Journal of Cystic Fibrosis 23 (2024) 262–268

Contents lists available at ScienceDirect

Journal of Cystic Fibrosis

journal homepage: www.elsevier.com/locate/jcf

Original Article

The gut microbiota in adults with cystic fibrosis compared to colorectal cancer

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ARTICLE INFO

Keywords:
Colorectal cancer
Cystic fibrosis
Gut microbiota
CFTR modulators
Dysbiosis

ABSTRACT

Background: Gut dysbiosis is implicated in colorectal cancer (CRC) pathogenesis. Cystic fibrosis (CF) is associated with both gut dysbiosis and increased CRC risk. We therefore compared the faecal microbiota from individuals with CF to CRC and screening samples. We also assessed changes in CRC-associated taxa before and after triple CF transmembrane conductance regulator (CFTR) modulator therapy.

Methods: Bacterial DNA amplification comprising V4 16S rRNA analysis was conducted on 84 baseline and 53 matched follow-up stool samples from adults with CF. These data were compared to an existing cohort of 430 CRC and 491 control gFOBT samples from the NHS Bowel Cancer Screening Programme. Data were also compared to 26 previously identified CRC-associated taxa from a published meta-analysis.

Results: Faecal CF samples had a lower alpha diversity and clustered distinctly from both CRC and control samples, with no clear clinical variables explaining the variation. Compared to controls, CF samples had an increased relative abundance in 6 of the 20 enriched CRC-associated taxa and depletion of 2 of the 6 taxa which have been reported as reduced in CRC. Commencing triple modulator therapy had subtle influence on the relative abundance of CRC-associated microbiota ($n = 23$ paired CF samples).

Conclusions: CF stool samples were clearly dysbiotic, clustering distinctly from both CRC and control samples. Several bacterial shifts in CF samples resembled those observed in CRC. Studies assessing the impact of dietary or other interventions and the longer-term use of CFTR modulators on reducing this potentially pro-oncogenic milieu are needed.

1. Introduction

Cystic fibrosis (CF) is one of the most common, life-limiting, autosomal recessive conditions affecting Caucasian populations [1]. It is caused by biallelic mutations to the CF transmembrane conductance regulator (*CFTR*) gene, resulting in the absence or dysfunction of the CFTR anion channel which is expressed in epithelial cells [1]. This complex multisystem disease is associated with numerous physiological effects and complications, including lung infections, inflammation, exocrine pancreatic insufficiency (PI), CF-related diabetes (CFRD), CF-related liver disease (CFRLD) and gut dysbiosis [1,2]. Life expectancy is anticipated to increase significantly with the introduction of highly effective CFTR modulators. Therefore, reducing the risk of complications in an ageing CF population, such as bowel cancer, is a priority.

A 10 -fold increased incidence of colorectal cancer (CRC) has been reported in people with CF (pwCF) [3]. The increased prevalence and earlier onset of CRC in pwCF may reflect an oncogenic milieu driven by intestinal inflammation, reduced *CFTR* expression, altered mucus secretion, a higher fat, lower fibre diet and gut microbiota alterations [4]. CF-related gut dysbiosis is characterised by reduced diversity and an increase in pro-inflammatory bacteria, concurrent with a reduction in

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https://doi.org/10.1016/j.jcf.2023.12.004
Received 16 June 2023; Received in revised form 6 December 2023; Accepted 6 December 2023
Available online 15 December 2023

bacteria considered anti-inflammatory [2].

The gut microbiota may impact on cancer development through several mechanisms, including upregulation of gut inflammation, epithelial damage and cell turnover [5]. Outside of CF, a CRC-associated microbiota has been characterised [6] and a recent systematic review identified a number of taxonomic shifts in the CF gut microbiota, which may mirror those observed in CRC [2]. Despite these findings and growing concerns that CF gut dysbiosis could be associated with CRC risk, no study has directly compared the gut microbiota in pwCF to people with CRC (pwCRC). Here, we compare the faecal microbiota from adults with CF to pwCRC and controls. We hypothesise that the faecal microbiota in pwCF shares features of the CRC-associated microbiota.

