Alistipes drives T cell-mediated IL-17 responses and microglial dysfunction in a Parkinson's disease mouse model

HARVARD MEDICAL SCHOOL

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Background

Emerging evidence implicates the gut microbiome in Parkinson's disease (PD) pathogenesis and progression. To identify changes in the gut microbiota associated with disease progression in the α-synuclein 3KL model of PD, we utilized individual antibiotics to deplete specific bacterial populations. Metronidazole slowed progression of motor dysfunction, which we link to depletion of *Alistipes*. *Alistipes* species have been widely associated with PD, yet the mechanisms underlying their detrimental effects remain unclear.

1. Antibiotic study design and motor phenotype

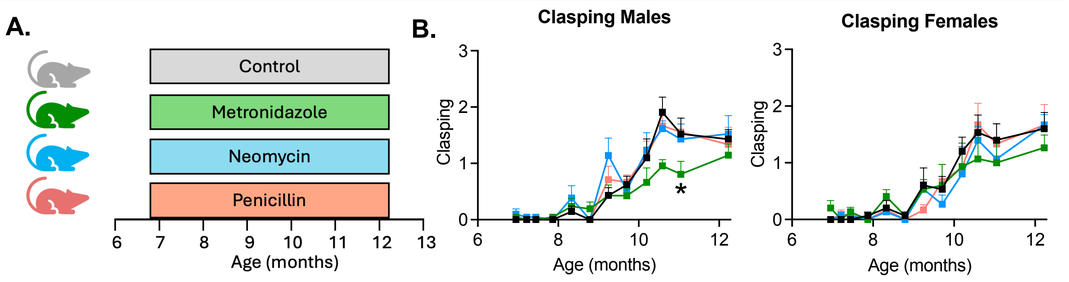

Figure 1. Metronidazole slows clasping phenotype in 3KL mice. **A.** Study design, 3KL male and female mice were treated with low dose metronidazole, neomycin, or penicillin (100 mg/L) from 7-12 months of age. **B.** Clasping phenotype was analyzed for the duration of the trial, with metronidazole significantly slowing the progression of clasping in male 3KL mice, and trending in female.

2. Effect of metronidazole on peripheral immunity

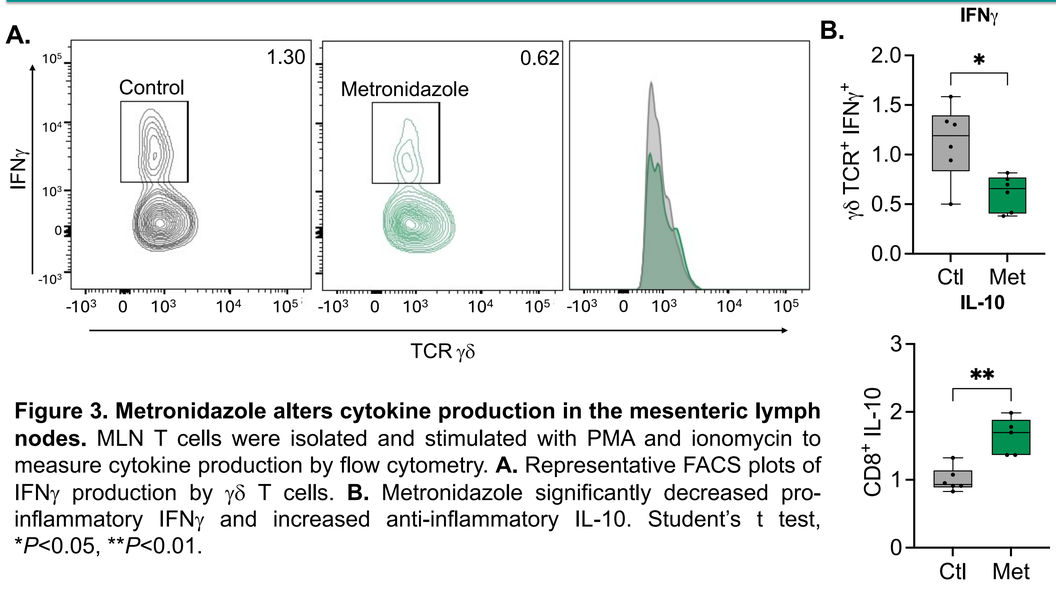

Figure 3. Metronidazole alters cytokine production in the mesenteric lymph nodes. MLN T cells were isolated and stimulated with PMA and ionomycin to measure cytokine production by flow cytometry. **A.** Representative FACS plots of IFNγ production by γδ T cells. **B.** Metronidazole significantly decreased pro-inflammatory IFNγ and increased anti-inflammatory IL-10. Student's t test, **P*<0.05, ***P*<0.01.

