Autism and the Impact of the Intestinal Microbiome:
Exploring the Relationship of the Intestinal Micro Flora to Diet, Digestion and Disease

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Hawaii Autism Foundation
Family Talk
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Objectives

• The participant will be able to discuss the general makeup of the intestinal microbiome in health.
• The participant will be able to delineate potential mechanisms that cause an alteration in the stable microbiome, predisposing to disease.
• The participant will recognize potential pathways to minimize disruption of the microbiome.
• The participant will be able to determine how medical treatments and dietary interventions may affect the intestinal microbiome and the potential “brain-gut” impact and potentially autism.
Disclosures

Timothy Buie has the following financial relationships to disclose

• Honoraria:
  Autism Speaks, Nutricia, Crestovo

• Funding support:
  AAP, Newman’s Foundation, Nutricia, Autism Research Institute

• I do not intend to speak about specific commercial products and will acknowledge any funded research.
### Identified Prevalence of Autism Spectrum Disorder

**ADDM Network 2000 – 2012**

**Combing Data from All Sites**

<table>
<thead>
<tr>
<th>Surveillance Year</th>
<th>Birth Year</th>
<th>Number of ADDM Sites Reporting</th>
<th>Prevalence per 1,000 Children (Range)</th>
<th>This is about 1 in X children...</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>1992</td>
<td>6</td>
<td>6.7 (4.5 – 9.9)</td>
<td>1 in 150</td>
</tr>
<tr>
<td>2002</td>
<td>1994</td>
<td>14</td>
<td>6.6 (3.3 – 10.6)</td>
<td>1 in 150</td>
</tr>
<tr>
<td>2004</td>
<td>1996</td>
<td>8</td>
<td>8.0 (4.6 – 9.8)</td>
<td>1 in 125</td>
</tr>
<tr>
<td>2006</td>
<td>1998</td>
<td>11</td>
<td>9.0 (4.2 – 12.1)</td>
<td>1 in 110</td>
</tr>
<tr>
<td>2008</td>
<td>2000</td>
<td>14</td>
<td>11.3 (4.8 – 21.2)</td>
<td>1 in 88</td>
</tr>
<tr>
<td>2010</td>
<td>2002</td>
<td>11</td>
<td>14.7 (5.7 – 21.9)</td>
<td>1 in 68</td>
</tr>
<tr>
<td>2012</td>
<td>2004</td>
<td>11</td>
<td>14.6 (8.2 – 24.6)</td>
<td>1 in 68</td>
</tr>
</tbody>
</table>

Autism is Heterogeneous

- Co-morbidity is commonly discussed regarding:
  1) Genetic syndromes
  2) Associated psychopathology
  3) Medical Conditions
    - A) Epilepsy
    - B) Gastrointestinal Disorders
    - C) Food Allergy, Immune Dysregulation
    - D) Catatonia
Prevalence of GI issues in autism

- Meta-analysis (1980-2012) of 15 qualified studies
- Number of pooled patients = 961

McElhanon BO et al. Pediatrics. 2014 May;133(5):872-83

So, newer data support a more consistent prevalence of **45-70%** of children with autism have GI issues.
## GI Findings in Children with Autism

Others have built on the suggestion that many children with autism suffering GI symptoms have a variety of GI findings including:

<table>
<thead>
<tr>
<th>Condition</th>
<th>References</th>
</tr>
</thead>
</table>
Clinical Work and Research Merge

Lack of Association between Measles Virus Vaccine and Autism with Enteropathy: A Case-Control Study

Mady Hornig¹, Thomas Briese¹, Timothy Buie², Margaret L. Bauman³, Gregory Lauwers⁴, Ulrike Siemetzki¹, Kimberly Hummel⁵, Paul A. Rota⁵, William J. Bellini⁵, John J. O’Leary⁶, Orla Sheils⁶, Errol Alden⁷, Larry Pickering⁸, W. Ian Lipkin¹*
Autism Consensus Statements

Key Statement (Statement 1): Individuals with ASDs who present with GI symptoms warrant a thorough evaluation, as would be undertaken in individuals without ASDs who have the same symptoms or signs.

Statement 6: Individuals with ASDs and GI symptoms are at risk for problem behaviors. When patients with GI disorders present with behavioral manifestations, the diagnostic evaluation can be complex.
The changing face of gut microbes
Microbiomes

mi·cro·bi·ome
ˌmīkrōˈbīōm/

noun
plural noun: microbiomes

the microorganisms in a particular environment (including the body or a part of the body).