2. Methods

2.1. Study design

Igloo-CF was a prospective observational cohort study, with two time points (baseline and follow-up). PI adults (≥18 years old) with CF and able to give informed consent were eligible. Those post lung transplant, prognosis <6 months, pregnant, significant gastrointestinal (GI) pathology, such as inflammatory bowel disease, short bowel syndrome or malignancy, were excluded. This data comprises a subset of Leeds participants who provided a fresh stool sample at one or more time points and consented to their samples being used in future research. Stool samples were collected a minimum of 2 weeks post intravenous (IV) antibiotics. Data were collected April 2019 to June 2021, with a COVID-19 related study pause March to December 2020. Paired stool samples were collected a median of 7 months apart (range 4 to 20 months). All participants with CF provided voluntary informed consent with ethical approval from the London Bromley Research Ethics Committee (18/LO/2241).

2.2. Clinical data

Clinical data was prospectively collected including demographics, annual days of IV antibiotics (categorised as low: 0–13 or high: ≥14 days), percentage predicted forced expiratory volume in 1 second ($ppFEV_1$), rate of decline in $ppFEV_1$ based on age and sex categories for PI adults with CF [7] and lung disease category. Genotype, body mass index (BMI), CFRD, CFRLD (defined as the presence of cirrhosis or fatty liver on ultrasound with variable elevation in liver function tests) and sputum microbiology status for *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex (BCC) were recorded. CFTR modulator status comprised: no modulator, single (Ivacaftor), double (Tezacaftor/-Ivacaftor or Lumacaftor/Ivacaftor) or triple therapy (Tezacaftor/Lumacaftor/Ivacaftor).

CF stool microbiota were compared to an existing observation study, which comprised a series of routinely collected guaiac faecal occult blood test (gFOBT) samples from the NHS Bowel Cancer Screening Programme (NHSBCSP) Southern Hub (Guildford, UK) between October 2016 and August 2019 [6]. Data are based on information collected and quality assured by Public Health England (PHE) Population Screening Programmes. Access to the data was facilitated by the PHE Office for Data Release. A sub-set of this data were employed: those with complete basic clinical data and were either 'blood-negative' - defined as controls – or had a blood positive sample and subsequent diagnosis of CRC on colonoscopy (CRC group). At the time of the study, NHSBCSP offered bi-annually screening for individuals aged 60–74 years old [8]. Those aged over 74 years had to initiate screening by requesting a kit [8]. An estimated 2 % of individuals will have a positive gFOBT and will require follow up, most commonly with a colonoscopy. Of those scoped, approximately 10 % will be diagnosed as having CRC [9]. Fewer than 2 % are estimated to have undetected CRC with a negative gFOBT test [10].

2.3. Stool sample collection, storage and processing

Fresh CF stool samples were collected and frozen at −80 °c. Just prior to DNA extraction, samples were defrosted overnight in a fridge, swabbed and processed as outlined in supplementary methods. DNA was extracted using the DNeasy 96 PowerSoil Pro-QIAcube HT Kit, according to kit protocol [11]. Details of how the CRC and control gFOBT samples from the NHS Bowel Cancer Screening Programme were processed can be found in the previous publication [6].

2.4. 16S rRNA sequencing

The bacterial DNA was amplified employing V4 16S rRNA analysis. Library preparation was conducted in line with the Earth Microbiome Project 16s Illumina Amplicon methodology, using 20 ng starting DNA per sample [12]. The CF stool samples were sequenced on one lane of an Illumina NextSeq2000. Samples were sequenced alongside 16S samples from other studies, for 2 × 150 bp sequencing, with 10 bp single index read. Details of the gFOBT sample sequencing methods are presented in Young et al. [6].

2.5. Bioinformatic and statistical analysis

Raw sequence reads were trimmed of adapters using cutadapt v3.4 [13]. Further processing was conducted inside the QIIME2 v2021.2 environment unless otherwise stated [14]. Reads paired were merged, quality-filtered, denoised and representative sequences chosen using DADA2 [15]. Read tables from the CF dataset were merged with the existing CRC/control dataset. Taxa were assigned to representative sequences using the BLAST+ algorithm [16] against the SILVA v132 database (Quast) by the QIIME2 feature classifier [17,18].

Alpha-diversity was described using Shannon Index and beta-diversity with principal coordinate analysis (PCoA) of Bray-Curtis distances. To test for differences in the Shannon Index between groups, the Kruskal-Wallis test was conducted. For beta-diversity, differences between groups were tested by performing PERMANOVA analysis of Bray-Curtis distances using Adonis [19]. Taxonomic calls were exported from QIME2 for further analysis. Linear discriminant analysis Effect Size (LEfSe) was conducted to test for biologically and statistically significant taxa differences between groups [20]. Taxa were compared at genus level. Shigella was considered part of the Escherichia genus. A p-value of <0.05 was regarded as significant.

