Altered serotonergic and GABA signaling in the gut in a mouse model of impaired maternally inherited UBE3A

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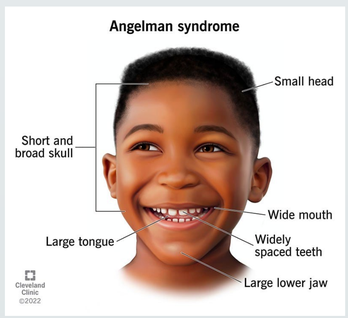
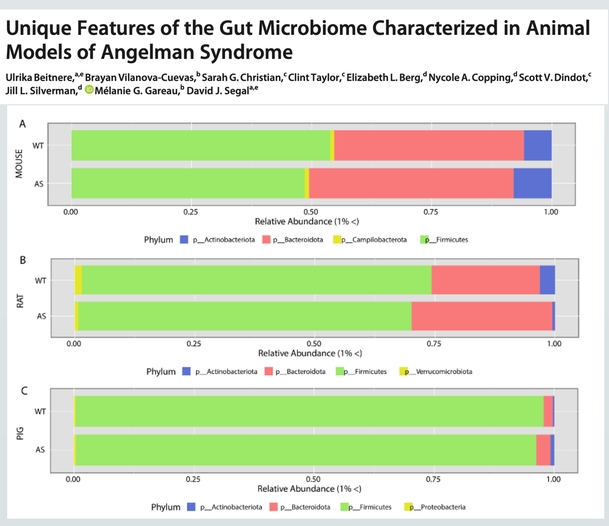
Introduction

- Patients diagnosed with Angelman Syndrome (AS) are characterized by the loss of function mutation of the maternal Ubiquitin Ligase E3A (UBE3A).[1]
- Genomic imprinting of paternal chromosome in neurons
  - Deficient neuronal UBE3A expression.[1]
- UBE3A codes for ubiquitin ligase E6-Associated Protein (E6-AP) critical for ubiquitin-proteasome pathway function
  - impaired neuronal degradation of proteins.[1]



Clinical Symptoms include:

- **Neurological impairments-** Neurodevelopmental delays, deficits in movement and coordination, epilepsy, and seizures
- **Concurrent Gastrointestinal issues-** Constipation, poor feeding due to hypotonia of throat, Reflux



Phylum-level differences between AS and WT microbial compositions by animal model.

- Microbiota dysbiosis across three different animal models (rat, mouse, pig) demonstrating differences arising due to genetic deletions resulting in AS.[2]
- Differences between AS & WT across three different animal models consistent with patients with chronic constipation.[2]
  - Decrease of Bifidobacterium & increase of Bacteroides

Hypothesis

Gut-brain impairments, including gastrointestinal motility delays and altered serotonergic and GABA signaling, will be present in AS-modeled mice

