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Changes of gastric microflora and metabolites in patients with chronic atrophic gastritis

Yumei Ma[1,2†], Jianming Jiang[3,4†], Zhufeng Yang[2,5†], Yongzhang Li[1,6†], Haiyan Bai[3,7], Zongxiu Liu[1,3], Shuo Zhang[1,3], Zheng Zhi[3,7*] and Qian Yang[3,7*]

Abstract

Background Chronic atrophic gastritis (CAG) is related to the body's microbial and metabolic systems. Combined studies of microbiome and metabolomics can clarify the mechanisms of disease occurrence and progression. We used 16S rRNA sequencing, metagenomics sequencing and metabolomics sequencing to depict the landscapes of bacterium and metabolites, construct correlation networks of different bacterium and metabolites describe potential pathogenic mechanisms of chronic atrophic gastritis.

Methods The gastric juices of 30 non-atrophic gastritis (NAG) patients and 30 CAG patients were collected. Gastric microflora was analyzed by 16S rRNA sequencing and metagenomics sequencing. Gastric metabolites were analyzed by LC–MS analysis. Different bioinformatics methods were used to analyze the data of microbiome and metabolome, and to analyze the relationship between them.

Results In atrophic gastritis, bacteria diversity decreased. The genera with a mean decrease in Gini greater than 1.5 included *peptostreptococcus, fusobacterium, prevotella, sphingomonas and bacteroides*. KEGG pathway included renal cell carcinoma, proximal tubule bicarbonate reclamation, citrate cycle and aldosterone synthesis and secretion with significant enrichment of differential metabolites. *Peptostreptococcus*, *fusobacterium, prevotella and sphingomonas* were in pivot positions of the correlation network of differential metabolites and differential bacterium. Viral carcinogenesis, glycine serine and threonine metabolism, RNA polymerase, galactose metabolism and retinol metabolism were enriched in chronic atrophic gastritis based on the metagenomic sequencing data.

Conclusion *Peptostreptococcus, fusobacterium, prevotella, sphingomonas and bacteroides* were the essential features that distinguish atrophic gastritis from non-atrophic gastritis, and caused disease by altering various metabolic pathways. Viral carcinogenesis, glycine serine and threonine metabolism, RNA polymerase, galactose metabolism and retinol metabolism may be related to the occurrence and progression of CAG.

Keywords Chronic atrophic gastritis, Non atrophic gastritis, Microbiome, Metabonomics, Random forest algorithm

†Yumei Ma, Jianming Jiang, Zhufeng Yang and Yongzhang Li have contributed equally to this work.

*Correspondence:
Zheng Zhi
hbzyxyzz@163.com
Qian Yang
hbszyangqian@163.com
Full list of author information is available at the end of the article

Introduction

Modern medicine believes that chronic gastritis includes chronic non-atrophic gastritis (NAG) and chronic atrophic gastritis (CAG), and the occurrence of CAG is usually a relatively long evolution process under the action of a variety of causes [1, 2]. The pathological process progresses from normal mucosa to NAG to CAG to intestinal metaplasia and eventually to gastric cancer [3]. Most intestinal gastric cancer occurs on the basis of CAG [3–6]. As a kind of precancerous lesion of gastric cancer [7–9], CAG is a hot topic in both clinical and scientific research fields.

CAG is a chronic stomach disease characterized by atrophy and reduction of the inherent glands of the gastric mucosa, with or without fibrous replacement, intestinal metaplasia, and/or atypical hyperplasia [10–13]. Because the natural glands of the gastric mucosa will occur physiologically atrophy or decrease, so this disease is more common in the elderly [14–16]. However, with the continuous increase of living pressure, the constant change of diet, as well as the continuous progress and popularization of electronic gastroscopy and pathological diagnosis, the discovery rate of clinical chronic atrophic gastritis is gradually increasing, and the affected population is becoming younger and younger [14–16]. The causes and mechanisms of its pathogenesis mainly include [1, 6, 17–19]: (1) the incidence is generally positively correlated with age. For example, the lack of gastric mucosa nutrition factors such as gastrin and epidermal growth factor, or the insensitivity of gastric mucosa sensory nerve endings to the above factors will lead to gastric mucosa atrophy; (2) *Helicobacter pylori* (*HP*) infection is the main cause, and more than 90% of patients with CAG suffer from *HP* infection; (3) Long-term drinking of strong tea, spirits, coffee, eating too hot and too cold and other physical factors can also lead to repeated damage of gastric mucosa; (4) Long-term oral administration of large amounts of non-steroidal anti-inflammatory drugs inhibited the synthesis of prostaglandin in gastric mucosa and damaged the mucosal barrier; (5) Bile reflux can destroy the mucosal barrier and cause chronic inflammatory changes of gastric mucosa.

At present, the main treatment plan is to target the causes of chronic atrophic gastritis, which mainly uses the eradication of HP infection, proton pump inhibitors, gastric modynamics drugs, gastric mucosal protective agents and other symptomatic treatment, and regular electronic gastroscopy follow-up, which cannot fundamentally solve the precancerous lesions of gastric cancer, and the clinical manifestations of CAG are diverse [20]. The more common clinical manifestations are dull or burning pain in the upper abdomen, heating, gastric distension, nausea, etc., which are difficult to diagnose clinically and eradicate the precancerous state of gastric cancer. Therefore, it is necessary to further explore the pathogenesis of chronic atrophic gastritis and explore new diagnostic markers and therapeutic targets.

With the development of DNA sequencing technology and bioinformatics methods, the study of human microbe has entered another new stage [21, 22]. Transcriptomics and proteomics are widely used in the study of gastrointestinal diseases. However, these does not fully explain the pathogenesis of the disease. Diseases of the digestive system are closely related to the body's microbial and metabolic systems. Combined studies of microbiome and metabolomics can clarify the mechanisms of disease occurrence and progression.

In this study, a total of 30 patients with CAG and 30 patients with NAG were included, and 16S rRNA sequencing, metagenomics sequencing and metabolomics sequencing were performed. We first analyzed the diversity and abundance of bacteria in CAG samples and NAG samples by 16S rRNA sequencing. The key pathogenic genera were screened by random forest algorithm, and the correlation network among genera was constructed. We also elucidated the possible pathogenetic pathways through the enrichment analysis of bacterium. In terms of metabolomics, we screened the differentially expressed metabolites of CAG samples and NAG samples, and selected key metabolites by random forest algorithm, which can be used as potential diagnostic markers. We constructed a network of correlations between differential metabolites and differential bacterium. In metagenomics, we highlight the results of differential genera and functional enrichment analyses. Our study may provide clues to elucidate the pathogenic mechanism of chronic atrophic gastritis and identify potential key pathogenic bacteria and pathogenic metabolites.

