Microbial Clues to a Genetic Puzzle: The Gut Microbiome in CTNNB1 Syndrome

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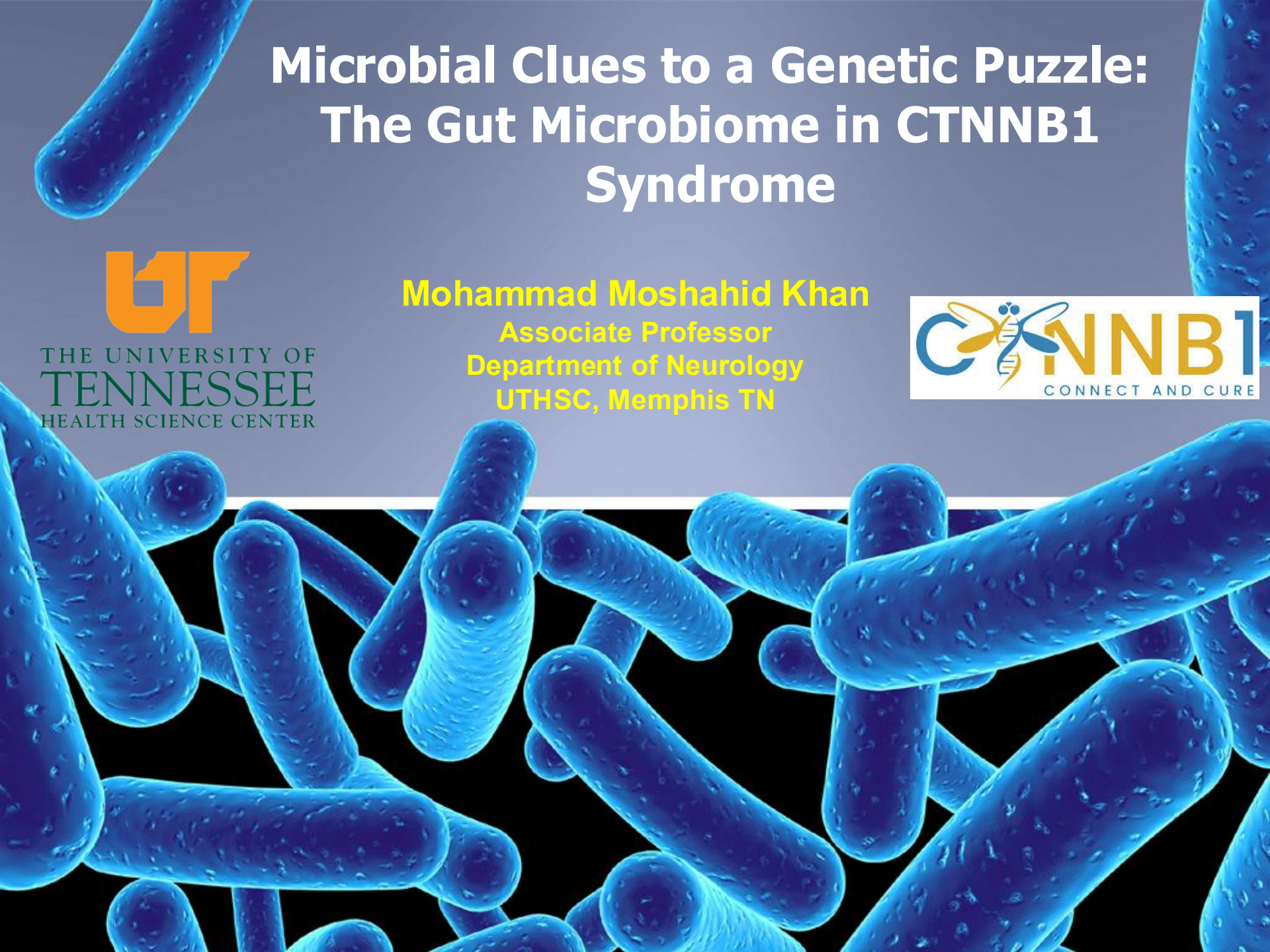
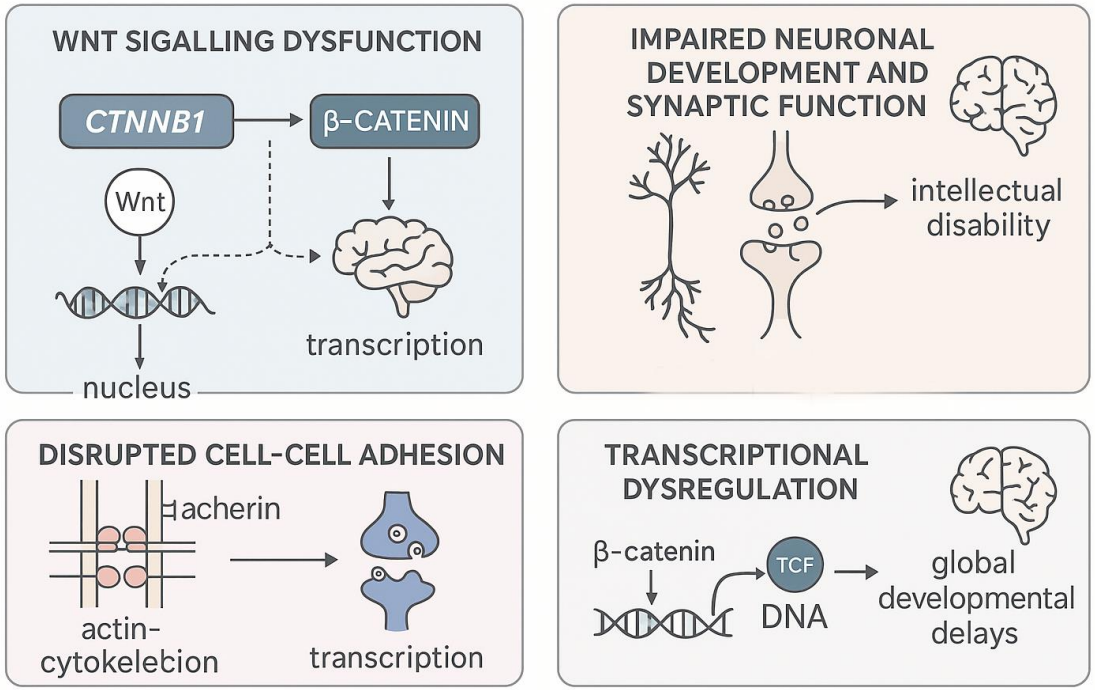
CTNNB1 CONNECT AND CURE



CTNNB1 Syndrome

- CTNNB1 syndrome is a rare neurodevelopmental disorder, affecting children worldwide.
- The disease is caused by a *de novo* loss-of-function mutations in the CTNNB1 gene, encoding β-catenin.
- Prevalence — 2.6–3.2 per 100,000 births.
- Characterized by global developmental delay, intellectual disability, spasticity, and speech impairment.
- Currently no disease-modifying therapies or targeted interventions.
- The precise pathophysiological mechanisms underlying CTNNB1 syndrome remains undefined.

Mechanisms underlying CTNNB1 Syndrome



- ✓ β-Catenin mediates canonical Wnt signaling, translocating to the nucleus to regulate gene expression via TCF/LEF transcription factors.
- ✓ Disrupted Wnt/β-catenin signaling impairs neuronal development, synapse formation, and neuroplasticity.
- ✓ Loss of β-catenin compromises cell-cell adhesion, particularly at adherens junctions, affecting tissue integrity.
- ✓ Aberrant β-catenin-TCF interaction leads to transcriptional dysregulation, contributing to neurodevelopmental deficits.

