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Original article

Association of circulating short chain fatty acid levels with colorectal adenomas and colorectal cancer

Flavia Genua [a], Bojana Mirković [b], Amy Mullee [c], Miroslav Levy [d], William M. Gallagher [a], Pavel Vodicka [e], David J. Hughes [a, *]

[a] Cancer Biology and Therapeutics Laboratory, UCD School of Biomolecular and Biomedical Science, UCD Conway Institute, University College Dublin, Dublin, Ireland
[b] Department of Medicine, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin, Ireland
[c] Department of Health and Nutrition, IT Sligo, Sligo, Ireland
[d] Department of Surgery, First Faculty of Medicine, Charles University and Thomayer Hospital, Prague, Czech Republic
[e] Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University, Prague, Czech Republic, Department of Molecular Biology of Cancer, Institute of Experimental Medicine of the Czech Academy of Sciences, Prague, Czech Republic

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SUMMARY

Background & aims: Short chain fatty acid (SCFAs) are bacterially derived metabolites suggested to have protective roles against colorectal cancer (CRC) development. However, there is sparse evidence from epidemiological studies in this context. Here, we assessed whether circulating SCFA concentrations varied in patients with colorectal adenomas (CRA) and CRC.

Methods: Levels of seven SCFAs were extracted from plasma samples and determined by gas chromatography for 213 individuals from Ireland and the Czech Republic (CRC, n = 84; CRA, n = 66; controls, n = 63).

Results: In the Irish CRA/CRC cohort, only levels of 2-MethylButyric acid were significantly higher in cancers compared to the adenoma and control groups (p-values = 0.016 and 0.043). Using regression analysis, we observed that levels of Acetic and Propionic acid were associated with an increased CRC risk in the Czech cohort (Odd Ratio (OR): 1.02; 95% Confidence interval (CI): 1.00–1.03; OR: 1.29; 95% CI: 1.05–1.59, respectively), while i-Valeric and Valeric acid levels were associated with a decreased cancer risk (OR: 0.92; 95% CI: 0.86–0.99; OR: 0.67; 95% CI: 0.44–1.00). In the Irish cohort, levels of SCFAs were not associated with CRC risk.

Conclusions: The association with colorectal neoplasia varied between the studied SCFAs. Future studies need to confirm these findings and address the mechanism of how these acids may promote or prevent colorectal carcinogenesis.

1. Introduction

Colorectal cancer (CRC) is the second leading cause of cancer related death and the third most commonly diagnosed in the world with 1.8 million new cases in 2018 [1]. As most CRCs arise from precursors lesions, the screening and early detection of colorectal adenomas (CRAs) with higher malignant potential represent a valid strategy to prevent the more advanced disease stages [2]. It is well established that dietary and lifestyle factors play a role in colorectal carcinogenesis and consequent to the adoption of a Western lifestyle, the global burden of CRC is expected to keep increasing over the next two decades [3]. Recently, accumulating evidence implicates dysbiosis of the human gut microbiota as an important aetiological risk factor in CRC development and prognosis [4,5].

In this context, diet has been recognized as a key element in impacting the functional relationship between gut microbiota and the host [6]. One of the beneficial functions of gut bacteria is the saccharolytic fermentation of indigestible fibre and resistant starch into short-chain fatty acids (SCFAs) [7], which are the main energy

* Corresponding author. Cancer Biology and Therapeutics Group, School of Biomolecular and Biomedical Science, UCD Conway Institute, University College Dublin, Dublin D04 V1W8, Ireland.
E-mail address: david.hughes@ucd.ie (D.J. Hughes).

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sources for colonocytes and thus vital to gastrointestinal health [8]. Among the SCFAs, Acetate, Propionate and Butyrate are the most abundant [9], while Butyrate is the favourable energy source for colonocytes [10]. SCFAs have been shown to modulate immune/inflammatory processes and inhibit the NF-κβ signalling pathway, affecting key mechanisms in tumour suppression such as cell cycle arrest and apoptosis [11]. Furthermore, SCFAs help to maintain gut barrier function, while enhanced Butyrate production may preserve this gastrointestinal epithelial lining through increasing expression of tight junction proteins [12]. Additionally, Butyrate and other SCFAs can bind and activate PPARγ (peroxisome proliferator-activated receptor gamma), a nuclear transcription factor that antagonizes NF-κβ signalling. A study conducted in human HT-29 colonic epithelial cells showed that Butyrate reduced inflammation by inhibition of NF-κβ activation and up-regulation of PPARγ expression [13].