Materials and methods

Study design

A total of 30 chronic atrophic gastritis (CAG) patients and 30 non-atrophic gastritis (NAG) patients attending Hebei provincial hospital of Traditional Chinese Medicine from January 2020 to December 2022 were selected as subjects. This study was a case–control study. Inclusion criteria: (1) patients aged between 18 and 65 years; (2) Patients in the CAG group met the diagnostic criteria for CAG, and patients in the NAG group met the diagnostic criteria for NAG. The diagnosis of the two diseases was based on gastroscopy and pathological biopsy results. (3) The patients fully understood the significance of this study and voluntarily sign the informed consent; (4) The patients had reading ability and good follow-up compliance. Exclusion criteria: (1) with autoimmune gastritis; (2) with gastric and duodenal ulcers and upper

gastrointestinal bleeding; (3) with gastric mucosa of severe dysplasia and mucosal pathological diagnosis suspected malignancy; (4) with serious organic diseases; (5) with mental illness cannot cooperate with researchers; (6) pregnant or lactating women; (7) have been enrolled in other clinical studies. Patients in the CAG group ranged in age from 34 to 65 years old, with an average age of (52.8 ± 8.29) years old, including 15 males and 15 females, with an average BMI of (24.19 ± 3.32) Kg/m^2. Patients in the NAG group ranged in age from 30 to 63 years old, with an average age of (52.1 ± 7.13) years old, including 12 males and 18 females, with an average BMI of (23.69 ± 2.62)Kg/m^2. There was no significant difference in baseline clinical data between the two groups ($P > 0.05$). All patients were yellow, and there was no significant difference in dietary habits.

Sample collection

Gastroscope models used in this study were GIF-H260, GIF-Q260 and GIF-HQ290 (Olympus, Japan). Under the direct vision of the gastroscope, disposable tubes were inserted through the gastroscopic biopsy orifice into the mucous lake at the lower part of the stomach (the fundus junction). Gastric fluid were extracted via syringe connected to the disposable tube. Gastric juices extracted were placed into the 20 ml sterile cryopreservation tubes and conserved at − 80 °C in the refrigerator.

Liquid chromatography-mass spectrometer (LC–MS) analysis

Sample preparation

After adding methanol to the gastric fluid sample, the protein was incubated and precipitated. The resulting supernatant was transferred to vials and kept at − 80 °C until UHPLC-QE Orbitrap/MS analysis. Weigh the sample and transfer it into a 1.5 mL EP tube. Add two small steel beads and 400 μL of methanol–water solution (4:1, v/v, containing 4 μg/mL L-2-chlorophenylalanine). Precool the mixture at − 40 °C for 2 min, then grind it in a grinding machine for 2 min (60 Hz). After grinding, perform ultrasonic extraction in an ice-water bath for 10 min, followed by incubation at − 40 °C for 30 min. Centrifuge the mixture at 4 °C and 1200 rpm for 10 min, then transfer 300 μL of the supernatant to an LC–MS vial and air-dry. After drying, reconstitute the residue in the vial with 300 μL of methanol–water (1:4, v/v), vortex for 30 s, ultrasonicate in an ice-water bath for 3 min, and incubate at − 40 °C for 2 h. Centrifuge the reconstituted extract at 4 °C and 1200 rpm for 10 min, collect 150 μL of the supernatant, filter through a 0.22 μm organic phase syringe filter, transfer to an LC vial, and store at − 80 °C for subsequent LC–MS analysis. Quality control (QC) samples were prepared by mixing equal volumes of extracts from all samples.

Liquid chromatography-mass spectrometry (LC–MS) analysis

Metabolomics data analysis was performed by Shanghai Luming Biotechnology Co., Ltd. The analytical system consisted of an ACQUITY UPLC I-Class Plus ultra-performance liquid chromatograph coupled with a QE Plus high-resolution mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a heated electrospray ionization (HESI) source. Both positive and negative ion modes were analyzed using an ACQUITY UPLC HSS T3 column (1.8 μm, 2.1 × 100 mm). The mobile phase comprised (A) water (0.1% formic acid, v/v) and (B) acetonitrile (0.1% formic acid, v/v) with the following gradient: 0.01 min, 5% B; 2 min, 5% B; 4 min, 30% B; 8 min, 50% B; 10 min, 80% B; 14 min, 100% B; 15 min, 100% B; 15.1 min, 5% B; 16 min, 5% B. The flow rate was 0.35 mL/min, and the column temperature was maintained at 45 °C. All samples were stored at 10 °C during analysis. The mass scan range was m/z 100–1200. Full MS resolution was set to 70,000, and MS/MS resolution to 17,500, with collision energies of 10, 20, and 40 eV. Instrument parameters included: spray voltage, 3800 V (+) and 3200 V (−); sheath gas flow, 35 arbitrary units; auxiliary gas flow, 8 arbitrary units; capillary temperature, 320 °C; auxiliary gas heater temperature, 350 °C; S-lens RF level, 50.

Data preprocessing and statistical analysis

LC–MS raw data were processed using Progenesis QI V2.3 (Nonlinear Dynamics, Newcastle, UK) for baseline filtering, peak identification, integration, retention time correction, peak alignment, and normalization. Key parameters included a precursor tolerance of 5 ppm, product tolerance of 10 ppm, and a 5% product ion threshold. Metabolites were identified using the Human Metabolome Database (HMDB), LipidMAPS (V2.3), Metlin, and a custom database based on accurate m/z values, secondary fragments, and isotopic patterns. Peaks with > 50% missing values (intensity = 0) within a group were removed, zero values were replaced with half of the minimum value, and compounds with database matching scores below 36 (out of 80) were discarded. Positive and negative ion data were merged into a single matrix.

Principal component analysis (PCA) was performed using R packages to evaluate sample distribution and analytical stability. Orthogonal partial least squares-discriminant analysis (OPLS-DA) and partial least squares-discriminant analysis (PLS-DA) were applied to distinguish metabolic differences between groups. Model validity was assessed via sevenfold cross-validation

and 200-response permutation testing (RPT). Variable importance in projection (VIP) values from OPLS-DA were used to rank metabolites contributing to group discrimination. A two-tailed Student's t-test was employed to validate significant differences (VIP > 1.0, p < 0.05).

High-throughput 16S ribosomal RNA gene sequencing

Microbial DNA was extracted from gastric fluid samples using Mobio PowerSoil®DNAIsolationKit. A universal primer was used to amplify bacterial 16S ribosomal DNA (341 F: 5′ -actcctacgggaggCAGCAGCAG-3′;806R: 5′ -GGACTACHVGGGTWTCTAAT-3′) targeting the v3-v4 region of 16S ribosomal DNA amplification target product. SolexaPCR and ImageJ were used for quantitative analysis. After mixing the samples, the library was constructed with the recovered products of 1.8% agarose gel. Double-terminal sequencing was performed using a MiSeq sequencer. The original data was spliced, the spliced sequences were filtered for quality, and the chimera was removed to get high-quality tags sequences. The sequences were clustered at the 97% similarity level, and 0.005% of all sequence numbers were used as the threshold for filtering operational taxonomic units (OTUs). Species annotation was performed by RDP Classifier software, and the reliability threshold was 0.8. PyNAST software was used to analyze the phylogeny of dominant OTUs. Microbial diversity analysis was performed using Mothur software. The dilution curve was used to evaluate whether the sequencing volume covered all groups in the sample. Based on the R language platform, PCA and principal coordinate analysis (PCoA) were performed to show Beta diversity. LefSe linear discriminant analysis was conducted. The significance of LefSe results was tested using metastats analysis.

