A correlational study of Weifuchun and its clinical effect on intestinal flora in precancerous lesions of gastric cancer

Yanqin Bian¹,²†, Xi Chen¹,³†, Hongyan Cao³, Dong Xie¹, Meiping Zhu⁴, Nong Yuan¹, Lu Lu¹, Bingjie Lu¹, Chao Wu¹, Nisma Lena Bahaji Azami¹, Zheng Wang¹, Huijun Wang⁵, Yeqing Zhang⁵, Kun Li⁵, Guan Ye⁵* and Mingyu Sun¹*

Abstract

Background: Weifuchun (WFC), a Chinese herbal prescription consisting of Red Ginseng, Isodon amethystoides and Fructus Aurantii, is commonly used in China to treat a variety of chronic stomach disorders. The aim of the paper was to determine the effect of WFC on intestinal microbiota changes in precancerous lesions of gastric cancer (PLGC) patients.

Methods: PLGC patients of H. pylori negative were randomly divided into two groups and received either WFC tablets for a dose of 1.44 g three times a day or vitacoenzyme (Vit) tablets for a dose of 0.8 g three times a day. All patients were treated for 6 months consecutively. Gastroscopy and histopathology were used to assess the histopathological changes in gastric tissues before and after treatment. 16S rRNA gene sequencing was carried out to assess the effects WFC on intestinal microbiota changes in PLGC patients. Receiver Operating Characteristics (ROC) analysis was used to assess the sensitivity and specificity of different intestinal microbiota in distinguishing between PLGC patients and healthy control group.

Results: Gastroscopy and histopathological results indicated that WFC could improve the pathological condition of PLGC patients, especially in the case of atrophy or intestinal metaplasia. The results of 16S rRNA gene sequencing indicated that WFC could regulate microbial diversity, microbial composition, and abundance of the intestinal microbiota of PLGC patients. Following WFC treatment, the relative abundance of Parabacteroides decreased in WFC group when compared with the Vit group. ROC analysis found that the Parabacteroides could effectively distinguish PLGC patients from healthy individuals with sensitivity of 0.79 and specificity of 0.8.

Conclusions: WFC could slow down the progression of PLGC by regulating intestinal microbiota abundance.

*Correspondence: yege@sphchina.com; mysun248@hotmail.com
†Yanqin Bian and Xi Chen contributed equally to this work
¹ Key Laboratory of Liver and Kidney Diseases (Ministry of Education), Institute of Liver Diseases, Shuguang Hospital, Shanghai University of Traditional Chinese Medicine, 528 Zhangheng Road, Pudong New District, Shanghai 201203, China
² Central Research Institute, Shanghai Pharmaceuticals Holding Co., Ltd., Building 4, No. 898, Halei Road, Pudong New Area, Shanghai 201203, China
³ Full list of author information is available at the end of the article

© The Author(s) 2021. Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.
Background
Among all cancers, gastric cancer ranks fifth in terms of incidence and third in terms of mortality worldwide [1]. More than 70% of new gastric cancer cases are found in developing countries. In addition, 42.6% of the global incidence and 45% of all gastric cancer-related deaths occur in China [2], which is attributed to the low screening and diagnosis rates of early gastric cancer. Early detection of precancerous lesions of gastric cancer (PLGC) to halt their further development can effectively reduce the incidence of gastric cancer.

Recent research has established that gastric cancer is associated with bacterial dysbiosis within the stomach, especially Helicobacter pylori (H. pylori) in the stomach. There is increasingly compelling evidence that the microbiome can affect gastrointestinal carcinogenesis [3], especially gut microbiota, which plays a role in gastric cancer formation, development and response to treatment [4]. Recent advances in metagenomics and bioinformatics have provided new insights on the microbial ecology in gastric tumor. By using advanced sequencing technology, more intestinal flora involved in gastric cancer occurrence and cancer treatment can be found.

So far, no one drug has been shown to be effective in treating PLGC with the exception of anti-H. pylori therapy. In China, vitacoenzyme tablets were approved for the treatment atrophic gastritis and esophageal epithelial hyperplasia. The tablets are a compound preparation from plant Soybean, and their main components are riboflavin and riboflavin derivatives. In fact, vitacoenzyme was included in several studies involving chronic atrophic gastritis (CAG) [5] and gastric precancerous lesions (GPL) [6, 7]. These studies found that vitacoenzyme had limited effect in protecting gastric mucosa. In our previous research, we found that WFC could inhibit inflammation of H. pylori infected gastric epithelial cells by blocking NF-kappaB pathways [11]. In our previous research, we found that WFC could inhibit inflammation and increase pepsin secretion by inhibiting MAPK signaling pathway [12]. Studies also demonstrated that WFC was antispasmodic and analgesic, and its functions included regulating gastrointestinal motility [13], inhibiting gastric acid secretion, protecting the gastric mucosa [13, 14], and improving histological endoscopic findings and symptoms [10] of PLGC.

However, there is not enough evidence in clinical trials regarding WFC ability to relieve PLGC and its mechanism is still unknown. More large scale randomized and control trials are needed to investigate WFC’s effectiveness on PLGC. In this study, we evaluated WFC’s effect on PLGC and assessed histopathological changes using a randomized and controlled trial. The stool samples of patients were collected to analyze the intestinal microbial abundance by high-throughput sequencing 16SrRNA. The results elucidate the probable mechanism of action of WFC in regulating intestinal microbial balance and treating atrophy and intestinal metaplasia (IM) to alleviate PLGC.

