

Effect of fecal microbiota transplantation from children with autism in germ-free mice: Importance of genetic background and gastrointestinal symptoms

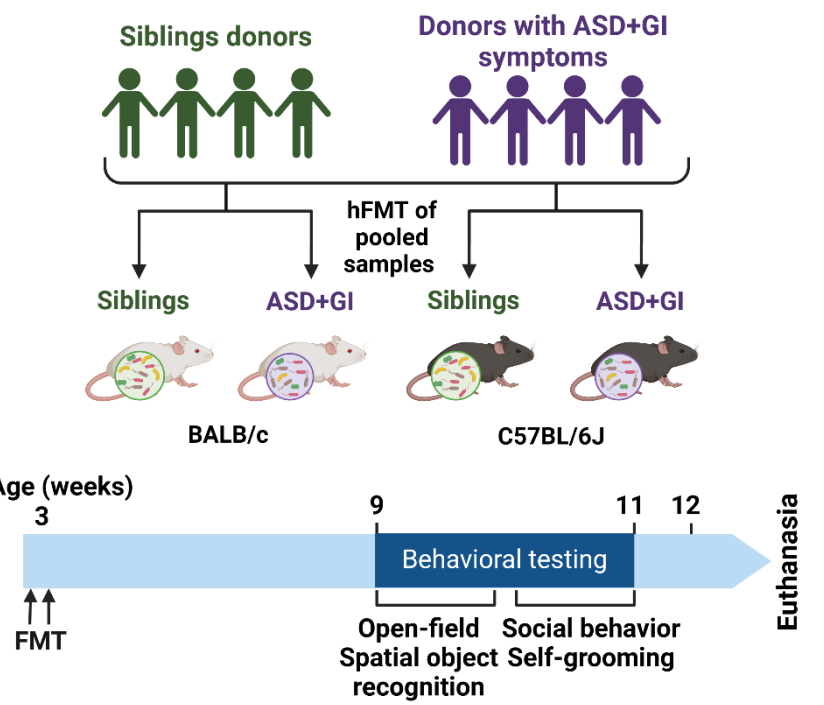
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Introduction

Autism spectrum disorder (ASD) is a multifactorial neurodevelopmental condition characterized by repetitive behaviors and impairments in social interaction and communication, often coexisting with anxiety, cognitive deficits, and gastrointestinal (GI) symptoms. Altered gut microbiota composition has been observed in individuals with ASD and murine models. Such microbiota alterations may exacerbate symptoms of ASD, as they can impact the immune system, contributing to dysregulated serotonin and kynurenine pathways, and increased neuroinflammation. This study evaluates the impact of human fecal microbiota transplantation (hFMT) from children with ASD and GI symptoms or from their neurotypical siblings to two strains of germ-free (GF) mice, focusing on autism-related behaviors, kynurenine and serotonin metabolism and immune parameters.

Methods



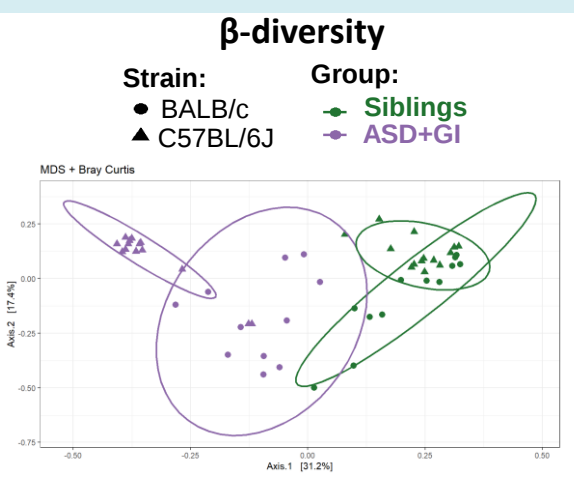
Experimental timeline:

