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Analysis of fecal short chain fatty acid concentration in miniature dachshunds with inflammatory colorectal polyps

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ABSTRACT. Short chain fatty acids (SCFAs) play an important role in the maintenance of colonic homeostasis, and their depletion has been reported in various gastrointestinal disorders. Inflammatory colorectal polyps (ICRPs) are a recently recognized disease specific to miniature dachshunds (MDs), and fecal dysbiosis with a reduction of SCFA-producing bacteria has been reported with this disease. Therefore, this study was performed based on the hypothesis that a reduced SCFA concentration associates with the development of ICRPs. We recruited 11 ICRP-affected MDs and 25 control MDs. Their fecal SCFA concentrations and bacterial proportions were quantified using high performance liquid chromatography and quantitative real-time PCR, respectively. The feces of ICRP-affected MDs contained lower amounts of propionic acid and lower proportions of *Bifidobacterium* than the feces of control MDs. Furthermore, fecal proportions of *Bifidobacterium*, Firmicutes and *Lactobacillus* exhibited significant positive correlations with fecal concentrations of total SCFAs and/or propionic acid; fecal *Escherichia coli* proportions correlated negatively with fecal concentrations of total SCFAs, as well as acetic, propionic and butyric acid. This result indicates an association between fecal dysbiosis and fecal SCFA concentrations; these phenomena may contribute to ICRP pathogenesis in MDs. Potential therapeutic targeting of the reduced propionic acid concentration using probiotics, prebiotics or SCFA enemas merits further study.

KEY WORDS: fermentative end product, high performance liquid chromatography, inflammatory colorectal polyp, microflora, miniature dachshund

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Colorectal polyps frequently occur in dogs; most of these polyps are neoplastic [32, 41]. By contrast, miniature dachshunds (MDs) in Japan commonly present with inflammatory colorectal polyps (ICRPs) [26]. ICRPs in MDs usually develop multiple polyps with severe inflammatory infiltration (predominantly with neutrophils and macrophages) [40], and respond well to immunosuppressive therapies such as prednisolone, cyclosporine and leflunomide [4, 26]. Therefore, ICRPs in MDs are speculated to be a novel breed-specific form of canine inflammatory bowel disease (IBD) [27].

The gut microbiota plays a crucial role in the maintenance of gastrointestinal health, in both humans and dogs [11, 12]. These microbes contribute to the mucosal barrier that defends against pathogen invasion, induces mucosal immune responses, supports digestion and provides nutritional support to enterocytes in the form of short chain fatty acids (SCFAs) [34]. SCFAs, predominantly comprising acetate, propionate and butyrate, are essential to colonic homeostasis, and are produced via fermentation of carbohydrates, peptides and glycoprotein precursors [2, 7]. Butyrate is the most effective SCFA in inhibiting colonic inflammation, carcinogenesis and oxidative stress; improving colonic defense barrier function; promoting satiety; and serving as a

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main energy substrate for colonocytes [8]. The anti-inflammatory mechanism of SCFAs has been described as the suppression of nuclear factor-kappa B reporter activity, inflammation-related gene expression and cytokine release [8]. Furthermore, a recent study revealed that SCFAs, particularly butyrate, induce the differentiation of colonic regulatory T (Treg) cells via upregulation of histone acetylation in the promoter and enhancer regions of the *Foxp3* gene [5]. Several studies have reported SCFA depletion in human IBD [13, 36, 42].

We recently reported a reduction in the fecal proportions of the SCFA-producing bacterial taxa, such as Lachnospiraceae, in ICRP-affected MDs [16]. Therefore, we hypothesized that a reduced SCFA concentration associates with the development of ICRPs. However, an alteration in the microbiota composition may not directly correlate with a functional change to the microbiota itself [39]. Hence, the objective of the present study was to compare the fecal microbiota and SCFA concentrations of ICRP-affected MDs with those of control MDs.

