Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.47750/jptcp.2023.30.03.066

Application of Metagenomics approaches for Crohn's Disease Istanbul University

Omar Sajer Naser[1]*, Elmira Davasaz Tabrizi[2]*, Dr. Ercan Arican[3]*
[1]Department of Molecular Biology and Genetics, program of biotechnology, Istanbul University, Istanbul, Turkey;
[2,3]Department of Molecular Biology and Genetics, Istanbul University, Istanbul, Turkey;

***Corresponding author:** Omar Sajer Naser, Department of Molecular Biology and Genetics, program of biotechnology, Istanbul University, Istanbul, Turkey, Email: omarsajer89@gmail.com

Submitted: 11 November 2022; Accepted: 08 December 2022; Published: 10 January 2023

ABSTRACT

Crohn's Disease (CD) also called the Chronic inflammation of the intestinal system, as well known as regional enteritis or regional ileitis generally occurs in the terminal segment of the ileum, the section of the small bowel farthest from the stomach (Barbara A. Schreiber, 2022). The study's aim was to identify the differences in the gut microbiome composition between patients with and without CD, as well as compare them with healthy control samples (a total of 35 human blood samples). This study is focusing on the mutations/variations in innate immunity and adaptive immunity genes, which increase the risk of inflammatory bowel disease. With that, it aimed to evaluate the effects of mutations on microbiota balance and dysbiosis observed in CD patients (Petagna L et al., 2020). As indicated in the results, we found the first principal coordinate which shows a percentage of 55.09% for the PC1 while showing a percentage of 22.26% for the C2. Moreover, the other principal coordinate shows a percentage of 55.09% for the PC1 while showing a percentage of 9.39% for the C2. Additionally, In the taxonomic classification, we found that bacterial community was found in samples C1 and C2 as similar to a CD. According to the results, all the microorganisms present are bacteria, but with differences in the phylum, order, family, genus, and species. Furthermore, it can be inferred that at the phylum level, the dominant bacteria are proteobacteria, at the class level, the dominant ones are gammaproteobacteria while at the order level, the dominant ones are pseudomonadellas.

Keywords: *Crohn's disease. Gastrointestinal. Metagenomics*

.

INTRODUCTION

Crohn's disease is an illness of the gastrointestinal tract marked by inflammation that affects any area of the digestive system. This condition has a gradual, debilitating course and is more common worldwide. Several factors have been attributed to the development of CD, including a susceptibility to genetic issues, changes in the gut microbiome, environmental exposures and immune system dysregulation. However, no definite cause of CD is currently known. In about 47% of patients, theCD has systemic extraintestinal manifestations (EIM) which significantly impair their quality of life (QOL). CD has a significant impact on patient long-term outcomes; these complications can include bowel problems such as fistulas and strictures. After 10 years of diagnosis, about half of CD patients experience problems with their bowels (Roda Get al., 2020). The Harvey-Bradshaw Index (HBI) and CD Activity Index (CDAI) are two indices regularly used as measures of the disease's activity. Traditional classifications of CD include mild, moderate, and severe disease (Ahmed M, 2021). Increased metabolic demands are also correlated with active gut inflammation, particularly in instances of intestinal resection or active CD of the small intestine (Fiorindi C et al.,2020).

In genetically susceptible hosts, environmental variables influence the likely risk of CD development and progression. In Western countries, smoking is considered one of the main risk factors for celiac disease and increases the likelihood of developing the disease. Table 1 lists some environmental risk factors for celiac disease. Table 1 Significant effect on CD incidence by ethnicity (Kondo K et al.,2019).

TABLE 1: Some of the environmental risk factors for Crohn's disease.

Environmental factors	Association
Smoking	There is a strong link between the beginning of illness and the progression of the disease.
Appendectomy	There is a positive correlation with illness initiation but no correlation with disease progression.
Low dietary vitamin D	There is a relationship between disease onset and progression.
Oral contraceptive use	There is a strong positive correlation with disease beginning but no relationship with disease progression.
Postmenopausal hormone use	There is no link between the development of the disease and the progression of the illness.
NSAID use	Positive correlation with illness start and a substantial relationship with disease progression.
Antibiotic use	Positive relationship with illness start and progression.
Depression and psychosocial stress	There is a positive correlation with illness initiation but no correlation with disease progression.
Low dietary fibre	There is a negative correlation with illness initiation but no correlation with disease progression.
High dietary fat	There is no link between the beginning of the disease and the progression of the illness.
High dietary protein	There is no link between the beginning of the disease and the progression of the illness.

