Metabolic Markers and Microecological Characteristics in Patients with Chronic Gastritis

Zhu-Mei Sun1, Jie Zhao1, Fu-Feng Li1*, Yi-Qin Wang1, Peng Qian1, Wei-Fei Zhang1, Yi-Ming Hao1, Xiao-Yan Pang 2, Shun-Chun Wang3*

1 Basic Medical Department, Traditional Chinese Medicine of Shanghai University, Cai Lun Road, Pu dong district, Shanghai 201203, China
2 School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Life Sciences Building 800 Dong chuan Road, Shanghai, 200240
3 Academy of Traditional Chinese Medicine

*Corresponding authors, Tel +86 021 51322346,
E-mail: li_fufeng@yahoo.com.cn

Abstract

Objective: We tested the metabolic components and micro ecological indexes of tongue coating in patients with chronic gastritis by metabonomics study and microbiological techniques to explore the metabolic pathways about tongue coat micro ecological characteristics. Methods: Tongue coating was collected from 70 patients with chronic gastritis and 20 normal controls. The samples were subjected to liquid chromatography and mass spectrometry (LC–MS) and 16S rRNA denatured gradient gel electrophoresis (16S rRNA–DGGE). The statistical techniques used were principal component analysis and partial least squares–discriminate analysis. Results: (1) Ten markedly different metabolites that mainly included UDP–D-galactose, 3-ketolactose, and vitamin D2 were observed on the tongue coating of the chronic gastritis group by metabolite detection analysis based on LC–MS technology. (2) Eight significantly different strips were observed in the chronic gastritis group based on 16S rRNA–DGGE technology. We selected the most pronounced strips of No. 8 and No. 10, for sequencing. The sequencing of strip No. 10 showed 100% similarity to that of Rothia mucilaginosa. The sequencing of strip No. 8 showed 96.2% similarity to that of Moraxella catarrhalis. The species observed may be new and has not
been previously reported. **Conclusion:** Changes in glucose metabolism can be the basis of tongue coating formation in patients with chronic gastritis. Tongue coating indicates a close relationship between the body energy metabolism and intestinal micro flora changes, thus providing a theoretical basis for non-invasive diagnosis.

**Keywords:** chronic gastritis, tongue coating, 16S rRNA–DGGE, LC–MS

**Introduction**

Traditional Chinese medicine (TCM) has always attached great importance to the function of tongue coating in syndrome differentiation. Two of these emphasize that tongue coating is indispensable: (1) syndrome differentiation according to the theory of Zang-fu organs and (2) syndrome differentiation according to the state of Qi, blood, and body fluid. Tongue coating refers to the layer of fur-like substance covering the surface of the human tongue, which consists of stomach Qi caused by Qi of the spleen and stomach. TCM believes that the tongue coating is a very sensitive scale that reflects physiological and pathological changes in the organs, especially the spleen and the stomach. <Xing Se Jian Mo> stated that the differentiation of the tongue proper and coating is that “the tongue coating is formed by stomach-Qi and the five organs are all supplied by the stomach, so the tongue coating can be used to inspect cold, heat, asthenia and excess.” Clinical practice shows that the tongue itself changes slowly in the development of chronic gastritis, but tongue coating changes rapidly and markedly, which helps evaluate the severity of the disease [1]. This phenomenon explains that changes of tongue coating are closely related with the diseases. So we want to explore the metabolic markers and composition of microorganisms in tongue coating in patients with chronic gastritis, to establish a theoretical basis for noninvasive detection and an experimental basis for an objective research in tongue inspection.

