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Profile of intestinal fungal microbiota in acute pancreatitis patients and healthy individuals

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Abstract

Objective The gut is involved in the development of acute pancreatitis (AP). Increased focus is being given to the role of gut microbiota in the pathogenesis of AP. Nevertheless, there is currently no available evidence regarding the composition of fungal microorganisms in the intestines of patients with AP.

Methods In this study, we sequenced ITS rRNA gene amplicons and examined the intestinal fungal microbiota in feces from 11 AP patients (the test group) and 15 healthy people (the control group). Additionally, we examined the relationship between fungus and clinical and biochemical markers.

Results Results showed a decline in alpha diversity in AP patients. The overall fungal microbiota in the test group was significantly different from that of the control group ($P < 0.05$). In both groups, the fecal fungal microbiota was dominated by Ascomycota and Basidiomycota phyla. At the genus level, the abundance of *Candida* was significantly higher in the test group and the abundances of *Penicillium*, *Auricularia*, unclassified Eurotiomycetes, *Epicoccum* and *Vishniacozyma* were significantly lower. Furthermore, AP patients had a significant decrease in the GMHI score and a significant increase in the MDI index. The co-abundance networks of gut fungus in AP patients showed more interactions and mostly positive correlations than in the control group. There was a strong positive link between *Aspergillus* and WBC counts, while There was a strong link between unclassified Rozellomycota and IL-6.

Conclusion Our study provides the first empirical evidence that AP patients have different fecal fungal microbiota, which raises the possibility that mycobiota contribute to the etiology and progression of AP.

Keywords Fungi, Acute pancreatitis, Candida, Fungal diversity, Gut microbiota

Introduction

Acute pancreatitis (AP) is a medical condition characterized by the inflammation of the pancreas. It causes pancreatic auto-digestion by prematurely activating digestive enzymes in pancreatic acinar cells. This inflammatory condition can range from moderate local inflammation to severe systemic involvement [1]. Early multiple organ failures and infectious complications cause substantial mortality and morbidity in severe AP (SAP) patients [2]. Particularly, fungal infections pose a significant obstacle to therapeutic therapy. It has been reported that systemic infection and deep organ fungal infection have

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been a result of fasting and water deprivation, insufficient enteral nutrition due to parenteral nutrition input, disruption of the intestinal mucosal barrier, and a shift in intestinal flora, leading to intestinal mucosal villus atrophy and microvillus shedding [3].

Recent investigations have indicated gut microbiota dysbiosis in AP patients compared to healthy controls. Research consistently shows that the microorganisms causing sepsis and pancreatic infection typically originate from the intestines, particularly in patients with SAP [4]. However, while the bacterial fraction has been extensively studied in both health and disease, relatively little information is available on the other microbial fractions, particularly the gut mycobiota. Fungi are a normal component of the human microbial community. Metagenomic sequencing confirmed a population of 0.01–0.1% of fungi in the human gut microbiota [5]. Fungi comprise a small portion of the gut microbiota, but recent evidence has shown their ecological association with disease pathogenesis, as shown by their role in pancreatic tumorigenesis by modulating host immunity [6]. With the advancement of next-generation sequencing and other technologies, fungi-related research has also gradually increased. We hypothesized that there was a modified fungal microbiota in the intestines of patients with AP and that this alteration could be linked to the severity of the condition.

In this study, we performed ITS rRNA gene amplicon sequencing to analyze the composition of fungal communities in the feces of individuals with AP. We examined a potential association between differential fungi and clinical and laboratory indicators including CT severity index (CTSI), C-reaction protein (CRP), Interleukin-6 (IL-6), white blood cell (WBC), Procalcitonin (PCT), D-dimer and Platelet count (PLT). The results would enhance our understanding of the composition of the gut fungal microbiota and its potential involvement in the pathogenesis and progression of AP.

Materials and methods

Clinical trial design and sampling

A total of 11 AP patients (8 males and 3 females aged 34–69 years), who presented within seven days of the onset of symptoms, were recruited from the Jiading Branch of Shanghai General Hospital (Shanghai, China) and placed in the test group between January 2021 and May 2022. Regarding the revised Atlanta categorization, each patient fulfilled the prerequisites for further practice. Patients with cancer, metabolic, hepatic, immunosuppressive diseases, and chronic pancreatitis were excluded. In the meantime, the control group consisted of 15 healthy volunteers, (10 males and 5 females aged 25–60 years). The control group was required to meet specific criteria for inclusion, which included the absence of pregnancy, no medical treatment that could impact intestinal function, and no prior history of chronic metabolic, cardiovascular, or gastrointestinal diseases. The general data revealed no statistically significant differences between the groups ($P > 0.05$).

This research was approved by the research ethics boards of Shanghai General Hospital, and written informed consent was obtained from all the patients before sample collection. We collected the fecal samples from patients within 48 h after admission to the hospital and from healthy volunteers. All samples were frozen immediately after sampling and stored at -80 ℃.

DNA extraction/isolation and PCR amplification

The E. Z.N.A.® Soil DNA Kit (Omega Bio-tek, United States) was used to extract DNA from each fecal sample. The procedure was followed exactly as directed by the manufacturer. The extracted DNA was kept at -20°C for further examination. The quality and concentration of DNA were determined by 1.0% agarose gel electrophoresis and a NanoDrop® ND-2000 spectrophotometer (Thermo Scientific Inc., USA) and kept at -80 ℃ before further use. For high throughput, ITS library preparation and sequencing, the ITS1 region of the ITS rRNA gene was amplified from the genomic DNA. PCR was initially performed using the primer set ITS1F (5'-CTTGGTCA TTTAGAGGAAGTAA – 3') and ITS2 (5'-GCTGCGTT CTTCATCGATGC – 3'). The PCR reactions were run in ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA) using the following protocol: 3 min of denaturation at 95℃, followed by 35 0.5 min denaturation cycles at 95℃, 0.5 min of annealing at 55℃, and 45 s of elongation at 72℃, with a final 10 min extension at 72℃. The PCR product was extracted from 2% agarose gel and purified, then quantified using Quantus™ Fluorometer (Promega, USA).

Sequencing

Purified amplicons were pooled in equimolar amounts and paired-end sequenced on an Illumina PE250 platform (Illumina, San Diego, USA) by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China) according to normal protocols.

Data processing

Raw FASTQ files were de-multiplexed using an in-house perl script, and then quality-filtered by fastp version 0.19.6 and merged by FLASH version 1.2.11. The optimized sequences were then grouped into operational taxonomic units (OTUs) with 97% sequence similarity using UPARSE 11. The most abundant sequence for each OTU was selected as a representative sequence. To minimize the effects of sequencing depth on the alpha and beta diversity measure, the number of ITS rRNA

gene sequences from each sample was rarefied to 31,814, which still yielded an average Good's coverage of 99.98%, respectively. RDP Classifier version 2.13 was used to compare the taxonomy of each OTU representative sequence to the ITS rRNA gene database (e.g. Unite8.0/its_fungi) with a confidence threshold of 0.7. The number of shared OTUs in both groups was determined and displayed using a Venn diagram.