Taxa were also compared against a meta-analysis of multiple metagenomic datasets compiled by Young *et al.* [6] of the 20 most discriminant taxa identified as enriched and 6 depleted in CRC faecal samples.

Raw sequence data is available from the European Nucleotide Archive, accession number PRJEB53976 (http://www.ebi.ac.uk/ena/data/view/PRJEB53976).

3. Results

3.1. Clinical and demographic data

Following quality control filtering and feature assignment by DADA2, there were between 20,469 and 271,279 reads per CF sample (median 127,917). Four samples failed library preparation quality control and five samples with < 35 reads were removed. This resulted in the inclusion of 84 baseline and 53 matched follow-up CF stool samples, which were compared to 491 controls and 430 CRC samples [6]. Males comprised 54 (64 %) of CF samples, compared to 289 (67 %) of CRC and 205 (42 %) of control samples. Mean age was 35 years (SD 9.84), 67.0 years (SD 4.5) and 68.1 years (SD 5.0) in CF, control and CRC samples respectively.

Clinical characteristics for CF participants are presented in Table 1. For those on CFTR modulators at baseline, participants had been taking single therapy for a median of 6 years (range 1–8), double therapy 453

Table 1
Clinical and demographic characteristics of the cystic fibrosis participants.

	n (%)
Age categories:	
18–28 years	20 (24 %)
29–39 years	44 (52 %)
40–50 years	14 (17 %)
51+ years	6 (7 %)
***CFTR* mutation:**	
F508del homozygous	54 (64 %)
F508del heterozygous	25 (30 %)
Other mutations	5 (6 %)
Microbiology:	
Chronic *Pseudomonas aeruginosa* growth	49 (58 %)
Burkholderia cepacia complex growth	11 (13 %)
Diagnosis of cystic fibrosis-related diabetes?	
Yes	23 (27 %)
No	61 (73%)
Diagnosis of cystic fibrosis-related liver disease?	
Yes	34 (40 %)
No	50 (60 %)
Rate of lung function decline	
Slow	37 (49 %)
Fast	38 (51 %)
$ppFEV_1$	55 (37.25)
Lung disease category	
Mild lung disease (> 80 $ppFEV_1$)	19 (23 %)
Moderate (40–79 $ppFEV_1$)	47 (56 %)
Severe (< 40 % $ppFEV_1$)	18 (21 %)
Intravenous antibiotic received in the last 12 months category	
Low (0–13 days)	32 (39 %)
High (≥ 14 days)	51 (61 %)
BMI category	
Underweight (<18.5 kg/m^2)	2 (2 %)
Healthy weight (18.5- 24.9 kg/m^2)	50 (60 %)
Overweight (25–29.9 kg/m^2)	26 (31 %)
Obese (≥30 kg/m^2)	6 (7 %)

days (range 28–1184) and triple therapy for 106 days (range 23–476) prior to sample collection. For paired data, there were six participants who commenced double therapy between time points, taking for a median of 107, range 3 to 427 days prior to follow-up stool collection. For the 23 participants who commenced triple therapy between baseline and follow-up (pre and post triple therapy group), five were taking double therapy at baseline and the rest no modulators. At follow-up, they had received triple therapy for a median of 191 days (range 27 to 496).

3.2. Faecal microbiota diversity in control, colorectal cancer and cystic fibrosis samples

While alpha-diversity was significantly lower in CRC faecal samples compared to controls ($p = 0.01$), both had a significantly higher alpha-diversity than CF samples ($p<0.001$), Fig 1a. Similarly, beta-diversity, represented by PCoA, demonstrated that CF stool samples clustered distinctly from both CRC and controls (Fig. 1b). Overall, the clustering of CF samples was so distinct that there was no obvious gradient as to whether a CF sample either did or did not resemble the control or CRC cohort.

3.3. Faecal microbiota diversity between cystic fibrosis samples

There was no clear clinical or demographic variable explaining diversity differences between the baseline CF stool samples, including the presence of CFRD, CFRLD, proton pump inhibitor (PPI) use, age category, sex, genotype, *P. aeruginosa* or BCC lung growth, lung disease severity, BMI category and IV antibiotic usage in the preceding year (Adonis test result $p > 0.05$ and Kruskal-Wallis for Shannon Diversity metrics $p > 0.05$ for all variables). There was no statistically significant difference ($p > 0.05$) in Shannon diversity between individuals taking CFTR modulators ($n = 25$) or by CFTR modulator type (single $n = 5$,

