Bacteriophages in Gut Microbiome Interactions in Patients with Inflammatory Bowel Disease: Differences by Sex and The Disease Type

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Research Article

Keywords: IBD, Gut Microbiome Interactions, Bacteriophages, Fungi, Bacteria, Regulatory Candidates

Posted Date: March 2nd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1240997/v2

Abstract

Background: Inflammatory bowel disease (IBD), known as the disease of the century, is a complex condition that affects millions of people worldwide. IBD is influenced by numerous factors such as genetics, lifestyle, and the gut microbial community, yet the role of microorganisms in driving and controlling the disease remains poorly understood. As we know, preceding studies have mainly focused on assessing gut bacteria and less on bacteriophages or fungi, and no study on interactions of the gut microbial community in patients with IBD has looked at bacteriophages in addition to bacteria and fungi by sex. No distinct microbial regulatory candidate has been proposed so far.

Results: Here, metagenomic data were obtained from 456 stool samples of 84 white race volunteers (40 females and 44 males) with no treatment history before sampling. Participants were studied by sex and the disease type using bioinformatics methods. Differences in interactions of bacteriophages, bacteria, fungi, and archaea in the gut of males and females with Crohn's disease were remarkable, indicating the necessity for different therapies for both groups. While, little difference was seen in the gut microbiome relations in females and males with ulcerative colitis.

Conclusions: The fungal strain *Malassezia globose* CBS 7966 beside the bacterial species *Bacteroides stercoris*in ulcerative colitis and *Parabacteroides* phage YZ-2015b in Crohn's disease were the sex-independent regulatory candidates. Uncultured crAssphage was recommended as a sex-dependent regulatory candidate for IBD in men. However, the fungus *Wickerhamomycesciferrii* which had proposed as regulatory candidate in Crohn's disease, was age-dependent in females. Four bacteriophages, such as Escherichia phage pro147, were suggested for study candidates in the metabolism of IBD.

Introduction

Inflammatory bowel disease (IBD) is a set of illnesses that cause inflammation of large and small bowels. The most prevalent conditions are Ulcerative Colitis (UC) and Crohn's Disease (CD), which differ based on the severity of symptoms and degree and site of intestinal involvement. Besides large and small bowels, the CD can affect the mouth, esophagus, stomach, and rectum, while the UC broadly affects the large bowel and rectum [1]. Relationships between IBD and polymorphism in protein-coding gene VDR (Vitamin D Receptor) [2], the risk of being infected with IBD in dysbiosis, and the role of beta carotene in preventing or delaying such disorders [3] are evidence to indicate IBD is influenced by genetics, the gut microbiome, and environmental factors [4]. In a 2014 study in Ontario (Canada), Eric I. Benchimol and colleagues reported the incremental rate of IBD incidence in children and adults (except for those over 65). As noted, the rate of IBD incidence has ascended to 543.3 per 100000 from 1999 to 2008. During these years, according to the Ontario Health Data Platform (OHDP), 74817 cases with positive IBD have been recognized, of which 39147 (52.3%) are females. Out of all patients with IBD, 35610 are diagnosed with positive CD, of which 53.7% are females, 36169 are with positive UC, where 50% of them are females, and ultimately 3038 are with unclassified IBD, of which 54.1% of diagnosed cases are females [5]. In a 30-year study by Lophaven SN et al. in Denmark, the incidence of sex-

independent UC and CD was incremental, and the rate of IBD in females was significantly higher than that of males [6]. In their 2018 study, Shailja et al. studied the IBD incidence in advanced western countries by comparative analysis. They found no meaningful difference in the UC incidence in females and males up to 45 (except for the age range of 5 to 9 years). There were more risk factors for UC in males over 45 than females. For females under 14, as opposed to males, the risk factors for CD were fewer, while at higher ages, females were more suffering from CD risk factors than their male counterparts. A demographic study of IBD in 17 groups from 16 regions of Europe, North America, Australia, and New Zealand through pooled analysis showed a sex-dependent age distribution of the IBD disease. However, more research is required for knowing the mechanisms involved in such sex-dependent differences [7]. The disease is formed by disrupting the epithelial barrier, transferring bacteria from the lumen to the mucosal tissue, and triggering immune responses and ultimately acute inflammations. In this process, alongside bacteria, the gut archaea, fungi, and viruses competing with each other presumably play a role that has been largely overlooked in studies [8]. The large and diverse bacteriophages can regulate the gut bacterial communities. The role of phages to alleviate the IBD symptoms remains unclear, while the positive effect of bacteriophages in FMF of some patients with IBD has been reported [9]. In their 2020 study, Nishiyama et al. discovered a bacteriophage in stool samples of patients with UC that contaminates helpful bacteria in intestinal homeostasis and causes the IBD in patients [10]. In a 2019 study, Ungaro et al. observed eukaryotic viruses in the biopsy of the ileum of a group of patients with IBD with no history of undergoing therapy sessions [11]. In their 2017 study, Sharpton and colleagues recognized some bacterial pathways involved in the IBD by studying changes in the microbiome between healthy and infected mice [12]. Schirmer and co-workers studied the gut bacteria to examine the pattern of variation in the abundance of bacterial species inter or between IBD patients and healthy participants [4]. Fang et al. (2018) studied the stool microbiome of a CD patient. They reported that the frequency of the dominant race of *Escherichia coli* helps investigate the phase of disease progression [13]. CD and UC were not homogeneous at the genetic and microbial levels [14]. Considering the multifactorial and incremental rate of the IBD incidence, variations in clinical observations of the infection, and responses to the therapy in various sexes [15], additionally, the unclear function of microorganisms in the illness process and differences in the proportion of intestinal microorganisms, including fungus, bacteria, archaea, and viruses have been examined in control and case groups about disease type and participants sex. All the samples were selected from a single race. For the first time, the differential correlation between viruses and other microorganisms of the gastrointestinal tract in CD and UC in males and females was evaluated. The results showed some differences in the frequency of the gut microorganisms and the correlation between them depending on the sex or the type of the disease.

Methods

Dataset

The study of bacteria and viruses in stool samples accurately represents the gut microbiome, especially the large bowel [16]. Accordingly, we studied the entire and normalized concentration genome of 456

stool samples from 84 white race volunteers, including 40 females and 44 males with no history of consuming antibiotics or chemotherapeutic or immunosuppressive agents. Data were extracted as qualitatively controlled, complete raw metagenomic data from the NIH-funded Human Microbiome Project (HMP2) available at the IBDMDB database [4] at https://ibdmdb.org/tunnel/public/HMP2/WGS/1818/rawfiles and categorized into six distinct groups (Supplementary 1).