Is there any other potential mechanism underlying CTNNB1 syndrome?

Could gut microbiome dysbiosis be a hidden driver of neurodevelopmental deficits in CTNNB1 syndrome?

Is targeting the gut microbiome a promising therapeutic avenue for CTNNB1 syndrome?

Is the Gut the unsung hero of the body?

🧠 The **brain:** Obviously, it's me! I'm the boss. I control everything."

❤️ Heart: Without my beat, you're all done!

🫀 Liver: I clean up your messes and keep the system running smooth. I deserve a raise!

💩 Gut: You're all full of it! I do everything — digest your food, absorb nutrients, get rid of waste, make hormones, and protect you from germs!

All others: 🤣

😡 Gut: You laugh? I'm shutting down.

A few miserable, bloated, and cranky days later, 🥴
Total system crash!

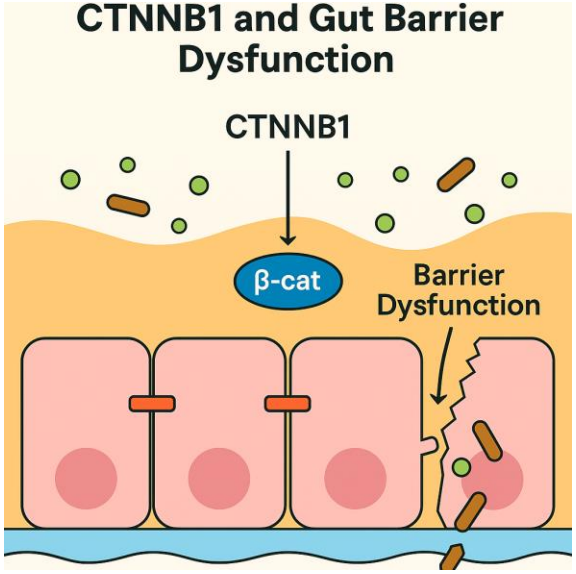
All organs: We underestimated the gut.

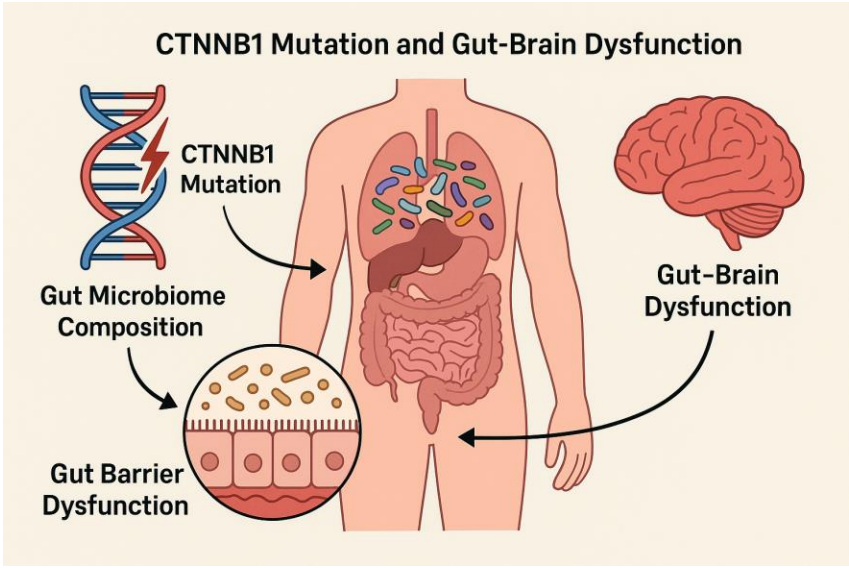
From that day on, they agreed: **Never mess with the gut, again**

All disease begins in the gut
Hippocrates (460 BC – 370 BC)

- The human body hosts about 40 trillion bacterial cells, outnumbering the approximately 30 trillion human cells within us.
- There are a **hundred times** more microbial genes present in our microbiome than our own human genes (~23, 000).
- Our genes turn on and off in response to what our microbes do.
- The gut is more than digestion; it's a multitasking superhero! Hormone production, immune defense, waste management, brain connection.

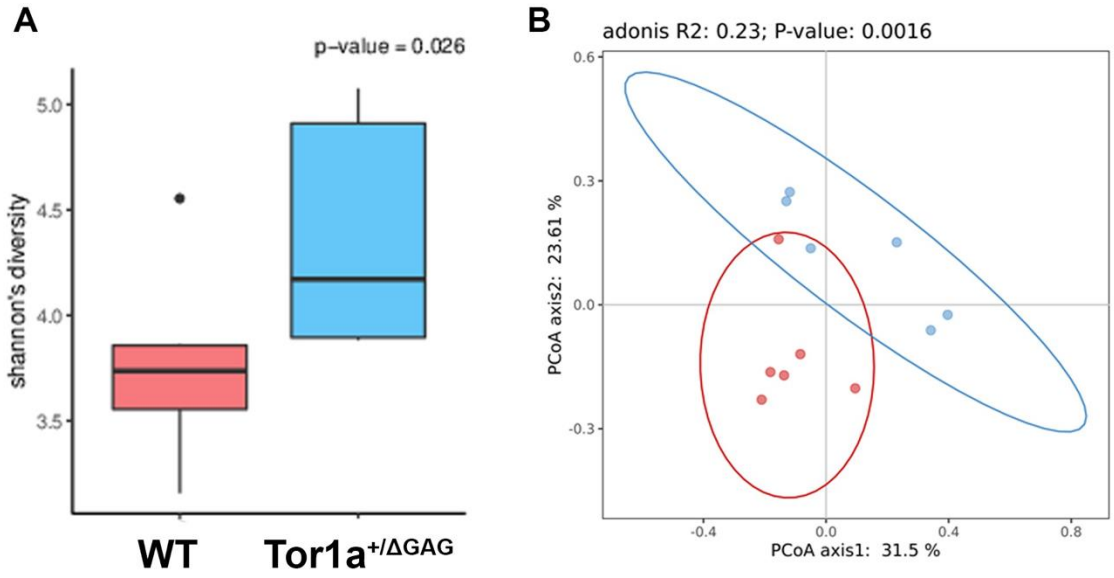
CTNNB1 is expressed in the gut epithelium, where it plays a critical role in maintaining gut barrier integrity; its dysfunction may contribute to increased intestinal permeability and microbial dysbiosis.

