

## *Escherichia coli* Dysbiosis Correlates With Gastrointestinal Dysfunction in Children With Cystic Fibrosis

Lucas R. Hoffman,<sup>1,2,6</sup> Christopher E. Pope,<sup>1</sup> Hillary S. Hayden,<sup>2</sup> Sonya Heltshe,<sup>1,6</sup> Roie Levy,<sup>3</sup> Sharon McNamara,<sup>6</sup> Michael A. Jacobs,<sup>2,3</sup> Laurence Rohmer,<sup>2</sup> Matthew Radey,<sup>2</sup> Bonnie W. Ramsey,<sup>1,6</sup> Mitchell J. Brittnacher,<sup>2</sup> Elhanan Borenstein,<sup>3,4,7</sup> and Samuel I. Miller<sup>2,3,5</sup>

Departments of <sup>1</sup>Pediatrics, <sup>2</sup>Microbiology, <sup>3</sup>Genome Sciences, <sup>4</sup>Computer Science and Engineering, and <sup>5</sup>Medicine, University of Washington, Seattle; <sup>6</sup>Seattle Children's Hospital, Washington; and <sup>7</sup>Santa Fe Institute, New Mexico

**Cystic fibrosis gastrointestinal disease includes nutrient malabsorption and intestinal inflammation. We show that the abundances of *Escherichia coli* in fecal microbiota were significantly higher in young children with cystic fibrosis than in controls and correlated with fecal measures of nutrient malabsorption and inflammation, suggesting that *E. coli* could contribute to cystic fibrosis gastrointestinal dysfunction.**

**Keywords.** cystic fibrosis; microbiota; *Escherichia coli*; inflammation; malabsorption.

The genetic disease cystic fibrosis (CF) is caused by mutations in the CF transmembrane conductance regulator (CFTR), which regulates epithelial cell ion and water permeability. CFTR mutations lead to chronic respiratory infections and dysfunction at gastrointestinal (GI) mucosal surfaces, resulting in substantial morbidity and mortality. The GI tract is often the site of the earliest manifestations of CF disease, including severe and recurrent intestinal obstruction, malabsorption of fat, protein, and fat-soluble vitamins, and resulting growth failure due to insufficient pancreatic enzyme secretion [1]. Because early growth has been correlated with overall morbidity and mortality in CF [1], there is great interest in understanding the determinants of growth success and failure. The advent of oral pancreatic enzyme and vitamin replacement therapies has improved CF outcomes, but both early growth failure and

intestinal obstruction persist [1], indicating that other factors likely contribute to these morbidities.

Recently, a small number of studies indicated that the GI microbiota of people with CF could be abnormal [2–4]. These studies focused on older people with CF who had received substantial amounts of antibiotics, lacked non-CF controls, and/or used technologies that either detected only individual bacteria (such as culture or species-specific polymerase chain reaction) or that could not distinguish microbes at the species level. To rigorously identify CF-specific GI microbiota that are not due to treatments, a study would optimally compare samples from very young children with and without CF using metagenomic tools. There are several characteristics inherent to the CF GI tract that could select for altered microbiota; these include ion and fluid abnormalities, accumulation of mucus, malabsorption that alters the GI luminal nutrient pool, and possible defects in mucosal immune function that all result from CFTR dysfunction. The GI tracts of people with CF are also frequently inflamed and may have impaired motility [1]. All of these properties may result in CF-specific GI microbiota, which could in turn further alter subsequent nutrient processing, inflammation, and mucosal immunity, potentially resulting in adverse long-term health consequences.

We collected fecal samples from young children with CF and pancreatic insufficiency ranging in age from several weeks to 5 years, and similar-aged, healthy children without CF, and analyzed their microbiota as a first step in probing this hypothesis. Demographic information is presented in [Supplementary Table 1](#). Using metagenomic phylogenetic analysis (MetaPhlan [5]) of metagenomic shotgun sequences generated with the Illumina HiSeq platform, we compared the microbiota in these samples among children with and without CF.

### METHODS

Methodologic details are included in the online [Supplementary Data](#). This study was approved by the Seattle Children's Hospital Institutional Review Board; informed consent was obtained for all study subjects.

### RESULTS AND DISCUSSION

We analyzed 44 samples from 12 children with CF, and 39 from 12 children without CF. A striking difference in microbiota constituency was immediately evident during the comparative

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Correspondence: Lucas R. Hoffman, MD, PhD, Pulmonary Division, University of Washington, 4800 Sand Point Way NE, MS OC.7.720, Seattle, WA 98105 (lhoffm@u.washington.edu).