"we depend on a vast army of microbes to stay alive: a microbiome that protects us against germs, breaks down food to release energy, and produces vitamins"

the combined genetic material of the microorganisms in a particular environment.
More than microbiomes, an integrated garden (flora)

- Archaea—single celled organisms
- Fungome—fungal proteins implicated in pathogenesis
- Virome—the collection of viruses in and on the human body. Viruses in the human body infect both human cells as well as other microbes such as bacteria.
- Metabolome—the total number of metabolites present within an organism, cell, or tissue. These metabolites are products of digestion and also products of the microbiota
THE HUMAN MICROBIOME

Bacteria, fungi, and viruses outnumber human cells in the body by a factor of 10 to one. The microbes synthesize key nutrients, fend off pathogens and impact everything from weight gain to perhaps even brain development. The Human Microbiome Project is doing a census of the microbes and sequencing the genomes of many. The total body count is not in but it's believed over 1,000 different species live in and on the body.

25 SPECIES
in the stomach include:
- Helicobacter pylori
- Streptococcus thermophilus

500–1,000 SPECIES
in the intestines include:
- Lactobacillus casei
- Lactobacillus reuteri
- Lactobacillus gasseri
- Escherichia coli
- Bacteroides fragilis
- Bacteroides thetaiotaomicron
- Lactobacillus rhamnosus
- Clostridium difficile

600+ SPECIES
in the mouth, pharynx and respiratory system include:
- Streptococcus viridans
- Neisseria sica
- Candida albicans
- Streptococcus salivarius

1,000 SPECIES
in the skin include:
- Pityrosporum ovale
- Staphylococcus epidermidis
- Corynebacterium jeikeium
- Trichosporon
- Staphylococcus haemolyticus

60 SPECIES
in the urogenital tract include:
- Ureaplasm parvum
- Corynebacterium aurimucosum

SOURCES: NATIONAL INSTITUTES OF HEALTH, SCIENTIFIC AMERICAN; HUMAN MICROBIOME PROJECT

Dean Tweed • POSTMEDIA NEWS / IMAGE: Fotolia
Cycle of healthy microbiota transmission and host development

Interferences

- **Antibiotics**: Taken by girl and boy, gravid woman, prepartum and postpartum, infant.
- **C-section**: Baby does not go through birth canal.
- **Washing of skin with antibacterials**: Intrapartum, baby (vernix), baby, girls, women.
- **Oral ingestion of antibacterials**: Children, women, men.

MG Dominguez-Bello & MJ Blaser 2015;7::307fs39
MA Fischbach & JA Segre Cell 2016;164:1288-1300
Development of the gut microbiota

• Fetal intestine: “sterile”  Or maybe not!
• Initial colonization determined by:
  – Delivery mode (caesarian section vs. vaginal)
  – Diet (breast feeding vs. formula feedings)
  – Hygiene (exposure to pathogens)
  – Medication (antibiotics)
• Temporal changes over the first years of life

P LaRosa et al. PNAS 2014;111:12522-7 (premature babies)
Gut diversity in 15 vaginally delivered and 9 caesarian section infants

Changing gut microbiota diversity
(330 Danish infants, 9-36 mos old)

Subject index (N=1-79), sorted after increasing ratio at 36 months

Low gut microbiota diversity in during the first month of life precedes asthma at school age

Asthma 7y

Reduced gut diversity (FLVR) at 100 days predicts childhood asthma.
Antibiotic effects

- **Monthly** microbiome, metabolomic and genomic testing on 39 infants for 3 years.
- Supports vaginal vs. C-sections biome differences (C-section often lacking protective Bacteroides species)
- Antibiotic exposure diminished diversity and promoted antibiotic resistance genes

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Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability

Moran Yassour et al

*Science Translational Medicine* 15 Jun 2016:
Vol. 8, Issue 343, pp. 343ra81
Reduced bacterial diversity (dysbiosis): an emerging theme across diseases

- Microbiota affected by:
  - Infections
  - Antibiotics
  - Xenobiotics
- Diabetes mellitus
- Obesity
- Cancers: gastric, colonic
- Inflammatory bowel diseases
- Necrotizing enterocolitis
- Irritable bowel syndrome, colic
Limitations of available studies

• Only within the last 2-3 years is it possible to evaluate the microbiome to species rather than phyla (families of organisms) using current technology

• Even now, proposals looking only at species without metabolic or proteomic studies are finding difficulty getting funded
Microbial composition of IBS vs. controls

Adults: M Rajlic-Stojanovic et al. Gastroenterology 2011;141:1792-1801
Children: D Saulnier et al. Gastroenterology 2011;141:1782-1791
Twin Studies in Colitis-Microbiome

- Patients with UC had different gene expression profiles and lower levels of biodiversity than their healthy twins, as well as unusual aerobic bacteria.