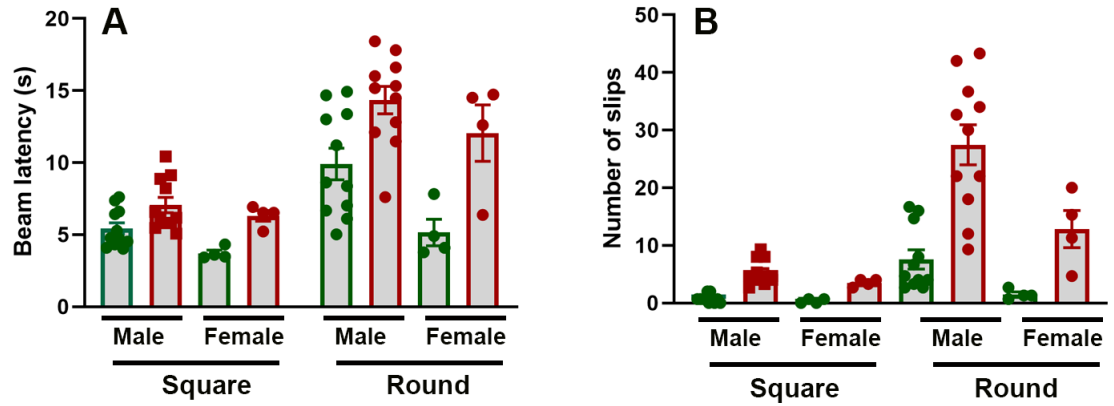


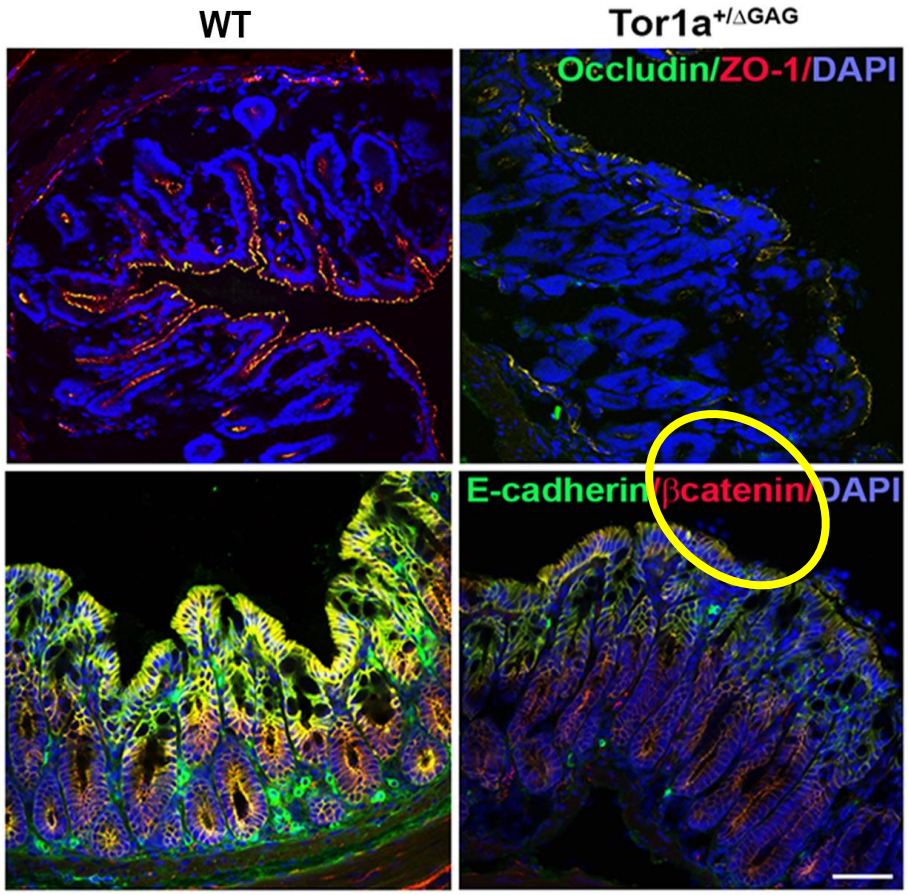


Gut dysbiosis is a common feature across a spectrum of rare genetic neurological disorders

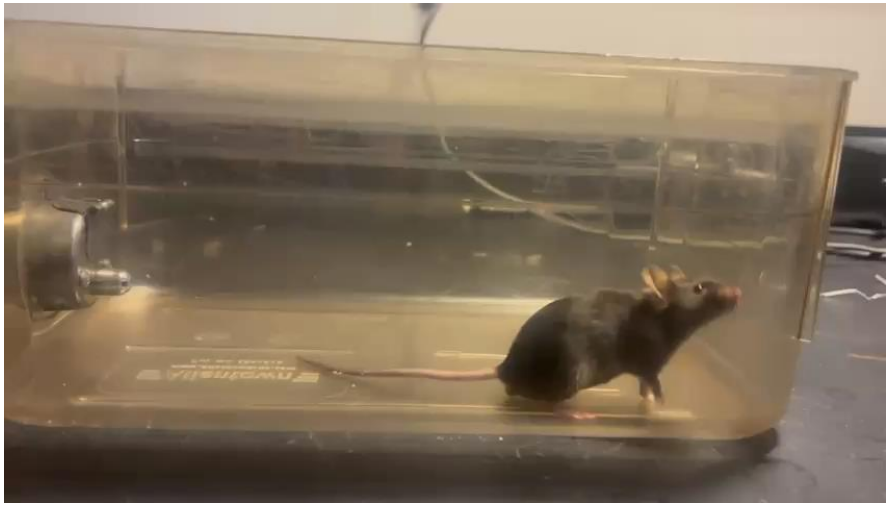
DYT1 dystonia: Primarily a childhood-onset disorder caused by a mutation in the *TOR1A* gene.