Metagenomics sequencing

Very short sequences, low quality sequences, splice sequences and host DNA sequences generated from Raw Reads were filtered prior to in-depth analysis of the data. We used standard Microbiome Helper steps for metagenomic sequencing data quality control and filtering. Firstly, the quality of the original data was detected by Fast QC tool to determine the size of the data and get the basic information such as Q20, Q30, error rate and GC content of the sequencing data. Then, the low quality sequences were removed by KneadData tool, Trimmomatic and Bowtie2. Finally, high quality microbial DNA sequences are obtained. DIAMOND software was used to compare the representative sequences of the non-redundant gene set with the NR library of NCBI, and the annotations of P < 1e-5 were selected to screen the proteins with the highest sequence similarity, so as to obtain functional annotation information.

Statistical analysis

Comparisons of continuous variables were made via the independent sample t test or the separate variance estimate t test. Differences between categorical variables were assessed using the Chi-square test. Spearman rank correlation analysis was used for the relationship between metabolites or between species and metabolites. False discovery rate (FDR, Benjamini-Hochberg) was used to adjust the original P values of multiple tests. SPSS version 22.0 and R version 3.5.2 were used to analyze the data.

Results

Diversity analysis of CAG group and NAG group

Alpha diversity analysis revealed there were significant differences in chao1, observed species, shannon and simpson index between the chronic atrophic gastritis (CAG) and non atrophic gastritis (NAG) groups (P < 0.05, Fig. 1A–D). PCoA and permutational multivariate analysis of variance (PERMANOVA) test for Beta diversity revealed a significant difference in the composition and abundance of gastric juice microbiota between groups (Unweighted Unifrac P = 0.039 and Bray–Curtis P = 0.028) (Fig. 1E and F). The top *tenamplicon sequence variants* (ASV) with the most significant differences between the CAG and NAG groups were *prevotella* (ASV6), *sphingomonas* (ASV12), *peptostreptococcus* (ASV15), *atopobium* (ASV29), *leptotrichia* (ASV44), *dialister* (ASV74), *sphingomonas* (ASV148), *fusobacterium* (ASV241), *tannerella* (ASV324), *catonella* (ASV478) (P < 0.05, Fig. 1G). Linear discriminant analysis (LDA) showed the differences on taxa abundance between groups. *Prevotella, rothia, peptostreptococcus* and *atopobium* were more enriched in NAG groups, while *ralstonia, sphingomonas, muribaculaceae and ruminococcus* were more enriched in CAG groups (Fig. 1H).

Identification of key pathogenic bacterial genera

We selected the top 50 genera with highest abundance, and calculated spearman correlation coefficient based on the relative abundance. |Spearman cor|> 0.8 and P-value < 0.01 were used as the screening criterion, and a interaction network was established to show the relationships among genera in CAG group (Fig. 2A). We show correlations among the top 30 abundance genera in CAG group (Fig. 2B). Random forest algorithm showed the importance of bacterial genera in describing the intrinsic characteristics of the CAG and NAG groups. The genera with a mean decrease in Gini greater than 1.5 included *peptostreptococcus, fusobacterium, prevotella, sphingomonas* and *bacteroides* (Fig. 2C).

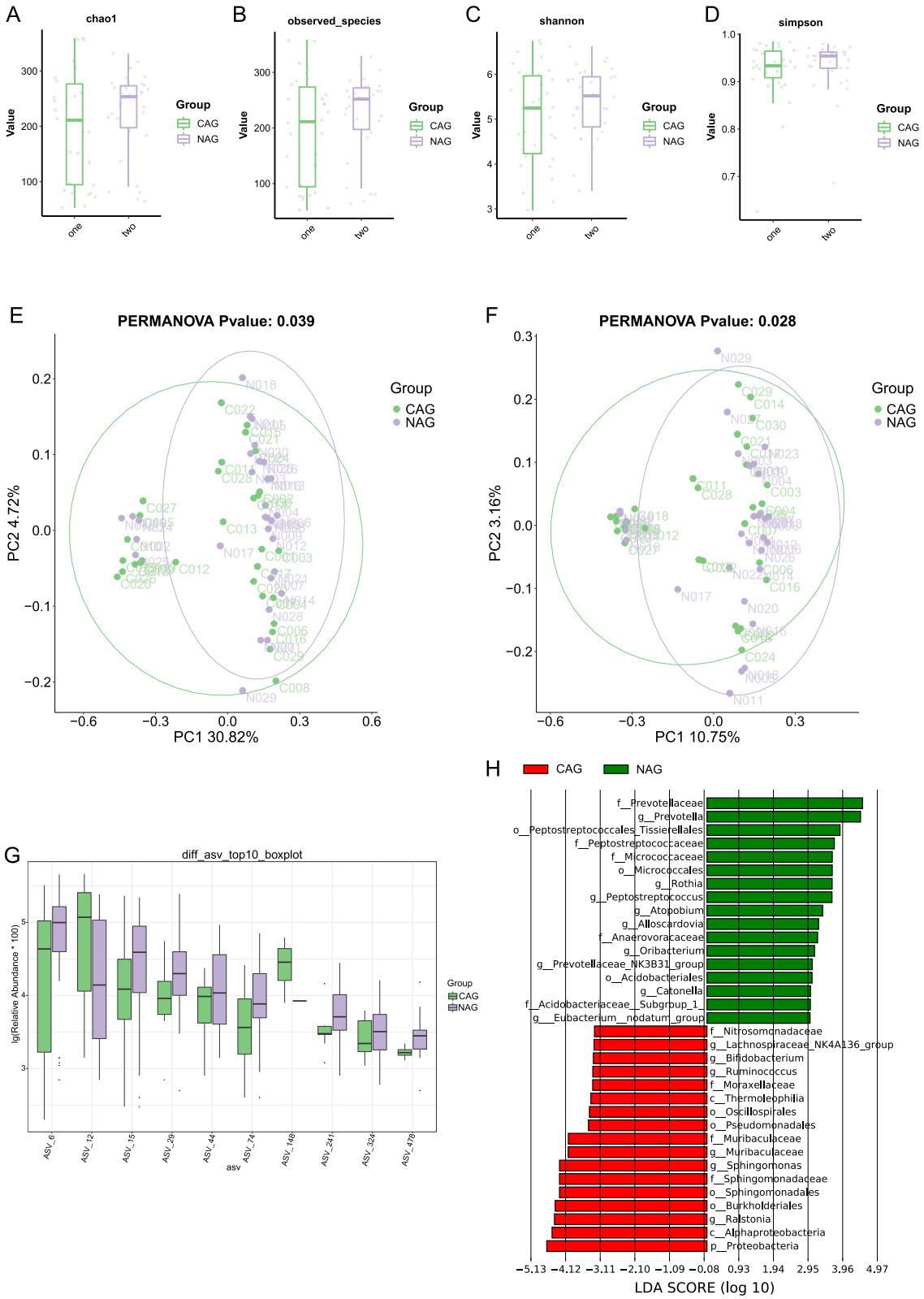

Fig. 1 Diversity analysis of chronic atrophic gastritis group and non atrophic gastritis group. **A**–**D** The differences of the diversity indexes between the two groups. **E** Principle coordinate analysis (PCoA) for Beta diversity. **F** Permutational multivariate analysis of variance (PERMANOVA) test for Beta diversity. G The top 10 amplicon sequence variants with the most significant differences between groups. H Linear discriminant analysis of the two group. C represented for chronic atrophic gastritis group, while N represented for non atrophic gastritis group

