The gastrointestinal (GI) system and gut microbiome in Autism Spectrum Disorders (ASD)

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Outline

- Historical link between ASD and gut, possible connections
- Evidence for role of gut microbiome in ASD
- Measurement Challenges
- Future Directions
‘All disease starts in the gut’

Hippocrates ~460-370 BC
Autism Spectrum Disorder

- Repetitive behaviors and interests
- Social and communication Impairments
  - Inclusion of sensory interests and aversions
  - Severity levels

DSM-5
APA 2013
“Autisms”

Eytan Nisinzweig, a young man with autism
GI Link with Autism

• Leo Kanner 1943: 6 of the 10 children with autism “presented severe feeding difficulty from the beginning of life”
GI Symptoms

• Most common GI issues (median across 144 autism studies)
  – Constipation (22%)
  – Diarrhea (23%)
  – Abdominal pain/discomfort (14%)
  – Bloating / flatulence (13%)
  – Nausea / vomiting (6%)
  – Any symptom (47%)

• Food sensitivities, preferences, mealtime behaviors, toileting problems are common

Holingue et al 2017
Why study the gut in autism?

- Complementary and Alternative Treatments/Medicine (CAMs)
Complementary/Alternative Treatments/Medicine (CAMs)

• Developed outside of Western mainstream medicine
• Many aim to ‘heal the gut’ to prevent/treat ASD symptoms
• Over 70% families report one+ CAMs
• Very little data on safety or efficacy

Christon et al 2010
Why study the gut in autism?

• CAMs
• Treating comorbidities, improving quality of life
• Gut as a risk factor for
  – Exacerbation of ASD symptoms
  – Incidence of ASD
Possible Conceptual Diagram

ASD  

GI issues
Possible Conceptual Diagram

Gut  →  ASD
Possible Conceptual Diagram

Gut → Discomfort

Distress → ASD

Pain → “Biology”
Evidence for role of gut microbiome in ASD
The Human Microbiome

“The microbiome is defined as the collective genomes of the microbes (composed of bacteria, bacteriophage, fungi, protozoa and viruses) that live inside and on the human body.”

**Microbe:** Microorganisms [e.g. bacteria, virus]

**Microbiota:** Microorganisms in particular site

**Microbiome:** Combined genetic material of microorganisms in particular environment
The Human Microbiome

The diversity of microbes varies by anatomic site
The Human Microbiome

Health, diet, hygiene, genotype… →
Composition, complexity, and function of our microbial communities
Roles of gut microbiome

• Bacteria communicate with each other and with host (Us!)
• Produce vitamins and nutrients
• Influence host gene expression, immune cell responses
• Regulate gut motility, intestinal barrier homeostasis, nutrient absorption, fat distribution
• Gut-brain communication, brain function, behavior
Synergistic Relationship

“Microbiota normally has a balanced compositional signature that confers health benefits... disruption of this balance confers disease susceptibility”

Microbiome is Dynamic

“...The microbiome is a **fluctuating** collection of genes and gene products; **environmental perturbations**, such as antibiotic treatment and infection, can readily alter the microbial composition and function of each community...The human metagenome is relatively **plastic or malleable**, which makes the microbiome an **attractive target for manipulation** by cell or gene therapy”

Measuring the Gut Microbiome

- Collecting samples of the actual gut is often not feasible or ethical.
- Most studies examine the **fecal microbiome**.
- DNA is extracted, sequenced to determine which microbes are present.
Evidence for role of gut microbiome in ASD
Maternal gut bacteria promote neurodevelopmental abnormalities in mouse offspring

Sangdo Kim1*, Hyunju Kim1*, Yeong Shin Yim2, Soyoung Ha1, Koji Atarashi3, Tze Guan Tan4, Randy S. Longman5, Kenya Honda3, Dan R. Littman6,7, Gloria B. Choi2 & Jun R. Huh1†

Maternal Immune Activation

Behavioral Abnormalities

Cortical patches
Maternal gut bacteria promote neurodevelopmental abnormalities in mouse offspring