A number of studies regarding the objectiveness the material basis of tongue coating have been reported. These methods mainly include cell biochemistry, immunology, molecular biology, proteomics, and other relative
methods and technologies worldwide \cite{2-7}. These methods and achievements in tongue coating research have opened up a broad prospect for the objective research in tongue inspection in TCM; it was not enough for tongue coating. Metabonomics is an emerging science that studies all metabolites and the variation of metabolic pathways in biological systems. The related technology exhibits high throughput, high sensitivity, and high accuracy. Metabonomics emphasizes the importance of the human body as a whole system, which is consistent with the holistic view, system view, and treatment based on syndrome differentiation in TCM. Thus, metabonomics has wide application potential in TCM research \cite{8-9}. Gao Jie \cite{10} found differential metabolites by analyzing the sera metabolite profiles of patients suffering from cirrhosis and cirrhosis with hepatocellular carcinoma, which may be an effective diagnostic method for hepatocellular carcinoma. Zhao Hui et al. \cite{11} found the levels of C-telopeptide, osteocalcin, bone alkaline phosphates, were shown to have sharply increased in the breast cancer. The study of metabolic biomarkers of some diseases has aroused people considerable interest; however, most samples are collected from blood, urine, or tissue extracts rather than tongue coatings. TCM thinks that the tongue coating is a very sensitive scale that reflects the physiological and pathological changes in the organs, especially the spleen and the stomach. We had done the metabonomics study of TCM greasy tongue coating in previous work \cite{12}. Therefore, this paper investigates metabolic markers from the tongue coating of patients with chronic gastritis to determine the relationship between the metabolic changes in tongue coating and chronic gastritis. In addition, the tongue contains tenfold more microorganisms than human cells in the intestine. These microorganisms play an important function in human health, disease, and drug metabolism. Many scientists believe that the physiological and pathological characteristics of the human body result from the action of the human gene and the microbial population gene in the human body. The 16S rRNA gene denatured gradient gel electrophoresis (DGGE) fingerprint technique effectively explores the
complex microbial community structure. This technique has been widely applied in various biological intestinal flora and environmental bacterium complex structure research. The 16S rRNA gene DGGE fingerprint techniques can rapidly and accurately analyze the stability of the samples, exhibit good reproducibility, and characterize the microbial composition of a DNA fingerprint. Tongue diagnosis of traditional Chinese medicine shows potential application value in exploring the metabolic pathways and micro ecological changes of chronic gastritis with metabonomics and micro ecology technology,

1 Data and methods

1.1 Subjects

1.1.1 Case selection: The participants mainly included patients from Putuo Hospital, which is affiliated with Shanghai University of Traditional Chinese Medicine. All patients underwent a gastro scope examination and were diagnosed with chronic gastritis. The people in the normal controls consisted of teachers and students from the Shanghai University of Traditional Chinese Medicine, who completed regular physical examination reports and filled out the health questionnaires \[13\]. The subjects were divided into two groups: the chronic gastritis group (70 cases) and the normal controls (20 cases).

Ethics Statement: All samples were obtained as part of a diagnostic carried out after the people gave written informed consent. The study was approved by the local ethics committee of the Putuo hospital (No.2012-32)

1.1.2 Criteria for diagnosis: The diagnosis criteria of chronic gastritis from the seminar views of Digestive Disease Branch of Chinese Medical Association \[14\] national chronic gastritis in 2000.

1.1.3 Case standard

1.1.3.1 Inclusion criteria The inclusion criteria were as follows: (1) consistent with the diagnosis criteria for chronic gastritis; (2) confirmed by gastric endoscopy; (3) aged between 20 and 75 years.

1.1.3.2 Exclusion criteria The exclusion criteria were as follows: (1) suffering from duodenal ulcer, gastric ulcer, gastric bleeding, gastric, and intestinal
diseases; (2) suffering from the disease of the liver, heart, kidney, lung, brain, and other organs or psychiatric patients simultaneously; (3) pregnant or lactating women; (4) Allergic constitution or multiple drug allergy sufferers

1.1.4 Eliminate criteria: Recently taking antibiotics (within one month) patients

1.2 Methods

1.2.1 Acquisition and processing of tongue coating samples: Patients were asked to rinse their mouths with saline. We used small spoons to scrape the tongue coating and placed the scrapings into a sanitized eppendorff tube that had been filled with 2 ml saline. The tongue coating samples were stored at room temperature for 1 h to 2 h and then centrifuged for 10 min at 4 °C and 3000 rpm, we began to count the cells. We ensured that \(10^6\) /ml cells were placed. We finally collected the supernatant and preserve it at -80 °C.

