Author's response to reviews

Title: Transglucosidase improves the gut microbiota profile of type 2 diabetes mellitus patients: A randomized double-blind, placebo-controlled study

Authors:

Makoto Sasaki (msasaki@aichi-med-u.ac.jp)
Naotaka Ogasawara (nogasa@aichi-med-u.ac.jp)
Yasushi Funaki (momomaru@aichi-med-u.ac.jp)
Mari Mizuno (mari@aichi-med-u.ac.jp)
Akihito Iida (iida@aichi-med-u.ac.jp)
Chiho Goto (chiho@nagoya-bunri.ac.jp)
Satoshi Koikeda (skoikeda@amano-enzyme.ne.jp)
Kunio Kasugai (kuku3487@aichi-med-u.ac.jp)
Takashi Joh (tjoh@med.nagoya-cu.ac.jp)

Version: 4 Date: 26 February 2013

Author's response to reviews: see over
To,
The Editor-in-Chief

BMC Gastroenterology

MS: 5703470318684932

February 26, 2013

Dear Editor:

We have enclosed our revised manuscript (5703470318684932) entitled “Transglucosidase improves the gut microbiota profile of type 2 diabetes mellitus patients: A randomized double-blind, placebo-controlled study” by Makoto Sasaki, Naotaka Ogasawara, Yasushi Funaki, Mari Mizuno, Akihito Iida, Chiho Goto, Satoshi Koikeda, Kunio Kasugai, and Takashi Joh.

We hope that you will find our revised manuscript acceptable for publication in BMC Gastroenterology, and that the contents of our paper will be of interest to your journal’s readership.

Thank you for your consideration.

Sincerely,

Makoto Sasaki, M.D., Ph.D.
Department of Gastroenterology,
Aichi Medical University School of Medicine
1-1 Yazakokarimata, Nagakute,
Aichi 480-1195, Japan
Phone: +81-561-62-3311
Fax: +81-561-62-1508
E-mail: msasaki@aichi-med-u.ac.jp
**Answer to Reviewer 1**

Thank you for your important comments. They were extremely helpful in improving the quality of our manuscript.

1. *No data on body weight and blood glucose (or HbA1c) level after TGD treatment were presented in the manuscript. These data were very important and should be reported. Furthermore, the authors should examine the relation between changes in body weight or blood glucose level and the gut microbiota.*

   Thank you very much for your suggestion. We have included a new figure (Figure 1) that presents the HbA1c levels and BMIs before and after the TGD treatment. In the Results, we have added some text as follows (page 9, lines 3–7):

   “The HbA1c level and BMI before and after the treatment are shown in Figure 1. In the placebo group, HbA1c levels were increased in 11 patients (69%) after the treatment; levels were higher but not significant than those in the TGD-treated group (14 [39%]; p = 0.07). In the placebo group, the BMI had increased in 9 patients (56%) after the treatment; the BMIs were higher than that in the TGD-treated group (14 [39%]).”

   Furthermore, we have described the relationship between the changes in HbA1c level or BMI and gut microbiota. In the Results, we have revised some text as follows (page 10, lines 7–11):

   “In the group with an increase in Bacteroidetes-to-Firmicutes ratio, 50% (12/24) and 48% (11/23) of the patients had decreased HbA1c levels and BMIs, respectively, after the TGD treatment; however, in the group without an increased Bacteroidetes-to-Firmicutes ratio, only 22% (2/9) and 33% (3/9) of the patients had decreased HbA1c levels and BMIs, respectively, after the TGD treatment.”

2. *Based on the data by cluster analyses, the authors described that TGD modulated the gut microbiota profile in patients with T2DM. Were the
changes in the microbiota by TGD statistically significant? In other words, did the author evaluate the differences in bacterial diversity between before and after TGD treatment?

Thank you very much for your comment. In this study, we compared the Bacteroidetes-to-Firmicutes ratios before and after the TGD treatment. As shown in the Results, we have revised some text as follows (page 10, lines 3–5):

“After 12 months of TGD therapy, the Bacteroidetes-to-Firmicutes ratios in both TGD groups significantly increased compared with that before the TGD treatment and were also significantly higher than that in the placebo group, indicating improvement of gut microbiota.”

