Gastrointestinal Problems in Children with Autism: Recognition of the problem and a potential link with serotonin

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History of GI Issues in ASD

- 1943: Leo Kanner described autism in his seminal paper
  - 7/11 children described to have "feeding or dietary issues"
    - Supportive of association between ASD & GI problems
  - These issues all related to autistic behavior
    - A theme throughout history

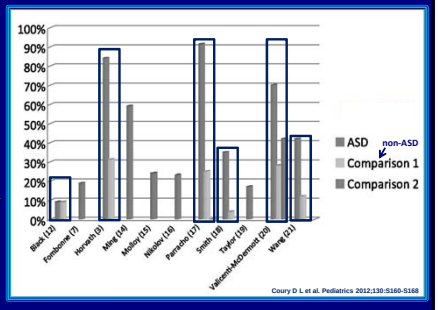
Kanner L. Autistic disturbances of affective contact. Acta Paedopsychiatr 1968; 35: 100-36

My Roles in Autism

- Pediatric Gastroenterologist at Columbia
  - Kids with ASD and Gastrointestinal (GI) issues
- Physician-scientist
  - "The second brain": enteric nervous system
    - The nervous system in the intestine
  - Roles of enteric nervous system development: serotonin and oxytocin:
    - motility disorders (how well the intestine moves)
    - intestinal inflammatory disease
    - ASD & gut function
  - Clinical/translational studies:
    - Association between GI disorders & difficult behaviors in ASD
    - Intestinal serotonergic signaling pathways in ASD
    - Utility of a GI screening tool for children with ASD

Gastrointestinal disorders are more common in children with ASD

- 9-91%[1-10]
- high rate of GI disorders in children with ASDs
- ASDs >> typical
  - all but one study
- Multicenter retrospective prevalence study[11]
  - >14,000 individuals
  - <age 35
  - 2-3x more common



[1]Black C et al. BMJ. 2002;325(7361):419–421; [2]Fombonne E et al. Mol Psychiatry.2001;6(2):133–134; [3]Horvath K et al. Curr Gastroenterol Rep. 2002;4(3):251–258; [4]Molloy CA et al. Autism. 2003;7(2):165–171; [5]Nikolov RN et al. J Autism Dev Disord. 2009;39(3):405–413; [6] Parracho HMRT et al. J Med Microbiol. 2005;54(pt 10):987–991; [7]Smith RA et al. Autism.2009;13(4):343–355; [8]Taylor RB et al. BMJ 2002;v 324(7334):393–396; [9]Valicenti-McDermott M et al. J Dev Behav Pediatr. 2006;27(suppl 2):S128–S136; [10] Wang LW et al. J Dev Behav Pediatr. 2011;32(5):351–360; [11]Kohane IS et al.PLoS One 2012;7(4):e33224.

Objectives

- GI Conditions in Autism
  - Prevalence
  - Types
  - Presentations
- Serotonin as a link between the brain and intestinal abnormalities in ASD

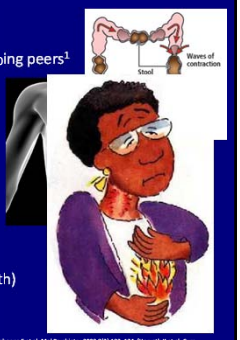
GI symptoms are common in autistic spectrum disorder (ASD)

- Meta-analysis confirms reason for this concern.
  - Overall: OR 4.42
    - 95% CI, 1.90–10.28
  - Constipation: OR 3.86
    - 95% CI, 2.23–6.71
  - Diarrhea OR 3.63
    - 95% CI, 1.82–7.23