Bioinformatics analysis

Bioinformatic analysis of the gut mycobiota was carried out using the Majorbio Cloud platform (https://cloud.majorbio.com). Mothur v1.30.2 was used to generate rarefaction curves based on the OTU information. The number of common OTUs in both groups was calculated and a Venn diagram was used to display the results. Alpha diversity, which represents the diversity of the microbiome community, was obtained by analyzing the ACE estimator, Chao estimator, Shannon-Wiener diversity index, and Simpson diversity index using Mothur v1.30.2. The larger the Chao or ACE index, the higher the gut flora abundance, whereas community diversity increases with a higher Shannon or Simpson index. Nonmetric multidimensional scaling (NMDS) plots based on the Bray-Curtis distances and principal coordinates analysis (PCoA) to visualize the structural diversity of the gut fungal flora in the discovery group. Using an Adonis analysis, the equivalent statistical significance of the beta diversity was determined independently. Using a Wilcoxon rank-sum test, significant compositional differences between the groups were compared at each taxonomic level of the mycobiota communities. To identify the fungal taxa and predominant fungi that are specifically related to AP, the significantly abundant taxa (phylum to genera) of fungi between the two groups (LDA score > 2, $P < 0.05$) were identified using the linear discriminant analysis effect size (LEfSe) method (http://huttenhower.sph.harvard.edu/LEfSe).

Table 1 Clinical data of the patients with AP and control subjects

	AP ($n = 11$)	Control ($n = 15$)
Male/female	8/3	10/5
Median age (range) (y)	45.09(34–69)	45.33(25–60)
BMI	27.876 ± 4.235	25.543 ± 3.281
CTSI score	3.18 ± 1.779	
WBC (*10^9/L)	11.261 ± 3.202	
PCT (ng/mL)	0.302 ± 0.500	
C-reactive protein levels (mg/L)	108.351 ± 52.962	
IL-6 (pg/mL)	79.742 ± 72.354	
D-dimer (μg/mL)	2.750 ± 1.862	
PLT (*10^9/L)	197.55 ± 46.168	

Statistical analysis

Non-parametric Mann-Whitney U tests were used to determine if there were significant differences between the groups. SPSS for Windows, version 20 was used to conduct a student's t-test. Spearman correlation analysis was used to evaluate the correlation between the abundance of distinct fungus and laboratory indicators. If the correlation coefficient between two nodes was greater than 0.6 or less than − 0.6, and the P-value was less than 0.05, the correlation was deemed statistically robust.

Results

Clinical characteristics of the study population

Table 1 displays the demographic and clinical characteristics of eleven AP participants and fifteen control subjects. In terms of age, gender, hypertension percentage, and body mass index (BMI), there was not a statistically significant difference found between the two groups ($P > 0.05$). Table 1 displayed clinical characteristics such as CTSI, CRP, IL-6, WBC, PCT, D-dimer, and PLT.

OTU clustering and alpha diversity analysis of gut mycobiota

Using Illumina high-throughput sequencing technology, a total of 1,916,597 raw reads were collected from 26 samples. From these, 1,486,419 high-quality sequences were selected, with an average of 57,170 sequences per sample. We produced 678 OTUs using 97% as the similarity cutoff. The Venn diagram showed that 173 of the 678 OTUs were shared by both groups, whereas 140 OTUs were unique to the test group, and 365 were specific to the control group (Fig. 1a). The microbial richness of the fecal sample reached near saturation at the sequencing depth used, as seen by the subsequent rarefaction curves. Given that the OTU-based Shannon-Wiener curve had already reached a plateau, it may be concluded that our sequencing depth was satisfactory (Fig. 1b).

The evaluation of alpha diversity includes the following: Chao, Shannon, Ace, Simpson, Coverage, and Sobs (also known as observed species). Indicators of community variety, the Shannon and Simpson indices, showed statistically significant changes ($P < 0.05$). Further analysis revealed no statistically significant variation in the coverage index, a measure of community coverage. These findings suggest that the diversity of intestinal fungi in patients with AP was significantly lower compared to the control group (Fig. 1c-e). There were no significant differences in Ace, Sobs, and Chao in response to community richness ($P > 0.05$) (Fig. 1f-h).

Analysis of the beta diversity based on OTU levels

To examine the similarity or dissimilarity in the general community structure between the afflicted and healthy groups, the species diversity was analyzed by intergroup

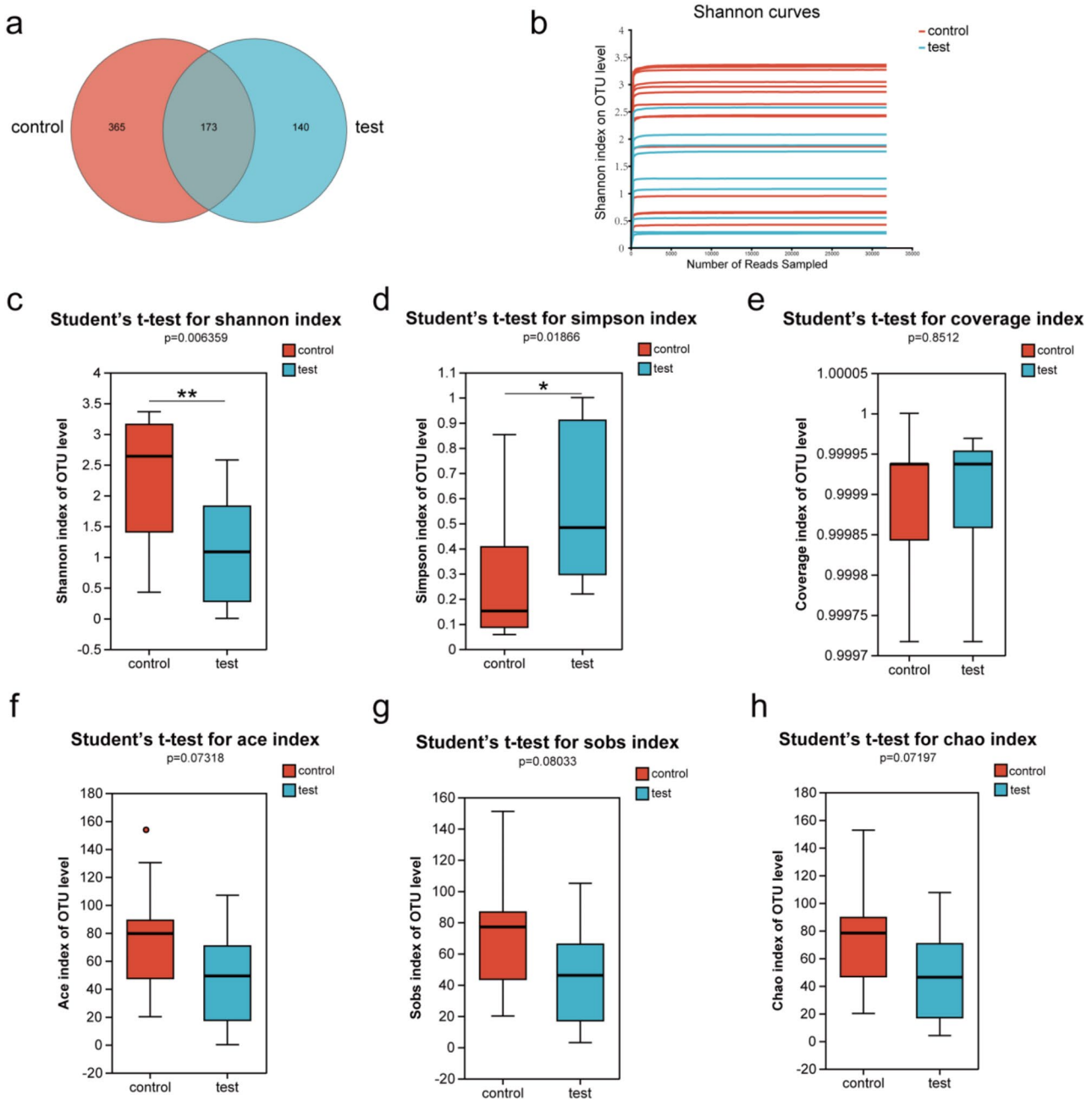

Fig. 1 Alpha diversity analysis of fecal mycobiota of healthy volunteers and AP patients. **(a)** Venn diagram demonstrates the shared and unique OTUs in both groups. **(b)** Shannon diversity curve of a single sample in each group. **(c-h)** The index values of alpha diversity (**c**: Shannon index; **d**: Simpson index; **e**: Coverage index; **f**: Ace index; **g**: Sobs index; **h**: Chao index)