3. Effect of low dose chronic antibiotic exposure on the 3KL microbiome

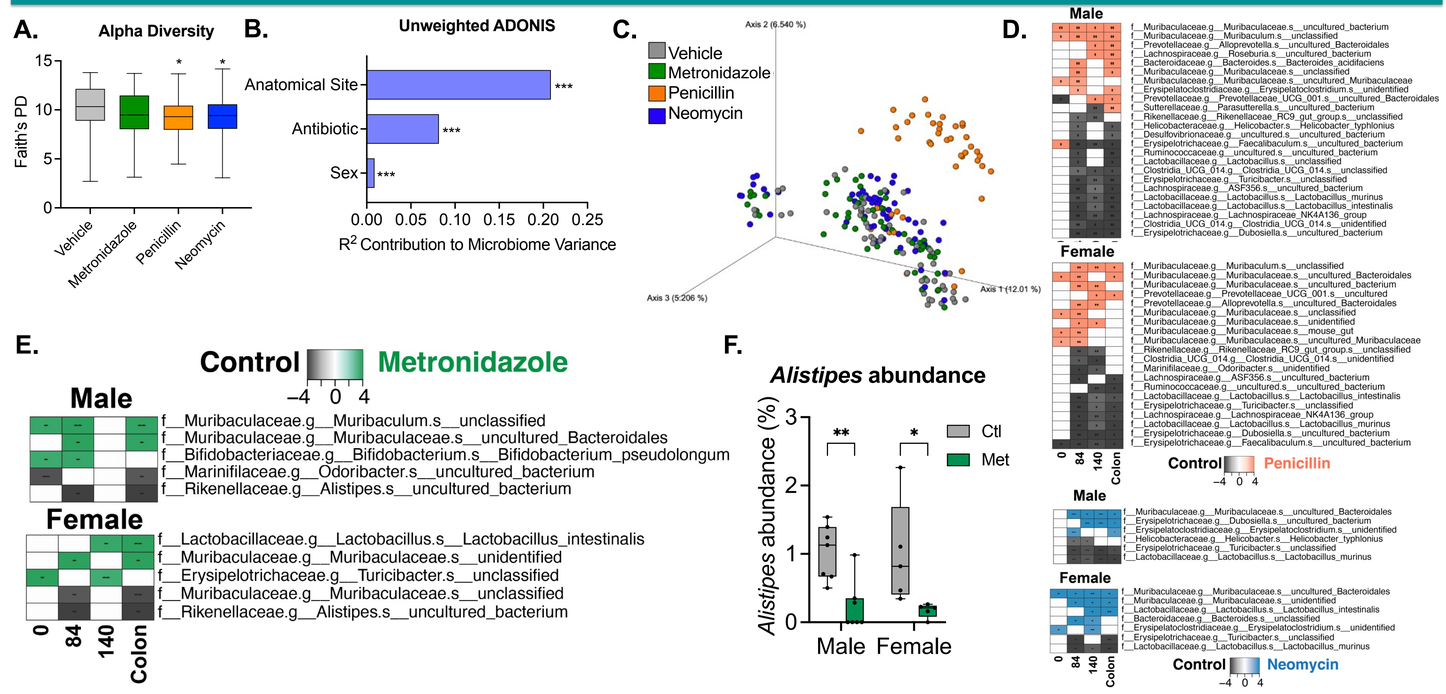

Figure 2. Antibiotics reshape the microbiome, and metronidazole specifically reduces *Alistipes* abundance in the colon. A. Alpha diversity is significantly reduced by neomycin and penicillin, with no effect from metronidazole as measured by Faith's PD. **B.** Microbial beta diversity is driven by anatomical site, antibiotics, and sex as shown by unweighted ADONIS analysis. **C.** Jaccard Emperor PCoA of antibiotic effects on beta diversity**. D.** Heatmaps identifying microbial species that are altered by penicillin and neomycin. **E.** Heatmap of microbial species altered by metronidazole. **F.** Abundance of *Alistipes* in male and female 3KL colon samples identified by 16s rRNA sequencing

