Effect of HFD on the microbiome and microglial in APP/PS1 mice

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Abstract

Background: The gut microbiota is altered in Alzheimer's disease (AD) patients and may contribute to AD by secreting microbial metabolic products that affect immunologic and/or neuronal function. High fat diet increases AD risk and affects the gut microbiota. Whether the Western diet affects AD pathogenesis via modulating microbiota has not been extensively investigated in animal models. We hypothesize that the Western high-fat high-sugar diet worsens AD by shifting gut microbiota, while a Mediterranean diet might not. **Methods and Results:** We administered six months of high saturated fat high sugar Western diet, a high unsaturated fat/high fiber Mediterranean diet, and a control diet to WT and APP/PS1-21 mice. We identified increased amyloid plaque (Aβ) burden in the hippocampus and increased splenic CD4+IFNγ+ in Western diet-fed APP male mice vs. Control diet-fed. We further profiled the microglial gene expressions and identified Western diet, vs. control diet, impaired microglial phagocytosis and dampened immune responses by downregulation of GM-CSF signaling and type I interferon signaling. We sequenced mouse stool samples of study days d0, d7 and d180 for 16S rRNA microbiome analyses to study the potential role and impact of the gut microbiome in a timely manner. We found that both Western and Mediterranean HFDs increased *Lactobacillus* in male and female mice, and that both diets decreased *Oscillospiraceae* UCG-003 in female mice. However, only Mediterranean diet increased *Akkermansia*, *Lachnoclostridium*, *Parabacteroides*. Next, we correlated APP mice gut microbiota with Aβ and identified significant ASVs at day 7, day 180, and at combined time points. Microbial data of same mouse were pooled from d7 and d180 to increase sample size in statistics and interpreted with caution. We identified *Lachnospiraceae ASV87 (BLASTn core.nt.db Clostridium sp., 99.73% identity) and Ruminococcaceae ASV574 (BLASTn core.nt.db Ruthenibacterium lactatiformans, 97.595 identity)* positively correlated with Aβ in Western-fed males on d180, with *Oscillospiraceae NK4A214 ASV553 (BLASTn core.nt.db Flintibacter butyricus 94.38% identity*), *Muribaculum ASV398 (BLASTn rRNA.type.db Muribaculum intestinale 99.73% identity*), *Muribaculaceae ASV235 (BLASTn core.nt.db Muribaculum gordoncarteri, 99.73% identity*) and *Muribaculaceae* ASV11 *(BLASTn rRNA.type.db Muribaculum intestinale, 92.76% identity*), *Odoribacter ASV485 (BLASTn core.nt.db Odoribacter splanchnicus, 100% identity*), and *Rikenella ASV506 (BLASTn rRNA.type.db Rikenella microfusus, 88.65% identity)* negatively correlated with Aβ on d180. We also identified *Colidextribacter ASV152* negatively correlated with plaque in Mediterranean-fed group, female data on d180 (data not shown). Next, we correlated APP mice microbiota with splenic CD4+IFNγ+ due to its consistent increase in western vs control in both male and female APP/PS1 mice. We found *Eubacterium coprostanoligenes* ASV117, and *Lactobacillus murinus* ASV530 positively correlated with CD4+IFNγ+, and that *Muribaculaceae* ASV491, ASV456, *Alistipes* ASV141, *Parabacteroides distasonis* ASV293, and *Lachnoclostridium dorea* ASV54 negatively correlated with splenic CD4+IFNγ+ on d180 of Western-fed male mice. **Conclusions:** Overall, the consistent findings are yet to be examined in animal models to reveal ASV-level microbiota effects on Alzheimer's disease pathological and immunological progression.

Research Outline

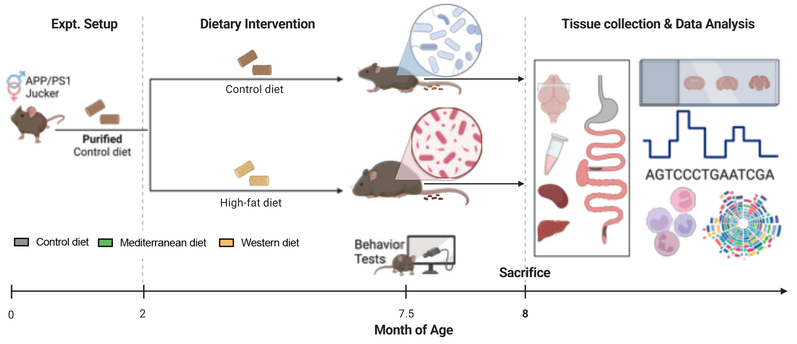

Figure 1. Project Schematic. (Generated online at BioRender.org) **We hypothesize that specific microbiota-diet interactions influence AD pathogenesis by modulating immune and metabolic responses. Aim 1:** Identify microbes and microbial functions altered by high fat diet (HFD) and linked to Aβ pathology and neuroinflammation in APP/PS1 mice.

