

# Recent Advances in Characterizing the Gastrointestinal Microbiome in Crohn's Disease: A Systematic Review

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**Background:** The intestinal microbiota is involved in the pathogenesis of inflammatory bowel disease. A reduction in the diversity of the intestinal microbiota as well as specific taxonomic and functional shifts have been reported in Crohn's disease and may play a central role in the inflammatory process. The aim was to systematically review recent developments in the structural and functional changes observed in the gastrointestinal microbiome in patients with Crohn's Disease.

**Results:** Seventy-two abstracts were included in this review. The effects of host genetics, disease phenotype, and inflammatory bowel disease treatment on the gastrointestinal microbiome in Crohn's disease were reviewed, and taxonomic shifts in patients with early and established disease were described. The relative abundance of Bacteroidetes is increased and Firmicutes decreased in Crohn's disease compared with healthy controls. Enterobacteriaceae, specifically *Escherichia coli*, is enriched in Crohn's disease. *Faecalibacterium prausnitzii* is found at lower abundance in Crohn's disease and in those with postoperative recurrence. Observed functional changes include major shifts in oxidative stress pathways, a decrease in butanoate and propanoate metabolism gene expression, lower levels of butyrate, and other short-chain fatty acids, decreased carbohydrate metabolism, and decreased amino acid biosynthesis.

**Conclusions:** Changes in microbial composition and function have been described, although a causative role remains to be established. Larger, prospective, and longitudinal studies are required with deep interrogation of the microbiome if causality is to be determined, and refined microbial manipulation is to emerge as a focused therapy.

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**Key Words:** Crohn's disease, microbiota, microbiome, dysbiosis

The pathogenesis of Crohn's disease, an inflammatory bowel disease (IBD), involves interactions between the host genome, gastrointestinal (GI) microbiota, and mucosal immune system. The initiating and perpetuating stimuli for immune dysregulation in IBD are not explained fully by genetic predisposition; other modifying factors must therefore be present.<sup>1</sup> Genetic loci associated with the development of IBD can perturb the delicate relationship between the microbiota and the host leading to a dysregulated immune response and intestinal inflammation.<sup>2</sup> The microbiome therefore plays a critical role in the pathogenesis of Crohn's disease.<sup>3</sup>

The GI microbiota of healthy humans is dominated by 4 major bacterial phyla: Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria.<sup>4,5</sup> There is some debate about the temporal

stability of the microbiota in healthy subjects.<sup>5–8</sup> Stability is challenged by factors such as the environment,<sup>9</sup> travel,<sup>8</sup> proximity to other humans and animals,<sup>10</sup> diet,<sup>11,12</sup> antibiotics,<sup>8,13</sup> and smoking.<sup>14,15</sup> Taxonomic changes or imbalance in a microbial community, termed dysbiosis, can be associated with the development of disease. Dysbiosis of the GI microbiota has been implicated in numerous conditions affecting human health including obesity,<sup>16,17</sup> colorectal cancer,<sup>18,19</sup> liver disease,<sup>20</sup> irritable bowel syndrome,<sup>21–23</sup> and IBD.<sup>24,25</sup> However, the critical question remains as to whether changes in the composition of the microbiome precede, follow, cause, or only correlate with the onset of disease. Low-resolution methods used to define the GI microbiota in the past such as quantitative PCR, fluorescent in situ hybridization, denaturing gradient gel electrophoresis, terminal restriction fragment length polymorphism, and phylogenetic microarray have differing levels of discrimination limiting the ability to make accurate and specific comparisons across studies. The recent development of the next generation sequencing techniques has allowed for unbiased high-resolution description of the composition, function, and ecology of the microbial community and has improved the understanding of the role of the GI microbiota in health and disease.<sup>5,26</sup>

Of the many conditions believed to be associated with dysbiosis of the GI microbiome, Crohn's disease has received the most interest. Broad patterns have begun to emerge in patients

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with Crohn's disease including reduction in within-sample biodiversity ( $\alpha$ -diversity) and decreased representation of several taxa in the Firmicutes phylum and an increase in the Gammaproteobacteria.<sup>25,27</sup> More specific taxonomic shifts have been reported in Crohn's disease<sup>24</sup>; however, interpretation of these changes across different studies is difficult because of heterogeneity of study populations, differences in sample site (e.g., stool versus mucosa), and technical differences in methodology and study design.

