**Author’s response to reviews**

**Title:** A systematic review and integrative approach to decode the common molecular link between levodopa response and Parkinson's disease

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Response to Reviewers comments:

Manuscript # MGNM-D-17-00060 entitled "An integrative approach to decode the common molecular link between levodopa response and Parkinson's disease"

We are thankful to the reviewers for their valuable time and efforts in thorough review of the manuscript. We have tried our best to address all the concerns/suggestions raised by the reviewers and believe that the revised version of the manuscript shall meet the journal’s publication requirements.

Mara Hutz (Reviewer 1):

This manuscript by Guin et al presents the results of a study that aims to disclose an integrative approach between levodopa response and Parkinson's disease susceptibility. The structure of the manuscript is organized, clear, and easy to follow. All the important aspects and literature are included and the methods and results are nicely presented.

However, I have also some comments and suggestions that, if considered, in my opinion they would help to improve the quality of the manuscript:
1. All Tables should be careful reviewed. I checked the original papers from some studies that have their data on the tables presented but, they do not match.

We are thankful to the reviewer for highlighting the presence of errors in the tables. As suggested, the tables are thoroughly revised and the respective corrections have been incorporated (highlighted in red) in table 1 and 2.

2. The criteria to choose results from the original works are not clear when univariate and multivariate analyses are presented.

We appropriately understand the reviewer’s concern and would like to thank for the same. Here we would like to clarify that all the baseline univariate significant allelic/genotypic associations with ADR/s and with L-dopa efficacy are reported in the table 1 and table 2, respectively. As per reviewer’s suggestion, this clarification is incorporated in the manuscript as well.

Page No: 16; Line No: 368-370

“All the baseline univariate significant allelic/genotypic associations with ADR/s and with L-dopa efficacy are reported in the table 1 and table 2, respectively.”

3. The limitations of the study should be discussed in the Discussion section.

We completely understand the importance of limitations in any scientific study and thank the reviewer for highlighting the same. We would like to mention that some limitations of the study were already discussed in the manuscript, which has been further modified and incorporated in the manuscript as below:

Page No: 14; Line No: 315-331

“Although significant findings have been observed in our study, several limitations exist. The papers included in the systematic review presented high heterogeneity in terms of diagnosis, response criteria, drugs administered with different doses and genotyping techniques. As suggested by Schumacher-Schuh et al. (2014)[67] the phenotypic heterogeneity in terms of adverse effects lacks clinical instrument to adequately measure the ADR, whereas in terms of efficacy, several response rating scales have been incorporated. In GWAS studies, the assayed SNPs are usually to mark a genome region that influences the studied phenotype, however, we have annotated these SNPs to their respective genes to identify the proteins that play a role in the biological processes which ultimately influences the phenotype. Motor fluctuation, a common ADR of levodopa, lacks clear clinical classification and hence assessment. A regular record of patient motor state could be preferred. Genetic heterogeneity is another source of variability between studies because different markers in the same genes were employed for these
associations; moreover, patients with different genetic backgrounds may not be strictly comparable. One major limitation of network biology is the quality and the coverage of the interactions. The rate of discovery of false positives and false negatives are high which shows the need to rank the reported interactions for further validation.

Marco Cosentino (Reviewer 2): This is a systematic review of genetic studies about L-DOPA response and adverse reactions in Parkinson's disease. A huge amount of information is critically analyzed and summarized providing a useful framework for hypothesis development and testing with potential clinical implications. Concerns are as follow:

4. Title and abstract - Please make clear that biomarkers are genetic in nature.
We thank and support the reviewer’s assertion on biomarkers being genetic in our study. The same modifications has also been made in the manuscript as below:
Page No: 1; Line No: 20-21
“Here, we aim to identify predictive genetic biomarkers for levodopa response (LR) and determine common molecular link with disease susceptibility”

5. In particular, regarding the title, please also include "systematic review,” as this is an important information for potentially interested readers.
We are thankful to the reviewer for highlighting the significance of systematic review in the title. We have modified the title as per the suggestion.
Page No: 1; Line No. 1
“A systematic review and integrative approach to decode the common molecular link between levodopa response and Parkinson’s disease”

6. In the abstract, the meaning of PPI is not explained.
We acknowledge and thank you for your comment. PPI is protein-protein interaction. The abbreviation has been included in abstract.
Page No: 2; Line No: 24-26
“Protein-protein interaction (PPI) analysis using STRING and functional enrichment using WebGestalt was performed to explore the common link between LR and PD.”