- Patients with UC had lower percentages of potentially protective bacterial species than their healthy twins (lactose fermenters).

Twin Study Indicates Loss of Interaction Between Microbiota and Mucosa of Patients With Ulcerative Colitis

PATRICIA LEPAGE, et al, GASTROENTEROLOGY 2011;141:227–236
Core microbiome in obese and lean twins: Missouri Adolescent Female Twin Study

with restoration after gastric bypass: H Zhang et al. PNAS 2009;106:2365-70
Low diversity (less modular) gut microbiota in IBD and in obesity

![Graph A] (Modularity vs. Number of Reads Sampled)

- **Lean, Healthy**
- **Obese**
- **IBD**

![Graph B] (Frequency of Difference in Modularity)

124 Danish & Spanish adults
42 BMI >30
25 IBD

S Greenblum et al., PNAS 2012;109:594-599
Abnormal Microbiome in Autism

• A growing number of studies point to altered microbiome in populations of children with autism.
• A consistent pathogen has not been reported
• Patterns of microbe alterations are being described and the effects vetted, high Clostridial species, diminished Bacteriodetes

Potential Etiologic Factors of Microbiome Disruption in Autism, Clinical Therapeutics, Buie, T
Volume 37 Number 5, 2015
Duodenal Microbiome in Autism

CORRELATION BETWEEN INTESTINAL DISACCHARIDASES AND MICROBIOME IN CHILDREN WITH AUTISM
Rafail I. Kushak, Timothy M. Buie et al.

• In autism group, 17 out of 21 subjects and in controls 18 out of 19 subjects were lactase deficient.
• In samples from autism subjects, the relative abundance of genus Bacteroides, Faecalibacterium, and Clostridium showed a statistically significant positive correlation with lactase activity.
• The duodenal microbiome in neurotypical children was different than in children with autism.

Conclusion: There are differences at the genus and species level in the duodenal microbiota in children with autism that could be influenced by maldigestion of lactose or nutritional differences in food consumption.
Paper accepted JPGN 10/2016
Gut Microbiome Clusters by Diet:

Ley et al, Science 2008;320:1647-1651
Athletes had a higher diversity of gut micro-organisms, representing 22 distinct phyla, which in turn positively correlated with protein consumption.
Control of Brain Development, Function, and Behavior by the Microbiome

Pathways linking the microbiome and central nervous system

Intestinal lumen

Intestinal barrier

Vagus nerve

Vagal stimulation

Circulatory system

Neurotransmitters
Hormones
Metabolites
Immune signaling

Immune system

MAMPs
Metabolites

CNS

TR Sampson & SK Mazmanian Cell Host Microbe 2015:17:564-576
Is there a way to fix anything?

• The microbiome is relatively established early in childhood at 2-3 years of age (setting point).
• Subsequent to this, there are disruptions like antibiotic exposures or illness but following these, over weeks to months, the microbiome migrates closely back to it’s previous baseline (whether healthy or unhealthy at the time).
• Establishing a healthy microbiome by this setting point should be our goal.
Definitions

**Prebiotic**: pre·bi·ot·ic /praˌbīˈatik/
Noun: **prebiotic**
a non-digestible food ingredient that promotes the growth of beneficial microorganisms in the intestines

**Probiotic** pro·bi·ot·ic /prōbīˈädik/
Adjective: denoting a substance that stimulates the growth of microorganisms, especially those with beneficial properties (such as those of the intestinal flora). Beneficial bacteria

**Xenobiotic**: xen·o·bi·ot·ic /zenəbīˈätik,
Noun: substances that are foreign to the body or to an ecological system.
Nature’s first Functional Food

**WHAT’S IN HUMAN MILK**

Human milk oligosaccharides (HMOs) are food for friendly bacteria like *Bifidobacterium infantis*. Shorter chain HMOs in particular are almost entirely consumed by this microbe.

