Reviewer #1: The tongue diagnosis is one of important method in the TCM diagnosis, clinically TCM doctor differentiate TCM syndrome often rely color of tongue coatings, therefore clarify the formation mechanism and the features of different color of tongue coatings by microbiota and metabolic differences is significance. 1. In the introduction, authors should further describe (1) the definition and significance of TCM tongue diagnosis and the advance in the researches; (2) why have focused on yellow and white tongue coatings and their microbiota and metabolic differences? 2. The author needs to specify how to control the effects of drugs such as antiviral drugs, Chinese medicine, etc. and foods, as well as the conditions and the procedures for collecting the color and sample of the tongue coatings. 3. In the discussion, authors should further explain (1) why dose CHB patients with yellow tongue coating were mainly those of amino acid metabolism, whereas what metabolism was involved in the white tongue coating? (2) what is the formation mechanism of tongue coatings colors. 4. Why do the discovery of metabolites in tongue coatings only use LC-MS, not GC-MS, or both? 5. The sample size of 53 CHB patients (28 yellow tongue coating, 25 white tongue coating) in this study was smaller, the results have showed the characteristic microbiotas and metabolites of yellow and white tongue coating should be validated with more samples. 6. This study was only demonstrated the tongue coating of CHB with a smaller samples, which cannot provide the basis to understand the modern theoretical mechanism of TCM tongue diagnosis and facilitate therapeutic strategies for individualized treatment. 7. The English should be further polished.

Thank you for the kindly suggestions for this article. We have added the description of definition and significance of the tongue diagnosis in the introduction, and explained the reasons for choosing yellow coating and white coating as the objects of this study.

We did not restrict the intake of antiviral drugs or Chinese medicines when patients were collected. The proportion of medication for the chronic hepatitis B (CHB) patients with the yellow or the white tongue coatings was supplemented in table 1. There was no statistical difference between the two groups. In addition, a principal component analysis (PCA) plot based on OTU distributions showed that the overall compositions of tongue coating microbiota were not significantly shifted by age, gender, BMI and antiviral treatment (Fig.S1). All the tongue coating samples were collected in the

morning before breakfast to avoid potential interference from food and drinks.

We specified the conditions and the procedures of the tongue coatings collection in methods (Sample collection). All the tongue coating samples were collected in the morning before breakfast. The tongue color of all participants was photographed by the tongue diagnostic information acquisition system in a stable light source (Daosh Co., Shanghai, China). Tongue coating samples collection reference a published article[1], which adopted the LC/MS method, therefore, we adopted this method in this study. In the future, the combination of GC/MS and LC/MS detection will be added to further validate the study.

Because of the relevant literature reports are limited, the relationship between amino acid metabolism and the CHB patients with yellow tongue coating is unclear. The functional analysis of tongue coating flora and metabolites in CHB white tongue coating patients lacked correspondence, the underlying reason might be the microbiotas functionality in the CHB white tongue coating patients were most likely related to genetic information processing, which association with metabolites is relatively little. We have added the above statement in discussion. We have found the CHB patients with yellow or white tongue coatings had microbiota and metabolite differences in this study, but these phenomena cannot explain the mechanism of the formation of the color of tongue coatings.

The samples were small in this study, in the future, we will expand the sample size to further verify the results of this study. We add the above statement in the deficiencies of this study and the future work part.

We have modified the statement of "provide the basis to understand the modern theoretical mechanism of TCM tongue diagnosis" to "This study explores the micro-features between different tongue coatings, which will promote our understanding of the TCM tongue diagnosis".

We have repolished the English language in American Journal Experts, the Certificate Verification Key: 6289-A3A8-BE16-6379-CCEP.

1 Sun ZM, Zhao J, Qian P, Wang YQ, Zhang WF, Guo CR, Pang XY, Wang SC, Li FF, Li Q. Metabolic markers and microecological characteristics of tongue coating in patients with chronic gastritis. BMC Complement Altern Med 2013; 13: 227 [PMID: 24041039 PMCID: 3852724 DOI: 10.1186/1472-6882-13-227]

Reviewer #2: Does age affect the microbiota of tongue coatings? Had patients received any treatment for HBV before research?

There is high diversity in the oral microbiome within and between individuals, besides factors specific to the host, age and race/ethnicity, environmental factors also contribute to the variability of the oral microbiome[1-2]. In this study, Age, gender and body mass index (BMI) did not significantly differed among groups (table 1).

