

# Contribution of the Intestinal Microbiome to the Development of Hirschsprung's-Associated Enterocolitis (Proposed Research)

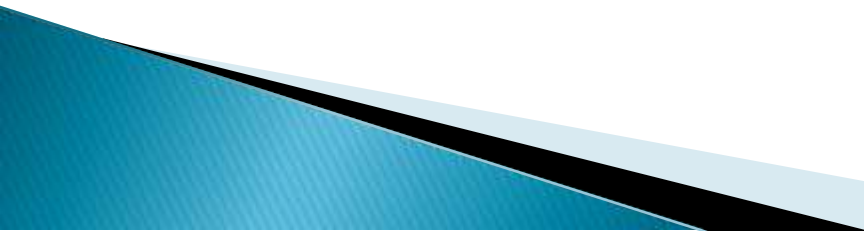
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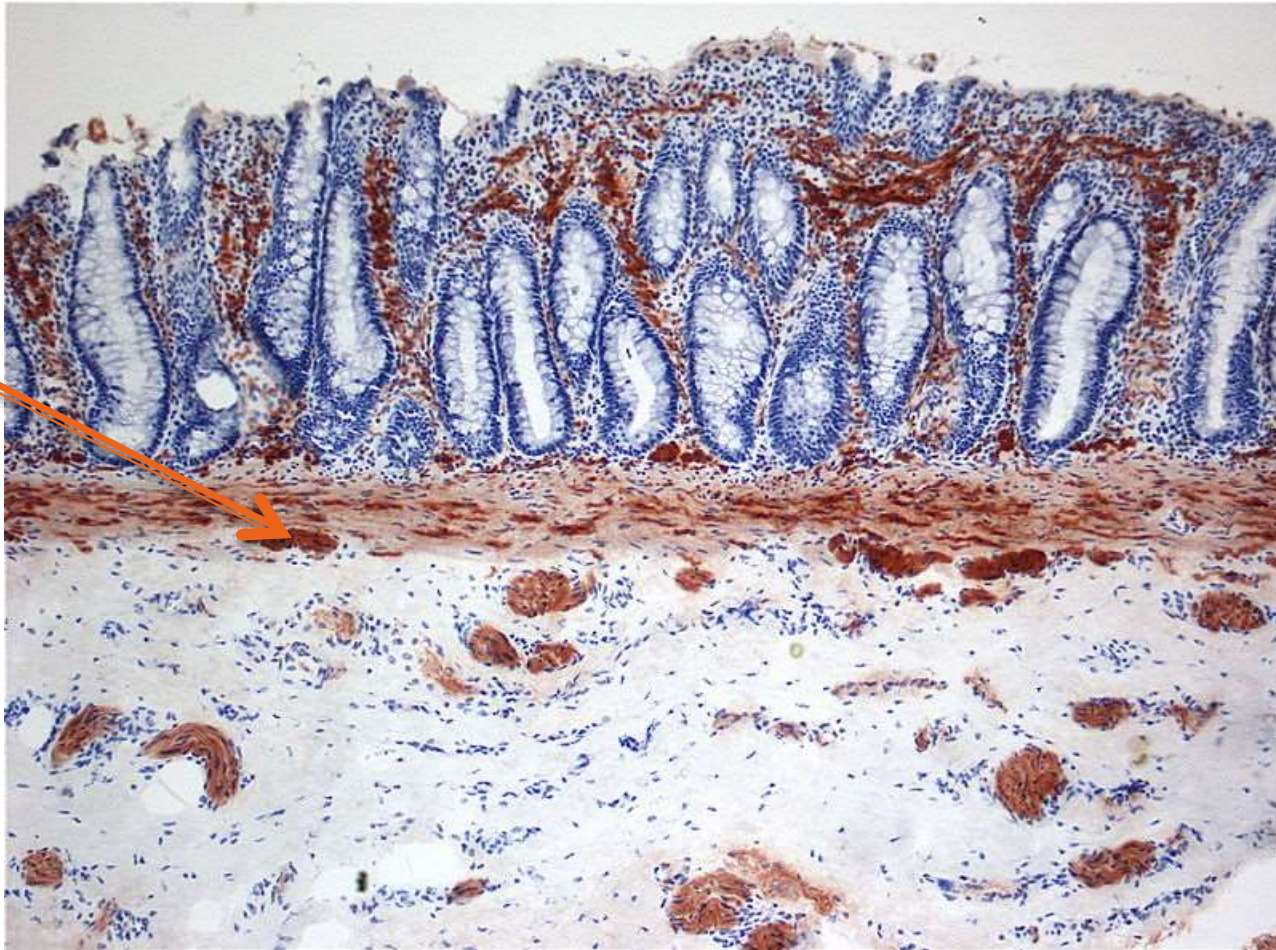
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# Hirschsprung's disease (HD)