Accompanying the compositional shifts of the GI microbiota that are seen in Crohn's disease are specific dysfunctions of microbial metabolism and bacterial protein signaling. Microbial function seems to be more consistently perturbed than composition.<sup>25</sup> In IBD, major shifts in oxidative stress pathways and decreased carbohydrate metabolism and amino acid biosynthesis have occurred in favor of nutrient transport and uptake.<sup>25</sup> Metaproteomic studies have revealed an imbalance of intestinal bacterial functions in Crohn's disease with quantifiable increases in several bacterial proteins, largely derived from *Bacteroides* species.<sup>28</sup>

It is hoped that characterizing these compositional and functional changes will lead to specific microbial manipulation as a therapy for Crohn's disease. Both diversion of the fecal stream and antibiotic therapy have been shown to delay recurrence of Crohn's disease postoperatively.<sup>29–31</sup> Fecal microbiota transplantation has been proposed as a therapy for IBD, but to date, there is limited evidence to support its efficacy in Crohn's disease.<sup>32,33</sup> Probiotics has not been shown consistently to benefit Crohn's disease.<sup>34</sup> Understanding in detail the compositional and functional changes in the GI microbiome and when these occur in the disease process will facilitate the development of efficacious microbial therapies.

This systematic review of the literature aims to characterize the compositional and functional changes observed in Crohn's disease from the time of diagnosis to the changes surrounding surgery.

## METHODS

This systematic review adheres to the relevant criteria from the PRISMA statement (Preferred Reporting Items for Systematic Reviews and Meta-Analyses).<sup>35</sup> The methods used, including identification, screening, eligibility, and inclusion, were agreed by authors (E.K.W. and M.A.K.) in advance.

An electronic search of the English language medical literature was conducted using Medline (EBSCOhost) and PubMed to identify published articles on the GI microbiome and Crohn's disease. The final search date was July 2014. This search strategy used a combination of the following prespecified MeSH headings and keywords alone or in combination: Crohn's disease, inflammatory bowel disease microbiota, microbiome, and dysbiosis. Boolean operators ("not," "and," "or") were also used in succession to narrow or widen the search. The search was restricted to English language and human studies.

Handsearching of abstracts from relevant international conferences was undertaken to obtain conference abstracts that would not

be identifiable through electronic searching. In addition, handsearching of the reference lists of relevant reviews and included studies was undertaken to identify further relevant references.

## Inclusion Criteria

Studies examining the GI microbiota/microbiome through investigation of intestinal tissue or feces in patients of any age with Crohn's disease or subjects with a family history of Crohn's disease were included. In studies examining a mixed IBD cohort, only results from the Crohn's disease subset were reviewed.

## Exclusion Criteria

Non-English language studies and animal studies were excluded. Studies examining a mixed IBD cohort where there was no separate analysis for the Crohn's disease subset of patients were excluded.

## RESULTS

A total of 287 nonduplicated articles were identified in the search. After abstract review, 64 fulfilled the inclusion criteria and were included in this review, a further 6 articles were identified from review of reference lists and 3 abstracts from review of recent conferences (Fig. 1).

## Crohn's Disease Risk Loci and the Gastrointestinal Microbiome

Host genetics seem to have an impact on the development of Crohn's disease. As many as 140 nonoverlapping genetic risk loci have been associated with Crohn's disease.<sup>36</sup> A potential link between host genetics and the GI microbiome has long been suspected. *NOD2* was the first identified Crohn's disease susceptibility gene.<sup>37</sup> *NOD2*, expressed in Paneth cells located predominantly in the terminal ileum, stimulates an immune reaction on recognizing muramyl dipeptide, a cell wall peptidoglycan constituent of Gram-positive and Gram-negative bacteria.<sup>38</sup> Patients with Crohn's disease with a *NOD2* variant have been shown to exhibit increased adaptive immune responses to microbial antigens absent in patients without this variant.<sup>39,40</sup> Small studies have found that *NOD2* is independently associated with shifts in the temporal development and composition of the GI microbiota.<sup>41,42</sup> Frank et al<sup>43</sup> demonstrated that the risk alleles at the *NOD2* and *ATG16L1* loci were associated with significant compositional shifts in their gut microbiota including decreased levels of Faecalibacterium and increased levels of Escherichia.<sup>44</sup> However, conflicting results were found recently by Kennedy et al<sup>44</sup> who found in their study of 40 patients with Crohn's disease of which 58% were found to have an *NOD2* variant that the *NOD2* allele was not associated with significant taxonomic shifts in gut microbiota. These studies suggest that Crohn's disease-associated loci may well affect the GI microbiome; however, consistent taxonomic shifts have not been demonstrated. With larger genome-wide studies and meta-analyses, the effects of host genetics on GI microbiota are likely to become clearer.

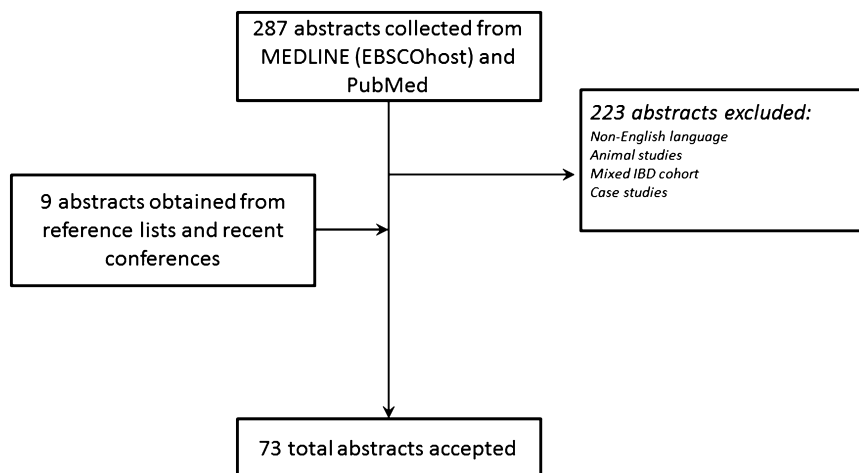


FIGURE 1. Flowchart illustrating published studies and abstracts included in systematic review.

