Author’s response to reviews

Title: MicroRNA-125a is over-expressed in insulin target tissues in a spontaneous rat model of Type 2 Diabetes

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Author’s response to reviews: see over
Dear Dr Norton,

We were very pleased to learn that our manuscript (MS: 3928681742263711) was preliminarily accepted for publication in BMC Medical Genomics and have addressed the revisions required by reviewer 1 as well as the editorial points raised. We hope that you find these to your satisfaction. The responses to the reviewer’s comments which required changes to the manuscript are indicated in blue font after the comment.

Yours sincerely

Cecilia Lindgren
Editorial points:

1) Please format the manuscript according to the guidelines for a medical manuscript. These guidelines can be found at the following link:
http://www.biomedcentral.com/bmcmedgenomics/ifora/

The abstract should include the following sections: Background, Methods, Results and Conclusions.

A methods section has now been included in the abstract.

Manuscript sections should include (in the following order): Abstract; Background; Methods; Results; Discussion; Conclusions; Abbreviations (if any); Competing interests; Authors' contributions; Acknowledgements; References; Figure legends (if any); Tables (if any); Description of Additional files (if any).

This has now been corrected and the methods section has now been moved to its correct position. A separate “conclusion” section (page 13) has been added. A “figure and table legends” section has been added (page 27).

2) Ethical approval- Please state the institutional body which gave ethical approval for the animal experiments. Experimental research that is reported in the manuscript must have been performed with the approval of an appropriate ethics committee. A statement to this effect must appear in the Methods section of the manuscript, including the name of the body which gave approval, with a reference number where appropriate.

This sentence has now been added to the Methods section: “Animal procedures were approved by the ethical review panel of the University of Oxford and UK Home Office licences” in paragraph 1 page 4.

3) Data availability and deposition - Nucleic acid sequences, protein sequences, and atomic coordinates should be deposited in an appropriate database in time for the accession number to be included in the published article. In computational studies where the sequence information is unacceptable for inclusion in databases because of lack of experimental validation, the sequences must be published as an additional file with the article. Where appropriate, authors should adhere to the standards proposed by the Microarray Gene Expression Data Society (http://www.mged.org) and must deposit microarray data in one of the public repositories, such as ArrayExpress (http://www.ebi.ac.uk/arrayexpress), Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/projects/geo/) or the Center for Information Biology Gene Expression Database (CIBEX; http://cibex.nig.ac.jp).

This has been done and the reference number for the submission is GSE17060 This has now been added in the results section (paragraph 3, page 6) as: “The entire datasets (for miRNA and
MicroRNA-125a is overexpressed in insulin target tissues in a spontaneous rat model of Type 2 Diabetes. Blanca M Herrera, Helen E Lockstone, Jennifer M Taylor, Quin F Wills, Pamela Kaisaki, Amy Barrett, Carme Camps, Cristina Fernandez, Jiannis Ragoussis, Dominique Gauguier, Mark I McCarthy and Cecilia M Lindgren


We would also request that you go through the manuscript formatting checklist one more time and ensure that your revised manuscript conforms to all of the points. The link to the formatting checklist is provided at (http://www.biomedcentral.com/info/ifora/medicine_journals). It is important that your files are correctly formatted.

The formatting of the files has been double-checked and now meets the standards required for publication in the BMC medical Genomics journal.

Reviewer 1: David Arthur Carlyle Simpson

Discretionary Revisions

4. The authors have fully addressed this comment regarding enrichment of miR-125a target genes and performed the suggested analyses, which I am pleased to see have yielded some useful information. The relationship between change in miR125a expression and predicted target gene enrichment is weak (not detected for Targetscan or PicTar) and rather confusing (more overlap in adipose which has a lesser fold change; enrichment amongst upregulated genes). This is perhaps not surprising given the difficulty of target gene prediction and the many influences on gene expression in the diabetic condition. It would be helpful to mention these issues in the discussion.

Paragraphs 3 & 4 of the discussion have been amended to say:

“Contrary to expectation, it was surprising to find an even stronger enrichment among the up-regulated genes. One possibility is that these are not direct target genes themselves, but represent changes further downstream of the miRNA regulation. No enrichment was observed with the target gene lists predicted by the other two algorithms, which may be due to differences in the target-gene prediction methods implemented or to the effects of long-term exposure to hyperglycaemia further affecting the control of gene expression. These results show that the relationship between miRNAs and gene expression is not a simple one.”

Though the enrichment of miR-125a target genes predicted by miRanda is an interesting finding, it raises a number of questions, including why both down and up-regulated genes should show significant enrichment of miR-125a target genes, and why stronger enrichment was observed for the tissue with lower fold induction of miR-125a. The difficulty of predicting genuine target genes combined with other influences on gene expression could provide some explanation.”
Additional comments:

2. In the amended supplementary table 2 what does red and blue colouring denote? If genes altered within a particular pathway are changed in different directions, can the authors comment on how this should be interpreted.

*Apologies for not indicating this in the table, the table legend has now been corrected to say: “Functional profiling and gene-symbols of differentially expressed genes in GK compared to BN rats in adipose tissue (n=1075) and liver (n=233) using GENECODIS. Red represents up-regulation, blue-down-regulation of genes in the categories of interest mentioned in the manuscript text”. This has also been added to the list of table legends on page 27.*

**Reviewer 2: Fude Fang**

No Further changes were requested by reviewer 2.