HFD-derived Alterations in Gut Commensals were Presence-Driven as well as Abundance-Driven

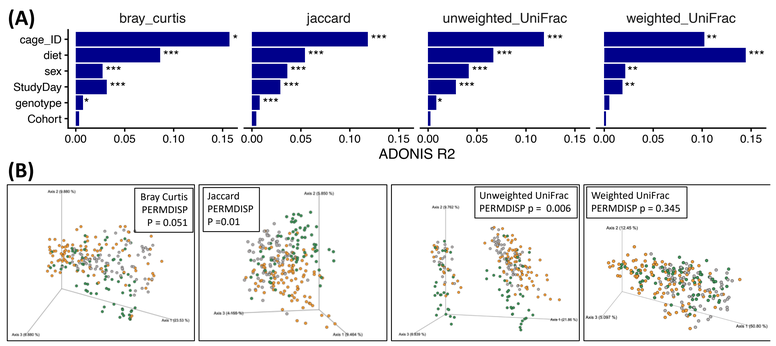

Figure 3. (A) Effect of host factors on total microbial beta diversity tested by ADONIS. Beta index ~ Diet + StudyDay + Genotype Sex Cage + Cohort (B) PCoA of microbial samples at a rarefaction sequencing depth of 4,000 reads/sample. Permutation n= 999 * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

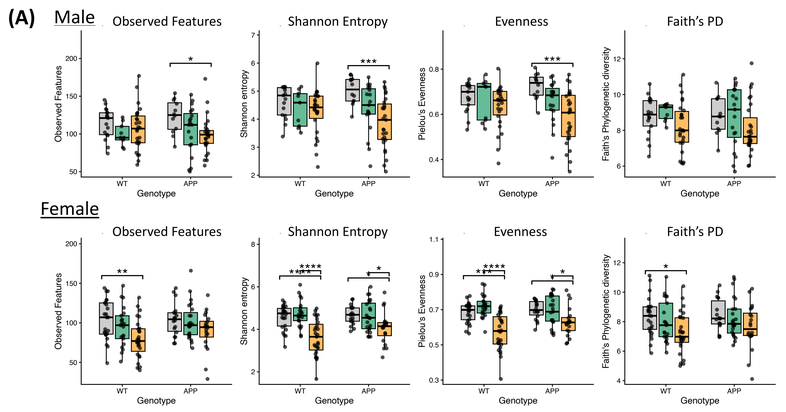

Figure 4. **Alpha diversities across genotype and diet** in (A) males and (B) females. Mouse n > 6 per group of sex-genotype-diet. Kruskal-Wallis test was applied to test genotype and diet effects, multiple comparisons were corrected using Benjamini-Hochberg method. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

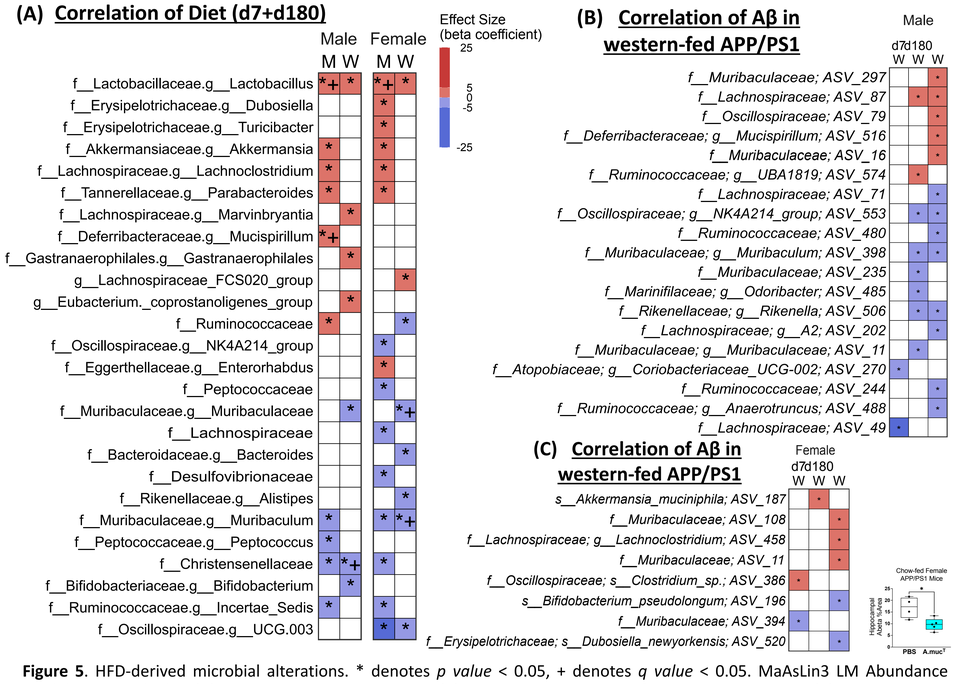

Figure 5. HFD-derived microbial alterations. * denotes *p value* < 0.05, + denotes *q value* < 0.05. MaAsLin3 LM Abundance Model were applied to (A) Genera ~ diet + reads + cage. (B) Western group, ASV ~ Aβ %area + reads + cage (C) Mediterranean group, ASV ~ Aβ %area + reads + cage. M abbreviated for Mediterranean and W for Western.