- Age (weeks): 3, 9, 11, 12
- Events: FMT (at 3 weeks), Behavioral testing (from 9 weeks), Euthanasia (at 12 weeks)
- Groups: Siblings, ASD+GI (for both BALB/c and C57BL/6J strains)
- Tests: Open-field, Spatial object recognition, Social behavior, Self-grooming recognition

Results

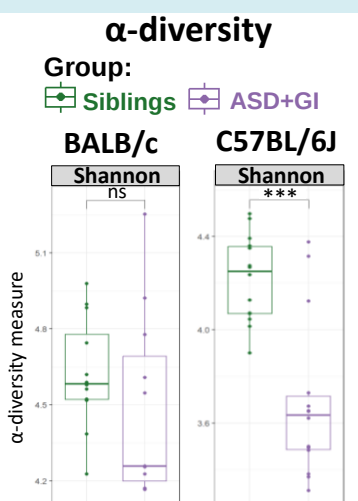
Microbiota

β-diversity



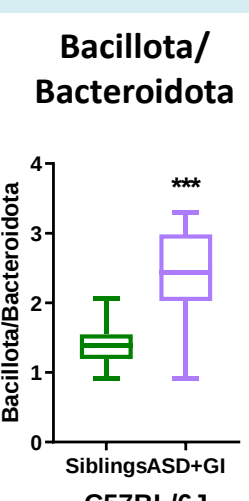
Strain: ● BALB/c ▲ C57BL/6J
Group: ● Siblings ● ASD+GI

α-diversity



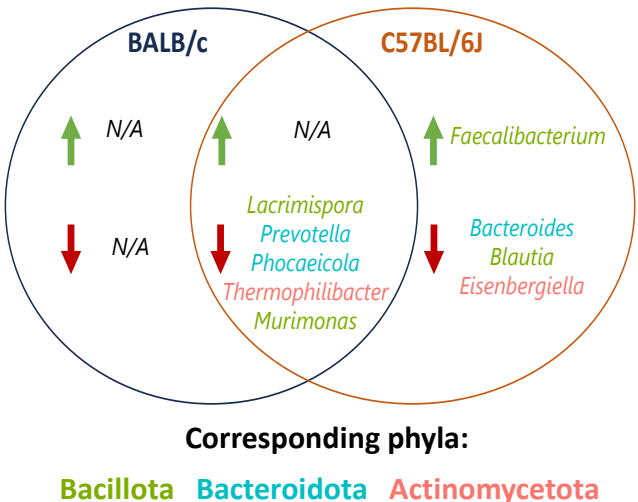
Group: ● Siblings ● ASD+GI
BALB/c C57BL/6J
Shannon