## Microbiota as the Cause of IBD in Phenotypically Normal but Genetically At-risk Individuals

Establishing a causal relationship between the GI microbiome and Crohn's disease requires consideration of the impact of both host genotype and environmental exposures. An important step to understanding pathogenesis and inferring causality is demonstrating that the microbial changes suspected of precipitating disease precede the onset of inflammation. Investigators have approached this question in part by examining the GI microbiota of healthy siblings of patients with Crohn's disease.

Twin studies by Dicksved et al<sup>45</sup> and later by Willing et al<sup>46,47</sup> have revealed conflicting results. In the study by Dicksved et al, 10 twin pairs (4 concordant and 6 discordant for Crohn's disease) and 8 healthy twin pairs were studied. The fecal microbial communities in the discordant twins were least similar when compared with healthy twin pairs or twin pairs concordant for Crohn's disease suggesting that the diseased individuals had a different microbial community structure than their healthy twin.<sup>45</sup> Willing et al<sup>46</sup> found that microbial community structure when measured in colonic and ileal tissue was no more similar ( $P = 0.29$ ) in 4 twin pairs concordant for Crohn's disease compared with 6 twin pairs discordant for disease. In examining the tissue and fecal microbiota of a larger group of twin pairs, Willing et al<sup>47</sup> found that biopsy specimens from concordant but not discordant twin pairs grouped together, indicating some influence of genetics on mucosal-associated microbiota.

Dysbiosis has been demonstrated in unaffected relatives of patients with Crohn's disease when compared with healthy controls.<sup>48–50</sup> Discordance exists as to whether this dysbiosis is similar to or distinct from that seen in patients with Crohn's disease. Hedin et al<sup>49,50</sup> have shown shared aspects of intestinal dysbiosis between patients with Crohn's disease and their unaffected siblings at a species level in both the tissue and feces. In fecal studies, lower relative abundance of *Faecalibacterium prausnitzii* ( $P = 0.048$ ), *Clostridia cluster IV* ( $P = 0.003$ ), and *Roseburia*

*spp.* ( $P = 0.09$ ) (changes widely found to be associated with Crohn's disease) were found in healthy siblings compared with controls.<sup>49</sup> When the mucosal microbiota from 21 patients with Crohn's disease, 17 of their healthy siblings, and 19 unrelated healthy controls was compared, the microbial diversity and species richness of the core microbiota in Crohn's disease and unaffected siblings were lower than in healthy controls. The species that contributed most to the dissimilarity between healthy siblings and healthy controls was *Faecalibacterium prausnitzii*.<sup>50</sup> Joosse et al<sup>48</sup> in a large study of the fecal microbiome involving 68 patients with Crohn's disease, 84 unaffected relatives, and 55 healthy controls found that unaffected relatives of patients with Crohn's disease have a different composition of their microbiota from healthy controls characterized by microorganisms with mucin degradation capacity.

Healthy siblings of patients with Crohn's disease may manifest microbiological abnormalities associated with Crohn's disease, which are distinct from their genetic risk. This dysbiosis in otherwise healthy siblings of patients with Crohn's disease may implicate microbiological processes in Crohn's disease pathogenesis and risk.

## New-onset Crohn's Disease

A potential criticism of much of the literature to date is that studied populations have included those with established disease, often of many years duration, who have received active medical and surgical treatment. The confounders of drug therapy, surgery, and chronic disease have made it difficult to relate taxonomic shifts to the onset of disease rather than a consequence of long-standing inflammation or drug effects. Examining the GI microbiome of newly diagnosed patients with Crohn's disease may help resolve this issue.

