Gut Bacteria, Diet, Essential Fatty Acids, Probiotics, and Fecal Transplants

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Financial Disclaimer

• Prof. James Adams is a part-time consultant for Crestovo, a company which is developing standardized full-spectrum microbiota for fecal transplant therapy.
GI problems

• Gastrointestinal problems are common in children and adults with autism

• Most studies show about 30-50% have chronic constipation, diarrhea, or alternating diarrhea/constipation
Gut Problems associated with worse symptoms (all four areas) – Adams et al 2011
Chaidez et al 2013

Investigated 499 children ages 2-5 years with ASD vs. 324 controls;

• Children with ASD 8x as likely to have 1 or more frequent GI problems compared to controls (15% constipation, 13% diarrhea for ASD vs. 4%/2% for controls)

• Found ASD children with GI problems had increased irritability, social withdrawal, stereotypy, and hyperactivity
Autism and gut microbiome
We Are not Alone

The Human Microbiome Project says the human body has 100 trillion microscopic life forms living in it.

You call this living?

What is the role of human gut microbiota?

- Break down plant polysaccharides
- Promoting GI motility
- Producing vitamins
- Competing against pathogen

- GI problems
- Producing toxins
- Disrupting immune system
- Competing against commensal bacteria
Abnormal Oral Antibiotic Use

- Five studies reported 2-3x higher usage of oral antibiotics during infancy of children with autism vs. controls, usually for treating ear infections.
- Commonly used oral antibiotics eliminate almost all of the normal gut bacteria.

Biotin

• A study of vitamin levels in 55 children with autism vs. 44 controls found that the primary difference was children with ASD had 20% lower levels of biotin, p<0.001. (Adams et al 2011)

• Also, the degree of improvement due to a vitamin/mineral supplement was primarily associated with low levels of biotin and vitamin K. (Adams et al 2012)

• Both biotin and vitamin K are primarily made by beneficial gut bacteria.
Antibiotic Therapy

A small open-label treatment study by Sandler et al with a potent non-absorbable antibiotic (Vancomycin) found temporary improvement in gut function and behavior, but the gains were lost when the treatment was stopped.


ARI Survey of Parent Ratings of Treatment Efficacy:

<table>
<thead>
<tr>
<th></th>
<th>% Worse</th>
<th>% No Change</th>
<th>% Better</th>
<th>Number of Reports</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antifungals</strong>&lt;sup&gt;C&lt;/sup&gt;: Diflucan</td>
<td>5%</td>
<td>41%</td>
<td>55%</td>
<td>330</td>
</tr>
<tr>
<td><strong>Antifungals</strong>&lt;sup&gt;C&lt;/sup&gt;: Nystatin</td>
<td>5%</td>
<td>46%</td>
<td>49%</td>
<td>986</td>
</tr>
<tr>
<td>Antibiotics (not recommended)</td>
<td>31%</td>
<td>57%</td>
<td>12%</td>
<td>1799</td>
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</table>
Yeast in autism?

Many anecdotal reports of yeast overgrowth in children with autism, and limited research evidence. However, Adams et al 2011 did not find elevated yeast (by culture or miocroscopically) in study of 58 children with autism vs 39 controls, and yeast overgrowth very rare on endoscopies (<10%) per anecdotal physician reports.
<table>
<thead>
<tr>
<th></th>
<th>ASD</th>
<th>Controls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultured Yeast</td>
<td>28%</td>
<td>13%</td>
<td>n.s.</td>
</tr>
<tr>
<td>Microscopic Yeast</td>
<td>71%</td>
<td>85%</td>
<td>n.s.</td>
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Gut Bacteria Research (continued)

Two small studies by Finegold et al found some limited evidence of abnormal anaerobic bacteria, primarily increases in clostridia. A study by Parracho et al also found increased amounts of clostridia.


One study of 58 children with autism vs. 39 controls found some abnormalities in gut bacteria, including decreased levels of bifidobacteria (an important beneficial bacteria) in children with autism compared to controls.

DNA-based methods

Two small studies using DNA-based methods to investigate gut bacteria have been conducted, but yielded dissimilar results – much larger studies are needed.

• Finegold et al (2011) found increased levels of *Desulfovibrio* bacteria in children with autism.

• Williams et al (2012) found *Sutterella* bacteria in half of the 23 children with autism but not in any of the 9 controls.


Hypothesis

Children with autism (GI problems) have different microbial communities in the gut.