B McElhanon et al. Pediatrics 2014;133;872

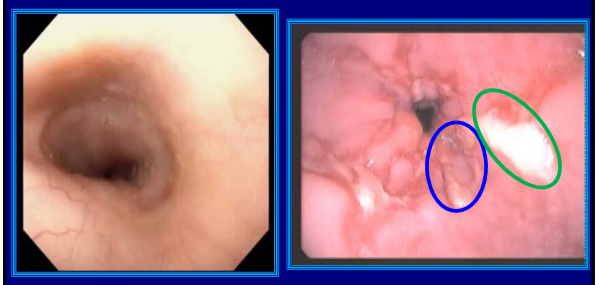
GI problems are common in ASD

- Motility abnormalities:
  - 3.5x more common than normally developing peers[1]
  - Constipation
  - Diarrhea
- Abdominal pain
  - Gastro-esophageal reflux disease (GERD)
  - Esophagitis, gastritis, duodenitis
- Others
  - nutrition
    - food/texture aversion
  - allergy/food sensitivity
  - dysbiosis (small bowel bacterial overgrowth)
  - Inflammatory bowel disease[12]
    - 0.83% versus 0.54%



[1]CDC; Autism and Developmental Disabilities Monitoring (ADDM) Network; [2]Black C et al. BMJ. 2002;325(7361):419–421; [3]Fombonne E et al. Mol Psychiatry.2003;8(2):133–134; [4]Horvath K et al. Curr Gastroenterol Rep. 2002;4(3):251–258; [5]Molloy CA et al. Autism. 2003;7(2):165–171; [6]Nikolov RN et al. J Autism Dev Disord. 2009;39(3):405–413; [7]Parracho HMRT et al. J Med Microbiol. 2005;54(pt 10):987–991; [8]Smith RA et al. Autism.2009;13(4):343–355; [9]Taylor RB et al. BMJ 2002;v 324(7334):393–396; [10]Valicenti-McDermott M et al. J Dev Behav Pediatr. 2006;27(suppl 2):S128–S136; [11] Wang LW et al. J Dev Behav Pediatr. 2011;32(5):351–360 ; [12]Kohane IS et al.PLoS One 2012;7(4):e33224; [13]Gorrindo et al. Autism Res 2012; 5: 101–8.

Esophagitis



Normal Esophagitis

Gastrointestinal Symptoms cause difficult behaviors

- Intensity correlates with behavior severity
- Comorbidities can arise as a result of GI discomfort[1-5]
  - irritability, anxiety, social withdrawal
  - regression
  - sleep disturbance, pica
- Presentations of GI pain in ASD[6]
  - abnormal posturing
  - self-injury to the abdomen or other areas
    - Hand and wrist biting, picking
  - vocal groaning or screaming
  - Aggression
  - Vocal tics
- GI problems are significantly associated with specific behaviors in ASDs[7]
  - self-injury, aggression, vocal tics
  - Subtle unexplained behaviors
    - sleep disturbance, tapping on chest, pointing to abdomen, increased irritability, change in eating patterns
  - GERD, gastritis and overall GI problems

[1]Maenner MJ et al. J Autism Dev Disord 2012; 42: 1520–5; [2] Horvath K et al. Curr Gastroenterol Rep 2002; 4: 251–8; [3]Nikolov RN et al. J Autism Dev Disord 2009; 39: 405–13; [4]Mazurek MO et al. J Abnorm Child Psychol 2013; 41: 165–76; [5] Gorrindo P et al. Autism Res 2012; 5: 101–8.[6] Buie T et al. Pediatrics 2010; 125 suppl 1: S19–29; [7]Buie T, Margolis K et al. Submitted

Self-injurious behaviors improved with treatment with an acid blocker

From files of Tim Buie, MD

Self Injurious Behaviors are Associated with GI Distress

From files of Tim Buie, MD

Joanna: Sandifer syndrome

- Usually in infants/toddlers
  - Adolescence in children with developmental disorders
    - nodding and rotation of the head
    - neck extension
    - gurgling
    - writhing movements of the limbs
    - severe hypotonia
- Joanna's diagnoses:
  - GERD
  - Erosive esophagitis despite standard PPI dosing
- Behaviors eliminated with bid dosing of PPI and sucralfate

A brain-gut connection in ASD

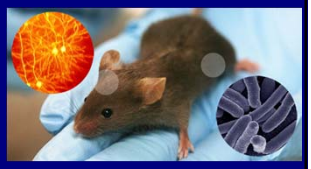
- GI problems may result from genetic and/or environmental risk factors for ASD
  - Stratify subpopulations of individuals
- Environmental
  - Maternal inflammation
  - Intestinal microbiome
- Genetic
  - C-met
  - CHD8
  - SERT G56A

