Biochemical and Biophysical Research Communications 445 (2014) 269–274

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Characterization of the intestinal microbiome of Hirschsprung's disease with and without enterocolitis

Zhilong Yan [a,1], Valeriy Poroyko [b,1], Song Gu [a], Zheng Zhang [c], Liya Pan [d], Jing Wang [c], Nan Bao [a], Li Hong [d,*]

[a] Department of Surgery, Shanghai Children's Medical Center, Shanghai Jiaotong University School of Medicine, China
[b] Department of Surgery, Chicago Comer Children's Hospital, University of Chicago, United States
[c] Pediatric Translational Medicine Institute, Shanghai Children's Medical Center, Shanghai Jiaotong University School of Medicine, China
[d] Department of Clinic Nutrition, Shanghai Children's Medical Center, Shanghai Jiaotong University School of Medicine, China

ARTICLE INFO

Article history:
Received 27 December 2013
Available online 10 February 2014

Keywords:
Hirschsprung's disease
Hirschsprung-associated enterocolitis
Metagenomics

ABSTRACT

Hirschsprung's disease (HD) is a congenital malformation of the gastrointestinal tract characterized by the absence of the distal enteric nervous system. Hirschsprung-associated enterocolitis (HAEC) is severe life threatening complication of HD. The disease pathogenesis is still unclear, but evidences suggest that the intestinal microbiota may play important role in the development of HD and HAEC. Because microbial abundance and diversity might differ in HD patients with enterocolitis, we sought to generate comparative metagenomic signatures to characterize the structure of the microbiome in HD patients with and without enterocolitis. Our experimental design is to enroll four HD patients (two with enterocolitis and two without enterocolitis). The microbiome was characterized by 16S rRNA gene, and the data obtained will be used to taxonomically classify and compare community structure among different samples. We found that the structure of the microbiome within HAEC patients are differ from those without enterocolitis. This study helps us to understand microbial contributions to the etiology of Hirschsprung-associated enterocolitis.

1. Introduction

The human intestine contains a vast population of microbes which are essential for the control of intestinal epithelial homeostasis, mucosal inflammation, intestinal development and human health [1]. Given the importance of the microbiome in human health, understanding the role of microbial communities in human health is emerging as one of the most important and fascinating biomedical challenges of our times. The microbes are organized into complex communities adapted to inhabit niches of human body [2–4]. Such ecosystems carry a broad range of functions, and the rise of pathogens within such communities cause infection and inflammation, which causes a series of challenges in biomedical researches. Recent microbiome diversity works seem to point to a new perspective in which the etiology is attributed to a shift in the global balance of the microbial flora rather than to the specific appearance of individual pathogens [5–8].

Hirschsprung's disease (HD), usually diagnosed in newborns, is a congenital malformation of the gastrointestinal tract characterized by the absence of the distal enteric nervous system and is a birth defect that affects about 1 out of 5000 individuals [9]. Older infants and children with HD usually present chronic constipation. Hirschsprung-associated enterocolitis (HAEC) is severe life threatening complication of the congenital HD, which can lead to distension, diarrhea, fever and even death. The etiology of HAEC remains controversial, however, the role of microbiome in the development of HAEC has been proposed and HAEC is thought to be in part due to bacterial overgrowth caused by stool retention [10]. It has been documented that a loss of essential homeostatic balance can lead to pathologic infections, such as *Clostridium difficile*, which is associated with HD and HAEC [11]. However, the association of *Clostridium* species as a cause of HAEC remains controversial [12,13]. Recently, Shen et al. [14] quantify *Lactobacilli* and *Bifidobacteria* from 30 HD infants and 15 controls and found a statistically significant decrease in the levels of probiotic bacteria in the HD patients.

Recent advances in DNA sequencing technology enable scientists to generate billions of nucleotide bases at a relatively lower cost. This deep sequencing based method has revealed an unex-

* Corresponding author. Fax: +86 21 50891405.
E-mail address: lihongscmc@aliyun.com (L. Hong).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2014.01.104
0006-291X/

pectedly high diversity of human gut microbiome. Up to date, we still do not know how many of these microbes contribute to HAEC and how common or exclusive are the HD with and without enterocolitis. In this work, to better understand show microbiomes related to HAEC and HD patients, we have undertaken a comparative sequencing survey of the intestinal microbiomes of the HAEC and HD patients.