1.2.2 Liquid chromatography–mass spectrometry (LC–MS) analysis: Tongue coating samples were prepared by centrifugation at 3000 rpm for 10 min, the supernatant was transferred into a 2ml auto sampler vial equipped with a conical low-volume insert for analysis. We placed 150µl of supernatant in 150µl of acetonitrile and vibrate the supernatant for 30s. The samples were centrifuged at 12000 rpm for 3 min. The supernatant was then transferred into a freeze drier. The frozen and dried samples were dissolved in 150µl of 80% acetonitrile. In a typical experiment, a 20µl aliquot of tongue coating was injected into a 2.1 mm × 100 mm HSS-T3 1.7µm column by using Water ZQ2000 Series for LC–MS (Wares, USA). The column was maintained at 45 °C and eluted with a linear gradient of 2% to 90% B at a flow rate of 600µl/min (where A 5 Mm AcNH4+0.1%FA and B acetonitrile) for 0 min to 10 min. After holding the solvent content to 100% methanol for 3 min, the column was returned to its starting condition. The column elution was separated such that approximately 250µl/min eluent was introduced to electro spray ionization mass spectrometry. Mass spectra were obtained on a full-scan operation in positive ion mode. The
capillary voltage was set at 3.6 kV, and the cone voltage was optimized at 30 V. The source temperature was set at 120 °C, the desolation gas temperature was 300 °C, and a nebulization gas flow was 600 L/h. Data profiling of positive ions from m/z 70 to m/z 1000 was recorded at 1 s/scan during analysis. The tune mixture solution (Agilent, USA) was employed as the lock mass (m/z 118.09, 622.05 or 922.02) at a flow rate of 30 μL/min via a lock spray interface for accurate mass measurement.

**Date extraction:** The typical total ion current chromatograms were unsuitable for pattern recognition because of the overlapping peak profiles. However, significant visual differences were observed between the patients with chronic gastritis and normal controls. The results of the overall data analysis were presented in the flowchart. Prior to peak resolution, the LC–MS data, which comprise a two-way matrix (retention time × mass-to-charge ratio) for each sample, are exported and stored under the Analytical Instrumental Association. The output data are stored in a two-dimensional matrix. Retention time is included in one direction and mass-to-charge ratio (m/z) in another direction.

For the LC–MS data, pattern recognition methods such as principal component analysis (PCA), partial least squares–discriminate analysis (PLS–DA), and orthogonal partial least squares–discriminate analysis (OPLS–DA) were employed to identify the biochemical pattern in fur tongue and suggest variables that can be used as biomarkers for chronic gastritis.

1.2.3. Polymerase chain reaction (PCR) -DGGE spectrum analysis of 16S rRNA V3 region

We performed bacterial genomic DNA extraction for 20 normal controls and 20 patients (selected the cases with the thickest and greasiest tongue coating from 70 patients). The tongue coating samples were washed by 0.1 M sodium phosphate buffer (pH 7.0) twice and then broken by the conventional bead-beater method[16]. We used phenol chloroform to extract the genomic DNA. The bacterial 16S rRNA gene V3 tongue variable regions of amplification system were discussed in the literature[17]. PCR was also presented in the
After the 16S rRNA gene region V3 amplification products were concentrated and measured (EnSpire Multilabel Plate Reader, PerkinElmer). We selected 200V for complete gel electrophoresis (Dcode DGGE, Bio-Rad). Dyeing with SYBR Green was then performed. The gel imaging system (Tanon-3500, Tanon) and Image J were used to obtain the DGGE digitalized map for multivariate statistical analysis (PCA, PLS–DA) to determine any significant difference between the strips in each group.