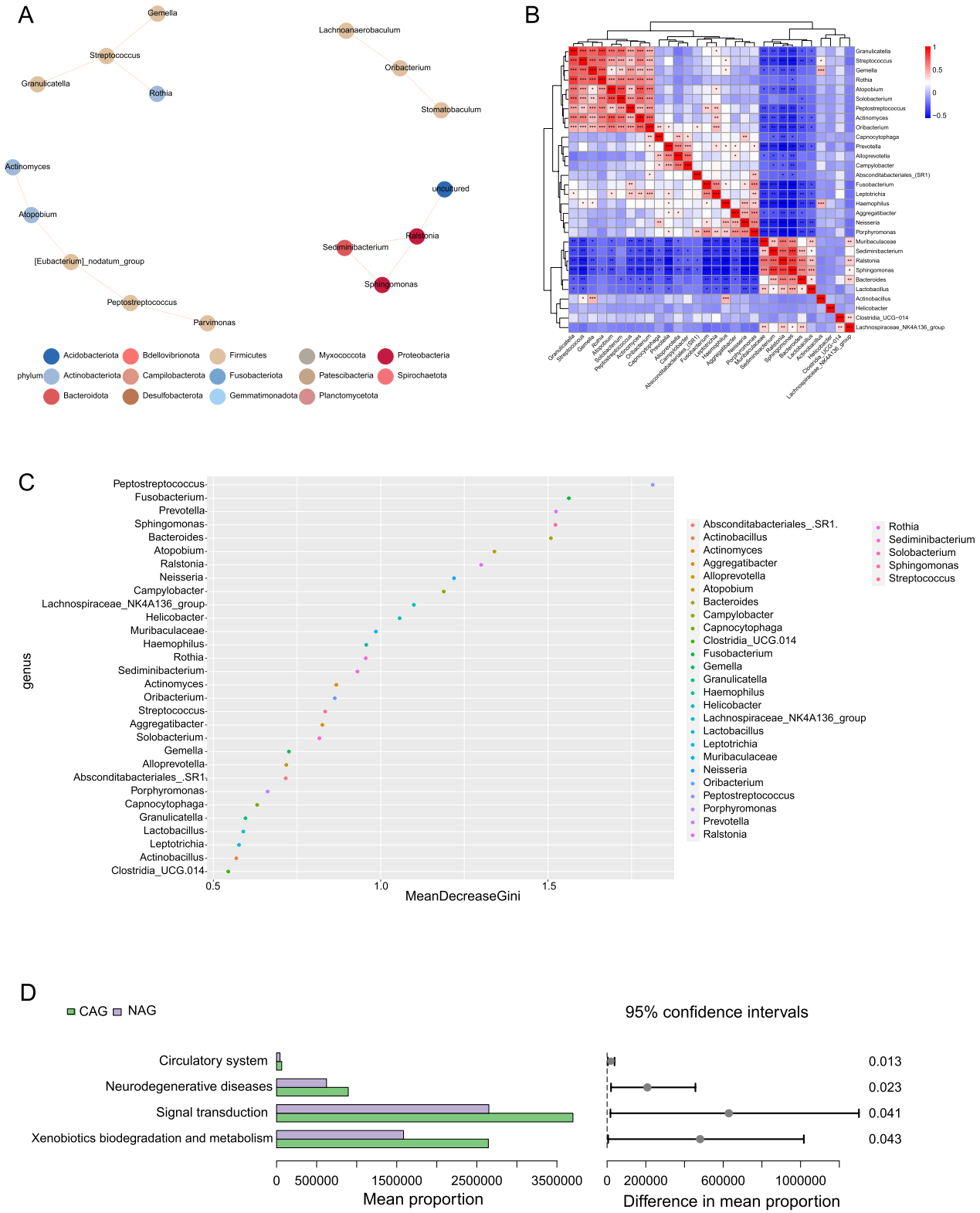

Fig. 2 Screening of key pathogenic bacterial genera. **A** Bacterial genus correlation network diagram. Red lines represented positive correlation and green lines represented negative correlation. The thicker the line, the higher the correlation between genera. **B** Correlation heat map between genera with top 30 abundance. **C** Mean decrease Gini of genera. **D** KEGG analysis between the two groups. The left bar was the mean abundance of pathways in each group, and the right was the 95% confidence interval and corresponding significance P value for comparison of differences between groups. C represented for chronic atrophic gastritis group, while N represented for non atrophic gastritis group

Identification of differentially expressed metabolites

Using VIP > 1, FDR < 0.05 and $|\log_2 FC| > 0$ as criteria, we screened out metabolites that were differentially expressed between the CAG and NAG groups (Fig. 3A and B). A total of 168 metabolites with differential abundance were obtained, among which 81 metabolites had up-regulated abundance and 87 metabolites had down-regulated expression in chronic atrophic gastritis samples (Table S1). The Correlation between differential metabolites and differential metabolite response strength data was calculated based on pearson correlation analysis, and the network was constructed by selecting the metabolite relationship pairs satisfying conditions P-value < 0.05 and correlation > 0.95 (Fig. 3C). Pearson correlation analysis was performed for the top 20 metabolites with significant differences ranked by VIP in chronic atrophic gastritis group (Fig. 3D). We performed KEGG enrichment analysis of differential metabolites. KEGG pathway included renal cell carcinoma, proximal tubule bicarbonate reclamation, citrate cycle and aldosterone synthesis and secretion with significant enrichment of differential metabolites (Fig. 3E).

Construction of microbial and differential metabolite correlation network

We used the random forest algorithm to screen differentially expressed metabolites that distinguish CAG from NAG gastritis, and the importance of the differentially expressed metabolites is shown in Fig. 4A. We constructed a correlation network of differential metabolites and differential bacterium, as shown in Fig. 4B. *Peptostreptococcus, fusobacterium, prevotella and sphingomonas* were in pivot positions and correlated with many kinds of metabolites.