MATERIALS AND METHODS

Animals and sample collection

This study included MDs referred to the Veterinary Medical Center of the University of Tokyo for investigation of chronic hematochezia and/or tenesmus, and diagnosed with ICRPs, between October 2012 and October 2014. The diagnosis of ICRP was confirmed by colonoscopic and histopathological exams, as described previously [26, 40]. We excluded dogs that had received antibiotics within four weeks prior to sampling. As controls, we recruited healthy MDs undergoing routine exams. These subjects presented no clinical signs of gastrointestinal disease or abnormalities, as determined by fecal examination and rectal palpation. No control MD had been treated with antibiotics or abdominal surgery within one year preceding this study. Detailed descriptions of ICRP-affected MDs and control MDs are listed in Supplementary Tables 1 and 2, respectively. Naturally passed feces were collected from each dog into sterile plastic tubes, and frozen at −80°C within an hour of defecation, until further analysis.

All procedures were conducted according to the Guidelines of the Animal Care Committee of the Graduate School of Agricultural and Life Sciences, the University of Tokyo. Written informed consent was obtained from the owners of each dog. The sampling was performed opportunistically and no additional manipulations were needed besides the necessary procedures for the diagnosis of ICRP or health examination. Thus, an approval from the Animal Care Committee of the Graduate School of Agricultural and Life Sciences, the University of Tokyo was not required.

Fecal characteristics

Fecal pH was measured by directly inserting the glass electrode of an H-7 HP pH meter (Horiba Seisakusho Co., Ltd., Tokyo, Japan) into feces. Fecal moisture content was determined by overnight oven drying of each sample at 103°C; mean values were calculated using three separate aliquots of each fecal sample. Approximately 0.3 g of feces were diluted at ratios of 1:4 to 1:10 (w/v) in distilled water, vortexed for 1 min and centrifuged at 2,000 ×*g* for 5 min. The supernatants were filtered via a 0.45 μm syringe filter. The supernatants were subjected to SCFA analysis (see below).

Measurement of SCFAs

SCFA concentrations in the supernatants were measured using high performance liquid chromatography (HPLC) [23, 24]. A mixture of 100 μl of the supernatant and 200 μl of crotonic acid (0.5 mM), an internal standard, were pre-labelled with 2-nitrophenylhydrazide using a Short- and Long-Chain Fatty Acid Analysis Kit (YMC Co., Ltd., Kyoto, Japan). The SCFA derivatives were extracted with n-hexane and diethyl ether, and subsequently evaporated to dryness. The residue was reconstituted with methanol, and filtered through a 0.2 μm syringe filter. Ten microliters of this filtrate was injected into an HPLC system with a YMC-Pack FA column (250 × 6.0 mm; YMC Co., Ltd.).

The HPLC system (JASCO, Tokyo, Japan) consisted of two pumps (PU-980), a column oven (CO-965), an autosampler (AS-950), a UV-VIS detector (UV-970) and an integrator (LCSS-905). We performed HPLC under the following conditions. The column oven temperature was 50°C, the mobile phase consisted of acetonitrile-methanol-water (30:16:54 v/v, pH 4–5 adjusted by 0.01 N HCl), the flow rate was 1.2 m*l*/min and the eluate absorbance was monitored online at a wavelength of 230 nm.

To construct SCFA calibration curves, we prepared standards of acetic, propionic, butyric, isobutyric, lactic, valeric, isovaleric and crotonic acids at 0.1–5.0 mM (0.1, 0.2, 0.5, 1.0, 2.0 and 5.0 mM). Figure 1 shows a typical chromatogram of standard solutions and a fecal sample from a control dog. The correlation coefficients of

