

Peer Review File

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Review Comments

Pulse-taking is widely used for diagnosis and treatment in traditional Chinese medicine (TCM). Protein complexes in serum perform various biological functions. Balanced constitution is a major constitution in TCM, but people with balanced constitution have some unified characteristics of unbalanced constitution. In the manuscript “Serum protein complex profiling reveals heterogeneity of Balanced constitutional population in traditional Chinese medicine through blue native PAGE”, the authors used BN PAGE to analyze protein complexes in serum samples from 25 people with balanced constitution.

A number of improvements need to be made before the manuscript can be accepted.

- (1) There are many examples of grammatical errors or irregular writing throughout the text. The language of this paper needs to be polished by a native English speaker.

Comment 1: There are many examples of grammatical errors or irregular writing throughout the text. The language of this paper needs to be polished by a native English speaker.

Reply 1: Thanks for your comment. The manuscript has been polished with the assistance of native English speakers.

Changes in the text: In the entire manuscript.

- (2) The blood biochemistry and symptom questionnaire of the 25 patients should be listed in a supplementary table.

Comment 2: The blood biochemistry and symptom questionnaire of the 25 patients should be listed in a supplementary table.

Reply 2: Thanks for your comment. The main characteristics of 25 volunteers were listed in supplementary Table 1.

Changes in the text: The added content was in Results part of manuscript and as follows:

The main characteristics of the 25 volunteers in type A or type B were shown in supplementary Table 1. No significant differences were found in sex, age, body mass index (BMI), heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting plasma glucose (FPG), total cholesterol (TC), total triglyceride (TG), glutamic oxaloacetic transaminase (AST), glutamic-pyruvic transaminase (ALT), blood urea nitrogen (BUN), creatinine (CR), and hypersensitive C-Reactive Protein (hr-CRP).

- (3) In Figure 1, what is the meaning of “*”? And in Figure 2, what is the meaning of “A’”, “B’” and “B’’”?

Comment 3: In Figure 1, what is the meaning of “”? And in Figure 2, what is the meaning of “A’”, “B’” and “B’’”?*

Reply 3: The "" in Figure 1A corresponded to the "*" sample in Figure 2A, that is, the sample numbered #6.*

"Type A'" was a subtype of type A, and the overall pattern of gel band in type A' was consistent with that of type A, but there was one more band located at molecular weight of 242 kDa in type A'. Similarly, "type B'" and "type B'" were two subtypes of type B.

Changes in the text: *The text has been revised in Results part of manuscript and as follows:*

In type A, there were 15 bands ranging from 146 kDa to 1236 kDa, labelled as A1-A15, respectively. We also noticed a subtype of type A, named as type A', whose protein complex pattern was very similar to that of type A except an additional band located at 242 kDa. This additional band was set as A12' as its molecular weight was between A12 and A13. There were 16 bands in type B, labeled as B1-B16. Similar to type A, type B contained subtypes B' and B''. Compared with type B, type B' had two additional bands B10' and B13', while type B'' has two additional bands (B13'' and B14') instead of B14 (Fig. 2C).

- (4) How can balanced constitution be explained? It does not equate to being healthy.

Comment 4: How can Balanced constitution be explained? It does not equate to being healthy.

Reply 4: According to the Classification and Determination of Constitution in TCM, there are two main types of Balanced constitution. One type is the population that fully meets the health standards, but the number of such people is very small; and the other type is the people whose eight unbalanced constitutions convert scores are all less than 15 points, which is the majority of the Balanced constitution. Although the latter is not equate to health, there is no obvious unbalanced constitutions' characteristics in the population, so it can still meet the description of the Balanced constitution. The typical characteristics of Balanced constitution are: healthy fair complexion, full of energy, good sleep and appetite, normal bowels movement and urination, easygoing and strong ability to adapt to the natural and social environment.

Our study supplemented and improved the current TCM constitutional classification. TCM constitutions were based on the standards defined in the "Classification and Determination of Constitution in TCM," which were added to the Methods.

Changes in the text: *We've added the measurement of TCM constitution type in Methods part:*

The constitution type of participants was evaluated using the TCM Constitution Scale. The survey consists of 60 questions, and there were 5 options for each questions (not all, few, sometimes, often, always), corresponding to 1-5 points. There were nine subscales used to assess nine TCM constitution types individually, including Balanced constitution, Qi-deficiency constitution, Yang-deficiency constitution, Yin-deficiency constitution, Phlegm-damp constitution, Damp-heat constitution, Blood-stagnation constitution, Qi-stagnation constitution and Inherited Special constitution. Firstly, we calculated the score by summing the scores for each subscale. Then the constitution convert scores of each subscale were calculated by applying the following formula (original score - the number of questions)/(the number of questions × 4) × 100. If the constitution convert scores of Balanced constitution was higher than 60 or the constitution convert scores of the eight unbalanced constitutions were less than 40 points, Balanced constitution was claimed. If the score of any constitution subscale was ≥ 40 points, unbalanced constitutions were diagnosed.

- (5) In the paper, all blood samples were collected from individuals with balanced constitution. Why were individuals with unbalanced constitution not chosen?

Comment 5: *In the paper, all blood samples were collected from individuals with balanced constitution. Why were individuals with unbalanced constitution not chosen?*

Reply 5: *Balanced constitution is the most popular constitution. From a clinical perspective, many people with Balanced constitution are in the sub-health state and specific medical intervention is needed. The individual differences and the heterogeneity of the population may explain the occurrence of diseases in the population of Balanced constitution.*

We plan to analyze the proteomics characteristic of the population with an unbalanced constitution in a separate study, which may help to deepen the understanding of individual difference and classification principles of TCM constitution.

Changes in the text: *No changes in the manuscript.*