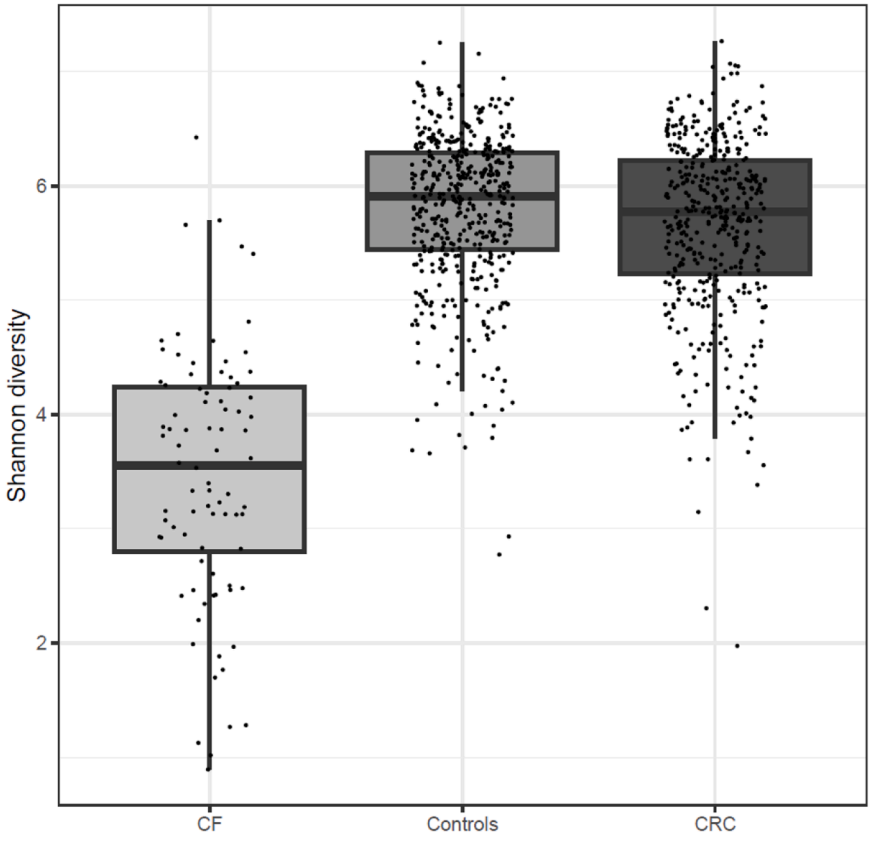

Fig. 1a. Box plot of Shannon Index between CF, control and CRC samples.

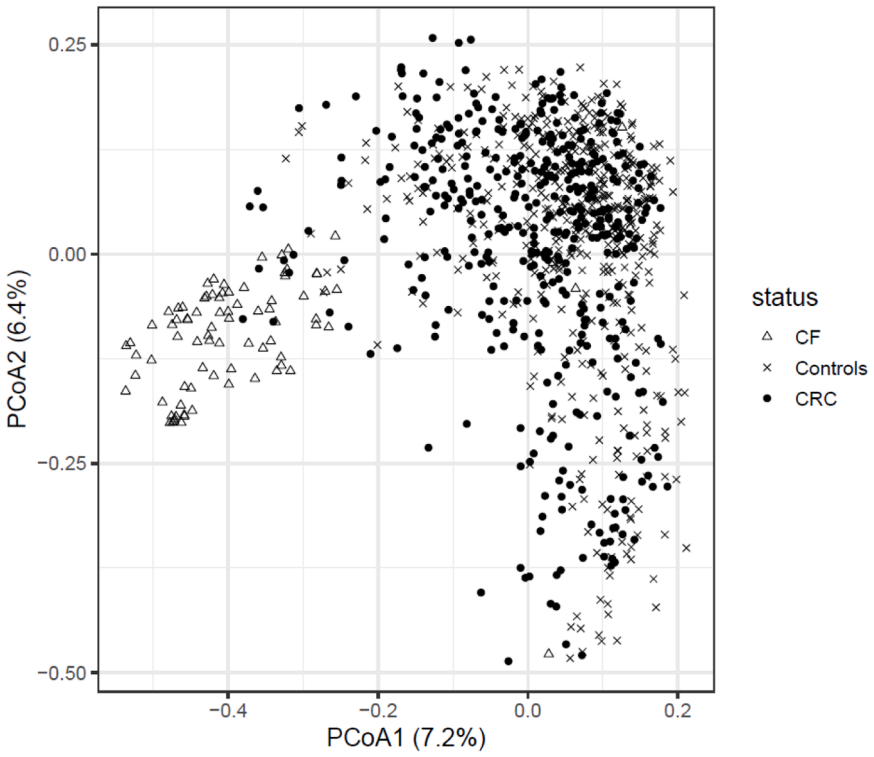

Fig. 1b. Bray–Curtis PCoA plot comparing CF, control and CRC samples.

double $n = 15$ or triple $n = 5$) compared to those not taking modulators ($n = 59$), Fig. S1. Visual inspection of Bray-Curtis PCoA plots indicated there was no clear pattern or distinct clustering according to CFTR modulator status at baseline.

