Polyp compared to non-polyp and fecal microbiome in pediatric Familial Adenomatous Polyposis

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S Septer
CA Lawson
S Umar
Adenomatous polyps: familial / sporadic

• Sporadic adenomatous polyps
  • 65% of all CR polyps
  • 2/3rd CRC arise in CR adenomatous polyps
  • CRC is 3rd leading cause of cancer

• Familial adenomatous polyposis
  • incidence: 8:10,000
  • APC gene mutation (5q21)
  • Innumerable adenomatous polyps - colon
  • CRC at mean age 36 yrs
Microbiome - adenoma interrelationship

• Individuals with sp.CRC frequently harbor abnormalities in gut microbiome (↓beneficial, ↑procacinogenic/proinflammatory bacteria)

• ↑Proteobacteria, ↑Fusobacteria ↓Bacteroidetes in adenomata / rectal mucosa / fecal microbiota of pts cf healthy controls
  
  Shen XJ et al. Gut Microbes. 2010
  Sanapareddy N et al. The ISME journal. 2012
  Brim H et al. PloS one. 2013
  McCoy AN et al. PloS one. 2013;

• Bacterial biofilms in FAP patients’ colonic mucosa harbor E coli and B fragilis with rel. overexpression of colibactin (clbB) and B fragilis toxin (bft) oncotoxins
  
  Dejea CM et al. Science. 2018
Aims:

• The aim of this study was to determine (a) if differences exist in the microbiome of polyp tissue (T) compared to adjacent non-polyp (H) mucosa, (b) between stool in individuals with FAP (S) compared with cohabiting unaffected family members (HS) and (c) between stool and tissue (mucosa) related microbiome.
Pediatric patient (< 21) with FAP (clinical diagnosis +/- APC mutation) + scheduled screening colonoscopy + co-habiting unaffected 1st degree relative (control)
## Results

<table>
<thead>
<tr>
<th>Subject / Control</th>
<th>Subject Gender</th>
<th>Subject Age at Procedure</th>
<th>Subject APC Mutation</th>
<th>Study Year</th>
<th>Subject</th>
<th>Control</th>
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</table>
Methods II

• Isolation of Genomic DNA and 16S V4 region amplification using 515F/806R primers; Amplicon sequencing on IonS5™ XL to generate raw reads.

• Quality filtering on the raw reads; Reference database (Silva database, https://www.arb-silva.de/) using UCHIME algorithm to detect and remove chimeric sequences.

• Sequences with ≥97% similarity were assigned to the same OTUs. Alpha diversity through 6 indices, including Observed-species, Chao1, Shannon, Simpson, ACE and Good-coverage. Beta diversity through ANOSIM and Linear Discriminant Analysis.
Results

Relative Abundance

Alpha Diversity

- Others
- Tenericutes
- Acidobacteria
- Euryarchaeota
- Cyanobacteria
- Verrucomicrobia
- Actinobacteria
- Fusobacteria
- Bacteroidetes
- Proteobacteria
- Firmicutes

T = Polyp
H = Healthy Tissue
S = Stool
HS = control stool
U = Unknown Tissue
1 = 1st year
2 = 2nd year
3 = 3rd year
## Results

### Beta Diversity

<table>
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<th></th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
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</table>

- **R** = 0.349, **P** = 0.001

### ANOSIM

- **R** = -0.03, **P** = 0.564
- **R** = 0.349, **P** = 0.001
- **R** = 0.391, **P** = 0.001

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**Between**

**H1H2H3**

**T1T2T3**

**Rank Value**

**R** = -0.03, **P** = 0.564

**Between**

**S1S2S3**

**T1T2T3**

**Rank Value**

**R** = 0.349, **P** = 0.001

**Between**

**HS1HS2HS3**

**T1T2T3**

**Rank Value**

**R** = 0.391, **P** = 0.001
Results

Linear Discriminant Analysis & Effect size (LEfSe)
### Results / Discussion

<table>
<thead>
<tr>
<th>Polyp (T)</th>
<th>non-polyp (healthy) mucosa (H)</th>
<th>(subject) stool (S)</th>
<th>(control) stool (HS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proteobacteria (P)</td>
<td>Firmicutes Bacteroides (P)</td>
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<td>Feacalibacterium Roseburia</td>
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</tbody>
</table>

1. Firmicutes Bacteroides (G)
2. Firmicutes Bacteroides
3. Fusobacteria, Proteobacteria
Conclusions

• There are differences in microbiome composition not only between polyps and synchronous tissues but also between tissues and stool samples collected from the same patient.

• Tissue and fecal bacterial populations are not only distinct but may differ in how they are enriched / distributed over time.

• Pediatric patients with FAP harbor complex microbiotic-polyp microenvironment perturbations suggesting a dysbiotic relationship.