Conclusions

- GI issues are common in children with autism
  - prospective, population-based studies needed to confirm whether GI problems are more prevalent in subsets of ASD
- GI conditions in autism may worsen behaviors and other co-morbidities
  - GI conditions should be ruled out
  - Critically needed:
    - Testing and treatment algorithms
    - Cause & effect studies
- Aggression or self injurious behaviors may require psychopharmacological or behavioral management
  - Medical etiologies should also be evaluated

A brain-gut connection in ASD: emerging research in environmental risks

- maternal immune activation
  - Inflammation is a potential non-genetic cause of autism[1]
    - epidemiological studies link maternal infections & elevated pro-inflammatory markers to increased autism risk in offspring
  - Mouse models of maternal immune activation[2]
    - ASD-related behavioral abnormalities, intestinal immune cell abnormalities and increased intestinal permeability



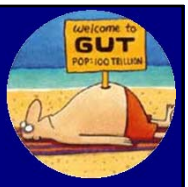
- Mice are not people ☹
  - Unknown whether there there is an increased prevalence of GI conditions in this population

[1]Mekinian A et al. Ann Rheum Dis 2013 Feb;72(2):217-22.[2]Hsiao EY et al. Cell 2013 Dec 19;155(7):1451-63

Etiology of GI Problems in ASD

- Medications
  - Selective Serotonin Reuptake Inhibitors:
    - Paxil, Prozac
      - Abdominal pain, nausea, gastritis, ulcers, GI bleed
      - Diarrhea → constipation
      - Decreased → increased appetite
  - Antipsychotics:
    - Increased appetite
    - Constipation
- Supplements
  - Probiotics: bloating, nausea, cramping
  - Fish oil: nausea, abdominal cramping
  - appropriate dosing
  - trial without supplements

A brain-gut connection in ASD: microbiome

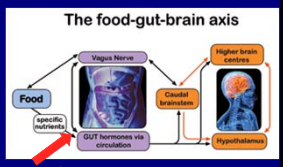


- Gut microbiota
  - Ensemble of microorganisms that reside in the intestine
  - contains tens of trillions of microorganisms
  - >1000 different species of known bacteria
    - weigh up to 4.4 pounds
  - >3 million genes
    - 150x more than human genes!
  - Like an individual identity card!
    - 1/3 common to most people
    - 2/3 are specific to individuals
    - Affected by many factors

- Why is the gut microbiota so important?
  - Directly impacts on our health
    - Helps to digest certain foods
    - Vitamin production (B and K)
    - Helps to combat aggression from "bad" or harmful bacteria
    - Helps to preserve gut permeability

A brain-gut connection in ASD: microbiome

- Microbiome alterations may alter behavior[4,5]
  - germ-free mice inoculated with selective bacteria alter anxiety/depressive behaviors
- Altered composition of the intestinal microbiota in ASD[1-3]
  - Desulfovibrio species in exclusively ASD
  - Sutterella species in kids with ASD & GI comorbidities
- Intestinal Consequences of altered microbiota
  - Impaired carbohydrate digestion[6,7]
  - Metabolomic differences
- Future of microbiome research
  - Which gut bacteria make a difference?
    - probiotics
  - Role of the metabolome
  - Is GI the chicken?
    - Motility differences and dietary preferences alter bacterial flora

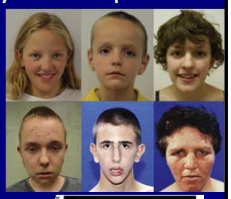


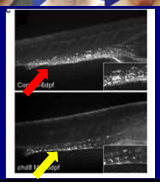
microbiota

[1]Finegold SM et al. Anaerobe.2010;16(4):444–453. [2]Finegold SM et al. Med Hypotheses. 2011 Aug;77(2):270-4. [3]Williams BL et al. MBio. 2012 Jan 10;3(1). [4]Heijtz RD et al. PNAS 2011; 108: 3047–52. [5]Bravo JA et al. Proc Natl Acad Sci U S A 2011; 108: 16050–5. [6]Williams BL et al. PLoS One. 2011;6(9):e24585. [7]Kushak RI et al. Autism. 2011 May;15(3):285–94.