We cut the target strips on DGGE by aseptic operation blade and put the fragments in sterile double-distilled water at 4 °C overnight. We then performed 16S rRNA V3 PCR gene amplification. The PCR reaction system and procedure were the same as those described above. After the PCR products were collected by a Gel Extraction Kit (Omega Bio-tek) and purified, we connected them with pGEM-T Easy Vector (pGEM-T Easy Vector System, Promega) and transformed to inhale the sensitive cells by chemical technology(Trans5 α, TransGen Biotech). We selected the white colony and rapidly extract plasmid DNA by the 0.5%Triton-X100 cracking method for PCR amplification. The PCR reaction conditions and reaction system were the same as previously mentioned. The amplified PCR product was subsequently analyzed by DGGE and cloned similarly with those in the original tapping DGGE strip location.

Results

1 Clinical data

1.1 General conditions

The 70 cases were consisted of patients in LongHua Hospital from 2009 to 2010. The hospital is affiliated with Shanghai University of Traditional Chinese Medicine. Informed consent was obtained from the patients. In the 70 cases (36 male, 24 female), 20 cases had atrophic gastritis, 50 cases had superficial gastritis, their mean ages were 43.95 ± 13.36 years. The 20 cases in the normal controls included the teachers, graduate students (9 male, 11 female). The mean ages were 44.6 ± 14.78 years. No significant difference in gender
and ages (P>0.05) were observed between the two groups.

2. Metabolomics results of the chronic gastritis group and the normal controls

2.1 Chromatographic analysis and comparison between the chronic gastritis group and the normal controls

The typical base peak ion chromatogram between the chronic gastritis patients and the normal controls were compared. Differences in the peak height were observed between the two groups (Fig. 1), which indicated that the content and composition were different. We then analyzed the samples by using multivariate statistics tools for further research.

Fig.1. Typical UPLC/MS metabolic fingerprinting total ion chromatogram of human tongue coating samples from the chronic gastritis group and the normal controls

2.2 PCA and PLS–DA analysis of the metabolic profiles of tongue coating samples from the chronic gastritis group and the normal controls
PCA appeared partially overlapped and was mainly on the left quadrant. Further research by PLS–DA showed that the sample points were completely separated, which indicates that the two groups’ metabolic pathways were truly different (Fig.2). To improve the accuracy of the PLS discriminated model, we used the OPLS–DA to analysis the results by removed some redundant information such as environmental factors, gender, and diet.

![Fig.2. PCA and PLS-DA analysis of the metabolic profiles of tongue coating samples from the chronic gastritis group and the normal controls. Red represents the normal controls, and black represents the chronic gastritis group.](image)

**2.5 OPLS–DA comparison between the chronic gastritis group and the normal controls**

We compared the tongue metabolic fingerprint differences between the chronic gastritis group and the normal controls by OPLS–DA. There were many different quadrants between two groups (Fig. 3). A large number of mass-to-charge ratios of metabolites were found based on the corresponding load diagram. We evaluated the potential biomarkers by variable importance projection (VIP). A total of 50 different metabolites were found between the two groups after we had computed the VIP value (VIP value >2). We evaluated 10 metabolites with the largest VIP value and identified them (Table 1) by referring to the database and past biochemical studies of tongue coating cells.
Fig.3. Scoring chart by OPLS–DA analysis for the greasy coating group and the normal group. Black denotes the chronic gastritis group, and red represents the normal group.

Table 1. Compounds and relative contents of the components of potential markers in the chronic gastritis group and the normal group