Methods
Quality and quantity analyses of Weifuchun
WFC tablet was kindly provided by Huqingyu-tang Pharmaceutical Co., Ltd. (Hangzhou, China). Quality and quantity analyses of the aqueous extract were performed with UPLC TOF-MS. HPLC-grade acetonitrile, methanol, and formic acid were purchased from Fisher Scientific (Santa Clara, USA). Naringin, ginsenoside Rb1, and oridonin were identified in WFC by UPLC TOF-MS. The following conditions were used to analyze naringin, oridonin, and ginsenoside Rb1: system, Acquity UPLC system (Waters, USA), which consists of a solvent degasser, a binary pump, an auto-sampler and a column oven; column, Acquity UPLC BEH C18 RP column (1.7 μm, 100 mm × 2.1 mm i.d.; Waters, USA); mobile phase A, 0.1% formic acid in water; mobile phase B, 100% acetonitrile; flow rate, 0.3 mL/min; wavelengths, 210 nm for ginsenoside Rb1, 254 nm for oridonin and 280 nm for naringin; injection volume, 10 μL; MS/MS detector,
Acquity Synapt G2 Q-TOF tandem mass spectrometer connected to the UPLC system by an ESI interface and controlled by MassLynx version 4.1 (Waters, UK). Samples were analyzed in the positive model. Data were collected and analyzed by Waters MassLynx version 4.1.

**Trial oversight**
This randomized and controlled trial was conducted in the outpatient clinics of Shuguang Hospital and Shanghai TCM-Integrated Hospital affiliated to Shanghai University of Traditional Chinese Medicine. All subjects (patients and health volunteers) provided written informed consent before enrollment. The trial was approved by the institutional review board at Shuguang Hospital and was conducted in accordance with the provisions of the Declaration of Helsinki and the CONSORT guidelines. An independent data and safety monitoring board reviewed the progress of the trial.

The study protocol, which describes the study in more detail, can be found in the clinical trial registry (https://register.clinicaltrials.gov) with the identifier NCT03814629. The study was approved by the Ethics Committees of Shuguang Hospital affiliated to Shanghai University of Traditional Chinese Medicine (No. 2016-478-29-01). Recruitment and data collection occurred between October 2015 and September 2017. Patients with a previous histological diagnosis of CAG with or without IM/dysplasia according to diagnosis criteria [15] and H. pylori (-) were considered eligible subjects, male or female, between 18 and 70 years. Participants with H. pylori positive infection without radical treatment, peptic ulcer or severe dysplasia (suspected malignant transformation), severe systemic diseases such as cardiovascular and cerebrovascular disease, hepatic diseases, kidney or lung disease, or with other tumors, were excluded. Participants were excluded if they had an allergic constitution or allergies to any known ingredients in WFC. Finally, patients with other diseases interfering with the study or patients unwilling to undergo repeated endoscopy after treatment were also not included.

The TCM standard for diagnosing syndromes was worked out with reference to the standard for diagnosing the type of spleen and stomach deficiency in the guidelines of diagnosing and treating CAG. Major symptoms were stomach pain or discomfort or stomach symptoms remission after warm or press operation. Minor symptoms included: (1) anorexia; (2) loose stools; (3) physical and mental fatigue; (4) shortness of breath and lazy speech; (5) stomach distention after eating; (6) belching; (7) chest distress; (8) stomach pain and fear of being pressed; and (9) light-colored tongue with small and wiry pulse. Patients with one of the major symptoms and two or more minor symptoms were diagnosed as suffering from the syndrome of spleen and stomach deficiency.

**Trial design and treatment**
Before endoscopy, patients were randomly assigned in a 1:1 ratio to receive either WFC therapy or vitacoenzyme. Computer-generated randomization was performed in a blinded manner, with status concealed from all the patients and the primary physician, endoscopist, pathologist, and statistician. After randomization, endoscopy was performed. The patients started the assigned trail medication within 1 week after endoscopy.

Each subject received either WFC tablets (1.44 g) (Hangzhou huqingyutang pharmaceutical co. LTD. Hangzhou, China, lot number 16066129) or vitacoenzyme tablets (0.8 g) (Beihai sunshine pharmaceutical co. LTD, Guangxi, China, lot number 102029), taken orally after meal 3 times a day for 6 months. Before randomization, H. pylori status was determined by rapid urease test (RUT) or by pathology. Positive subjects received standard eradication therapy. H. pylori status was re-evaluated at the end of the 6th month. If required, the status would be re-evaluated by 13C-urea breath test at 4 weeks after the cessation of therapy.

**Screening measures**
The demographics of participants were collected, including age, gender, course of disease, and current and past gastric disease treatment. The histologic diagnosis and grading were made according to the updated Sydney system [16].

**Histological scores**
The criteria to evaluate the histopathology were made according to the updated Sydney system [16]. Each gastric tissue was evaluated separately for the following items: chronic inflammation (CI), acute inflammation (AI), atrophy, IM, and dysplasia. Atrophy was defined as loss of glands and graded as absent (0), mild (1), moderate (2), or severe (3). IM was graded as absent (0), mild (1), moderate (2), or severe (3) according to the proportion of the gastric mucosa replaced by the metaplastic tissue. Presence and severity of dysplasia defined by atypical cytological and architectural derangement, subcategory of mild (1), moderate (2), and severe (3) grade adhered to the diagnosis for gastric neoplasia.
High-throughput sequencing
Total DNA was extracted using the QIAamp DNA Stool Mini Kit. All extractions yielding >2 ng/μ of total DNA, as indicated by NanoDrop 2000 UV–Vis Spectrophotometer measuring. Each DNA sample was amplified for the V3 region of 16S rRNA gene and libraries were sequenced in a single run of the Illumina MiSeq sequencing platform at NovelBio Biomedical technology Co., LTD.

OTU clustering
Bacterial 16S rRNA reads were analyzed using the Quan titative Insights into Microbial Ecology(QIIME) software package [17]. Operational taxonomic units(OUT) were created by single-linkage clustering the reads using Swarm [18] and removing OTUs comprised of only a single or pair of reads. Representative sequences from each OUT were aligned using the PyNAST aligner [19]. All OTUs were tested for correlations between the proportional abundance of OUT and the post-PCR ampli con concentration of a sample according to the methods listed elsewhere [20].