Computational Methods

Data from participants of the control and patient groups were segregated by sex to assess the frequency and correlation between the gut microbiome in various sexes in both IBD diseases (UC and CD). For a more accurate study of the samples, the control subjects were classified based on the mean age of the patient groups for both sexes. Then, the age-related microbes were identified in the control group of males and females.

Assessment of microbial prevalence

The microorganisms with the greatest mean frequency levels were found in the samples from patients and controls of both sexes. Each group was purged of microbes with zero standard- deviation. The variations in the frequency of each organism were investigated in the control-patient groups for both sexes, groups suffering the identical illness from two various sexes, and groups experiencing multiple ailments from the identical sex. The instances with significantly different mean frequency percentages in the two groups (pertaining to the Z-test with = 0.05) and the proportion of the mean frequency percentage throughout the two groups, exceeding two or less than 1 / 2, were included. Age-related organisms were excluded from them when establishing the microbes with remarkable discrepancies in frequency. These differences between males and females in the control group were compared to identify sex-linked microorganisms. On a 32-core server with 256 GB of RAM and the CentOS 7 operating system, MetaPhlan2 [17] and MiCop[18] were used to determine the frequency percentages of bacteria and Archaea in each sample as well as the frequency percentages of fungus and viruses. Outlier microorganisms were removed using discordant software [19] in an R platform. The correlation and differential correlation among viruses and other microorganisms were computed using various frequencies from the preceding phase in control and diseased groups for each sex, groups suffering identical illness from different sexes, and groups with identical sex experiencingmultiple diseases. Differential correlation networks, in which Spearman rank correlation with was used Benjamini& Hochberg method & 0.05 as a threshold, have been considered. Shared edges and nodes between the networks of the investigated groups were retrieved from their differential correlation links. The values of the differential correlations were rounded. The networks were marked with edges equivalent to or higher than 0.5 (edges equivalent to or lower than -0.5), and sex-linked microbeswere tagged. Edges with pairwisemicroorganism relationships were taken into account.

DiffCorr software [20] evaluated correlation and differential correlation between viruses and other microbes in the R platform. Eventually, the differential correlation networks in the previously described

groups were created using the Cytoscape program [21].

Results

Samples Stratification

The mean age of IBD(UC & CD) males(females) was 21.17(30.63) yrs.Table 1 shows the data about the examined specimens, which were divided into six categories based on the patient's gender and the control group or illness type. The overall number of participants, samples collection, mean age, and specimen variation were all provided for each group.

Table 1. Samples information by gender and age.

GROUPS:	Control		Case-IBD			
	non-BD		UC		CD	
Sex	Male	Female	Male	Female	Male	Female
Number of the**Volunteers**	14	11	8	15	22	14
Number of the**Samples**	93	71	52	76	110	54
(mean&standard deviation) **Age**of the**Samples**	41.51 18.45	22.72 14.96	17.06 5.60	35.41 16.17	23.12 18.71	23.93 13.05

Microbes discovered and their frequency percentages

There were 394 bacterial species, 2 Archaea species, 569 virus species (from 46 families), and 14 fungus strains found in all of the patient and control feces collections. At least one of the six categories examined, six bacterial species, four viral families, five virus species, and four fungi strains were determined as microorganisms with the greatest mean frequency. Figures 1, 2, and 3 depict their flowchart (see Supplementary 2).The most common fungus was *Enterocytozoonbieneusi*H348, which was found in all groups at a frequency of at least 50%. After that, strain *Malassezia globose* CBS 7966 had the highest mean frequency in the UC groups of males (28.5%) and UC females (12.43%), as well as the CD group of females (10.44%),while in the CD groups,*Wickerhamomycesciferrii*strain was in the second place, males (15.26%), females control (14.29%),and male control (3.78%). Given anmean

frequency of 13.59 percent, 12.81 percent, 10.65 percent, and 10.12 percent, *Faecalibacteriumprausnitzii* was the most prevalent bacterial species in the female control group, UC females, male control group, and CD females, respectively. At the same time, the highest mean percentage of abundance in males with UC belonged to *Bacteroides stercoris* (11.18%) and in males with CD to *Bacteroides uniformis* (8.62%).Only*Methanobrevibactersmithii*, with a mean frequency of 10.99 percent, 4.88 percent, and 2.49 percent, was detected in the male and female control groups and UC females, respectively; no archea was identified in the other groupings. With 46.86 % and 43.99 % frequency in DC and UC male patients, respectively, and more than 32.5 percent in each control and UC female group, the viral family of *Siphoviridae* was found to be the most prevalent. In contrast, it was 25.2 percent in the male control group.But unclassified bacterial viruses with 42.53% and 29.05% had the highest mean percentage in the control groups of males and CD of females, respectively. When compared to other viral species, uncultured crassphage exhibited the greatest mean frequency, with about 27% in both the female control and diseased groups and 41.22% in the male controls.In contrast, its mean frequency was substantially decreased in the diseased male groups.The highest mean frequency in the CD group of males was 17.24% of Bacteroides phage B124-14 species, whereas the mean frequency percentage was substantially decreased in the other groups. The mean incidence was about 9% among UC males and females. The highest mean frequency in the UC males group was 11.5% of *Parabacteroides* phage YZ-2015b viral species.Twenty-four age-related microorganisms were discovered in the control groups, including two fungi, ten bacterial species, and twelve virus species (see TableS14 Supplementary 3). By comparing the different sex of the same disease or different diseases of the same sex,or control and disease groups of the same sex, 127 age-independent microbes were identified with different frequencies.For example, the CD males group differed significantly from the controls in the mean frequency of eight bacterial species, one fungal strain, and Twenty-two viral strains (p-value <0.05).Table 2 contains all of the research group's data (details in Table S16Supplementary 3).Twenty-three sex-related microorganisms were found in the control groups, including one fungal strain, ten bacterial species, and twelve viral species. Among which, the following are worth mentioning: Fungal strain of *Saccharomyces cerevisiae* with an mean frequency of 6.79% and 1.58% across the female and male controls, respectively; *Salmonella* phage RE-2010 with an mean frequency of 3.48% and 0.13% among the female and male controls, respectively; Shigella phage SfIV species with an mean frequency of 1.56% and 0.44 % among the female and male control, respectively; and Enterobacteria phage cdtI species with an mean frequency of 1.18% and 0.41% across the female and male controls, respectively (see Table S15inSupplementary 3).