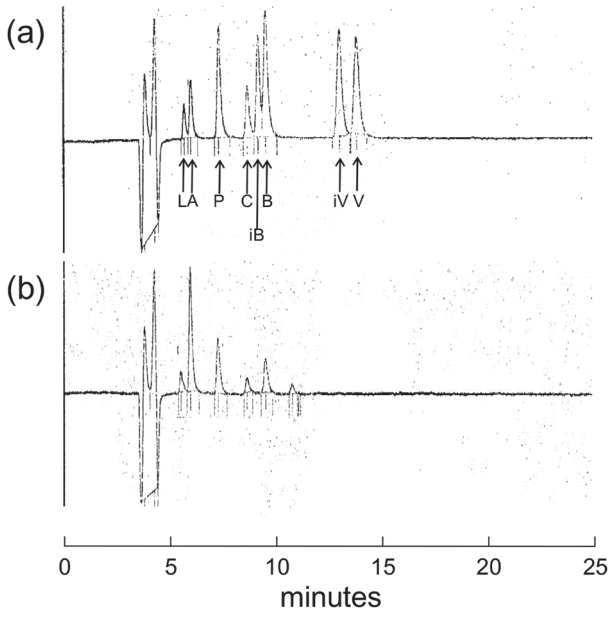

Fig. 1. Chromatograms of mixture of standard solutions (5.0 mM each) of eight short chain fatty acids (a), and a fecal sample from a control dog (b). A, acetic acid; B, butyric acid; C, crotonic acid; iB, isobutyric acid; iV, isovaleric acid; L, lactic acid; P, propionic acid; V, valeric acid.

Table 1. Primer sequences used in the present study

Target		Primer sequences (5′–3′)	Annealing	References
All Bacteria	Forward	CCTACGGGAGGCAGCAG	59°C, 30 sec (2-step)	[18]
	Reverse	ATTACCGCGGCTGCTGG		
Bacteroidetes	Forward	CCGGAWTYATTGGGTTTAAAGGG	60°C, 20 sec	[25]
	Reverse	GGTAAGGTTCCTCGCGTA		
Bifidobacterium	Forward	TCGCGTCCGGTGTGAAAG	60°C, 20 sec	[30]
	Reverse	CCACATCCAGCATCCAC		
Blautia	Forward	TCTGATGTGAAAGGCTGGGGCTTA	56°C, 20 sec	[35]
	Reverse	GGCTTAGCCACCCGACACCTA		
Clostridium perfringens	Forward	AAAGATGGCATCATCATTCAAC	55°C, 20 sec	[43]
	Reverse	TACCGTCATTATCTTCCCCAAA		
Enterococcus	Forward	CCCTTATTGTTAGTTGCCATCATT	61°C, 20 sec	[20]
	Reverse	ACTCGTTGTACTTCCCATTGT		
Escherichia coli	Forward	GTTAATACCTTTGCTCATTGA	55°C, 20 sec	[19]
	Reverse	ACCAGGGTATCTAATCCTGTT		
Faecalibacterium	Forward	GAAGGCGGCCTACTGGGCAC	63°C, 15 sec	[6]
	Reverse	GTGCAGGCGAGTTGCAGCCT		
Firmicutes	Forward	GCAGTAGGGAATCTTCCG	58°C, 20 sec	[3]
	Reverse	ATTACCGCGGCTGCTGG		
Fusobacteria	Forward	KGGGCTCAACMCMGTATTGCGT	59°C, 15 sec	[35]
	Reverse	TCGCGTTAGCTTGGGCGCTG		
Lactobacillus	Forward	AGCAGTAGGGAATCTTCCA	58°C, 20 sec	[20]
	Reverse	CACCGCTACACATGGAG		
Ruminococcaceae	Forward	ACTGAGAGGTTGAACGGCCA	59°C, 30 sec (2-step)	[6]
	Reverse	CCTTTACACCCAGTAAWTCCGGA		
Turicibacter	Forward	CAGACGGGGACAACGATTGGA	63°C, 20 sec	[35]
	Reverse	TACGCATCGTCGCCTTGGTA		

the calibration curves were 0.9922–0.9984. Recovery tests were performed by adding known amounts (10 μmol) of each SCFA to 10 m*l* aliquots of calibration standard solution (1.0 mM); the recovery ranged from 95.9 to 118.0%. All procedures of the SCFA concentration analysis were performed in duplicate. The concentration of SCFA was normalized to fecal dry weight.

