The Microbiome In Colorectal Polyps

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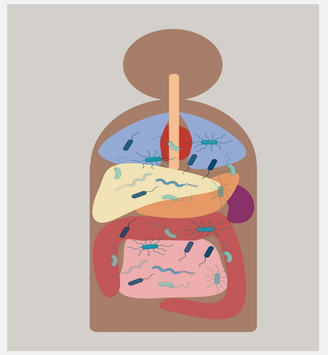
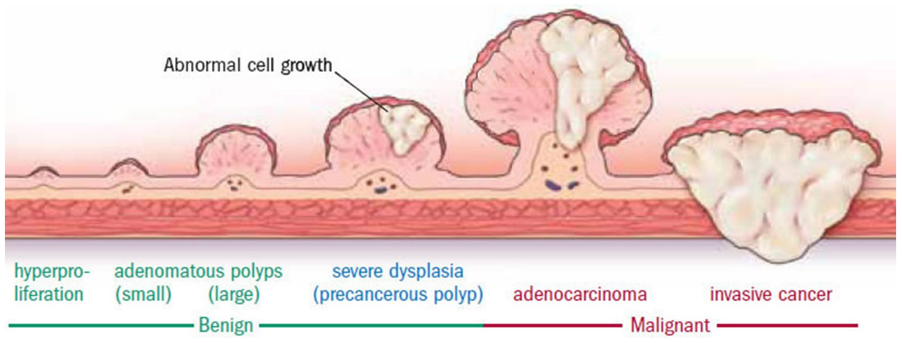


Background

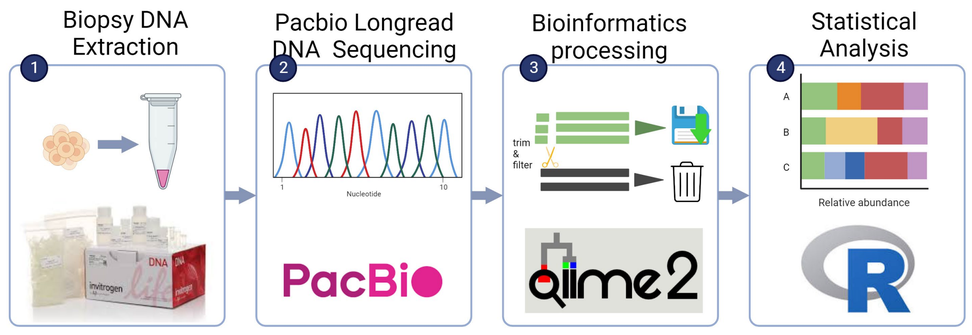


- Bowel polyps are benign but excessive proliferation of bowel epithelial cells.
- 5% of polyps grow into malignant colorectal cancers unless excised.
- Polyp surveillance includes histological risk factors such as polyp size and family history, but do not yet include genetic and environmental factors that are conducive to polyp transformation to cancer.
- Including these factors may improve disease risk assessment thus identifying at-risk patients earlier and reduce unnecessary colonoscopies.
- Different stool microbes have already been correlated with gut health (*e.g.*, butyrate-producing bacteria) and malignant bowel cancers (*e.g.*, *Fusobacterium nucleatum, Bacteroides fragilis, Porphyromonas*)[1,2].
- Less is known about microbiome differences between people with pre-cancerous polyps and those without directly at the gut mucosa.

Aim

Identify bacteria at the gut mucosa that are differentially expressed between people with and without pre-cancerous bowel polyps.

Methods



- Mucosal biopsy DNA from colonoscopy patients with (n = 36) or without (n = 39) polyps was sequenced with Pacbio[3] primers designed to amplify the V1-V9 region of the 16S rRNA gene.
- The resulting fastq files underwent filtering using dada2[4] and taxonomic assignment from the SILVA[5] database using the Qiime2[6] platform.
- Alpha and Beta diversity measures, Composition analyses, Differential Expression analyses and Partial Least Squares Discriminant Analyses were performed using the R platform[7] to examine for differences in microbiome profile between people with polyps and those without.