Statistical analysis
According the clinical report and previous research results [9, 21], we calculated the sample size of 35 patients in each group of the trial population. We allowed for a 15% initial dropout rate, and a further 10% loss to follow-up, resulting in the enrollment of 47 patients in each group. An interim analysis was not planned. The statistical analyses were performed by using IBM SPSS Statistics 21.0. Data were expressed as mean± standard or median (range) for continuous variables, and frequencies (percentages) for categorical variables. Student’s t-test or Mann–Whitney test or Chi square test was used to compare baselines including demographic data and basic evaluating variables. For comparison of variations from baseline to endpoint, paired t-test was performed on variables with normal distributions, and wilcoxon signed-ranks test on non-normal variables. ANOVA test was used to compare microbial abundances between groups. Also, Chi-square test or Fisher’s exact test was used for atrophy and intestinal metaplasia disappearance rate and symptom disappearance rate. All statistical tests were two-sided and assumed to be statistically significant at a level of P < 0.05.

Results
Naringin, ginsenoside Rb1, and oridonin contents in the WFC formula
Naringin, ginsenoside Rb1, and oridonin are the major constituents of aqueous extract of Radix Ginseng Rubra, Fructus aurantii, and Isodon amethystoides, respectively.

Baseline characteristics of participants
Of the 87 patients who were screened, a total of 79 patients underwent randomization (Fig. 1). Of these patients, 70 were included in the intention-to-treat population (36 in the WFC treatment group and 34 in the vitacoenzyme group) after the exclusion of 9 patients, including 3 who underwent additional surgery after endoscopic resection, and 4 who did not receive assigned treatment, and 2 who did not meet other eligibility requirements. Demographics (age, gender and course of disease), histopathology (histological score) and clinical symptom (aggregate score) were similar in the two groups (Table 1). We included 60 patients who had undergone gastroscopy at 6-month follow-up in the histologic analysis.

Histology and clinical symptom
After treatment, the results of gastroscopy and histopathology improved both in the WFC group and in the vitacoenzyme group (Vit group), especially with regard to atrophy and IM (Fig. 2A–C). A large number of neat glands, less IM, reduced intercellular congestion edema and inflammatory cell infiltration was observed in histopathological findings in WFC group compared with the Vit group (Fig. 2B). The total change value of pathology aggregate score in WFC group remarkably increased compared with the Vit group (Fig. 2C). Patients with gastric mucosal atrophy or with IM in mild grade or moderate grade were the majority before treatment. But after treatment, patients with non-gastritis (normal) grade or mild grade in the WFC group were more than those in the Vit group, suggesting that WFC could improve atrophy and IM. The total effective rate for alleviating atrophy degree was 80% in the WFC group and 23.33% in the Vit group, respectively. And the total effective rate for alleviating IM degree was 73.33% in the WFC group and 26.67% in the Vit group, respectively (total effective rate of alleviating the atrophy and IM degree = the alleviated atrophy and IM degree/ total cases × 100%) (shown in Tables 2 and 3).

As shown in Table 4, effective rate of alleviating clinical symptom in the WFC group (86.67%) was higher than in the Vit group (23.33%) (total effective rate for alleviating clinical symptom = (total clinical symptom score before treatment-total clinical symptom score after treatment)/ before treatment × 100%), indicating that WFC could dramatically improve clinical symptoms.
The taxonomic composition of intestinal microbiome
Sixty-six feces samples from all participants (28 samples from patients before and after treatment with WFC, 28 samples from patients before and after treatment with Vit drug, and 10 samples from healthy volunteers) were collected and sequenced the variable region V3 of the 16SrRNA using the Illumina MiSeq platform. A total 6,122,474 sequences ranging from 86,389
to 102,665 sequences per sample (mean = 92,764.758; median = 92,591) were obtained after quality control analyses. From these data, we identified a total of 62,453 OTUs.

The intestinal microbiomes across all 66 samples included sequences that corresponded to 3 dominant (> 1.00%) Phyla: Firmicutes (52.74%), Bacteroidetes (39.10%) and Proteobacteria (6.44%). These Phyla comprised 7 dominant (> 1.00%) class and 19 dominant (> 1.00%) genera. Top 6 dominant (> 3.00%) genera were Bacteroides (24.51%), Lachnospiraceae (unclassified) (15.87%), Faecalibacterium (6.25%), Prevotella 9 (6.07%), Lachnoclostridium (3.37%), and Parabacteroides (3.00%). The mean relative proportion of dominant (> 1%) phyla, class and genera in the five groups were shown in Fig. 3. All these genera are commonly found in the feces of individuals with and without PLGC, although in different proportions [22–26].

A variable number of OTUs from these 19 dominant (> 1.00%) genera were included in the core microbiome, which could comprise the stable and consistent members and associations in the whole community [27, 28]. The least stringent definition of the core (presence in at least 50% of the samples) identified 127 OTUs of commensal and pathogenic bacteria; while a more stringent definition (presence in at least 95% of the samples) included 8 OTUs of the following genera: Bacteroides, Lachnospiraceae (unclassified), Faecalibacterium, Lachnoclostridium, Parabacteroides, Streptococcus, Escherichia-Shigella, and Lachnospiraceae.