Hansen et al<sup>51</sup> investigated the mucosa-associated microbiota of 25 patients with newly diagnosed IBD (Crohn's disease 13 and ulcerative colitis 12). All patients had active colitis. Patients who had received systemic antibiotics or steroids in 3

months before their diagnosis or immunosuppression at any time were excluded. No distinct clustering within any phenotypic group was observed with principal component analysis of weighted Unifrac distances; however, microbial diversity (when measured by Shannon, Simpson, or phylogenetic diversity) was significantly reduced in Crohn's disease when compared with ulcerative colitis and healthy controls. Pyrosequencing studies by Hansen et al<sup>51</sup> have shown *Faecalibacterium* to be found in higher abundance in children with Crohn's disease compared with controls (16.7% versus 8.4% of all reads, respectively,  $P = 0.02$ ). This is in marked contrast with numerous reports demonstrating a reduction of *Faecalibacterium* in both newly diagnosed and established Crohn's disease in adults and children.<sup>24,47,52–56</sup>

Gevers et al<sup>24</sup> have provided the largest and most detailed examination of the microbiome in newly diagnosed children with Crohn's disease to date. In this study, 447 patients aged 3 to 17 years with Crohn's disease and 221 controls were included. Mucosal tissue from the terminal ileum and rectum were analyzed from all participants, with a subset also providing fecal specimens. Microbiome profiling was performed using 16S rRNA gene sequencing on the Illumina MiSeq platform. Overall diversity in microbial composition was not different between Crohn's disease and control samples. However, several specific microbes were found to be significantly associated with subject's disease phenotype. Taxa identified as significant biomarkers for disease included Enterobacteriaceae, Bacteroidales, Clostridiales, Pasteurellaceae (*Haemophilus sp.*), Veillonellaceae, Neisseriaceae, and Fusobacteriaceae. The increased relative abundance of these taxa was found to be associated with increasing severity of Crohn's disease as measured by the Pediatric Crohn's Disease Activity Index.<sup>24</sup> The microbial composition as determined from ileal samples was found to be both sensitive and specific for the classification of Crohn's disease on receiver-operating characteristic analysis (area under the curve = 0.85).<sup>24</sup> This study suggests that in new-onset Crohn's disease, early gut microbial signatures may offer a unique potential for accurate diagnosis.

## Disease Activity and Relapse

Several studies have attempted to define the microbial dysbiosis in Crohn's disease based on disease activity. Populations have been heterogeneous, and both sampling and laboratory methods have varied greatly limiting interpretation. Table 1 summarizes the publications, which have described differences in microbial composition between patients with Crohn's disease and healthy controls. Studies including 6 or more patients where patients with Crohn's disease are compared with healthy controls have been included (Table 1). Studies examining the postoperative Crohn's microbial environment were not included in this table but are discussed in the subsequent section.

Microbial biodiversity is reduced in Crohn's disease,<sup>27</sup> attributed mainly to a reduction of diversity within the Firmicutes phylum,<sup>66</sup> and although there are some inconsistencies in the characterization of specific compositional changes across studies, some consistent patterns have emerged. Crohn's disease seems to

be associated with specific taxonomic shifts. At a phylum level, the relative abundance of Bacteroidetes is increased and Firmicutes is decreased in Crohn's disease compared with healthy controls.<sup>63,64</sup> Enterobacteriaceae, of the Proteobacteria phylum, are also increased in relative abundance<sup>25</sup> with *Escherichia coli* specifically being enriched in the tissue and feces of patients with Crohn's disease relative to healthy controls. *Faecalibacterium*, especially *F. prausnitzii*, which has been found to exhibit anti-inflammatory properties,<sup>52</sup> appears to be at significantly lower abundance in patients with Crohn's disease leading to speculation that this species may have a protective or therapeutic role in the prevention or treatment of Crohn's disease.<sup>48,53,54,56,66</sup> *F. prausnitzii* supplementation is currently being investigated for its anti-inflammatory potential and demonstrated benefit in animal models.<sup>78</sup>

Significant differences exist between the microbial communities depending on sample origin (stool versus tissue),<sup>25</sup> but there do not appear to be significant differences between different biopsy sites or inflamed and noninflamed segments of bowel in Crohn's disease.<sup>24,57,77</sup> Haberman et al,<sup>57</sup> in their study of 243 children and adolescents with newly diagnosed Crohn's disease, 73 disease controls (patients with ulcerative colitis), and 43 healthy controls, combined host-transcriptomic and microbial profiling to define core gene expression profiles and microbial communities in the ileum of patients with Crohn's disease. Gene expression patterns differed between patients with Crohn's disease, ulcerative colitis, and controls, but when patients with Crohn's disease with an active ileitis were compared with those with a normal ileum but colonic Crohn's disease, a similar pattern was observed. This suggests that gene expression profiles in Crohn's disease are independent of clinical inflammation. In the same study, the ileal microbial community was examined. In the ileum of non-IBD controls and ulcerative colitis patients, there was an abundance of the Firmicutes phyla, which was not observed in the ileum of patients with Crohn's disease, a pattern well described in the literature. In patients with Crohn's disease, there was a shift toward Fusobacteria, Gemellaceae, and Proteobacteria expansion, which is again a microbial pattern observed in adults with long-standing disease. Similar microbial profiles were observed in the ileum of patients with Crohn's disease irrespective of the distribution of disease or the presence or severity of endoscopic and histologic ileal inflammation.<sup>57</sup>