Two Swedish studies found that adhering to a Mediterranean diet lowered the risk of developing late-onset Crohn's disease by a significant margin. However, more research is needed to determine if dietary changes have any effect on overall microbial diversity within the digestive system (Khalili H et al., 2020).

For more than a generation, the source of CD has remained a mystery. In addition, by combining direct patient experience with molecular biology, molecular genetics, and gene sequencing, scientists have made significant progress in the knowledge of the underlying pathways that predispose to the disease. The etiology of celiac disease is largely influenced by genetics. Siblings of CD patients were approximately twice as likely to have the disease than the normal community, and the risk was actually significantly higher among relatives, first-degree (incidence rate 7.8),

second-degree (incidence rate 7.8) and third-degree relatives. In addition, examination of more than 300 twin pairs showed that identical twins were more closely matched for disease phenotypes (37%) than fraternal twins (7%). Surprisingly, about one-third of first-degree relatives had impaired gastrointestinal function, including increased permeability or markers of inflammation such as the neutrophil protein S100A12. Despite this large genetic effect, finding disease-causing genetic mutations has been a challenge (Segal A w, 2019).

Currently, the 110 genes associated with CD and UC that overlap with IBD originate from 163 loci. This range includes 30 genes strictly associated with CD; the length of these genes include innate immune response, adaptive immune response, autophagy and epithelial barrier maintenance. Additionally, the first line of defense against pathogens involves the epithelial cells' TLR recognition and caspase activation recruitment domain 15 (NOD2) combination. The next best defense involves NOD2/CARD15 pathway with nucleotide-binding oligomerization domains and a pathogenic component (caspase activation recruitment domain 15). β-defensin HBDs inhibit pathogen growth and alter the production of specific microbial peptides. Patients with this condition display disrupted immune signaling pathways and altered production of these molecules (Ahlostrom, 2022).

People with CD possess abnormal lysozyme granules in their Paneth cells. These changes are due to the genetic polymorphism T300A in the ATG16L1 gene. The ATG16L1 protein works to fight bacterial infections by enhancing NOD2 immunoglobulin-2 production at sites of bacterial infection. Variations in NOD2 can slow down the ability to recruit ATG16L1 to sites of bacterial infection, which affects autophagy (Gammoh, 2020). The aim of this study was to investigate biomarkers that could be used to analyze risk levels in IBD patients, especially CD patients, and to identify in advance those who are at risk of disease exacerbation. First, human IBD patients were examined for mutations at expected loci, including nucleotide-binding oligomerization domain protein 2 (NOD2), innate and adaptive immunity genes IL-23R.

METHODS

This study took place in two hospitals and one clinic in order to collect the blood samples of Crohn's disease patients. Istanbul University has provided this research by informed consent allowing collecting blood samples, storing, and analysis them in the Molecular Biology and Genetic Department. 25 blood samples have been collected including both genders in the average age between 20-65, additionally, 10 healthy samples collected in order to compare with the disease. In addition, the hospitals and the clinic have provided the research with informed consent which allowed us to explain the study to the patients and have their acceptance.

All participants signed the written informed consent which included research information and the goals. Within 1 year following their initial consent, the research respondents were given surveys included information regarding inflammatory bowel diseases(IBD) plus the CD particularly, disease phenotypic features, surgical histories, and treatment history. Furthermore, only individual surveys were used to obtain a smoking history and a family background of CD.

Apply the blood separation kit according to the instructions in the kit manual. The resulting DNA extracts were quantified using NanoDrop. DNA concentration (ng/ml blood) and purity [absorbance at 260/280 (A260/A280)] were measured spectrophotometrically in clean DNA centrifuge tubes using a NanoDrop 2000 instrument. Pure DNA has an A260/A280 ratio ranging from 1.8 to 2.0. Put the PCR tube in a T100 thermal cycler, and make some changes to the scheme in Table 2 to react according to the change of DNA concentration. TM set between 55-68°C in a volume of 25 μl as shown in Table 2.

Apply samples according to company kit protocol. Reactions were performed in a final volume of 25 μl. PCR tube containing 11.5 μl total DNA, 12.5 μl Taq PCR Master Mix, 1 μl forward primer, and 1 μl reverse primer. GeneRuler DNA Ladder Mix for determining the size and approximation of DNA fragments on agarose gels.

TABLE 2: PCR Analysis Protocol.

