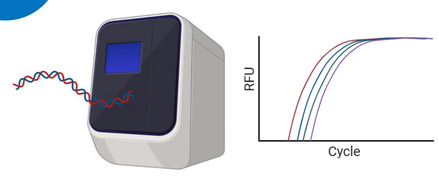
Histological analysis

Histological evaluation of colon inflammatory cell infiltration



In vivo FITC-dextran intestinal permeability assay

Gene expression



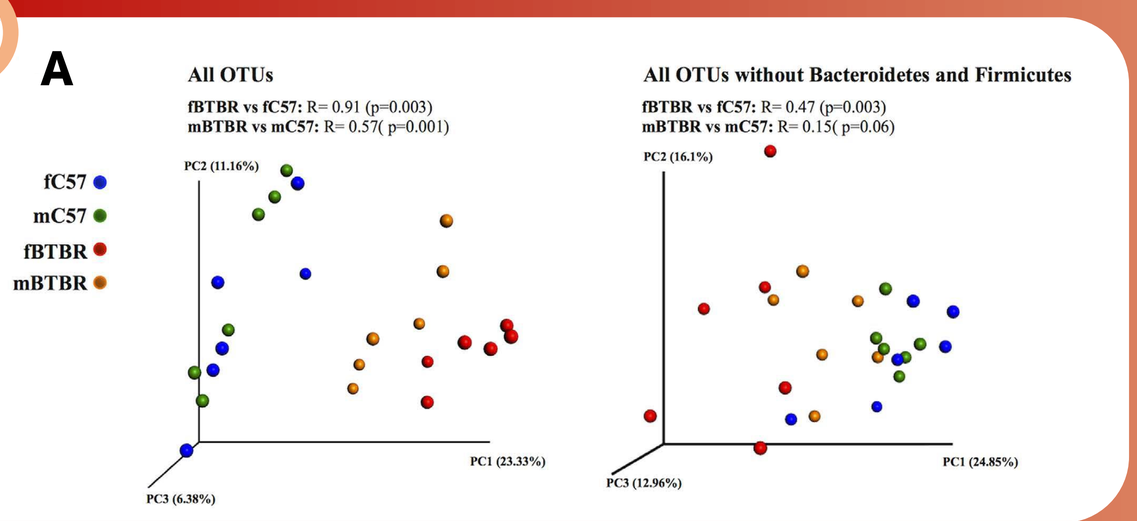
RT-PCR from colon RNA samples
Amplification of zonuline-1, occludin, TNF-α, IL-6, IL-10, BDNF...

Microbiota analysis

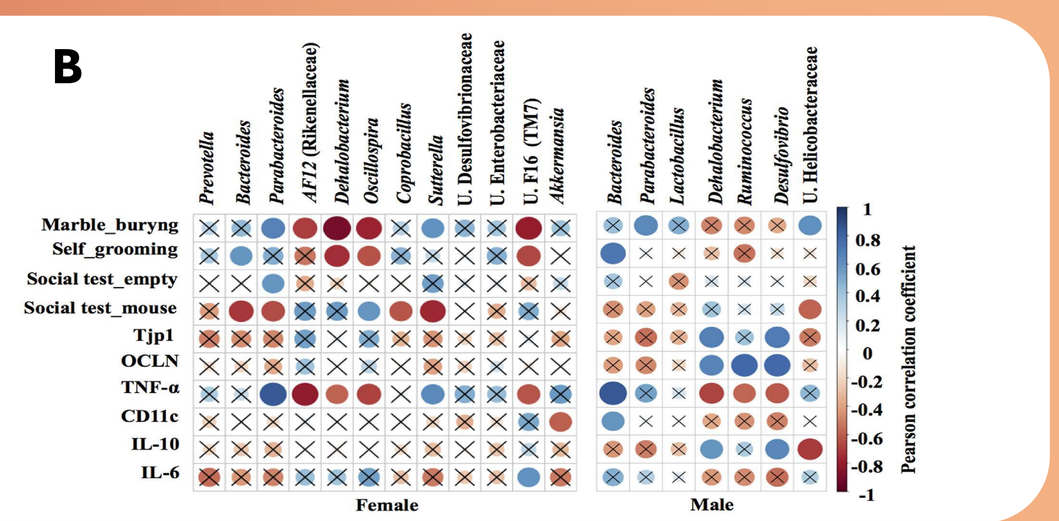
DNA isolation — 16S rDNA extraction — DNA sequencing