4. Microglial transcriptome shifts driven by metronidazole

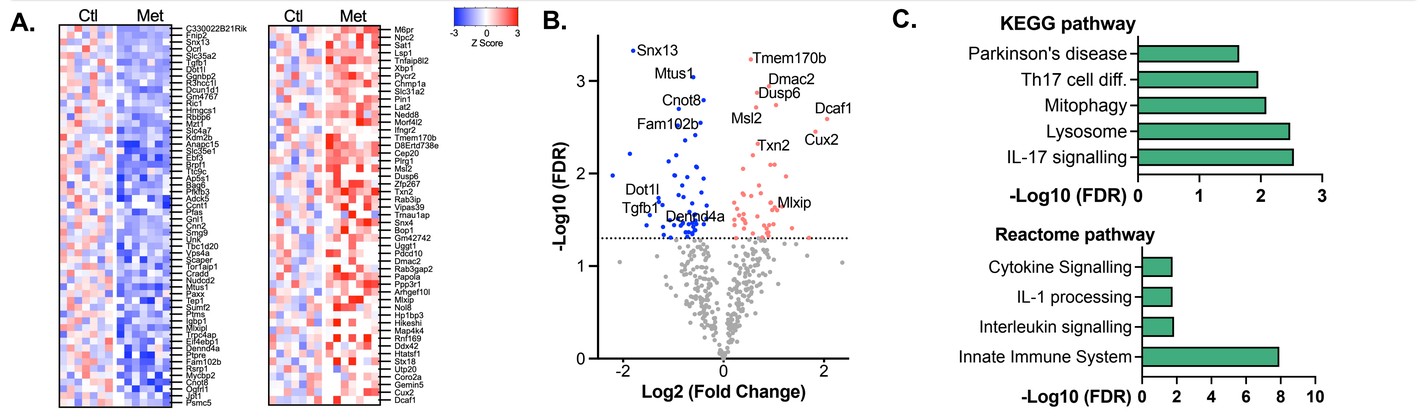

Figure 4. Metronidazole alters the microglial RNA signature and pathways. Microglia were isolated by FACS sorting and underwent bulk RNA sequencing. **A.** Heatmap of down- and up-regulated genes altered by metronidazole **B.** Differentially expressed gene volcano plot, DEGs identified by DESeq2. **C**. KEGG and Reactome pathways from significantly altered genes identified by StringDB.

5. *Alistipes* accelerates motor deficits in 3KL male mice

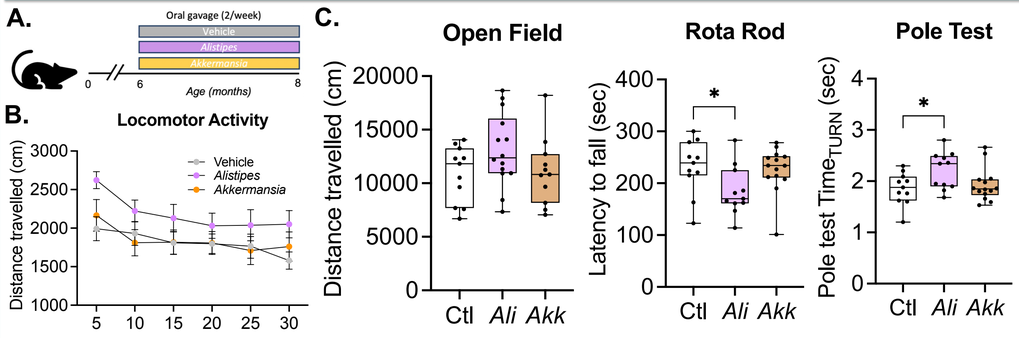

Figure 5. *Alistipes* accelerates motor deficits in 3KL male mice. A. Study design, 3KL male and female mice are administered BHI broth (vehicle), *Alistipes muris* or *Akkermansia muciniphila* in BHI broth from 6-8 months of age. **B.** *Alistipes* mice have a non-significant increase in locomotor activity. **C.** *Alistipes* colonized mice have significantly reduced latency to fall on the rota rod and increased turn time on the pole test. One-way ANOVA, **P*<0.05.