<table>
<thead>
<tr>
<th>t_R/min</th>
<th>m/z</th>
<th>VIP</th>
<th>Compounds</th>
<th>Normal Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.59</td>
<td>566.0</td>
<td>2.38</td>
<td>UDP-D-galactose</td>
<td>↑</td>
</tr>
<tr>
<td>4.06</td>
<td>396.8</td>
<td>3.13</td>
<td>Vitamin D2</td>
<td>↓</td>
</tr>
<tr>
<td>4.07</td>
<td>340.1</td>
<td>3.25</td>
<td>3-Ketolactose</td>
<td>↑</td>
</tr>
<tr>
<td>4.08</td>
<td>379.2</td>
<td>3.39</td>
<td>Metarhodopsin</td>
<td>↑</td>
</tr>
<tr>
<td>8.14</td>
<td>334.2</td>
<td>3.59</td>
<td>Prostaglandin A2</td>
<td>↓</td>
</tr>
<tr>
<td>8.18</td>
<td>318</td>
<td>2.22</td>
<td>Leukotriene A4</td>
<td>↑</td>
</tr>
<tr>
<td>8.56</td>
<td>302.2</td>
<td>2.85</td>
<td>17alpha-methyltestosterone</td>
<td>↓</td>
</tr>
<tr>
<td>8.86</td>
<td>177.9</td>
<td>3.03</td>
<td>Pyrophosphate</td>
<td>↑</td>
</tr>
<tr>
<td>9.17</td>
<td>127</td>
<td>2.64</td>
<td>Piperideine-2-carboxylate</td>
<td>↓</td>
</tr>
<tr>
<td>9.25</td>
<td>310.0</td>
<td>3.00</td>
<td>Ribulose-1,5-bisphosphate</td>
<td>↑</td>
</tr>
</tbody>
</table>

3 Comparison of micro ecology between the chronic gastritis group and the normal controls

3.1 Comparison of 16S rRNA gene region V3 PCR PCR–DGGE diagrams between the chronic gastritis group and the normal controls

We processed the PCR product of the bacterial 16S rRNA gene V3 region from two groups of tongue coating samples by DGGE. The atlas was shown in Fig 4. Observation of the DGGE atlas revealed numerous bacterial DGGE atlas strips and some main strips in the two groups.
Fig. 4. PCR–DGGE atlas of 16S rRNA gene V3 region between the chronic gastritis group and the normal controls

Fig. 5. Comparison of PCR–DGGE diagrams of 16S rRNA gene V3 region between the normal controls and the chronic gastritis group, the arrow indicates the most significantly different strips between the two groups. Blue represents high brightness in the gastritis group, whereas red represents high brightness in the normal controls.

The DGGE atlases of the two groups were different (Fig. 5). Bacteria of these types of strips represented may indicate an association with the occurrence and development of chronic gastritis, which deserves further study. We simultaneously found eight strips in the two groups which were markedly
different. (Fig 5) Strips 1, 3, 4, 7, and 8 were abundant in gastritis patient samples, whereas strips 2, 6, and 10 were abundant in normal samples. The bacteria that these strips represent can be associated with chronic gastritis. PCA of the data of the two groups revealed that the samples basically gathered in two different regions (Fig. 6(a)). When we set the data of the two groups as the model (Fig. 6(b)), we found that they can be separated.

![Fig6. PCA and PLS-DA of the 16S rRNA gene V3 region of the normal group and the chronic gastritis group](image)

(a) PCA results (b) PLS-DA results

### 3.2 DGGE strip sequencing results

We first chose strips No. 8 and No. 10, the most markedly different strips among the samples for sequencing. Sequencing of strip No. 8 indicated that its nearest neighbor is *Moraxella catarrhalis* which demonstrated a 96.2% similarity. Sequencing of strip No. 10 showed 100% similarity with *Rothia mucilaginosa*.

### Discussion

Chronic gastritis is a common clinical disease with high incidence in various diseases of the digestive system (nearly about 90% of patients who have undergone gastroscopy). The incidence rate gradually increases with aging. Long-term pain causes severe discomfort to patients. The diagnosis of chronic gastritis is handled by gastroscopy and pathological sections. Chinese medicine thinks that the tongue can reflected the peoples’ physiological and
pathological information. We can determine the severity of the diseases and treatment based on syndrome differentiation by observing the tongue coating. Therefore, metabolic markers from the tongue coating should be explored. In recent years, extensive research in tongue coating has been conducted, and a certain degree of success has been achieved using advanced scientific knowledge and modern techniques [19-26]. The combination of metabonomics and microecology provides a new noninvasive technique and direction for the exploration of metabolic and microecological figures of tongue coating in patients with chronic gastritis.