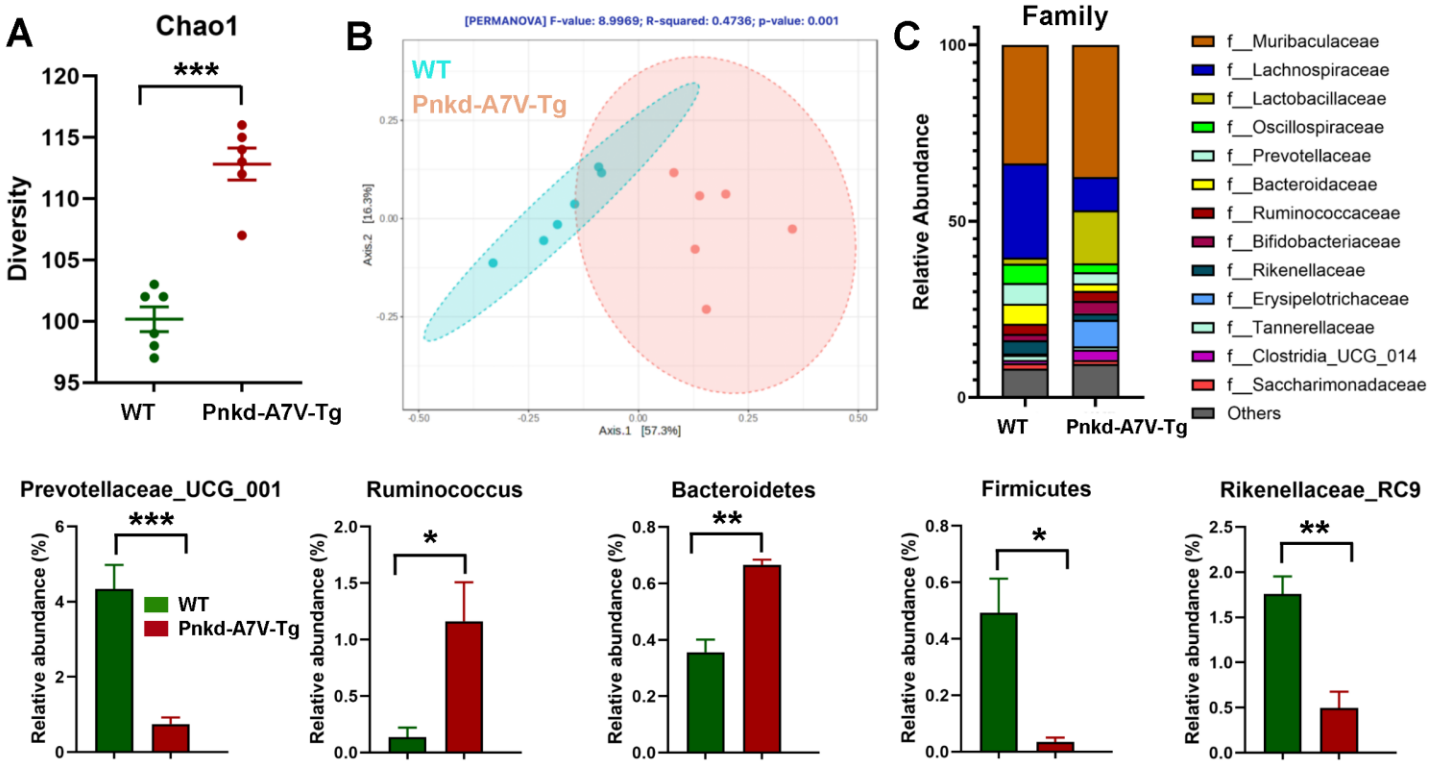


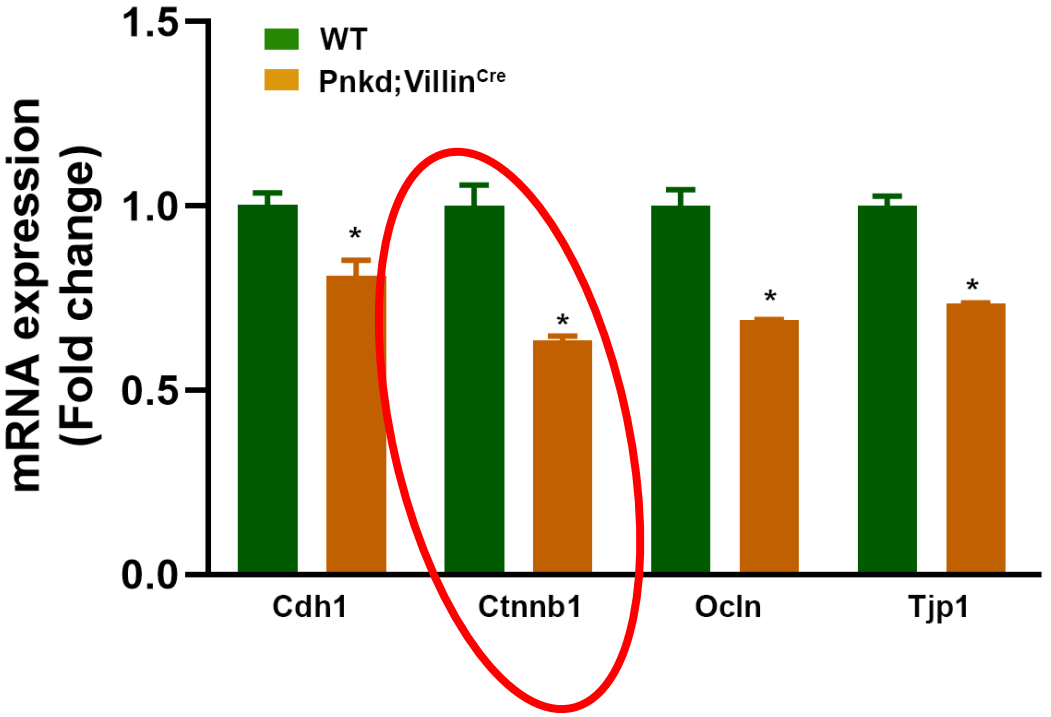

WT
Tor1a+/ΔGAG
Occludin/ZO-1/DAPI
E-cadherin/βcatenin/DAPI

PNKD: A rare genetic neurological disorder marked by early developmental delays and episodic involuntary movements.



Diverse gut microbiome profiles are associated with *Pnkd* mutation in mice.




mRNA expression
(Fold change)
1.5
1.0
0.5
0.0
WT
Pnkd;Villin^Cre
*
*
*
*
Cdh1
Ctnnb1
Ocln
Tjp1

From Gut to Brain: Microbiome-based clues to neurodevelopmental deficits in CTNNB1 Syndrome

1. **Identify an association of *CTNNB1* mutations and gut microbial signatures.**

2. **Discover therapeutically relevant microbiome-based molecular pathways.**

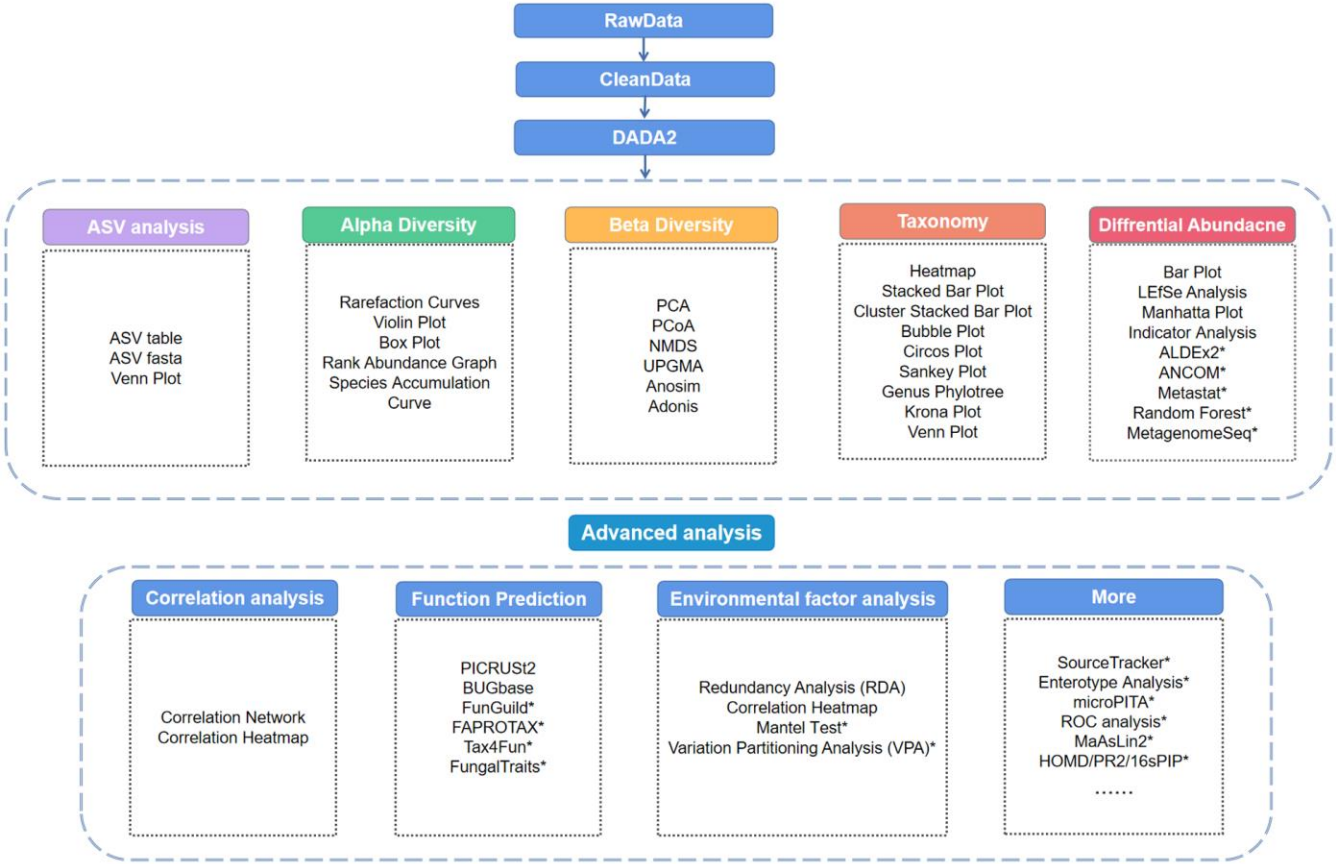
3. **Identify and test novel therapeutic strategies.**

Is there any association with *CTNNB1* mutations and gut microbiome?