Disease remission and relapse seem to be associated with taxonomic changes in both tissue and feces, including loss of population diversity leading to a predominance of pathogenic species, such as *Clostridia difficile*, *Bacteroides vulgates*, and *E. coli* before relapse in IBD.<sup>27</sup> Andoh et al<sup>58</sup> in their analysis of the fecal microbiota profiles of 160 patients with Crohn's disease identified differences in the gut microbiota associated with disease activity when assessed using the Crohn's Disease Activity Index. Haberman et al<sup>57</sup> demonstrated that although there was no difference in the ileal microbial profile of patients with Crohn's disease with and without deep ulceration, an increase in the clinical Pediatric Crohn's Disease Activity Index was associated with

**TABLE 1.** Documented Microbial Alterations in Patients with Crohn's Disease

Author	Population	N	Sample Type/Site	Method	Taxa Enriched in CD	Taxa Decreased in CD
Haberman et al <sup>57</sup>	Pediatric	243	Tissue	Illumina	<i>Proteobacteria</i> , <i>Neisseriaceae</i> , <i>Gemellaceae</i> , <i>Fusobacteriaceae</i> , <i>Veillonellaceae</i> , <i>Pasturellaceae</i> , <i>Enterobacteriaceae</i>	<i>Firmicutes</i> , <i>Erysipelotrichaceae</i> , <i>Lachnospiraceae</i> , <i>Clostridiales</i> , <i>Bifidobacteriaceae</i>
Gevers et al <sup>24</sup>	Pediatric	447	Tissue	Illumina	<i>Veillonella</i> , <i>Haemophilus</i> , <i>Escherichia</i> , <i>Fusobacterium</i>	<i>Dialister</i> , <i>Bilophila</i> , <i>Sutterella</i> , <i>Rikenellaceae</i> <i>Bacteroides</i> , <i>Lachnospiraceae</i> , <i>Coprococcus</i> , <i>Ruminococcus</i> , <i>Erysipelotrichaceae</i> , <i>Dorea</i> , <i>Ruminococcaceae</i> , <i>Faecalibacterium</i> , <i>Oscillospira</i>
Juste et al <sup>28</sup>	Adult	6	Feces	2D-DIGE	<i>Bacteroides vulgatus</i> , <i>Ruminococcus obeum</i>	<i>Roseburia faecis</i> , <i>Faecalibacterium prausnitzii</i>
Andoh et al <sup>58</sup>	Adult	160	Feces	T-RFLP	<i>Desulfovibrio</i> , <i>Lawsonia</i>	<i>Faecalibacterium Coprococcus</i> , <i>Roseburia</i> , <i>Dorea</i>
Hedin et al <sup>49</sup>	Adult	22	Feces	qPCR		<i>Faecalibacterium prausnitzii</i> , <i>Clostridia cluster IV</i> , <i>Roseburia</i>
Kennedy et al <sup>44</sup>	Adult	40	Feces	Illumina	<i>Enterobacteriaceae</i>	<i>Faecalibacteria</i>
Lopez-Siles et al <sup>56</sup>	Adult	45	Tissue	qPCR	<i>Escherichia coli</i>	<i>Faecalibacterium prausnitzii</i>
Ran et al <sup>59</sup>	Adult	8	Tissue	T-RFLP	<i>Firmicutes</i> , <i>Actinomycetaceae</i>	<i>Bacteroides</i>
Kabeerdoss et al <sup>55</sup>	Adult	20	Feces	TTGE		<i>C. leptum</i> , <i>Faecalibacterium prausnitzii</i>
Hansen et al <sup>51</sup>	Pediatric	13	Tissue	Pyro	<i>Faecalibacterium (Faecalibacterium prausnitzii)</i>	
Morgan et al <sup>25</sup>	Adult	121	Tissue	Pyro	<i>Clostridium</i> , <i>Enterobacteriaceae (Escherichia/Shigella)</i>	<i>Roseburia</i> , <i>Phascolarctobacterium</i> , <i>Ruminococcaceae (Faecalibacterium)</i>
Fujimoto et al <sup>54</sup>	Adults	47	Feces	T-RFLP		<i>Faecalibacterium prausnitzii</i>
Docktor et al <sup>60</sup>	Pediatric	40	Tongue and buccal mucosa brushings	PMA	<i>Spirochaetes Synergistetes Bacteroidetes</i>	<i>Fusobacteria</i> , <i>Firmicutes</i>
Kaakoush et al <sup>61</sup>	Pediatric	19	Feces	Pyro	<i>Bacteroidetes Proteobacteria</i>	<i>Clostridia</i> , <i>Coprococcus</i> , <i>Roseburia</i> , <i>Ruminococcaceae</i> ,
Thomazini et al <sup>62</sup>	Adult	8	Tissue (rectum)	Culture	<i>Escherichia coli</i>	
Frank et al <sup>43</sup>	Adult	35	Tissue	CI	<i>Enterobacteriaceae</i> , <i>Ruminococcus gnavus</i>	
Andoh et al <sup>63</sup>	Adult	31	Feces	T-RFLP	<i>Bacteroidetes</i>	<i>Clostridium</i>
Walker et al <sup>64</sup>	Adult	6	Tissue	CI, qPCR	<i>Bacteroidetes</i> , <i>Enterobacteriaceae</i>	<i>Firmicutes</i> , <i>Bacteroides vulgatus</i>
Joossens et al <sup>48</sup>	Adult	68	Feces	DGGE	<i>Ruminococcus gnavus</i>	<i>Dialister invisus</i> , <i>Clostridium cluster XIVa</i> , <i>Faecalibacterium prausnitzii</i> , <i>Bifidobacterium adolescentis</i>
Willing et al <sup>47</sup>	Adult	29	Feces	Pyro	<i>Ruminococcus gnavus</i> , <i>Enterobacteriaceae</i>	<i>Faecalibacterium</i> , <i>Roseburia</i>
Verma et al <sup>65</sup>	Adult	12	Tissue	qPCR	<i>Eubacterium Methanobrevibacter</i>	<i>Ruminococcus</i> , <i>Lactobacillus</i> , <i>Bifidobacterium</i> , <i>Bacteroidetes</i>