Results

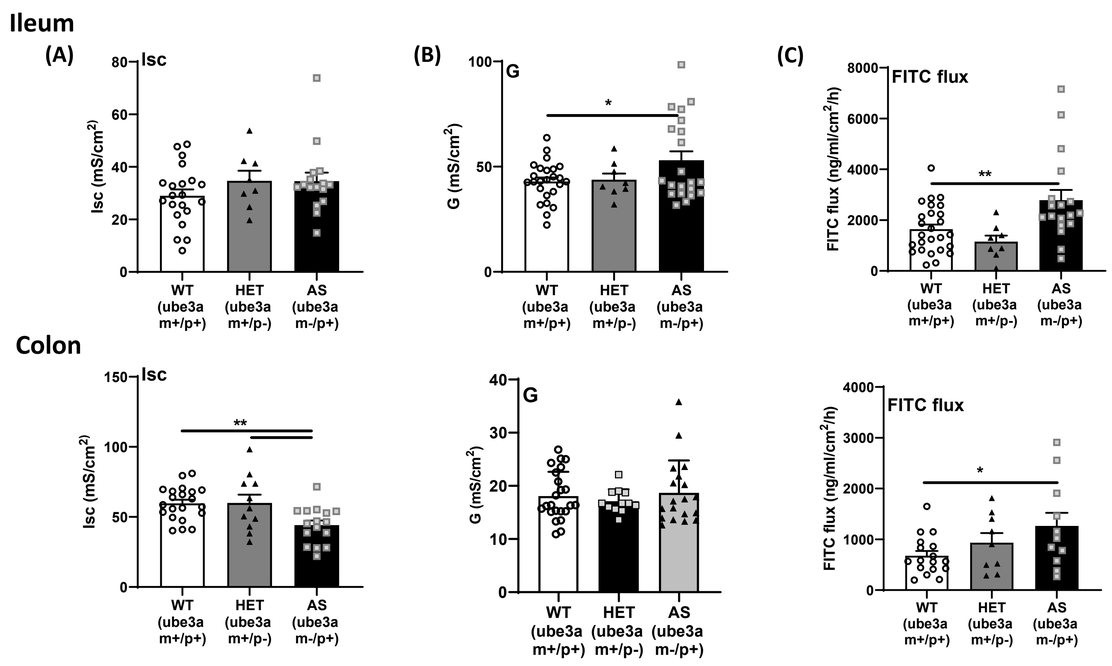


Figure 1. Ussing chamber studies. AS, HET, and WT mice were compared. Ussing chamber studies assessed (A) short circuit current [Isc], (B) conductance (G) and (C) FITC flux in the ileum and distal colon. Altered ion transport and permeability was observed in ileum and colon in AS mice. Students T-test, *$p < 0.05$, **$p < 0.01$.

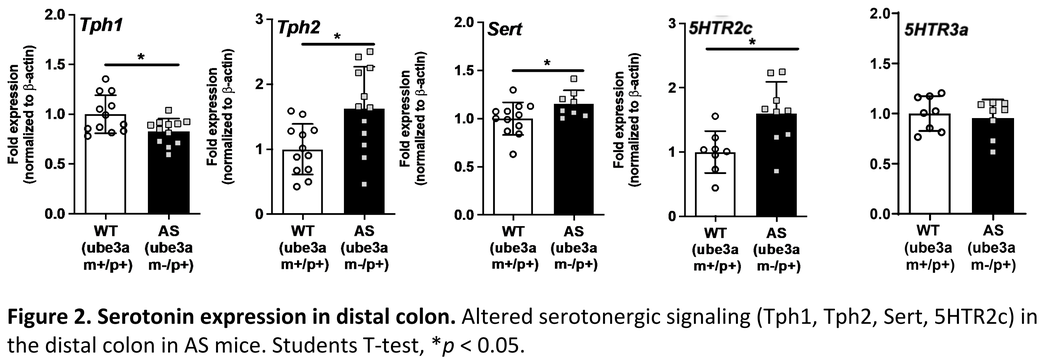


Figure 2. Serotonin expression in distal colon. Altered serotonergic signaling (Tph1, Tph2, Sert, 5HTR2c) in the distal colon in AS mice. Students T-test, *$p < 0.05$.

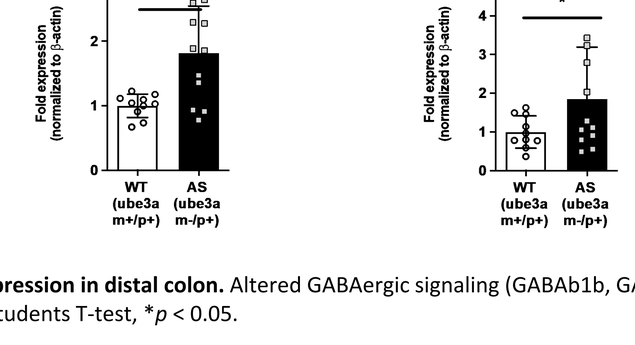


Figure 3. GABA expression in distal colon. Altered GABAergic signaling (GABAb1b, GABAa2a) in the distal colon in AS mice. Students T-test, *$p < 0.05$.

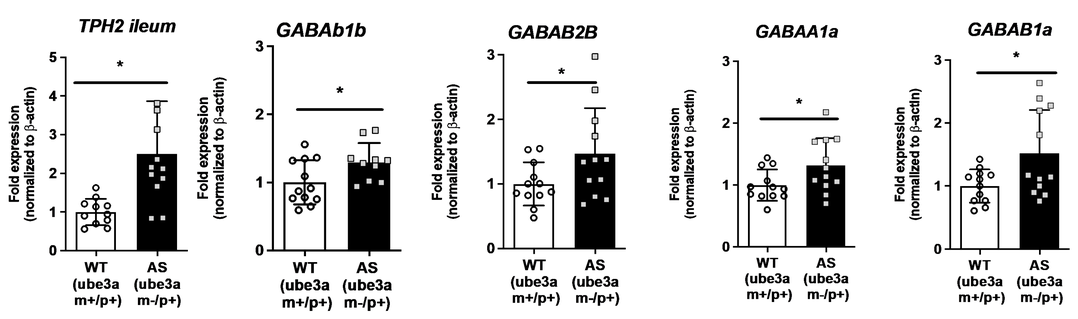


Figure 4. Serotonin and GABA expression in ileum. Altered serotonergic signaling (Tph2) and GABAergic signaling (GABAb1b, GABAb2b, GABAa1a, GABAb1a) in the ileum of AS mice. Students T-test, *$p < 0.05$

