Author’s response to reviews

Title: Using KASP Technique to screen LRRK2 G2019S mutation in a large Tunisian cohort

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Author’s response to reviews:

Dear Dr Pasini,

Herewith enclosed, please find a novel version of the paper entitled "Using KASP Technique to screen LRRK2 G2019S mutation in a large Tunisian cohort" (MGTC-D-16-00265). We have carefully reviewed the comments and have revised the manuscript accordingly. Our responses are given in a point-by-point manner below. Changes to the manuscript are highlighted in yellow and lines’ numbers correspond to the revised version of our manuscript.

We hope the revised version is now suitable for publication and look forward to hearing from you in due course.

Sincerely,

Prof. Riadh Gouider
Response to Dr. Jia Nee Foo (Reviewer 1):

Thank you for your review of our paper. We have answered each of your points below.

1. Most of the manuscript states that 250 PD patients were analyzed in this study, but in Methods under KASP genotyping assay, it states that 254 patients were analyzed for a total of 472 samples. Is this a mistake, or are the 4 additional samples duplicates or genotyping controls? This should be made clear.

We apologize for this mistake. The total number of PD patients is 250. The genotyping controls have been specified in ‘KASP genotyping assay’ paragraph in Methods (lines 112-116). All samples were duplicated.

2. Please also make clear that there is no overlap between these samples and those described in previous studies (in Table 4). Were the control subjects recruited from the same hospital?

The discussion has been modified to clarify that there was not any overlap between these samples and those described in previous studies in table 4 (table 3 in the new version of the manuscript - line 186-189). The control subjects were recruited from different primary care clinics and not from the same hospital. Controls were selected following these criteria (good mental and physical health; individuals with personal or familial psychiatric disorders or cognitive impairment history, were excluded).

3. The authors mentioned that the genotypes of 60 samples were validated by Taqman and Sanger sequencing but did not mention the results. Were the genotypes 100% concordant, and if not, why?

The genotypes were 100% concordant. This detail has been added in the manuscript (lines 157-159).

4. Are the genotypes in Hardy Weinberg equilibrium? Please state the HWE P-values in cases.

The genotypes are in Hardy-Weinberg equilibrium. In cases the p(WT) = 0.816 and q(G2019S) = 0.184, Chi-square=0.038 and P<0.05. This information has been added in the text (lines 143-145 and 152-153).

5. The authors should discuss further the advantages of using the KASP method for genetic testing in clinic, in terms of cost, speed, equipment required and ease of genotyping one or few samples each time.

We have included the advantages of using KASP method in the discussion from line 211 to line 235.

Response to Dr. Nicolas Dupré (Reviewer 2):
Thank you for your review of our paper. We have answered each of your points below.

1) The authors should describe in more detail the advantages and disadvantages of the KASP technique

As requested by the first reviewer too, we have included the advantages of using KASP method in the discussion from line 211 to line 235. The only reported disadvantage of KASP is the fact that it is a uniplex technique and could not be used for multiple screening.

2) The authors should give more examples of the recent use of KASP in other neurological disorders

The recent use of KASP in various neurological disorders has been discussed in the last part of the manuscript (lines 236-248).

3) The results of the genotyping of 60 DNA samples by TaqMan and Sanger should be given

The genotypes were 100% concordant. This detail has been added in the manuscript (lines 157-159).

4) The authors should comment as to whether KASP could be useful in more diverse populations, to screen more variants. What does the literature indicate? This would provide a larger applicability to their study

According to the literature, the KASP assay could be conducted on large number of samples to screen several SNP within a short period of time. This information has been added into the discussion part (lines 216-219).

5) Please provide technical readouts of the KASP technique in a figure

Technical readouts of the KASP technique were detailed in several publications before (Please see: the LGC group website https://www.lgcgroup.com/kasp, the KASP genotyping chemistry user guide and manual, Yuan et al 2014 (doi: 10.5147/pggb.2014.0144), He et al 2014 (doi: 10.1007/978-1-4939-0446-4_7)). Therefore, we think that it will be redundant to add these details as a figure in our article.

6) Table 3 could be deleted, since it provides little usefull information

Table 3 has been deleted from the manuscript.

7) In the discussion, testing strategy for LRRK2 mutation in their population should be detailed (younger? family history? all cases? symptomatic vs asymptomatic?)

These details have been mentioned in the discussion (lines 197-205).
Response to Dr. Nicolas L. Dzamko (Reviewer 3):

Thank you for your review of our paper. We have answered each of your points below.

1) The conclusion states that compared to other techniques the KASP assay was reliable, time and cost efficient. However no data to support this conclusion is given in the paper. What other techniques? What was the time and cost saving?

The advantages of KASP assay comparing to Taqman and Sanger sequencing have been included in the discussion from line 211 to line 235.

2) It is mentioned in the methods that KASP results were verified by Taqman assay and Sanger sequencing. No data is provided. What Taqman assay was used? How was Sanger sequencing performed? Were all subjects the same genotype or were any differences encountered? Were the controls identified that had the G2019S mutation also genotypes as such by the "other methods"?

The genotypes were 100% concordant. This detail has been added in the manuscript (lines 157-159). In the methods section, we have detailed the Taqman assay (lines 124-132) and Sanger sequencing (line 133-141). Only 60 patients from 250 were sequenced using Taqman and Sanger.

3) Please give details of KASP primers and catalogue numbers of Taqman assays

These details have been given in methods section (lines 106-111 and 124-132).

4) In the discussion, it is important to note that the controls with G2019S mutation are currently under the general age-at onset for PD and thus rather than healthy controls they may actually be at risk carriers that could develop PD in ~10 years or could likely even be in the prodromal phase of PD.

We would like to thank the reviewer for his pertinent comment. We have added this point in the discussion (lines 180-183).

5) Please look at the following paper which I think also uses KASP PCR to screen for LRRK2 mutations and if this paper is not the first as claimed then please amend. Journal of Human Genetics (2015) 60, 357-362

The paper of Pihlstrøm et al 2015 (Journal of Human Genetics (2015) 60, 357-362) used KASP PCR to screen only for the common LRRK2 variant rs1491942 (1000G MAF = 0.3). We mentioned that our work was the first one to use KASP for the LRRK2 G2019S (rs34637584) screening. To avoid ambiguity, we have modified the abstract and the background part. We have discussed this point from line 236 to line 240.