comparison. The principal co-ordinates analysis (PCoA) was employed to measure beta diversity, utilizing the bray_curtis distance metric. The Adonis analysis revealed a substantial distinction in the gut fungal community between the two groups ($R^2 = 0.1361$; $P = 0.0160$) (Fig. 2a). The ANOSIM analysis also yielded consistent outcomes ($R = 0.1664$; $P = 0.017$) (Fig. 2b). We confirm this outcome using PCoA with both weighted and unweighted UniFrac distances (Figure S1c and d).

Moreover, by employing non-metric multidimensional scaling (NMDS) in conjunction with the bray_curtis distance to assess beta diversity, Adonis and ANOSIM analyses ($R^2 = 0.0909$; $P = 0.0250$; $R = 0.1664$; $P = 0.0170$, respectively) revealed a distinction in the intestinal fungal communities of the two groups (Figure S1a and b). This finding suggests that the disparities between the groups were more pronounced than those within the groups, suggesting that the classification of the control (healthy)

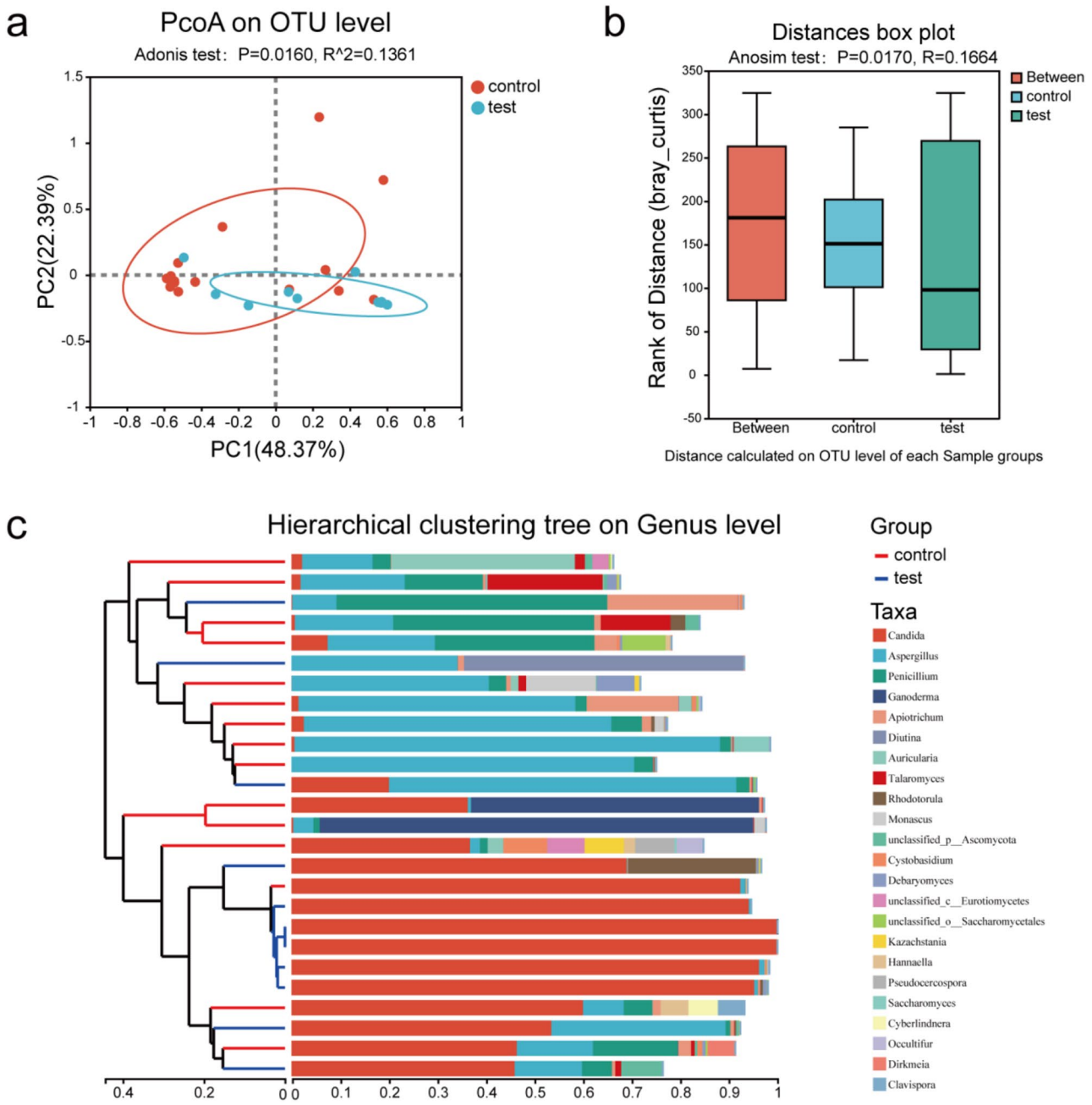

Fig. 2 Analysis of beta diversity based on OTU and genus levels. **(a)** PCoA plots of beta diversity based on Bray-Curtis distances Adonis analysis in different groups. **(b)** PCoA plots of beta diversity based on Bray-Curtis distances ANOSIM analysis in different groups. **(c)** A hierarchical clustering tree was created to show the relationships among all the samples

and test (AP) groups was significant for the purposes of this research. At the OTU level, hierarchical cluster analysis partitioned the fungi into discrete branches (Fig. 2c). The examination of colony typing in relation to the dominant colony structure of the two groups indicated a statistically significant distinction in the typing composition (Figure S1e and f).

Acute pancreatitis altered gut mycobiota taxonomic composition

When accounting for the species of all OTUs, a total of 8 phyla were found in the test group, while only 6 phyla were detected in the control group. Ascomycota and Basidiomycota dominated the fungal microbiome of both populations. The percentage of dominating flora in the test group was 92.3% and 7.201%, respectively, whereas they were 77.78% and 21.05% in the control group. The

relatively average abundance of the fungal microflora at the phylum level is shown in Fig. 3a. At the class level, the numbers of fungi in Saccharomycetes (67.22%) were the largest in the test group and the number of other classes like Eurotiomycetes (21.83%), Tremellomycetes (4.00%) and Microbotryomycetes (2.53%) were less than 50%. Additionally, the dominating classes detected in the control group were Eurotiomycetes (45.30%), Saccharomycetes (23.97%), Agaricomycetes (14.54%) and Sordariomycetes (4.76%) in descending order (Fig. 3b). The composition of gut flora in both groups had a similar organization at the order and family levels, as observed at the class level. The bar charts that are specific to the community are displayed in Fig. 3c and d. At the genus level, the fungal microbiota was dominated by *Aspergillus* in the control group, followed by *Candida*, *Ganoderma*, *Penicillium*, *Auricularia*, and *Talaromyces* with proportions of 28.65%, 19.30%, 9.92%, 9.26%, 3.03% and 2.87% respectively. In the test group, *Candida* was the most dominant fungus, followed by *Aspergillus*, *Penicillium*, *Diutina*, *Apiotrichum*, and *Rhodotorula*, with proportions of 61.34%, 15.18%, 5.98%, 5.23%, 2.82%, and 2.47%, respectively. The relatively average abundance of the mycobiota at the genus level is shown in Fig. 3e. The composition of these two groups of intestinal flora at the species level was analyzed to further understand the species-specific effects of AP on the intestinal flora (Fig. 3f).