### Table 1 Baseline characteristics of participants (n = 60)

|                          | WFC (n = 30) | Control (n = 30) | Total (n = 60) | $\chi^2$ | t   | P       |
|--------------------------|-------------|-----------------|----------------|---------|-----|--------|
| Agea                     | 50.37 (12.50) | 53.90 (10.61)   | 52.13 (11.53)  | 1.2     | 0.243 |        |
| Sex                      |              |                 |                |         |      |        |
| Male                     | 16 (53.33)   | 15 (50.00)      | 31 (51.67)     | 0.268   | 0.796 |        |
| Female                   | 14 (46.67)   | 15 (50.00)      | 29 (48.33)     |         |      |        |
| Course of diseasea       | 5.57 (4.94)  | 5.87 (5.10)     | 5.72 (4.93)    | 0.2     | 0.818 |        |
| Pathology                |              |                 |                |         |      |        |
| Chronic Gastritisb       |              |                 |                |         |      |        |
| Mild                     | 7 (23.33)    | 10 (33.33)      | 17 (28.33)     | 1.888   | 0.596 |        |
| Moderate                 | 20 (66.67)   | 19 (63.33)      | 39 (65.00)     |         |      |        |
| Severe                   | 2 (6.67)     | 1 (3.33)        | 3 (5.00)       |         |      |        |
| Active Gastritisb        |              |                 |                |         |      |        |
| Mild                     | 11 (36.67)   | 7 (23.33)       | 18 (30.00)     | 1.765   | 0.414 |        |
| Moderate                 | 3 (10.00)    | 2 (6.67)        | 5 (8.33)       |         |      |        |
| Severe                   | 0 (0.00)     | 0 (0.00)        | 0 (0.00)       |         |      |        |
| Atrophyb                 |              |                 |                |         |      |        |
| Mild                     | 14 (46.67)   | 19 (63.33)      | 33 (55)        | 3.000   | 0.254 |        |
| Moderate                 | 12 (40)      | 10 (33.33)      | 22 (36.67)     |         |      |        |
| Severe                   | 4 (13.33)    | 1 (3.33)        | 5 (8.33)       |         |      |        |
| Intestinal metaplasiab   |              |                 |                |         |      |        |
| Mild                     | 16 (53.33)   | 21 (70.00)      | 37 (61.67)     | 2.739   | 0.493 |        |
| Moderate                 | 10 (33.33)   | 7 (23.33)       | 17 (28.33)     |         |      |        |
| Severe                   | 3 (10.00)    | 2 (6.67)        | 5 (8.33)       |         |      |        |
| Dysplasiab               |              |                 |                |         |      |        |
| Mild                     | 6 (20.00)    | 4 (13.33)       | 10 (16.67)     | 2.632   | 0.235 |        |
| Moderate                 | 0 (0.00)     | 0 (0.00)        | 0 (0.00)       |         |      |        |
| Severe                   | 0 (0.00)     | 0 (0.00)        | 0 (0.00)       |         |      |        |
| Aggregate scorec         | 5.70 (2.00)  | 4.97 (1.63)     | 1.6            |         | 0.125 |        |
| Clinical Symptoms        |              |                 |                |         |      |        |
| Aggregate scorec         | 15.32 (5.71) | 15.92 (5.43)    | 3.5            | 0.929   |      |        |

a Years, mean(SD)

b The number of cases meeting the diagnosis were counted and showed as cases (percent); absent cases were included when $P$ value were calculated through two-sided $X^2$ test

c Aggregate score represented sum of all grade score including absent, mild, moderate and severe; absent, 0; mild, 1; moderate, 2; severe, 3
Pathogenic representatives from *Bacteroides*, *Lachnospiracea*, *Faecalibacterium*, *Lachnoclostridium* and *Streptococcus* genera have been consistently associated to gastric cancer [29–32]. Unlike the above 5 dominant genera, *Parabacteroides* could be more related to the gastric and intestinal disease such as dyspepsia [33], and *Escherichia-Shigella* and *Lachnospiracea NK4A136 group* were involved in gastrointestinal inflammation and immunity [34, 35].

**Univariate, receiver operating characteristics (ROC) curve**

As shown in Fig. 4, microbial abundances of all the eight dominant bacterial genera was compared between various groups based on the ANOVA, and between WFCB vs WFCA and VitB vs VitA by the paired t-test. All candidates, except for *Parabacteroides*, were not significantly
Table 3  Comparison of gastric mucosal intestinal metaplasia level

| Group | n  | Intestinal metaplasia level (n) | Change of intestinal metaplasia level after treatment (n) | Effective rate (%) |
|-------|----|---------------------------------|----------------------------------------------------------|--------------------|
|       |    | Non-gastritis  Mild  Moderate  Severe  3 levels  2 levels  1 level  0 level |                                                          |                    |
| WFC   |    |                                 |                                                          |                    |
| WFCB  | 30 | 1 16 10 3 2 2 18 8             |                                                          | 73.33**            |
| WFCA  | 30 | 14 15 1 0                      |                                                          |                    |
| Vit   |    |                                 |                                                          |                    |
| VitB  | 30 | 1 17 10 2 0 1 7 22             |                                                          | 26.67              |
| VitA  | 30 | 5 10 14 1                      |                                                          |                    |

WFCB, patients before treatment with WFC; WFCA, patients after treatment with WFC; VitB, patients before treatment with vitacoenzyme; VitA, patients after treatment with vitacoenzyme; **, P < 0.01, compared with Vit

Table 4  Comparison of clinical symptom cumulative score

| Group | n     | Before treatment | After treatment | Effective rate (%) | t     | P       |
|-------|-------|------------------|-----------------|--------------------|-------|---------|
| WFC   | 30    | 15.32±5.71       | 6.27±2.19       | 86.67              | 23.231| < 0.01  |
| Vit   | 30    | 15.92±5.43       | 13.69±4.12      | 23.33              |       |         |

WFC, Weifuchan Group; Vit, Vitacoenzyme Group;

Fig. 3  Microbial profiles (mean relative proportion) of most abundant (> 1%) phyla, class and genera by comparison for groups. Health: healthy volunteers; WFCB: PLGC patients before treatment with WFC; WFCA: PLGC patients after treatment with WFC; VitB: PLGC patients before treatment with vitacoenzyme; VitA: PLGC patients after treatment with vitacoenzyme
Fig. 4  Comparisons for bacterial abundance. Bacterial relative abundance differences were observed for 8 core genera in each group. TSS: Total sum normalization. *, p < 0.05
different between groups. Compared with Health group, the relative abundance of *Parabacteroides* was significantly higher in WFCB group. On the other side, abundance of *Parabacteroides* declined observably in WFCA group. This decrease was not observed in the Vit group. The arithmetic mean ± standard deviation of the relative abundance (raw count number reads) were 0.76 ± 0.76 (14729.04 ± 14767.28) for Health group, 1.86 ± 1.01(35775.48 ± 20532.81) for WFCB group and 0.98 ± 0.79 (19062.95 ± 15363.4) for WFCA group. 8 candidate genera were selected to analyze ability to assess PLGC using ROC curve. The results were shown in the Fig. 5 and in Table 5. The area under the curve (AUC) was > 0.7 and p value < 0.05 only for *Parabacteroides* (AUC=0.7714, p value=0.026). The other 7 candidate genera were all < 0.7 in AUC, suggesting that *Parabacteroides* may be an important candidate in the assessment of PLGC development and the therapeutic effect of WFC on PLGC.