6. *Alistipes* increases splenic Th17 γδ T cells and IL-17 production

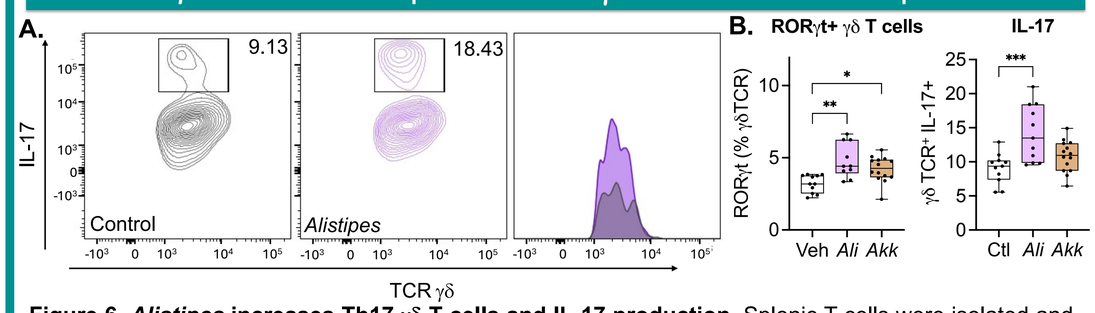

Figure 6. *Alistipes* increases Th17 γδ T cells and IL-17 production. Splenic T cells were isolated and stimulated with PMA and ionomycin to measure cytokine production by flow cytometry. **A.** Representative FACS plots of IL-17 production by γδ T cells. **B.** *Alistipes* significantly increased RORγt+ γδ T cells and IL-17 production. One-way ANOVA, **P*<0.05, ***P*<0.01, ****P*<0.001.

7. *Alistipes* upregulates inflammatory microglial genes and pathways

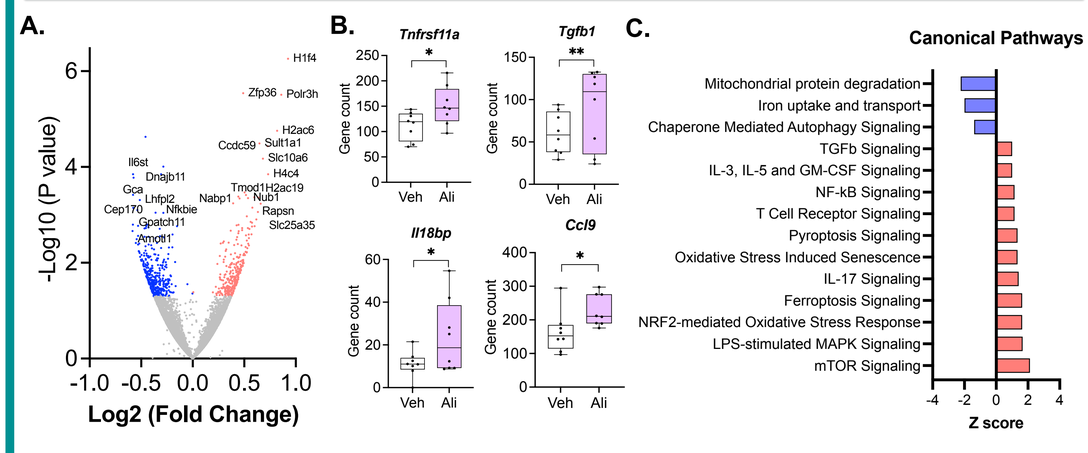

Figure 7. *Alistipes* modulate microglia. Microglia were isolated by FACS sorting and underwent bulk RNA sequencing. **A.** Volcano plot of differentially expressed genes, DEGs identified by DESeq2. **B.** Key inflammatory genes that are upregulated by *Alistipes* exposure including *Tnfrs11a (*TNF Receptor Superfamily Member 11a), *Tgfb1* (Transforming Growth Factor Beta 1), *Il18bp* (IL-18 Binding Protein), and *Ccl9* (C-C Motif Chemokine Ligand 9). **C.** Pathway activation analysis of FACs sorted microglial transcriptional profiles from 3KL male mice colonized with *Alistipes* using IPA functional pathway analysis with Z-score scale inferring the activation state of implicated biological functions. **P*<0.05, ***P*<0.01