Sangdoo Kim¹*, Hyunju Kim¹*, Yeong Shin Yim², Soyoung Ha¹, Koji Atarashi³, Tze Guan Tan⁴, Randy S. Longman⁵, Kenya Honda³, Dan R. Littman⁶,⁷, Gloria B. Choi² & Jun R. Huh¹†

Maternal Immune Activation

Antibiotic

Healthy pups
Maternal gut bacteria promote neurodevelopmental abnormalities in mouse offspring

Sangdoo Kim^1*, Hyunju Kim^1*, Yeong Shin Yim^2, Soyoung Ha^1, Koji Atarashi^3, Tze Guan Tan^4, Randy S. Longman^5, Kenya Honda^3, Dan R. Littman^6,7, Gloria B. Choi^2 & Jun R. Huh^1†

- Gut bacteria that induce Th17 cells ✔
- Pro-inflammatory stimulus ✔
- Pregnancy ✔

All required for increase in IL-17a which promotes MIA-induced neurodevelopmental abnormalities
Maternal Immune Activation + Specific Bacteria in Mom’s Gut → ASD in child
Maternal Immune Activation 

+ 

Specific Bacteria in Mom’s Gut 

? 

ASD in child
Dissertation Aim

Antibiotic Exposure In Pregnant Women \(\rightarrow\) ASD in offspring
Dissertation Aim

Antibiotic Exposure In Pregnant Women

X

Maternal Immune Activation

ASD in offspring
ASD human microbiome studies
Findings Inconsistent

Clostridium, Sutterella, Lactobacillus, Desulfovibrio

Prevotella, Coprococcus, and Veillonellaceae

Bacteroidetes/Firmicutes ratio

Finegold et al 2002; Song et al 2004; Williams et al 2012;
Wang et al 2013; Tomova et al 2014; Williams et al 2011
Reasons for inconsistencies across studies

• Differences in how microbiome data was collected, stored, analyzed
• Unadjusted confounding
• Heterogeneity of ASD
Dissertation Aim

To estimate associations of gut microbiome signatures including diversity metrics and relative abundance of taxa with ASD co-occurring conditions.

ASD co-occurring conditions  →  Gut microbiome
• Recruit 30 boys 3-6 years with ASD from Kennedy Krieger Institute, Center for Autism and Related Disorders

• Stool kit: Omnigene Gut
• Questionnaires:
  • Child behavior checklist
  • The short sensory profile
  • GI questionnaire
  • Bristol Stool Scale

• Samples will be processed and sequenced at JHU with 16s rRNA technology
Statistical Analysis

Estimate associations between co-occurring issues/behavior and microbial profiles, within ASD

Exemplar Hypotheses:

- Anxiety $\sim$ **Prevotella** within ASD
- Somatic Complaints $\sim$ **Prevotella** within ASD
- Sleep problems $\sim$ **Prevotella** within ASD
Measurement Issues

• Assessing GI symptoms/conditions in ASD is challenging
Evaluation, Diagnosis, and Treatment of Gastrointestinal Disorders in Individuals With ASDs: A Consensus Report

abstract

Autism spectrum disorders (ASDs) are common and clinically heterogeneous neurodevelopmental disorders. Gastrointestinal disorders and associated symptoms are commonly reported in individuals with ASDs, but key issues such as the prevalence and best treatment of these conditions are incompletely understood. A central difficulty in recognizing and characterizing gastrointestinal dysfunction with ASDs is the communication difficulties experienced by many affected individuals. A multidisciplinary panel reviewed the medical literature with the aim of generating evidence-based recommendations for diagnostic evaluation and management of gastrointestinal problems in this patient population. The panel concluded that evidence-based recommendations are not yet available. The consensus expert opinion of the panel was that individuals with ASDs deserve the same thoroughness and standard of care in the diagnostic workup and treatment of gastrointestinal concerns as should occur for patients without ASDs. Care providers should be aware that problem behavior in patients with ASDs may be the primary or sole symptom of the underlying medical condition, including some gastrointestinal disorders. For these patients, integration of behavioral and medical care may be most beneficial. Priorities for future research are identified to advance our understanding and management of gastrointestinal disorders in persons with ASDs. Pediatrics 2010;125:S1–S18
Gastrointestinal Symptoms in Autism Spectrum Disorder: A Review of the Literature on Ascertainment and Prevalence