Case-control studies have shown variance in the relative abundance of SCFAs in several human diseases, including gastrointestinal disorders [14], while a cross-sectional study reported a significant reduction of total SCFAs in stool samples of CRC patients compared with healthy controls [15]. However, little is known about the distribution of SCFAs in pre-cancerous lesions, including CRAs (particularly in those with high grade dysplasia, which are associated with a high malignancy potential).

According to epidemiology studies, an increased risk of CRC has been associated with a reduction of Acetate, Propionate and Butyrate [10], that represent the major source of energy for the colonocytes [16,17].

To address whether there was a pattern of SCFA levels associated with increasingly dysplastic neoplasia from adenoma to cancer, we evaluated circulating plasma concentrations of seven SCFAs in patients with CRA (n = 66) or CRC (n = 84), and 63 control individuals from two different study cohorts (Irish and Czech). Potentially, SCFA levels may serve as indicators of epithelial colonic tissue integrity or its progression from adenoma to cancer.

2. Materials and methods

2.1. Clinical characteristics

This study included plasma samples donated by 213 individuals from two European studies, from the Republic of Ireland and Czech Republic. The Irish cohort (n = 128) included patients with CRC (n = 26), high grade dysplasia (HGD, n = 18), or tubular and tubulovillous adenomas (TA/TVA, n = 48), diagnosed at the Departments of Gastroenterology and Surgery, The Adelaide & Meath Hospital, in Dublin, Ireland. Controls (n = 36) were individuals positive for the immunochemical Faecal Occult Blood Test (FIT), but where no colorectal neoplasia was detected upon consequent colonoscopy ('colonoscopy-negative' controls). The Czech cohort (n = 85) included CRC patients (n = 58) and blood donor controls (n = 27) from the Department of Surgery, Thomayer Hospital in Prague, Czech Republic. All CRCs were classified according to the tenth revision of the International Classification of Diseases (ICD-10). The clinical data, including age at diagnosis, sex, pTNM (Tumour stage, Regional lymph node involvement, and distant metastasis) staging, histological grade of the tumour, and primary tumour localization were taken from patient medical records (see Table 1 for the summary of the clinical characteristics of our study cohorts).

All patients gave informed consent in accord with the 1964 Helsinki Declaration and all patient samples were coded to protect participant identity. The study was approved by the Ethical committee of the St. James's Hospital and Federated Dublin Voluntary Hospitals Joint Research Ethics Committee (Ireland, reference 2007-37-17), and the Ethical Committee of the Thomayer Hospital in Prague (Czech Republic, reference NT13424-4/2012).

2.2. Sample collection and SCFA measurements

The blood samples were collected within one day of surgery or colonoscopy in 6 mL VACUTAINER® tubes (Cruinn Diagnostics, Dublin, Ireland) with EDTA. Within 4 h of collection, bloods were centrifuged at 2000×*g* for 10 min to separate the top plasma layer, which was then stored at −80 °C in cryovials. Levels of 7 SCFAs (Acetic, Propionic, i-Butyric, Butyric, 2-Methylbutyric, i-Valeric, Valeric) from all plasma samples were determined using the hollow fibre (16 cm fibre; Membrana GmbH, Wuppertal, Germany) supported liquid membrane extraction coupled with gas chromatography (GC) as described previously [18,19]. Identification of SCFAs in the analysed samples was done by comparison of retention times of chromatogram peaks in the sample with the retention times of peaks obtained by running the SCFA standards in a standard mixture on the GC apparatus. The concentration of SCFAs in the analysed sample was calculated using 2-Ethylbutyric acid as the internal standard to correct for variation in sample and injection volume, and a standard mixture of SCFAs and 2-Ethylbutyric acid. Linearity range and enrichment factor of the method for all the SCFAs was previously described in [19]. Human plasma was diluted 1:15.

2.3. Statistical analysis

A comparison between the baseline levels of the seven SCFAs was conducted in the control groups of the two cohorts applying the Mann–Whitney U test. In the Irish cohort, differences in SCFA levels among four groups (CRC, HGD, TA/TVA, and colonoscopy-negative controls) were analysed using non-parametric tests (Kruskal–Wallis, Mann–Whitney U test) for all SCFAs, except for Butyric acid levels as these were normally distributed (ANOVA t-test). In comparisons of SCFA levels between CRC patients and control subjects in the Czech cohort, the ANOVA t-test was used to assess i-Butyric, i-Valeric and Valeric acids (data normally distributed), while the Mann–Whitney U test was applied for Butyric, 2-Methylbutyric, Propionic and Acetic acids. Normality was assessed applying the Shapiro–Wilk test. A binary multivariate logistic regression analysis with adjustment for sex and age was also performed to estimate the odds ratios (OR) and 95% confidence intervals (95% CI) for modelling an association between SCFAs and CRC development risk in the Czech cohort. In a second regression model conducted in a smaller cohort of samples (53 cancers and 26 controls), due to missing data for 5 cases and 1 control subject, we further adjusted the analysis for covariates known to be associated with CRC risk including sex, age, smoking and alcohol consumption. Unfortunately, there was insufficient dietary questionnaire data available for all subjects (30/58 cancers and 25/27 controls) to include in the regression analysis, and this information was not sufficiently detailed to calculate grams/day of intake of major food groups such as meat or vegetables.