Results

Diversity Measures: How different are the two groups?

Fig 1. Boxplot of Shannon alpha diversity for proximal gut mucosa samples. Shannon diversity reflects the number of species living in a habitat (richness) and their relative abundance (evenness); greater Shannon indexes reflect higher microbial diversity. There is no significant difference in Shannon diversity between the samples with polyps and those without.

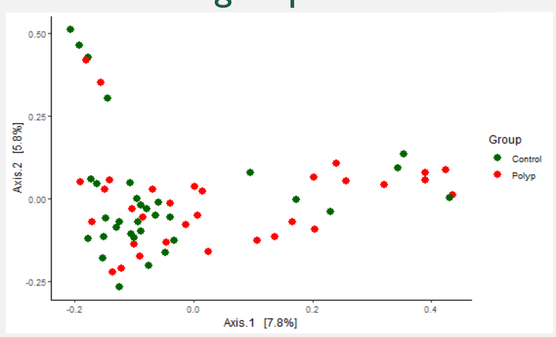


Fig 2. Principal Coordinates Analysis as a measure of beta diversity for proximal gut mucosa samples. PCoA reflects any similarities between groups: Points that are closer together represent microbial communities that are similar in sequence composition. There is no significant separation of samples with polyps and those without, suggesting overall similar communities between the two groups.

Composition Plot: Which bacteria comprise the two groups?

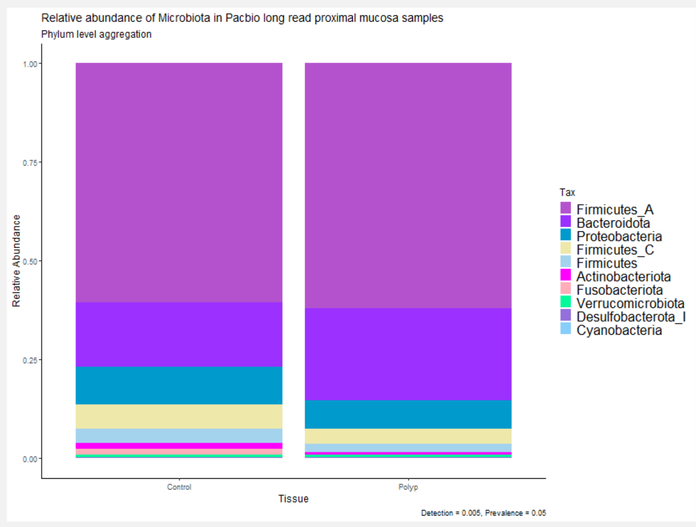
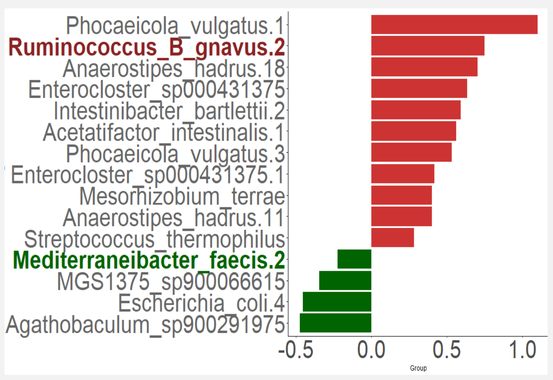


Fig 3. Composition Plot for proximal gut mucosa samples. ASV abundances are agglomerated to the Phylum level of taxonomy. Bacteria were included that showed a detection rate of 0.005 and a prevalence of 0.05. Samples from polyp patients show a higher relative abundance of Bacteroidota, but lower relative abundance of Proteobacteria and Firmicutes from Classes Negativicutes (Firmicutes_C) and Bacilli (Firmicutes), compared to controls.

Differential Expression Analyses: Which bacteria distinguish the two groups?