Microbiome Profiling

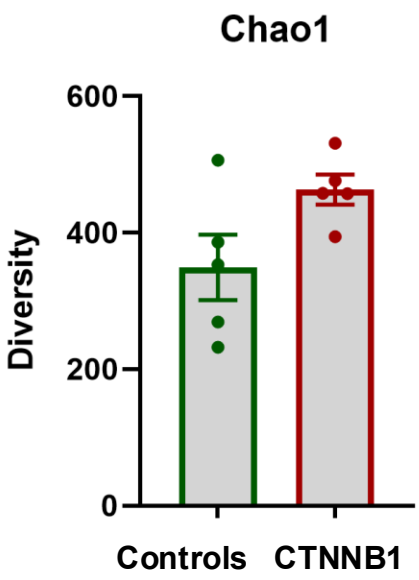
- Collected fecal samples from CTNNB1 patients and isogenic controls

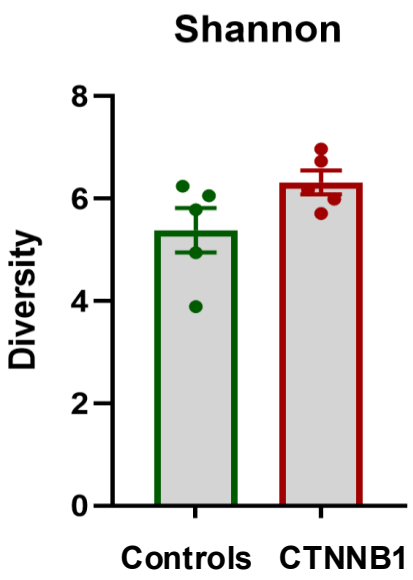
- DNA isolation → 16S rRNA → taxonomic & functional analysis

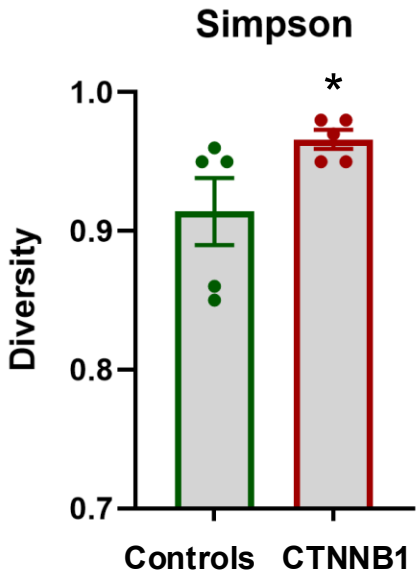


Altered gut microbial compositions in CTNNB1 patients

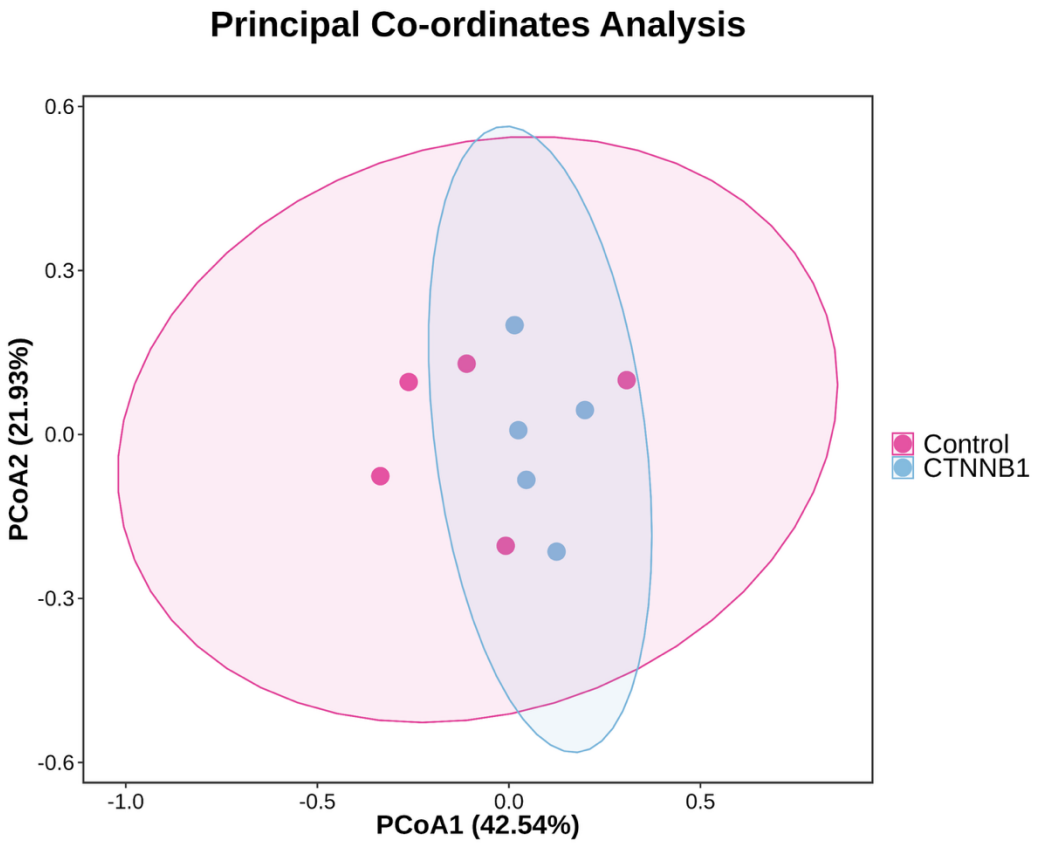
- ✓ Higher Chao1 and Shannon indices indicate increased microbial richness and diversity.
- ✓ Simpson index indicates changes in community evenness