Long term goal

Identify specific microbes that can be targeted for diagnosis and for treatment
Study design

Neurotypical kids (20)

Kids with autism (19)
Taxonomy

95% Identity
97% Identity
Higher microbial diversity found in the gut of neurotypical children

(Kang et al. 2013 Plos One)
Top 10 most abundant genera

- **No significant difference here**

- *Akkermansia* very abundant in Autism group, contribution to low diversity
Veillonellaceae, Prevotella, and Coprococcus are all lower in ASD (p<0.05); all 3 are carbohydrate-digesting bacteria, suggesting a decreased ability to digest carbohydrates.

(Kang et al. 2013 Plos One)
Prevalence of the genus *Prevotella*

Differences between children with autism and neurotypical more pronounced than differences in GI symptoms


http://www.plosone.org/article/info:doi/10.1371/journal.pone.0068322
The genus *Prevotella*

- Gram-negative rods, anaerobes, and non-spore forming
- Major metabolic end products are succinic and acetic acids.

- *Prevotella* degrade a broad spectrum of **plant polysaccharides**.
- Prevalent in African children (De Filippo et al. 2010)
- One of noteworthy genera in the enterotype study (Arumugam et al. 2010)
Prevotella cluster possible protection against pathogens
The *Prevotella* cluster

- *Desulfovibrio, Oscilibacter, and Coprococcus*
- Consume dietary fiber and produce short chain fatty acids (SCFA), which are 60-70% of food for intestinal cells
- Adams et al 2011 found children with ASD had 27% lower amounts of SCFA in stool, p<0.00002 - consistent with less *Prevotella*
- May relate to increased intestinal permeability/leaky gut

The *Enterobacteriaceae* cluster

- *Salmonella, Escherichia/Shigella, and Citrobacter*
- Potential pathogenic bacteria
- Low abundance in African children- low occurrence of GI diseases
Prevotella & Diet

A study of African children on traditional neolithic diet (rich in carbohydrate, fiber, non-animal protein). Mostly whole grains (millet grain, sorghum), legumes (black-eyed peas), and vegetables.

53% of gut bacteria were Prevotella, vs. undetectable Prevotella in European children on traditional western diet.
Gut microbiota and the host

Metabolites

Short chain fatty acids

Fermentation substrate

colonocytes

immune cells

brain

liver

energy

Immune system

Satiety

Lower cholesterol and fattiness
2nd cohort study

Preliminary results confirm:

1) Decreased diversity of gut bacteria in children with autism
2) Decreased *Prevotella* in children with autism
$\text{Prevotella copri}$

$\text{Log}_{10}$(relative abundance in %)

-3 -2 -1 0 1 2

Cohort #1

Cohort #2

Combined

$P=0.002$

$P=0.010$

$P=0.0002$

Autistic

Neurotypical

4/20 10/19 5/18 13/17 9/38 23/36
Children with autism have less diverse microflora, regardless of whether or not they had GI symptoms.

*Prevotella* was much lower in children with autism.

- Potential “health specific” biomarker
- Suggests impaired ability to digest carbohydrates
- Consistent with previous measurements of less short-chain fatty acids in stool (less food for intestinal wall)
Treatment Implications

• Broad-spectrum probiotic probably needed
• Unfortunately, most commercial over-the-counter “probiotics” only contain a few bacteria, and not the ones needed by children with autism, and fail to implant long-term
• Suggests transplant of beneficial bacteria from healthy donor may be useful
**Probiotics for ASD?**

- Parracho et al 2010 – randomized, double-blind, placebo-controlled cross-over study with *lactobacillus plantarum* (45 Billion CFU/day)

- 62 participants recruited, 23 dropped out at baseline due to difficulty collecting stool samples, 22 dropped out during the study (including 3 for adverse events – skin rash, diarrhea, weight loss), so only 17 completed the study.

- Slight improvement in formed stools during probiotic vs. placebo:

<table>
<thead>
<tr>
<th>Stool consistency</th>
<th>258</th>
<th>270</th>
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<tbody>
<tr>
<td>Soft</td>
<td>18.6 %</td>
<td>19.3 %</td>
</tr>
<tr>
<td>Formed</td>
<td>73.3 %</td>
<td>64.8 %*</td>
</tr>
<tr>
<td>Hard</td>
<td>8.1 %</td>
<td>15.9 %*</td>
</tr>
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</table>

- No significant effect on abdominal pain (rare problem), bloating (rare), flatulence (common)

- Slightly better improvement on “problem behavior”, but probably not statistically significant
2nd Probiotic Study

West et al 2013 – open-label study of Delpro (mixture of 5 probiotics, 30 billion CFU/day) for 6-month treatment

• *Lactocillus acidophilus, Lactobacillus casei, Lactobacillus delbruecki, Bifidobacteria longum, Bifidobacteria bifidum*; 6 billion CFU/day each

