Author’s response to reviews

Title: Targeted next-generation sequencing identifies novel variants in candidate genes for Parkinson’s disease in Black South African and Nigerian patients

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Response to reviewers’ comments

27 November 2019

To: The Editor: Dr. Matteo Pasini

BMC Medical Genetics

Dear Dr. Pasini

Submission of revised manuscript ‘Targeted next-generation sequencing identifies novel variants in candidate genes for Parkinson’s disease in Black South African and Nigerian patients’ (Ms. Ref.: MGTC-D-19-00107R1)

We thank the reviewers for their constructive comments on our manuscript and the opportunity to submit a revised version. Our responses to the questions and comments are provided below.

Reviewer reports:

Pyotr A. Slominsky (Reviewer 2):

1. Line 109-110. It is very interesting that in your sample there are two patients with a purely Mendelian inheritance of the disease. But for these patients, another analysis option must be applied - a direct analysis of cosegregation of the mutation and disease. You must at least exclude this patients from Table 1 and discuss this patients separately.

Response: In order to clearly distinguish these two patients (s43_059 and s94_069) from the rest we have now mentioned them specifically in Table 1. In addition, in Table 3 we have labelled them with an asterisk and have moved them to the top of the Table. We did not omit them from the Tables as this may cause some confusion but we do discuss them separately in the Results (page 11, lines 219 to 231) and Discussion (page 13, lines 265 to 277) sections. We hope the reviewer finds this acceptable.
2. Table 2. You use Meta Score to evaluate mutations in two ways - LR and SVM. First, you must indicate that in both cases, a pred score is used. The critical levels of these score must be mentioned (https://softgenetics.com/PDF/GeneticistAssistant-Variant-Reference-Fields.pdf). But not all variants described in Table 1 correspond to these score - thus, all variants in the PINK1 and PRKN genes do not pass the Meta score value. As a result, the number of potentially significant mutations will change dramatically.

Also in some cases a mutation frequency in patients is very close to GnomAD MAF. It is necessary to discuss.

Response: Yes, the reviewer is quite correct. We used a cut-off score of &gt;0.8 and inserted the following sentence into the Methods section on page 9, lines 165 to 166. “We used a score of &gt;0.8 as a cut-off for including the variant into our list of rare “pathogenic variants” as recommended by Liu et al. 2016”.

Therefore, the variants in PINK1 and PRKN did not pass these criteria and we are left with 54 variants. All references and work related to the PINK1 and PRKN variants have now been omitted throughout the manuscript, figures and tables.

With regard to the Gnomad MAF, the following sentence has been added to the Results section on page 12, lines 234 -236. “In some cases, the MAF of the variant in African controls in GnomAD was ≥0.01, similar to the frequency observed in the patients (Table 2), and those variants were therefore excluded.”

3. Table 3. The problem of multiple mutations in one patient remains not sufficiently discussed. It is necessary to prioritize mutations taking into account both the Meta score, allele frequency and the biological function of the corresponding proteins. Nevertheless, choose a priority missense variant for each patient, taking into account all factors.
Response: The following sentences have been added to the Results section on page 12, lines 232 to 238 “We attempted to prioritise one possible pathogenic variant per patient based on MAF (\(<0.01\)), pathogenicity prediction scores (\(>0.8\)) and evidence of prior association of the gene/protein with PD or Parkinsonism (Table 2; Additional file 10: Table S6). In some cases, the MAF of the variant in African controls in GnomAD was \(\geq0.01\), similar to the frequency observed in the patients (Table 2), and those variants were therefore excluded. The prioritised variants are shown in bold and in green font in Table 3. In a few individuals, one variant could not be prioritised over others as more than one variant fulfilled these criteria.”

We have inserted an additional Supplementary file 10: Table S6 which provides evidence linking the gene/protein, in which a variant was found in the patients, to PD or Parkinsonism.

4. Given all the factors, modeling makes sense only for one protein, ATP13A2. In this regard, this piece of work needs to be reduced.

Response: The modelling (as well as all other associated data) for the PINK1 and PRKN variants have now been removed from the manuscript.
Mathias Toft (Reviewer 3):

The revised manuscript has improved substantially from the previous submission and now reads well.

I have only one minor comment: In my previous review of the paper I mentioned a lack of clarity about the aims of the study. The manuscript now includes a statement about this at the end of the introduction: «The primary goals of the present study was to use this panel to determine whether a common pathogenic mutation was present, and to characterise the genetic variation in known and novel PD genes, in a group of Black South African and Nigerian PD patients." In my opinion also the abstract would prefer from a similar statement and a statement of the results of this. It could also be considered to remove some of the rather technical statements about variant filtering etc from the abstract to make it more readable to a broader audience.

Other than this the paper is fine and I support the publication of this rare report of a genetic study from Africa.

Response: We thank the reviewer for the positive comments. The aim of the study and more details on the findings have been added to the Abstract. In addition, the technical details on filtering have now been omitted from the Abstract.