Comparison of microbial diversity and abundance based on metagenomics sequencing

In order to further analyze the influence of microorganisms on chronic atrophic gastritis, we selected samples for metagenomic sequencing. Metagenomic sequencingresults showed that a total of 1589 bacteria genera were identified in both CAG and NAG samples, and the top 15 bacteria genera with the highest proportion were shown in Fig. 5A. We have shown the top 30 bacteria genera with the most significant differences, including peptostreptococcus, fusobacterium, prevotella, and bacteroides, which coincided with the results of 16S rRNA sequencing to a certain extent (Fig. 5B). In terms of alpha diversity and beta diversity, there was no statistical significance between the two groups (Fig. 5C–F). We used the LEfSe to identify bacterium with significant differences in relative abundance between the two groups. Haemophilus was significantly enriched in non atrophic gastritis samples. *Odoribacter, mycoplasmataceae, mycoplasma, tenericutes, mycoplasma, mycoplasmatales, splanchnicus, odoribacteraceae, CHU740, F0091* and *NPS 308* were significantly enriched in atrophic gastritis samples (Fig. 5G). We show the top 10 species with the most significant differences (Fig. 5H).

Functional enrichment analysis

GO pathway enrichment analysis showed that different strains were significantly enriched in cellular process, metabolic process, biological regulation and other biological processes. The different strains were significantly enriched in cellular component items such as cell part, membrane and macromolecular complex. The different strains were significantly enriched in antioxidant activity, binding, catalytic activity and other molecular function items (Fig. 6A). Based on the KEGG database, we used the LEfSe tool to analyze the relative abundance of microbial metabolic pathways in each sample. A total of 7 bacteria-specific metabolic pathways with significant relative abundance differences were identified between the two groups by LEfSe tool. Biotin metabolism and Olfactory transduction were significantly enriched in NAG group. Viral carcinogenesis, glycine serine and threonine metabolism, RNA polymerase, galactose metabolism and retinol metabolism were enriched in chronic atrophic gastritis based on the metagenomic sequencing data (Fig. 6B).

Discussion

The ratio of human bacteria to human cells is close to 1:1, and the genes they carry are known as the second set of human genome [23–26]. Bacteria not only exert extensive influence on human living environment, but also co-evolve with human beings and interact with human bodies almost from the fetal stage, participating in all aspects of human function [27–30]. Our understanding

(See figure on next page.)

Fig. 3 Identification of differentially expressed metabolites. **A** and **B** The Volcano and heatmap of differentially expressed metabolites between atrophic gastritis group and non atrophic gastritis group. **C** Differentially expressed metabolites correlation network diagram. The thickness of the lines represents the degree of relevance, the red line represents positive correlation, and the blue line represents negative correlation. D Correlation heatmap of the top 20 metabolites with most significant differences. E KEGG analysis based on differentially expressed metabolites

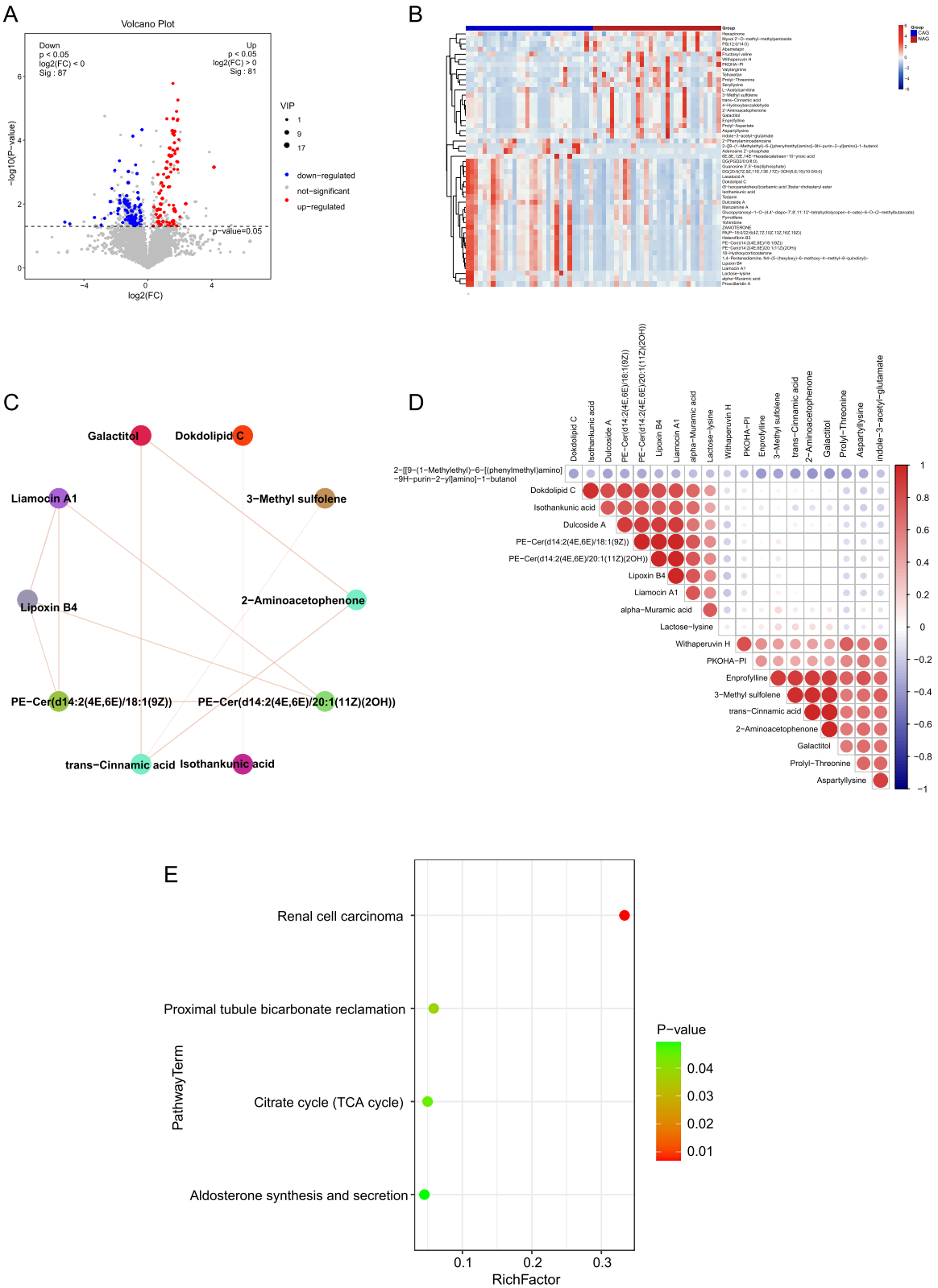

Fig. 3 (See legend on previous page.)

