Dog and human inflammatory bowel disease rely on overlapping yet distinct dysbiosis networks

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Inflammatory bowel disease (IBD) is an autoimmune condition that is difficult to diagnose, and animal models of this disease have questionable human relevance1. Here, we show that the dysbiosis network underlying IBD in dogs differs from that in humans, with some bacteria such as Fusobacterium switching roles between the two species (as Bacteroides fragilis switches roles between humans and mice)2. For example, a dysbiosis index trained on humans fails when applied to dogs, but a dog-specific dysbiosis index achieves high correlations with the overall dog microbial community diversity patterns. In addition, a random forest classifier trained on dog-specific samples achieves high discriminatory power, even when using stool samples rather than the mucosal biopsies required for high discriminatory power in humans2. These relationships were not detected in previously published dog IBD data sets due to their limited sample size and statistical power3. Taken together, these results reveal the need to train host-specific dysbiosis networks and point the way towards a generalized understanding of IBD across different mammalian models.

Dogs are commonly used as large animal models for drug discovery and safety assessment. The usefulness of dogs as a model for inflammatory bowel disease (IBD) is unexplored as yet, but is not unreasonable, as dogs have been useful for studying spontaneously occurring disorders similar to those affecting people4. For example, dogs live in a close relationship with and share an environment with their owners and are therefore frequently exposed to similar environmental factors, including enteropathogens and toxins. It is well recognized that dogs and humans suffer from similar spontaneous and lifestyle-associated diseases such as obesity, allergies, diabetes mellitus and cancer, and are often treated with similar antibiotics and drugs. IBD in humans is a chronic autoimmune disease of multifactorial aetiology and has limited treatment options. Similarly, canine idiopathic IBD is a commonly observed chronic inflammatory enteropathy that occurs spontaneously, with similar multifactorial aetiology, due to an interplay between an aberrant host immune system, genetics, environmental factors and gut microbiota1. Common clinical signs are vomiting, diarrhoea and weight loss. Histological evaluation of intestinal biopsies reveals diffuse or multifocal inflammatory cell infiltration (most commonly lymphoplasmacytic, followed by eosinophilic and neutrophilic), with concurrent changes in the mucosal architecture (for example, villus atrophy and fusion)5. Enteric protein loss may be observed in severe cases. In a subset of dogs, invasive and adherent Escherichia coli have also been described, and these share common features with strains isolated from humans with Crohn’s disease4. Clinical signs may be controlled by single or combination therapy, including dietary modifications, antibiotics and immuno-suppressants. However, clinical relapse occurs frequently, and lifelong therapy may be needed. Previous small-scale studies have revealed dysbiosis in the small and large intestines of dogs with IBD, with some changes in bacterial taxa similar to those observed in humans with IBD5. For example, in both humans and dogs with IBD, increases in Proteobacteria, specifically Enterobacteriaceae6, and decreases in Firmicutes, including Faecalibacterium and Blautia8, have been reported. However, no detailed studies comparing the changes in gut microbiota in humans and canines with IBD have been reported to date. The aim of this study was to describe, in detail, the microbiome changes in a large group of dogs with IBD, compare these to the microbiome of humans with IBD, assess host similarities and differences, and train a dysbiosis index composed of non-IBD- and IBD-associated bacteria.

**Results and discussion**

As expected based on results from human studies2,9, but not previously clearly established in canine studies8,10, dog IBD cases and controls differ substantially in both microbial community diversity and structure (Fig. 1). IBD dogs had a significantly lower alpha diversity (Mann–Whitney test, \( P = 0.003 \)) than non-IBD affected dogs (Fig. 1a). However, alpha diversity in this population did not correlate with age, fat intake, weight or protein intake (Supplementary Fig. 1), nor with body condition scores (\( P > 0.05 \), Supplementary Methods). Clear separation (using permutational multivariate analysis of variance (PERMANOVA) grouping samples by disease status, \( P = 0.001 \), Supplementary Methods) between IBD dogs and controls was observed using unweighted UniFrac (Fig. 1b), and the biplot shows the most abundant taxa driving these overall patterns. Consistent with ref. 10, antibiotic history did not reveal differences within IBD-affected dogs (PERMANOVA \( P = 0.501 \), abx = 35, no abx = 12). On the contrary, a significant effect was observed in non-IBD dogs (PERMANOVA \( P = 0.01 \); abx = 8, no abx = 77). Finally, when combined, the disease effect was significantly stronger (PERMANOVA \( P = 0.001 \)) than the history of antibiotic usage (pseudo-F on IBD groups 1.99; pseudo-F based on antibiotics groups 9.46).

A random forests classifier11 trained on the dog data achieved an area under the curve (AUC) of 0.93 (Fig. 1c, see discriminant operational taxonomic units (OTUs) in Supplementary Table 1), demonstrating excellent classification accuracy compared to human stool samples, for which the AUC was only 0.63 using a much larger training set12 and achieved only AUC = 0.86 even when using mucosal biopsies. Consequently, in dogs, but not in humans, high classifier accuracy is achievable for IBD using only stool samples.