3. *The authors should add the data of placebo group in Figure 3 which shows the fecal bacteria structure before and after treatment.*

Thank you very much for your suggestion. We had unintentionally used the wrong method to compare the 2 different dendrograms; we analyzed all the samples in 1 dendrogram (Figure 3). As the presented in the Results, the bacterial lineages were constant within each individual, and there were no significant differences between the T2DM patients and healthy volunteers. As shown in the Results, we have revised some text as follows (page 9, lines 17–21):

“Most of the healthy individuals (6/10) and T2DM patients (45/60) were classified into cluster I, indicating no significant difference in fecal bacterial communities between the healthy individuals and T2DM patients. In the placebo and TGD groups, the bacterial communities were generally similar before and after the treatment. These results suggest that bacterial lineages were constant within each individual (Figure 3).”

**Answer to Reviewer 2**

Thank you for your important comments. They were extremely helpful in improving the quality of our manuscript.
Major compulsory revisions

1. In the Subjects and Methods section the Authors stated that the enrollment criteria and study design were previously described. Are the patients enrolled in this study the same of reference 1? Please specify and add more details about the study design and the enrollment criteria.

   Thank you very much for your suggestion. We have described our study design in detail (page 6, lines 7–9; page 6, lines 12–15; page 6, lines 17–19).

2. The Authors did not provide any explication about the evaluation of the sample size, about the number of healthy controls and about the choice of transglucosidase dosage. Please provide them.

   Thank you very much for your suggestion. We agree with the reviewer that a larger number of controls would have been better and a matched-pairs sample would have been best. We referred to the study of Ley et.al. (Nature 2006, 444(7122):1022-1023), which showed a significant difference in microbiota between 12 obese people and 12 lean people. We decided the transglucosidase dosages based on our previous experience. We have revised some text as follows (page 6, lines 14–15):
   “Based on our previous in vitro and in vivo experiments [2, 8], we used TGD dosages of 300 and 900 mg/day”

3. The Authors should describe what kind of diabetes medication (including the dosage) are used throughout the study.

   Thank you very much for your suggestion. We have changed Table 1 to include the ongoing diabetes therapies. We have also modified the Results section accordingly (page 8, line 1, from the bottom).

4. In the Results section the Authors did not specify the difference in Bacteroides/Firmicutes ratio between diabetic patients and healthy controls, but in the Discussion section They stated that smaller Firmicutes populations were observed in T2DM patients than in the healthy population. Please provide more details.
Thank you very much for your suggestion. We have included data regarding the Firmicutes population in the T2DM patients and healthy population. In the Results section, we have added some text as follows (page 10, lines 1–3):

“However, the mean size of the Firmicutes populations (49.0%) in the T2DM patients was significantly smaller than that in the healthy patients (58.6%; p < 0.05).”

5. The major limitation of this study is that the Authors dis not show any correlation between gut microbiota modification and metabolic state of the patients. Does modification of gut microbiota correlate with reduced levels of HbA1c?

Thank you very much for your question. We have described the relationship between the changes in HbA1c level or BMI and gut microbiota. In the Results, we have revised some text as follows (page 10, lines 7–11):

“In the group with an increase in Bacteroidetes-to-Firmicutes ratio, 50% (12/24) and 48% (11/23) of the patients had decreased HbA1c levels and BMIs, respectively, after the TGD treatment; however, in the group without an increased Bacteroidetes-to-Firmicutes ratio, only 22% (2/9) and 33% (3/9) of the patients had decreased HbA1c levels and BMIs, respectively, after the TGD treatment.”

Minor essential revisions

6. Please provide a table showing individual results for any patients and not only mean values and HbA1c levels before and after treatment.

Thank you very much for your suggestion. We have now included a new figure (Figure 1) that presents the HbA1c level and BMI before and after the TGD treatment.

Discretionary revisions

7. The abstract should specify how many healthy controls the study included.

Thank you very much for your suggestion. In the Abstract, we have revised some text as follows (page 3, lines 10–13):
“Comparisons of fecal bacterial communities were performed before and after the TGD treatment and were performed between T2DM patients and 10 healthy individuals, using the terminal-restriction fragment length polymorphism analysis”

8. In the Subjects and Methods section the Authors stated that patients received treatment after every meal. Please specify that patients received treatment 3 times a day after main meals.

Thank you very much for your suggestion. In the Methods section, we have revised some text as follows (page 5, lines 14–16):

“The patients were randomized into 3 groups according to the treatment received as follows: 100 mg of TGD, 300 mg of TGD, and placebo 3 times a day after the main meal for 12 weeks”