Differential correlation among microbes with varying percentages of frequency

It was discovered that 94 microbes, including 31 bacterial species, two fungus strains, and 61 virus species, showed a differential correlation with other microbes in at least two separate groups. Table 3 displays some of them (see supplementary 4).Interestingly, among males and females with Crohn's disease, there are 312 differential correlations between different pairs of 41 microbes, including one fungal strain, seven bacterial species, and 33 viral species. In all of these, except for five microbes, which included three bacterial species and two viral species, the mean percentage of frequencies in males with

Crohn's disease was substantially greater than that of females suffering from this condition (0.05=α >*p*-value). Females with ulcerative colitis and their control group showed the least differential correlation in the mean frequency percentage between intestinal microbes, equivalent to 1. These were discovered among two different virus species.

Table 2. Comparison of the number of microorganisms based on various percentages of frequency among both groups (*p*-value <0.05).

Groups		CD-IBD	UC-IBD	Non-IBD	
Sex		Male	Female	Male	Female
Non-IBD	Male	8 B,1 F,22 V			10 B,1 F,12 V
Non-IBD	Female		3 B,1 F,7 V		
UC-IBD	Male	9 B,2 F,16 V	11 B,1 F,6 V	8 B,8 V	
CD-IBD	Female	7 B,1 F,33 V	9 B,9 V		6 B,1 F,8 V

Abbreviations : B, bacteria; F, fungus; V, virus; UC, Ulcerative Colitis; Crohn Disease, CD.

Table 3- Microbes with differential Correlation in some of the studied Groups.

Groups		microbe 1	abundance		microbe 2	abundance		r1	r2	(diff
NONF	CDF	B *Clostridium_ramosum*	0.014	0.001	V *Salmonella* phage SSU5	0.52	0.018	0	0.6	0.00
NONM	CDM	V Escherichia phage TL-2011c	0.005	0.016	B *Bacteroides_sp_1_1_6*	0.053	0.711	0.6	-0.1	5.81
NONM	UCM	B *Megamonas_unclassified*	0.032	0.377	V unculturedcrAssphage	41.215	6.444	0.5	-0.1	0.02
CDM	UCM	V Enterobacteria phage WPhi	0.32	0.103	B *Fusobacterium_nucleatum*	0.445	0.005	0.6	0	0.00
CDM	UCM	V Shigella phage 75/02 Stx	0.04	0.006	B *Proteus_mirabilis*	0.563	0	0.5	1	0
CDM	UCM	B *Anaerostipes_unclassified*	0.021	0.003	V Lactobacillus phage Lc-Nu	0.08	0.001	-0.1	0.6	9.43
CDM	CDF	V Enterobacteria phage phi80	0.064	0.02	B *Lachnospiraceae_bacterium_6_1_63FAA*	0.058	0	0.6	0	0.00
CDM	UCM	V Shigella phage 75/02 Stx	0.04	0.006	B *Fusobacterium_nucleatum*	0.445	0.005	0.5	0	0.01
CDM	UCM	V Staphylococcus phage SA1	0.009	0.003	B *Fusobacterium_nucleatum*	0.445	0.005	0.5	0	0.00
CDM	UCM	V Escherichia phage pro147	0.494	0.172	V Streptococcus phage TP-J34	0.753	0.125	-0.1	0.6	6.07
CDM	UCM	V Escherichia phage TL-2011c	0.016	0.004	B *Fusobacterium_nucleatum*	0.445	0.005	0.5	0	0.00
CDF	UCF	V Streptococcus phage SpSL1	0.011	1.02	V Parabacteroides phage YZ-2015b	23.665	11.29	-0.2	0.3	0.04
NONM	CDM	B Megamonas_unclassified	0.032	2E-04	V uncultured crAssphage	41.22	13.73	0.5	-0.1	0.00
NONM	CDM	V Cafeteria roenbergensis virus BV-PW1	9E-05	0.015	V Enterobacteria phage mEp235	0.005	0.018	0.7	-0.1	2.13

abundance □ (□): abundance of the microbe in the group □ (□).

r1 : The correlation between microbe 1 & microbe 2 in group □ ;

r2 : The correlation between microbe1 & microbe 2 in group □.

r1-r2: The differential correlation between microbe 1 & microbe 2 in group □ & □. *p*-value: *p*-value for r1-r2.

Abbreviations:B, bacteria; F, fungus; V, virus; CDM, CD in males; CDF, CD in females; UCM, UC in males.

UCF, UC in females; NONM, non-IBD in males; NONF, non-IBD in females.

Table 4- Outline of Differential Correlation Networks between Microbes in the Studied Groups.

Group I	Group II	Number of Edges	Number of Nodes	Number of Microbes	Type of Microbes
CDM	CDF	312	41=33 V+7 B+1 F	5	2 V,3 B
CDM	NONM	115	30=22V+7B+1F	12	7 V,5 B
CDM	UCM	59	24=14V+8B+2F	3	1 F,2 B
UCF	UCM	2	4 =3 B+1 F	3	2 B, 1 F
UCF	NONF	1	2 =2 V	1	1 V
UCM	NONM	6	11=5V+6B	4	1 V,3 B
CDF	UCF	9	11 =8 V+2 B	4	3 V,1 B
CDF	NONF	12	9 =7 V+2 B	7	6 V,1 B

Number of Edges :Number of edges in differential correlation network between **Groups I,II.**

Number of Nodes :Number of nodes in differential Correlation network between **Groups I,II.**

Number ofMicrobes:Numberofmicrobes in differential correlation network that their abundance in GroupI is lowerthanGroup **II.**

TypeofMicrobes: Type ofmicrobes in differential correlation network that their abundance in GroupI is lower than Group **II.**

Abbreviations:B,Bacteria; F,fungus;V,virus; CDM, CD in males; CDF, CD in femals;UCM, UC in males; UCF, UC in females;NONM, non-IBD in males; NONF, non-IBD in females.

Only in one viral species, the mean frequency percentage in the female control group was significantly higher than females experiencing ulcerative colitis (p-value <α= 0.05). Interestingly, unlike Crohn's disease in ulcerative colitis, there were only a few(two) differential correlations between microbes in males and females.Table 4 provides further information. There were 44 shared edges found in the related groups of microorganisms in differential correlation networks (between 15 bacteriophage nodes and one fungus node), evident in at least two network systems. Tables 5 and 6 illustrate some of which and other edges may be regulatory candidates for CD or UC illness (see Supplementary 4).Figures4, 5, 6, and 7 depict the microbial differential correlation networks of mean frequency percentage with greatness 0.5 or above (in associated groups).

Table 5- Regulatory Candidate Microbes for Crohn's Disease.

