Author's response to reviews

**Title:** The G-Protein beta 3 subunit 825 TT genotype is associated with epigastric pain syndrome-like dyspepsia

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**Author's response to reviews:** see over
Dear the Editor,

Thank you for considering our manuscript (MS: 4814861213084556) “The G-Protein beta 3 subunit 825 TT genotype is associated with epigastric pain syndrome-like dyspepsia”. We have responded to the reviewers’ comments and criticisms and have provided a point-by-point response. We have also got the check of our English by a native English speaker. Please find the edited revised points by red ink. We hope that you will now find the manuscript suitable for publication in BMC Medical Genetics. Thank you for your time and attention in the handling of our manuscript.

Sincerely,

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This is an interesting study evaluating the role of GNB3 825T polymorphism in patients with functional dyspepsia and concluded that the homozygous GNB3 825T allele influences the susceptibility to EPS-like dyspepsia. However, there are a few limitations in the study.

1. How was sample size estimated? What was the power of the sample size used for subgroup analysis?
   We regret not having addressed this point in detail. In the healthy Japanese population, approximately 20% are expected to be homozygous for the 825T allele. Assuming that approximately 5% of subjects have symptoms of dyspepsia, a 20% increase in the prevalence of a genotype would be of clinical relevance. Thus, setting \( \alpha = 0.05 \) and \( \beta = 0.80 \), 826 asymptomatic controls and 44 subjects with dyspepsia would be sufficient to identify a clinically relevant difference. The actual number of enrolled subjects (68 FD cases and 761 controls) has a power of 93% to detect the assumed difference. For the subgroup analyses for EPS (68 cases), PDS (43 cases), and EPS excluding PDS (28 cases), the power to assumed difference is 79%, 76%, and 61%, respectively.

We have now added the following section in the Methods.

**Power of the Study**

In this study, we assessed the potential association of symptoms of dyspepsia with GNB3 C825T allele status. In the healthy Japanese population, approximately 20% are expected to be homozygous for the 825T allele. Assuming that approximately 5% of subjects have symptoms of dyspepsia, a 20% increase in the prevalence of a genotype would be of clinical relevance. Thus, setting \( \alpha = 0.05 \) and \( \beta = 0.80 \), 826 asymptomatic controls and 44 subjects with dyspepsia would be sufficient to identify a clinically relevant difference. The actual number of enrolled subjects (68 FD cases and 761 controls) has a power of 93% to detect the assumed difference. For the subgroup analyses for EPS (68 cases), PDS (43 cases), and EPS excluding PDS (28 cases), the power to assumed difference is 79%, 76%, and 61%, respectively.

2. “Our data may explain familial aggregation of patients with functional gastrointestinal disorders” – how many patients with dyspepsia included in the study had family history of dyspepsia? Were there patients from same family included in the study?

Thank you for your comment. We had not taken familial histories and we can not investigate this aspect with the subjects in the present study. Therefore, we added a reference to previous reports. We want to raise the possibility of the hereditary factors in the generation of dyspeptic symptoms. We rewrote the sentence as follows:

“Involvement of the genotype for the generation of EPS-like symptoms demonstrated in this study may, in part, explain the previous findings of familial

3. In the Abstract, methods and results are clubbed together. Also, Result section does not present the data adequately.
We apologize for the incorrect organization and presentation. We have now corrected these sections.

4. Numbers in the table does not add up to the number of subjects included as mentioned in the study.
Thank you for your comment. We are sorry for the unclear statements. The number of controls is 761 and that of FD subjects is 68, for a total of 829 subjects. FD subjects were categorized as EPS (43) or PDS (40); 15 subjects had both EPS and PDS. We have now added footnotes in Tables.

5. Adding 350 (in which UGI and EGD performed) and 318 (in which no test was performed) controls the total is 668; further addition of 136 FD (in which UGI and EGD were performed) and 25 FD (in which no test was performed) total is 171. But when calculated from table 1, FD, EPS and PDS the total is 151. Please clarify such number discrepancy.
We are sorry for the unclear statements. In this study, UGI was performed in 350 subjects and EGD was performed in 136 subjects. No subjects underwent both examinations. Neither UGI nor EGD was performed in 343 subjects comprising 318 controls and 25 FD cases.
   In 350 subjects who underwent UGI, 325 were controls and 25 were FD cases.
   In 136 subjects who underwent EGD, 118 were controls and 18 were FD cases.
We now include these breakdowns in the Methods.