Program Name	Initial Denaturation	Amplification			Final Elongation	Cooling
		Denaturation	Annealing	Elongation		
Cycles	1	25-35			1	1
Temperature [°C]	94	94	61.3*	72	72	4
Time	3 min.	30 sec.	30 sec.	1 min.	10 min.	Unlimited time

* In the protocol, the temperature suggested between 55-68°C and in result of our gradient PCR the best recommended temperature is 61.3°C.

RESULTS AND DISCUSSION

The preprocessing

Filter out short reads and trim excessively long tails.

Filtered reads clustered with 100% identity to CD-HIT-DUP.

Identify chimeric reads.

Secondary clusters are recruited into the primary cluster.

Remove noisy sequences in clusters of size x or less. Here x is calculated statistically.

Remaining representative reads from non-chimeric clusters are clustered using a greedy algorithm into OUTs at a user –specified OUT cutoff (e.g. 97% ID at specific level).

Clustering

Filter out ambiguous reads and trim long tails.

Identify chimeras and removing chimeras

Reference files (RDP) – http://www.monthur.org/wiki/RDP_reference_files

Generate a distance matrix.

Clustering (method: Average neighbor).

Taxonomic assignment

Use representative sequences from each OUT to assign taxonomy.

Construct phylogenetic trees from aligned and filtered representative sequences of OUTs.

Produce publication-quality graphic results through statistical analysis and visualization.

Experimental Methods and Workflow

Experiment Overview

The Illumina (NGS) workflow consists of 4 basic steps, as shown in Figure 1:

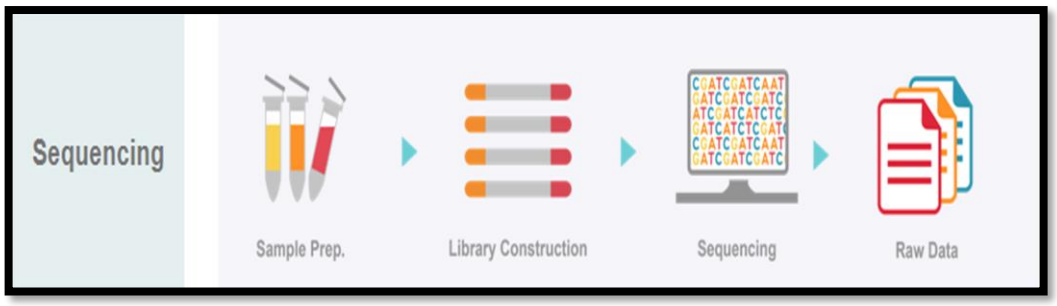

FIGURE 1: Trial overview

Sample Preparation

DNA and RNA are taken from the samples for constructing the library. After quality control is performed, the acceptable samples are put into library construction.

Library Construction

DNA or cDNA samples are randomly fragmented and then joined with 5' and 3' ligation primers. Alternatively, "tagmentation" combines the two steps into one by first fragmenting the sample with a ligation reaction. After that, the resulting DNA is PCR amplified, gel purified and placed into a library.

Sequencing

During cluster creation, libraries are loaded onto surface-bound oligonucleotides that are lined up in flow cells. Fragments are captured on these lawns and then amplified into distinct clonal clusters by bridge amplification. Once the clusters are complete, templates are sorted.

Illumina SBS methods use a terminator-based approach that reversibly identifies individual bases as they are incorporated into the DNA strand. By including all four reversible terminators in every sequencing cycle, natural competition reduces sequence bias and significantly reduces raw error rates. Compared to other techniques, this dramatically decreases the chance of sequence context-sensitive errors even in homopolymers and regions with multiple repeats.

Raw data

Convert sequence data to raw data for analysis.

Generation of Raw Data

Illumina sequencers use sequencing software to generate raw images, with integrated primary analysis software called RTA (real-time analysis) for system control and base calling. BCL (base calling) binaries were converted to FASTQ using the Illumina bcl2fastq package. The adapter will not be read trimmed.

Based on the results, the DNA concentration of the CD sample set was determined to be in the range of 8 ng/ml - 40 ng/ml. A 1465 bp fragment was then amplified using universal DNA primers.

Calculate total bases, reads, GC (%), Q20 (%), and Q30 (%) for Crohn's disease samples. For example, in the control sample, 118,302 reads were generated with a total read base of 35.6 Mbp. GC content (%) is 52.56%, Q30 is 79.21%, as shown in Table 3.

TABLE 3: Raw data Stats for Crohn's disease.