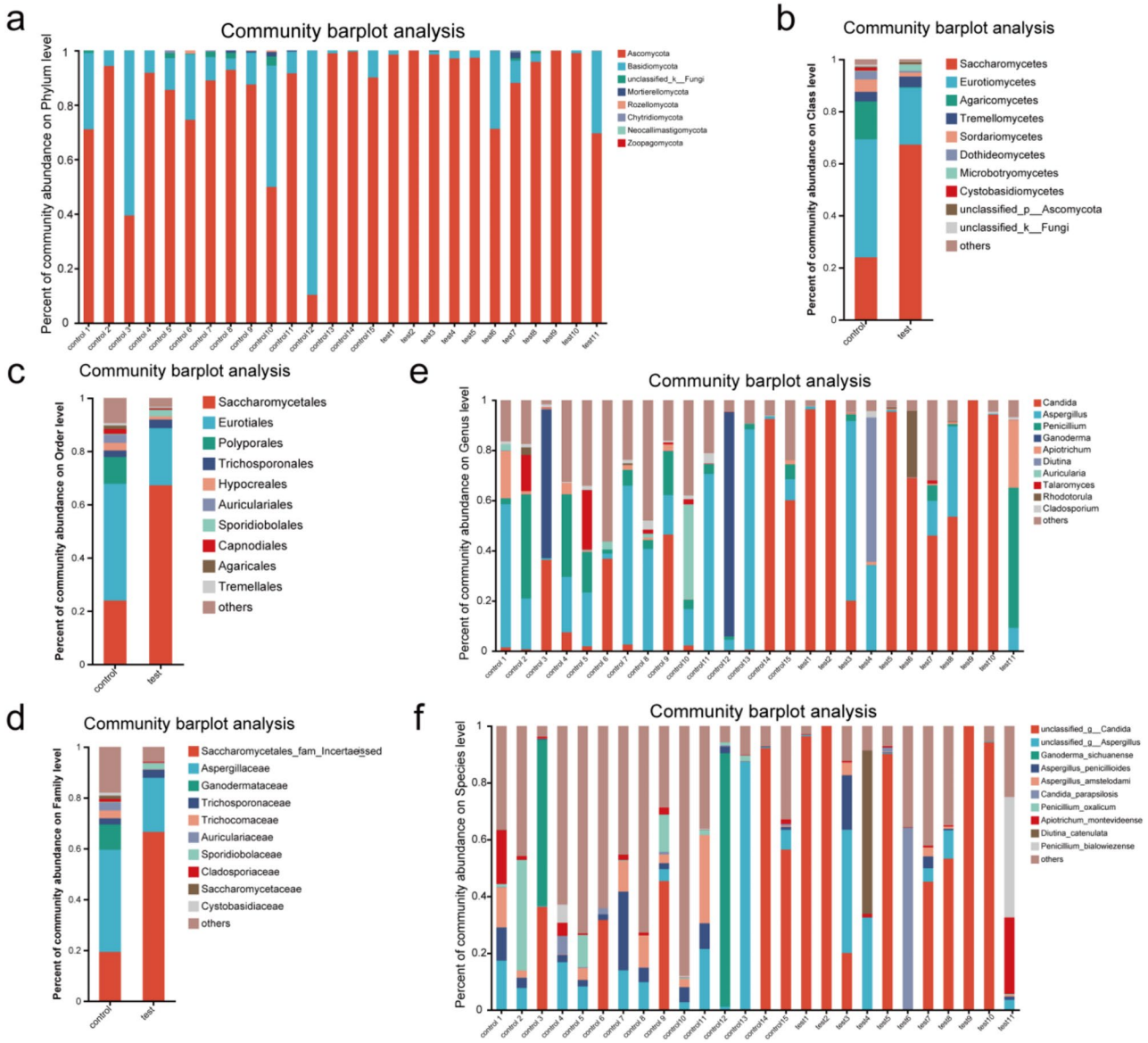

Fig. 3 Flora composition and comparison of fungal microbiota composition in both groups. **(a-f)** Microbiota composition at the (**a**) phylum, (**b**) class, (**c**) order, (**d**) family, (**e**) genus and (**f**) species level

The clustered heatmap of the highest abundance features ($n = 20$) shows similar results. At the phyla level, the abundance of Basidiomycota, Rozellomycota and Chytridiomycota was found higher in the control group (Fig. 4a). Figure S2 shows the similarity or difference relationship between the community structure of the disease group and the healthy group by Community heatmap analysis at the class, order, and family level. At the genus level, the abundance of *Ganoderma*, *Aspergillus*, *Auricularia*, *Talaromyces*, *Exophiala* and *Trichoderma* was detected higher in healthy participants, while the high abundance of *Candida*, *Diutina* and *Rhodotorula* were detected in AP patients. At the species level, the abundance of *Ganoderma sichuanense*, unclassified Monascus, *Talaromyces rugulosus*, unclassified Auricularia, *Penicillium oxalicum* and *Aspergillus amstelodami* was higher in the control group while a higher abundance of unclassified Candida, *Penicillium bialowiezense*, *Candida parapsilosis*, *Diutina catenulata*, *Rhodotorula mucilaginosa* and *Aspergillus fumigatus* was found in the test group.

The Circos graph effectively showed the link between species and samples at the phylum, genus, and species levels, helping understand the diseased and healthy groups' species distributions (Figure S3). The heatmap analysis on the OTU level of fungal microbiota showed a distinct microbiome difference between the two groups. There were 8 OTUs that were determined to be distinct between the sample groups. Out of these OTUs, one was found to be more prevalent in the test group compared to the control group. This particular OTU belongs to the genus *Candida*. The test group exhibited lower levels of 7 genera, including *Aspergillus*, *Penicillium*, *Auricularia*, *Cladosporium*, and unclassified Eurotiomycetes (Figure S4).

Significant shifts in the fungal microbiota between the groups

The Wilcoxon rank-sum test revealed significant differences in the fungal composition between the groups. Ascomycota were substantially more abundant in the test group than in the control group at the phylum level. In contrast, the abundance of Basidiomycota was significantly reduced in the test group compared to the control group (Fig. 5a). The test group exhibited a significantly higher abundance of *Candida* at the genus level compared to the control group. Conversely, the levels of *Penicillium*, *Auricularia*, unclassified Eurotiomycetes, *Epicoccum* and *Vishniacozyma* were significantly lower in the test group compared to the control group (Fig. 5b). Results obtained at the species level exhibit a resemblance to the outcomes obtained at the genus level (Fig. 5c).

To establish the unique fungal taxa and prominent fungus in AP patients, a LEfSe was utilized to reveal the maximum difference in fungal microbiota structures between the groups. This analysis showed that the abundance of *Candida* was significantly higher in the test group than in the control group. The LEfSe analysis drew conclusions similar to those mentioned previously (Fig. 5d).