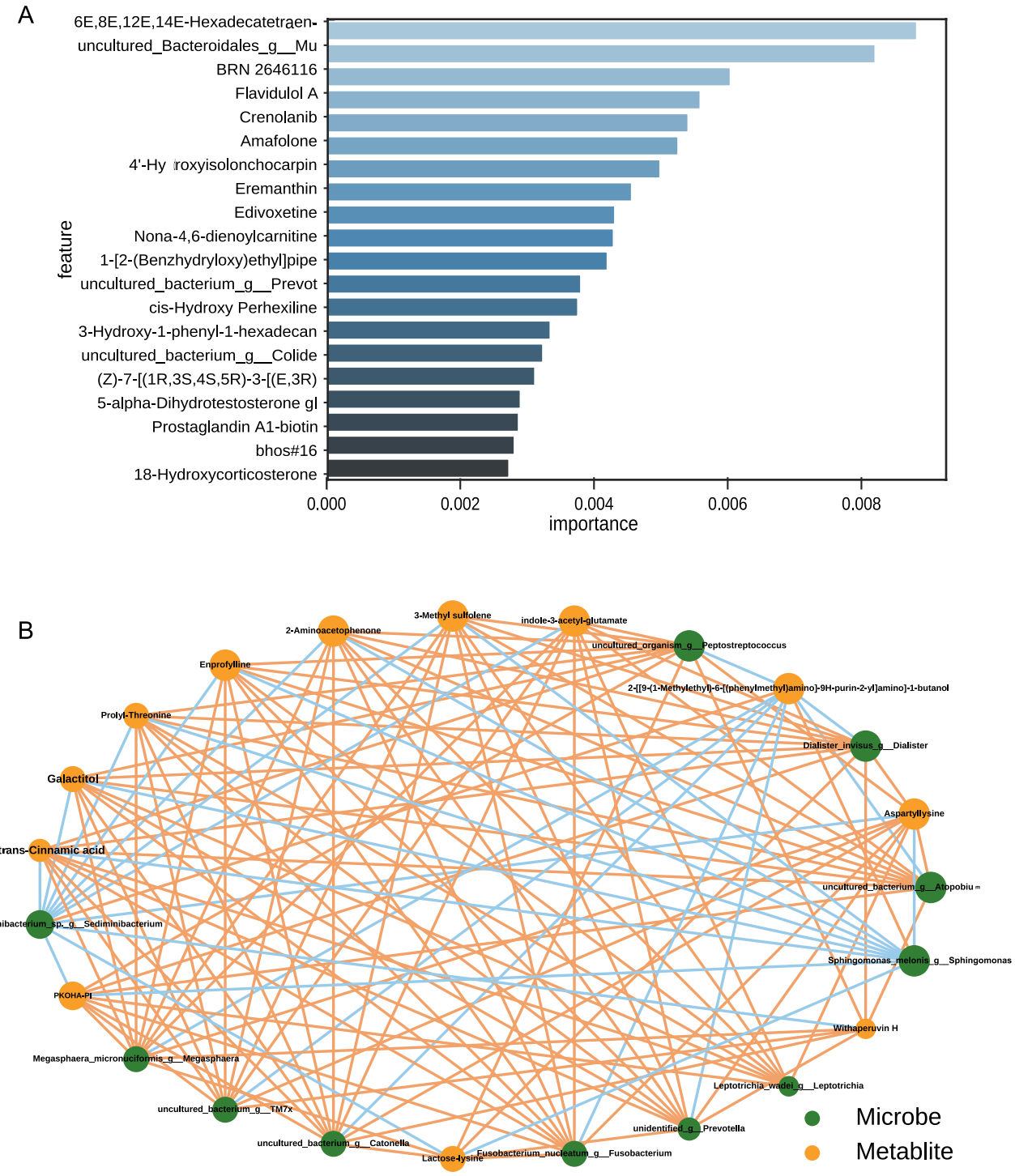

Fig. 4 Construction of microbial and differential metabolite correlation network. **A** The identification of the metabolites that characterize the disease via random forest algorithm. **B** Microbial and differential metabolite correlation network. The yellow lines represented positive correlation and the blue lines represented negative correlation. The thickness of the line represented the level of the correlation coefficient