7. Figure 1 - It is unclear the meaning of "articles overlap with ADR", since the 11 articles are from a set of papers already defined as "levodopa response related". Moreover, were such papers related to ADR, why they were not moved in the proper group?

We appreciate and thank the reviewer for pointing out this clarifying comment. From the ‘Levodopa response related’ articles (n=30), only 8 L-dopa response studies have been included (tabulated in Table 2). Figure 1 has been modified accordingly, where the criteria for exclusion of the remaining studies have been detailed.

8. Why were full texts unavailable? Is it possible that so high number of unavailable full texts (22 out of 74) might have biased the results of the analysis?

We completely agree with the reviewer on the possibility of biasness due to unavailability of full text articles. The unavailable articles were searched again, a few full texts were extracted and the figure (figure 1) has been modified accordingly. Unavailability of articles is one of the limitation of the study.

9. Please check labels for grammar.

We agree that English language and grammar is an inevitable part of the manuscript and thank the reviewer for mentioning the same. As simple and easy to understand language plays very important role in scientific literature, we have thoroughly checked and revised the grammatical errors including tenses at appropriate places.

10. PPI analysis - I wonder whether in all the selected studies and in particular in GWAS studies it is actually possible to identify individual proteins. Indeed, assayed SNPs should be usually just considered to mark a genome region that may influence the selected outcome. Such limitation should be taken into account and discussed.

We acknowledge the reviewer for this comment. We agree that in GWAS studies, the assayed SNPs are usually to mark a genome region that influences the studied phenotype. Here, the SNPs assayed in the GWAS studies have been annotated to their respective genes to identify the proteins that play a role in the biological processes which ultimately influences the phenotype/pathophysiology of the disease. Since the aim of our study was to identify predictive
genetic biomarkers for levodopa response and determine common molecular link with disease susceptibility, therefore identifying proteins was important. The approach has been used to identify proteins from the other selected studies as well. This limitation, as suggested by the reviewer, has been incorporated in the manuscript under the discussion section.

Page No: 14; Line No: 320-324

“In GWAS studies, the assayed SNPs are usually to mark a genome region that influences the studied phenotype. However, we have picked up the annotated genes corresponding to the significantly associated SNPs from the respective studies, to identify the proteins that play a role in the biological processes which ultimately influences the phenotype.”

11. Tables 1 and 2 - ORs should be included wherever possible.

We agree with the reviewer and completely understand the importance of OR in genetic association studies. Hence, all the reported OR (Odds ratio) with 95% CI (Confidence interval) mentioned in the original articles have been incorporated in table 1 and table 2. Wherever these inferential statistics are not reported, they were calculated using reported genotypic/allelic frequencies. This additional column in table 1 and 2 has been highlighted in red.

Jolanta Dorszewska (Reviewer 3): Manuscript is the search for more effective PD therapy based on genetic background. Methodology of research is very detailed described.

My remarks:

12. among non-motor functions lack of genetic determinants of depression, such as the genetic variations of SPR gene, Oczkowska et al. 2015.