To evaluate the abundance network of gut fungal communities in both test and control groups, the top 50 species in terms of total abundance at the genus level were chosen and analyzed using Spearman's correlation analysis to reveal the relationships between species. The size of the graph nodes represents species abundance, while different colors signify species. Positive and negative correlations are indicated by red and green connecting lines, respectively. The correlation coefficient increases with line thickness, and the more lines, the closer the species are to other species. Our analysis revealed a higher frequency and stronger correlation of gene interactions within the test group compared to the control group. In the test group, fungal genera were largely positively linked compared to the control group (Fig. 6a and b).

Using the gut microbiome health index (GMHI) and microbial dysbiosis index (MDI), we found that the GMHI was considerably greater in the healthy group compared to the diseased group (Fig. 6c). Additionally, the healthy group had a much lower MDI than the afflicted group (Fig. 6d). These findings suggest that the presence of fungal species linked to good health in the gut of the control group may be more abundant. In addition, we discovered that the Shannon and Simpson indices had a positive correlation with the GMHI, despite the fact that this result did not indicate a statistically significant difference (Fig. 6e and f).

Association of gut mycobiota with clinical and laboratory indicators

The study employed Spearman correlation analysis to evaluate the association between the dominating fungus of the top 50 and clinical variables. Our findings indicate that all genera, with the exception of *Candida* and unclassified Eurotiales, showed a positive correlation with CTSI. A generally negative association was found between PCT and fungus, as well as between D-dimer and fungus dominant microorganisms. Among them, *Aspergillus* showed a notable positive association with WBC levels, while unclassified Rozellomycota had a strong positive correlation with IL-6 (Fig. 7).

This finding was further validated by linear regression analysis, which involved examining the relationship between clinical variables and the proportion of different fungal species in the community (Fig. 8a and e). Furthermore, the presence of Saccharomycetales showed a positive correlation with the WBC, in addition to the

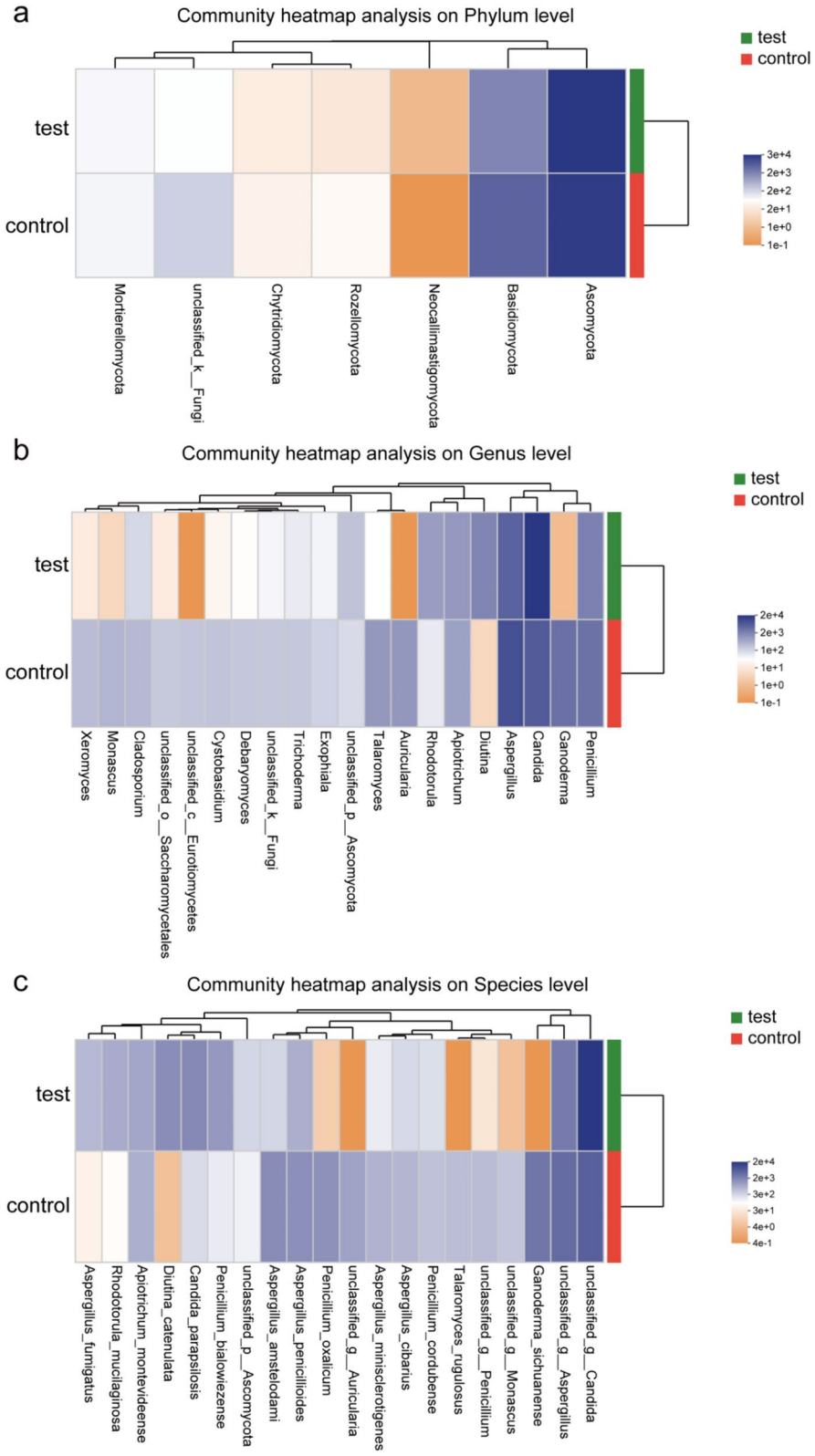

Fig. 4 Group clustering heat map of top 20 features in different taxa. **(a)** Phylum, **(b)** Genus, **(c)** Species