Groups		microbe 1	abundance		microbe 2	abundance		r1	r2	lfdr (difference)	P-value	(r1-r2)
|	?		|	?		|	?					
NONM	CDM	V Escherichia phage pro147	0	0.494	V Escherichia phage pro483	0.014	0.527	0	0.9	0	0	-0.9
NONM	CDM	V Pseudomonas phage OBP	0	0.001	V Yersinia phage L-413C	0.01	0.395	0	0.8	9.61731E-15	6.66134E-16	-0.8
NONM	CDM	V Pseudomonas phage OBP	0	0.001	V Escherichia phage pro483	0.014	0.527	0	0.7	1.83419E-10	2.22327E-11	-0.8
NONM	CDM	V Shigella phage Ss-VASD	0.003	0.015	B *Bacteroides_sp_1_1_6*	0.053	0.711	0.7	-0.1	7.70581E-11	9.0068E-12	0.8
NONM	CDM	V Escherichia virus P2	0.003	0.631	V Escherichia phage pro483	0.014	0.527	0.3	0.9	2.16671E-11	2.43872E-12	-0.6
NONM	CDM	B *Lactococcus_lactis*	0	0.001	V Escherichia virus P2	0.003	0.631	0.6	0	3.72058E-05	7.89213E-06	0.6
NONM	CDM	V Escherichia virus P2	0.003	0.631	F *Wickerhamomycesciferrii*	3.777	15.26	0.4	0.8	0.000347001	8.11171E-05	-0.4
NONM	CDM	V Enterobacteria phage Wphi	0.002	0.32	F *Wickerhamomycesciferrii*	3.777	15.26	0.3	0.6	0.002516671	0.000664575	-0.3
NONM	CDM	V Escherichia phage pro147	0	0.494	F *Wickerhamomycesciferrii*	3.777	15.26	0.3	0.6	0.00631852	0.001723343	-0.3
NONF	CDF	V Enterobacteria phage 9g	0.027	0	V Enterobacteria phage IME10	0.108	0.002	0.1	1	0	0	-0.9
NONF	CDF	B*Dysgonomonas_unclassified*	0	0.308	V Escherichia virus HK022	0.046	0.174	0	0.8	7.43169E-09	4.95446E-10	-0.8
CDM	CDF	V Escherichia virus P2	0.631	0.078	F *Wickerhamomycesciferrii*	15.26	4.907	0.7	0.1	1.72E-05	3.51431E-06	0.6
CDM	CDF	V Enterobacteria phage Wphi	0.32	0.003	F *Wickerhamomycesciferrii*	15.26	4.907	0.7	0.3	0.006423056	0.001970493	0.4
CDM	CDF	V Yersinia phage L-413C	0.395	0.017	F *Wickerhamomycesciferrii*	15.26	4.907	0.7	0.3	0.016519712	0.005520001	0.4
CDM	CDF	V *Parabacteroides* phage YZ-2015b	5.709	23.67	F *Wickerhamomycesciferrii*	15.26	4.907	-0.1	-0.5	0.040209746	0.014956064	0.4
CDM	CDF	V Escherichia phage pro147	0.494	0.106	F *Wickerhamomycesciferrii*	15.26	4.907	0.7	0.3	0.019046455	0.006503668	0.3
CDM	CDF	*V Pseudomonas phage phiKZ*	*1.375*	*0.404*	*F Wickerhamomycesciferrii*	*15.26*	*4.907*	*-0.6*	*-0.1*	*0.002884078*	*0.000812466*	*-0.5*
CDM	CDF	*V Streptococcus phage SpSL1*	*0.482*	*0.011*	*F Wickerhamomycesciferrii*	*15.26*	*4.907*	*-0.1*	*0.4*	*0.004704784*	*0.00139996*	*-0.5*
CDM	CDF	*V Salmonella phage SE2*	*0.026*	*0*	*V Bacillus phage AR9*	*0.039*	*0*	*0.1*	*1*	*0*	*0*	*-0.9*
CDM	CDF	*V Yersinia phage L-413C*	*0.395*	*0.017*	*V Streptococcus phage SpSL1*	*0.482*	*0.011*	*-0.2*	*0.7*	*2.50604E-08*	*4.40085E-09*	*-0.8*
CDM	CDF	*V Enterobacteria phage Phi1*	*0.004*	*0.067*	*V Parabacteroides phage YZ-2015b*	*5.709*	*23.67*	*0.2*	*-0.3*	*0.004649867*	*0.001377948*	*0.5*
CDM	CDF	*V Escherichia virus P2*	*0.631*	*0.078*	V Bacteroides phage B124-14	*17.239*	*4.46*	*-0.5*	*0*	*0.003000757*	*0.000856314*	*-0.5*
NONM	CDM	B Bacteroides_sp_1_1_6	0.053	0.711	V Parabacteroides phage YZ-2015b	13.95	5.709	-0.1	0.4	0.002318	0.00045	-0.5
NONM	CDM	V Escherichia phage pro147	3E-04	0.494	V Enterobacteria phage If1	0.018	0.409	-0	0.9	0	0	-0.9
NONM	CDM	F Wickerhamomyces ciferrii	3.777	15.26	V Enterobacteria phage If1	0.018	0.409	0.2	0.69	9.31E-05	1.52E-05	-0.5

abundance |(?): abundance of the microbe in the group | (?).

r1 : The correlation between microbe 1 & microbe 2 in group |;

r2 : The correlation between microbe 1 & microbe 2 in group ?.

r1-r2: The differencial correlation between microbe 1 & microbe 2 in group | & ?. *p*-value: *p*-value for r1-r2.

Abbreviations :B, Bacteria; F, fungus; V, virus; CDM, CD in males; CDF, CD in females.

NONM, non-IBD in males; NONF, non-IBD in females.

Table 6-Regulatory Candidate Microbes for Ulcerative Colitis Disease.

Groups		microbe 1	abundance		microbe 2	abunda
NONM	UCM	V *Bacteroides* phage B40-8	0.016	1.907	B *Bacteroides_stercoris*	2.76
NONM	UCM	B *Collinsella_intestinalis*	0	0.146	V *Bacteroides* phage B40-8	0.016
NONM	UCM	B *Clostridium_citroniae*	0.002	0.001	V *Salmonella* phage RE-2010	0.134
UCF	UCM	B *Megamonas_rupellensis*	0.005	0.089	F *Malassezia globosa* CBS 7966	12.426
UCF	UCM	B*Akkermansia_muciniphila*	0.422	0.093	B *Bacteroides_stercoris*	4.922
NONF	UCF	V *Streptococcus* virus Sfi19	0	0.053	V Enterobacteria phage 9g	0.027
CDF	UCF	Streptococcus phage SpSL1	0.011	1.02	Enterobacteria phage If1	0.285

abundance | (‖): abundance of the microbe in the group | (‖).

r1 : The correlation between microbe 1 & microbe 2 in group | ;

r2 : The correlation between microbe 1 & microbe 2 in group ‖.

r1-r2: The differencial correlation between microbe 1 & microbe 2 in group | & ‖. *p*-value: *p*-value for r

Abbreviations :B, Bacteria; F, fungus; V, virus; UCM, UC in males; UCF, UC in females;

NONM, non-IBD in males; NONF, non-IBD in females.