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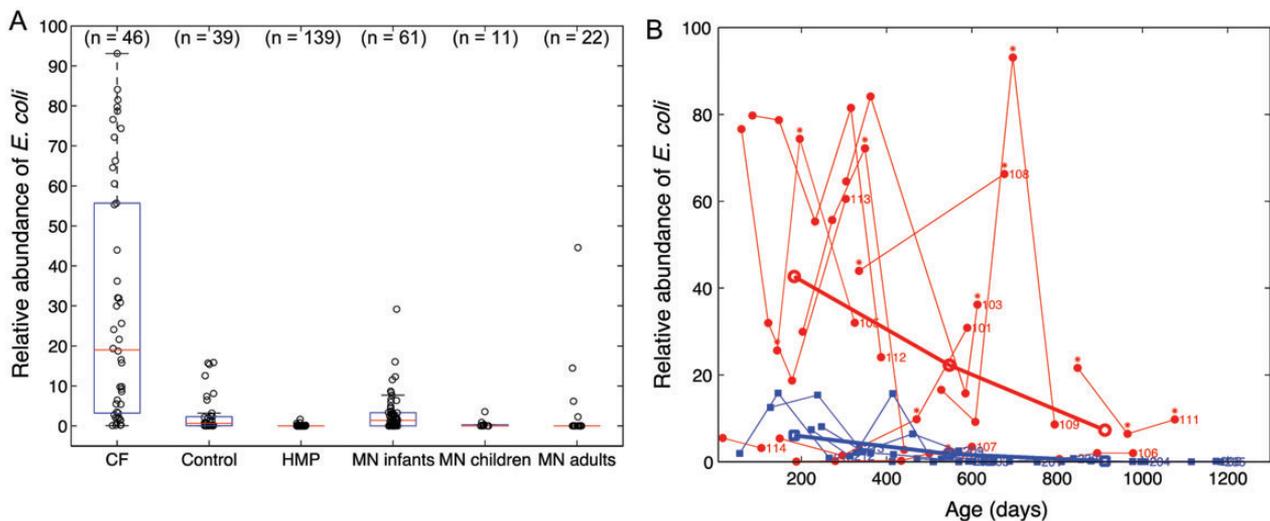
phylogenetic analysis of the CF vs non-CF fecal samples (Supplementary Table 2). As shown in Figure 1, the gram-negative bacterium *Escherichia coli* was markedly more abundant in the samples from children with CF than in those without CF.

*Escherichia coli* usually comprises <5% of bacterial cells in human fecal samples by approximately 1 month of age, generally declining to <1% and remaining as such thereafter [6]. Accordingly, the abundances of *E. coli* were significantly higher than those reported in previous microbiota studies of pediatric populations (Figure 1) [7]. Relatively high abundances of *E. coli* in the large intestine have been associated with inflammation and GI carcinogenesis in inflammatory bowel disease [8], functionally linking dysbioses in which this species is overrepresented with GI disease.

A multivariate mixed effect model accounting for repeated measures was used to assess whether the abundance of *E. coli* correlated with clinical characteristics. Because antibiotic treatment has also been associated with a transient increase in *E. coli* abundance in the GI tract, and because fecal microbiota of antibiotic-treated children largely return to pretreatment configurations within 15 days [9], the 12 samples taken within 30 days of antibiotic treatment (all of which were from 7

subjects with CF, Supplementary Tables 1 and 4) were excluded for this analysis. Of the variables included in the model (CF, age, breast or formula feeding, table food diet, fecal levels of the inflammatory marker calprotectin, and fecal fat content), calprotectin and the fractional amount of fat in the stool each independently correlated significantly with *E. coli* abundance (both  $P < .02$ ; Supplementary Table 3). Whereas CF was associated with *E. coli* abundance in a univariate model ( $P < .0001$ ), this association was lost after adjusting for fecal fat and calprotectin because of their strong association with CF.

Chronic respiratory infection of children with CF is often reflected by clonal expansion of individual lineages of specific bacterial species in the lung. To determine whether *E. coli* fecal populations in children with CF were similarly clonal, we performed a computational multilocus sequence typing (MLST) analysis using reads from the metagenomic shotgun data for 8 CF and 10 non-CF samples (each sample from a different subject) that mapped to the 7 *E. coli* housekeeping genes in the MLST scheme. This analysis showed that, unlike respiratory infections, both CF and non-CF gut *E. coli* populations were just as commonly comprised of individual or multiple concurrent lineages. Furthermore, analysis of *E. coli* gene content from the