- ▶ Congenital malformation of the GI tract characterized by the absence of the distal enteric nervous system (ganglion cells)
  - ▶ Main genetic cause of functional intestinal obstruction
  - ▶ Classification (L-HD, S-HD)
  - ▶ Most cases in the newborn period
  - ▶ Major susceptibility gene is *RET*
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# Histopathology of HD


Aberrant  
acetylcholine  
esterase(ACHE)  
positive fibres



# Epidemiology of HD

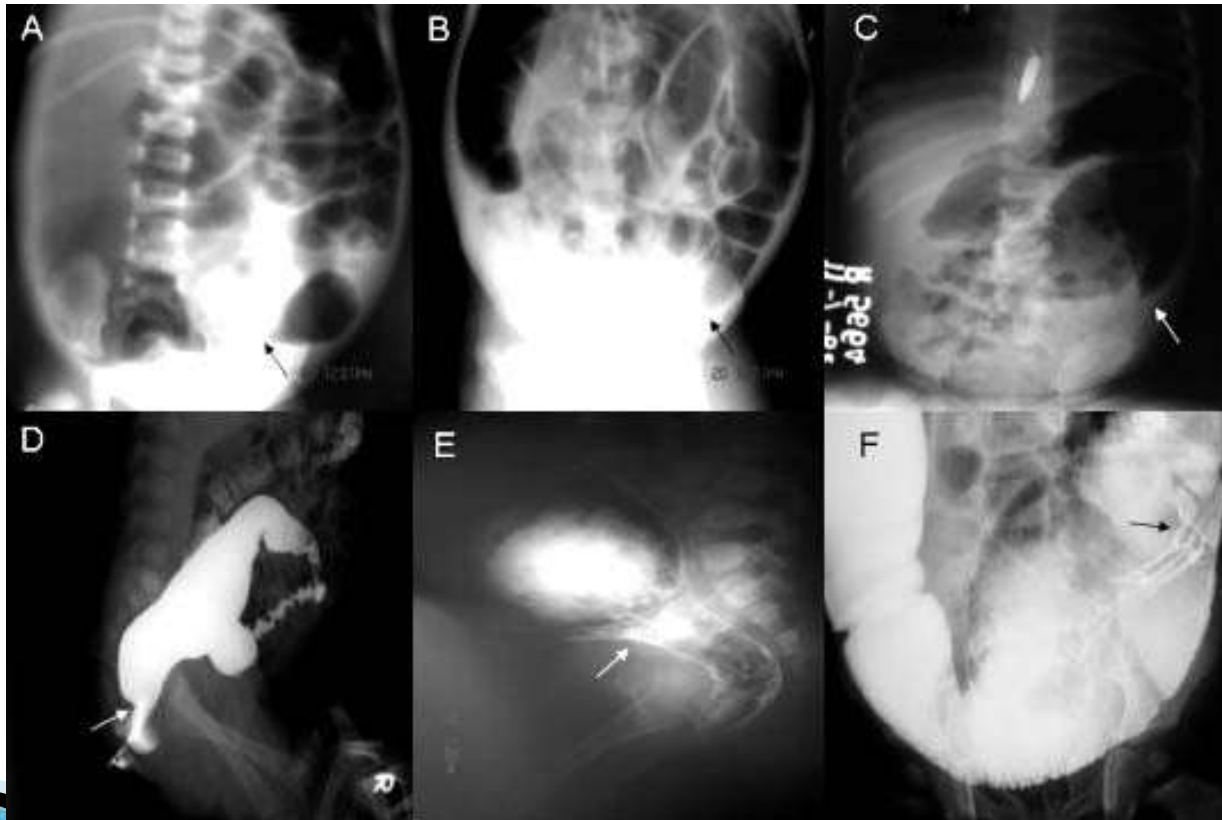
- ▶ 1 / 5000 live-births
  - ▶ Variation between ethnic groups
  - ▶ Sex bias
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# Symptoms of HD


- ▶ Intestinal obstruction
  - ▶ Delayed passage of meconium
  - ▶ Abdominal distention
  - ▶ Vomiting
  - ▶ Neonatal enterocolitis
  - ▶ Absence of relaxation of internal sphincter
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# Abdominal Radiography