Changes in faecal diversity between paired baseline and follow-up CF stool samples was conducted. When measured with Bray-Curtis distance, there were similar distributions both within and between participants, Fig. S2. Moreover, no demographic and clinical variable, including single, double or triple therapy, were associated with beta-diversity ($p > 0.05$ Adonis test). When specifically focusing on Shannon diversity changes pre- and post- triple therapy, changes were highly intra-individual, Fig. S3. Days of IV antibiotics between time points did not appear to explain this variation.

3.4. Faecal microbiota taxa in control, colorectal cancer and cystic fibrosis samples

There were a number of taxa differences between CRC, CF and control samples (Fig. 2). Control samples had a higher relative abundance of 26 genera compared to CRC and CF samples, which included *Faecalibacterium* and *Roseburia* (Fig. 2). There were 16 genera enriched in CRC samples, including *Escherichia,* and *Parvimonas,* compared to control and CF samples (Fig. 2). The CF samples had an increased relative abundance of 17 genera compared to control and CRC samples, which included *Blautia* and *Bifidobacterium* (Fig. 2.).

3.5. Comparison of colorectal cancer and cystic fibrosis faecal samples to CRC-associated microbiota meta-analysis

To contextualise comparisons, the 26 taxa previously identified in a meta-analysis as being associated with CRC [6] were compared with these data (Table 2). For the CF samples, 6 of the 20 enriched CRC-associated taxa identified in the meta-analysis, were enriched in CF samples compared to controls (*Peptostreptococcus, Blautia, Lachnoclostridium, Streptococcus, Hungatella* and *Veillonella*), and 2 (*Faecalibacterium* and *Anaerostipes*) of 6 depleted taxa were also depleted in CF samples compared to controls, Table 2.

3.6. Faecal microbiota differences between CF samples compared to CRC-associated meta-analysis taxa

Taxa differences between demographic and clinical characteristics in baseline CF stool samples were compared to the 26 meta-analysis CRC-associated taxa. None of the 26-CRC associated taxa were statistically significantly different between: sexes, CFRD, CFRLD status, age categories, BMI categories, PPI use, high or low IV antibiotic exposure in the last year. Those homozygous for F508del had an enrichment of *Gemella* compared to other *CFTR* mutations. Participants with chronic *P. aeruginosa* in their sputum had a lower relative abundance of *Streptococcus* compared to those who were *Pseudomonas* negative. An absence of BCC lung colonisation was associated with enrichment of *Flavonifactor* in the stool. Participants with moderate lung disease had a significantly higher relative abundance of *Faecalibacterium*, compared to those with mild or severe lung disease.

Those on CFTR modulators at baseline ($n = 25$) had a higher relative abundance of two of the 26 CRC-associated bacteria (*Streptococcus* and *Veillonella*) compared to those not ($n = 59$). Both *Bifidobacterium* and *Prevotella7* were enriched in those not on CFTR modulators ($n = 59$) compared to those taking double therapy ($n = 15$). Numbers of participants on single ($n = 5$) and triple ($n = 5$) therapy at baseline were too low to analyse. However, the 23 participants who commenced triple therapy between time points had at baseline (pre-triple therapy) an increased relative abundance of 4 (*Blautia, Veillonella, Streptococcus and Lachnoclostridium)* of the 20-CRC associated enriched taxa, compared to controls, and depletion of 3 (*Collinsella, Anaerostipes, Faecalibacterium*) of the 6 taxa associated with reduced abundance in CRC compared to controls (Table S1).