Chronic gastritis causes pathophysiologic changes with corresponding metabolite changes. Analysis and comparison of these metabolites with those from the normal group to determine biological markers for some diseases can result in an efficient diagnostic method. Kettunen et al [27] used nuclear magnetic resonance high-throughput method to process 117 metabolic markers, including lipoprotein subclasses, amino acids, lipids, and the maximum of genome-wide association. They finally found 31 gene regions related to metabolite concentration in the blood. Of the 31 gene regions, 11 sites have no reported relationship with metabolic measurement, including the new site affecting cardiovascular disease risk markers and the potential biological markers with type II diabetes. Kleberg [28] demonstrated that the increased expression of PEAK1 can catalyze the proliferation of pancreatic tumor cells, which makes PEAK1 a biomarker and a small-molecule therapeutics target. Kinase PEAK1 has been observed in the early stages of pancreatic cancer. Metabonomic studies on chronic gastritis are rarely reported. The determination of metabolic syndrome biomarkers from biological samples (blood, urine, tissue extract) can help distinguish high-risk groups with chronic gastritis and achieve one grade of prevention. To simplify the process of clinical diagnosis, the present study can help in the early detection, early diagnosis, and early treatment of the disease and achieve two grades of prevention. In addition, this study can identify chronic gastritis in the early
stages, help understand the history of the disease, and achieve three grades of prevention. LC–MS shows significant advantages compared with other metabolomics methods. First, LC–MS exhibits high resolution and sensitivity, which ensures accuracy of results. Second, the pretreatment of samples involves a simple procedure. We can detect selected target metabolites within a short period, simultaneously analyze similar compounds in a complex matrix, and show the compounds that are unstable and difficult to separate during the preprocessing stage. Therefore, if we have no complete knowledge of the compound structures, we can identify the known and unknown compounds from biological samples by this method, which is suitable for the metabolite profile analysis.

Tongue metabolic fingerprinting in this study shows that the potential markers are mostly related to sugar metabolism, such as 3-keto lactose, a galactic metabolite. UDP-D-galactose is a nucleoside two-sugar phosphate that is a metabolic product of galactose and involved in the synthesis of oligosaccharides. 2-D-RNA DNA is the basic raw material of the RNA and DNA genetic materials in the life system and used as an ingredient in some vitamins and coenzymes. “Spleen takes charge of transportation” means that spleen transports cereal essence to nourish the limbs and bones, which are the foundation of birth and the source of Qi and blood. This process is equivalent to energy looping in the human body.

Phlegm, blood stasis, indigestion, and other pathological factors often cause spleen and stomach disorders. The transpiration of stomach Qi changes the tongue coating, which indicates its possible relation with the abnormal energy circulation. These materials are mostly the products of energy metabolic development, similar to the results of cell chemical structures. The change in glucose metabolism is one of the mechanisms of tongue coating formation in patients with chronic gastritis, which suggests that the incidence of chronic gastritis relates to energy metabolism changes.

Human ecology is an emerging branch of the science of life, which mainly
studies the interaction and connection between normal flora and the host. The four major microecological areas in the human body are the oral cavity, gastrointestinal tract, skin, and vaginal tract. The human body has approximately $10^{10}$ bacteria and 78% of them exist in the gastrointestinal tract. These bacteria interact with the body and achieve an inner balance. Once the balance is broken, bacterial pathogenesis occurs. One example is diarrhea, which is induced by intestinal dysbacteriosis. This pathogenesis is consistent with that in Chinese medicine. The micro ecological study of tongue coating has been frequently reported in recent years and proved the close association between tongue coating and microorganisms. A study [31] found that spleen–stomach damp heat syndrome leads to the reduction in Gram-positive cocci and the increase in Gram-negative bacilli from tongue coating. Yumin Sun et al. [32] found that the dominant bacteria in white coating as well as yellow coating were hemolytic streptococcus type A and catarrhal cuscus, whereas the dominant bacteria in white sticky coating as well as yellow sticky coating were hemolytic streptococcus type A and yellow pharyngeal bacteria. Yuefei Jiang et al. [33] found that the diversity of the flora on the tongue coating in patients with diarrhea-type irritable bowel syndrome and spleen–stomach damp heat syndrome is higher than that in the normal group and the spleen-deficiency group. Jing Wang et al. [34] observed microecological imbalance on the tongue coating in patients with acute pancreatitis, and changes in tongue coating are related to the severity of illness. The amount of bacteria on the thick coating is more than that on the thin coating, which mainly consist of G-anaerobes. Anaerobic bacteria increase, whereas aerobic bacteria decrease on the thick coating.