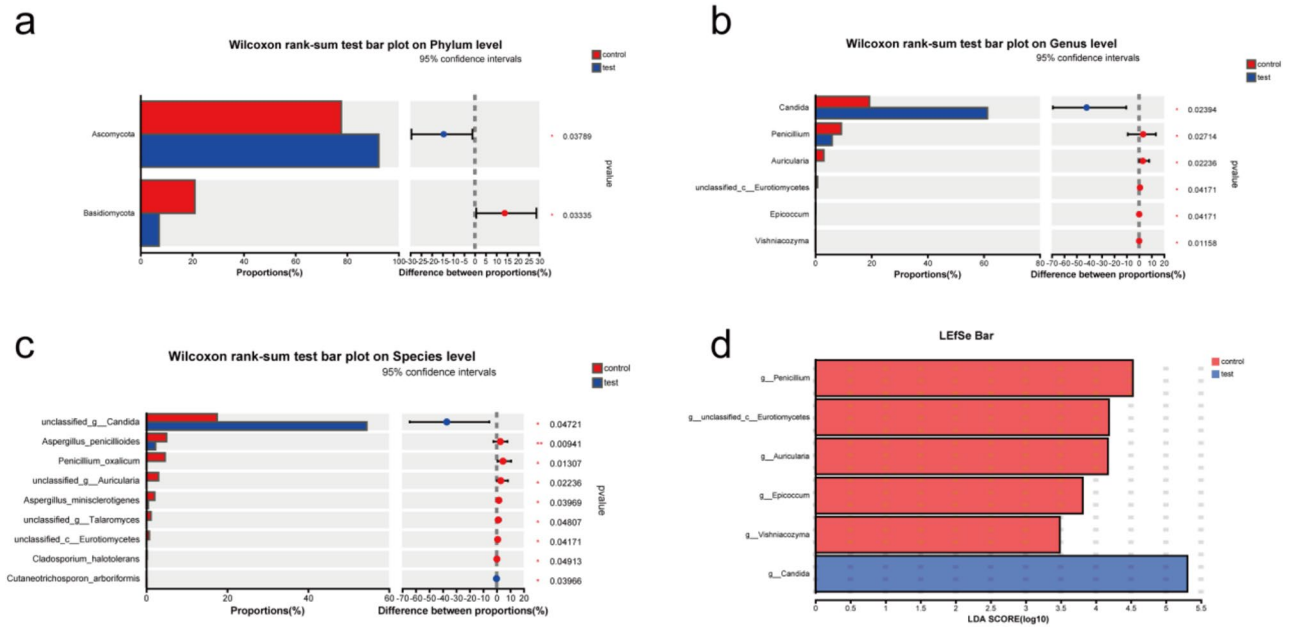

Fig. 5 Flora composition and comparison of gut mycobiota composition in both groups. **(a)** Differences in fungi composition at the phylum level. **(b)** Differences in fungi composition at the genus level. **(c)** Differences in fungi composition at the species level. **(d)** Mycobiota differences at the genus level, as assessed by Linear discriminant analysis effect size

Aspergillus (Fig. 8b). And IL-6 had a favorable correlation with *Diutina*, *Dirkmeia*, and *Acremonium*, in addition to the unclassified Rozellomycota (Fig. 8f-h). However, the unclassified Hypocreales and *Cystobasidium* showed a negative correlation with CRP (Fig. 8c and d). Through the utilization of ordinal regression analysis, a statistical examination was conducted to assess the relationships between alpha diversity and clinical variables, as well as between beta diversity and clinical variables. The investigation revealed that none of the obtained results exhibited a significant difference. The Shannon index had a positive correlation with CTSI and WBC and a negative correlation with PCT (Figure S5a-c). Additionally, PCoA had a positive correlation with IL-6 and WBC, while it displayed a negative correlation with PCT (Figure S5d-f).

Discussion

Numerous investigations have demonstrated that there is a correlation between intestinal microecology and acute pancreatitis [7]. Previous research found that the presence of disrupted gut microbiota in both individuals with AP and animal models [8]. The impairment of the intestinal barrier function and imbalance of the intestinal flora can result in enterogenic endotoxemia, which is the primary cause of subsequent infections in individuals suffering from AP. The gut fungal microbiota and the bacterial microbiota interact in a mutually beneficial and antagonistic way [9]. The prevalence of fungal infections has been gaining more and more attention lately [10]. While the healthy pancreas is generally resistant to fungal invasion, inflamed glands are more susceptible to infection, which is proportional to the extent of necrosis [11]. Furthermore, the timely identification of fungal infections in individuals afflicted with AP, coupled with the implementation of suitable therapeutic interventions, has the potential to enhance the overall prognosis [12, 13]. A meta-analysis has elucidated that pancreatic fungal infection (PFI) is a prevalent occurrence among individuals afflicted with necrotizing pancreatitis (NP), and it is intricately linked to heightened mortality rates, an elevated rate of admission to the critical care unit, and an extended duration of hospitalization [14]. Henceforth, the current investigation was concerned with scrutinizing the alterations in fungal composition in the fecal samples of individuals diagnosed with AP and comparing them with those of healthy volunteers. Additionally, the study aimed to explore the potential correlation between these changes and clinical indicators.which suggested a possible role of fungi in the development of AP.

In this study, there was no significant difference in the abundance of fungal microbiota between AP patients and healthy controls, but the diversity of gut fungal microorganisms was reduced in AP patients compared to controls. Several studies have revealed a correlation between individuals afflicted with inflammatory bowel disease and a notable decline in the diversity of gut fungi [15, 16]. The phenomenon of beta diversity demonstrates the capacity of AP to modulate the composition of gut fungi. Comparing the two groups' fungal community compositions and alterations allowed us to delve deeper

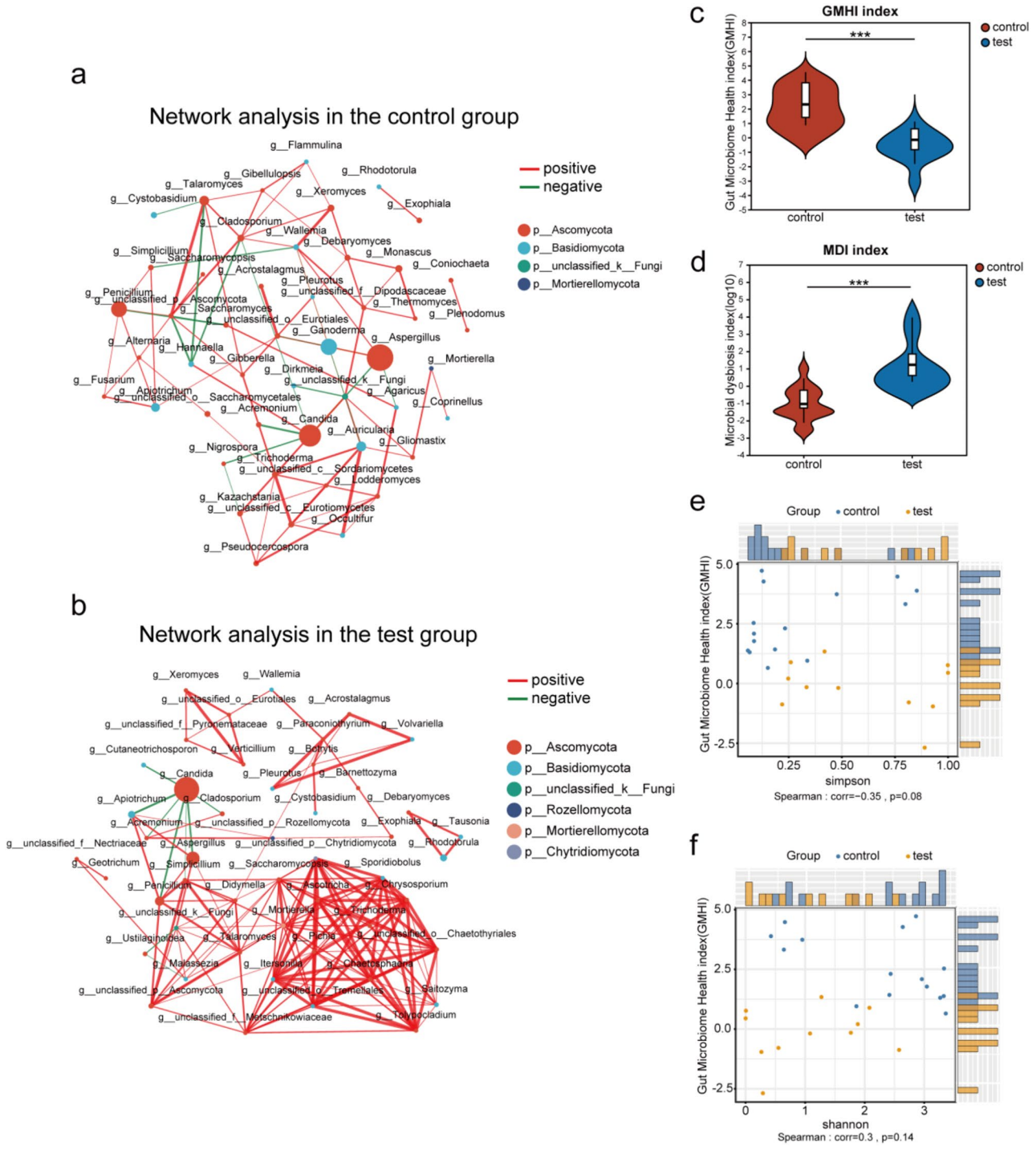

Fig. 6 Network analysis in the control and test group. **(a)** The distinctive gut fungal co-abundance networks in the control group. **(b)** The distinctive gut fungal co-abundance networks in the test group. **(c)** GMHI between the control group and the test group. **(d)** MDI between the control group and the test group. **(e)** The correlation analysis between GMHI and Simpson diversity. **(f)** The correlation analysis between GMHI and Shannon diversity

into the impacts of AP on gut fungi. The results showed that Ascomycota and Basidiomycota were dominated at the phylum level in the two sample groups. At the genus level, the fungal microbiota was primarily composed of *Aspergillus*, *Candida*, and *Penicillium*. Among them, the abundance of *Candida* increased in the test group, while *Penicillium* abundance was decreased with a statistically significant difference. The majority of research has consistently identified *Candida* as the sole fungal infection in patients with acute pancreatitis [17]. *Candida*, a common