- ▶ A, D – rectosigmoid, B, E – midsigmoid, C, F – descending colon



# Treatment of HD

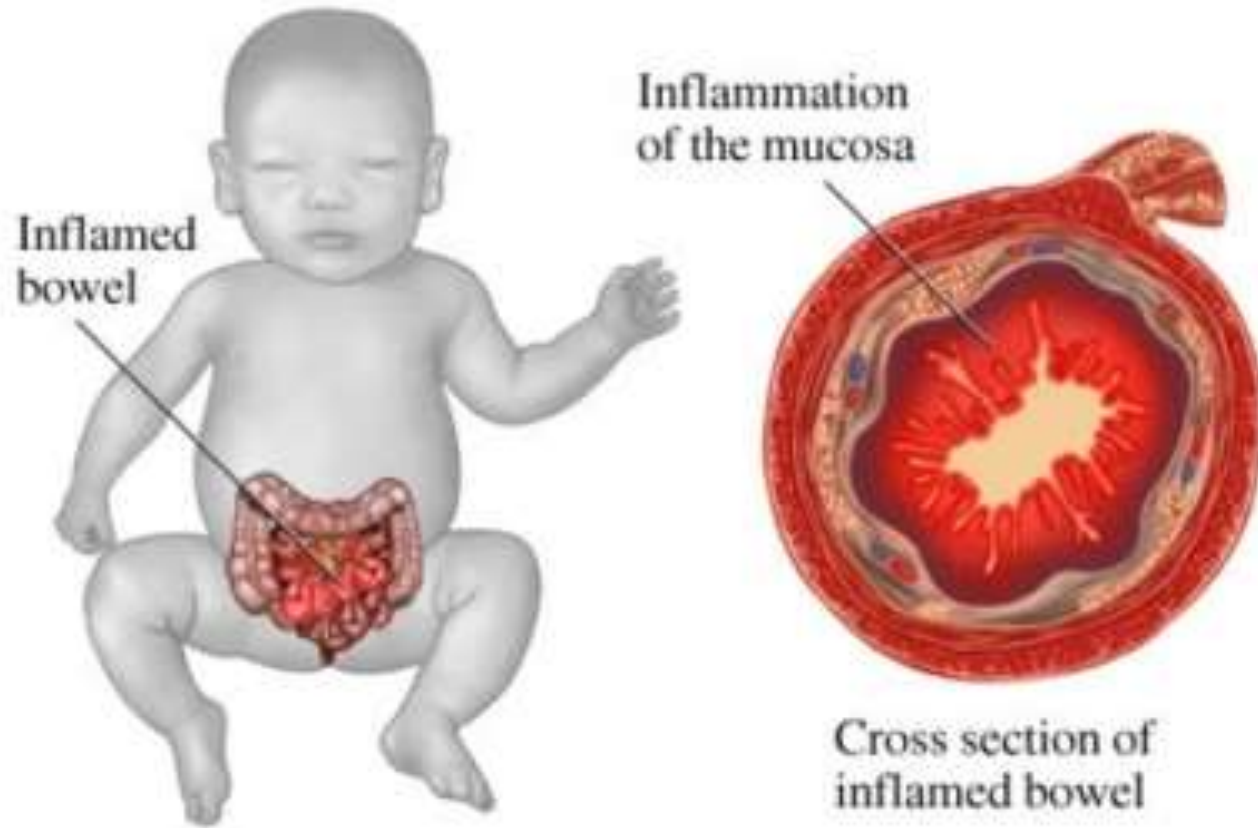
- ▶ Surgery – remove aganglionic section
  - ▶ Intestine is brought to the surface, affected part is removed
  - ▶ The colostomy is closed and healthy intestine is reattached
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# Hirschspung's–Associated Enterocolitis (HAEC)

- ▶ Most serious complication of HD
  - ▶ Inflammation of the mucosa of the colon or small intestine
  - ▶ One–third of children with HD
  - ▶ Most common reason for hospitalization in HD
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# HAEC