8. Effect of *Alistipes* on the gut microbiome

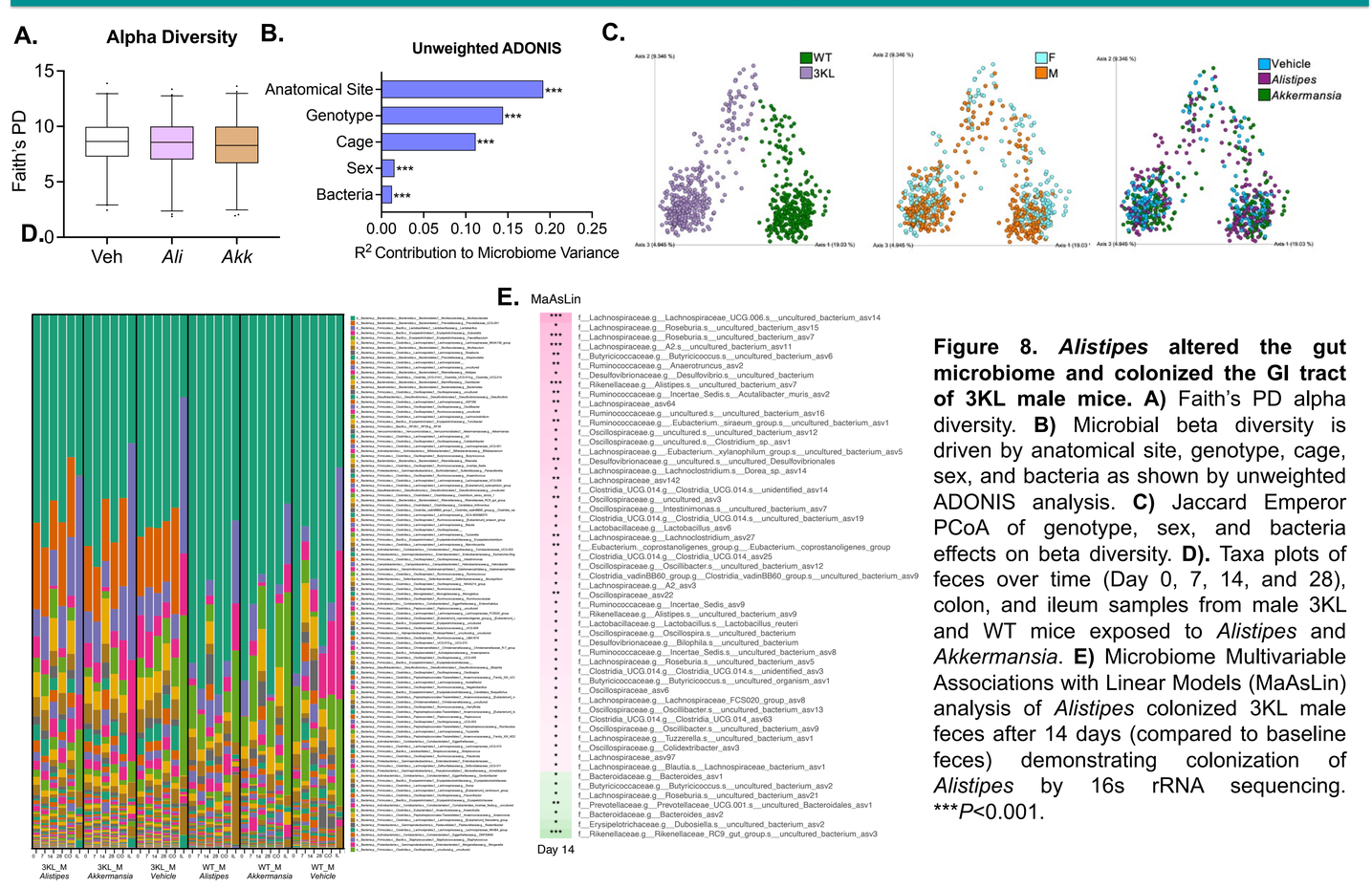

Figure 8. *Alistipes* altered the gut microbiome and colonized the GI tract of 3KL male mice. A) Faith's PD alpha diversity. **B)** Microbial beta diversity is driven by anatomical site, genotype, cage, sex, and bacteria as shown by unweighted ADONIS analysis. **C)** Jaccard Emperor PCoA of genotype, sex, and bacteria effects on beta diversity. **D).** Taxa plots of feces over time (Day 0, 7, 14, and 28), colon, and ileum samples from male 3KL and WT mice exposed to *Alistipes* and *Akkermansia*. **E)** Microbiome Multivariable Associations with Linear Models (MaAsLin) analysis of *Alistipes* colonized 3KL male feces after 14 days (compared to baseline feces) demonstrating colonization of *Alistipes* by 16s rRNA sequencing. ****P*<0.001.

9. IL-17 blockade negates motor impairment and inflammatory response to *Alistipes*