Due to the small number of CRC cases analysed in the Irish cohort, we further explored the association between SCFA levels and colorectal neoplasia risk by grouping the neoplasia cases (CRC, CRA and HGD). Analyses were conducted using IBM SPSS Statistics v.24 (SPSS inc., Chicago, IL, USA) and R (RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/). To be cautious, we have also presented q-values for the multiple-testing correction using a False Discovery Rate (FDR)

Table 1
Clinical characteristics of the studied cohort of patients.

Cohort	Irish (n = 128)				Czech (n = 85)	
Diagnosis	Control	TA/TVA	HGD	CRC	CRC	Control
Total, n of patients	36	48	18	26	58	27
Sex n (male/female)	17/19	30/18	11/7	13/13	40/18	12/15
Age at diagnosis, median ± IQR (years)	58 ± 7	61.5 ± 11	59 ± 7	65.5 ± 23	64 ± 15	56 ± 10
Location n (colon/rectum) (missing)	–	34/13 (1)	10/8	22/4	21/18	–
T staging n (T0/T1/T2/T3/T4/T5) (missing)	n.a	n.a	n.a	1/3/3/8/5/0/1 (8)	0/16/15/19/7/1 (0)	n.a
N staging n (N0/N1/N2/N3) (missing)	n.a	n.a	n.a	15/2/3/1 (5)	37/15/4/2	n.a
M staging n (M0/M1/M2/M3) (missing)	n.a	n.a	n.a	6/3/12/0 (5)	49/8/0/1	n.a
Cancer histology type (adenocarcinoma/mucinous adenocarcinoma) (missing)	n.a	n.a	n.a	19/0 (2)	50/8 (0)	n.a
Smoking (yes/no) (missing)	n.a	n.a	n.a	n.a	33/20 (5)	8/18 (1)
Alcohol consumption (yes/no) (missing)	n.a	n.a	n.a	n.a	36/17 (5)	23/3 (1)

IQR, interquartile range; CRC, colorectal cancer; TA/TVA, tubular and tubulovillous adenoma; HGD, High Grade Dysplasia; TNM staging, Tumour stage, regional lymph node involvement, and distant metastasis; -, missing; n.a, not applicable. Controls were individuals positive for the immunochemical Faecal Occult Blood Test (FIT) but negative after colonoscopy (Irish cohort) or blood donor patients (Czech cohort).

Online Calculator © 2016 (Carbocation Corporation, Boston MA, USA) based on the Benjamini-Hochberg method. P- and q-values ≤ 0.05 were considered statistically significant.

3. Results

3.1. Differences in SCFA levels among pathology groups

In the two studied cohorts, baseline levels of SCFAs were similar except for i-Butyric acid, which was significantly higher in the Irish cohort compared to the Czech (p-value = 0.007) (Supplementary Fig. 1).

In the Irish cohort, only levels of 2-Methylbutyric acid were significantly different across the pathology groups (p-value = 0.05). However, the FDR-adjusted q-value (= 0.35) did not retain significance (Table 2). All the boxplots with the respective p-values and q-values are shown in Supplementary Fig. 2. Considering that the p-values are derived from a clear hypothesis-driven approach with a small number of comparisons of related SCFA metabolites, we base our interpretation on the observed p-values and present the q-values as a conservative balance. Furthermore, the comparison of 2-MethylButyric acid concentration between two independent groups was significantly higher in cancers compared to other pathology groups (CRC vs. HGD p = 0.019; CRC vs. TA/TVA p = 0.016, CRC vs. control p = 0.043) (Fig. 1). In the Czech study, levels of Acetic and Propionic acids were significantly higher in cancer compared to control (p-values = <0.001, 0.006, respectively), while Butyric, Valeric and i-Valeric acids levels were lower in cancers than in controls (p-values = 0.05, 0.013, and 0.012, respectively: Fig. 2). Multiple testing adjustment retained the statistically significant estimations for the varying levels of Acetic, Propionic, Valeric and i-Valeric acids (q-values = 0.007, 0.02, 0.02, 0.02, respectively: Fig. 2) but not for Butyric acid (q-value = 0.07: Fig. 2).