**Figure 1.** A, Box-and-whisker plot of the relative abundance of *Escherichia coli* in all fecal samples from young children in the study with and without cystic fibrosis (CF), revealing markedly higher abundance of *E. coli* in fecal samples from subjects with CF. *Escherichia coli* abundances in other non-CF populations from published and archived data are included for comparison, including 139 adult samples from the Human Microbiome Project (<http://www.hmpdacc.org/>) and 94 samples from a multinational microbiome study of healthy children and adults [7], partitioned into infants (aged  $\leq 1$  year), children (aged 1–3 years), and adults (aged  $>3$  years). MetaPhlAn (see Methods) was used to analyze all samples. Similar results were obtained with 16S ribosomal RNA gene pyrosequencing (Spearman correlation = 0.896,  $P < .001$ ). The relative *E. coli* abundances for the CF samples were significantly different from those obtained for all of the non-CF analyses shown ( $P < .01$ ). B, Temporal pattern of *E. coli* abundance in fecal samples from children with (red) vs without (blue) CF. Relative abundance is plotted by age of source subject; 2 samples from a child older than 3 years and for which no there were comparison control samples are omitted for clarity. Subject numbers for children with CF are shown. The bold lines illustrate the average *E. coli* relative abundance for all CF (red) vs all non-CF (blue) samples within a year of age (ie, age 0 days to 365 days, 366 to 730 days, etc), with each average plotted at the midpoint for each year. Red asterisks indicate those samples from subjects with CF that were collected within 30 days after antibiotic treatment; 2 subjects with CF (113 and 114) never had any antibiotics recorded over their lifetimes. Abbreviations: CF, cystic fibrosis; HMP, Human Microbiome Project; MN, multinational.

metagenomic data did not demonstrate any unique virulence-associated features, such as enrichment for specific adhesins or other virulence genes, in the CF vs non-CF populations. Therefore, although *E. coli* was more abundant in CF fecal samples, there were no unique epidemiologic features of CF GI *E. coli* populations to suggest specific pathogenic characteristics in these organisms, at least at this early stage of disease.

Previous, qualitative studies of CF fecal microbiota have identified dysbioses characterized by relatively sparse representation of the genus *Bifidobacterium* [2–4] and lower overall diversity [3] compared to samples from people without CF. However, the subjects in these studies were generally much older than infants, leading the authors to speculate that those differences were due to the high burden of antibiotics to which their subjects were exposed. In 2 recent studies of fecal microbiota of infants [10] and older children and adults [4] with CF, bacterial taxa identified as *Escherichia* were also found to be abundant, although not quantified; no comparison with non-CF samples was made; and no analyses of the relationships with diet, treatments (including antibiotics), fecal fat, or measures of inflammation were presented. In contrast, the focus of this study on young children, the correlations with malabsorption and inflammation, and the exclusion in the statistical analyses of samples collected after recent antibiotic treatments indicate that the observed dysbiosis resulted from characteristics of the CF GI tract lumen itself, rather than therapy or diet. However, these results did not identify whether the *E. coli* dysbiosis shown here in young children with CF was the direct result of CFTR dysfunction, a general property associated with malabsorption and inflammation in the GI tract, or both.

The correlation with fecal fat content could reflect nutritional selection for *E. coli* that can metabolize the protein or lipids that are poorly absorbed, and are therefore abundant, in the CF GI tract [1]. Alternatively, because fecal fat content serves in part as a marker of GI tract CFTR dysfunction, the relationship with *E. coli* abundance may actually be due to other dysfunctional features characteristic of the CF GI tract, such as decreased motility or mucus accumulation [1]. The chief source of nutrients for *E. coli* in the healthy GI tract has been shown to be mucus-associated sugars [11], and accumulated mucus could therefore result in the expansion of mucus-metabolizing *E. coli* populations.

The higher fecal measures of calprotectin, indicative of GI inflammation, observed among the children with CF in this study are in agreement with findings from previous studies [1]. It is tempting to speculate about whether the observed higher *E. coli* abundance is the source or product of GI inflammation. Elevated *E. coli* abundances (but not as strikingly high as in this study) have been observed among people with diverse inflammatory bowel diseases [8]. The collective evidence from these and other studies suggest that the changes that occur in the

intestinal lumen during inflammation can both increase the ability of *E. coli* to colonize the gut mucosa and further induce inflammation [12]. As *E. coli* populations may have expanded in the GI tracts of the children with CF because of their abilities to utilize the higher levels of fat and mucus present, *E. coli* abundance may serve as both a marker and cause of CF GI dysfunction and disease.

Regardless of the mechanism, high *E. coli* fecal abundance could contribute to GI inflammation [8], alter lipid metabolism and absorption [13], and worsen CF GI disease and malnutrition. Thus, early measures of *E. coli* dysbiosis could predict severe GI disease, subsequent poor growth, and related CF clinical outcomes. The identification of *E. coli* dysbiosis in these young children could therefore represent an opportunity for intervention and improved outcomes for people with CF through therapeutic manipulation of their GI microbiota. This exploratory study highlights the need for additional research to determine the *E. coli* abundance in the fecal microbiota of a larger population of children with CF, changes in those microbiota as children age, the causes of the observed dysbiosis, and its impact on early CF clinical outcomes.

## Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (<http://cid.oxfordjournals.org/>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

## Notes

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**Potential conflicts of interest.** All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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