Discussion

Because males and females with IBD have distinct medical manifestations, prevalence patterns, and therapeutic responses, separate volunteered specimens must be separated depending on illness type and sex. These findings suggest that in terms of gender differential, this is the first research to look at the connections between various microorganisms in the intestinal tract of IBD individuals, including fungi, archaea, bacteria, and viruses at the species (or strain) scale. As regulatory candidates for IBD, two fungus strains, one bacterial species,and two viral species were recognized.Also as candidate sex-related microorganism in non-IBD participants, one fungal strain and one viral species were found. We were unable to identify any Archaea that were linked with inflammatory bowel disease. There were apparent disparities among males and females in their connection with intestinal microbiomes in Crohn's disease.

Still, there were more similarities between males and females' intestinal microbiome relationships in ulcerative colitis.

Fungi

Based on our result for the frequency of microbes, in both diseased and control groups, the fungal biodiversity in fecal specimens was smaller than the bacterial biodiversity; this is in agreement with a previous study [22]. *Microsporidia, Ascomycota, and Basidiomycota* were among the fungi studied in this research (see table S8supplementary 2). Four *Enterocytozoonbieneusi*H348,*Wickerhamomycesciferrii, Malassezia globosa*CBS 7966, and *Saccharomyces cerevisiae*S288C fungal strains were found in substantial prevalence in certain diseased or control groups, as shown in Figures 1, 2, and 3, suggesting their significance in the gut microbiota. There has also been evidence of *Ascomycota* and *Basidiomycota*divisionsduring studies of normal subjects [22]. The relative prevalence of *S. cerevisiae, M. restricta,* and *C. Albicans* from *Saccharomyces, Malassezia,* and *Candida* was higher than any other fungal strains [22]. Inflammatory bowel disease is linked to the fungus *Malassezia globosa*from*Malassezia*, which may generate indole ligands (a cytoplasmic transcriptional regulator in epithelial cells) for AhR receptors. *Malassezia* is the most frequent fungus found on the human epidermis, accounting for more than ten different species.Furthermore, several species have been found in other regions of the body, particularly the gut, indicating that the fungus is widespread in this organ [1]. IBD patients had a greater mean frequency of the fungus *Malassezia globosa*CBS 7966than control groups in this research. Nevertheless, it was more prevalent among males with UC than males with CD and females experiencing UC (see Table S22 in Supplementary4). Table 6 shows that this strain may be a regulatory candidate for UC illness based on the results.Study[23] found that the most frequent intestine *Microsporidian* in Iranian patients receiving IBD therapy was the *Enterocytozoonbieneusi*, which belongs to the genus *Microsporidia*. In research,*E.bieneusi* was found nearly exclusively in individuals with normal immune systems and immunodeficiency [24]. With more than 47% prevalence, it was the most frequent intestinal *Mycobiom* in our research (Figures 1, 2, and 3).Furthermore, this strain was shown to be age-dependent in the females' group (see Table S14 in Supplementary 3) (Females under 30 in the control group had a substantially greater prevalence than females over or equal to 30 in the same group).*Wickerhamomycesciferrii*is a fungus that produces sphingolipid derivatives and shares its sphingoid bases with the human model in stereochemistry [25]. Due to its controlling the gastrointestinal tract's inflammatory responses, higher sphingolipid levels in various gastrointestinal tract regions are linked to IBD [26].Nevertheless, it was found as an age-related fungus in our research on females groups (see Table S14 in Supplementary 3). This implies that in the control group, the frequency of this fungus was substantially greater in females under the age of 30 than in females beyond the age of 30 in the same group. IBD metabolism in males may be studied using the existence of this fungus in the intestinal tract. There is no doubt that this fungus is a regulatory candidate in CD illness, as shown in Table 5. It has been reported that the sugar content of *Saccharomyces cerevisiae*'s wall differs in different strains may promote anti-inflammatory or pro-inflammatory characteristics in the host; moreover, its frequency is higher in CD compared to the control group [27]. As a result, the *Saccharomyces cerevisiae*S288C strain found in participant stools was shown to be sex-dependent in our research (see Table S15 in

Supplementary 3). This implies that the females control group had a substantially greater incidence than males in the same group.

Bacteria

It can be concluded from Figures 1, 2, and 3 that because of their high prevalence at least in one of disease or control groups, the bacterial species, like *Faecalibacteriumprausnitzii, Bacteroides vulgatus, Bacteroides stercoris, Bacteroides uniformis,* and*Prevotellacopri* are essential in the gut microbiota.Individuals with IBD have had a high frequency of *Faecalibacteriumprausnitzii*and *Bacteroides vulgatus*species[4] in agreement with our result. However, According to TableS14 in Supplementary 3, both of these microbes were shown to be age-related in females. This implies that in the control group, the percentage of frequency among females under the age of 30 was substantially higher)lower(than the percentage among females above the age of 30 in the same group, respectively.According to our findings, the frequency of *Roseburia intestinalis*, which was previously seen alternately in some IBD patients [4], was lower among IBD females (UC and CD) than among their control group. At the same time, it was greater among males with ulcerative colitis than the control group (see Table S9 in Supplementary2).There was a large amount of *Bacteroidesstercoris*species in the fecal matter of CD patients [28], and research [29] found a connection between *Bacteroides stercoris* species and UC. In our research, its mean percentage was greater throughout females with CD than in UC. In contrast, the percentage was greater in males suffering IBD (UC and CD) than in their controls (see Table S9in Supplementary2). *Bacteroides stercoris*isa regulatory candidate for UC, as shown in Table 6.*Bacteroidesuniformis* was less common in patients with UC in research [29] compared to the control group. In contrast, its frequency in females was found to be age-dependent in our investigation (see Table S14 in Supplementary 3). This implies that in their control group, females below the age of 30 had a reduced percentage of incidence versus females over the age of 30.

It was similarly lower in UC-affected males than CD-affected ones.According to recent research [64], *Prevotellacopri* is more prevalent in recently confirmed RA patients; however, in our result, it was shown to be less prevalent in males experiencing CD than those with UC and control(see Table S9 in Supplementary2).