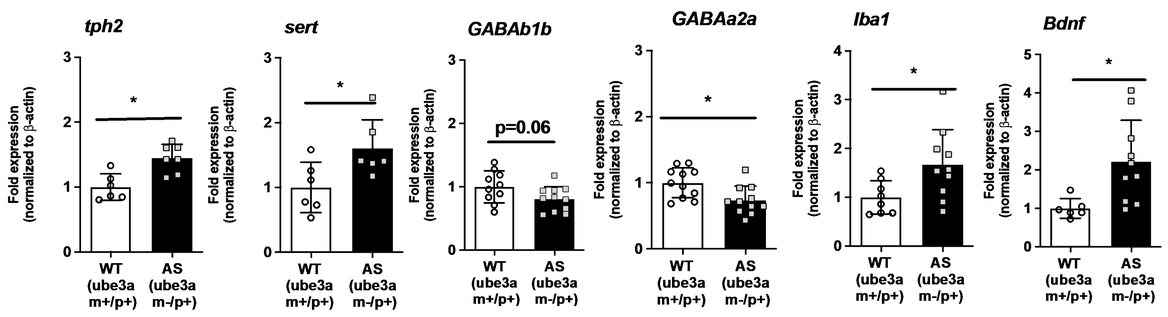


Figure 5. Serotonin and GABA expression in Hippocampus. Altered serotonergic signaling (Tph2, Sert), GABAergic signaling (GABAb1b, GABAa2a), neuroinflammation (Iba1) and neurogenesis (Bdnf) in the HC in AS mice. Students T-test, *$p < 0.05$

Summary

- Decreased secretory state in colon coupled with increased permeability in colon and ileum in AS mice suggestive of altered motility and permeability in the gut
- Altered serotonin and GABA gene expression in the gut and brain in AS mice suggestive of altered neuronal signaling

Conclusion

AS mice show evidence of constipation mediated by altered enteric nerves, which may provide insight to the mechanisms of constipation in AS patients.

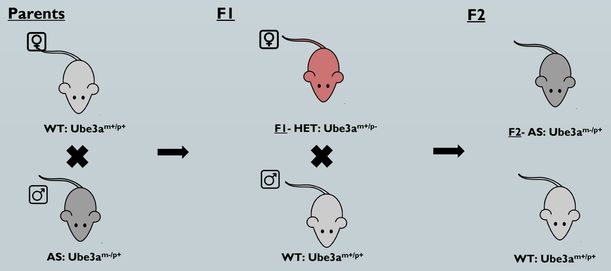
Future Directions & Speculations

- Butyrate is a short-chain fatty acid (SCFA) that beneficially modulates the gut microbiome.
- We will administer a trial of Tributyrin (butyrate) as a therapeutic strategy to improve GI symptoms and MGB axis signaling by beneficially modulating the gut microbiome in AS mice

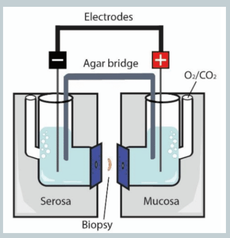
We hope to determine whether these findings are common across other neurodevelopmental/ degenerative diseases and provide a better understanding of the signaling pathways involved in constipation across species.

Methods

Mouse model of AS: Ube3a$^{m-/p+}$



Ussing Chamber



Gut tissues (ileum & distal colon) of AS & WT mice analyzed for

- **Isc**- active ion transport
- **G**- tight junction permeability
- **FITC**- macromolecular permeability

qPCR

Ileum, distal colon, Hippocampus (HC) tissues of AS & WT mice analyzed for

- Serotonergic signaling
- GABA signaling

References

1. Beitnere U, Vilanova-Cuevas B, Christian SG, Taylor C, Berg EL, Copping NA, Dindot SV, Silverman JL, Gareau MG & Segal DJ. Unique Features of the Gut Microbiome Characterized in Animal Models of Angelman Syndrome. mSystems. 2023 Feb 23;8(1). doi: 10.1128/msystems.00608-22.

2. Glassman LW, Grocott OR, Kunz PA, Larson AM, Zella G, Ganguli K & Thibert RL. Prevalence of gastrointestinal symptoms in Angelman syndrome. Am J Med Genet A. 2017 Oct;173(10):2703-2709. doi: 10.1002/ajmg.a.38401.

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