Author's response to reviews

Title: Transcriptome signatures in Helicobacter pylori-infected mucosa identifies acidic mammalian chitinase loss as a corpus atrophy marker Short title: The transcriptome of corpus atrophy

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Author's response to reviews: see over
Reviewer's report
Title: Transcriptome signatures in Helicobacter pylori-infected mucosa identifies chitinase loss as a corpus atrophy marker Short title: The transcriptome of corpus atrophy
Version: 1 Date: 28 July 2013
Reviewer: George Sachs

This reviewer finds the figures somewhat confusing and at least would like to see a standard heat map of individual genes reflective of corpus and antrum rather than the relatively arbitrary grouping used in figure 2, which is also too small.

Authors’ comments:
We have presented the results as groups of genes based on the gene ontology (GO) annotation derived from the GO consortium. This kind of analysis can lead to an easy biological interpretation in terms of function, and provides much more overview than a list of individual genes. However, we have now also added the details of individual genes in Supplementary data, which will allow the reader to explore the results gene by gene.

Figure 3 has an abundance of data which is very hard to follow and also should be divided into 3 separate figures with more adequate legends.

Authors’ comments:
We agree with the reviewer that the figure 3 is a bit complicated. In this figure, we attempt to simultaneously present the gene expression profile clustering together with their functional enrichment. We argue that there is a clear benefit for the reader to capture the results simultaneously, and we therefore keep the arrangement as is.

Figure 4 should name the genes analyzed rather than A, B etc. Nevertheless the actual data are very impressive and this criticism is directed at making the ms more transparent for the average reader.

Authors’ comments:
We agree with the reviewer, we have now put name of the genes into the figure.

Results: These are generally well classified and interesting in the division between naïve and atrophy at the outset. They very correctly identify corpus atrophy gene depletion and increase. Some of the genes should be named here e.g. ATP4B, gastrin etc. Again here an improvement in figure 2 would improve readability.

Authors’ comments:
See the comments to figure 2 above. We have added supplementary data which will be useful for the readers.

The corpus overexpressed genes are entirely expected except for genes related to angiogenesis and clotting. Also, the immune response genes in corpus atrophy are of interest and some should be enumerated. It is also worth explaining the similarity here between atrophy and Hp infection. It is also clear that loss of function genes relate to parietal cell genes as stated here.
Authors’ comments:
We agree with the reviewer that there are interesting findings worth exploring. However, in the interest of space, we do not add these discussions to the present manuscript.

Again figure 4 is difficult to follow. The upregulation of antrum specific genes is very clear in the text, less so in the figures. The somatostatin gene could also be discussed in addition to gastrin as could the gastrin receptor and chromogranin as reflecting ECL cells.

Authors’ comments:
Figure 4 has been updated, see above. Again, in the interest of space, we have not added more items to the discussion.

Reviewer's report
Title: Transcriptome signatures in Helicobacter pylori-infected mucosa identifies chitinase loss as a corpus atrophy marker Short title: The transcriptome of corpus atrophy
Version: 1 Date: 4 August 2013
Reviewer: Fumitaka Oyama

Reviewer's report:
Major comments:
1. The authors should present gene lists of both up- and down-regulated genes with fold change as a new Table of “Altered expression of mRNA in stomach tissues by DNA microarray analysis.”

Authors’ comments:
We agree with the reviewer and we now have provided full information of gene expression fold changes and statistic in Supplementary data

2. Microarray results should be confirmed by an independent gene expression profiling method. Quantitative data are essential for this kind of work. The authors carried out qPCR on the decreased expressions of the genes. However, the increased expressions of the genes were only poorly characterized. They should carry out qPCR on the up-regulated genes with fold change and show as Table of “Altered expression of mRNA in stomach tissues confirmed by qPCR analysis.” Furthermore, the authors should show how they selected the primers and validation of their gene specific primers for qPCR.

Authors’ comments:
We agree that the genes with increased expression are poorly described. However, in this manuscript we have focused on the genes with loss of expression in atrophy. The increasing genes will be studied in forthcoming manuscripts.

