Reviewer's report

Title: The three CYBA variants (rs4673, rs1049254 and rs1049255) are benign: new evidence from a patient with CGD

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Reviewer: Amit Rawat

Reviewer's report:

I applaud the authors for the extensive immunological and genetic analysis performed in the patient and his relatives. I have the following comments about the manuscript.

1. The title of the manuscript is rather confusing. “The three CYBA variants are not pathogenic: new evidence from a patient with CGD”. Which CYBA variants? Kindly modify the title to make it more clear and precise.

2. The CYBA variants alluded to in the manuscript are SNPs and NOT mutations and are not associated with Chronic granulomatous disease. Two of these variants i.e. c.214T>C (rs4673) and c.521T>C (rs1049254) result in synonymous codon changes whereas the c.*24G>A (rs1049255) in the 3' UTR is associated with decreased gene expression. And it is only the haplotype with the 3 variants that is associated with decreased oxidase activity and NOT individual variants.

3. The algorithm for lab diagnosis of a patient with suspected CGD is to perform a NBT and DHR. If these are abnormal further testing invovles evaluating the individual NADPH oxidase component proteins by flow cytometry or western blotting.

4. The DHR in the patient's mother and sister was clearly suggestive of a carrier state for X-linked CGD and the b558 by flow cytometry showed a bimodal pattern also suggesting a carrier state for X-linked CGD. b558 expression was also absent in the patient. In this situation the putative gene to be sequenced is the CYBB gene which is responsible for X-linked CGD. What was the rationale for sequencing the CYBA gene associated with autosomal recessive CGD when all the prior investigations clearly suggested an X-linked CGD?
5. b558 estimation involves estimation of both gp91phox and p22phox. In this complex situation it would have been better to test individually for p22phox and gp91phox by either flow cytometry or western blotting. This would also confirm whether the haplotype with the 3 variants in the CYBA gene resulted in a decreased expression of the encoded protein i.e p22phox.

6. The father with all the 3 variants in the homozygous state was stated to have a normal DHR. What was the SI compared to a healthy control who did not have the haplotype in question?

7. Since the variant in the CYBB is a novel intronic change, it would be customary to screen at least 50 healthy controls (100 alleles) to conclusively prove that this is indeed a mutation and a not a SNP.

8. Would the authors also like to comment how did they infer the pathogenic nature of this mutation? Were any functional studies performed to confirm this? Were some in-silico methods for prediction of the splice site variants used?

9. There are errors of grammar and syntax which need to be addressed.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No
Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
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Not relevant to this manuscript

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