Response letter

ESPS Manuscript NO: 34445

Subject: Ketogenic diet imposes significant effect on imbalanced gut microbiota in infants with refractory epilepsy

Dear Editors and Reviewers,

Many thanks for kind comments on our submission, and your work would be so important for us because these comments could make our article published with higher quality. And point-to-point responses are shown as follows, please kindly check it. Looking forward to your valuable comments, and we would like to do corresponding revision as you suggest.

Reviewer #1

This is an important study in regards to refractory epilepsy in pediatric patients correlating GM-changes to seizure frequency after KD and compared to controls.

Comment 1: However, while this may provide a foundation for future research, I believe more of that future research needs to be currently added before this study can be more groundbreaking--eg. demonstrate a benefit in a refractory epilepsy patient by changing the GM to that of normal control independent of KD (such as with fecal transplant or antibiotic therapy), or show and on-off-on correlation with KD and GM

Response 1: Many thanks for this professional suggestion. It is a pilot study for imbalanced GM of children with refractory epilepsy, and GM change after KD treatment. This will be a additional and significant reference for understanding GM and KD therapy in epilepsy therapy.

As you mentioned, it will be necessary to clarify if clinical improvement was mediated by GM. Based on findings in this study, metagenomic research is also on-going to investigate detailed GM imbalance of children with refractory epilepsy. Then species-level classification and gene/functional network could be constructed, to

understand potential contribution of GM to symptom mitigation. Animal model will be considered in future: transplanting of feces(collected from diseased children) to germ-free mice, and determining if GM dysbiosis could induce epilepsy associated symptoms. If the result is positive, mice will be divided into two subgroups: GM of one group will be deprived by broad-spectrum antibiotics and KD treatment will be performed for each group. Inter-group comparison could indicate whether GM mediate KD therapy. If GM could not induce any symptoms, KD, probiotics and other methods will be applied to change GM, then evaluate if changing GM is important in ameliorating epileptic symptoms.

Reviewer #2

The authors report for the first time that gut microbiota from epileptic infants differ significantly from that of healthy patients and that the ketogenic diet, even after one week, can alter the composition of gut microbiota.

Comment 2 : The study is interesting and deserves to be repeated after a longer period of the ketogenic diet to obtain more significant results.

Minor concerns:

Abstract Results, last line:

What did the authors mean by: "kept continued to increase" in the study context?

Material and Methods:

Please give the composition of the ketogenic diet

Results:

Please, give statistical data for the Shannon index, and PCA

Table 1 and supplementary Table 3 are partially redundant and could be grouped.

Sampling date can be removed and replaced with the medical treatment.

The statistical differences concerning age and gender can be given in the text.

The word "effective" is not clear. The reader must understand that the improvement of clinical symtoms results from the effect of the ketogenic diet.

Response 2: Appreciated for your kind and professional comments. Further research plan is detailed in Response 1 and metagenomics analysis is on-going now. Based on these pilot study, animal-model will be applied in our lab, to improve understanding of GM mediation in mitigation of epilepsy.

"Kept continued to increase" represents "increased" after treatment, and this has been corrected in revision. Composition of KD was added in updated manuscript, please kindly check it.

Statistical data of Shannon index and PCA was detailed in supplementary table 2 and 3, respectively. Table 1 has been removed and correlated sample information is detailed in Supplementary Table 1. Statistic difference of gender and age was also summarized in the main text: "Both gender and age had no statistical significance for affecting GM, with p-value 0.069 and 0.234 respectively." Sampling date was also replaced by medical treatment, and detailed in supplementary table 1.

The word "effective" mainly represents control of seizure frequency, and it was categorized into three classes: seizure-free, reduction 50-90%, reduction <50%. To promote the understanding of results, "effective" was replaced by specific improvement: i.e. reduced seizure frequency.