

# Do the gut flora play a role in African Americans with colon cancer?

Hassan Brim, Ph.D.  
Howard University



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# Colon Cancer in African Americans

- High incidence and high mortality
- Very aggressive and usually detected at late stages
- Different factors might be responsible
- Analysis of known TSG and oncogenes
- A high rate of MSI

# Ways to understand the causes

- High throughput experiments: genomics and epigenomics
- Genomics: aCGH study of 30 AA CRC
- Chromosome X amplification and implications
- Tumors clustering, maximum likelihood vs Parsimony??, Cloud computing as a solution?
- Epigenomics: 12 AA CRC and 12 AA adenoma study. Clustering, differential methylation vs cumulative??

# Diet and Gut Flora

- Diet's role is unquestionable: patterns of colon cancer in western world, Japanese Americans vs Japanese, African Americans vs Africans
- SLC5A8 study: 82% of AA colon adenomas show methylation of this gene, a butyrate transporter

# From single bacteria to microbiomics

- *Enterococcus faecalis*: induce polyp formation in Mi mice abd genomic polyploidy (Dr. Huycke, OSU)
- Enterotoxigenic *Bacteroides fragilis*: induces polyp formation in Min mice through a pSTAT3 pathway (Dr. Sears, JHU)
- *Helicobacter pylori*: Macrophages/Nitric oxide/DNA methylation, Our study found a correlation with high size polyps.

# Gut Flora Analysis

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DNA was extracted from 4 stool samples, PCR amplified using universal 16S rDNA primers and cloned.

384 clones were sequenced from each stool sample DNA and identified using a rDNA database

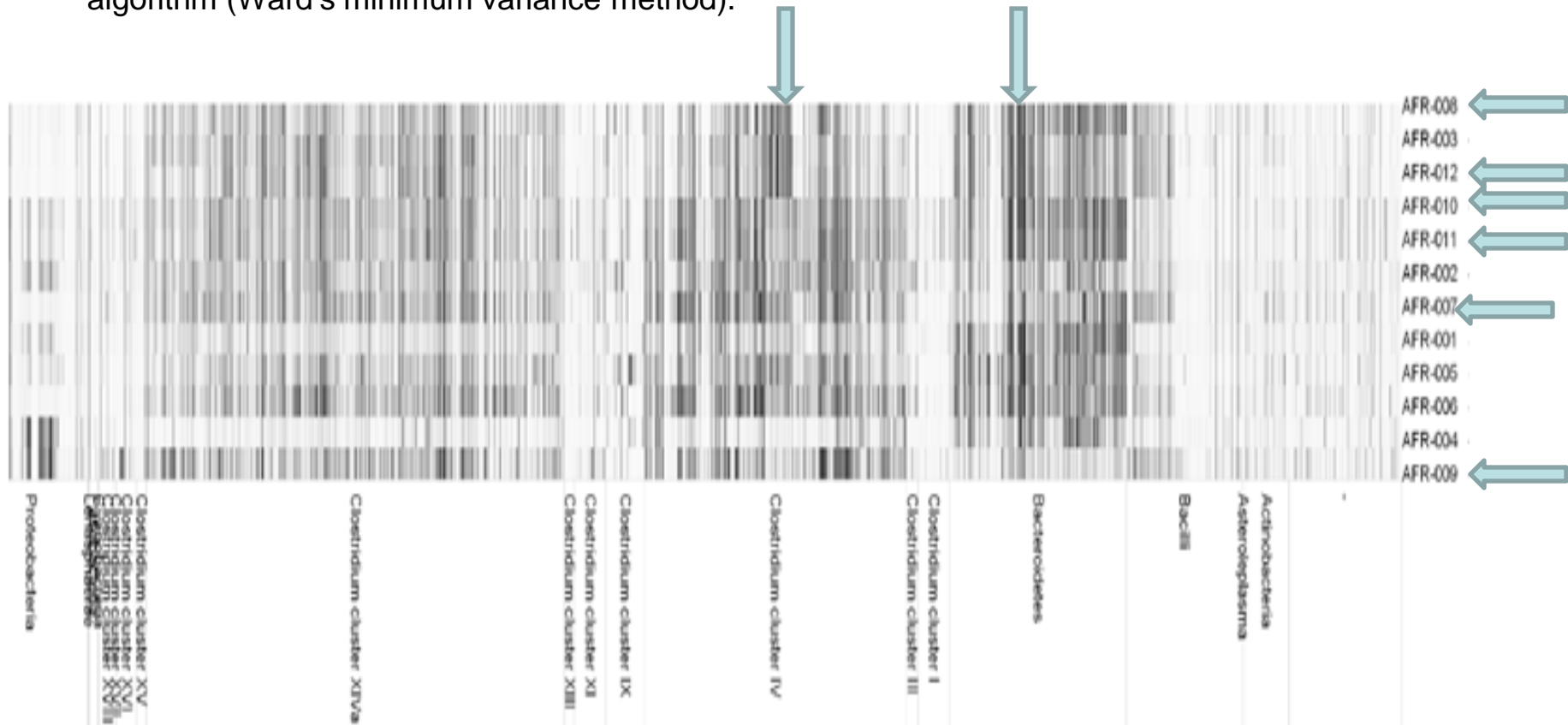
	<i>HB01</i> <i>(Polyp patient)</i>	<i>HB02</i>	<i>HB03</i> <i>(Polyp patient)</i>	<i>HB04</i>
<i>Bacteroides</i>	31.5%	4.8%	30.2%	10.2%
<i>Clostridia</i>	65%	93.3%	65%	68.5%

*H-H Partnership: Research Project*

# HIT Chip Analysis of DNA Stool Samples from Healthy and Adenoma Patients

Stool DNA from 12 patients (6 healthy AFR001-006 and 6 with adenoma AFR007-012) were used for 16 S rDNA amplification. The resulting PCR products were fragmented and fluorescently labeled before the hybridization to a Human Intestinal Tract (HIT) Chip.