Sample ID	Total reads bases (bp)	Total reads	GC (%)	AT(%)	Q20 (%)	Q30 (%)
CD	40,935,398	135,998	52.4	47.6	90.88	81.82
CD	55,149,220	183,220	51.8	48.2	91.67	83.02
CD	41,969,634	139,434	52.58	47.42	90.3	81.18
CD	55,181,728	183,328	51.78	48.22	90.5	81.57

Total read bases: The total number of bases sequenced.

Total Reads: Total number of reads. For Illumina paired-end sequencing, this value refers to

Reading 1 and Reading 2.

GC (%): GC content.

AT (%): AT content.

Q20(%): Percentage of bases with a phred quality score greater than 20.

Q30(%): Percentage of bases with a phred quality score greater than 30.

Thirty-five participants were included in the study cohort, including 10 healthy controls and 25 patients with Crohn's disease. Patients are not first-degree relatives. Their microbiomes in the gut were characterized in a longitudinal study. General information was collected directly from patients, including their age, diseases they had, their family history of IBD, smoking history of IBD and whether they were taking any medications to aid in recovery.

Gene amplicon sequence analysis was performed to determine bacterial communities in human blood samples from IBD (specifically Crohn's disease) and healthy groups. Merge and filter raw data to obtain clean data. Representative sequence OUTs were performed using the FASTQ file format and read quality control was checked using QIIME. To remove representative reads from non-chimeric clusters, they were clustered into OUT using a greedy algorithm with an application-specific OUT limit of 97% IDs at the species level. Each OTU was analyzed by blast using QIIME to determine phylum annotations in taxonomic order (kingdom, phylum, class, order, family, genus, and species).

Summary of Produced Data

Raw Data Statistics

Calculate the total number of bases, reads, GC (%), Q20 (%), and Q30 (%) for 10 samples. For example, 118,302 reads were generated in C1 with a total read base of 35.6 Mbp. The GC content (%) is 52.56%, and the Q30 is 79.21%. The comparison between the healthy group and the Crohn's disease group is shown in Table 4.

TABLE 4: Raw date statistics for the healthy group and crohn's disease patients.

Sample ID	Total read bases (bp)	Total reads	GC(%)	AT(%)	Q20(%)	Q30(%)
Control 1	35,608,902	118,302	52.56	47.44	89.13	79.21
Control 2	53,534,656	177,856	52.38	47.62	90.98	82.31
CD1	40,935,398	135,998	52.4	47.6	90.88	81.82
CD2	55,149,220	183,220	51.8	48.2	91.67	83.02
CD3	41,969,634	139,434	52.58	47.42	90.3	81.18
CD4	55,181,728	183,328	51.78	48.22	90.5	81.57

From Table 4, it can be concluded that the genetic material in blood samples obtained from Crohn's disease patients is higher than that obtained from healthy patients. This is evidenced by the presence of nitrogenous base pairs in the AT and CG forms, as described above. Numerous findings support the existence of higher genetic material. One of Vrakas et al. (2017), on gut bacterial composition and bacterial translocation in Italian inflammatory bowel disease, supports this. This suggests that the live commensal bacteria in the gut are present in the peripheral blood, they can induce inflammation there.

Principle Coordinate Analysis of Samples (Unweighted Unifrac)

PCoA is a technique that extracts and visualizes a few key pieces of data from multidimensional data. This process maps the different measurements taken into the distance matrix to new axes of rotation that separate it into multiple orthogonal sections. Each section then takes on a new set of axes that measures the variation of a specific measurement. The first axis measured by PC1 shows 55.09% of variation, while the second axis measured by C2 shows 22.26% variation. Figure 2 shows how two-dimensional calculations organize the data into different sections. It also demonstrates C2's measurement results compared to PC1's.

This analysis maximizes the linear correlation between distances in a low-dimensional space. The analysis uses distances collected from many different samples; it works best with small sample sizes.