Viruses

Family scale

Figures 1, 2, and 3 demonstrate that the *Siphoviridae*, Unclassified bacterial viruses, *Myoviridae*, and unclassified *Microviridae*viral families were found to be the most prevalent, whereas the *Siphoviridae* and *Myoviridae*, *Podoviridae*, and *Inroviridae* viral families based on research [9] were found to be the most common in normal participants. Among subjects with CD and the control group, the most common viral families found were those belonging to the *Siphoviridae*, *Myoviridae*, and *Podoviridae* ones, according to ref. [30].

Species-level

It can be shown in Figures1, 2, and 3 that a greater frequency was found for five virus types, including uncultured crAssphage, *Parabacteroides* phage YZ-2015b, Bacteroides phage B124-14, *Streptococcus* virus phiAbc2, and BeAn 58058 virus.UnculturedcrAssphage as CrAss-like phages, which infect bacteria of the order Bacteroidales, are the most abundant bacteriophage family in the human gut and play an essential role in the viral nucleus of a person [31]. Researchers found that it was more common among UC participants than in the control group [32], whereas in our research, Uncultured crAssphage was less common in IBD(CD &UC)malesthan in those with control group (see Table S16 in Supplementary3). Table 3 and Figure4suggest it as a regulatory candidate in IBD male patients.*Parabacteroides* phage YZ-2015b was shown to be more prevalent among individuals with colon cancer [33], but in our research, it was more(less) common in females(males) with CD than in those with control group (see Table S16Supplementary3). Table 5and Figur 4 show that it can be recommended as a regulatory candidate for CD females (males).Bacteroides phage B124-14's host is *Bacteroides fragilis*[34], a bacterium seen in high concentrations in patients with recurrent Crohn's disease [35]. This bacteriophage has been linked to CD illness, as shown in Table 5. *Streptococcus* virus phiAbc2 infects *Streptococcus thermophilus*, a bacteria often found in dairy products [36]. Using a mouse model of IBD, this bacteria decreased inflammation while maintaining the intestinal mucosal barrier [37]. Nevertheless, the proportion of its occurrence among females was shown to be age-dependent in our research (see Table S14 in Supplementary 3). This implies that in thefemales' control group, the frequency was substantially greater in females below the age of 30 than in those above the age of 30.*Chlorocebus aethiops* and *Oryzomys sp.* are the bacterial hosts of the phage BeAn 58058 virus, according to reference [34]. It was more frequent among females with IBD than their controls in our research, and it was greater in males with UC than in those with CD and controls (see Table S10 in Supplementary2).

Exploration of age-dependent microbes

According to the wide age range of the participants in our study (Supplementary1)and Introducing some age-related microbes in IBD patients treated in the hospital [38], Seeking a more

in-depth look at the interactions between gut microbiomes, we first discovered and subsequently eliminated these microbes mentioned above from our targeted various groups (see Table S14 in Supplementary 3).

Exploration of sex-linked microbes

One of the motivations for including sex-linked microorganisms in animal studies may be the varied reactions of males and females to the same illness therapy and the responses of sexual hormones with microbial metabolites [39]. Consequently, in our research on sex separation, we found sex-linked microorganisms (see Table S15 Supplementary 3). The *Saccharomyces cerevisiae*S288C strain and the Enterobacteria phage cdtI bacteriophage are some examples.

Evidence of correlation and Differential correlation throughout the human intestinal microbiota between case-control (sex-separated) and case-case groups

A differential correlation was found between the Escherichia phage TL-2011c and *Bacteroides_sp_1_1_6* throughout the control and the CD for males, as shown in Table 3. According to the virus-host DB database [34], *Bacteroides_sp_1_1_6* is a host for Escherichia phage TL-2011c. On the other hand, according to Table 1 of the study [40], the species *Bacteroides sp. 1 1 6* is equivalent to *B. thetaiotaomicron* proposed for the therapy of Crohn's disease [41].A differential correlation was found between the uncultured crAssphage and *unclassified Megamonas*throughout the control and the UC for males, as shown in Table 3. While the relationship between uncultured crAssphage and UC has been documented in research [32], and confirmation of UC in individuals with a higher prevalence of certain species, such as *Megamonas*, compared to the controls has been documented [42].To compare females with CD to those without, we used data from Table 3 to see whether there was any differential correlation in the types of *Salmonella* phage SSU5 and *Clostridium ramosum* bacteria. Fermentation products and SCFA found in *Clostridium ramosum* are toxic to *Salmonella* strains [43]. It may also cure IBD patients by reducing the number of Tregs in the body [44]. *Salmonella enterica subsp. enterica serovar* Typhimurium str has been found as a host for *Salmonella* phage SSU5 in the virus-host DB database [34]. This bacterium may worsen illnesses and cause the development of goblet and Paneth cells inside the intestinal tract. *Clostridium ramosum*, on the other hand, has been linked to increased uptake of fatty acids and intestinal epithelial cells multiplication [45].Shigella phage 0.75 Stx and *Proteus mirabilis* had a differential correlation in the CDM-UCM groups, as shown in Table 3. On the other hand, *Proteus mirabilis* is critical in the development of Crohn's disease [46]. Viral host database records indicate that the Shigella phage 75/02 Stx is thought to be hosted by the *Enterobacterales*order member *Shigella sonnei*, while *Proteus mirabilis* species is also a member of this order [34].*Malasseziaglobosa*CBS 7966 and *Megamonasrupellensis* had a differential correlation in males and females with UC, as shown in Table 6. Additionally, it has been evidenced that *Malassezia globose* was formerly connected with a pathogenic bacterial species favoring host [47], and its negative correlation with many bacteria has been shown in UC patients (not seen on CD) [1].On the other side, one of the criteria used to diagnose UC is the prevalence of the genus *Megamonas* [48].A differential correlation was found between the*Wickerhamomycesciferrii* and the Escherichia virus P2 throughout the CD for males and females, as shown in Table 5.Even though this fungus encodes a protein that is homologous with an endogenous virus-like element (EVE) from the Partiviridae family (NCBI: txid1520125). EVE is a viral intervention cycle that has subsequently been discovered as a fungus' antiviral defense mechanism [49]. Further study is needed to determine whether this is the cause for the association between fungus and viruses.Table 3 shows that a differential correlation was found between males experiencing UC and the control group between the *Bacteroides* phage B40-8 and the *Bacteroides stercoris*. While *Bacteroides fragilis* is listed as a host for this phage in the virus-host DB database [34], that belongs to the same family as *Bacteroides stercoris*and has been linked to UC illness [29]. Nevertheless, *Bacteroides fragilis* was more prevalent among males with CD and females suffering UC than their controls in our research (see Table S9in Supplementary2).Men suffering CD and UC had differential correlations between Lactobacillus phage Lc-