Our study included 20 cases of chronic gastritis and 20 cases of normal people to analyze 16S rRNA gene V3 region by polymerase chain reaction–denaturing gradient gel electrophoresis (DGGE). DGGE mapping and multivariate statistical analysis indicated a difference in bacterial composition between the two groups of samples. We also identified the most
different strips from these two groups by statistical analysis. We then recycled and sequenced the most pronounced strips (Nos. 8 and 10) and determined that their nearest neighbors were *M. catarrhalis (M. mora* bacteria) and *R. mucilaginosa*.

*R. mucilaginosa* is normal flora existing in human nasopharyngeal [35], oropharynx, and upper respiratory tract that can be isolated from the nasopharyngeal cavity and bronchial secretions. However, information regarding *R. mucilaginosa* is limited. The species was first reported in 1900 by Migula and was called *Micrococcus mucilaginosus* at the time. In 1907, the species was renamed to *Streptococcus salirarius* by Andrews and Gordon. *R. mucilaginosa* is a type of facultative anaerobic and Gram-positive coccus that comes in pairs or sets on smears, appears white in blood culture medium, and is non-hemolytic. *R. mucilaginosa* adheres to the surface of the culture medium and exists in the human oral cavity. This type of bacteria is considered an opportunistic pathogen. The bacterial catalyses is weakly positive or negative, and all enzymes and coagulates are negative. *R. mucilaginosa* is active in respiration and fermentation and can decompose galactose, fructose, galactose, glycerol, malt sugar, mannose, and sucrose, among others.

The results of our experiment showed that brightness gradually decreases from the normal group to the chronic gastritis group. The preliminary experiment of cell chemistry indicated that chronic gastritis occurs because of the broken balance of energy metabolism in tongue epithelial cells, activating its pentose shunt and enhancing oxidation. Consequently, unusual hyperplasia and differentiation of cell metabolism occur. The tongue coating is composed of epithelial cells, bacteria, and food residues. When epithelial cells are metabolically active, the amount of oxygen in the environment after metabolism can breed anaerobic bacteria. Facultative anaerobic bacteria perform aerobic and anaerobic fermentation simultaneously. These bacteria can grow in aerobic or anaerobic environment but thrive better in aerobic environment. Most pathogenic bacteria have such nature. The facultative
anaerobic bacteria detected in this experiment can decompose sugars, such as glucose, fructose, and galactose, which proves that the formation of chronic gastritis is associated with sugar metabolism. Comparison of microbial composition from the chronic gastritis group and the normal group showed that patients with chronic gastritis are related to inner energy metabolism and intestinal micro flora changes.

In addition, the brightness of the bacterial strip exhibiting 96.2% similarity with *M. catarrhalis* in the chronic gastritis group is higher than that in the normal control, which may have not been reported yet. This kind of bacteria, which is closely related to the formation of chronic gastritis, deserves further investigation.

**Conclusion**

We observed the metabolic components and micro ecological indexes of tongue coating in patients with chronic gastritis. This study has demonstrated that the changes in the metabolic patterns and micro ecological indexes were associated with the chronic gastritis, and could be identified by metabonomic method based on LC-MS analysis and microbiological techniques based on DGGE and multivariate statistics. The PLA-DA and OPLS-DA score plots suggested distinct differences between the normal controls and chronic gastritis group. Nine specific variables and two bacterial strips were used in the data analysis. Tongue coating indicates a close relationship between the body energy metabolism and intestinal micro flora changes, thus providing a theoretical basis for non-invasive diagnosis.