**Discussion**

Gastric cancer develops through a multistep process triggered by *H. pylori* and progression from superficial gastritis to atrophic gastritis, IM, and dysplasia [36]. Atrophy, IM or dysplasia is considered as PLGC and require accurate surveillance programs. PLGC belong to the “stomach distension” and “epigastralgia” category in traditional Chinese medicine. WFC is a clinical effective prescription for body discomfort including distension and fullness of the stomach, belching and poor appetite, constipation or diarrhea, lassitude and weakness, dizziness and emaciation, sallow complexion. In this study, the results showed that WFC could significantly improve clinical symptoms and gastric mucosa pathology, especially atrophy and IM.

The etiology of *H. pylori*-positive has been well described over the past few decades [37], but *H. pylori* eradication cannot completely eliminate the recurrence risk of gastric cancer [38], suggesting there is another

![Fig. 5](image-url)  
**Fig. 5** Receiver operating curve analysis for selected microbial biomarker of PLGC. 8 selected microbial biomarkers of PLGC were tested by ROC analysis

| Out                                      | Area  | Lower bound | Upper bound | Cut off value | Sensitivity | Specificity | p value |
|------------------------------------------|-------|-------------|-------------|---------------|-------------|-------------|---------|
| Bacteroides                              | 0.66  | 0.44        | 0.88        | 3.494         | 0.43        | 1           | 0.178   |
| Lachnospiraceae; Other                   | 0.58  | 0.33        | 0.82        | 89550.33      | 0.93        | 0.2         | 0.520   |
| Faecalibacterium                         | 0.53  | 0.29        | 0.77        | 96980.75      | 0.21        | 0.9         | 0.815   |
| Lachnoclostridium                        | 0.55  | 0.31        | 0.79        | 19030.36      | 0.71        | 0.5         | 0.682   |
| Parabacteroides                          | 0.77  | 0.57        | 0.97        | 18354.22      | 0.79        | 0.8         | **0.026**|
| Streptococcus                            | 0.58  | 0.34        | 0.81        | 4847.02       | 0.57        | 0.8         | 0.520   |
| Escherichia-Shigella                     | 0.55  | 0.31        | 0.79        | 3969.2        | 0.43        | 0.8         | 0.682   |
| Lachnospiraceae NK4A136 group            | 0.66  | 0.45        | 0.88        | 11207.62      | 0.43        | 1           | 0.178   |

Only *Parabacteroides* have statistical difference in ROC curve
factor affecting the progression of gastric cancer. It has been reported that the alternations of fecal microbiota involved in the process of H. pylori-related gastric lesion progression [39]. Resident microbes can induce inflammation, leading to cell proliferation and altered stem cell dynamics, which can lead to alternations in DNA integrity and immune regulation and promote carcinogenesis [40]. In this study, we observed gut microbes alteration in PLGC population with H. pylori negative and the effect of WFC on this population. The result found that 8 types of microbes may dominate the bacterial community of PLGC at genus level. Further analysis found that only the abundance of Parabacteroides was significantly different in Health group vs WFCB group and in WFCB group vs WFCA group. AUC was >0.7 for Parabacteroides in ROC analysis which could effectively distinguish PLGC patients from healthy individuals, with sensitivity of 0.79 and specificity of 0.8, suggesting the importance of Parabacteroides in PLGC occurrence.

Parabacteroides genera belong to the Bacteroidetes phyla and the Porphyromonadaceae family. Among the gut Parabacteroides, Parabacteroides Distasonis (P. distasonis) is defined as one of the 18 core members in the gut microbiota of humans and thought to have important physiological functions in hosts [41]. Results from animal studies proved the protective role of P. distasonis in colonic tumorigenesis and maintenance of intestinal epithelial barrier in AOM-treated mice [42]. A study from 736 American Gut Project sample found that the abundance of P. distasonis is relatively lower in patients with obesity, inflammatory bowel diseases, nonalcoholic fatty liver, and multiple sclerosis [43–45]. However, some studies found the relative abundance of Parabacteroides are increased in heart diseases [46], leukemia [47, 48] and early hepatocellular carcinoma [49]. The phenotype of cancer cachexia is associated with increased levels of Parabacteroides [50]. The studies suggested Parabacteroides may perform many biological functions in the human body.