Disruptive CHD8 Mutations Define a Subtype of Autism in Early Development

- Chromodomain Helicase DNA Binding Protein 8
  - Remodels chromatin
  - pivotal role in vertebrate early development & morphogenesis
- First gene mutation to show a very strong penetrance linked to a subtype of autism
  - First direct relationship between a gene mutation & ASD
- 6,176 children with ASD
- 15 had a CHD8 mutation
  - all had similar characteristics in appearance
    - Large heads and wide-set eyes
- Interviewed families of all cases with CHD8 mutations
  - sleep disturbance & gastrointestinal problems
- To confirm the findings, researchers disrupted the CHD8 gene in zebrafish
  - developed large heads & wide set eyes
  - The fish had fewer enteric neurons & were constipated
- ASD currently diagnosed on behavior
  - Results could lead to a "genetics-first approach"
- Short term: clinicians can provide targeted treatment



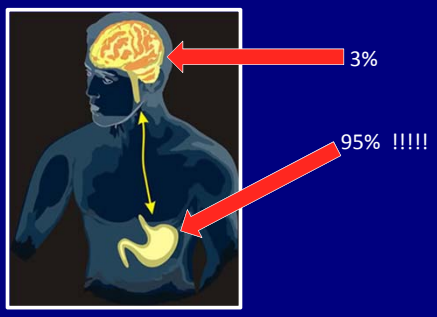


Bernier a et al. Cell. 2014 Jul 17:158(2):263-76.

A brain-gut connection in ASD: Genetic risk factors

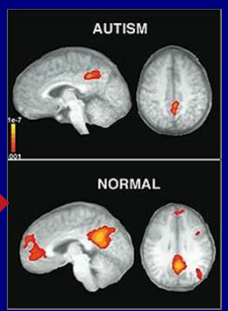
- C-met
- CHD8
- SERT G56A

A Brain-Gut Connection in Autism: Serotonin



A brain-gut connection in ASD: Genetic risk factors

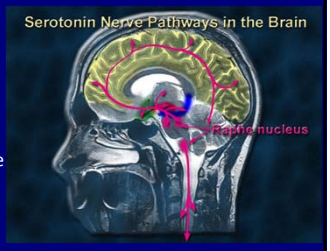
- *c-Met* susceptibility gene
  - Hepatocyte growth factor
    - membrane receptor essential for embryonic development
  - leading candidate gene for autism risk
    - Influences strength of connections between brain regions involved in social behaviors
    - common variants in MET appear more frequently in ASD
    - Expression altered in the brains of people with autism
- *c-Met* promoter variant rs1858830[1,3]
  - single nucleotide polymorphism that increases the risk for ASD
    - distinctively associated with individuals with ASD & GI dysfunction



[1] Rudie J.D. et al. *Neuron* **75**, 904-915 (2012); [2] Campbell D.B. *et al. Proc. Natl. Acad. Sci. U. S. A.* **103**, 16834-16839 (2006); [3]Campbell DB et al. Pediatrics 2009; 123: 1018–24; [4] Campbell D.B. *et al. Ann. Neurol.* **62**, 243-250 (2007)

Serotonin in the Brain & Gut: Critical for Brain Function

- Brain/Central Nervous System (CNS)
  - Neurotransmitter
    - Sleep, mood, appetite
  - One of the most widely distributed & earliest systems to develop in mice & humans[1]
    - Innervates almost all areas of the brain
    - Serotonergic neurons in human brain from the fifth gestational week[2]
    - Overlap between humans & mice



[1]Azmitia EC et al. *J Clin Psychiatr* 1991, 52(Suppl):4-16. [2] Sundstrom E et al.*Brain Res Dev* 1993, 75:1-12 [3] Lauder JM et al. *Dev Neurosci* 1978, 1:15-30.[4] Gaspar P et al. *Nat Rev Neurosci* 2003, 4:1002-1012.