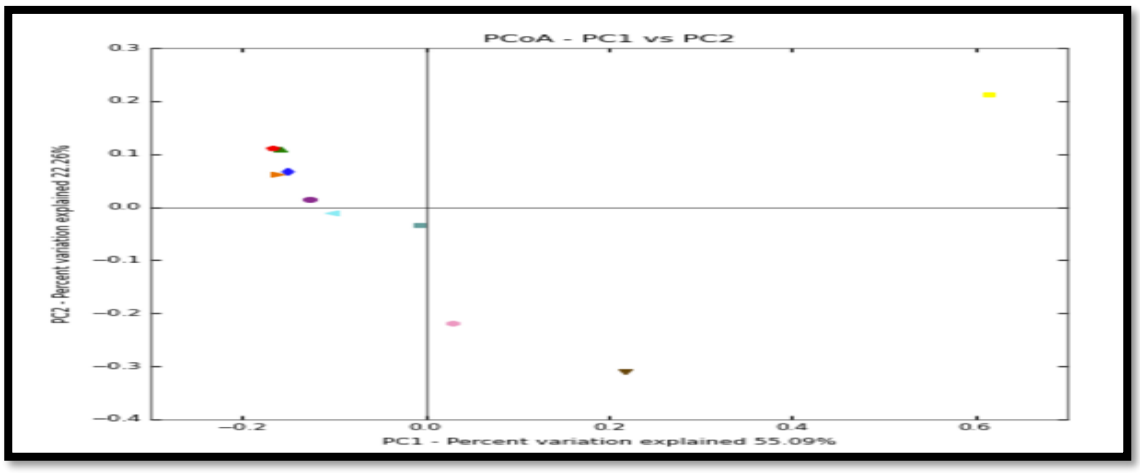

FIGURE 2: The principle coordinate.

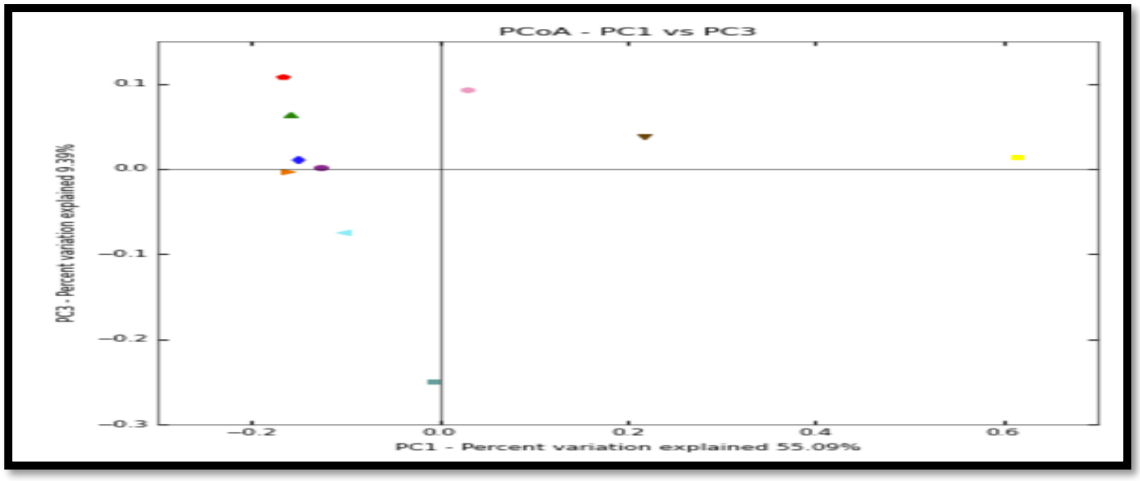

FIGURE 3: The principle coordinate

The principal coordinate shows the percent of 55.09% for the PC1 while shows the percent of 9.39% for the C2 as shown in figure 2.