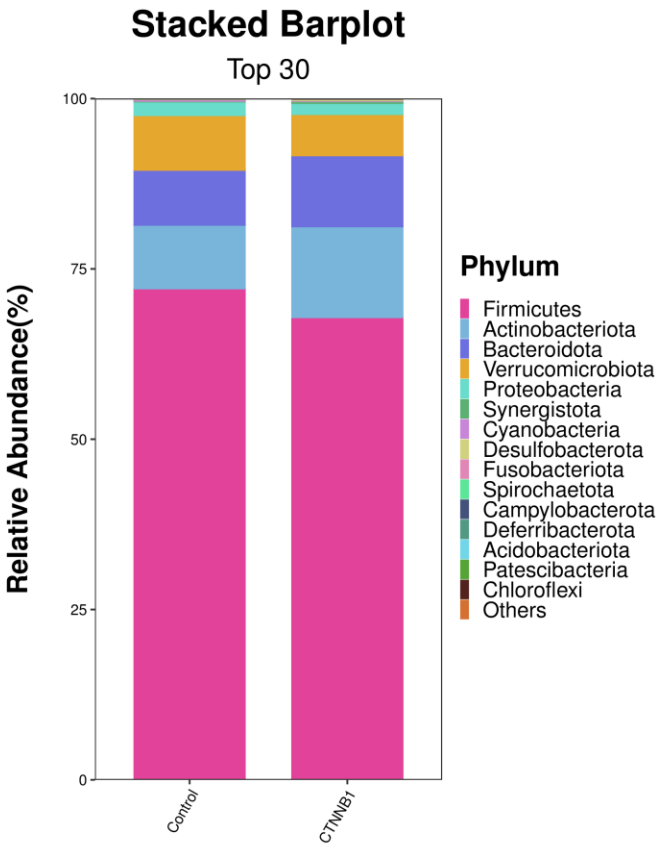


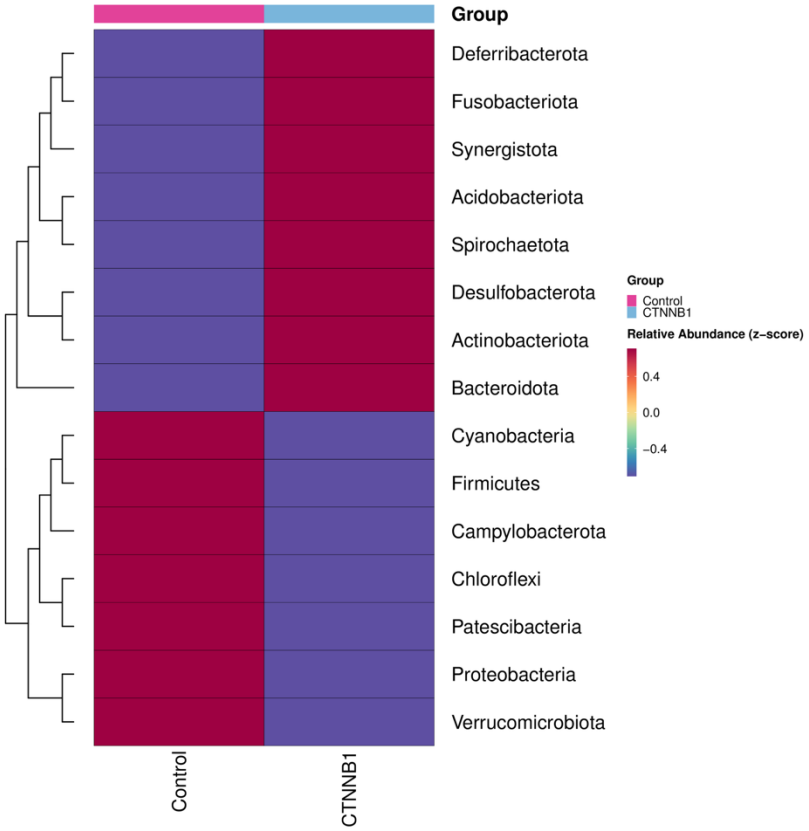


- ✓ Beta diversity analysis revealed separation between groups, suggesting distinct microbial community.

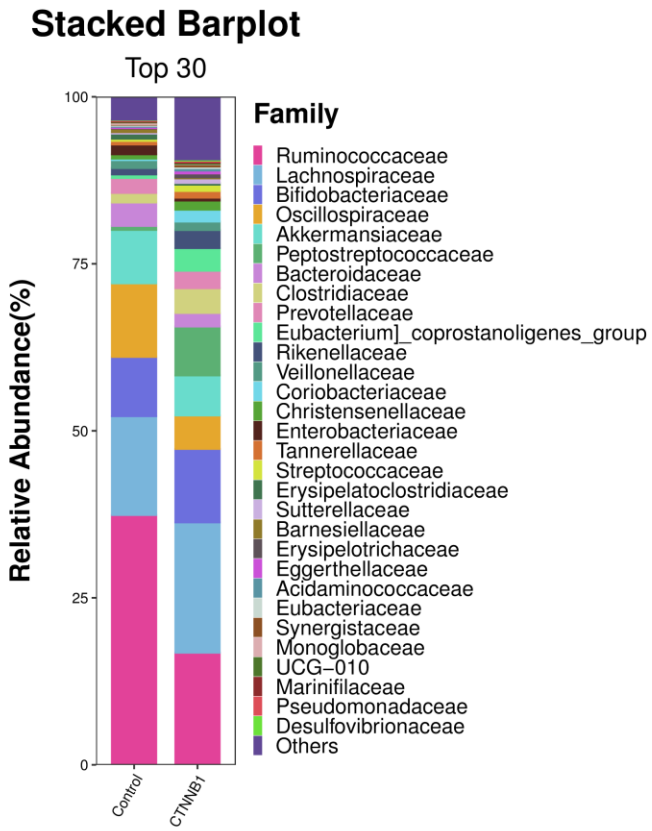


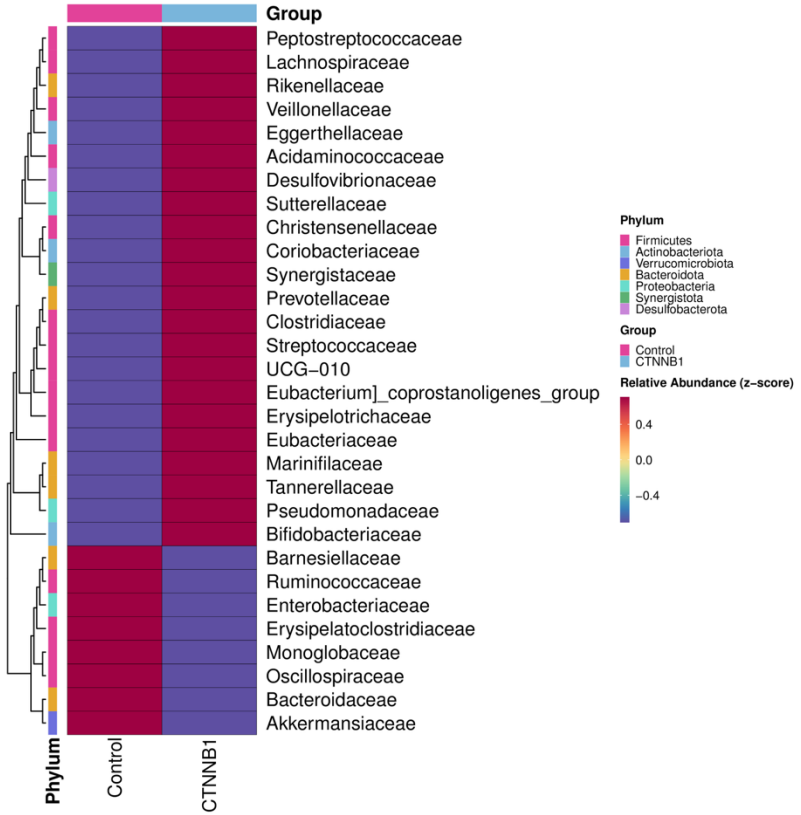
- ✓ Reduced Firmicutes alongside increased Bacteroidota and Actinobacteria - gut microbial imbalance.
- ✓ Decreased Verrucomicrobiota, especially Akkermansia -impaired gut mucosal integrity and disrupted host-microbe interactions.