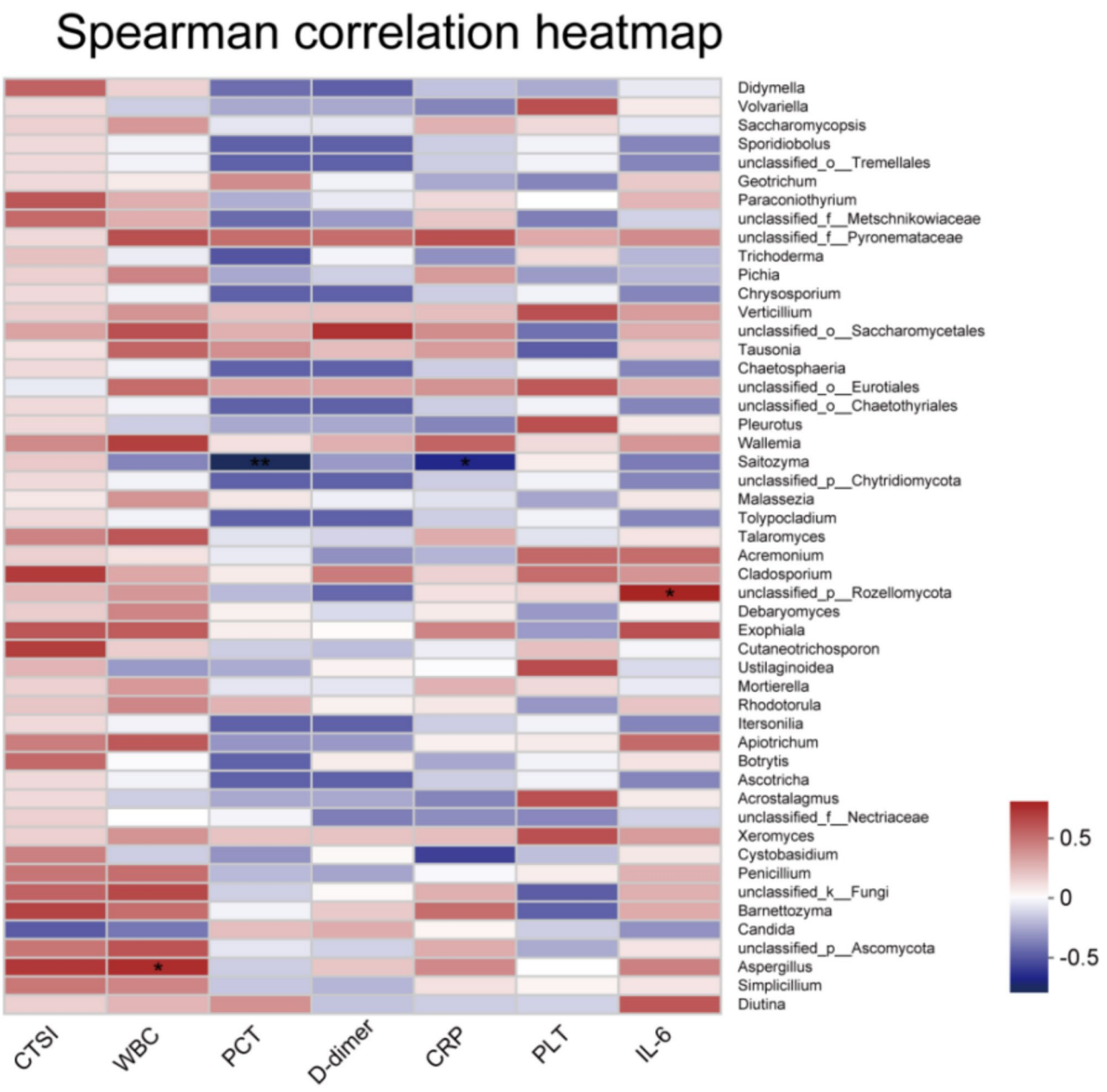

Fig. 7 Spearman correlation analysis between the fungus with clinical and laboratory indicators

resident of the human microflora, can be found in various parts of the body, including the skin, gastrointestinal tract, genitourinary tract, and even the respiratory tract [18]. Candidaemia has been identified as a linked consequence of necrotizing pancreatitis and is correlated with high mortality. In addition, individuals with recurrent *Clostridioides difficile* infection (CDI) exhibit a dysbiosis of the intestinal fungal flora, which can be effectively treated with fecal microbiota transplantation (FMT). According to a study, recipients who successfully underwent FMT exhibited a greater relative abundance of *Saccharomyces* and *Aspergillus* following the procedure. Conversely, a high abundance of *Candida albicans* in the feces of the donor was linked to a decrease in the effectiveness of FMT [19]. Furthermore, CDI patients exhibited a decrease in *Aspergillus* levels when compared to healthy controls [20]. Diarrhea may also find relief with the rise of *Aspergillus* [21].

Through the analysis of the variations in the composition of intestinal fungi between the two groups, it was observed that the AP group exhibited increased levels of *Cutaneotrichosporon arboriformis*, along with *Candida*, in comparison to the control group. *Penicillium oxalicum*, unclassified Auricularia, unclassified