Recent evidence from in vivo and vitro confirmed that P. distasonis possessed a strong ability to transform primary bile acids into secondary bile acids and enhancing the level of succinate in the gut [26, 51]. The gut microbiota and the bile acid pool played pivotal roles in maintaining intestinal homeostasis. Interplay between bile acid and the gut microbiota promoted gastrointestinal carcinogenesis [52]. It was also reported that bacterial metabolites, including secondary bile acid, had the potential to cause direct DNA damage or to provoke inflammation, which in turn promoted carcinogenesis [53]. Bile acids could promote gastric IM by upregulating CDX2 and MUC2 expression via the FXR/NF-κB signaling pathway [54]. Succinate, the intermediates of the mitochondrial pathway known as the Krebs cycle, had extensive evidence for “non-metabolic” signaling functions or metabolic reprogramming leading to altered immune cell and transformed cell function in the initiation of carcinogenesis [55, 56]. Additionally, Parabacteroides in the gut could use type VI secretion systems (T6SSs) to antagonize symbiotic gut E. coli, facilitating colonization and cancer progression [57]. To sum up, Parabacteroides are multifunctional bacteria in the human gut and have the potential capacity to promote gastric carcinogenesis. In our study, the relative abundance of Parabacteroides in PLGC group was significantly high before treatment with WFC. In contrast, a decreased abundance of Parabacteroides was observed in PLGC group after treatment with WFC. The effect of WFC on gut Parabacteroides corresponded with the results of gastric histology in PLGC. Accumulating data suggested that gut microbiota had a role in the etiology of several types of cancer, including gastric cancer. However, data about intestinal microbiota correlation with PLGC were not enough. Further studies on the alteration of intestinal flora in PLGC development are needed. It has been reported that Parabacteroides play a predominant role in anti-obesity effects [58], but short of evidence for its effects on gastric cancer. Our research explore the relationship between PLGC pathology variation and Parabacteroides in clinical trial, which maybe reveal a novel and potential mechanism and will provide help for further studies regarding the mechanisms of action of WFC on chronic gastric disease. In our previous research, we found that WFC could improve histopathological changes of gastric mucosa of PLGC in rats induced by N-methyl-N'-nitro-N-nitrosoguanidine partly by inhibiting MAPK signaling pathway to increase pepsin secretion [12]. In this study, the results suggested that WFC could inhibit inflammation of gastric mucosa by regulating the abundance of gut microbiota. But further studies are needed to investigate causal relationships between WFC and intestinal flora in PLGC. The study had few limitations including the limited sample size, especially for collected feces for intestinal flora. Larger sample sizes are needed in order to confirm the role of Parabacteroides in the development of PLGC.

Conclusions
This study suggested WFC slowed down PLGC, which could be related to Parabacteroides abundance variation. The results will help elucidate the effects of WFC on PLGC and provide a treatment method for PLGC.

Abbreviations
PLGC: Precancerous lesions of gastric cancer; WFC: Weifuchun; CAG: Chronic atrophic gastritis; GPL: Gastric precancerous lesions; IM: Intestinal metaplasia; CI: Chronic inflammation; AI: Acute inflammation; CFDA: China food and drug administration; Health: Healthy volunteers; WFCB: Patients before treatment with WFC; WFCA: Patients after treatment with WFC; VitB: Patients before
treatment with vitacoenzyme; VitA: Patients after treatment with vitacoenzyme; OTUs: Operational taxonomic units.

Acknowledgements
We gratefully acknowledge Dr. Zhang and Dr. Guo from NovoBiov Biomedical technology Co., LTD, who provided us much technical help.

Authors' contributions
YB, XC, GY and MS conceived and designed the clinical trial; YB and XC wrote the paper; MS, MZ and NY recruited patients; XC, HC, DX, BL, LL and CW collected the samples; YB, XC, HC and ZW analyzed clinical data; HW and YZ finished the drug quality and quantity analyses; NLBA helped proofread the manuscript; GY and MS critically revised the paper for important intellectual content and gave final approval for publication of the paper. All authors read and approved the final manuscript.

Funding
This work was supported by the major Project of Shanghai Municipal Science and Technology Commission (No. 15DZ1900104, 1940192300). The fourth batch of outstanding TCM talents of the State Administration of Traditional Chinese Medicine (2017-124), Innovation course of Postgraduate students in Shanghai University of Traditional Chinese Medicine (2017); Outstanding TCM talents of Shanghai University of Traditional Chinese Medicine (2020); Outstanding TCM reserve talents of Shanghai University of Traditional Chinese Medicine (2020); Shanghai Key Laboratory of Traditional Chinese Clinical Medicine, Key Disciplines of Liver and Gall Bladder Diseases and Key Laboratory of Chronic Deficiency Liver Disease of State Administration of Traditional Chinese Medicine of the People's Republic of China. The study authors were independent of the funder. The funding sources had no involvement in the study.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding authors on reasonable request.

Declarations
Ethics approval and consent to participate
The study was approved by the Ethics Committees of Shuguang Hospital affiliated to Shanghai University of Traditional Chinese Medicine (No. 2016-478-29-01) and performed in accordance with the Declaration of Helsinki. All patients were fully informed of the study and informed consent were obtained from all patients.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1 Key Laboratory of Liver and Kidney Diseases (Ministry of Education), Institute of Liver Diseases, Shuguang Hospital, Shanghai University of Traditional Chinese Medicine, 528 Zhangheng Road, Pudong New District, Shanghai 201203, China. 2 Arthritis Institute of Integrated Traditional Chinese and Western Medicine, Shanghai Academy of Traditional Chinese Medicine, Guanghua Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai 200052, China. 3 Department of Infectious Disease and Gastroenterology, Shanghai TCM Integrated Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai 200080, China. 4 Department of Gastroenterology, Shuguang Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China. 5 Central Research Institute, Shanghai Pharmaceuticals Holding Co., Ltd, Building 4, No. 898, Halie Road, Pudong New Area, Shanghai 201203, China.

Received: 30 May 2021 Accepted: 31 October 2021 Published online: 20 November 2021