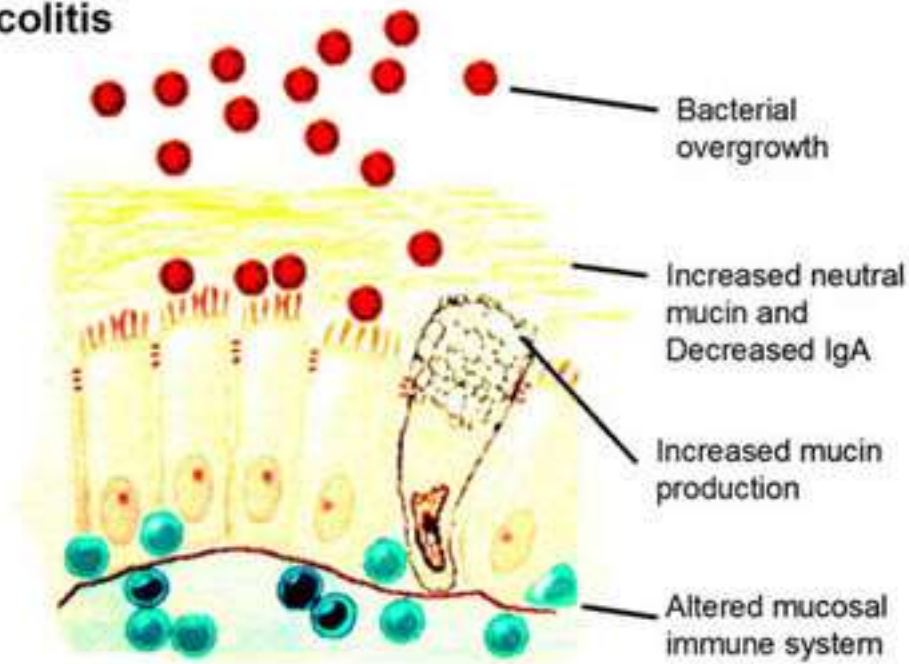


# Symptoms of HAEC

- ▶ Bloating abdomen
  - ▶ Severe diarrhea
  - ▶ Vomiting
  - ▶ Fever
  - ▶ Lethargy
  - ▶ Poor feeding
  - ▶ Rectal bleeding
  - ▶ Shock
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# Pathophysiology

## Pathophysiology of Hirschsprung-Associated Enterocolitis




# Treatment of HAEC

- ▶ Rectal irrigation and IV antibiotics (for serious cases)
- ▶ Oral antibiotics and rectal irrigation (for mild cases)

# Objective of Our Research

- ▶ To determine the contribution of the normal intestinal microbiome to the development of HAEC

# Evidence that Supports Our Hypothesis

- ▶ Levels of bifidobacteria and lactobacilli are decreased in HD
  - ▶ Overgrowth of *Clostridium difficile*
  - ▶ Antibiotic therapy has been shown to prolong life of EdnrB mice
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# Broader Impact

- ▶ This is the first longitudinal study of detailed microbiome composition in children with HD
- ▶ This research might help to understand other serious intestinal inflammatory diseases

# Materials and Methods

- ▶ Five patients with HAEC in Massachusetts General Hospital
  - ▶ Availability of samples (may take a year)
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# Procedure

Collection of fecal samples

Prior to antibiotic treatment

After antibiotic treatment

3 months after antibiotic treatment

6 months after antibiotic treatment

Freezing and pulverization of samples

Extraction of genomic DNA: Bead-beating technique and phenol-chloroform extraction

Pyrosequencing of 16S rRNA gene

Classification and comparison of samples structure

# Bead-Beating Technique

Mix extraction buffer with wet weight of sample

Add glass beads

Blend the sample in Bead-Beater for 2 min

Add sodium dodecyl sulfate (SDS)