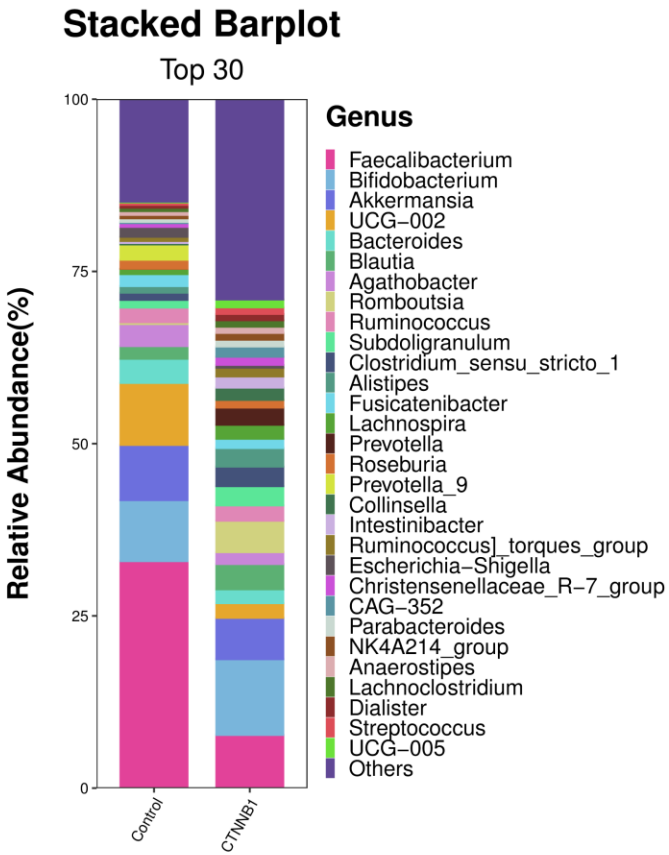


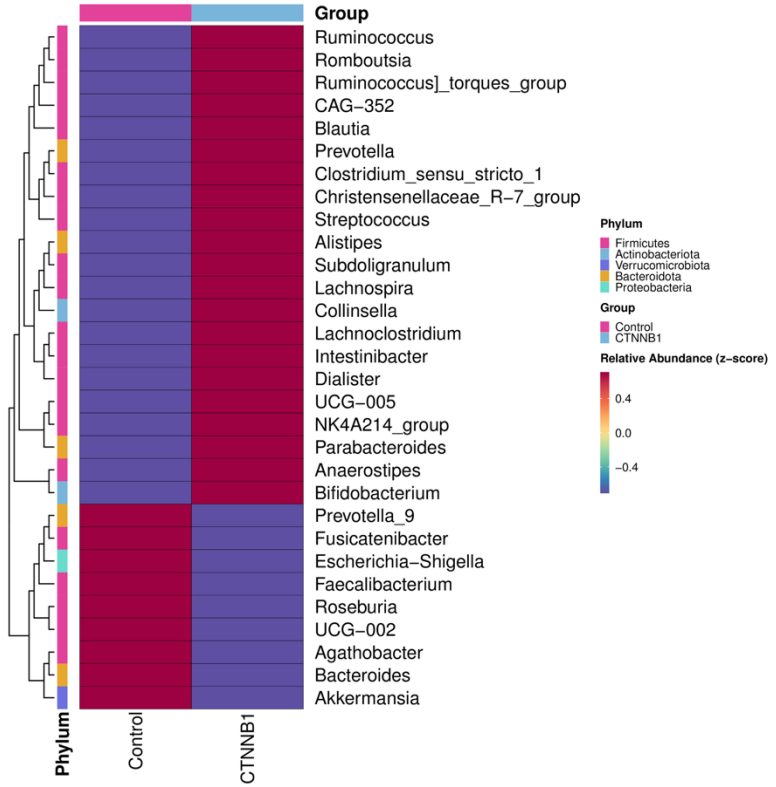
Gut microbiome profile at Family level



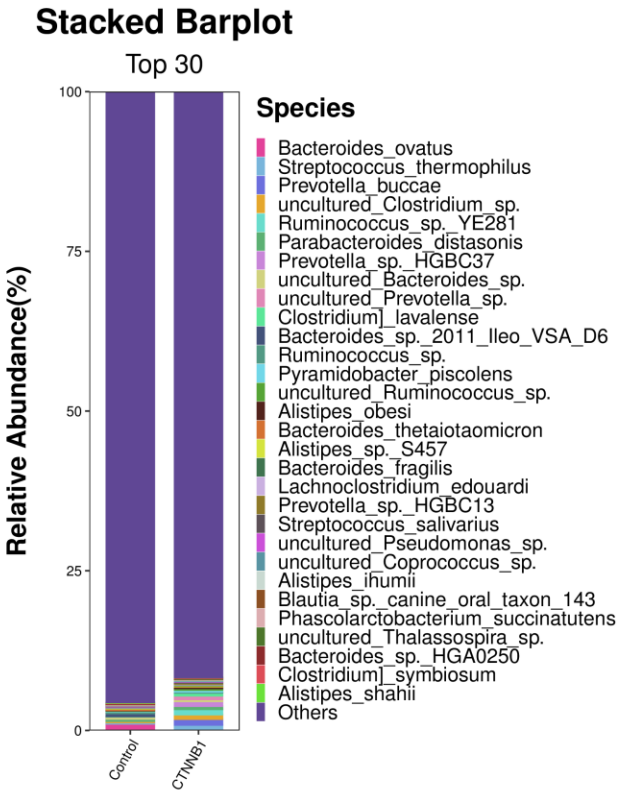


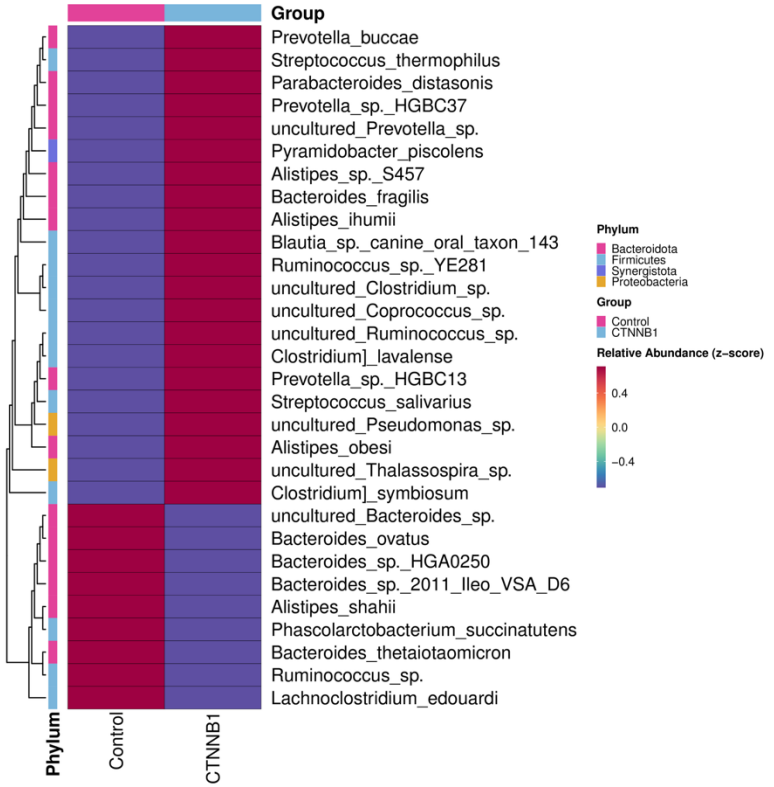
Gut microbiome profile at Genus level





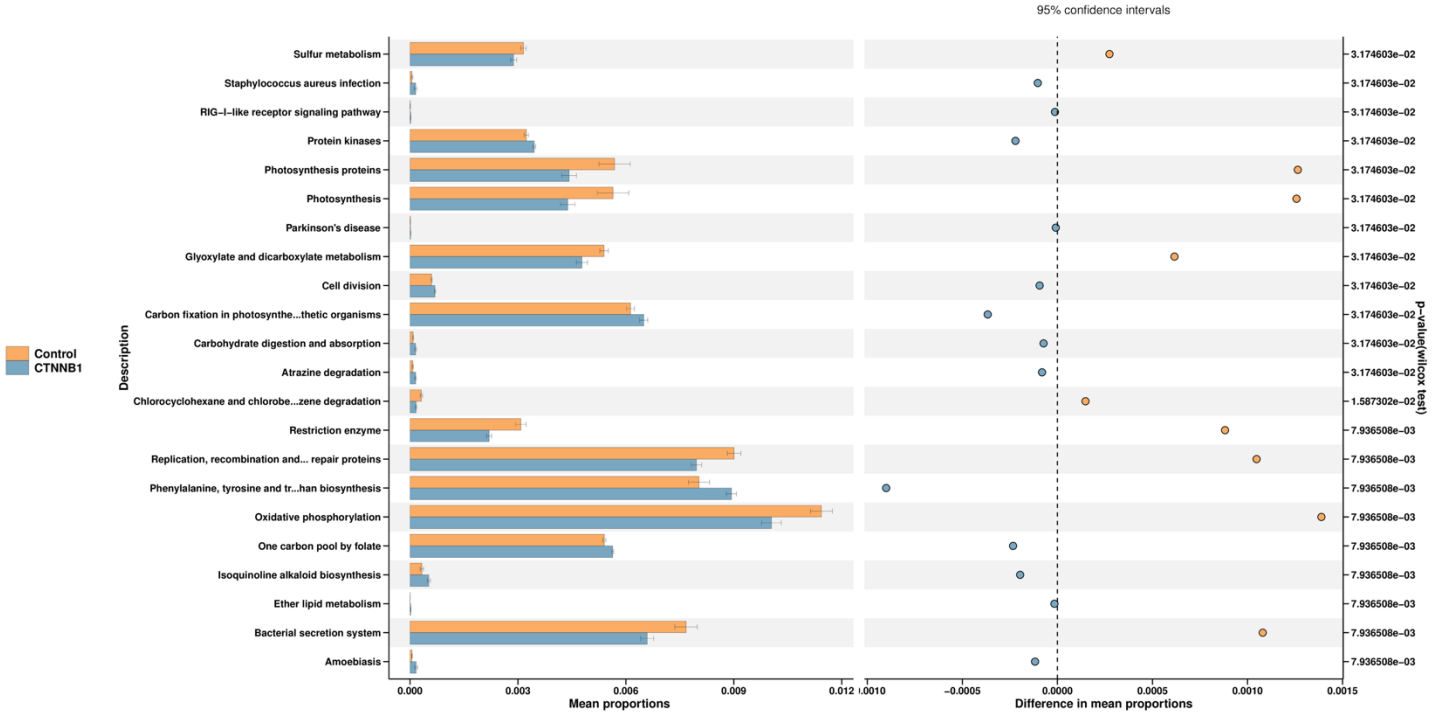
Gut microbiome profile at Species level





PICRUSt2-based functional prediction:

- Energy metabolism and oxidative phosphorylation, indicating changes in microbial ATP production and redox balance.
- Cofactor and vitamin metabolism (e.g., B-vitamins, NAD^+ biosynthesis), suggesting potential impacts on host micronutrient availability.
- Genetic information processing, including pathways for DNA repair, recombination, and protein kinases, pointing to increased microbial stress responses or replication activity.
- Sulfur and lipid metabolism