Quantitative real-time polymerase chain reaction (qPCR)

DNA was extracted from the feces (approximately 0.2 g) of each dog using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Selected bacterial taxa within the fecal microbiota were analyzed by qPCR assays using the THUNDERBIRD SYBR qPCR Mix (TOYOBO Co., Ltd., Osaka, Japan) and Thermal Cycler Dice Real Time System (Takara Bio Inc., Otsu, Japan). Information on the primers used in our analyses is depicted in Table 1 [3, 6, 18–20, 25, 30, 35, 43]. The qPCR assays were performed in duplicate using a 20-μl reaction volume/well. The amplification conditions were as follows: 2-step PCR (95°C for 60 sec, 40 cycles of PCR [95°C for 15 sec and 59°C for 30 sec] and finally dissociation); or, 3-step PCR (95°C for 60 sec, followed by 40 cycles of denaturation [95°C for 15 sec], annealing [55–63°C for 15–20 sec], extension [72°C for 40 sec] and finally dissociation). The qPCR data were analyzed by the Thermal Cycler Dice Real Time System software version 4.01A (Takara Bio Inc.). The amplification efficiencies, derived from standard curves based on a 10-fold dilution series of representative cDNA samples, were 83.3–107.5%. Ct values were determined as second derivative maximum cycles. Nuclease-free water was used as a negative control. A sample with a known Ct value (as a positive control) was included with all sample runs, to control for run-to-run Ct variation. The concentration of amplified DNA from each bacterial taxon was normalized to the total concentration of amplified DNA from all bacteria.

Statistical analysis

Statistical analyses were performed using commercially available software (JMP Pro version 11.0.0, SAS Institute, Cary, NC, U.S.A.). The normality of the data was checked using the Shapiro-Wilk test. Differences in gender were tested using Fisher's exact test. Differences in age, body weight and fecal parameters between ICRP-affected MDs and control MDs were determined using Welch's *t*-test or the Mann-Whitney *U*-test where appropriate. Correlations between the abundance of fecal bacterial DNA and SCFA concentrations were evaluated using the Spearman's rank correlation coefficient. Statistical significance was defined as $P<0.05$.

Table 2. Summary statistics and evaluated markers for ICRP-affected and control dogs

	ICRP	Control	*P*-value[a)]
Sex			
Male (neutered)	5 (4)	17 (8)	0.274
Female (spayed)	6 (4)	8 (5)	
Age (months)	119 (48–159)	103 (24–197)	0.786
Body weight (kg)	5.15 (3.85–7.50)	6.10 (3.90–7.75)	0.392
Body condition score[b)]	5 (5–7)	5 (3–7)	0.237
Fecal parameters			
Dry matter (%)	20.32 (9.24–38.37)	33.22 (15.84–46.18)	<0.001
pH	6.80 (5.90–8.65)	6.40 (5.50–7.40)	0.051
Total SCFA[c)]	181.73 (0.00–535.75)	249.91 (122.89–501.59)	0.070
Acetic acid[c)]	134.09 (0.00–316.16)	136.65 (43.69–334.17)	0.241
Propionic acid[c)]	0.00 (0.00–125.54)	50.04 (0.00–137.44)	0.030
Butyric acid[c)]	5.88 (0.00–57.30)	9.62 (0.00–39.32)	0.163
Isobutyric acid[c)]	0.00 (0.00–0.00)	0.00 (0.00–1.20)	0.254
Lactic acid[c)]	0.00 (0.00–109.97)	33.21 (0.00–170.12)	0.079
Valeric acid[c)]	0.00 (0.00–0.00)	0.00 (0.00–0.07)	0.254
Isovaleric acid[c)]	0.00 (0.00–0.00)	0.00 (0.00–0.67)	0.171
Acetic acid (%)	69.41 (57.94–100.00)	59.15 (32.47–87.47)	0.004
Propionic acid (%)	4.88 (0.00–34.53)	23.98 (0.00–34.88)	0.049
Butyric acid (%)	3.45 (0.00–11.14)	4.02 (0.00–15.12)	0.661
Isobutyric acid (%)	0.00 (0.00–0.00)	0.00 (0.00–0.21)	0.527
Lactic acid (%)	3.96 (0.00–31.05)	16.85 (0.00–67.53)	0.304
Valeric acid (%)	0.00 (0.00–0.00)	0.00 (0.00–0.03)	0.527
Isovaleric acid (%)	0.00 (0.00–0.00)	0.00 (0.00–0.12)	0.364