Changes in the prevalence of the 26 CRC-associated faecal taxa between paired baseline and follow-up CF samples were assessed. For the sixteen participants not on CFTR modulator therapy at either time point, the relative abundances of *Butyricimonas* and *Prevotella* were higher at baseline while *Streptococcus* increased at follow-up. In the 23

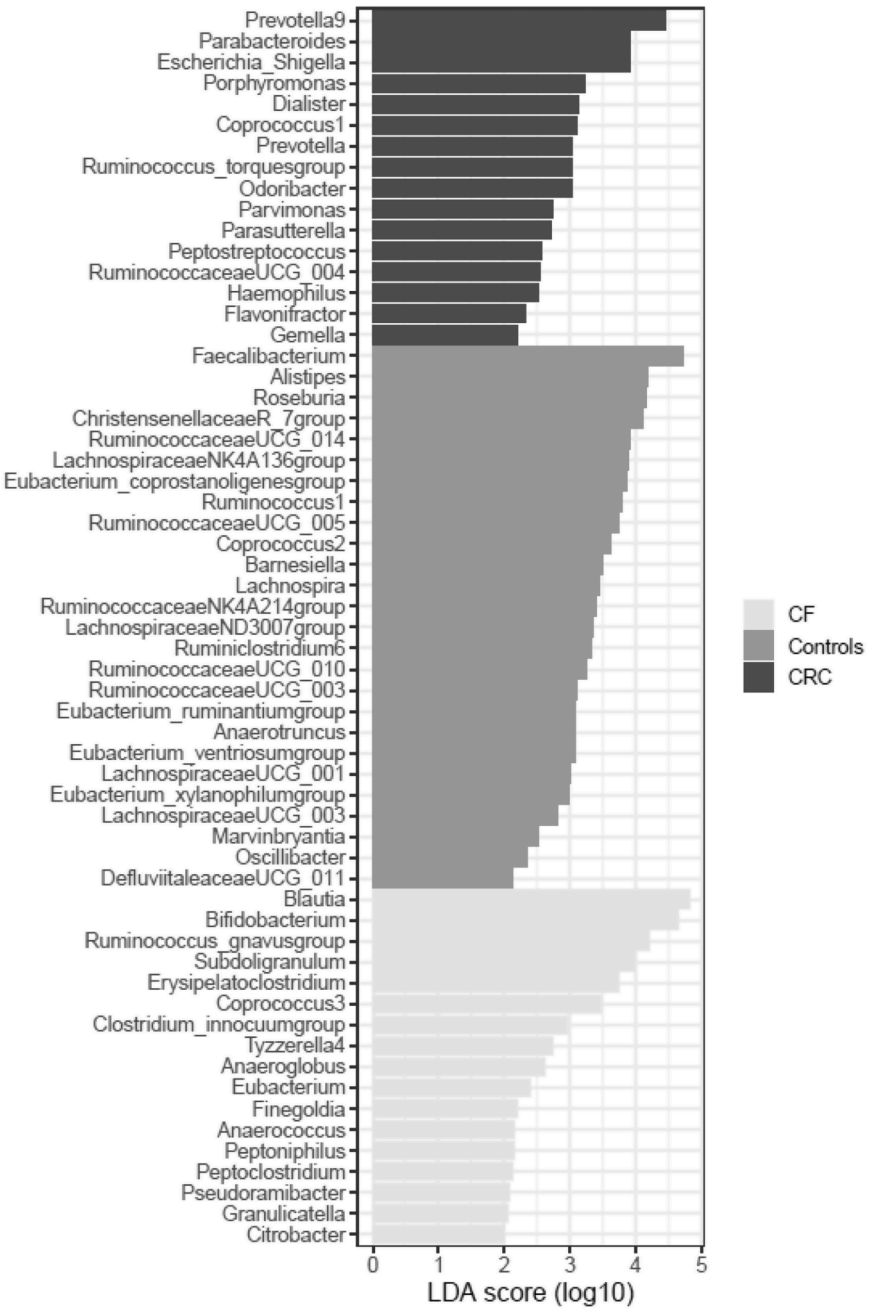

Fig. 2. Statistically significant enriched taxa between colorectal, cystic fibrosis and control faecal samples.

participants who commenced triple therapy between time points, none of the taxa changes at follow-up matched the 26 CRC-associated faecal microbiota meta-analysis taxa. However, when comparing these follow-up samples directly to controls, only 3 (*Blautia, Veillonella, Streptococcus*) of the 20-CRC associated enriched taxa had an increased relative abundance and 2 depleted (*Anaerostipes, Faecalibacterium*) of the 6 depleted taxa associated with CRC, differing from baseline, Table S1. Numbers of other CFTR modulator combinations were too small for analysis.

4. Discussion

The incidence of CRC in pwCF is likely to rise as lifespan increases, highlighting the need to better understand its aetiology and modifiable risk factors. In the non-CF population, gut dysbiosis has been associated with CRC but data remains limited in CF. Therefore, we compared the gut microbiota in pwCF to pwCRC and controls.