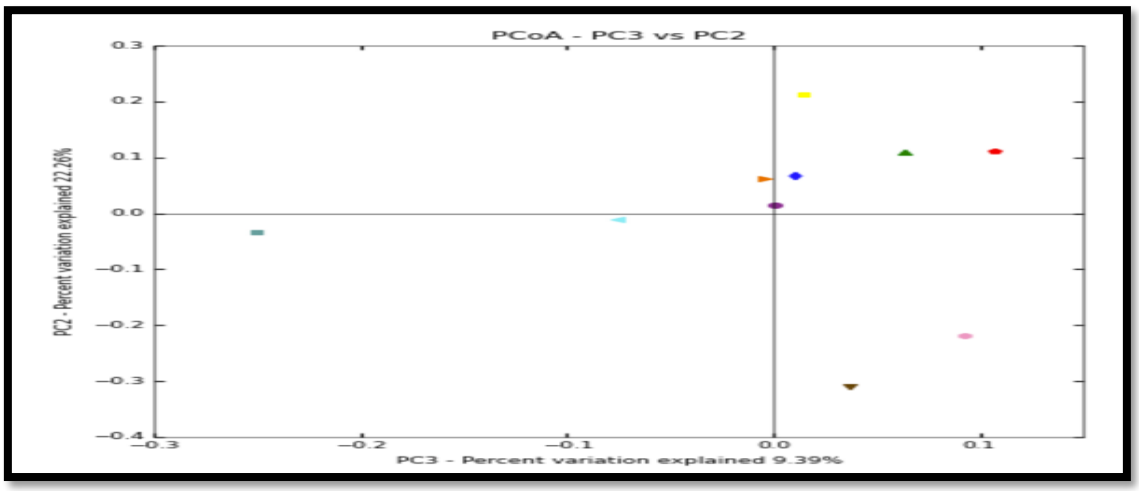

FIGURE 4: The principle coordinate

J Popul Ther Clin Pharmacol Vol 30(3):e649–e658; 10 January 2023.

From Figure 4, the second largest amount of fluctuation is explained by the second principal coordinate, where PC3 has a percentage of 9.39% and PC2 has a percentage of 22.26%.

In addition, Figure 5 shows the results of the community richness and diversity that indicated CD 6 has the highest Shannon and gini-simpson value indices of 0.48 and 1.2 respectively. The least values of the two indices go with CD -3 gini-simpson index of 0.25 and Shannon index of 0.45. According to (Konopiński, 2020), association and dissociation are true among data obtained in research results, however, error rate may be negatively associated with population heterozygosity.

SampleName	OTUs	Chao1	Shannon	Gini-Simpson	Good's Coverage
C1	16.0	16.0	1.07677108046	0.412548246126	0.999883027255
C2	23.0	23.2	1.06172674112	0.396738686805	0.99986891263
CD.1	19.0	21.0	1.02804153552	0.389458223947	0.999654845112
CD.3	17.0	19.0	0.580587146161	0.206066258667	0.999785094129
CD.6	24.0	26.5	1.20314489707	0.463996935976	0.999511957052
CD.7	21.0	24.0	0.839711863996	0.264745416993	0.999886410965

FIGURE 5: Shows the community richness and diversity.

• OTU: In the operational definition of a species or species group, an OTU is typically used when DNA sequence data is not available.

• Chao1: Returns the Choa1 diversity estimate derived from the identified OTU.

• Shannon: The Shannon index takes species number and consistency into account.

• The Gini-Simpson index: The Gini-Simpson index is a measure of the probability that two individuals chosen at random belong to the same species in a habitat.

• Commodity Abundance: Abundance is calculated as C1-(s/n),

o What is the number of unique OTUs and n is the number of individuals in the sample.

The index provides a relative assessment of the sample's proportional representation of the larger context.

Figure 6 presents the taxonomic classification of all the microbial communities present in the samples used through phylum, order, family, genus, and species. According to the results, all the microorganisms present are bacteria, but with differences in the phylum, order, family, genus, and species. However, the paradox here is that bacterial community was found in samples C1 and C2. Since it is not expected, it may be explained that they already have symptoms of IBD but they have yet to examine it, or the symptoms are not acute.

TABLE 6: Taxonomic classification of all the microbial community.