- **Milk**
  - Macro- and micronutrients
  - Water
  - Lactose

- **Macro-/micronutrients**
  - HMOs
  - Proteins
  - Lipids

- **HMOs**
  - Proportion eaten by *B. infantis*
  - Chain length
  - Other HMOs of longer lengths

A Petherik. Nature 2010;468:S5-S7


T Gura. Science 2014;345:747-749
Human milk oligosaccharides

- Large component of breast milk (5-10 g/L)
- Complex mixture of galacto-oligosaccharides
- Bifidogenic properties
- Concentration affects microbiome composition
- Normally, not present in infant formulas

T Gura. Science 2014;345:747-749
Benefits of prebiotics for infants

Systematic review of 11 RCT’s (n=1,459)

- Results in softer, more frequent stools
- Increases #’s of Bifidobacter & Lactobacilli
- Reduces stool pH
- No effect on weight accretion
- Clinical impact **not** assessed as an outcome
  - might protect against harmful bacteria
  - may reduce infection rates

Fiber

• 269 incident cases of CD (incidence, 8/100,000 person-years)
• 338 cases of UC (incidence, 10/100,000 person-years).

• High fiber intake in the highest quintile was associated with a 40% reduction in risk of CD

• This apparent reduction appeared to be greatest for fiber derived from fruits

• Fiber from cereals, whole grains, or legumes did not modify risk

• Neither total intake of dietary fiber nor intake of fiber from specific sources appeared to be significantly associated with risk of UC.
Effects of prebiotics on obesity

Mechanisms of action of probiotics:

Colonization resistance:
- Competitive exclusion

Barrier function: tight junctions
- Reduce macromolecular permeability, bacterial translocation

Metabolic effects:
- Bacteriocins
- Decrease pH
- Quorum sensing

Innate & adaptive immunity:
- IgA, IgG, IgM, Tregs
- Mucins, TFF

Modulation of signal transduction:
- NF-κB
- IFNγ
- MAPK
- DC
- TC
- PC

P Sherman et al. Nutr Clin Pract 2009;24:10-14
Probiotics in experimental colitis: timing matters!

Probiotics reduce symptoms of functional abdominal pain in childhood

S Guandalini et al. JPGN 2010;51:24-30 (VSL#3)
A Gawronska et al. APT 2007;25:177-184 (LGG)
Subject global assessment IBS symptoms after 4 wks:

$p = 0.0118$

PJ Whorwell et al. Am J Gastroenterol 2006;101:1581-90
Probiotics are not all alike

<table>
<thead>
<tr>
<th>Indication and probiotic strain</th>
<th>Recommended dosage</th>
<th>Evidence level</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment of acute infectious diarrhea</td>
<td>Saccharomyces boulardii: 10^9 CFU per capsule of 250 mg, 2-6 capsules per day</td>
<td>1b</td>
<td>[27,30]</td>
</tr>
<tr>
<td></td>
<td>Lactobacillus rhamnosus GG or L paracasei B 21060: 10^9 CFU twice-daily</td>
<td>2b</td>
<td>[29]</td>
</tr>
<tr>
<td>Prevention of antibiotic-associated diarrhea</td>
<td>S boulardii: 1 g or 4×10^9 CFU per day</td>
<td>1b</td>
<td>[43]</td>
</tr>
<tr>
<td></td>
<td>L rhamnosus GG: 10^{10}-10^{11} CFU twice-daily</td>
<td>1b</td>
<td>[43]</td>
</tr>
<tr>
<td>Prevention of C difficile-associated diarrhea</td>
<td>S boulardii: 2-3×10^9 CFU for 28 days, followed up for another 4 weeks</td>
<td>1b</td>
<td>[47,48]</td>
</tr>
<tr>
<td>Coadjuvant therapy for H pylori eradication</td>
<td>L rhamnosus GG: 6×10^9 CFU twice daily</td>
<td>1b</td>
<td>[69]</td>
</tr>
<tr>
<td></td>
<td>S boulardii: 500 mg-1 g or 2-4×10^9 CFU per day</td>
<td>1b</td>
<td>[69]</td>
</tr>
</tbody>
</table>

Recommended dose and evidence level were obtained according to World Gastroenterology Organisation Global Guidelines for Probiotics and Prebiotics published in October 2011. These guidelines are available at http://www.worldgastroenterology.org/probiotics-prebiotics.html. CFU: colony-forming units.
Probiotics DO NOT Set Up Residence

30% of those supplemented established long term **engraftment**
Maldonado-Gomez et al., 2016, Cell Host & Microbe 20, 515–526 October 12, 2016
Gut-Brain Connections
L. reuteri MM4-1A impacts brain health

Maternal high-fat diet alters offspring gut microbiome, social behavior, PVN oxytocin levels, and VTA plasticity

Maternal Regular Diet (MRD) Offspring
- Normal
- Social
- Normal

Maternal High-Fat Diet (MHFD) Offspring
- Dysbiosis
- Impaired
- Impaired

Precision microbial reconstitution in MHFD offspring restores social behavior, oxytocin levels, and VTA plasticity