The CF faecal microbiota was found to be fundamentally distinct from both those with CRC and controls. There were, however, noteworthy taxa similarities between CF and the CRC-associated taxa. For example, we found an increased relative abundance of certain CRC-associated bacteria in CF samples compared to controls, namely *Peptostreptococcus, Blautia, Lachnoclostridium, Streptococcus, Hungatella* and *Veillonella* [6]. There was also a reduction in the relative abundance of beneficial bacteria, *Faecalibacterium* and *Anaerostipes,* in the CF samples compared to controls [6].

A recent study compared microbiota from colonic aspirate in individuals with and without colonic adenomas in pwCF (n = 21) and controls (n = 21) [21]. They reported a higher prevalence of colonic adenomas in pwCF compared to controls (67 % and 33 %), with CF samples having a lower bacterial diversity and clustering distinctly from control samples [21]. There was enrichment of *Bacteroides fragilis,*

Table 2
Comparison of cystic fibrosis and colorectal samples, relative to control samples, to 26 key CRC-associated taxa identified from meta-analysis [6].

Taxa	Meta-analysis on CRC-associated relative abundance*	Relative abundance in CRC samples compared to controls	Relative abundance in CF samples compared to controls
Parvimonas	Enriched	Enriched	NS
Fusobacterium	Enriched	Enriched	NS
Peptostreptococcus	Enriched	Enriched	Enriched
Escherichia	Enriched	Enriched (Escherichia / Shigella#)	Decreased (Escherichia / Shigella#)
Odoribacter	Enriched	Enriched	Decreased
Parabacteroides	Enriched	Enriched	Decreased
Blautia	Enriched	Enriched	Enriched
Lachnoclostridium	Enriched	NS	Enriched
Flavonifractor	Enriched	NS	NS
Gemella	Enriched	Enriched	NS
Prevotella	Enriched	Enriched	Decreased (OTUs *Prevotella9* and *Prevotella7*)
Streptococcus	Enriched	NS	Enriched
Oscillibacter	Enriched	Decreased	Decreased
Butyricimonas	Enriched	NS	Decreased
Eisenbergiella	Enriched	NS	NS
Solobacterium	Enriched	Enriched	NS
Hungatella	Enriched	Enriched	Enriched
Veillonella	Enriched	NS	Enriched
Enorma	Enriched	NS	NS
Anaerotruncus	Enriched	Decreased	Decreased
Intestinibacter	Depleted	NS	NS
Fusicatenibacter	Depleted	NS	NS
Collinsella	Depleted	NS	Enriched
Anaerostipes	Depleted	NS	Decreased
Bifidobacterium	Depleted	NS	Enriched
Faecalibacterium	Depleted	Decreased	Decreased

CF- cystic fibrosis, NS- no significant difference;.
* as presented in Young *et al.*, [6];.
# Escherichia/Shigella cannot be distinguished using these V4 16 s rRNA primers, in this study Shigella is considered to sit within the Escherichia genus, see methods.

potentially associated with CRC, in CF individuals with adenomas (n = 14) compared to those without (n = 7) [21]. It is difficult to directly compare this study to our own, due to differences in sample types and participant characteristics, including genotype, exocrine pancreatic function and organ transplant status [21]. Eight (38 %) of their CF participants had a solid organ transplant [21], which itself is associated with CRC risk [3].

A study by Dayama *et al.* [22] investigated the mucosal microbiota and host colonic mucosa oncogenic gene expression in 16 adults with CF, in the absence of CRC. They observed an enrichment of genes related to CRC, including tumour protein 53 [22]. The authors identified interactions between microbiota taxa and host genes, with *Clostridium* and *Pseudomonas* mostly negatively correlated with GI cancer genes and bacteria such as *Veillonnella* and *Fusobacterium* positively correlated [22].

The presence of certain gut bacteria in pwCRC is likely associative, with them thriving in the tumour-mediated environmental changes, while others may have a pathogenic role [23]. Certain bacteria exhibit pro-oncogenic characteristics by enhancing inflammation, epithelial damage, cell turnover and inducing gene mutations through the production of genotoxins [5]. In CF, the milieu of primary *CFTR* dysfunction, gut dysbiosis, a reduction in beneficial short chain fatty acid (SCFA) producing bacteria, diet and inflammation may further accentuate the CRC risk [4,24]. There is growing evidence that *CFTR* acts as a tumour suppressor gene [24].