TABLE 1 (Continued)

Author	Population	N	Sample Type/Site	Method	Taxa Enriched in CD	Taxa Decreased in CD
Schwartz et al <sup>53</sup>	Pediatric	40	Feces	qPCR	<i>Escherichia coli</i>	<i>Faecalibacterium prausnitzii</i> , <i>Bifidobacterium</i>
Kang et al <sup>66</sup>	Adult	6	Feces	PMA, qPCR	<i>Enterococcus spp.</i> , <i>Clostridium difficile</i> , <i>Escherichia coli</i> , <i>Shigella flexneri</i> , <i>Listeria sp</i>	<i>Eubacterium rectale</i> , <i>Bacteroides fragilis</i> group, <i>B. vulgatus</i> , <i>Ruminococcus albus</i> , <i>R. callidus</i> , <i>R. bromii</i> , <i>Faecalibacterium prausnitzii</i>
Fyderek et al <sup>67</sup>	Pediatric	22	Tissue	Culture	<i>Streptococcus Bifidobacteria</i>	
Willing et al <sup>46</sup>	Adult	14	Tissue	T-RFLP, CI, qPCR	<i>Escherichia coli</i>	<i>Faecalibacterium prausnitzii</i>
Andoh et al <sup>68</sup>	Adult	34	Feces	T-RFLP	<i>Bacteroides</i> , <i>Enterobacteriales</i>	<i>Clostridium cluster IV, XIVa</i>
Takaishi et al <sup>69</sup>	Adult	23	Feces	Culture	<i>Lactobacillus Enterococcus</i>	<i>Bacteroides fragilis</i> , <i>B. vulgatus</i> , <i>B. ovatus</i> , <i>Clostridium coccooides</i> , <i>C. leptum</i> , <i>Atopobium</i> , <i>Bacteroidaceae</i> , <i>Bifidobacteria</i> , <i>Veillonella</i>
Dicksved et al <sup>45</sup>	Adult	11	Feces	T-RFLP	<i>Bacteroides ovatus</i> , <i>Bacteroides vulgatus</i>	<i>Bacteroides uniformis</i>
Sepehri et al <sup>70</sup>	Adult	10	Tissue	ARISA, T-RFLP	<i>Clostridia</i>	<i>Bacteroidetes</i>
Manichanh et al <sup>27</sup>	Adult	6	Feces	FISH, CI		<i>Firmicutes (C. leptum)</i>
Scanlan et al <sup>71</sup>	Adult	16	Feces	DGGE		<i>Bacteroides</i>
Sokol et al <sup>72</sup>	Adult	13	Feces	FISH		<i>Clostridium leptum</i>
Bibilioni et al <sup>73</sup>	Adult	20	Tissue	FISH, CI	<i>Bacteroidetes Verrucomicrobia</i>	
Gophna et al <sup>74</sup>	Adult	6	Tissue	CI	<i>Bacteroidetes</i> , <i>Proteobacteria</i>	
Swidsinski et al <sup>75</sup>	Adult	20	Tissue	FISH	<i>Bacteroides fragilis</i> , <i>Bacteroidetes-Provetella</i>	<i>Eubacteria rectale</i>
Ott et al <sup>76</sup>	Adult	26	Tissue	CI		<i>Bacteroidetes</i> , <i>Eubacterium</i> , <i>Lactobacillus</i>
Seksik et al <sup>77</sup>	Adult	17	Feces	TTGE	<i>Enterobacteria</i>	<i>Clostridium Coccooides</i>

All changes in taxa in Crohn's disease subjects are in comparison with healthy controls.

ARISA, automated ribosomal spacer analysis; CI, clones; DGGE, denaturing gradient gel electrophoresis; 2D-DIGE, 2-dimensional differential gel electrophoresis; FISH, fluorescent in situ hybridization; PMA, phylogenetic microarray; Pyro, 454 pyrosequencing; qPCR, quantitative PCR.

a reduction in abundance of Firmicutes and Bacteroidetes and an increase of Proteobacteria.