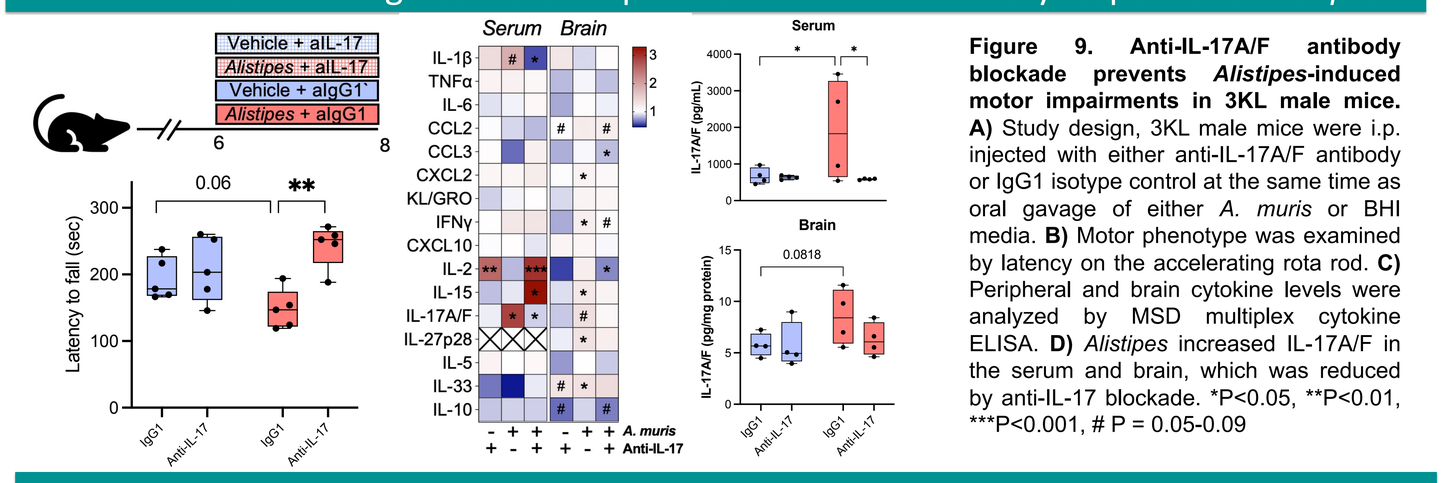

Figure 9. Anti-IL-17A/F antibody blockade prevents *Alistipes*-induced motor impairments in 3KL male mice. A) Study design, 3KL male mice were i.p. injected with either anti-IL-17A/F antibody or IgG1 isotype control at the same time as oral gavage of either *A. muris* or BHI media. **B)** Motor phenotype was examined by latency on the accelerating rota rod. **C)** Peripheral and brain cytokine levels were analyzed by MSD multiplex cytokine ELISA. **D)** *Alistipes* increased IL-17A/F in the serum and brain, which was reduced by anti-IL-17 blockade. *P<0.05, **P<0.01, ***P<0.001, # P = 0.05-0.09

Conclusions

These findings identify *Alistipes* as a detrimental microbiome component that exacerbates parkinsonian phenotypes through IL-17-mediated neuroimmune mechanisms. IL-17 blockade mitigated these effects, highlighting a microbiome-immune-brain axis driving progression.