3.2. SCFA levels and association with CRC

In the Czech cohort, concentrations of Acetic and Propionic acid were associated with an increased CRC risk (OR: 1.02; 95% CI: 1.00–1.03; OR: 1.29; 95% CI: 1.05–1.59, respectively), while i-Valeric and Valeric levels were associated with a decreased CRC risk (OR: 0.92; 95% CI: 0.86–0.99; OR: 0.67; 95% CI: 0.44–1.00) (Table 3). Levels of Butyric, i-Butyric and 2-MethylButyric acids were not significantly associated with CRC risk. After FDR correction, the associations remained significant for Acetic and Propionic acids (q-values = 0.007, 0.03, respectively) but not for i-Valeric and Valeric acids (q-values = 0.09, 0.08, respectively) (Table 3). In the fully adjusted model (for sex, age, smoking and alcohol consumption), levels of Acetic, Propionic and i-Butyric acids were also associated with an increased CRC risk (OR: 1.02; 95% CI: 1.00–1.04; OR: 1.31; 95% CI: 1.03–1.65; OR: 1.17; 95% CI: 1.00–1.38, respectively) (Table 3). However, while the OR point estimate did not differ by more than 10% from the first regression model (adjusted by age and

Table 2
Circulating Short Chain Fatty Acid levels (μM) in colorectal cancer, tubular/tubulovillous adenoma, high grade dysplasia and control in the Irish cohort.

Short Chain Fatty Acid (μM)	Ireland									q-value
	Control		TA/TVA		HGD		CRC		p-value	
	Median	IQR	Median	IQR	Median	IQR	Median	IQR		
Acetic Acid	164	201–117	158	206–113	146	191–98	198	387–147	0.21	0.70
Propionic Acid	14	37–7.6	13	27–8.9	12	30–7.8	11	16–8.3	0.89	0.90
i-Butyric Acid	8.8	11–6.4	7.2	9.1–4.4	7.0	9.2–4.9	9.5	11.-6.5	0.25	0.58
Butyric Acid[a]	9.8	2.9	9.4	2.6	10.2	2.29	10.18	3.24	0.41	0.71
2-MethylButyric Acid	13[a]	16–9.7	11[b]	17–5.8	10[c]	13–8.7	14[d]	18–7	**0.05**	0.35
i-Valeric Acid	18	24–14	19	26–11	16	23–7	17	24–10	0.56	0.78
Valeric Acid	4.1	6.9–2.6	4.7	6–3	5.1	5.7–3.2	3.9	6.3–2.5	0.72	0.84

Kruskal Wallis test.[a,b,c,d] Mann–Whitney test: different subscripts indicate statistical differences between groups. Statistically significant P-values are indicated in bold.
[a] Values were normally distributed (mean, standard deviation); ANOVA t-test. CRC, colorectal cancer; TA/TVA, tubular and tubulovillous adenoma; HGD, High Grade Dysplasia; IQR, interquartile range. Controls were individuals positive for the immunochemical Faecal Occult Blood Test (FIT) but negative after colonoscopy (Irish cohort). P-value was significant at ≤ 0.05. Colorectal cancer n = 26; high grade dysplasia n = 18; tubular/tubulovillous adenoma n = 48; controls n = 36.