We did not restrict the intake of antiviral drugs or Chinese herbs when patients were collected. The proportion of medication for the chronic hepatitis B patients with the yellow and the white tongue coating patients was supplemented in table 1. There was no statistical difference between the two groups. A principal component analysis (PCA) plot based on OTU distributions showed that the overall compositions of tongue coating microbiota were not significantly shifted by age, gender, BMI and antiviral treatment (Fig.S1).

1 Moon JH, Lee JH. Probing the diversity of healthy oral microbiome with bioinformatics approaches. *BMB Rep* 2016; **49**(12): 662-670 [PMID: 27697111 PMCID: PMC5346311]

2 Xu X, He J, Xue J, Wang Y, Li K, Zhang K, Guo Q, Liu X, Zhou Y, Cheng L, Li M, Li Y, Li Y, Shi W, Zhou X. Oral cavity contains distinct niches with dynamic microbial communities. *Environ Microbiol* 2015; **17**(3): 699-710 [PMID: 24800728 DOI: 10.1111/1462-2920.12502]

Reviewer #3: This paper investigates microbiota and metabolic differences in chronic hepatitis B (CHB) patients with yellow or white tongue coatings. This study confirmed the hypotheses that the yellow tongue coating had abnormal oral microbiota and metabolic compositions. It's a new finding to elucidate the alteration of the tongue coating with the clinical features. It's an interesting phenomenon and study. The pity is that I cannot discover the clinical application prospect in this manuscript. Since it's known that chronic hepatitis is prone to accompany by disorder of intestinal microbiota. And I don't believe this study might be helpful to solve the key problems in HBV.

It is well known that intestinal microbiotas are widely involved in the pathogenesis of chronic liver diseases. As part of the human microbiome, attention has been paid to the relationship between oral microbiota and intestinal microbiota, and many diseases have found the oral microbiota disruption accompanied with intestinal microbiota disorders[1-4]. A study reported that microbiota isolated from the salivary are strong inducers of T helper 1 (TH1) cells when they colonize in the gut, suggested that the oral cavity may serve as a reservoir for potential intestinal pathobionts that can exacerbate intestinal disease[5]. Patients with cirrhosis also found have impaired salivary defenses and worse inflammation. Salivary dysbiosis was greater in patients with cirrhosis who developed 90-day hospitalizations[6].
Tongue diagnosis is a characteristic diagnosis method of traditional Chinese medicine(TCM) with a long history, but lacks scientific explanation. TCM believes that the tongue is an important window of body change, which is of great significance for clinical treatment.Tongue diagnosis has the advantages of convenience and intuitiveness and represents the first-hand information for the TCM doctors to guide the diagnosis and treatment principles. The yellow and the white tongue coatings are the most common manifested tongue coating colors in clinic, which lead to different treatment strategies in TCM. Therefore, this research characterized the microbiota and metabolic compositions of yellow or white tongue coatings in CHB patients, and associated the tongue coating microbiota with metabolites and host physiological indices. This study could help to understand the old TCM

tongue diagnosis with modern theoretical mechanism and facilitate therapeutic strategies for individualized treatment.

1 Koren O, Spor A, Felin J, Fak F, Stombaugh J, Tremaroli V, Behre CJ, Knight R, Fagerberg B, Ley RE, Backhed F. Human oral, gut, and plaque microbiota in patients with atherosclerosis. Proc Natl Acad Sci U S A 2011; 108 Suppl 1: 4592-4598 [PMID: 20937873 PMCID: PMC3063583 DOI: 10.1073/pnas.1011383107]

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3 Xiao E, Mattos M, Vieira GHA, Chen S, Correa JD, Wu Y, Albiero ML, Bittinger K, Graves DT. Diabetes Enhances IL-17 Expression and Alters the Oral Microbiome to Increase Its Pathogenicity. Cell Host Microbe 2017; 22(1): 120-128 e124 [PMID: 28704648 PMCID: PMC5701758 DOI: 10.1016/j.chom.2017.06.014]

4 Qin N, Yang F, Li A, Prifti E, Chen Y, Shao L, Guo J, Le Chatelier E, Yao J, Wu L, Zhou J, Ni S, Liu L, Pons N, Batto JM, Kennedy SP, Leonard P, Yuan C, Ding W, Chen Y, Hu X, Zheng B, Qian G, Xu W, Ehrlich SD, Zheng S, Li L. Alterations of the human gut microbiome in liver cirrhosis. Nature 2014; 513(7516): 59-64 [PMID: 25079328 DOI: 10.1038/nature13568]

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