6. Results in the main manuscript are presented in sketchy manner.
Thank you for your comment. We have now provided more detailed presentation of the results.

7. Authors have stated that a total of 171 subjects were excluded from the study. It is not clear that how many were from control group and how many from FD group.
Thank you for your comment. In 171 subjects who were excluded from the study based on blood and UGI and EGD findings, 155 were from the control group and 16 were from the FD group. We now have mentioned this in the Methods.

8. Authors have used median and range for the presentation of data. Please specify that whether they have checked by any statistical method whether the data were normally distributed or not?
Thank you for your comment. Since the distribution of age was not normal ($p < 0.01$ by the Kolmogorov-Smirnov test), the data were summarized as median and range and subjected to the Mann-Whitney U-test. Now we mention this in the Methods and Results.

9. Method is presented after Result and Discussion sections
We apologize for the incorrect organization, and we have now moved the Methods section to the appropriate position.

10. **Clinical parameters should be presented in greater details.**
Thank you for your comment. We now have added the data for BMI, ALT, and blood pressure in Table 1 and summarized these in the Results.

11. **It is better not to use both polymorphism and genotype. It should be either polymorphism or genotype.**
Thank you for your comment. We now have unified these to “genotype”.

12. **In the abstract authors have used “association between GNB3 protein polymorphism and dyspepsia”. I think, it should be “GNB3 gene polymorphism”**.
Thank you for the comment. We have corrected this to “In the present study we clarified the association between GNB3 gene polymorphism”.

13. **In the Table-1 authors have not mentioned the unit of Age. Please mention “years”**.
Thank you for your comment. We added “years” in Table 1.

14. **All the patients were evaluated for IBS and GERD. Were all these subjects excluded from the study?**
Thank you for your comment. We did not exclude subjects with IBS and GERD from the study. IBS and GERD co-occur with FD and this issue is discussed in the third paragraph of the Discussion section.

“In the present study, 62 subjects with predominant symptoms of GERD were included. However, no association was found between the GNB3 C825T genotype and GERD symptoms. Furthermore, the analysis of data in the present study was not affected by concomitant GERD symptoms in some subjects.”

15. **I think Discussion is quite elaborate and written well. But is should be shortened.**
Thank you for your kind suggestion. We have now shortened the Discussion.

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.
Reviewer's report
Title: The G-Protein beta 3 subunit 825 TT genotype is associated with epigastric pain syndrome-like dyspepsia
Version: 1 Date: 13 October 2009
Reviewer: Durk Rimmer de Vries
Reviewer's report:

Minor essential revisions: in the introduction, a negative study by Andresen et al is cited with the goal to link functional gastrointestinal disorders to visceral hypersensitivity. This serves no purpose and the reference should be changed, earlier work has been done, e.g. using balloon distension at multiple levels in the GI tract, that proves the wanted point much more convincingly. Trimble Dig Dis Sci 1995, 40:1607-13 is a good example.
Thank you for your comments. We have changed the reference.

In the second paragraph G-proteins are called membrane receptors, which is not the case, they are the ligand to the GPCRs. Please change this.
We have corrected the sentence. Thank you for pointing this out.

Regarding the methods, I would like to know the response rate: how many people were asked to participate and how many accepted and declined, respectively?
Thank you for your comments. The questionnaire and blood samples were collected from 1000 consenting participants. We do not have data on how many people were approached, but we estimate that 80% of those who underwent annual health check-up at Healthcare Center of Social Insurance Shiga Hospital from December 2007 to April 2008 agreed to participate in the study. We have included this in the Methods.

I would also like to know if any control experiments were performed using e.g. restriction fragment length analysis to check the accuracy of the genotyping assay.
Thank you for your comment. We employed TaqMan SNP Genotyping Assay for the assaying the GNB3 gene polymorphism. We performed the same analysis in replicate in 12% of the samples (randomly selected) in different batches and confirmed the reliability of the data.