a) Welch's *t*, Mann-Whitney *U*, or Fisher's exact test between ICRP-affected MDs and control MDs. b) Represented using a 9-point scale. c) Data are shown in μmol/g fecal dry matter. Measurements, other than sex, are reported as medians (ranges). ICRP, inflammatory colorectal polyp; MD; miniature dachshund; SCFA, short chain fatty acid.

RESULTS

Animals

The present study observed 11 ICRP-affected MDs and 25 control MDs. Baseline characteristics such as sex, age, body weight and body condition score did not significantly differ between these two groups (Table 2).

Fecal characteristics

The fecal dry matter content for ICRP-affected MDs was significantly lower than that for control MDs ($P<0.001$) (Table 2). The fecal pH in ICRP-affected MDs was relatively higher than in control MDs, but did not show significant difference ($P=0.051$) (Table 2).

Fecal SCFA concentrations

The concentrations of isobutyric, valeric and isovaleric acids were barely detected in only one or two dogs, and did not differ significantly between the groups. Fecal propionic acid concentration in ICRP-affected MDs was significantly lower than that in control MDs ($P=0.030$) (Table 2). Concentrations of total fecal SCFA and acetic, butyric and lactic acids did not differ significantly between the groups (Table 2). When the composition of each SCFA was expressed as a percentage of the total fecal SCFA concentration, the proportion of propionic acid was significantly decreased ($P=0.049$), while that of acetic acid was significantly increased ($P=0.004$) (Table 2).

Proportions of selected bacterial taxa

The abundance of the *Bifidobacterium* in ICRP-affected MDs was significantly lower than that in control MDs ($P=0.048$) (Fig. 2). None of the other evaluated bacterial taxa differed significantly between the groups.

Correlation between fecal SCFA concentration and bacterial proportion

A positive correlation was observed between fecal total SCFA concentrations and proportions of Firmicutes, *Bifidobacterium* and *Lactobacillus* ($\rho=0.3761$ [$P=0.026$], $\rho=0.3279$ [$P=0.049$] and $\rho=0.3897$ [$P=0.019$], respectively) (Table 3). Firmicutes also exhibited a significant positive correlation with fecal propionic acid concentration ($\rho=0.4828$ [$P=0.004$]) (Table 3). The proportion of *Escherichia coli* tended to correlate negatively with total fecal SCFA concentration ($\rho=-0.3022$ [$P=0.073$]); it had significant negative correlation with acetic, propionic and butyric acids ($\rho=-0.4263$ [$P=0.010$], $\rho=-0.3559$ [$P=0.033$] and $\rho=-0.3451$ [$P=0.039$], respectively); whereas this proportion correlated positively with lactic acid ($\rho=0.3380$ [$P=0.044$]) (Table 3).