- Host–microbe signaling and pathways associated with infectious and neurodegenerative diseases (e.g., Parkinson's disease), highlighting a potential microbiome-mediated contribution to neurodevelopmental dysfunction.



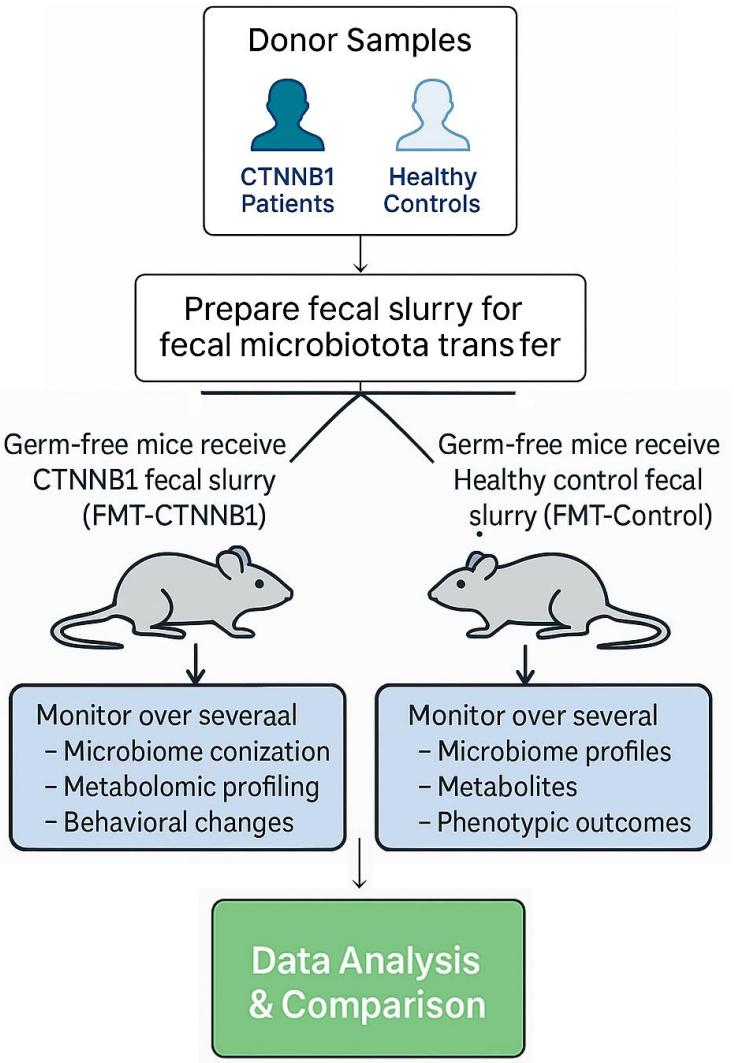
Goal of the research

1. **Identify an association of *CTNNB1* mutations and gut microbial signatures.**

2. **Discover therapeutically relevant microbiome-based molecular pathways.**

3. **Identify and test novel therapeutic strategies.**

Ongoing studies…..



Our ongoing and future studies aim to integrate metabolomics and mechanistic interventions to validate microbial activity and causality, with the goal of developing effective and safe microbiome-based therapeutic strategies for CTNNB1 syndrome!

NIH
National Institutes of Health

CDMRP
DEPARTMENT OF DEFENSE
CONGRESSIONALLY DIRECTED MEDICAL RESEARCH PROGRAMS

CHNNB1
CONNECT AND CURE

ALZHEIMER'S ASSOCIATION