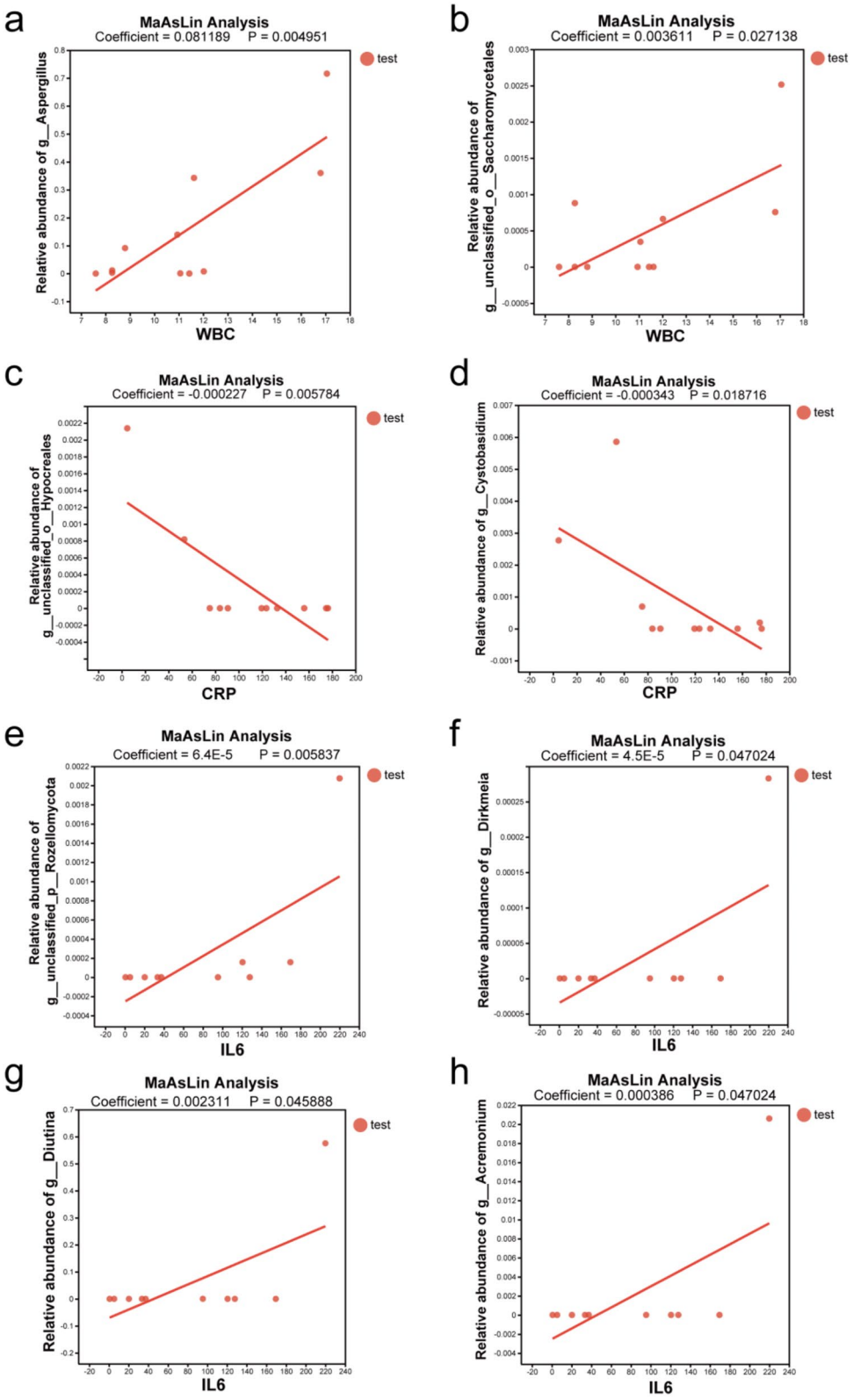

Fig. 8 Multivariate Association with Linear Models analysis between the clinical characteristics and species. **(a)** Between *Aspergillus* and WBC. **(b)** Between unclassified Saccharomycetales and WBC. **(c)** Between unclassified Hypocreales and CRP. **(d)** Between *Cystobasidium* and CRP. **(e)** Between unclassified Rozellomycota and IL-6. **(f)** Between *Dirkmeia* and IL-6. **(g)** Between *Diutina* and IL-6. **(h)** Between *Acremonium* and IL-6

Eurotiomycetes, *Epicoccum* and other fungi had a decrease. *Cutaneotrichosporon arboriformis* has been documented as a producer of a polysaccharide that exhibits immunological resemblance to the glucuronoxylomannan produced by *Cryptococcus* species, which are known to be pathogenic to humans. Antioxidant and genoprotective properties were discovered in *Penicillium oxalicum*, and polyketides extracted from the fungus were discovered to suppress the development of pancreatic cancers [22, 23]. The genus *Auricularia*, particularly the notable species *Auricularia auricula*, has been documented as a source of nutritious edible fungi abundant in bioactive compounds [24]. The polysaccharides derived from *Auricularia auricula* have been found to effectively reduce obesity and ameliorate non-alcoholic fatty liver disease in mice [25, 26]. The results of the study showed that there was a large amount of *Auricularia* predominantly in the 10th participant of the control group. The result exhibited significant individual variability. Similarly, *Ganoderma* has extraordinary value in nutrition, cosmeceuticals and medical treatments [27]. There was a large amount of *Ganoderma* predominantly in the 3th and 12th participants of the control group. Therefore, we believe that intestinal fungi can be influenced by diet [28]. Additional examination of the GMHI and MDI indices indicated a significant decrease in the GMHI index and a significant increase in the MDI index among AP patients. The analysis of co-abundance networks in both the control group and the AP group showed that there were higher interactions and predominantly positive correlations among gut fungus in AP patients compared to the control group. The findings have elucidated the intricate interplay between the modifications in gut fungi within individuals with AP. These alterations collectively engendered an intestinal microenvironment that exhibited a heightened propensity for the proliferation of pathogenic bacteria, while concurrently impeding the growth of advantageous bacteria.

It is well-established that the levels of inflammatory components such as leukocytes, CRP, and IL-6 are directly associated with the severity of acute pancreatitis. A meta-analysis demonstrated that IL-6 has the potential to be utilized in the early prediction of MSAP/SAP and in guiding clinical decision-making [29]. Utilizing correlation analysis of differential fungi and clinical indicators, we discovered that WBC had a positive association with Saccharomycetales and Aspergillus, and IL-6 had a positive correlation with *Diutina*, *Dirkmeia*, *Acremonium*, and unclassified Rozellomycota. *Cystobasidium* and unclassified Hypocreales were inversely associated with CRP.

This study has substantiated the modification of intestinal mycobiota, elucidated the presence of gut mycobiota dysbiosis in patients with AP, and further examined various species to identify potential fungi as clinical therapeutic targets. These fungi could be utilized for prophylactic antifungal therapy or supplementation with beneficial fungi to counteract systemic infections caused by fungal migration. Then we conduct a correlation analysis between the clinical indicators and the altered fungi, with the aim of facilitating the identification of significant fungi as potential targets and mitigating the likelihood of secondary infection, thus improving the prognosis of AP.

Some limitations exist in this investigation. To begin with, there was a noticeably tiny sample size. Our preliminary results indicate a significant difference between the two groups; however, further research with larger samples is needed to confirm these findings. This study was constrained by the lack of data concerning the participants' dietary habits, which precluded an analysis of the potential impact of diet on gut fungal composition. Furthermore, this study provided a preliminary correlation analysis between the fungal microflora and AP patients, without delving into any additional research on the underlying mechanisms. Additional prospective studies are required to gain a more comprehensive understanding of the underlying mechanisms of fungal infections in AP and to ascertain the potential benefits of preemptive treatment approaches, such as preventive antifungal therapy.

Conclusion

The current investigation discovered modified fungal microbiota in the feces of individuals suffering from AP. This finding offers fresh perspectives on the potential involvement of fungal microbiota in the onset and advancement of AP. Thus, by ensuring a harmonious gut fungal microbiome or introducing certain protective fungi through supplementation, the likelihood of fungal infections can be diminished and the outlook for acute pancreatitis can be enhanced.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13099-024-00675-z.

Supplementary Material 1

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Author contributions

YL and QJ designed the research work. MC and MF performed the research activities. SC conducted a statistical analysis. JW and MZ prepared figures and wrote the manuscript. MZ edited the manuscript submitted. All authors have read and given their approval for publication.

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Data availability
The raw sequencing reads have been uploaded to the NCBI Sequence Read Archive (SRA) database (Accession Number: PRJNA1027283).

Declarations

Ethical approval
This study followed the Helsinki declaration. This research was approved by the research ethics boards of Shanghai General Hospital, and written informed consent to participate in the study was obtained from all the patients.

Competing interests
The authors declare no competing interests.

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