Kingdo	Phylum	Class	Order	Family	Genus	Species	C1	C2	CD.1	CD.3	CD.6	CD.7
Bacteria	_Actinobacteria	_Actinomycetia	_Bifidobacteriales	_Bifidobacteriaceae	_Bifidobacterium	_Bifidobacterium longum	0	0	0	0	0	10
Bacteria	_Actinobacteria	_Actinomycetia	_Corynebacteriales	_Corynebacteriaceae	_Corynebacterium	_Corynebacterium appendicis	0	0	0	0	0	0
Bacteria	_Actinobacteria	_Actinomycetia	_Propionibacteriales	_Propionibacteriaceae	_Cutibacterium	_Cutibacterium granulosum	0	0	0	0	0	0
Bacteria	_Actinobacteria	_Actinomycetia	_Propionibacteriales	_Propionibacteriaceae	_Cutibacterium	_Cutibacterium namnetense	0	0	0	0	0	0
Bacteria	_Bacteroidetes	_Bacteroidia	_Bacteroidales	_Bacteroidaceae	_Phocaeicola	_Phocaeicola vulgatus	0	0	0	0	0	47
Bacteria	_Cyanobacteria	_	_Oscillatoriales	_Oscillatoriaceae	_Aerosakkonema	_Aerosakkonema funiforme	0	0	0	0	0	3
Bacteria	_Firmicutes	_Bacilli	_Lactobacillales	_Lactobacillaceae	_Lactobacillus	_Lactobacillus gallinarum	0	0	0	0	0	13
Bacteria	_Firmicutes	_Clostridia	_Eubacteriales	_Clostridiaceae	_Clostridium	_Clostridium saudiense	0	0	0	0	0	10
Bacteria	_Firmicutes	_Clostridia	_Eubacteriales	_Lachnospiraceae	_Kineothrix	_Kineothrix alysoides	0	0	0	0	0	4
Bacteria	_Firmicutes	_Clostridia	_Eubacteriales	_Lachnospiraceae	_Mediterraneibacter	_[Ruminococcus] gnavus	0	0	0	0	0	3
Bacteria	_Firmicutes	_Clostridia	_Eubacteriales	_Oscillospiraceae	_Acetivibrio	_Acetivibrio alkalicellulosi	0	0	0	0	0	7
Bacteria	_Firmicutes	_Negativicutes	_Selenomonadales	_Selenomonadaceae	_Megamonas	_Megamonas funiformis	0	0	0	0	0	100
Bacteria	_Proteobacteria	_Alphaproteobacteria	_Hyphomicrobiales	_Bradyrhizobiaceae	_Bradyrhizobium	_Bradyrhizobium valentinum	36	40	28	31	37	48
Bacteria	_Proteobacteria	_Alphaproteobacteria	_Hyphomicrobiales	_Methylobacteriaceae	_Methylorubrum	_Methylorubrum populi	0	0	0	0	0	8
Bacteria	_Proteobacteria	_Alphaproteobacteria	_Hyphomicrobiales	_Methylocystaceae	_Methylopila	_Methylopila henanensis	0	0	0	0	0	1
Bacteria	_Proteobacteria	_Alphaproteobacteria	_Hyphomicrobiales	_Rhizobiaceae	_Agrobacterium	_Agrobacterium fabrum	0	1	0	3	1	34
Bacteria	_Proteobacteria	_Alphaproteobacteria	_Sphingomonadales	_Sphingomonadaceae	_Sphingomonas	_Sphingomonas adhaesiva	0	2	0	0	0	0
Bacteria	_Proteobacteria	_Alphaproteobacteria	_Sphingomonadales	_Sphingomonadaceae	_Sphingomonas	_Sphingomonas kyeonggiensis	112	186	105	139	188	1,238
Bacteria	_Proteobacteria	_Alphaproteobacteria	_Sphingomonadales	_Sphingomonadaceae	_Sphingomonas	_Sphingomonas pituitosa	3	4	1	1	3	0
Bacteria	_Proteobacteria	_Betaproteobacteria	_Burkholderiales	_Burkholderiaceae	_Ralstonia	_Ralstonia pickettii	2,111	3,522	2,672	2,487	3,012	2,325
Bacteria	_Proteobacteria	_Betaproteobacteria	_Burkholderiales	_Oxalobacteraceae	_Glaciimonas	_Glaciimonas frigoris	0	2	0	0	1	0
Bacteria	_Proteobacteria	_Betaproteobacteria	_Burkholderiales	_Oxalobacteraceae	_Noviherbaspirillum	_Noviherbaspirillum suwonense	0	4	0	1	1	0
Bacteria	_Proteobacteria	_Deltaproteobacteria	_Syntrophobacterales	_Syntrophaceae	_Smithella	_Smithella propionica	0	0	0	0	0	3
Bacteria	_Proteobacteria	_Gammaproteobacteria	_Pasteurellales	_Pasteurellaceae	_Haemophilus	_Haemophilus paraphrohaemolyticus	0	0	0	0	0	0
Bacteria	_Proteobacteria	_Gammaproteobacteria	_Pseudomonadales	_Pseudomonadaceae	_Azotobacter	_Azotobacter armeniacus	0	0	0	0	2	0
Bacteria	_Proteobacteria	_Gammaproteobacteria	_Pseudomonadales	_Pseudomonadaceae	_Pseudomonas	_Pseudomonas guguanensis	2	11	7	5	6	0
Bacteria	_Proteobacteria	_Gammaproteobacteria	_Pseudomonadales	_Pseudomonadaceae	_Pseudomonas	_Pseudomonas monteilii	7	8	5	1	2	62
Bacteria	_Proteobacteria	_Gammaproteobacteria	_Pseudomonadales	_Pseudomonadaceae	_Pseudomonas	_Pseudomonas paralactis	6,274	11,463	8,768	20,598	6,980	22,493
Bacteria	_Proteobacteria	_Gammaproteobacteria	_Pseudomonadales	_Pseudomonadaceae	_Pseudomonas	_Pseudomonas punonensis	0	4	2	0	6	1
Bacteria	_Proteobacteria	_Gammaproteobacteria	_Pseudomonadales	_Pseudomonadaceae	_Pseudomonas	_Pseudomonas tolaasii	4	10	1	0	6	0
Bacteria	_Proteobacteria	_Gammaproteobacteria	_Xanthomonadales	_Xanthomonadaceae	_	_[Pseudomonas] geniculata	0	0	0	0	0	1