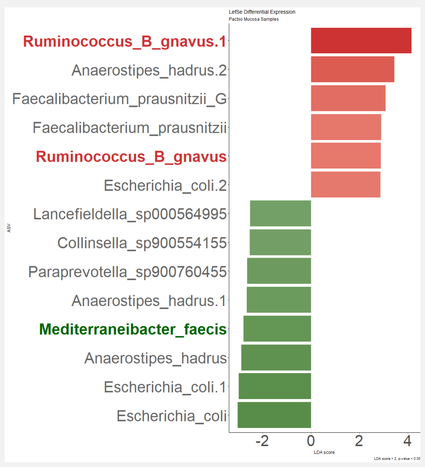


Fig 5 Boxplot of univariate (A) ANCOMBC and (B) LefSe differential expression analyses for proximal gut mucosa samples. Analyses were run to assess for individual gut microbes that may be differentially expressed between samples with polyps and those without.. Two methods were used to assess for method robustness. Although the two methods showed little overlap in identified bacteria, both methods suggest that Ruminococcus gnavus is more abundant, and Mediterraneibacter faecis less abundant, at the proximal gut mucosa in those with polyps compared to those without.

Partial Lease Squares Discriminant Analysis:

Which bacteria distinguish the two groups using multivariate methods?

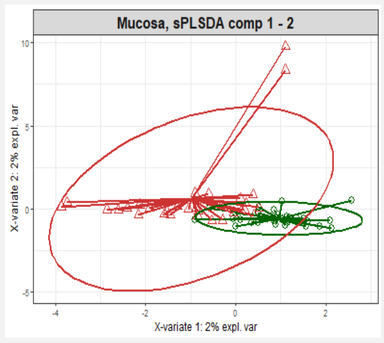


Fig 6. Sample plot of multivariate PLS-DA. PLS-DA is a multivariate method designed to find variables that best describe differences between groups. These results suggest that samples do not separate widely according to polyp status, suggesting similar bacterial compositions between the two groups.

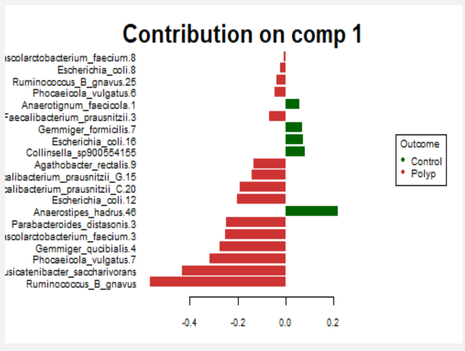


Fig 7. Loading plot of multivariate PLS-DA. This plot shows the relative importance of bacteria chosen by the PLS-DA method to separate the two groups, with the most important bacteria showing the longest bars at the bottom, and least important with the shortest bars up the top. This multivariate method agrees with the univariate method that Ruminococcus gnavus and Phocaeicola vulgatus is more associated with mucosa in polyp samples compared to controls.

Conclusions and Future Directions

- At the earliest stages of polyp progression to malignancy, differences in the gut microbiome are subtle.
- However both univariate and multivariate analyses of the data suggest that bacteria such as Phocaeicola vulgatus and Ruminococcus gnavus are relatively more abundant, and Mediterraneibacter faecis less abundant, at the proximal mucosa in people with polyps compared to those without.
- Future directions for this project will be to include distal biopsies in the analysis, and then assess for a multi-omic panel that includes other environmental factors such as tumour mutational burden and immune factors, which may affect polyp development to carcinogenesis.

Sydney Cancer Partners
Collaboration, Translation, Impact

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References

1. Zackular, JP., et al, Cancer prevention research 7.11 (2014): 1112-1121.
2. Baxter, NT., et al. Genome medicine 8.1 (2016): 1-10.
3. https://www.pacb.com/
4. https://benjjneb.github.io/dada2/
5. https://www.arb-silva.de/
6. https://www.pacb.com/
7. https://www.r-project.org/