• 33 participants started, 25 completed;

• 84% had moderate/severe constipation, and 56% had moderate/severe diarrhea

• 20% reduction in ATEC score, p<0.05; significant improvement in all 4 subscales – speech, sociability, sensory/cognitive, physical/behavior

• Diarrhea: 48% improved, 32% no change, 8% worse

• Constipation: 52% improved, 36% no change, 4% worse
3\textsuperscript{rd} Probiotic Study

Kaluzna-Czapinska et al 2012 – Open label study with 22 children with ASD

• Lactobacillus acidophilus (strain Rosell-11), 10 Billion CFU/day for 2 months

• Urinary d-arabinitol (metabolite of candida) decreased 50%

• Some reported improvements in ability to concentrate (71%), eye contact (41%), ability to follow orders (77%), ability to react to other peoples emotions (36%)
4th Probiotic Study

Tomova et al 2015: open label study involving 10 children with ASD given ‘Children Dophilus”
3 strains of Lactobacillus (60%), 2 strains of Bifidumbacteria (25%) and one strain of Streptococcus (15%) – 3x/day for 4 months

Effect of probiotic:
• Firmicutes significantly decreased, which resulted in the increase of the Bacteroidetes/ Firmicutes ratio to the level of the healthy individuals
• Bifidobacterium decreased significantly after the probiotic intervention to the level of the healthy subjects.
• Desulfovibrio — a suspected pathogen in autism, decreased significantly

No description of effect on symptoms
Fecal Transplant

- Approximately half of stool is bacteria.
- The US Food and Drug Administration (FDA) allows FMT for use in patients with a dangerous GI infection called clostridium difficile (CD) when other therapies fail. CD kills about 15,000 people in the US each year, and hospitalizes many more.
- A recent review of 27 published scientific case reports and studies involving 317 patients with life-threatening, antibiotic-resistant CD found that FMT therapy resulted in an overall cure rate of 92%, usually with a single dose, with usually little or no side effects.
- The amazing success of FMT therapy for CD infections has led to its investigation for treating many other GI problems.
Fecal Transplant (cont.)

• FMT in ulcerative colitis (UC) in sporadic cases can resolve symptoms within 6 weeks and maintain remission up to 13 yr (9 patients; Borody 1989, 2001, 2003, Bennet 1989). Occasional sporadic ‘cure’ differs from remission.

• And FMT in UC improved stool frequency and abdominal pain in 7/8 patients (88%) - degree of benefit varied (Brandt 2012).

• FMT for Crohn’s disease was reported to alleviate symptoms within 3 days in a few cases (Borody 1989)

• FMT for IBS and IBD involved a case series of 55 patients, with a cure rate in 20 (36%), decreased symptoms in 9 (16%) and no response in 26 (47%) (Borody 1989).
Fecal Transplant (cont.)

FMT for chronic constipation involved a series of 45 patients, and a combination of colonoscopic FMT followed by fecal enemas resulted in improvements in defecation, bloating, and abdominal pain in 40 (89%). At follow-up 9-19 months later, defecation remained normal in 60% (Andrews 1995).

Would it also help for chronic constipation in autism?
Probably, since we suspect chronic constipation is often due to pathogenic bacteria, but research is needed to be sure.
Microbiota Transplant for Autism

One of our collaborators, Dr. Thomas Borody, a gastroenterologist from Australia, has used FMT treatment nearly 5000 patients, including 9 children with autism treated with a culture of 20 gut bacteria, for which he reports:

"After 3 months of the cultured microbiota there was a substantial improvement in bowel function, and the parents noticed a substantial reduction in the odor of their stool. Several children started the study with abdominal cramps which disrupted their sleep, and by the end of the treatment their abdominal cramps were reduced and they were able to sleep throughout the night. Parents reported marked increase in vocabulary, clear improvement on task performance and new ability to listen to parents’ requests. “
Trial of MT for Autism

The FDA and ASU’s Human Subject Board approved our pilot study of 20 children with autism ages 7-17 to participate in a trial of MT.
Study Purpose

The purpose of the study is to evaluate the safety and tolerability of a combination of oral vancomycin (an antibiotic) followed by a microbiome transplant (transferring gut microbiome from a healthy person) on children with autism who have gastrointestinal problems.
Summary

• Low diversity of gut bacteria in children with ASD

• Suggests less ability to digest carbohydrates, less SCFA’s to feed intestinal cells, altered neurotransmitters, possibly less vitamins

• Suggests FMT may be useful for treating gut problems in ASD and possibly improving ASD symptoms
THANKS!

Many thanks to families who participated.

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