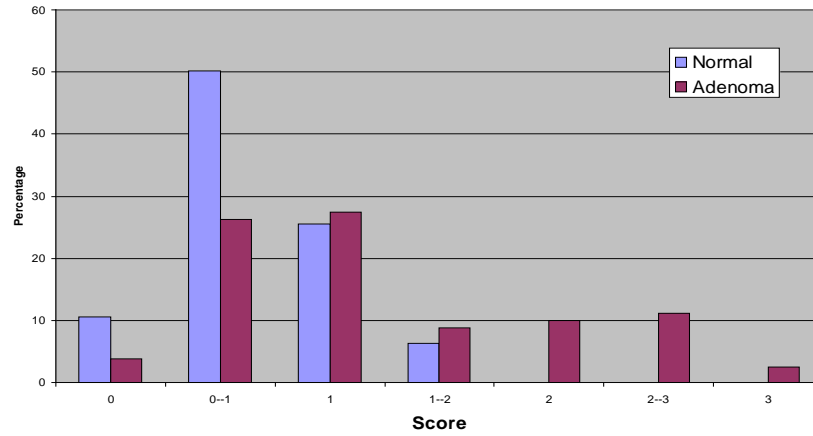
Hierarchical clustering of probe profiles was carried out by calculating a distance matrix between the samples based on the squared difference between each pair of profiles (Euclidian distance). The distance matrix was used in the hclust implementation in R of a hierarchical clustering algorithm (Ward's minimum variance method).



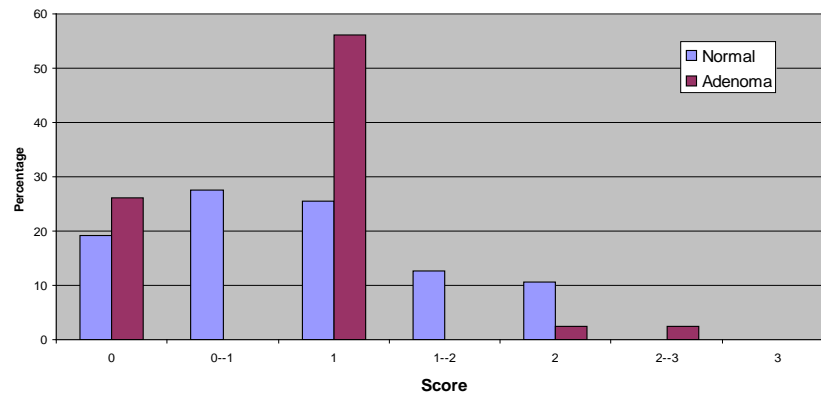




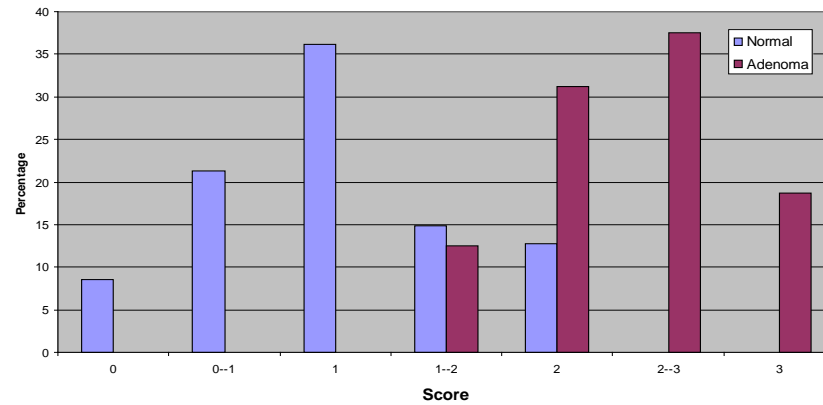
**pSTAT3 Nuclear Staining of Normal and Colon Adenoma Samples**



**pSTAT3 Cytoplasmic Staining in Normal and Colon Adenomas Samples**



**pSTAT3 Stromal Staining in Normal and Colon Adenomas Samples**



# ***bft* Detection in *C.diff* (CD) and Adenoma (CA) Stool Samples**

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- Stool DNA was used in a Touchdown PCR with 16S universal primers and primers specific for *Bacteroides*, *B. fragilis* and BFT gene
- Out of 82 samples (34 CD and 48 CA), only 7 had unamplifiable DNA.
- Positive for *B. fragilis* were 45
- Positives for BFT were 2 (one CD and one CA samples)
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# EIA

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- Detection of anti-BFT as evidence of ETBF exposure
- Preliminary data:
  - Bangladesh, acute diarrhea
    - 96% (64/67) patients with diarrhea anti-BFT+, day 7
    - 12.5% (2/16) endemic control patients anti-BFT+

# EIA Results with African American Serum Samples

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- EIA experiments for the detection of anti-BFT antibodies involving 87 serum samples from colon adenoma patients and 180 healthy individuals are underway.
- A similar experiment for the detection of anti- *H. pylori* antibodies is also being pursued.
- Metagenomic analysis