The lack of a single obvious clinical feature driving stool microbiota diversity variations between CF samples may relate to the presence of PI, chronic antibiotic exposure and physiological perturbances due to defective *CFTR* [2,25]. There may also be unidentified factors, including the impact of a higher fat and lower fibre diet in pwCF, which in the non-CF population has been associated with gut dysbiosis and increased CRC risk [26].

Only modest gut microbiota changes have been reported with single and double CFTR modulator therapy, with studies on triple therapy ongoing [27]. In this study, we did not find a consistent or drastic impact of CFTR modulators on either bacterial diversity or CRC-associated taxa. However when comparing triple therapy treatment to control samples at baseline and follow-up separately, levels of *Lachnoclostridium* were no longer significantly different to controls at follow-up and there was evidence of enrichment of *Collinsella,* which is often depleted in CRC [6]. Given this was not the primary focus on the paper, along with the relatively small sample size and length of time on modulators, future studies will need to focus on the impact of longer-term treatment, especially in children, where early intervention may prove more impactful.

Study limitations include the technical variations in initial stool sample handling and storage; this includes NHSBCSP gFOBT cards collecting three stool samples per person and being stored dried at room temperature compared to the CF samples, where the stool sample underwent a freeze thaw cycle before being swabbed. In addition, the DNA extraction method, sequence runs and sequencer differed between the CF samples compared to the gFOBT samples. This may have resulted in the degradation of *Bifidobacterium* species and increase in *Escherichia*'s relative abundance following exposure to oxygen in controls compared to CF samples [28,29]. Reassuringly, Young *et al.* [6] has previously demonstrated that prolonged storage at room temperature only minimally affected results compared to 'same day' DNA extraction. CF participants were, on average, appreciably younger than bowel screening participants. While the gut microbiota is considered relatively stable by adulthood, it may still have influenced results and comparability between the cohorts [30]. It is difficult to determine the extent to which the taxonomic shifts observed in CF stool samples are pro-oncogenic or represent an overridingly dysbiotic, pro-inflammatory gut microbiota with fewer SCFA-producing bacteria. However, these mechanisms are likely promotional for CRC. This needs elucidating in future research. Faecal microbiota transplantation (FMT) has proved effective in a small number of cases for the treatment of *C. difficile* infection in pwCF [31]. The wider impact of this intervention on restoring microbial gut health has not yet been determined in pwCF. In a mouse model, FMT was shown to both reverse dysbiosis and inhibit CRC progression [32].

In summary, the CF stool microbiota is severely dysbiotic and demonstrates several bacteria shifts resembling those associated with CRC. Future studies need to investigate whether longer-term CFTR modulator therapy and dietary intervention could positively modify these changes or whether other interventions, such as FMT, are required to restore microbial health.

CRediT authorship contribution statement

L.R. Caley: Data curation, Formal analysis, Project administration, Investigation, Writing – original draft. **H.M. Wood:** Data curation, Formal analysis, Methodology, Validation, Visualization, Project administration, Writing – review & editing. **D. Bottomley:** Methodology, Formal analysis, Writing – review & editing. **A. Fuentes Balaguer:** Methodology, Formal analysis, Writing – review & editing. **L. Wilkinson:** Methodology, Formal analysis, Writing – review & editing. **J. Dyson:** Methodology, Formal analysis, Writing – review & editing. **C. Young:** Methodology, Writing – review & editing. **H. White:** Funding acquisition, Supervision, Writing – review & editing. **S Benton:** Investigation, Resources, Writing – review & editing. **M. Brearley:** Investigation, Resources, Writing – review & editing. **P. Quirke:** Conceptualization, Funding acquisition, Methodology, Resources, Writing – review & editing. **D.G. Peckham:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

D.G.P speaker/board honoraria from Vertex. H.W: Received previous funding from Gilead, which was not directly related to this research project and from Health Education England for simulated placement delivery funding. L.R.C, H.M.W, D.B, A.F.B, L.W., J.D., C.Y., S.B., M.B., P.Q and declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

This work was supported by the UK CF Trust (SRC 012) and the CRUK Grand Challenge 'Optimisticc' grant (GN 1114017).

Acknowledgements

We would like to thank all the study participants and the research nurses Lindsey Gillgrass, Anne Wood and Lynette Haigh. PQ, HW, CY and DB are supported in part by the National Institute for Health and Care Research (NIHR) Leeds Biomedical Research Centre. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health and Social Care. PQ is an NIHR senior investigator emeritus.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcf.2023.12.004.

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