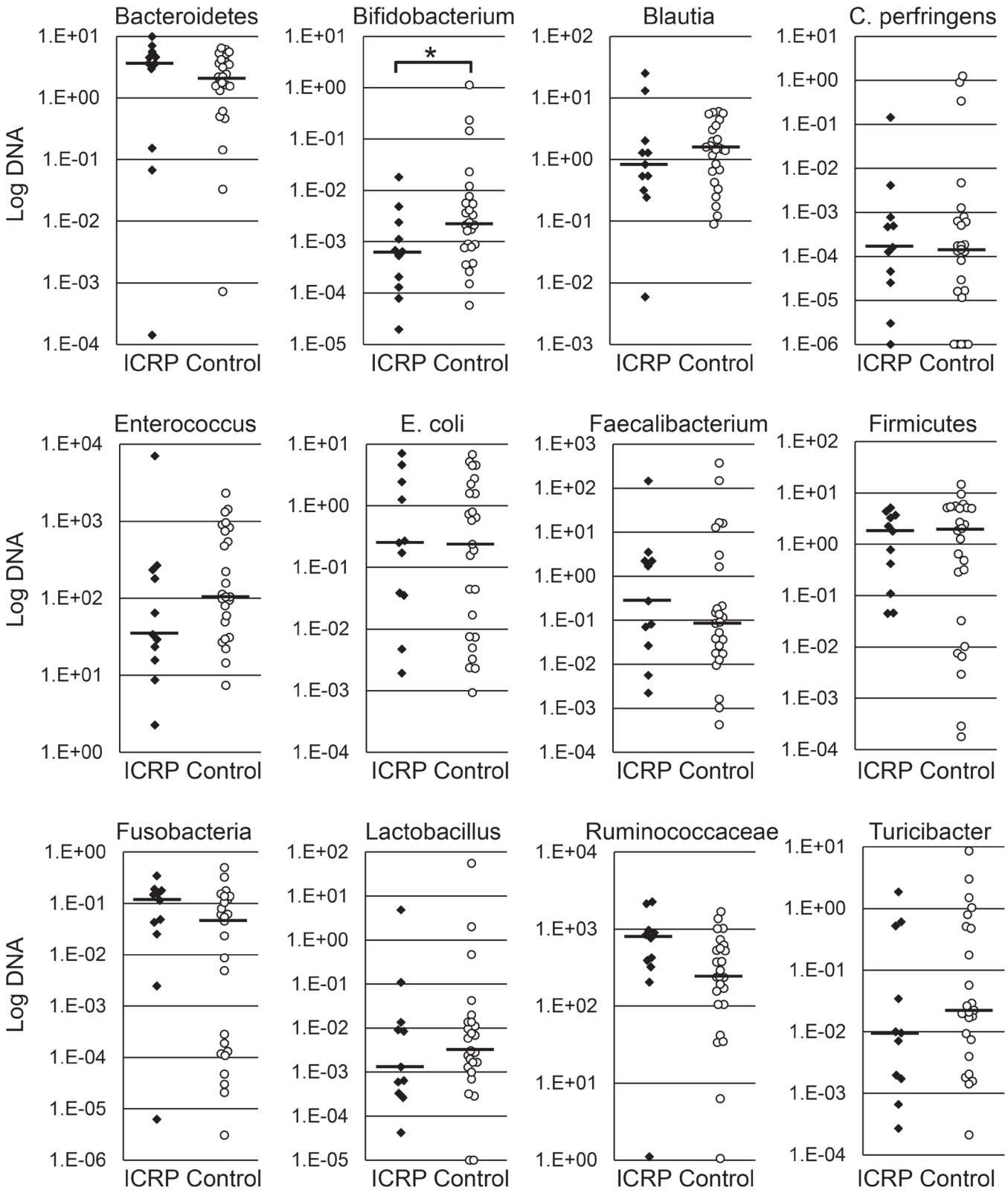

Fig. 2. Abundances of selected bacterial taxa. The horizontal lines represent the median value for each taxon. Data are expressed relative to the geometric mean of the abundance of all bacteria in each sample. Asterisk indicates statistically significant difference ($P<0.05$). ICRP, inflammatory colorectal polyp.

Table 3. Correlations between fecal SCFA concentrations and abundance of bacterial DNA