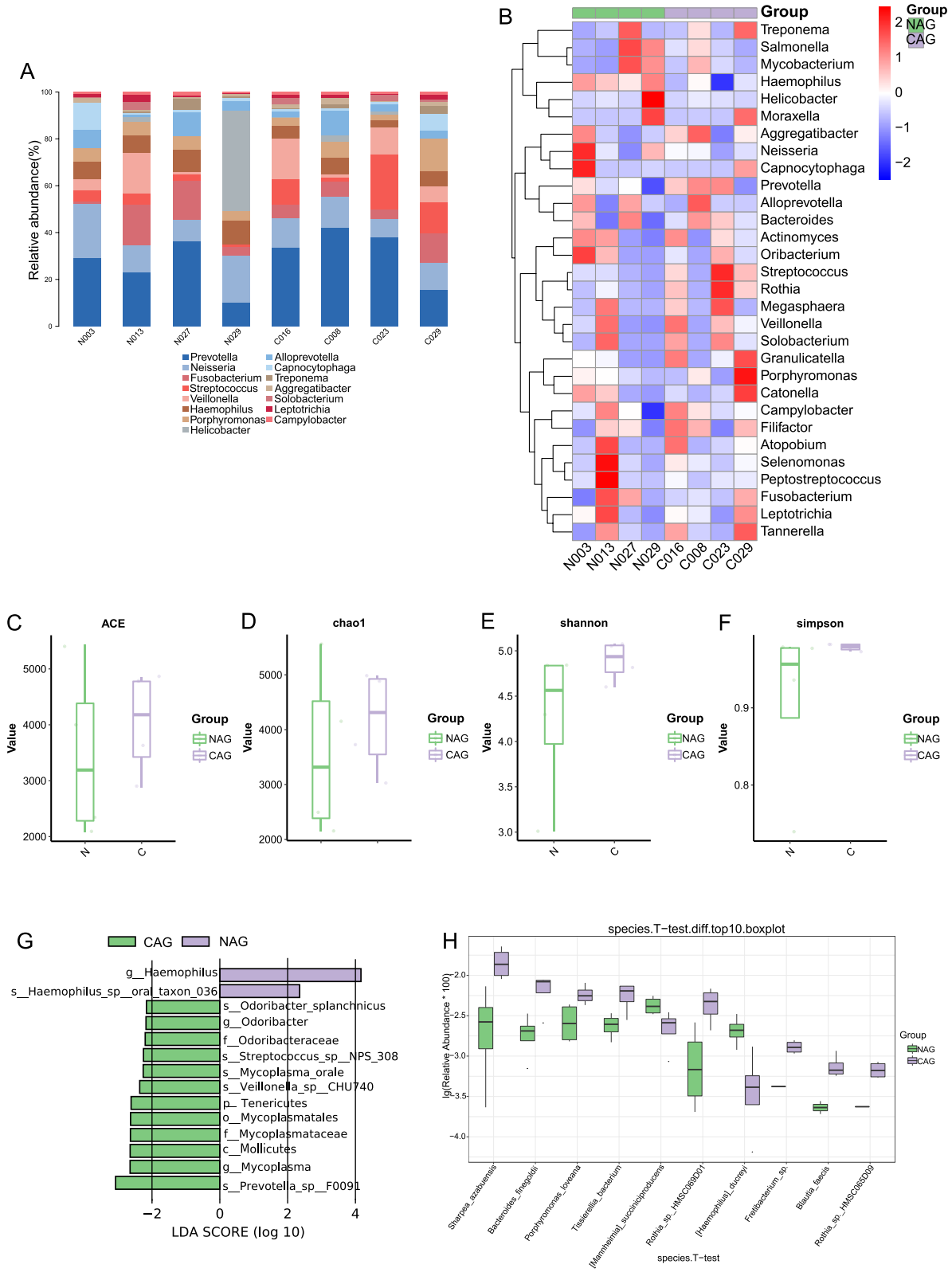

Fig. 5 Comparison of microbial diversity and abundance based on metagenomics sequencing. **A** Genus composition ratio of samples. **B** Heatmap of the top 30 genera with the most significant differences. **C**–**F** The differences of the diversity indexes between the two groups. **G** Linear discriminant analysis of the two group. **H** The top 10 species with the most significant differences

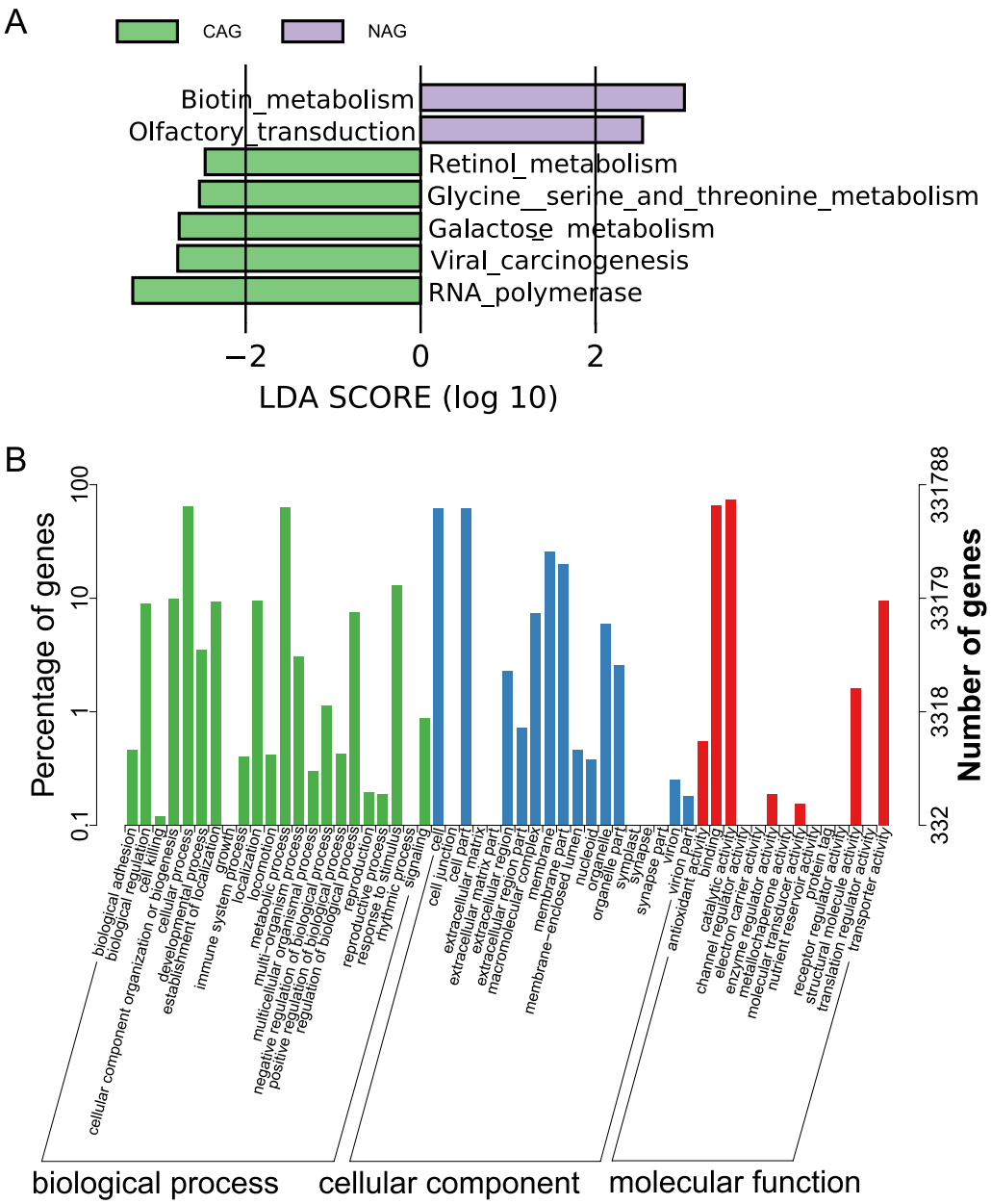

Fig. 6 Functional enrichment analysis of different microbial between groups. **A** Functional enrichment analysis based on KEGG. **B** Functional enrichment analysis based on GO

of microorganisms relevant to human survival and disease is very limited, often limited to the exploration of a particular species and its function. Human beings needed to rely on the culture of a single microorganism, so as to realize the systematic and in-depth study of human microorganisms. However, such research is a huge workload and progress is slow. Advances in sequencing technology and the development of molecular biology have freed human beings from this dependence, giving human beings the ability to fully describe the microbial landscape and explore the functions of microorganisms on the human body. Human digestive tract is colonized by trillions of microorganisms, which constitute a complex and huge microbial ecosystem and play an important role in maintaining human homeostasis. These microorganisms are very finely balanced, and the imbalance in their composition and function can lead to a variety of diseases, including gastrointestinal, neurological, respiratory, metabolic, cardiovascular, and malignant tumors.

16 s RNA sequencing and metagenomics sequencing sequencing are two different methods for studying microbiome. The former has advantages in genera identification, while the latter has advantages in species function. Both methods have been widely used in gastritis and gastric cancer to explore the role of human digestive tract microorganisms in stomach diseases. The structure of gastric microflora is complex, and there are many other microorganisms besides HP, and the imbalance of gastric microflora is closely related to gastric cancer [31, 32]. A comparative study was conducted on the gastric mucosal flora of all subjects by 16S rRNA sequencing. It was found that intestinal metaplasia and gastric cancer patients had obvious imbalance of gastric mucosal flora. At the same time, the study also found that, compared with patients with superficial gastritis, 21 bacterial categories were significantly enriched in the stomach of patients with gastric cancer, and the relative abundance of another 10 bacterial categories was significantly reduced in the stomach of patients with gastric cancer, and 5 core dominant species were enriched in the stomach of patients with gastric cancer. These include *Peptostreptococcus stomatis, Streptococcus anginosus, Parvimonas micra, Slackia exigua* and *Dialister pneumosintes* comprises a community that well classifies gastric cancer patients from gastritis patients (ROC, AUC =0.82), and this result is validated in another area subject [33, 34]. Ferreira et al. studied the gastric mucosal flora and found that compared with patients with chronic gastritis, the diversity of gastric flora Alpha and the relative abundance of HP decreased in patients with gastric cancer [35]. Some symbiotic bacteria, which usually colonize the gut, were enriched in the stomach of gastric cancer patients. In addition, the microecological imbalance index calculated by the relative abundance of differentially distributed bacteria between the two groups can effectively distinguish gastric cancer from chronic gastritis [35].