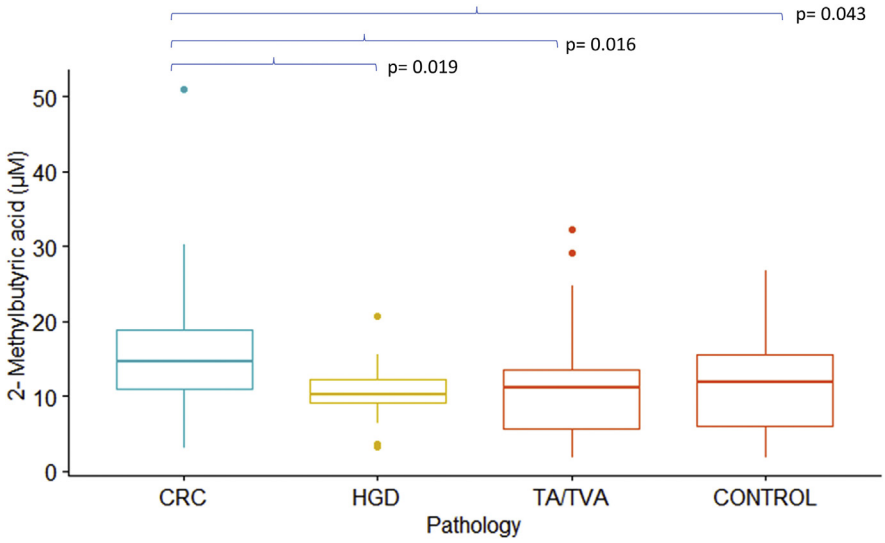

Fig. 1. Plasma 2-MethylButyric Acids levels (µM) from the Irish study across pathology groups. Mann Whitney test. P-value was significant at ≤ 0.05. The boxplots show the median and the interquartile range of 2-MethylButyric acid in plasma across the pathology group in the Irish cohort. CRC, colorectal cancer; TA/TVA, tubular and tubulovillous adenoma; HGD, High Grade Dysplasia. Controls were individuals positive for the immunochemical Faecal Occult Blood Test (FIT) but negative after colonoscopy. Colorectal cancer n = 26; high grade dysplasia n = 18; tubular/tubulovillous adenoma n = 48; controls n = 36.

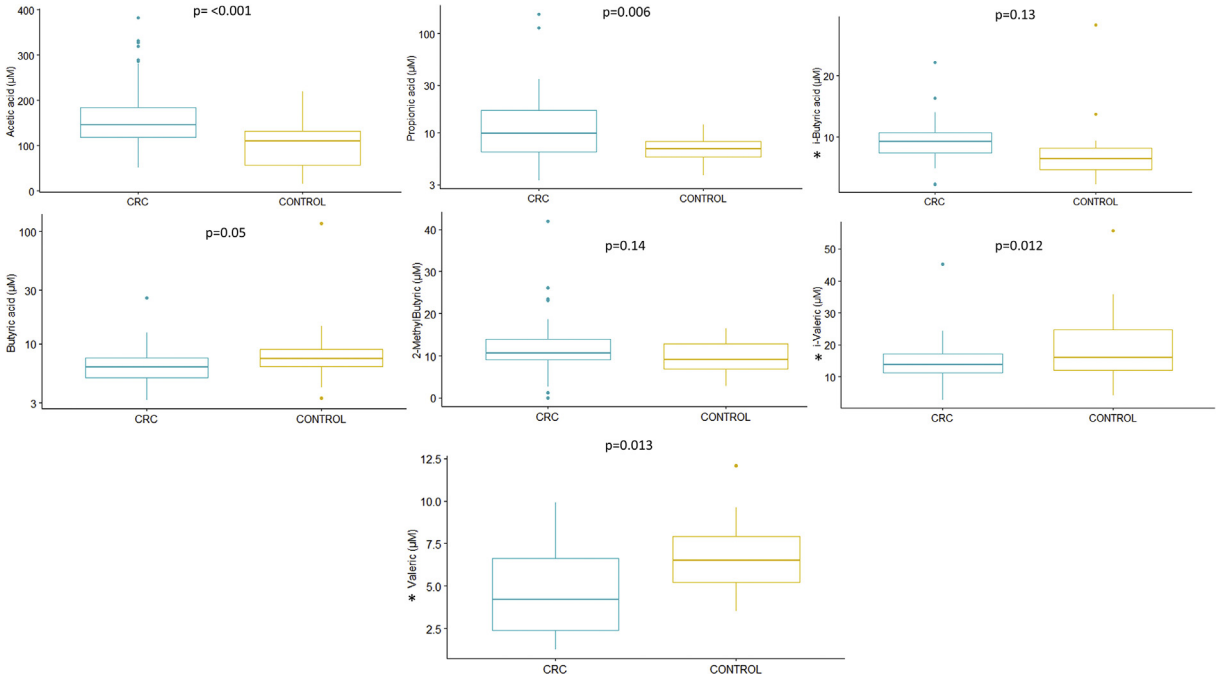

Fig. 2. Circulating Short Chain Fatty Acid levels (µM) in colorectal cancers and controls in the Czech study. Mann–Whitney U test. *Vvalues normally distributed, ANOVA t-test. The box plots show the median and interquartile range of plasma SCFAs. Levels of i-Valeric, Butyric and Valeric acids were significantly higher in controls than in cancers, while Propionic and Acetic Acids were higher in cancer than controls. Controls were represented by blood donor volunteers. P-value was significant at ≤ 0.05. FDR-adjusted q-values: Acetic acid = 0.007, Propionic acid = 0.02, i-Butyric acid = 0.15, Butyric acid = 0.07, 2-MethylButyric acid = 0.14, i-Valeric acid = 0.02, Valeric acid = 0.02. Colorectal cancer cases n = 58; controls n = 27.

sex) for all the acids, levels of Valeric and i-Valeric were not associated with CRC risk with the additional adjustment for smoking and alcohol consumption (p-values = 0.10, 0.08, respectively). Upon multiple testing adjustment, Acetic acid retained association with CRC risk (q-value = 0.01) (Table 3).