Nu and *Unclassifiable Anaeropsipes*, as seen in Table 3. The association of dysbiosis in IBD patients (especially UC) with *Unclassified Anaerostipes* was determined [50]. Furthermore, certain strains of *Anaerostipes* throughout the gastrointestinal tracts of patients experiencing Crohn's disease and ulcerative colitis were fewer than controls(in patients with CD less than UC) [51]. Although A probiotic bacteria present in normal females' genitals and urinary system, *Lacticaseibacillusrhamnosus* [52] is the host of Lactobacillus phage Lc-Nu to the virus-host DB database [34].As shown in Table 3, differential correlations between Enterobacteria phage WPhiand*Fusobacteriumnucleatum*were reported in males experiencing CD and UC in this research. *Escherichia* is a host for Enterobacteria phage WPhi, according to the ViralZonedatabase[53]. Escherichia phages have also been linked to an intestinal inflammatory response in the epithelium of UC individuals [29].Patients with acute leukemia's feces and plasma have been shown to have significant levels of Enterobacteria phage WPhi [55]. *Fusobacterium nucleatum* (a bacteria known to damage the intestinal barrier) produces a rise in its abundance in patient stools and when administered to a mouse model with colitis aggravation of colitis [54]. In both males and females experiencing CD, we found a differential correlation between Enterobacteria phage phi80 and the *Lachnospiraceae bacteria 6 1 63FAA*, as shown in Table 3. In addition, we identified Enterobacteria phage phi80 infected *Escherichiacoli*belonging to the *Proteobacteria*[34]. There was no rise in the prevalence of *Proteobacteria* in the investigation [56], but the *Lachnospiraceae* family experienced an increase in abundance. In the stools of Crohn's disease participants, *Escherichia coli* (AIEC) was a pathobiont.

Overall Statistical view

A significant number of differential correlations among intestinal microorganisms was shown in Table 4 to be associated with CD illness, particularly in males and females with CD, males with CD, and their controls (or those with UC). This may be partly because males and females are affected differently by Crohn's disease. It also shows a larger shift in the microbial interactions among males with CD than in controls or those with UC. In addition, UC condition was linked to the minimum number of differential correlations between intestinal microorganisms, particularly among females with UC and their controls, males and females experiencing UC, and males with UC and their controls. This indicates that UC is less complicated among males and females than Crohn's disease and that UC illness in males and females is more comparable.A maximum differential correlation among intestinal microorganisms and controls was observed in males sufferingCD, as seen in Table 4. Males with CD had substantially greater frequencies of the microorganisms involved in differential correlation (approximately three-quarters of them) than theircontrol. CD in males may be the most exaggerated form of IBD. According to Table 4, females experiencing UC had the fewest differential correlation among intestinal microorganisms and the controls. As a result, UC in females is the most basic form of IBD (see Table S22Supplementary4).Figures4, 5, and 6 show that, unlike males, females' intestinal microbial interactions had no shared edge among the UC, CD, and controls. Thus, UC and CD disease processes are independent in females. Table 4 shows that one fungal strain, 33 bacteriophages, and seven bacterial species have changed microbial interactions among males and females experiencing CD. Moreover, The microbial connections among males and females suffering UC, including one fungal strain and three species of

bacteria, have shifted in our research (e.g., *Akkermansiamuciniphila*,that a link between the reduction in its frequency and inflammation of the large intestine, has been reported [63].

Evidence of Bacteriophageimportance

Table 4 shows that bacteriophages had the greatest effect on alterations in the gut microbial interaction network in both patients and controls in our research. Bacteriophages were shown to be effective as only bacterial regulators in research [57]. Study [58], on the other side, linked bacteriophages to inflammatory aggravation among individuals with inflammatory bowel disease and, for instance, enhanced host immunity throughout participants with Crohn's disease and ulcerative colitis is associated with increasing Caudovirales order. The order Caudovirales was shown to be critical in the makeup of the gut microbiome in people with inflammatory bowel disease (IBD) [59]. A member of this order, the Yersinia phage L-413C, according to Table 5, has been identified as a potential regulation candidate for CD illness and is known to target *Yersinia pestis*, an *enterobacterial plague* pathogen [61]. *Streptococcus* phage Sfi19, *Streptococcus* phage SpSL1, *Enterobacteria phage If1*, and Escherichia phage pro147, which are covered by the UC and CD regulation sections, need to be studied more thoroughly (Tables 3, 5, and 6). As shown in research [60], Partitiviruses cooperate synergistically or competitively in horizontal transmission to its host fungal at the junction of its hyphae, which may indicate a correlation between two different viruses with the common host. More study is required to fully understand the impact of bacteriophages on inflammatory bowel disease since most prior investigations have focused on bacteria.

Alterations in microbial communications among UC and CD conditions

In *Bacteroides eggerthii*(seen in Figure 5), a decrease in the percentage is known as a stimulant of intestinal colitis [62].*Streptococcus thermophilus* is the host of *Streptococcus* phage TP-J34 [34]and has been found to have beneficial consequences on IBD [37].

Conclusion

To understand the disparity in responsiveness to the identical medication, we looked at the gut microbiome interactions in males with Crohn's disease vs. females. Our results revealed a greater resemblance between males and females suffering UC illness than those of other research groups in terms of microbial interactions. This study found that bacteriophages were the most prevalent microorganisms present in the microbial interactions, and prior research has indicated that FMT therapies may benefit from their administration .Candidate regulatory microorganisms for ulcerative colitis were identified as *Malassezia globosa*CBS 7966 (a fungus) and *Bacteroides stercoris*(a bacterium), as well as *Parabacteroides* phage YZ-2015b(a virus) for Crohn's disease. This studyalso proposed uncultured crAssphage(a virus)as sex-dependent IBDregulatory candidatein men. Although, *Wickerhamomycesciferrii* (a fungus) was proposed as regulatory candidate in Crohn's disease, it was age-dependent in females.we presented the *Saccharomyces cerevisiae*S288C (a fungus)and the Enterobacteria phage cdtI(a virus) as sex-dependent candidates in non-IBD participants.There are four bacteriophages such as *Streptococcus* virusSfi19, *Streptococcus* phage SpSL1, *Enterobacteria phage If1*,

and Escherichia phage pro147, suggested as research subjects for the metabolic processes of IBD among males and females.To learn more about the relationship between microbes and laboratory studies of its candidate's microbe, researchers need to look at aseptic mice (both normal and diseased),explaining their role in health or disease. Also, the study of intestinal microbial relationships in a Dataset of IBD patients of other races and its comparison with the results of this study and another suggestion is to investigate the relationship of these microbes and candidate microbes in other studies with the host, which may indicate the early stages of the disease.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The data used during the current study got through the Inflammatory Bowel Disease Multi'omics Database (http://ibdmdb.org), which is available to the public.