	Total SCFAs		Acetic acid		Propionic acid		Butyric acid		Lactic acid	
	ρ	*P*-value	ρ	*P*-value	ρ	*P*-value	ρ	*P*-value	ρ	*P*-value
Bacteroidetes	0.0183	0.916	0.0713	0.679	0.1012	0.557	0.0080	0.963	–0.2891	0.087
Bifidobacterium	0.3279	0.049	0.2867	0.090	0.1045	0.544	0.2607	0.125	0.1962	0.252
Blautia	0.0716	0.678	0.2378	0.163	–0.0106	0.951	0.0545	0.752	–0.1967	0.250
C. perfringens	0.1356	0.431	–0.0925	0.592	–0.0001	0.999	–0.0843	0.625	0.2007	0.240
Enterococcus	–0.0682	0.693	0.0342	0.843	–0.1230	0.475	–0.0925	0.592	–0.0649	0.707
E. coli	–0.3022	0.073	–0.4263	0.010	–0.3559	0.033	–0.3451	0.039	0.338	0.044
Faecalibacterium	–0.0023	0.989	0.0510	0.768	–0.0016	0.993	0.1741	0.310	–0.0682	0.693
Firmicutes	0.3761	0.026	0.1792	0.289	0.4828	0.004	0.0021	0.993	0.0219	0.892
Fusobacteria	–0.1145	0.506	–0.0435	0.801	–0.0584	0.735	0.2154	0.207	–0.1735	0.312
Lactobacillus	0.3897	0.019	0.2465	0.147	0.2186	0.200	0.2190	0.200	0.1058	0.539
Ruminococcaceae	–0.0695	0.687	0.1619	0.346	–0.1946	0.256	0.1297	0.451	–0.2169	0.204
Turicibacter	0.1475	0.391	0.1925	0.261	0.0866	0.615	0.2018	0.238	0.2079	0.224

DISCUSSION

This study revealed depleted fecal concentration of propionic acid in ICRP-affected MDs. On the contrary, fecal butyric acid concentrations did not differ significantly between the groups. In contrast, butyric and propionic acid abundances are likely to decrease in human IBD patients [13, 36]. This inter-species difference in reduced SCFA production patterns may be due to species-specific disease pathogenesis and/or dysbiosis.

Although the anti-inflammatory effect of butyrate has been described as the most potent [8], propionate and acetate have also been reported to exhibit anti-inflammatory activity. Tedelind *et al.* [38] ranked the potency of these compounds as: butyrate >propionate >acetate. Therefore, the decrease of propionic acid in MDs with ICRPs may contribute to the development of inflammation.

The major fecal SCFA products are, reportedly, acetic, propionic and butyric acids, which are commonly found in proportions of 60:20:20 (acetic: propionic: butyric) in humans [7, 44]. In contrast, the butyrate concentration in healthy dogs has been reported at a lower ratio (~10% of the total amounts of acetic, propionic and butyric acids) [9, 29, 33, 45]; that finding is consistent with the present results. The lowness of the fecal butyrate ratio therefore seems characteristic of dogs. The low fecal butyrate concentration in healthy dogs would mean that the observed reduction in the fecal butyrate concentration in ICRP-affected MDs was a small change and hard to measure; consequently, it may lead to the absence of statistically significant differences.

The fecal dry matter content was significantly decreased in ICRP-affected MDs, relative to this metric in control MDs. This depletion may result from excessive mucus secretion commonly observed in MDs with ICRPs [26, 37], which may lead to the dilution of fecal SCFAs in the gastrointestinal tract. Indeed, the fecal concentrations of total SCFA and acetic acid, as well as propionic acid, were significantly decreased in ICRP-affected MDs relative to control MDs, when the concentrations were normalized by fecal wet weight (Supplementary Table 3). The SCFAs are absorbed from the mucosal surface and metabolized by enterocytes, muscles and liver tissues [44]; the reduced SCFA concentration at the mucosal surface may thus induce systemic disorders, as well as colonic inflammation. Furthermore, the ICRP-affected MDs had relatively high fecal pH relative to control dogs (P=0.051, Table 2), which may be due to the decreased SCFA abundance. Low pH may reduce the growth and activity of potential pathogens [11].

