Author's response to reviews

Title: Genetic determinants of glucose-6-phosphate dehydrogenase activity in Kenya

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Author's response to reviews: see over
Dear Prof. Epplen,

Thank you for taking the time to consider our article "Genetic determinants of glucose-6-phosphate dehydrogenase activity in Kenya." We also wish to thank the peer reviewers, Dr. Sunil Parikh and Dr. Lorenz von Seidlein, for their helpful comments, which we address point-by-point on the pages that follow. Additionally, we have added a Conclusions section to this revised version of the manuscript as requested. We hope that we have adequately addressed all concerns raised and look forward to your reply to our revised submission.

Sincerely,

Shivang S. Shah, M.D., D.Phil.
(on behalf of the authors)
Minor revisions

1) Please explain what modifications were used for the Randox G6PD assay, as this may impact on the assay performance.

The modifications used for the Randox G6PD assay were simply a scaling down of the assay using 10-fold reduced volumes to facilitate use of a spectrophotometric plate reader to obtain assay read-out. Quality control checks were performed with positive and negative control samples to ensure the precision and accuracy of observed readings. (manuscript updated accordingly in Methods section).

2) How was the phenotypic assay cutoff chosen?

The phenotypic assay cutoff was chosen by using the empiric distribution profile of G6PD activity in males. As noted in the Supplementary Information (Figure S5), the cutoff is the intermodal minimum of the kernel density estimation of the probability density function for this distribution.

3) Please comment on the impact of waiting up to 4 days at 4 degrees on the G6PD phenotype assay.

In our lab we have found reliably consistent assay results even 1-2 weeks after initial collection with samples were stored in EDTA at 4 degrees, but we do agree that the most ideal situation would be to perform assays on the freshest samples possible. (manuscript updated accordingly in Methods section)

4) Please describe the demographics of the children studied in more detail. Were all healthy or any parasitemic? Was this community-based sampling? Ethnicity information?

The blood was obtained from a prospective birth cohort from healthy, non-parasitemic children from Kilifi of predominantly Mijikenda ethnicity. (manuscript updated accordingly in Methods section)

5) Figure S2 - needs a description of which gender corresponds to solid/clear Bars

This has been corrected in the revised manuscript.

6) Figure 4 - would cluster male and female genotypes together, as it is confusing have male A clustered with the female genotypes.
This has been corrected in the revised manuscript.

Discretionary

1) Have the author’s sufficiently credited the work of others? The association of 202 and 376 has been known for decades, and those that initially described this should be noted more transparently.

We have included more references to the original genetic association between 202A and 376G in the final section of Results, specifically citing two works by Hirono & Beutler and Beutler et al., published in PNAS and Blood, respectively, in 1988 and 1989.

2) Were the authors able to look for signatures of natural selection, given their extensive sequencing efforts?

Our limited genomic window did not permit these formal analyses, but we did find near complete homogeneity of the mutant 202 haplotype, in keeping with the hypothesized recent origin of this allele.
Reviewer's report:

1. Is the question posed by the authors well defined? Yes “... how genetic variation at G6PD impacts clinical outcomes...?”

   No actionable items.

2. Are the methods appropriate and well described? From my admittedly limited background in molecular genetics the methods appear appropriate.

   No actionable items.

3. Are the data sound? The data collection is appropriate – the data appear sound and should be published.

   No actionable items.

4. Does the manuscript adhere to the relevant standards for reporting and data deposition? Yes. The authors repeatedly refer to manuscripts in preparation. This is irritating for the reader. Please do your best to insert the relevant references?

   As this manuscript is still in preparation, regrettably we are not able to provide a specific reference.

5. Are the discussion and conclusions well balanced and adequately supported by the data? Yes – as far as I can see the discussion is reasonable and well written.

   No actionable items.

6. Are limitations of the work clearly stated? The authors seem less inclined to linger on the limitations of their work as one could hope. Specifically it is regrettable the authors apparently can’t use international units for the measurement of enzyme activity.

   Have added clarification for lack of use of international units in Methods section.

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished? The paper appears well referenced.

   No actionable items.
8. Do the title and abstract accurately convey what has been found? The abstract and title reflect the content of the paper.

No actionable items.

9. Is the writing acceptable? The paper is well written.

No actionable items.