In a longitudinal study of the fecal microbiome of 33 patients with Crohn's disease, Rajca et al<sup>79</sup> explored the changes in the gut microbiota predictive of clinical relapse after infliximab discontinuation. Lower rates of Firmicutes were seen in those who relapsed compared with nonrelapsers. A low rate of *F. prausnitzii* ( $P = 0.014$ ) and a low rate of *Bacteroides* ( $P = 0.030$ ) predicted relapse independently from systemic inflammation measured by C-reactive protein level ( $P = 0.0001$ ).<sup>79</sup>

### Treatment for Crohn's Disease Affects the Microbiome

One of the major difficulties in characterizing the microbiome in Crohn's disease is understanding the effect of treatments, including antibiotics, on the GI microbiota. Antibiotics have been shown to consistently and significantly reduce ecological diversity in patients with and without Crohn's disease.<sup>25,80</sup>

However, if courses of antibiotics are short, prompt recovery of the intestinal microbiota may be seen.<sup>81</sup> Repeated antibiotic exposures however can lead to long-term perturbation of the gut microbiota as demonstrated by Dethlefsen et al<sup>82</sup> when they examined the effect of repeat courses of Ciprofloxacin on the gut microbiota of 3 individuals over 10 months. Morgan et al<sup>25</sup> examined the effect of antibiotics on an IBD population and found that antibiotics were among the strongest factors associated with a reduction in ecological diversity with many individual bacterial clades being greatly reduced or nearly absent after administration of antibiotics, including *Collinsella*, *Dorea*, *Butyricoccus*, *Subdoligranulum*, and *Acetivibrio*.

The effect of nonantibiotic IBD treatments on the GI microbiome has not been extensively investigated; however, the current data are contradictory. One study has shown that mesalamine is associated with significant reductions in *Escherichia/Shigella* and that 5-aminosalicylic acid and immunosuppressant treatments are associated with modest increases in *Enterococcus*.<sup>25</sup>

However, other groups have not found that an effect of 5-amino-salicylic acid or azathioprine on the composition of the GI microbiota in Crohn's disease.<sup>14</sup> Lopez-Siles et al,<sup>56</sup> examined the mucosal-associated *F. prausnitzii* and *E. coli* abundance in 45 patients with Crohn's disease and found that although anti-TNF treatment diminished *E. coli* abundance, *F. prausnitzii* remained depleted, suggesting that anti-TNF therapy does not restore the levels of these 2 species to those found in the healthy gut.

If and how potent immunomodulators and biologics have a significant and sustained effect on the GI microbiome and how this may relate to their efficacy in Crohn's disease has not been explored in detail.

### Postoperative Recurrence

The postoperative setting is an ideal clinical environment in which to explore the dynamic changes in the GI microbiome regarding the presence or absence of disease recurrence. Neut et al<sup>83</sup> interrogated the microflora of the ileum using culture-based techniques in 61 patients with Crohn's disease at the time of surgery and at follow-up endoscopy; they then compared these findings to those seen in ileocelectomy and ileoscopy controls. In both Crohn's patients and controls, ileocelectomy induced a significant increase in bacterial counts and diversity in the neoterminal ileum. Postoperative increases in *E. coli* and enterococci were detected in Crohn's patients. Early endoscopic Crohn's recurrence was associated with high counts of *E. coli*, *Bacteroides*, and *Fusobacteria*.<sup>83</sup> Sokol et al<sup>52</sup> in their examination of the ileal mucosa-associated microbiota in patients with Crohn's disease at resection and 6 months postoperatively found that changes in *F. prausnitzii* concentration were associated with a higher risk of ileal postoperative recurrence. A lower proportion of *F. prausnitzii* on resected ileal Crohn's mucosa was associated with endoscopic recurrence at 6 months ( $P = 0.03$ ), suggesting that there may be a microbial signature, detectable at the time of resection, that can inform disease behavior, and the risk of recurrence postoperatively. These findings have also been replicated in smaller studies from Dey et al<sup>84</sup> and De Cruz et al.<sup>85</sup>

### Functional Studies of the Microbiome in Crohn's Disease

Characterization of the GI microbiota at a compositional level does not provide information about the microbial mechanisms and metabolism that may be disturbed in disease. Given the relative stability of the functional composition of the gut microbiota in health<sup>5</sup> and the extensive changes observed in IBD,<sup>25</sup> attention has focused on examining the functional potential of the metagenome. The transcriptomes of gut bacteria are dynamic and responsive to changes in the physiochemical conditions present in the colonic environment. Changes in microbial function between healthy controls and IBD appear more extensive and more consistent compared with changes in community structure.<sup>25</sup>