Serotonin, Autism & the Brain

- Serotonin important for pre- and postnatal human brain development[1]
  - Abnormal brain serotonin levels → abnormal connecting neural circuits[2]
  - Changes in serotonergic function & signaling associated with ASD[2]
    - Increased # serotonin axons branching in temporal cortex
      - associated with auditory sensation & language
- Humans undergo high brain-serotonin synthesis capacity during childhood
  - Functional neuro-imaging studies (PET scan) show diminished serotonin synthesis [3]

[1]Chugani DC. *Mol Psychiatr* 2002, 7(Suppl 2):S16-S17. [2]Chandana SR et al. *Int J Dev Neurosci* 2005, 23:171- 182.
[3] Chugani DC et al. *Ann Neurol* 1999, 45:287-295 [4]Rosemeyer R. *Molecular Autism* 2013, 4:37

Manipulation of serotonin homeostasis alters neuroanatomy & functions of the intestine……

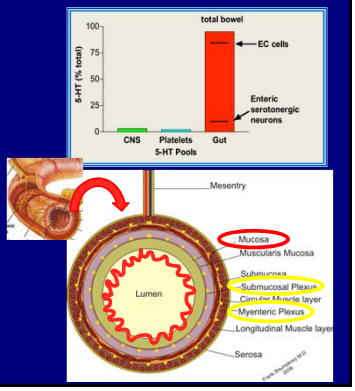
Research from our laboratory and others indicates that serotonin critically affects GI structure & function[1]

	TPH1 KO (no mucosal serotonin)	TPH2 KO (no neuronal serotonin)	SERT KO (increased serotonin)
Enteric nervous system growth		↓	
Gut speed	↓ (peristaltic reflex)	↓ ↓ ↓	↑ ↑
Inflammation	↓	↑	↑
Gut "leakiness"	↑		↑
Villus height/ crypt depth		↓	↑

[1]Gross-Margolis et al. Gut. 2013 Jun 7. [Epub ahead of print],
[2]Gross et al. Gastroenterology. 2012 Aug;143(2):408-17.
[3]Li Z, Gross-Margolis et al. J Neurosci. 2011 Jun 15;31(24):8998-9009
[4]Li Z, Gross-Margolis et al. J Neurosci. 2010 Dec 8;30(49):16730-40.

Serotonin is Critical for Gut Function!

- Intestine
  - 95% of the body's serotonin is located in the intestines!
  - Critical mediator
    - Enteric nervous system development
      - "Brain in the gut"
      - Control many functions!
    - intestinal motility
      - How fast, slow or coordinated gut movement is
    - intestinal secretion
      - Fluids that make stool softer

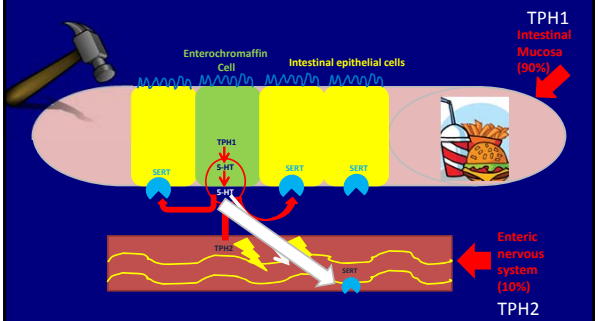


How do we put this all together?

- Is disruption in serotonin homeostasis a cause of both brain and gut abnormalities in ASD?
  - Abnormality in the serotonin transporter (SERT)
- Genome-wide association study for SERT-associated genetic abnormalities in ASD
- Several SERT coding variants identified as risk factors in children with ASD[2]
  - All result in overactive serotonin transporter activity
    - Take up (inactivate) serotonin with increased efficacy
- Most common coding variant: G56A
  - G56A transgenic mouse
  - SuperSERT mouse

[1]Campbell DB et al. Pediatrics 2009; 123: 1018–24; [2]Russo AJ et al. Biomark Insights 2009; 4: 181–90; 3Campbel DB et al. Ann Neurol 2007; 62: 243–50.