RESULTS

BTBR mice



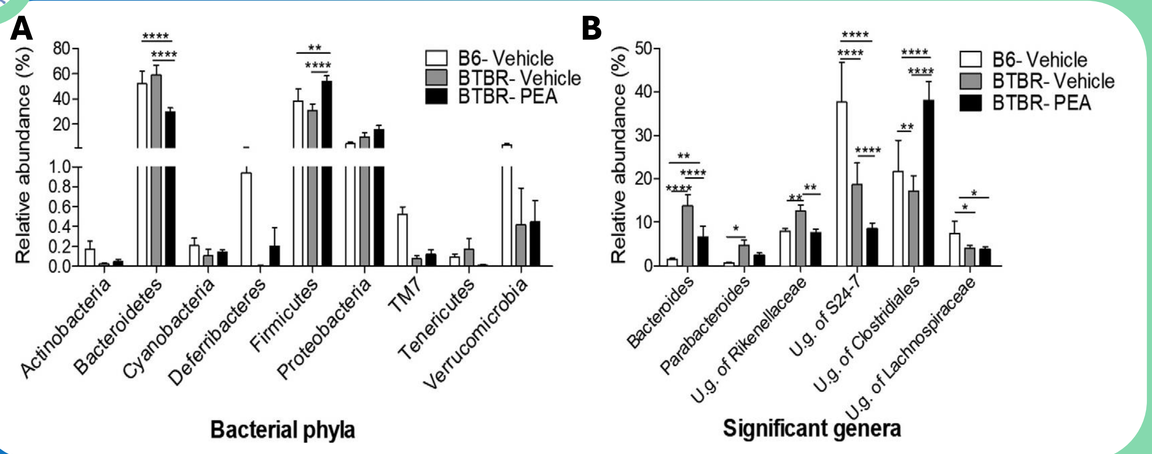
A) We found that Bacteroidota and Firmicutes taxa reassortment mainly marks the differences of gut microbiota between BTBR and C57 mice in both sexes according to ANOSIM results on Unweighted Unifrac analysis.



B) Pearson correlation showed that the increase of *Parabacteroides* and *Sutterella*, together with the decrease of *Dehalobacterium*, *Oscillospira* and an unclassified member of *TM7* were strongly associated with altered behaviour and TNF-α expression in female BTBR.

At the **behavioural level**, these mice displayed a lower sociability and an increase of repetitive and stereotypical behaviours compared to C57 mice. This phenotype could be in part due to the altered gut microbiota.

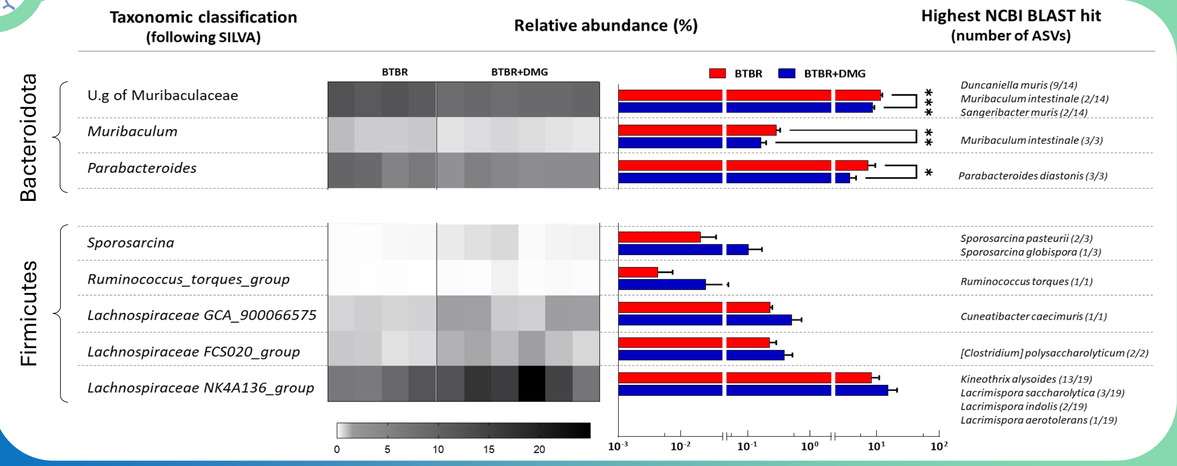
PEA in BTBR mice



A) PEA treatment strongly impacted the levels of definite identified phyla. Among the 9 phyla detected, Bacteroidota and Firmicutes were the most abundant and primarily affected by PEA treatment; B) At genus level, the increase of Firmicutes/Bacteroidota ratio upon PEA administration was mainly a consequence of the diminution of *Bacteroides*, U. g. of *Rikenellaceae*, U.g. of *S24-7* and the increase of U.g. of *Clostridiales*.

Behavioural level: Increase of sociability / decrease of repetitive and stereotypical behaviours. This phenotype could be linked to the altered gut microbiota.

DMG-vitB in BTBR mice



The DMG-vitB treatment in BTBR mice induced a new microbiota profile characterized by an increase of Firmicutes members, such as *Sporosarcina*, *Ruminococcus torques group* and *Lachnospiraceae*, and a decrease of the Bacteroidota members (U.g of *Muribaculaceae*, *Muribaculum* and *Parabacteroides*).

Behavioural level: Decrease of repetitive and stereotypical behaviours. This phenotype could be linked to the altered gut microbiota.

CONCLUSIONS

These studies underscore the **critical role of the microbiota-gut-brain axis in ASD pathogenesis** and highlight the **potential of PEA and DMG-vitB** as therapeutic interventions for improving **both central and peripheral alterations** in the BTBR mouse model. Further research into the microbiota-gut-brain axis may hold the key to developing novel treatments for individuals with ASD.

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