Bacillota/Bacteroidota



Bacillota/Bacteroidota
Siblings ASD+GI
C57BL/6J

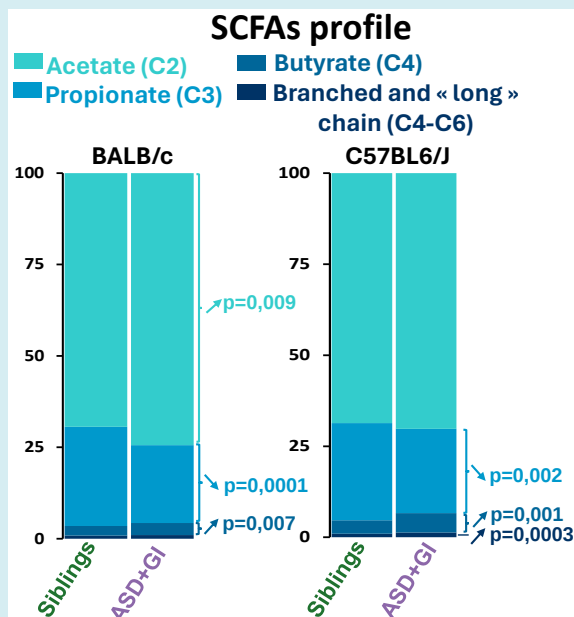
Differentially modulated genera



BALB/c C57BL/6J

Corresponding phyla: Bacillota Bacteroidota Actinomycetota

SCFAs profile



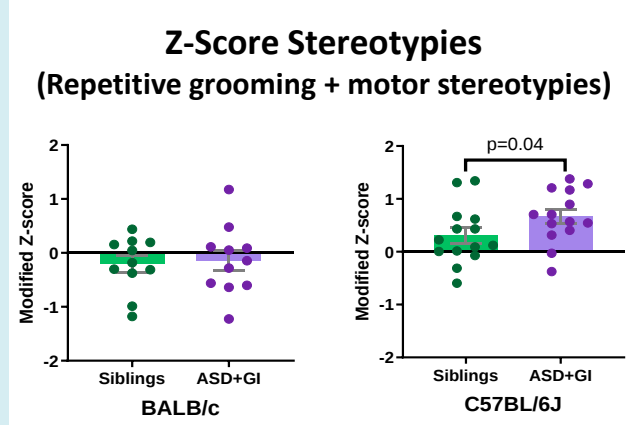
Acetate (C2) Butyrate (C4)
Propionate (C3) Branched and « long » chain (C4-C6)

BALB/c C57BL/6J
Siblings ASD+GI

→ Microbiota profiles differed between groups, with decreased α-diversity (p=0,0004) and increased Bacillota/Bacteroidota ratio (p=0,0002) in the ASD+GI group only in C57BL/6J mice.
→ Relative abundances of genera varied between groups, with some variability between strains.
→ In both strains of mice, the ASD+GI group had decreased propionate and increased butyrate cecal proportions. In BALB/c, acetate proportion was decreased in the ASD+GI group while BLCA proportion was increased in this group in C57BL/6J mice

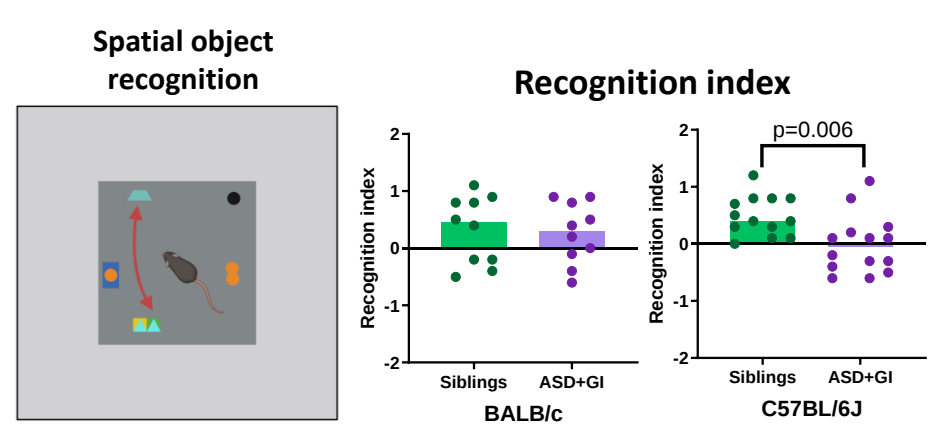
Behavior

Z-Score Stereotypies (Repetitive grooming + motor stereotypies)



Modified Z-score
Siblings ASD+GI
BALB/c C57BL/6J

Spatial object recognition

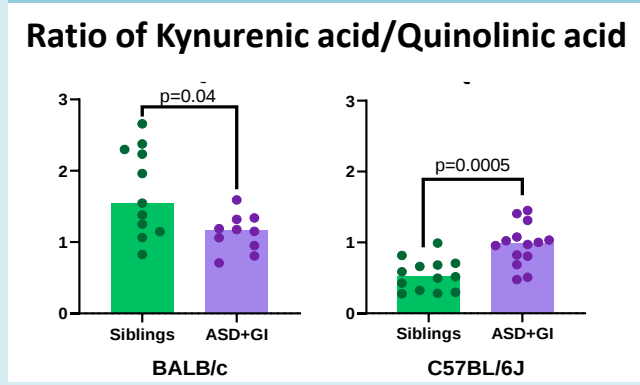


Recognition index
Siblings ASD+GI
BALB/c C57BL/6J

→ No effect of hFMT on behavior in BALB/c mice
→ FMT from ASD+GI group leads to increased repetitive behaviors and worsened spatial memory in C57BL/6J mice.