Intestinal Serotonin Mechanics



- TPH1 = produces mucosal serotonin
- TPH2 = produces neuronal serotonin
- SERT = inactivation of serotonin

GI problems are common in children with autism: Is 5-HT the Link?
SERT G56A ("SuperSERT") Transgenic Mouse

- Expresses the most common gain-of-function SERT coding variant in children with ASD
  - Core autism-related behavioral abnormalities
    - Altered social function & communication, repetitive behaviors
  - High blood serotonin levels
    - 30% individuals with ASD
  - Altered serotonin-related brain abnormalities
    - Altered firing of serotonergic neurons
    - $5HT_{1A}$ and $5HT_{2A}$ receptor hypersensitivity

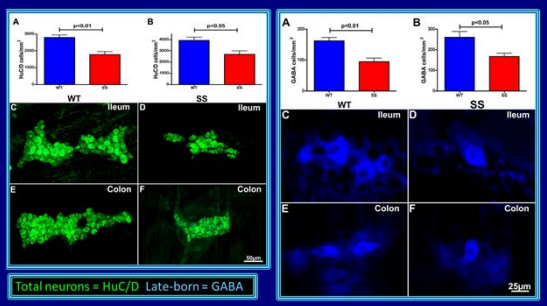
Veenstra-Vanderweele et al. PNAS 2012; 3;109(14):5469-74.

Hypothesis

Genetic abnormalities in the serotonin transporter (SERT), of the kind found in autism, also cause abnormalities in gut development & function

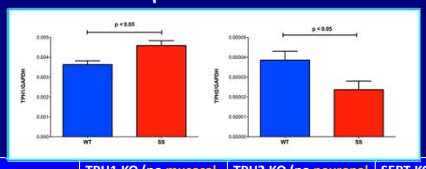
Could the G56A mutation be a brain-gut link in ASD?

Myenteric neurons are deficient in G56A (SuperSERT) mice



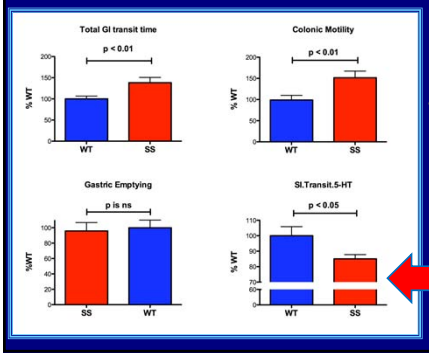
- The neurons responsible for intestinal motility are deficient in G56A mice compared to WT
- Intestinal motility: how fast the intestine moves → diarrhea vs. constipation

TPH1 expression is elevated but that of TPH2 is depressed in G56A mice



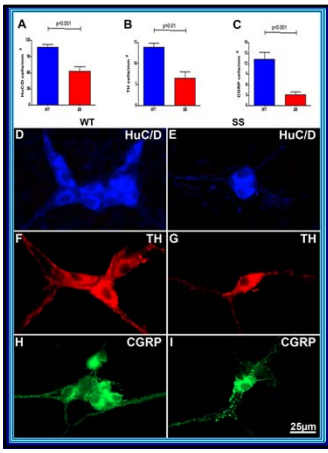
	TPH1 KO (no mucosal serotonin)	TPH2 KO (no neuronal serotonin)	SERT KO (increased serotonin)
ENS development/ neurogenesis			
Motility	(peristaltic		
Inflammation			
Permeability			
Villus height/ crypt depth			

Total GI transit and colonic motility are slower in G56A than in WT mice



- Stimulatory effect of exogenous 5-HT is blunted in G56A mice
  - Constipation & ASD

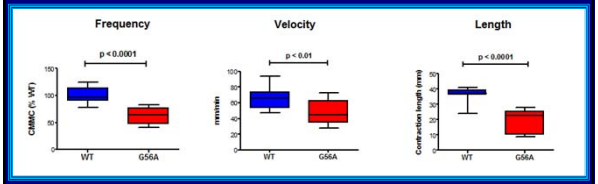
Total and late-born submucosal neurons are deficient in G56A (SuperSERT) mice



- The neurons responsible for intestinal secretion and peristalsis are deficient in G56A mice compared to WT
  - Secretion places water into stools to make them soft
  - Peristalsis creates effective movement for stools through the intestine