We thank the reviewer to rightly point out that non-motor symptoms of Parkinson’s disease (PD) is an important area. Non-motor symptoms affects the quality of life of PD patients greater than motor dysfunction [1]. Several non-genetic factors like disease severity and duration, gender, age at onset, daily levodopa dose increases the burden of non-motor symptoms [2, 3, 4, 5]. However, studies on genetic association with non-motor symptoms in PD are scarce. Oeda T et al. (2015) examined the GBA [glucocerebrosidase gene] mutations in PD patients and time to develop non-motor symptoms like dementia, psychosis and motor symptoms like dyskinesia and wearing off. They concluded PD patients with mutation developed dementia and psychosis earlier than those without mutations (p<0.001 and p=0.017) whereas no significant association was found for motor symptoms [6]. Non-motor complications have also been reported to be induced by L-dopa administration for a longer duration. Aquino C.C et al (2014) reports the fluctuating levels of
dopamine, via D1 and D2 receptors on the dopaminergic pathway, likely causes the non-motor symptoms [7]. As suggested by the reviewer, Oczkowska et al. (2015) detected PRKN variants, D394N positively correlated with the response to L-dopa therapy in the early period of the disease while the S167N polymorphism probably may be related with a better response to L-dopa therapy in late period of PD [8]. However, the genetic basis of non-motor complications associated with PD or L-dopa induced, still needs to be explored with further confirmation in studies on larger groups.

13. The large amount of data in the manuscript makes it unreadable. Maybe the summary should be presented graphically or additionally given genes important for PD therapy.

We are grateful to the reviewer and acknowledge that the large amount of data is difficult to read. Therefore, table 1 has been modified, which consists only the significant genes and their polymorphisms associated with L-Dopa induced ADR(s). Similarly, table 2 has also been modified, consisting of only the significantly associated genes and their polymorphisms with L-Dopa efficacy. The complete table of all the 38 articles on ADR have been represented in supplement table 8(a) and the complete table of all the 8 articles on L-Dopa efficacy have been provided in supplement 8(b). These supplementary files have been included in the manuscript as below.

Page No. 5, Line No. 113-115

“The methodological and demographic characteristics of the ADR studies and the drug efficacy related studies of levodopa are summarized in Table 1 and Table 2 respectively, with significant genetic association (p≤0.05) [complete table in Supplement 8(a) and 8(b)].”

Page No. 6, Line No. 127-129

“The different motor functioning assessment scales used are provided in Table 1 (supplement 8a).”

Page No. 7, Line No. 166-167

“The cumulative quality assessment score obtained by individual ADR studies are represented in Table 1 (supplement 8a) and that of drug efficacy in Table 2 (supplement 8b).”

Page No. 8, Line No. 175-178

“From total publications, 40 variants within 18 genes (HOMER1, ADORA2A, ANKK1, MTHFR, DRD2, SLC6A3, COMT, UGT1A, ACE2, BDNF, ABCC8, RYR1, DRD3, GRIN2A, SLC6A4, HTR2A, CYP2D6, CCK ) were found to have significant association (p≤0.05) with any type of levodopa induced ADR in PD (Table 1, supplement 8a).”
“rs4680 (COMT)[34], rs6280 (DRD3)[37], rs921451, rs3837091 (DDC)[38], rs28363170, rs3836790 (SLC6A3)[40] were the significant variants with reduced LR (Table 2, supplement 8b)”.

14. It would also be useful to list prognostic and predictive genes for PD or therapy.

We are thankful to the reviewer for this important comment. A summary of the significant genes and their polymorphisms associated with L-Dopa ADR(s) and with L-Dopa efficacy have been represented table 1 and table 2, respectively. The significantly associated predictive genes and their respective polymorphisms in PD are reported (in bold) in supplementary table 1.

Page No. 5, Line No. 113-115

“The methodological and demographic characteristics of the ADR studies and the drug efficacy related studies of levodopa are summarized in Table 1 and Table 2 respectively, with significant genetic association (p≤0.05) [complete table in Supplement 8(a) and 8(b)].”

15. Correct language and editorial errors e.g. page 13.

We thank the reviewer for their comment on language correction. We understand that simple and clear English language is significant for a good scientific article. As suggested, the manuscript has been thoroughly checked and revised. Editorial errors like ‘cause’, ‘proteins’, ‘established’, ‘tabulate’ and spacing in page 13 were also corrected.