References
1. Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65(2):87–108.
2. Wang W, Sun Z, Deng JF, et al. A novel nomogram individually predicting disease-specific survival after D2 gastrectomy for advanced gastric cancer. Cancer Commun (Lond). 2018;38(1):23.
3. Chen J, Domngue JC, Sears CL. Microbiota dysbiosis in select human cancers: evidence of association and causality. Semin Immunol. 2017;32:25–34.
4. Wong SH, Kwong T, Wu CY, et al. Clinical applications of gut microbiota in cancer biology. Semin Cancer Biol. 2019;55:28–36.
5. Zhang J, Wang H. MORRISIDE protects against chronic atrophic gastritis in rat via inhibiting inflammation and apoptosis. Am J Transl Res. 2019;11(9):6016–23.
6. Peng L, Xie YF, Wang CG, et al. Moxibustion alleviates gastric precancerous lesions in rats by promoting cell apoptosis and inhibiting proliferation-related oncogenes. Afr J Tradit Complement Altern Med. 2017;14(2):148–60.
7. Zeng JH, Pan HF, Liu YZ, et al. Effects of Weipixiao (胃病消) on Wnt pathway-associated proteins in gastric mucosal epithelial cells from rats with gastric precancerous lesions. Chin J Integr Med. 2016;22(4):267–75.
8. Chen X, Zhao YH, Zhang YQ, Ye G, Sun MY. Clinical applications and modern research progress of Weifuchun. Jiangxi Traditional Chin Med. 2016;47:77–80 (In Chinese).
9. Li HZ, Wang H, Wang GQ, et al. Treatment of gastric precancerous lesions with Weiyanans. World J Gastroenterol. 2006;12(33):5389–92.
10. Li Y, Xu JK, Uu XR. Clinical and pathological study of weiyan serial recipes in the treatment of gastric precancerous lesions. Zhongguo Zhong Yi Jie He Za Zhi. 2011;31(12):1635–48.
11. Huang X, Lu B, Zhang S, et al. Effect of Weifuchun on inhibiting inflammation of Helicobacter pylori-infected GE-1 cells and NF-kappaB signaling pathway. Zhongguo Zhong Yi Jie He Za Zhi. 2014;34(4):450–4.
12. Wang H, Wu R, Xie D, et al. A combined phytochemistry and network pharmacology approach to reveal the effective substances and mechanisms of Wei-Fu-Chun tablet in the treatment of precancerous lesions of gastric cancer. Front Pharmacol. 2020;11:559471.
13. Chen W, Chen WJ, Yang M. Effect of Wei Fuchun tablet on gastrointestinal function in functional dyspepsia rats. Chin J Modern Appl Pharm. 2019;36:629–32 (In Chinese).
14. Xu HB, Chen QE, Chen CJ. Effect of matrine combined with Weifuchun tablets on gastric acid secretion in patients with chronic atrophic gastri.

Wits. World Chin J Digestol. 2017;25:2139–43 (In Chinese).
15. Dinis-Ribeiro M, Areia M, de Vries AC, et al. Management of precancerous conditions and lesions in the stomach (MAPS): guideline from the European Society of Gastrointestinal Endoscopy (ESGE), European Helicobacter Study Group (EHSG), European Society of Pathology (ESP), and the Sociedade Portuguesa de Endoscopia Digestiva (SPED). Endoscopy. 2012;44(1):74–94.
16. Dixon NF, Genta RM, Yardley JH, et al. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am J Surg Pathol. 1996;20(10):1161–81.
17. Caporaso JG, Kuczynski J, Stombaugh J, et al. QiIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7(3):335–6.
18. Mahe F, Rognes T, Quince C, et al. Swarm: robust and fast clustering method for amplicon-based studies. PeerJ. 2014;2:e593.
19. Dinis-Ribeiro M, Areia M, de Vries AC, et al. Management of precancerous conditions and lesions in the stomach (MAPS): guideline from the European Society of Gastrointestinal Endoscopy (ESGE), European Helicobacter Study Group (EHSG), European Society of Pathology (ESP), and the Sociedade Portuguesa de Endoscopia Digestiva (SPED). Endoscopy. 2012;44(1):74–94.
20. Hansen MEB, Rubel MA, Bailey AG, et al. Population structure of human gut bacteria in a diverse cohort from rural Tanzania and Botswana. Genome Biol. 2019;20(1):16.
21. Deng X, Liu ZW, Wu FS, et al. A clinical study of weining granules in the treatment of gastric precancerous lesions. J Tradit Chin Med. 2012;32(2):164–72.
22. Sithik S, Pokromtiks J. Gut microbiota as a host defender and a foe: the 2 faces of commensal bacteroides thetaiotaomicron in inflammatory bowel disease. Inflamm Bowel Dis. 2019;25(6):e71.
23. Lopez-Siles M, Duncan SH, Garcia-Gil LJ, et al. Faecalibacterium prausnitzii: from microbiology to diagnostics and prognostics. Isme J. 2017;11(4):841–52.

24. Ley RE. Gut microbiota in 2015: prevotella in the gut: choose carefully. Nat Rev Gastroenterol Hepatol. 2016;13(2):69–70.

25. Yousef O, Lahi H, Kodaka A, Takken M, Ehsan H, et al. Stool microbiota composition differs in patients with stomach, colon, and rectal neoplasms. Dig Dis Sci. 2018;63(11):2950–8.

26. Wang K, Liao M, Zhou N, Bao L, Ma K, Zheng Z, et al. Parabacteroides distasonis alleviates obesity and metabolic dysfunctions via production of succinate and secondary bile acids. Cell Rep. 2019;26(1):22–35.

27. Backhed F, Fraser CM, Ringel Y, Sanders ME, Sartor RB, Sherman PM, et al. Defining a healthy human gut microbiome: current concepts, future directions, and clinical applications. Cell Host Microbe. 2012;12(5):611–22.

28. Shade A, Handelsman J. Beyond the Venn diagram: the hunt for a core microbiome. Environ Microbiol. 2012;14(1):4–12.

29. Liu X, Shao L, Liu X, Ji F, Mei Y, Cheng Y, et al. Alterations of gastric mucosal microbiota across different stomach microhabitats in a cohort of 276 patients with gastric cancer. EbiolMedicine. 2019;40:336–48.

30. He C, Peng C, Wang H, Ouyang Y, Zhu Z, Shu X, et al. The eradication of Helicobacter pylori restores rather than disturbs the gastrointestinal microbiota in asymptomatic young adults. Helicobacter. 2019;24(4):e12590.

31. Gantuya B, El-Serag HB, Matsumoto T, Ajami NJ, Oyuntsetseg K, Azzaya D, et al. Gastric microbiota in Helicobacter pylori-Negative and -Positive gastritis among high incidence of gastric cancer area. Cancers (Basel). 2019;11(4):504.

32. Qi YF, Sun JN, Ren LF, Cao XL, Dong JH, Tao K, et al. Intestinal microbiota composition differs in patients with stomach, colon, and rectal neoplasms. Sci Rep. 2019;9:23961.