2. Materials and methods

2.1. Patients and samples

The subject population consisted of four patients (two patients with HAEC and two with HD). This study was performed on patients treated at Shanghai Children's Medical Center between April 20, 2012 and August 14, 2012. The study was approved by the joint committee of ethics of the Shanghai Children's Medical Center affiliated to school of medicine, Shanghai Jiaotong University (SCMCIRB-K2012022). Written informed consent with a signature was obtained from the parents of each patient. Study cohort is comprised of 4 patients: 2 with HAEC (No. 1 male, age 2 month; No. 2 male, 6 month) and 2 patients with HD (No. 3 female, age 7 month; No. 4 male, 12 month). Specimens of intestinal content were taken during the surgery from different sites along the intestine of patient No. 1 (ileum, appendix, transverse colon, and rectum), No. 2 (appendix, ascending colon, sigmoid colon, and, rectum), No. 3 (proximal and distal colon), No. 4 (appendix, transverse colon, and rectum). Specimens of intestinal content were taken during the surgery from different sites along the intestine (Table 1). Samples were cooled to 4 °C (dry ice) immediately after collection, and then frozen at −80 °C within 30 min. All patients had not been given probiotics and antibiotics at least 5 days before sample collection. All the patients were confirmed by the pathologic diagnosis.

2.2. DNA extractions

DNA from different samples was extracted using the QIAamp DNA Stool Mini Kit (Qiagen, Inc., Valencia, CA, USA) according to manufacturer's instructions. At first, a minimum of 2 ml of Buffer ASL and 300 mg of samples was used in the protocol; and then a ratio of 700 μl of Buffer ASL per 100 mg of sample weight was used for larger volumes using no more than 1500 mg of samples and 10.5 ml of Buffer ASL; next, following the addition of Buffer ASL to each sample, 0.70 mm Garnet Beads were added to the suspension and vortexed for 10 s; at last, a second bead-beating was performed following the heating of the suspension in 0.1 mm Glass Bead Tubes, and vortexed for 10 min.

2.3. 16S rDNA sequencing

Using the total DNA from the 13 samples as a template, we amplified the V1–V3 region of the bacterial 16S rRNA. PCR pools were sequenced using MiSeq Libraries that were constructed with the Illumina Nextera XT kit. It has been sequenced on an Illumina MiSeq using 2×250 bp paired-end sequencing according to the manufacturer's instructions. Sequencing reads with primer sequences were removed. All sequences are available upon request.

2.4. Data analysis

We next clustered the sequences using the CD-hit-est based clustering method [15]. Operational taxonomic units (OTUs) were defined using a 97% sequence similarity cutoff, corresponding to species-level groupings. Next, sequences were grouped into various OTUs using Felsentein-corrected similarity matrices and the sequences within an OUT share at least 97% similarity. The Ribosomal Database Project (RDP) classifier [16] was used to classify the 16S rDNA into distinct taxonomic category by aligning sequences to a curated database of taxonomically annotated sequences. All 16S rDNA sequences were mapped to the RDP database using BLASTN in order to achieve taxonomic assignments. Sequences greater than 97% identity were used to associate a group of OUTs to specific species, while those with less than 97% identity were considered novel reads. Statistical significance of factors potentially contributing to compositional differences between samples was examined using the non-parametric permutation analysis of similarity (ANOSIM), analogous to the univariate ANOVA test.

3. Results

3.1. A deep look at the microbiome in HD and HAEC patients

We compared the intestinal microbiomes of HD children (ages 7 and 12 months) to that of HAEC children (ages 2 and 6 months) by taking intestinal contents from different sites along the intestine during the surgery (Table 1 and Fig. 1). DNA was isolated from the samples of different sites along the intestines of HD and HAEC patients. The deep sequencing technology was used to produce a substantial genomic data for the human intestinal microbiome. Specifically, we generated 16S rDNA data from 13 samples of four

Table 1
Characteristics of the participants in this work.

Subject No.	Patients No.	Gender	Age (months)	Diagnosis	Site	Grouping
1	1	Male	2	HAEC	Transverse colon	Proximal
2	1				Appendix	Proximal
3	1				Ileum	Proximal
4	1				Rectum	Distal
5	2	Male	6	HAEC	Ascending colon	Proximal
6	2				Sigmoid colon	Distal
7	2				Appendix	Proximal
8	2				Rectum	Distal
9	3	Female	7	HD	Distal of the colon	Distal
10	3				Proximal of the colon	Proximal
11	4	Male	12	HD	Transverse colon	Proximal
12	4				Appendix	Proximal
13	4				Rectum	Distal