Competing interests

The authors declare that they have no competing interests.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Authors' contributions

FF conceived the research study and designed some methods, selectedthedataset, implemented some methods and conducted all experiments, performed statistical analysis, generated figures, and drafted the manuscript. HL conceived the research study and designed some methods, implemented some methods and designed the statistical analysis, drafted and edited the manuscript.EA conceived the research studyand designed some methods, consulted and edited the manuscript. KK conceived the research study and designed some methods, contributed to data availability, coordinated metagenomic analysis, consulted and edited the manuscript.All authors read and approved the final manuscript.

Acknowledgements

We would like to acknowledge SeyedDavarSiadat, ParvizAbdolmaleki, and Hamid Moghimifor their comment and discussion to study design.

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Figures

Figure 1

See image above for figure legend.

Figure 2

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Figure 3

See image above for figure legend.

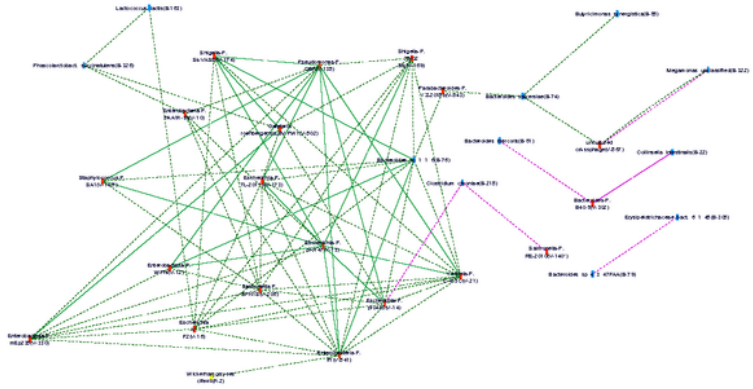

Figure 4

Differential correlation Networkbetween control and IBDpatients(CD & UC) in **males.**

Abbreviations:B,Bacteria;V, virus; CDM, CD in males; UCM, UC in males; NONM, non-IBD in males. ABS(differential correlation) in (CDM-NONM) €[0.5, 0.7] Green -Dash, ABS(differential correlation) in (CDM-NONM) €[0.8, 1.2] Green-Solid, ABS(differential correlation) in (UCM-NONM) €[0.5, 0.7] Purple- Dash, ABS(differential correlation) in (UCM-NONM) €[0.8, 1.2] Purple -Solid.

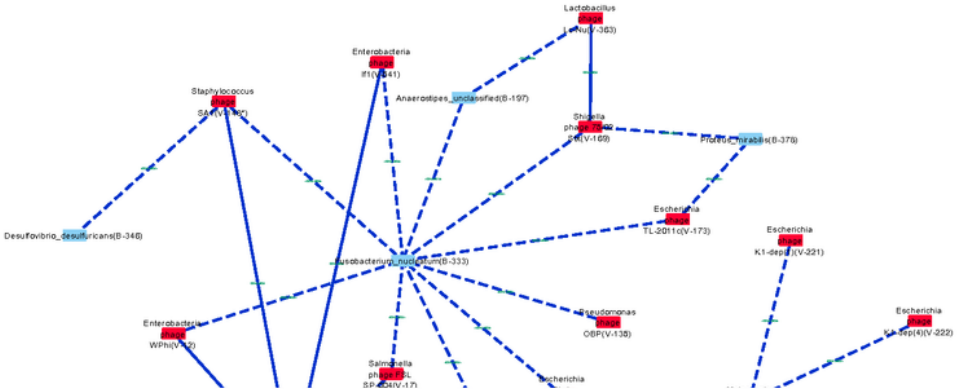

Figure 5

Differential Correlation Network between IBD patients(UC and CD) in **males**.

Abbreviations:B, Bacteria; F, fungus; V, virus; -P., Phage; CDM,CD in males; UCM,UC in males. .ABS(differential correlation) in (CDM-UCM) € [0.5, 0.7] Blue-Dash، ABS(differential correlation) in (CDM-NONM) €[0.8, 1.2] Blue-Solid·

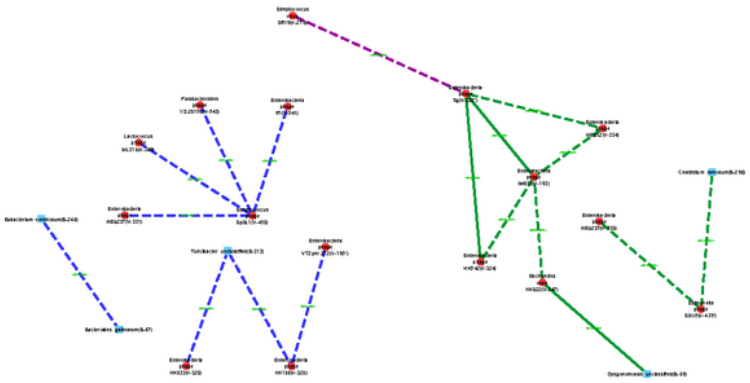

Figure 6

Differential Correlation Network between control and IBD patients(CD & UC) in **females** or between IBD patients(UC and CD) **in females**.

Abbreviations:B,Bacteria; F, fungus; V, virus; CDF, CD in females; UCF, UC in females; NONF, non-IBD in females.

Figure 7

Differential correlation Network between IBD Patients by subtype(CD & UC) and gender(**female & male**).

Abbreviations:B, Bacteria; F, fungus; V, virus; CDM, CD in males; CDF, CD in females; UCM, UC in males; UCF, UC in females.

ABS(differential correlation) in (CDM-CDF) €[0.5, 0.7] Blue-Dash, ABS(differential correlation) in (CDM-CDF)€[0.8, 1.2] Blue-Solid , ABS(differential correlation) in (UCF-UCM) €[0.5, 0.7] Purple-Dash.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementary1Samplesdata.xlsx
- Supplementary2Maxmicrobe.xlsx
- Supplementary3AgesexSig.diffrnt.abndanc.2groups.xlsx
- supplementary4diffcorrlnresultanalysis.xlsx