Products of bacterial activity have a regulatory effect on inflammation in IBD.<sup>86</sup> Microbial metabolism has been found to be altered in Crohn's disease.<sup>25,87</sup> Morgan et al<sup>25</sup> showed that

amino acid metabolism showed major perturbation in IBD. Metagenomic and metaproteomic studies have confirmed a decrease in butanoate and propanoate metabolism genes in ileal Crohn's disease<sup>25</sup> and lower overall levels of butyrate and other short-chain fatty acids (SCFAs).<sup>69,87</sup> Butyrate has been found to decrease TNF production and proinflammatory cytokine expression in intestinal tissue in patients with Crohn's disease offering a potential therapeutic role.<sup>88</sup> The reduction in SCFAs levels appears to be in excess of the decreases in SCFA-producing Firmicutes clades described in microbial composition studies in Crohn's disease suggesting that the expression of SCFAs may also be downregulated.<sup>87</sup>

Specific bacterial protein signals have been shown to be associated with IBD. The IBD metagenome is also associated with an increase in functions characteristic of auxotrophic and pathobiont bacteria (including Proteobacteria and Pasturellaceae), such as a decrease in biosynthesis of amino acids and an increase in amino acid transporter genes.<sup>24,25</sup> A number of studies have also demonstrated an increase in sulfate-reducing bacteria, such as *Desulfovibrio*, sulfate metabolism transport in IBD,<sup>25,89</sup> and a high amount of fecal tryptic activity in patients with Crohn's disease.<sup>90</sup> The IBD metagenome has been shown to be able to manage increasing levels of oxidative stress by increasing glutathione transport and riboflavin metabolism; these pathways may be amplified with increased disease severity.<sup>24,25,28</sup>

Juste et al<sup>28</sup> in a small study of 6 patients with Crohn's disease has provided the first evidence that quantifiable bacterial protein signals are associated with disease. In this study, an increase in *Bacteroides* proteins participating in the protection against oxidative stress (AhpC), protein synthesis, folding and repair (FusA, DnaK, and ClpB), energy saving, and the maintenance of a high carbon flux within both glycolysis and pentose phosphate pathways (PPi-dependent Pfk and TktA-TktB), and adhesion and colonization (PepD) were recognized. These findings may reflect common and significant signals in Crohn's disease; however, the sensitivity and specificity of these signals need to be explored in a larger well-defined population.

### CONCLUSIONS

Interactions between the host and the GI microbiome contribute to Crohn's disease pathogenesis; however, a causal link between specific aspects of the GI microbiome and Crohn's disease remains to be established.

Numerous studies, in heterogenous populations, have illustrated specific taxonomic changes associated with Crohn's disease, with similar changes observed in genetically at-risk but phenotypically normal individuals, implying a role of the host genome in the development of a proinflammatory microbiome. Despite promising correlations between shifts in microbial composition and Crohn's disease, the presence or absence of a single taxon has not been identified as causal or protective against the development of disease. This supports the view that compositional change (dysbiosis), rather than a single putative organism, may be responsible for disease.

One of the biggest challenges in defining the dysbiosis in Crohn's disease is correcting for microbial changes associated with patient age, long-standing disease, the severity of mucosal injury or inflammation, and drug exposure. This has led to an interest in examining the microbiome of newly diagnosed treatment-naïve pediatric populations. In these populations, specific microbial profiles have been described and are distinct from patients with ulcerative colitis and healthy controls. At least in a subset of patients, the microbial shifts observed are the same as those observed in more heterogeneous patient cohorts, which include adults with long-standing or refractory disease and previous exposure to drug treatment. This suggests that this dysbiosis may exist before, rather than as a result of, long-standing intestinal inflammation. In small studies of genetically at-risk but phenotypically healthy individuals, a Crohn's like dysbiosis has been observed, suggesting further that these proinflammatory microbial changes precede the onset of intestinal disease. These studies add to the growing evidence base suggesting a causative association between microbial dysbiosis and Crohn's disease.

The finding that dysbiosis is observed in the intestinal mucosa of patients with Crohn's disease in areas without inflammation or ulceration implies again that these microbial changes can be observed independent of inflammation.

Crohn's disease-specific host and microbe profiles have identified the ileum as the primary inductive site for all forms of Crohn's disease. Postoperative studies, directed at interrogation of the GI microbial community at the neoterminal ileum, the site of Crohn's disease recurrence, may therefore be informative. Such studies may detail a microbial signature associated with early postoperative disease recurrence and, in doing so, assist in identifying the potential role of the specific organisms in triggering disease. Larger studies in this area are needed.

Pediatric studies have suggested that the composition of a patient's GI microbiota may assist in the diagnosis of Crohn's disease and even in the prediction of future disease severity, but validation of these findings in large populations is required. Whether the GI microbiome can predict response to therapy in Crohn's disease is a critical question and one which has not been answered to date. More detailed metagenomic, metatranscriptomic, and metaproteomic studies in large well characterized and homogeneous populations are required to define more specifically the role of the GI microbiome in the multifactorial pathogenesis of Crohn's disease.

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