In the Irish cohort, levels of SCFAs were not associated with CRC risk (Supplementary Table 1). Similarly, there was no significative association with colorectal neoplasia risk when all the cases (CRC, CRA, HGD) were grouped (Supplementary Table 1).

4. Discussion

The present study was designed to examine the association of circulating plasma concentrations of the major SCFAs in patients

Table 3
Association of Short Chain Fatty Acid levels with colorectal cancer risk among the Czech cohort.

Short Chain Fatty Acids (μM)	Czech Republic					
	Model 1[a]			Model 2[b]		
	OR (95% CI)	p-value	q-value	OR (95% CI)	p-value	q-value
Acetic Acid	1.02 (1.00–1.03)	**0.001**	**0.007**	1.02 (1.00–1.04)	**0.002**	**0.01**
Propionic Acid	1.29 (1.05–1.59)	**0.01**	**0.03**	1.31 (1.03–1.65)	**0.02**	0.07
i-Butyric Acid	1.11 (0.95–1.30)	0.17	0.23	1.17 (1.00–1.38)	**0.05**	0.11
Butyric Acid	0.91 (0.77–1.08)	0.31	0.31	0.95 (0.82–1.09)	0.50	0.50
2-MethylButyric Acid	1.07 (0.94–1.22)	0.26	0.30	1.12 (0.95–1.32)	0.17	0.19
i-Valeric Acid	0.92 (0.86–0.99)	**0.04**	0.09	0.92 (0.84–1.01)	0.08	0.14
Valeric Acid	0.67 (0.44–1.00)	**0.05**	0.08	0.68 (0.42–1.07)	0.10	0.13

Colorectal cancer cases n = 53; controls n = 26. OR: Odds ratio; CI: Confidence interval. P-value was considered significant at level ≤0.05 (indicated in bold type).
[a] Multivariate logistic regression with adjustment for age and sex. Colorectal cancer cases n = 58; controls n = 27.
[b] Multivariate logistic regression with adjustments for sex, age and lifestyle factors (smoking and alcohol consumption).

with advancing neoplasia from adenomas to CRC. Overall, we observed that SCFA levels tend to significantly vary in the comparison between cancer and control groups. However, except for 2-Methylbutyric acid level in the Irish cohort, no statistically significant difference was observed in the concentration of other SCFAs between control and pre-cancerous lesion (HGD, TV/TVA) groups. As observed in the Czech cohort, levels of Butyric, Valeric and i-Valeric acid were significantly lower in the CRC patients than in the controls. This concurs with the hypothesis of a protective effect of higher SCFA levels against colorectal carcinogenesis. The assessment of SCFAs in faecal samples of CRC (n = 14) and colitis and haemorrhoids patients (n = 14) patients, showed lower Acetic and Propionic acid levels in the cancers compared to the other pathology groups [20]. Findings from other gastrointestinal disease studies reported reductions in different SCFAs in patients with intestinal pathologies. A case-control study showed that a large reduction in Butyric and Propionic acid concentrations was present in faecal samples from inflammatory bowel disease (IBD) patients (n = 8) compared to controls (n = 50) [14]. Similar results were found by Takahisci et al. [21] in a study conducted in faecal samples, where concentrations of Butyric acid and Propionic acid were significantly decreased in patients with IBD (n = 51), compared to healthy controls (n = 10).

In our study, levels of Propionic and Acetic acids measured in the Czech cohort were higher in the CRC group than the controls, while in the Irish cohort, levels of 2-Methylbutyric acid increased from control to progressive neoplastic stages (as assessed by the control, TA/TVA, HGD, and CRC groups).

In the modelling of CRC development risk in the Czech cohort, we observed that higher levels of Valeric and i-Valeric acid were associated with a decreased risk of CRC. These findings support the hypothesis that certain SCFAs may be protective against CRC [11]. However, higher concentrations of Acetic and Propionic acid were conversely associated with an increased CRC risk. While some of these findings did not retain significance after FDR multiple-testing adjustment, we contend that such corrections are over-stringent, as all our analyses were planned a priori, based on our stated hypothesis, and with a modest number of related SCFA metabolites. Thus, these data are sometimes discordant with the hypothesis of a protective role of SCFAs against CRC development,as they suggest that higher levels of some SCFAs may have cancer promotive effects.