33. Gantuya B, El-Serag HB, Matsumoto T, Ajami NJ, Oyuntsetseg K, Azzaya D, et al. Gastric microbiota in Helicobacter pylori-Negative and -Positive gastritis among high incidence of gastric cancer area. Cancers (Basel). 2019;11(4):504.

34. Qi YF, Sun JN, Ren LF, Cao XL, Dong JH, Tao K, et al. Intestinal microbiota is altered in patients with gastric cancer from Shanxi province, China. Dig Dis Sci. 2019;64(5):1193–203.

35. Bashir M, Prietl B, Tauschmann M, Mautner SI, Kump PK, Treiber G, et al. Bacterial dysbiosis is associated with gastric intestinal metaplasia by upregulating CDX2 and MUC2 expression in Barrett’s esophagus. Cell Mol Biol (Noisy-le-grand). 2016;62(3):349–56.

36. Bäckhed F, Fraser CM, Ringel Y, Sanders ME, Sartor RB, Sherman PM, et al. Defining a healthy human gut microbiome: current concepts, future directions, and clinical applications. Cell Host Microbe. 2012;12(5):611–22.

37. Nardone G, Rocco A, Malfertheiner P. Review article: Faecalibacterium prausnitzii: from microbiology to diagnostics and prognostics. Isme J. 2017;11(4):841–52.

38. Ley RE. Gut microbiota in 2015: prevotella in the gut: choose carefully. Nat Rev Gastroenterol Hepatol. 2016;13(2):69–70.

39. Yousef O, Lahi H, Kodaka A, Takken M, Ehsan H, et al. Stool microbiota composition differs in patients with stomach, colon, and rectal neoplasms. Dig Dis Sci. 2018;63(11):2950–8.

40. Wang K, Liao M, Zhou N, Bao L, Ma K, Zheng Z, et al. Parabacteroides distasonis alleviates obesity and metabolic dysfunctions via production of succinate and secondary bile acids. Cell Rep. 2019;26(1):22–35.

41. Backhed F, Fraser CM, Ringel Y, Sanders ME, Sartor RB, Sherman PM, et al. Defining a healthy human gut microbiome: current concepts, future directions, and clinical applications. Cell Host Microbe. 2012;12(5):611–22.

42. Shade A, Handelsman J. Beyond the Venn diagram: the hunt for a core microbiome. Environ Microbiol. 2012;14(1):4–12.

43. Gantuya B, El-Serag HB, Matsumoto T, Ajami NJ, Oyuntsetseg K, Azzaya D, et al. Gastric microbiota in Helicobacter pylori-Negative and -Positive gastritis among high incidence of gastric cancer area. Cancers (Basel). 2019;11(4):504.

44. Del CF, Nobili V, Vernoocchi P, Russo A, De Stefantis C, Gnani D, et al. Gut microbiota profiling of pediatric nonalcoholic fatty liver disease and obese patients unveiled by an integrated meta-omics-based approach. Hepatology. 2017;65(2):451–64.

45. Verdam FJ, Fuentes S, de Jonge C, Zoetendal EG, Erbil R, Greve JW, et al. Human intestinal microbiota composition is associated with local and systemic inflammation in obesity. Obesity (Silver Spring). 2013;21(12):E607–15.

46. Liu Z, Ma Z, Zhang H, et al. Felic acid increases intestinal Lactobacillus and improves cardiac function in TAC mice. Biomed Pharmacoch. 2019;120:104982.

47. Bindels LB, Neyrinck AM, Claas SP, et al. Symbiotic approach restores intestinal homeostasis and prolongs survival in leukemic mice with cachexia. Isme J. 2016;10(6):1456–70.

48. Bindels LB, Beck R, Schakman O, et al. Restoring specific lactobacilli levels restores rather than disturbs the gastrointestinal microbiota in asymptomatic young adults. Helicobacter. 2019;24(4):e12590.

49. Shi J, Li H, Liu P, Lin H, Li J, et al. Evaluation of the gut microbiota in patients with gastric cancer from Shanxi province, China. Dig Dis Sci. 2019;64(5):1193–203.

50. Bashir M, Prietl B, Tauschmann M, Mautner SI, Kump PK, Treiber G, et al. Bacterial dysbiosis is associated with gastric intestinal metaplasia by upregulating CDX2 and MUC2 expression in Barrett’s esophagus. Cell Mol Biol (Noisy-le-grand). 2016;62(3):349–56.

51. Ryan DG, Murphy MP, Frezza C, Prag HA, O’Neill LA, et al. Defining a healthy human gut microbiome: current concepts, future directions, and clinical applications. Cell Host Microbe. 2012;12(5):611–22.

52. Wang S, Dong W, Liu L, Xu M, Wang Y, Liu T, et al. Interplay between bile acids and the gut microbiota promotes intestinal carcinogenesis. Mol Carcinog. 2019;58(7):1155–67.

53. Louis F, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. Nat Rev Microbiol. 2014;12(10):661–72.

54. Yu JH, Zheng JB, Qi J, Yang K, Wu YH, Wang K, et al. Bile acids promote gastric intestinal metaplasia by upregulating CDX2 and MUC2 expression via the FXR/NF-κB signalling pathway. Int J Onco. 2019;54(3):261–70.

55. Ryan DG, Murphy MP, Frezza C, Prag HA, O’Neill LA, et al. Defining a healthy human gut microbiome: current concepts, future directions, and clinical applications. Cell Host Microbe. 2012;12(5):611–22.

56. Sajnani K, Islam F, Smith RA, Gopalan V, Lam AK. Genetic alterations in Krebs cycle and its impact on cancer pathogenesis. Biochime. 2017;135:164–72.

57. Coyne MJ, Comstock LE. Type VI secretion systems and the gut microbiota. Microbiol Spectr. 2019;7(2). https://doi.org/10.1128/microbiolspec.MB-0009-2018.

58. Wu TR, Lin CS, Chang CJ, et al. Gut commensal Faecalibacterium prausnitzii plays a predominant role in the anti-obesity effects of polysaccharides isolated from Hiratsuita sinensis. Gut. 2019;68(2):248–62.