**Acknowledgment**

This study was financially supported by the Funding of Shanghai Municipal Health Bureau (20114Y196, F49008,)and state Administration of Traditional Chinese Medicine of the Peoples Republic of China

**Competing interests**

We, the authors, have no competing interests related to the manuscript entitled" Metabolic Markers and Microecological Characteristics of Tongue
Coating in Patients with Chronic Gastritis"

Authors’ contributions
Fu-Feng Li, Shun-Chun Wang and Xion-yan Pang conceived the idea and designed the research. Fu-Feng Li, Yi-Qin Wang and Yi-Min Hao acquired and processed the data. Fu-Feng Li, Jie Zhao, Wei-Fei Zhang and Peng Qian performed the research and analyzed the results. Zhu-Mei Sun, Fu-Feng Li wrote the paper. All authors read and approved the final manuscript.

References


[29] Guowang Xu, Methods and Application of Metabonomics, Beijing, Science and


Fig. 1. The typical UPLC/MS metabolic fingerprinting total ion chromatogram of human tongue coating samples from chronic gastritis and normal groups.
Fig. 2. PCA and PLS-DA analysis of the metabolic profiles of tongue coating samples from the chronic gastritis group and the normal group. Red represents the normal group, and black represents the chronic gastritis group.
Fig. 3. Scoring chart by OPLS–DA analysis for the greasy coating group and the normal group. Black denotes the chronic gastritis group, and red represents the normal group.
(Thin white coating from the healthy group and greasy coating from the chronic gastritis group)

Fig. 5. Comparison of PCR–DGGE diagrams of 16S rRNA gene V3 region between the normal group and the chronic gastritis group

Note: The arrow indicates the most significantly different strips between the two groups. Blue represents high brightness in the gastritis group, whereas red represents high brightness in the healthy group with thin white coating.
(Thin white coating from the healthy group and greasy coating from the chronic gastritis group)

Fig. 5. Comparison of PCR–DGGE diagrams of 16S rRNA gene V3 region between the normal group and the chronic gastritis group

Note: The arrow indicates the most significantly different strips between the two groups. Blue represents high brightness in the gastritis group, whereas red represents high brightness in the healthy group with thin white coating.
Table 1. Compounds and relative contents of the components of potential markers in the chronic gastritis group and the normal group

<table>
<thead>
<tr>
<th>$t_R$/min</th>
<th>m/z</th>
<th>VIP</th>
<th>Compounds</th>
<th>Relative normal group</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.59</td>
<td>566.0</td>
<td>2.38</td>
<td>UDP-D-galactose</td>
<td>↑</td>
</tr>
<tr>
<td>4.06</td>
<td>396.8</td>
<td>3.13</td>
<td>Vitamin D2</td>
<td>↓</td>
</tr>
<tr>
<td>4.07</td>
<td>340.1</td>
<td>3.25</td>
<td>3-Ketolactose</td>
<td>↑</td>
</tr>
<tr>
<td>4.08</td>
<td>379.2</td>
<td>3.39</td>
<td>Metarhodopsin</td>
<td>↑</td>
</tr>
<tr>
<td>8.14</td>
<td>334.2</td>
<td>3.59</td>
<td>Prostaglandin A2</td>
<td>↓</td>
</tr>
<tr>
<td>8.18</td>
<td>318</td>
<td>2.22</td>
<td>Leukotriene A4</td>
<td>↑</td>
</tr>
<tr>
<td>8.56</td>
<td>302.2</td>
<td>2.85</td>
<td>17alpha-methyltestosterone</td>
<td>↓</td>
</tr>
<tr>
<td>8.86</td>
<td>177.9</td>
<td>3.03</td>
<td>Pyrophosphate</td>
<td>↑</td>
</tr>
<tr>
<td>9.17</td>
<td>127</td>
<td>2.64</td>
<td>Piperideine-2-carboxylate</td>
<td>↓</td>
</tr>
<tr>
<td>9.25</td>
<td>310.0</td>
<td>3.00</td>
<td>Ribulose-1,5-bisphosphate</td>
<td>↓</td>
</tr>
</tbody>
</table>