Encouraged by these results, we tested whether a dysbiosis (Supplementary Methods) network trained on stool samples alone could be used to interpret the pattern of IBD in dogs and whether this pattern overlapped the human network. In humans, we have previously found that substantially better correlation networks can

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be achieved from mucosal biopsies, because many taxa contributing to these networks are not seen in the stool. Accordingly, we used the techniques described in ref. 2 to generate correlation networks and a dysbiosis index for dog samples (see Supplementary Methods for more information). Using the network together with the biplots (Fig. 1b), we see that Gammaproteobacteria (specifically Enterobacteriaceae) are significantly associated with IBD, whereas various Firmicutes such as Clostridium and Ruminococcus are associated with non-IBD samples. When these features are compared with discriminant OTUs, obtained from the random forests classifier, we observe a general overlap of the lineages (Supplementary Tables 1 and 2). However, we also see OTUs that are not highlighted by the correlation network, specifically Erysipelotrichaceae Allobaculum and Lachnospiraceae Blautia producta; this is probably because these do not consistently co-occur with or co-exclude other taxa.

The human dysbiosis index failed to negatively correlate with alpha diversity in dog samples (Fig. 2a,b shows the correlation and Fig. 2c the principal coordinates analysis (PCoA) plot), but a dog-specific dysbiosis index showed a statistically significant negative correlation in the same samples (Fig. 2d,e shows the correlation and Fig. 2f the PCoA plot; for reference see the two groups in Fig. 2h). We also tested the index with previous data10 and, although the sample size was limited, we observed similar patterns (Supplementary Fig. 2). Similarly to humans, the dysbiosis index in dogs is negatively correlated with phylogenetic diversity ($r = -0.45$, $P < 0.001$). However, the list of ‘non-IBD’ (co-occurring in non-IBD samples) and ‘IBD’ (co-occurring in IBD samples) bacteria only partially overlaps between host species (Supplementary Table 2). A comparison of the correlation networks of the taxa for humans (as described in ref. 2) and the network generated for the dog data (Fig. 2g) revealed overlapping and discordant taxa. In particular, Fusobacterium appears to be associated with IBD$^2$ and colorectal cancer$^{12}$ in humans but not with non-IBD dog samples. Of note, we previously observed high levels of Fusobacterium sp. in dogs$^{13}$, but also in carnivores of multiple species$^{14,15}$, and noted...
Figure 2 | Human and dog dysbiosis index. a, b. Human dysbiosis index describing the dog samples grouped by disease status. c. Weighted UniFrac PCoA plot coloured by dysbiosis index. d, e. Dog dysbiosis index describing the dog samples grouped by disease status. f. Weighted UniFrac PCoA plot coloured by dysbiosis index. g. Correlation network used to determine the dysbiosis index in dogs, coloured by the bacteria associated with IBD and non-IBD samples. Co-exclusion edges are showed as dotted lines and co-occurrence edges as solid lines. h. Unweighted UniFrac PCoA plot of the dog data. Panels a, b, d and e all show 95% confidence intervals.

higher levels of Fusobacterium in dogs with more access to the outdoors, which may correlate with a wide intake of other immunomodulatory environmental bacteria. Given the limited adaptation (~8.9 Mya, when humans split from gorillas) of the human lineage (historically omnivorous) to a carnivorous diet, as compared to the base of the Carnivora 40–45 Mya (ref. 19), it is possible that this taxon has yet to be incorporated into the non-IBD portion of the network in humans. Nonetheless, it is important to remember that this is only a statistical association, and further research would need to be developed to properly validate this. Consistent between human and canine networks and former studies were findings of decreased Faecalibacterium and increased E. coli in IBD and these taxa seem to be important in this disease across animal hosts. Other taxa (Enterococcus and Allobaculum) from the canine network that were associated with IBD or non-IBD are generally consistent with previous results based on either small-scale studies or targeted PCR (ref. 10), but additional taxa were discovered here, such as Butyricoccus, which was associated with non-IBD dogs.

To measure the relative effect size of host species and IBD, we combined humans and dogs into a single PCoA plot, marked by clinical status (Supplementary Fig. 4). We demonstrate that, at a microbial level, the disease effect is smaller than the host effect (human versus dog, Supplementary Fig. 4a). Similarly, the disease effect is weaker than the species effect when analysing PICRUSt2 (human versus dog, Supplementary Fig. 4). We demonstrate that, at a compositional and predicted functional level, species is a more significant influence on the microbial community than disease (Supplementary Methods and Supplementary Fig. 3). In both dogs and humans, predicted pathways were relatively similar.
across IBD and non-IBD samples, with the most abundant pathways across both groups in both species including ‘housekeeping’ pathways such as transporters, ABC transporters, DNA repair and recombination proteins, ribosome, purine metabolism, transcription factors, peptidases, pyrimidine metabolism and chromosome (Supplementary Fig. 5). Although these most abundant pathways were not significantly associated with health or disease, the abundances of several lower-abundance pathways were significantly different across IBD and non-IBD samples in dogs, as shown in Supplementary Table 3. No pathways were significantly different between disease statuses in humans.