These studies on the relationship between gastric microbes and gastric cancer were conducted using marker gene analysis, namely 16S rRNA gene amplicon analysis. By designing PCR primers in the evolutionarily conserved region of bacterial 16S rRNA gene to amplify the highly variable region of the gene, high-throughput sequencing was used to reconstruct the structural composition of a specific microbiota, and bioinformatics was used to predict the functional characteristics of the biota. However, the identification of bacterial taxa by this method is mostly at the genus level, and the information of a large number of genes except 16S rRNA gene will be lost. Meanwhile, the bias of PCR primers and multiple PCR cycles may also bring large errors to the final results. Compared with marker gene analysis, metagenomics analysis of all the genomes in the sample has a higher resolution in the identification of the taxonomic structure and functional characteristics of the microflora, which can be accurate to the level of strains or even strains, and the annotation of microbial functional characteristics is more accurate and comprehensive. Park et al. confirmed by metagenomics that high expression of type IV secretion system gene may promote the occurrence and progression of gastric cancer [36]. Metagenomics studies confirmed that the abundance of HP decreased in atrophic gastritis samples, intestinal metaplasia samples and gastric cancer samples, whereas the abundance of other microorganisms increased. HP infection may inhibit colonization of carcinogenic intestinal flora [36].

In addition to microbiome, metabolomics can be used to assist clinical diagnosis, pre-diagnosis and efficacy evaluation by analyzing the changes of metabolites in response to pathological changes of the body, and provide ideas for the study of the pathogenesis of diseases. Therefore, some scholars also began to try to use metabolomics technology to further analyze and study stomach diseases. Zu et al. showed the metabolic differential profile in urine of rats with CAG. D-glutamine and D-glutamic acid metabolism may be involved in the occurrence and progression of diseases [37]. Tong et al. believed that before and after berberine treatment, metabolic pathways were changed and metabolic phenotypes were significantly different in rats with atrophic gastritis. Their study identified 15 metabolic markers associated with berberine treatment that may be key nodes in berberine's mechanism for treating chronic atrophic gastritis [38]. Of these, 17 metabolites returned to normal levels after treatment. Their study concluded that the possible mechanism of action of Shidan granule is related to tricarboxylic acid cycle and amino acid metabolism pathway [39].

In our study, 16S rRNA sequencing and metagenomics sequencing were used for the first time to demonstrate the microbiome landscape of gastric fluid samples from patients with CAG, and metabolomics analysis was combined to explore the correlation between microorganisms and metabolites. Our study found that compared with NAG, the diversity of bacterial Alpha and Beta in atrophic gastritis samples decreased. According to the random forest algorithm, *peptostreptococcus, fusobacterium, prevotella, sphingomonas* and *bacteroides* are the five most important bacteria genera to distinguish between CAG and NAG. Compared with NAG samples, 168 different metabolites in CAG samples were screened. These differential metabolites were significantly enriched in citrate cycle (TCA cycle), renal cell carcinoma, proximal tubule bicarbonate reclamation and aldosterone synthesis and secretion. We constructed a network of correlations between microbes with different

abundance and differentially expressed metabolites. We found that *peptostreptococcus, prevotella, sphingomonas and fusobacterium* are at the hub of the network and are associated with a variety of metabolites. This is highly consistent with the results of the previous random forest algorithm. This indicates that these four bacteria genera can characterize the essential characteristics of disease and lead to the occurrence of disease by changing the expression of multiple metabolites and metabolic pathways. In metagenomics studies, we focus on species differences and functional enrichment analysis. The results showed that different bacterium were significantly enriched in viral carcinogenesis, glycine serine and threonine metabolism, RNA polymerase, galactose metabolism and retinol metabolism.

There are some shortcomings in this study.First of all, the sample size of this study is small, and it is still necessary to further expand the sample size. Secondly, this study did not stratify the samples according to age and sex, which are all factors affecting the composition of microbiota and metabolic profile. Finally, this study did not carry out basic experiments to further explore the biological significance of specific strains and their related metabolites.

Conclusion

Peptostreptococcus, fusobacterium, prevotella, sphingomonas and bacteroides were the essential features that distinguish atrophic gastritis from non-atrophic gastritis, and caused disease by altering various metabolic pathways. Viral carcinogenesis, glycine serine and threonine metabolism, RNA polymerase, galactose metabolism and retinol metabolism may be related to the occurrence and progression of chronic atrophic gastritis.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-025-06458-7.

Supplementary Material 1Table S1 A total of 168 metabolites with differential abundance were obtained between chronic atrophic gastritis and non atrophic gastritis samples

Author contributions

Qian Yang and Jianming Jiang designed and conducted the study. Yumei Ma, Zheng Zhi, Zhufeng Yang, Yongzhang Li and Haiyan Bai analyzed the data. Yumei Ma, Zheng Zhi, Zhufeng Yang, Zongxiu Liu and Shuo Zhang wrote and revised the manuscript.

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Availability of data and materials

The datasets generated during 16 s and metagenomic sequencing of gastric fluid DNA samples have been deposited in NCBI Sequencing Read Archive (accession ID: PRJNA1040805 PRJNA1041479). Other datasets from the current study are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of Hebei provincial hospital of Traditional Chinese Medicine. The ethical approval number is HBZY2020-KY-042–04.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Reserch Center, Hebei Province Hospital of Chinese Medicine, 389 Zhongshan East Road, Shijiazhuang 050011, Hebei, China. [2]Hebei Key Laboratory of Integrated Chinese and Western Medicine for Gastroenterology Research, 389 Zhongshan East Road, Shijiazhuang 050011, Hebei, China. [3]Hebei Key Laboratory of Turbidity Toxin Syndrome, 389 Zhongshan East Road, Shijiazhuang 050011, Hebei, China. [4]Reserch Center of Turbidity Toxin Theory, Hebei University of Chinese Medicine, 389 Zhongshan East Road, Shijiazhuang 050011, Hebei, China. [5]Department of Gastroscopy Room, Hebei Province Hospital of Chinese Medicine, 389 Zhongshan East Road, Shijiazhuang 050011, Hebei, China. [6]Hebei Technology Innovation Center of TCM Spleen and Kidney Diseases, 389 Zhongshan East Road, Shijiazhuang 050011, Hebei, China. [7]Department of Gastroenterology, Hebei Province Hospital of Chinese Medicine, 389 Zhongshan East Road, Shijiazhuang 050011, Hebei, China.

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