The bacteriology of pouchitis: a molecular phylogenetic analysis using 16s rRNA gene cloning and sequencing

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Background

- 32% of patients with UC will eventually require colectomy

- Restorative proctocolectomy (IPAA) is the operation of choice

- Need for restorative proctocolectomy in some patients with FAP

Pouchitis

- Most common long term complication\(^1\)
- Occurs cumulatively in 20-50% with UC and 2-5% of patients with FAP\(^2,3,4\)
- Accounts for 10% of pouch failures\(^5\)

Bacterial dysbiosis in Pouchitis

- Bacterial dysbiosis probably fundamental to the inflammatory process
- Antibiotics effective\(^1\)
- Probiotics effective\(^2,3\)
- <50\% gut microbiota culturable\(^4\)

Aim

- Identify, compare and contrast the mucosal associated microbiota in UC and FAP pouchitis and non-pouchitis groups using 16s rRNA sequencing.
Method; Classification of patients

PDAI: Three components

1. Clinical

2. Endoscopic

3. Histology

Score of $\geq 7$ = pouchitis

Method; Pouchitis patient criteria

**Inclusion criteria**
- Symptomatic pouchitis with PDAI ≥7
- History of chronic pouchitis
- Antibiotic responsive pouchitis

**Exclusion criteria**
- Patients taking probiotics or antibiotics within 2 weeks
- NSAIDs, immunosuppressants
- Cuffitis, stricture etc
Method: non-pouchitis patient criteria

Inclusion criteria

- Good pouch function with PDAI 0
- No previous history of pouchitis

Exclusion criteria

- Patients taking probiotics or antibiotics within 2 weeks
- NSAIDs, immunosuppressants
- Cuffitis, stricture etc
**Method**

- 8 non-pouchitis UC patients
- 8 UC pouchitis patients
- 5 non-pouchitis FAP patients
- 3 FAP pouchitis patients
Lab method

- DNA extraction was performed on single biopsy specimens using the DNeasy blood and tissue kit
- 16s rRNA gene amplified with PCR using broad-range bacterial primers
- Sequences aligned and chimeric sequences removed; 3184 full-length sequences in total
- Sequences initially given a broad classification at the phylum and family levels
- Sequences divided into phylotypes
- Shannon diversity index calculated for each sample
Results; UC Vs FAP

Phylum level analysis

- Significantly greater bacterial diversity in the FAP group compared to the UC group (p= 0.009)
- Significant increase in Proteobacteria (p= 0.019)
- Significant decrease in Bacteroidetes (p= 0.001)
UC pouchitis Vs UC non-pouchitis

Phylum level analysis

No significant differences identified

Significant reduction in bacterial diversity in the UC pouchitis group compared to the UC non-pouchitis group (p= 0.009)
Results; UC Vs FAP
Family level analysis

**Proteobacteria**
- Comamonadaceae
  (p = 0.007)
- Moraxellaceae
  (p = 0.027)
- Alcaligenaceae
  (p = 0.03)

**Bacteroidetes**
- Bacteroidaceae
  (p = 0.013)
- Prevotellaceae
  (p = 0.023)

**Firmicutes**
- Ruminococcaceae
  (p = 0.007)

*significant differences
Results;
Species level analysis

- *Faecalibacterium prausnitzii*, detected in 6 out of 8 FAP patients but only 4 out of 16 UC patients (p=0.029).

- *Bacteroides vulgatus*, significantly increased in the FAP group compared to the UC group (p =0.031).

- No individual species or phylotype specifically associated with either UC or FAP pouchitis was found.
Conclusion

- UC pouch patients have a different, less diverse, gut microbiota than FAP patients.

- A further reduction in bacterial diversity but no significant dysbiosis occurs in those with pouchitis.

- The study suggests that a dysbiosis occurs in the ileal pouch of UC RPC patients which predisposes to, but may not directly cause, pouchitis.
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