Taken together, these results have important implications for translational medicine and for understanding IBD in dogs. Also, the major functional gene content was conserved across non-IBD and IBD humans and dogs. Although some significant predicted functions were identified between non-IBD and IBD dogs, not enough non-IBD individuals in the human sample population were analysed for proper statistical power. This, together with previous work, suggests similar functional changes within the microbiota of dogs and humans with IBD. Previous studies have already shown that some treatment approaches to IBD that target the microbiome are conserved across dogs and humans, such as antibiotics, dietary modulation and probiotics. For example, specific probiotic therapy has been shown to have similar effects on faecal and mucosa-adherent microbiota and host immune response (that is, increase of tight junction proteins, increase in beneficial mucosa-adherent bacteria) in humans, dogs and rat models of colitis.

Our study also revealed that the dysbiosis networks clearly differ in some key bacterial groups. A better understanding of the similarities of these microbial networks and functional changes may extend our ability to test therapeutic approaches across multiple host species.

Methods

Naturally passed faecal samples were analysed from 85 healthy dogs and 65 dogs with chronic signs of gastrointestinal (GI) disease, and inflammatory changes were confirmed by histopathology. All dogs were participating in different clinical studies, and leftover faecal samples were used for this study. The protocol for sample collection was approved by the Clinical Research Review Committee of the College of Veterinary Medicine, Texas A&M University (CRRC 09-06).

Dogs with histological signs of chronic GI disease (for example, vomiting, diarrhoea, anorexia and weight loss) were diagnosed with idiopathic IBD based on the World Small Animal Veterinary Association (WSAVA) criteria: (1) chronic (that is, more than 3 weeks) GI signs; (2) histopathological evidence of mucosal inflammation; (3) inability to document other causes of GI inflammation; (4) inadequate response to dietary, antibiotic and anthelmintic therapies; and (5) clinical response to anti-inflammatory or immunosuppressive agents. Histological samples were obtained endoscopically. The clinical status of each dog was evaluated using a published clinical canine IBD activity index (CIBDAI). Within the IBD dogs, 41 dogs had histologically confirmed inflammation in the small intestine, 18 dogs had histological changes in both small intestine and colon, and 5 dogs had only histological changes reported in the colon. Histological changes were predominantly of lymphoplasmacytic infiltrates, with a subset of dogs also showing eosinophilic and/or neutrophilic components. The mean (s.d.) clinical IBD activity index (CIBDAI) for IBD dogs was 6.4 (3.1).

Dogs were excluded if they had received antibiotics within the past two weeks of sample collection. Data on antibiotic history were nevertheless collected: 34/65 dogs with IBD had no history of prior antibiotics administration, while 13 dogs received antibiotics several weeks (more than two weeks) or months before sample collection. The remaining 18 dogs in the IBD group had no information about prior antibiotic use. In the healthy group (n = 85), 76 dogs had not received any antibiotics and 9 dogs had a history of antibiotic use, but not within the last two weeks of sample collection. No technical replicates were generated in this study.

Sample and animal information (age, weight, gender, breed, duration of clinical signs, histopathology, antibiotic usage) was obtained from clinical records. Also, if the owner provided the information, the exact diet (trade name and manufacturer) fed at the time of sample collection was recorded in the clinical records, and the dietary macronutrients (protein, fat and carbohydrate content) were recorded from the manufacturer’s data on the labels.

Body weights ranged from 2.9 to 55 kg (mean 22 kg; s.d. 14.9 kg), which was not significantly different (Mann–Whitney test; P = 0.087) from the healthy dogs (range 0.9–50 kg; mean 20.3 kg, s.d. 10.7 kg). Mean age (s.d.) was 5.4 (3.07) in the IBD group, which was not significantly different (Mann–Whitney test; P = 0.311) from the healthy dogs (4.7 (3.22)). There was a wide breed distribution in the IBD group (24 different breeds) and in the control group (42 breeds).

Dysbiosis network. The dysbiosis network was calculated as described in ref. 2, using Cytoscape. The index was calculated by taking the log transform of the abundance of the ratio of IBD-associated microbes to non-IBD associated microbes as determined by the correlation network.

This network was created by first scoring the co-occurrence and co-exclusion patterns in the samples. CONCOPT uses a compositionally adjusted version of the checkerboard score and the results are filtered to remove non-statistically significant relationships and to preserve the largest connected component only. The results are represented as a graph, where vertices are microbes and the edges are interaction types. Vertices are of one of two classes (IBD-associated and healthy-associated), as determined by the class where they were predominantly abundant, and edges between vertices have a weight given by their adjusted checkerboard score.
(negative values represent co-exclusions and positive values represent co-
occurrences). The specifics of this processing are described in the Jupyter notebooks
(see Supplementary Information).

Data availability. Raw sequences for the dog samples have been deposited in the
European Nucleotide Archive (ENA) under accession nos. ERP014919, and
equivalent processed OTU tables and metadata can be accessed through Qita
(https://qiita.microbio.me) under study no. 833, ‘Dog models of inflammatory bowel
disease’. Data for the human data set2 can be found at the Sequence Read
Archive (SRA) under accession no. SRP043010.

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Additional information

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Competing interests

The authors declare no competing financial interests.