The relative DNA abundances for several bacterial taxa correlated positively with fecal SCFA concentrations. This result indicates that dysbiosis may lead to the reduction of SCFA production in the gastrointestinal tract. *Bifidobacterium* was less abundant in ICRP-affected MDs and positively correlated with fecal total SCFA concentration, which may contribute to the pathogenesis of ICRPs. Similarly, Firmicutes correlated positively with fecal total SCFA and propionic acid concentrations. Depleted Firmicute abundances have been reported in gastrointestinal disorders including canine IBD and ICRPs in MDs [10, 16], although we did not observe a significant decrease in the present study. Firmicutes is the predominant bacterial phylum in the canine colonic microflora; this taxon contains SCFA-producing bacteria such as *Lactobacillus*, and the beneficial *Clostridium* cluster IV and XIVa [11]. Since a limited set of bacterial species were evaluated in the present study, other unevaluated Firmicutes may contribute to the observed reduction of fecal propionic acid in ICRP-affected MDs.

Escherichia coli abundance correlated negatively with fecal concentrations of total SCFA, as well as acetic, propionic and butyric acids. *Escherichia coli* is a commensal gut microbe, although some strains can be pathogenic and are associated with gastroenteritis [21]. Furthermore, Minamoto *et al.* [22] reported that fecal proportions of *E. coli* were increased in dogs with acute or chronic diarrhea. Therefore, an increase in *E. coli* abundance might indicate dysbiosis with the reduction of SCFA-producing bacteria, although in the present study, we did not observe a significant difference in the proportion of *E. coli* between ICRP-affected and control MDs.

Commensal microbe-derived SCFAs, particularly butyric and propionic acid, have been described as inducers of colonic Treg cell differentiation [5], which plays a central role in the suppression of inflammatory response. Since the fecal propionic acid concentrations in ICRP-affected MDs were depleted in the present study, we speculate that colorectal Treg cell abundance in

ICRP-affected MDs was reduced. However, Ohta *et al.* [27] reported elevated mRNA expression of IL-10 in the polypoid lesions of ICRP-affected MDs; IL-10 is a well-known anti-inflammatory cytokine produced by mononuclear cells, including Treg cells [31]. Further studies are warranted on the IL-10 cellular source in ICRPs, and the distribution of Treg cells in the polypoid lesion. Moreover, the association between colonic SCFAs and induction of Treg cells in dogs needs to be investigated.

An enema with SCFAs has been described as a treatment for patients with ulcerative colitis, a major form of human IBD [1, 8]. Because the colonic molar ratio of SCFAs may differ between animal species as noted above, a dog-specific enema composition should be prepared by exploring the efficacies of differing regimens. This method might be a novel therapeutic option for ICRPs in MDs, since such lesions are usually restricted to the colorectal mucosa [15, 26].

SCFAs have been described as anticarcinogenic [8]. Since ICRPs in MDs occasionally develop colorectal neoplasia [17], further investigations of the association between SCFA concentrations and colorectal neoplasia in dogs might help elucidate the tumorigenesis of ICRPs.

Heterogeneous samples of diseased and control dogs may have limited this study. Several dogs in each group had received immunosuppressive drugs and/or probiotics, predominantly prednisolone (Supplementary Tables 1 and 2). This heterogeneity may explain the discrepancy between the present study and previous studies in observed fecal microbiota proportion [16]. However, we believe that the administration of the drugs and/or probiotics did not interfere with the reduction of propionic acid observed here, for the following reasons. We previously reported that the oral administration of prednisolone did not directly affect the microbiota [14]. In addition, we found no significant difference in SCFA concentration between ICRP-affected MDs receiving immunosuppressive treatment and counterpart MDs not receiving immunosuppression (data not shown). In the present study, three ICRP-affected MDs and no control MDs had received probiotics. Since dietary information for several dogs was not available, our results may be confounded by dietary differences−particularly dietary fiber differences [28, 29]. However, as with the probiotics, only three ICRP-affected MDs had received a high-fiber diet (Supplementary Table 1) at recruitment. Since only a limited number of cases were recruited, we encourage further studies with larger sample sizes.

In conclusion, this study revealed that ICRP-affected MDs have low concentrations of propionic acid. We also found an association between fecal dysbiosis and fecal SCFA abundance, which may contribute to the pathogenesis of ICRPs in MDs. The usefulness of SCFAs as disease monitoring markers, and their significance as therapeutic targets, merits further investigation.

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