Increasing evidence suggests a more dynamic link between diet, microbiome composition and SCFA production in healthy versus disease states [16]. Thus, the role of certain SCFAs in either protection from or promotion of colorectal carcinogenesis remains controversial, as demonstrated by several studies in the literature. A cross-sectional study conducted in faecal samples of patients with intestinal disorders including celiac disease (n = 16), adenomatous polyposis (n = 9) and CRC (n = 19), showed that the control group (n = 16) had higher levels of Acetic acid and lower concentrations of Butyric, i-Butyric, Valeric and i-Valeric acids compared to CRC patients [15]. Furthermore, in 2013, Weir et al. found that the relative proportion of Acetic, i-Butyric, Valeric and i-Valeric acids were significantly higher in stool samples from CRC patients (n = 10), while Butyric acid was significantly higher in stool samples of healthy individuals (n = 11) [22]. In the same study, the pyrosequencing analysis of the V4 region of 16S rRNA gene confirmed that different bacterial genera were under-represented in the CRC samples, especially bacteria-producing Butyrate [22]. The role of Butyrate in CRC disease and progression is still to be clarified. It has been demonstrated that Butyrate can inhibit the growth of tumour cells via inhibition of histone deacetylases (HDAC), which alters the expression of target genes involved in proliferation, apoptosis and cell differentiation [23]. However, the same effect is not detectable in normal colonic epithelium cells or when non-cancerous colonic cell lines are incubated in vitro with this acid [24]. This divergence is described as the “Butyrate paradox”. While Butyrate represents one of the main energy sources for normal colonocytes, this acid is not properly metabolized in colon cancer cells due to the Warburg effect [25]. As demonstrated by the Warburg effect, cancerous cells are metabolically more active than non-malignant cells and aerobic glycolysis is favoured over oxidative metabolism. Consequently, Butyrate is accumulated into the nucleus and glucose represents the energy source, leading to important implications on CRC progression through proliferation and growth of cancer cells [26]. Nevertheless, a role for Acetate in progressing tumour growth is supported by PET imaging studies using [^{11}C] acetate where different studies have documented high acetate uptake in several solid tumour types, including hepatocellular carcinoma and lung cancer [27–29]. The nucleocytosolic acetyl-CoA synthetase enzyme, ACSS2, has been implicated as a key source of acetyl-CoA for tumours to metabolise acetate, supporting the hypothesis that cancer cells, unlike normal cells, can use this hydrocarbon fuel to grow and survive [30].

The relative changes in SCFA production depending on dysbiosis and on potential biosample (e.g., blood vs faeces) measurement issues - due to the possible increased translocation of SCFAs from the gut to the bloodstream caused by the higher epithelial permeability (“leaky gut”) during neoplastic transformation - might explain these results. In future, the assessment of faecal and circulating SCFAs in the same cohort of patients could help to understand whether any differences may be affected by the leakage of SCFAs into circulation across a compromised gut barrier. As some of the SCFA plasma levels were found to be lower in CRC patients, it seems that circulating levels do not simply reflect an increased

transition of these SCFAs from the gut to the bloodstream due to the higher epithelial permeability ("leaky gut") in CRC patients. However, as it is known that a compromised epithelial barrier facilitates bacterial translocation in CRC [31,32], the amount and pattern of circulating SCFAs might be affected by the over-abundance or the reduction of bacteria involved in SCFA production. Future studies need to address whether these acids are differentially contributing to – or protecting from - colorectal carcinogenesis or are simply a reflection of microbial dysbiosis and/or differential transition rates into the bloodstream across a compromised gut epithelial lining in patients with CRC.