Correlation of CD4 IFNγ + reads + cage in Western diet-fed APP/PS1 Males

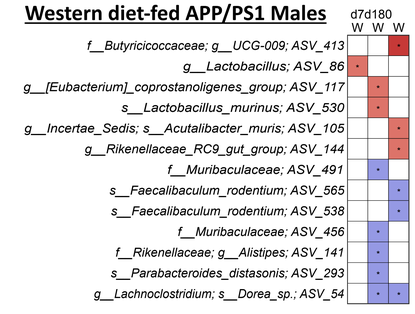

Figure 6. Gut microbial alterations related to splenic CD4+IFNγ.
MaAsLin3 LM Abundance Model were applied to correlations of taxa with splenic CD4+IFNγ+ cell population on the ASVs of western-fed mice, taxa~ IFNγ + reads + cage; * denotes *p value* < 0.05, + denotes *q value* < 0.05.

Amyloid Pathology & Peripheral Inflammation

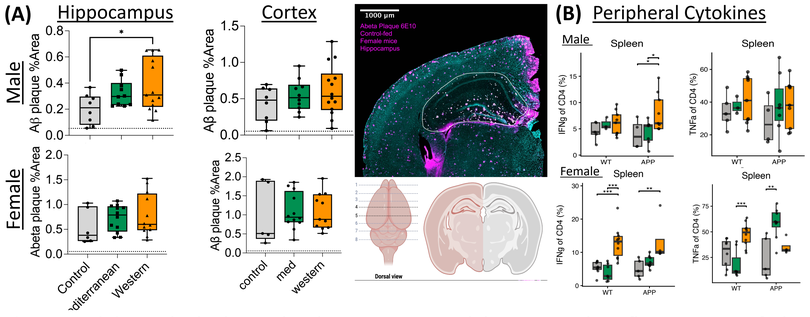

Figure 2. (A) Amyloid plaque burden was assessed by immunohistofluorescence of the hippocampus and cortex area of APP/PS1 mice of an APP/PS1 sire. Plaque burden is defined as the perc hippocampal/cortical area that were covered with 6E10 Aβ plaques. One-way ANOVA with Dunnett's post hoc. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (B) CD4+IFNγ+ and CD4+TNFα+ % of CD4+ T cells in the spleen were stratified by diet, genotype and sex groups. Two-way ANOVA performed on transformed data ln(1 +p). Tukey post hoc was performed between diet groups within each genotype. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ Data are plotted on the original scale.

Microglial Transcriptional Responses

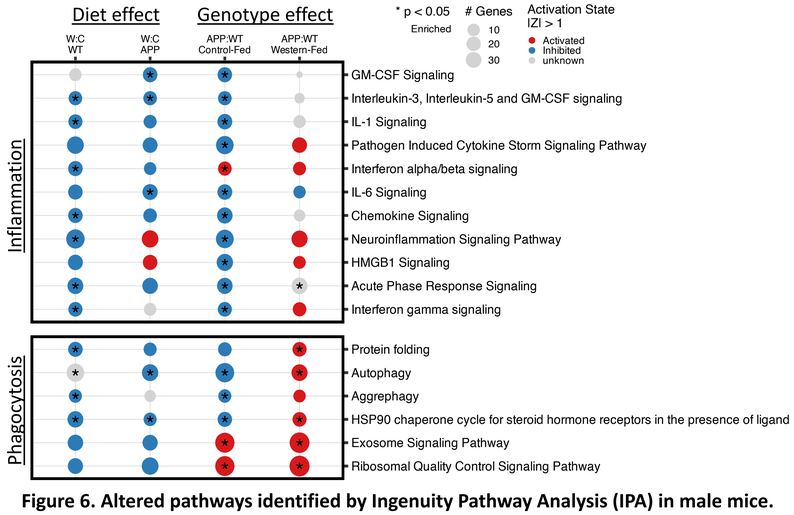

Figure 6. Altered pathways identified by Ingenuity Pathway Analysis (IPA) in male mice.

Conclusion & Next Step

1. Western high fat diet increased Aβ plaque burden and peripheral CD4+IFNγ+ in the spleen and cervical lymph node in APP/PS1 male mice; Western diet triggered neuroinflammation and was immunosuppressive in APP/PS1 male mice.
2. Western diet downregulated microglial GM-CSF signaling in male mice was consistent with our other project where we identified decreased GM-CSF after *Bacteroides fragilis* administration.
3. The two HFDs, Western diet and Mediterranean diet, reshaped the mouse gut microbiome both in abundance and prevalence. However, western diet decreased alpha diversity and evenness while Mediterranean diet did not. Specifically, Western diet reduced *Bacteroidota* genera and strict anaerobe *Oscillospiraceae*, with stronger and more numerous effects observed in females.
4. *Lactobacillus* genus increased in western diet vs control and *Lactobacillus murinus* ASV530 was positively correlated with CD4+IFNγ+ on d180 supporting a link between western diet-induced microbial remodeling and peripheral immune activation.
5. We will orally administer *Akkermansia muciniphila* in Western-det fed male APP/PS1 mice and examine host pathological response.

Acknowledgements

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