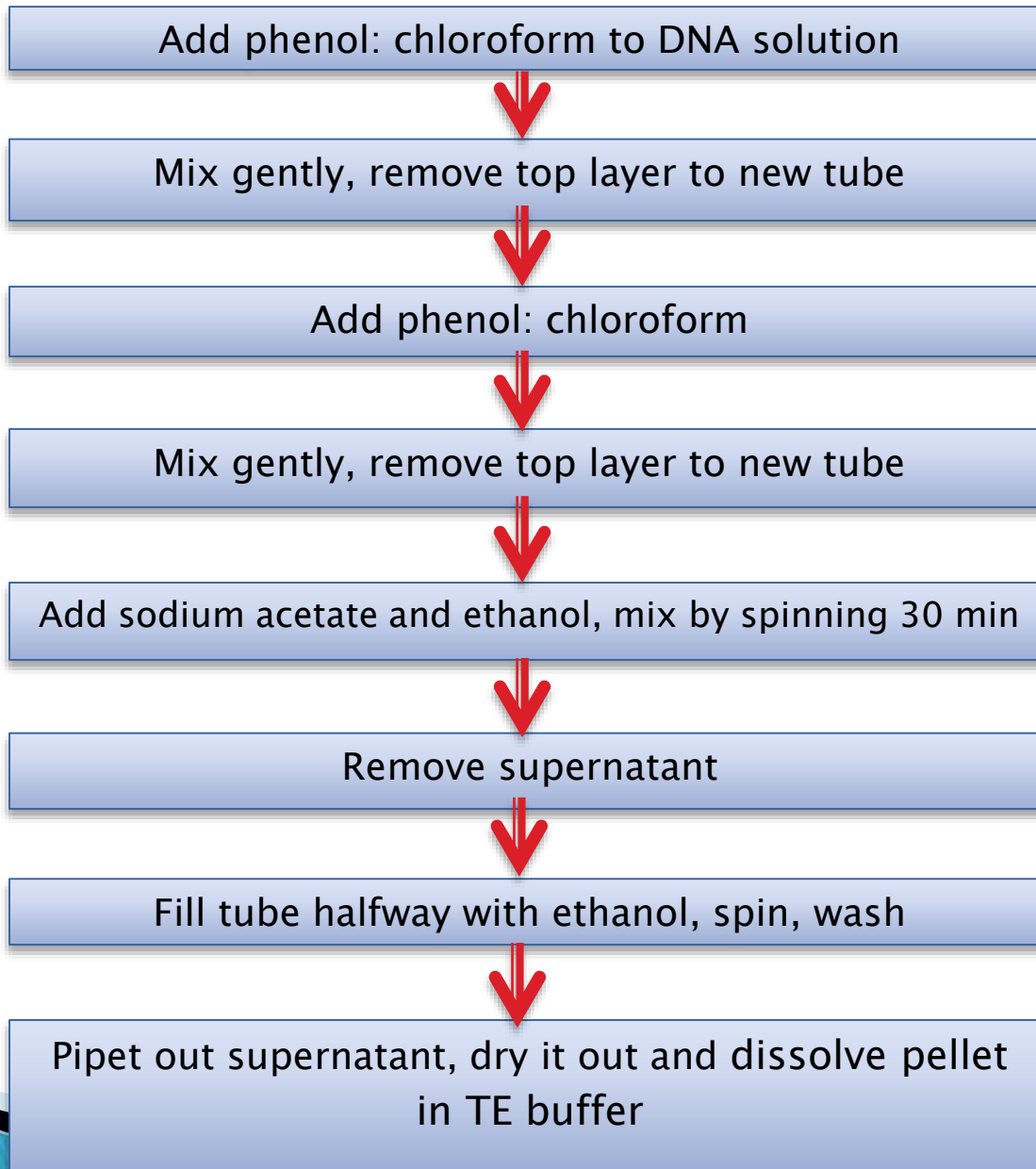
Blend the sample for 5 sec and incubate for 1 hour

Re-extract sample pellet, extract buffer, incubate, centrifuge


Transfer sample to ice for 5 min, centrifuge and add

Incubate for 2 hours, centrifuge the sample, resuspend pellet in TE buffer

# Phenol-Chloroform Extraction



# Discussion of Expected Results

- ▶ We predict that the structure and function of the microbiome of each patient before antibiotic treatment will be different from those after treatment
  - ▶ We expect variations between results of individual patients
  - ▶ These data will help to form strategies to positively impact the morbidity and mortality of HAEC
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# Future Direction

- ▶ Investigate if specific microbial population may contribute to the variations in disease phenotype

# Acknowledgements



- ▶ This project was supported by grants from the National Center for Research Resources (5P20RR016474-12) and the National Institute of General Medical Sciences (8 P20 GM103432-12) from the National Institutes of Health. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of NIH.
- ▶ We thank Dr. Jun Ren, Dr. Scott Seville, and the University of Wyoming INBRE Network for their support in this research.

# Work Cited

- ▶ Pseudomembranous colitis following resection for Hirschsprung's disease. J Pediatr Surg. 1992
- ▶ Detection of intestinal bifidobacteria and lactobacilli in patients with Hirschsprung's disease-associated enterocolitis. World J Pediatr. 2009
- ▶ Genomics approach to the analysis of bacterial communities dynamics in Hirschsprung's disease-associated enterocolitis: a pilot study. Pediatr Surg Int., 2009
- ▶ Murine model of Hirschsprung's disease-associated enterocolitis. I: phenotypic characterization with development of a histopathologic grading system. J Pediatr Surg, Sept 2002
- ▶ Amiel J, Sproat-Emison E, Garcia-Barcelo M, Lantieri F, Burzynski G, Borrego S, Pelet A, Arnold S, Miao X, Griseri P, Brooks A, Antinolo G. Hirschsprung disease, associated syndromes, and genetics: a review. J Med Genet, 2008
- ▶ <http://winchesterhospital.org/health-library/article?id=626205>
- ▶ <http://www.hirschsprungs.info/>