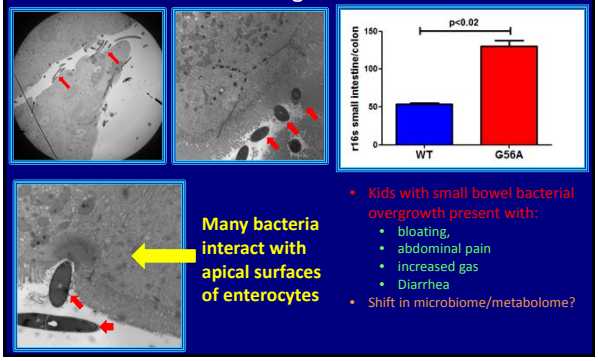
- Total neurons
  - HuC/D
- Late born neurons
  - CGRP and TH

The G56A mutation affects the ENS independently from the CNS



- Spatiotemporal mapping: *in vivo* method of measuring intestinal peristalsis
- Intestine isolated & placed in a measurement chamber without the brain
  - Gut motility changes measured are independent from brain influence
- Peristalsis is abnormal in The G56A mice
  - Frequency, velocity and length of contractions are all significantly less in G56A mice compared to WT littermates
- Peristaltic contractions help move stool out of the large intestine

Small Intestinal & fecal bacteria are more abundant in G56A > WT Mice: Small bowel bacterial overgrowth



Many bacteria interact with apical surfaces of enterocytes

- Kids with small bowel bacterial overgrowth present with:
  - bloating,
  - abdominal pain
  - increased gas
  - Diarrhea
- Shift in microbiome/metabolome?

So far……..

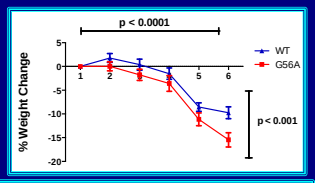
- Gastrointestinal problems are prominent in children with autism
- The serotonin transporter is the major breakdown mechanism for serotonin in the intestine
- G56A SERT genetic mutation is the most common SERT-based human mutation in ASD
- G56A mouse model of autism:
  - ASD-associated features in mice
  - Defects in ENS development
  - Abnormal gut movement
  - A brain-gut link!

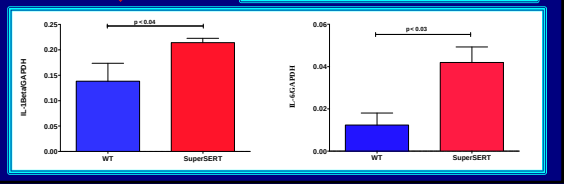
- Is there a way to fix the enteric nervous system defect and reverse the gut movement problems?

Severity of DSS-induced colitis is significantly greater in G56A > WT mice

- DSS causes an intestinal condition most similar to Ulcerative colitis in humans

Intestinal pro- inflammatory cytokines



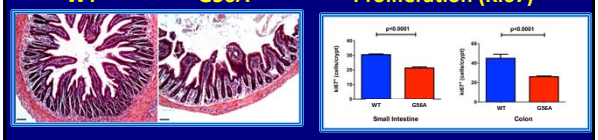


Conclusions

- GI problems are frequently associated with ASD
  - contribute significantly to problems behaviors
- There are several known brain-gut links in ASD
  - Environmental: maternal inflammation, microbiome
  - Genetic: c-met, CHD8 and G56A (SuperSERT)
- We evaluated ENS structure and GI function in a mouse with the most common SERT-based mutation (SuperSERT) found in ASD
  - ENS development, GI motility, susceptibility to intestinal inflammatory disease and intestinal epithelial permeability are abnormal

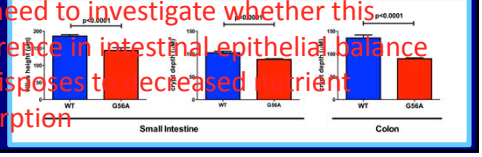
Villus height, crypt depth, and proliferation ↓ in G56A mice

WT **G56A** **Proliferation (Ki67)**



Villi **Crypts** **Crypts**

Villus height and crypt depth



- We need to investigate whether this difference in intestinal epithelial balance predisposes to decreased nutrient absorption

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