The relationship between dietary fibres and protection from CRC may be partly modulated by SCFA production [33,34]. It is well-established that dietary changes impact gut microbial composition and the switching from a high-fibre diet to a low-fibre diet may cause significant changes in the gut microbiota, thereby potentially increasing CRC risk [35]. Alterations in the microbiota could impact levels of bacterial metabolites including SCFAs, polyphenols, vitamins, tryptophan catabolites and polyamine [36]. Insoluble fibres might protect colonocytes by minimizing the exposure to ingested carcinogens compounds while SCFAs, especially Butyrate, provide energy to colonocytes and act as a tumour suppressor [37]. In 1991, Clausen et al. showed that the ratio of Butyrate to other SCFAs was reduced in patients with CRA ($n = 17$) than in controls ($n = 16$) [38], while a study conducted in stool samples of patients with familial adenomatous polyposis ($n = 20$) and healthy controls ($n = 11$) suggested that a decreased Butyrate production by colonic carbohydrate fermentation might promote CRC development in these patients [39]. A study addressing how dietary factors influence CRC risk, compared the colonic content in faecal samples of 12 Africans Americans and 10 Caucasian Americans, who consumed a high-fat diet, to 13 Native Africans, with a low-fat diet. As a result, Acetate, Propionate and Butyrate were lower in Africans and Caucasian Americans than in Native Africans, suggesting that the higher risk of colon cancer in Americans may be partly explained by a high-fat diet that promotes production by microbes of secondary bile acids - potentially carcinogenic - and less protective SCFAs [40]. Worldwide westernization of lifestyle and dietary patterns is associated with increasing CRC incidence [8,41]. In this context and related to the production of SCFAs, a large observational study from Europe showed that dietary fibre intake was inversely associated with CRC development risk [42,43]. Similarly, another prospective study conducted in a Scandinavian cohort reported that the intake of cereals was associated with a reduction in the incidence of CRC [44]. These data support the hypothesis that dietary fibre is important for CRC prevention, and that this may be partly mediated by SCFA production. A limitation of our study is the absence of sufficient dietary data in both the cohorts (none in the Irish and substantial missing and/or rudimentary data in the Czechs). However, Irish and Czech populations present similar dietary and lifestyle patterns, rich in processed and red meat, lacking in fruit and vegetable intake, and high smoking and alcohol consumption rates [45,46]. Furthermore, the incidence of excess weight and obesity is constantly growing in both countries [47,48]. Among European countries, Ireland and the Czech Republic show similar incidence and mortality rates for CRC, that are higher than the EU average [1,49]. All these considerations support the hypothesis that the microbiome composition and its contribution to SCFA production might be similar in both countries. In this context, an appreciable number of the samples tested in this study for the Irish cohort ($n = 50$), but only a small number for the Czech subjects ($n = 9$), were also included, along with a German cohort, in a study examining colorectal tumour tissue levels of *Fusobacterium nucleatum (Fn)*, a butyrate-producing microbe implicated in colorectal carcinogenesis [50]. Relative quantification of *Fn* DNA levels were similarly over-abundant in cancerous compared to matched mucosal tissue in all three cohorts examined, and levels did not significantly differ in the tumour only or matched tissue only comparisons between the different countries, suggesting similar bacterial levels (and consequently SCFA production) in healthy or disease tissue. A future prospective study exploring the microbial composition and SCFA levels in the same subjects (and at serial sampling times before cancer diagnosis) would be desirable to ascertain the relationship between the microbiome and SCFA production relevant to colorectal carcinogenesis.

A metagenome-wide association functional study (MWAS) of the microbiota associated in CRC and CRA patients compared to controls may better identify the metabolically active bacteria contributing to an alteration in SCFA levels [51]. Additionally, the optimisation of SCFA levels in the gut by increased dietary fibre intake and microbiome manipulation, needs further study as a potential prevention or treatment strategy for CRC [52,53]. As it has been shown that SCFAs in the colon are reduced compare to controls, the increase of SCFA intake through diet and probiotics might be protective against CRC [54].

A limitation of our study is the absence of microbiome metagenomic sequencing due to a lack of available matching tumour DNA or stool samples from the same patients. Analysis of the microbial content in the gut in correlation with SCFA levels might provide insight into the relationship of these bacterial metabolites with colorectal carcinogenesis.

To summarize, SCFAs may have both pro- and anti-carcinogenic effects depending on the stage of the development of tumours from a healthy colorectal tissue through advancing adenoma stages, or simply result from the increasingly dysplastic disease process. One of the strengths of this study is the assessment in plasma of SCFAs in patients with pre-cancerous lesions, as there are no similar studies in the literature. In future, the evaluation of these metabolites in a wider cohort of CRA patients will be essential to explore the role of SCFAs in different stages of colorectal neoplasia development. Furthermore, there is also a need for a large, prospective study to ascertain if levels of SCFAs are associated with CRA or CRC development before disease diagnosis. As SCFA levels may change depending on the degree of intestinal disease and circulating concentrations may be affected by the "leaky gut" in CRC patients, the use of these metabolites as screening biomarkers in plasma for CRC early detection in the clinical practice is not recommended. In future, the comparison between faecal and blood SCFAs taken from the same patients might help to better understand the role of these bacterial metabolites in CRC development and progression. Furthermore, the examination of the interactions of these metabolites with diet, individual lifestyle and microbiome data from patients might elucidate the role of SCFAs in CRC and intestinal diseases.

Author contribution

Conceptualization: DJH, FG. Samples collection: DJH, PV, ML. Experiments: BM, RD. Statistical analysis: FG, AM. Funding acquisition: DJH, PV. Writing—original draft: FG, DJH, AM. Writing—review and editing: RD, PV, ML, WMG.

Declaration of competing interest

WMG is a co-founder and Chief Scientific Officer in OncoMark Limited.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnesp.2021.09.740.

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