Furthermore, it can be concluded that at the phylum level, the dominant bacteria are Proteobacteria, at the class level, γ-proteobacteria are dominant, and at the order level, Pseudomonas are dominant. In contrast, a study by Ma et al. (2021).

At the phylum level, Firmicutes, Bacteroidetes, Proteobacteria, and Actinomycetes were the dominant bacteria in healthy people, patients with inflammatory bowel disease, and colon cancer patients, among which Firmicutes and Bacteroidetes were the most common.

CONCLUSION

In conclusion, patients with Crohn's disease had a different bacterial composition than healthy controls. It was concluded that blood samples obtained from CD patients contained higher genetic material than samples obtained from healthy patients. This was evidenced by the presence of nitrogenous base pairs in the AT and CG forms included in the findings, despite the relatively high presence of genetic material in healthy controls. The high abundance of bacterial DNA in the form of nitrogenous bases suggests a role for microbes in the development of CD. Although bacteria and genetic material were found in blood samples from healthy subjects, these factors were not examined due to study limitations, which could be explained by other factors.

Therefore, the presence of CD in healthy subjects may be associated with a possible genetic predisposition and the intake of excessive purine- and pyrimidine-rich foods in affected subjects. This study provides evidence for the critical role of microbes in causing IBD, especially CD.

According to statistical methods, CDs consistently had higher total read bases compared to controls. Thus, CD is a complex disease involving the host, microbes (in this case bacteria), and environmental factors. Employing a multidisciplinary approach linking genetic factors, risk factors, and microbes is critical to fully understanding the mechanisms underlying host-microbe interactions in disease development. In this study, bacteria were found to be the main cause of CD, but their taxonomic order varied by strain and species.

REFERENCES

1. Ahmed M., 2021. Crohn's Disease recent advances. IntechOpen. p. 106. DOI: 10.5772/intechopen.97103, Book.
2. Barbara A., Schreiber. 2022. Crohns Disease. The Editors of Encyclopaedia Britannica. https://www.britannica.com/science/Crohn-disease.
3. Fiorindi C. et al., 2020. Nutritional adequacy in surgical IBD patients, clinical nutrition ESPEN. Volume 14.
4. Konopiński M.K., 2020. Shannon diversity index: a call to replace the original Shannon's formula with unbiased estimator in the population genetics studies. Bioinformatics and Genomics,PeerJ 8:e9391 https://doi.org/10.7717/peerj.9391
5. Ma, et al., 2021. Metagenome Analysis of Intestinal Bacteria in Healthy People. Patients with Inflammatory Bowel Disease and Colorectal Cancer. Journal Frontieres in Cellular and Infectious Microbiology. Volume 11.
6. Petagna L. et al., 2020. Pathophysiology of Crohn's disease inflammation and recurrence. Biology Direct. Volume 15. Article number 23.
7. Roda G. et al., 2020. Crohn's disease. Nature Reviews Disease Primers. Volume 6. Article number 22.
8. Segal A W, 2019, Studies on patients establish Crohn's disease as a manifestation of impaired innate immunity, Journal of Internal Medicine, Volume 286, Issue 4.
9. Vrakas S. et al., 2017. Intestinal Bacteria Composition and Translocation of Bacteria in Inflammatory Bowel Disease. Journal pone. Volume 12. Issue 1.
10. Kondo K. et al., 2019. The association between environmental factors and the development of Crohn's disease with focusing on passive smoking: a multicenter case- control study in Japan. PLoS One. Volume 14.
11. Khalili H. et al., 2020. Adherence to a Mediterranean diet is associated with a lower risk of later- onset Crohn's disease: results from two large prospective cohort studies. BMJ Journals. Gut. Volume 69. Issue 9.
12. Ahlostrom G., 2022. Metagenomic analysis of crohn's disease. UPTEC X 22031. Uppsala University.
13. Gammoh N., 2020. The multifaceted functions of ATG16L1 in autophagy and related processes. Journal of Cell Science. Volume 133. Issue 20.