3. The authors wrote “Hypoxanthine#phosphoribosyl#transferase (HPRT1) which we have previously observed to be a good reference gene for normal stomach tissue (unpublished results).” Please include this data in main text or supplementary data. Furthermore, it is not clear the expression levels of HPRT1 itself can be changed among the healthy and diseased tissues. The authors
should show that HPRT1 expression is not altered significantly among their samples. In addition, authors should carry out qPCR by delta Ct method using other housekeeping genes such as #-actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Authors’ comments:
We have now included a citation that is confirming the suitability of HPRT1 as reference gene between normal and diseased gastric mucosa (see page 8): “All assays were performed in technical duplicates using a 7500 Real Time PCR System (Applied Biosystems). Hypoxanthine-phophoribosyl-transferase (HPRT1), which has previously been observed to be a good reference gene for normal stomach tissue [17], was used as a reference gene for all assays to normalize expression by the ΔCt method.”

The cited paper also states that GAPDH and B-actin are less suitable for this purpose, and therefore we were not using them in the study. We have observed the same results in our own unpublished results. In the manuscript, we remove the statement of unpublished results, and have replaced it with the following reference:


4. It is very interesting that acidic chitinase (we usually call it “acidic mammalian chitinase, AMCase” was strongly down-regulated in corpus atrophy. To conclude that “loss of acidic chitinase expression is a promising marker for corpus atrophy,” authors should analyze the levels of protein expression as well enzymatic activity of AMCase in the stomach tissues in this manuscript. The authors should make every attempt to convince the readers of the reliability of their data. For the identification of human AMCase using Western blotting, it is essential to show the antibody recognizing “human AMCase” certainly. This is because many peptide antibodies cross-react with mid-molecular cytoplasmic proteins. In the legend of Figure S5 “CHIA protein has a theoretical molecular weight of between 40 and 52 kDa.” The deduced molecular mass of the full length human AMCase is 49,911 Da. As far as I know, nobody has detected “human AMCase protein” in human tissues including lung and stomach by Western blotting. Please refer to the following articles on this matter.


c. Seibold MA, Donnelly S, Solon M, Innes A, Woodruff PG, et al. (2008) Chitotriosidase is the primary active chitinase in the human lung and is


Authors’ comments:
The comments of the reviewer are very valid, and we have therefore updated our discussion, page 21, where we propose the following: The reason for a difference between our results and mRNA results of previous papers may be that the previous studies used antrum samples, which we show lack mRNA for AMCase in contrast to corpus samples. The reason for a lack of protein expression may be due to the use of different antibodies in the western blot assay. Our results are supported by a very strong correlation between mRNA and protein results in all patient tissues where both methods were used. This correlation is now included in the manuscript, as supplementary fig S5.

The new wording: “In contrast to our results, two recent studies described very low or absent levels of AMCase mRNA and/or protein in human stomach [41, 42]. However, the mRNA tested in at least one of those studies was from antrum tissue [42], and we indeed show that AMCase is expressed only in the corpus (Fig. 4). Furthermore, different antibodies were used for the protein assays in our study and the conflicting study, which may explain the different protein results obtained. The specificity of our western blot assay is supported by a very strong correlation between mRNA and protein levels ($r^2 = 0.92$; Fig. S5).”.

Minor comments:
1. “acidic chitinase” should be “acidic mammalian chitinase” in title, abstract, etc. This is changed throughout the manuscript.

2. The authors should reconstruct the Table I in a logical manner. What are X and 0, 1, 2 and 3? Table I is updated accordingly.

3. Key words should be in Page 2. The key words are located just after the Abstract, and does not fit on page 2.

4. Please include “List of Abbreviations.” We have chosen not to include a list of abbreviations (an option according to the Author instructions).

5. Table II: Illumina ID should be GenBank Accession No. We have chosen to show the Illumina IDs instead in this table. Entrez gene IDs (and Illumina IDs) are found in the Supplementary data table.
6. Table IV: there are two AMCase isoforms of variant 1 and variant 2. The authors should show their GenBank Accession No. 
Again the details can now be found in Supplementary data.

7. Reference #16 did not mention “AMCase is expressed in human chief cells.”
This has been changed (page 21). “AMCase is a chitin-degrading enzyme which is active under acidic conditions, and has been shown to be expressed in chief cells of mammals [39, 40]”

8. The authors should cite and discuss the other’s work on the expression of AMCase in Helicobacter pylori-infected stomach tissues.
This has been included in the discussion section (see Major point 4 above).

9. The authors should include legend for Fig. S4. 
This has been included.

10. Typographical errors:
Page 5, 1st paragraph: 58C should be 58°C.
Page 6, 1st paragraph: (Fig S1). Two periods. Please remove one.
Title of Fig 3 should be “Hierarchical” instead of Hierarchial.
All are fixed