Kynurenine metabolism

Ratio of Kynurenic acid/Quinolinic acid

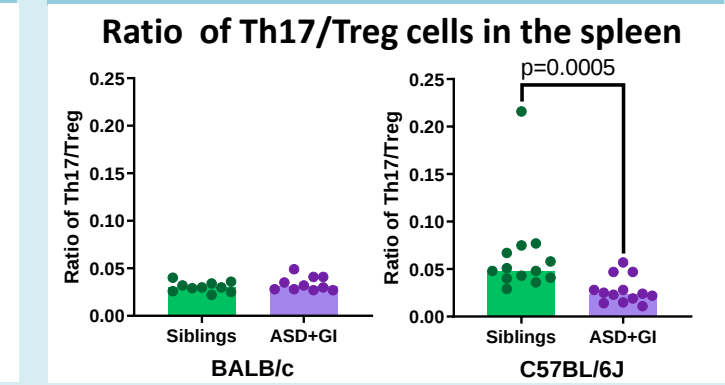


Ratio of Kynurenic acid/Quinolinic acid
Siblings ASD+GI
BALB/c C57BL/6J

→ KA/QA is decreased in ASD+GI in BALB/c, but increased in this group in C57BL/6J mice .

General inflammation

Ratio of Th17/Treg cells in the spleen

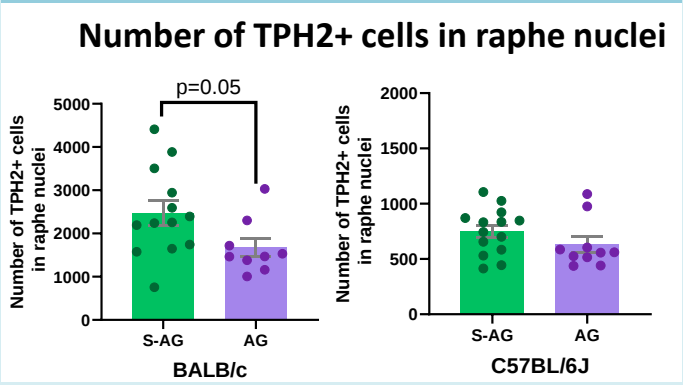


Ratio of Th17/Treg
Siblings ASD+GI
BALB/c C57BL/6J

→ Less inflammatory T cell profile in the ASD+GI group in C57BL/6J mice only.

Brain serotonin

Number of TPH2+ cells in raphe nuclei



Number of TPH2+ cells in raphe nuclei
Siblings ASD+GI
BALB/c C57BL/6J

→ Decreased serotonergic neurons in the ASD+GI group in BALB/c mice only.

Conclusion

- hFMT studies in mice such as this one allow to investigate the causality of microbiota alterations in ASD pathophysiology at the preclinical level.
- By investigating the effect of hFMT from ASD individuals on two strains of GF mice in the same experimental conditions, we highlight the importance of the genetic background of the recipient animals in studies on the microbiota-gut-brain axis.
- Microbiota composition and fermentation activity differed between groups, with some variability between strains.
- ASD-related behavioral differences between groups were found only in C57BL/6J mice while only BALB/c mice of the ASD+GI group showed decreased serotonergic neurons in the brain.
- C57BL/6J mice from the ASD+GI group displayed an increased ratio of KA/QA, a sign of decreased neurotoxicity, and decreased inflammatory T cell profile in the spleen. On the contrary, the KA/QA ratio was slightly decreased in the ASD+GI group in BALB/c mice.
- Overall, these results suggest that the microbiota interacts with the genetic background in its impact on behavior, brain function, and inflammation in the context of ASD.