MHFD Offspring + Lactobacillus reuteri
- Treated
- Social
- Restored

SA Buffington et al. Cell 2016;165:1762-1775
Probiotics increase neurotransmitters in male mice (*L. rhamnosus*, JB-1 for 4 wks)

**Glutamate+glutamine**

**N-acetyl aspartate + N-acetyl glutamate**

**GABA**

Magnetic resonance spectroscopy

Mouse Model

Microbiota Modulate Behavioral and Physiological Abnormalities Associated with Neurodevelopmental Disorders

Elaine Y. Hsiao,1,2,* Sara W. McBride,1 Sophia Hsien,1 Gil Sharon,1 Embriette R. Hyde,3 Tyler McCue,3 Julian A. Codelli,2 Janet Chow,1 Sarah E. Reisman,2 Joseph F. Petrosino,3 Paul H. Patterson,1,4,* and Sarkis K. Mazmanian1,4,*

Cell 2013
Gut microbiome and the brain in humans

Altered fecal microbiota (reduced *Faecalibacterium* spp.) in active major depressive disorder (n=29), versus inactive MDD (n=17) & healthy controls (n=30)

Reduced cognitive reactivity (rumination and aggressive thoughts) in healthy adults receiving probiotics
L Steenbergen et al. Brain, Behavior, and Immunity 2015;48:258-264

Autism and autism-spectrum disorders?
F Mangiola et al. World J Gastroenterol 2016;7:361-368
[based on mouse studies in: Cell 2013;155:1451-63]
Probiotics Affect Brain Function

Consumption of fermented milk product with probiotic modulates brain activity.

- Yogurt culture (Bifidobacterium Lactis, Streptococcus thermophiles, Lactobacillus bulgaricus, and Lactococcus lactis)

Probiotics modulate brain activity in women

FMPP denotes fermented milk product with 4 probiotic strains
Reduced activity in response to emotional faces attention task (n=12)
K Tillisch et al (E Mayer). Gastroenterology 2013;144:1394-1401
Let’s get to the fixing part
Prevention

• Evaluate C-Section Delivery, can we turn the pattern of elective, convenience delivery?
• Judicious antibiotic exposures to mother and baby
• ? Probiotics supplemented for “health”
• Focus families back onto breast feeding for as long as possible
• Fiber as food is back, fruit may be best
C-Section Rates Nationally

• The overall rate of caesarean section births in the U.S. was **32.7 percent in 2013**

• The WHO supports that approximately 10 percent to 15 percent of C-section deliveries are medically necessary

• It is important to evaluate why high rates are present, risk, monitoring concerns, medicolegal concerns
C-section Rates HI

- In 2014, the cesarean delivery rate was 27.2%
- In Hawaii, the rate of C-Section delivery has gradually risen where nationally the rate has begun to decline
- This rate is close to the national average currently
Antibiotics, birth mode and diet

- **Breast Milk** for 3 months or longer supported higher diversity of the microbiome for the first 2 years of life compared to formula fed infants.
- **C-Section Delivery** brought lower diversity compared with vaginal delivery for up to 2 years. (In another study performed by this group, diversity improved if infants born by C-section were exposed to maternal vaginal secretions).
- **Antibiotics** lowered diversity for up to 2 years. The worst impact on the microbiota was antibiotic exposure from 6-12 months of age.

Antibiotics, birth mode, and diet shape microbiome maturation during early life

- Nicholas A. Bokulich†,
Diet

Particular diet models:

• Moderate/low Carbohydrate
• Protein maximized, ? Fat (at least no high fat)
• Fruit based fiber
• Yogurt/Fermented sources offering probiotics
• Nutrient conscious: Vitamin D,C iron etc
• Unique restrictions need vetting
Non-caloric artificial sweeteners alter the gut microbiome in mice and man

Saccharin, sucralose, aspartame; effects reduced by antibiotics; effects transferrable to germ-free mice


Contrasting opinion (based on doses in animals)

B Magnuson. Calorie Control Council
Atlanta, GA, March 13, 2015

Dietary emulsifiers impact the mouse gut microbiota promoting metabolic s., colitis

B Chassaing et al. Nature 2015;519:92-96
Microbiota in immunity & inflammation

Y Belkaid & TW Hand, Cell 2014;157:121-141
Supplements

• Supplement all you can through good food
• Nutrient absorption is better when delivered as food
• Prebiotics and probiotics are available, watch for ingredients if restricted diet, watch for side effects
Resources: GI, Autism

http://www.gikids.org/

https://medlineplus.gov/autismspectrumdisorder.html
Thanks

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• Jeremy King MD
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