Calliope Holingue, Carol Newill, Li-Ching Lee, Pankaj J. Pasricha, and M. Daniele Fallin

There is no standard approach to measuring GI symptoms in individuals with ASD, despite postulated interactions. The objectives of this study were to (a) describe the range of GI symptom ascertainment approaches in studies of ASD, (b) describe the range of prevalence estimates across studies, and (c) assess associations between ascertainment approach and prevalence estimates. Studies published from 1/1/1980 to 1/31/2017 were collected via PubMed. Eligibility included studies with at least ten individuals with ASD that measured GI symptoms or conditions. We excluded review and hypothesis papers. We extracted information on study design, GI symptom ascertainment method, demographics, and ASD diagnostic criteria. From a subset of studies, we extracted GI symptom estimates. Out of a possible 386 titles, 144 were included. The prevalence range for constipation was 4.3–45.5% (median 22%), for diarrhea was 2.3–75.6% (median 13.0%), and for any or more than one symptom was 4.2–96.8% (median 46.8%). GI symptoms differed significantly by age of individuals, primary goal of study, study design, study sample, and who reported symptoms ($P<.05$). Due to small sample size, we were not able to test for associations between every GI symptom and study characteristic of interest, or examine associations between GI symptoms and intellectual or verbal disability. Studies used a broad range of methods to ascertain GI symptoms in ASD. GI symptoms varied widely across these studies, with significant differences by study characteristics. Our findings highlight the need for a reliable, valid GI assessment tool to be used consistently across studies of ASD. *Autism Res* 2018, 11: 24–36. © 2017 International Society for Autism Research, Wiley Periodicals, Inc.

**Lay Summary:** We reviewed studies having to do with autism spectrum disorder and the gastrointestinal system, dating back to 1980. We found that the median prevalence of constipation was 22.2%, diarrhea 13.0%, and any symptom 46.8%. All symptoms had a wide range of estimates across studies. GI symptoms were associated with characteristics of the study, including who measured the GI symptoms. We call for the development of a reliable and valid GI questionnaire for studies of ASD.

**Keywords:** co-morbid conditions; exposure assessment/exposomics; psychometrics
Dissertation Aim

To develop a reliable and valid questionnaire that assesses gastrointestinal (GI) symptoms in children with autism spectrum disorder for use in epidemiologic studies.
Study Population

• Registry of parents of child with ASD at Kennedy Krieger Institute
• Have previously consented to be contacted for research purposes
• Ages 3-18 years
• Recruitment, consent, data collection will all happen online
Methods

• Base off of Autism Treatment Network GI Inventory and Brief Autism Mealtime Behavior Inventory

• Focus groups and expert panel

• Administer our questionnaire + child behavior checklist

• Assess psychometric performance and revise

• Administer to independent sample, assess psychometric performance
Key Takeaways

• GI symptoms are elevated in ASD
• GI symptoms/disorders may be due to having ASD
• Gut may play a role in causing ASD
• Both directions are likely occurring, depends on the individual
• Accurate, reliable assessment, diagnosis, treatment of GI symptoms in ASD is critical
Impact and Implications

• Etiology of autism and mental illness

• Target for prevention and treatment
Future work

- Measurement and assessment
- Latent classes approaches to identifying ‘subgroups’ of ASD
- Incorporating multiple data types
- Animal and human studies should inform each other, inform mechanistic work
## Thank you!!

### Thesis committee
- Dani Fallin (Adviser)
- Li-Ching Lee
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- Jay Pasricha

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Shameless Plugs

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  › Tu W, 8:30am - 4:50pm

Auditors Allowed: No
Grading Restriction: Letter Grade or Pass/Fail
Contact: Michelle Carlson
Course Instructor:
  › Michelle C. Carlson
Frequency Schedule: One Year Only
Resources:
  › CoursePlus
  › Evaluations
Shameless Plugs

Gut-Mental Health Working Group

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References (2)


• Vuong HE, Hsiao EY. Emerging roles for the gut microbiome in autism spectrum disorder. Biol Psychiatry. Elsevier; 2016;


