Author’s response to reviews

Title: Risk variants of the α-synuclein locus and REM sleep behavior disorder in Parkinson's disease: a genetic association study

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Author’s response to reviews:

To

Chin-Hsien Lin
Editor of BMC Neurology

Date: 20.12.2017

Subject: Manuscript # NURL-D-17-00535 “Risk variants of the α-synuclein locus and REM sleep behavior disorder in Parkinson's disease: a genetic association study”

Dear Editor,

Please thank the reviewers for their helpful comments on our paper. Their comments have been carefully addressed as described below.

I hereby confirm that both authors have read the manuscript, the paper has not been previously published, and is not under simultaneous consideration by another journal. In addition, all persons involved in the writing of the manuscript have been included on the author list.

I hope that the revised manuscript can be accepted for publication in BMC Neurology.

Sincerely,

Kari Anne Bjørnarå, MD, PhD
Department of Neurology
Reviewer # 1:

1. RBD questionnaire usually is not able to predict RBD precisely. Therefore, the authors should discuss this point as the limitation of this paper.

We agree that this is a limitation, and have changed the term RBD to possible RBD (pRBD), in accordance to what is commonly used in studies based on RBD questionnaires, including recent papers based on PPMI data. Since RBD is so common in PD, a questionnaire with 96% specificity gives a reasonable likely correct diagnosis. Additionally, according to the DSM-5 criteria for RBD, a suggestive history is enough to diagnose RBD in patients with an established synucleinopathy. We have added a paragraph in the manuscript where we discuss this point.

2. In abstract, the authors described the "Indirectly, it also supports the hypothesis of RBD as relevant marker for a distinct subtype of the disorder." However, it remains unclear whether one point questionnaire can reveal the RBD in PD patients. Iranzo and Tolosa reported "there is a group of PD patients and bed partners who report that the frequency and intensity of their RBD symptoms (nightmares and vigorous behaviors) decrease with time and can even disappear" (Ref; Rapid eye movement sleep behavior disorder in Parkinson’s disease. In Non-Motor Symptoms of Parkinson's disease. 2nd edition. Oxford, 2014). Therefore, in this study, the authors might not be able to divide from the PD with REM to PD without REM. The authors should discuss this point.

This comment rises is an important question on whether RBD is a persistent feature in PD, and if one single assessment is enough. RBD symptoms may vary over time and may be influenced by medication. In contrast to the citation above from Iranzo and Tolosa, some studies find that RBD is associated with longer disease duration (Sixel-Doring, Trautmann et al. 2011, Bugalho and Viana-Baptista 2013). We have previously shown that RBD frequency is stable over time, with the majority of patients (77%) reporting the same symptoms after 3 years (Bjornara, Dietrichs et al. 2015). Regardless of this, we acknowledge the possibility of misdiagnosing possible RBD, and have discussed this issue in the revised manuscript.

3. In this study, the participants were not confirmed the pathological diagnosis. Therefore, non-RBD group might include non-synucleopathy parkinsonism, such as progressive supranuclear palsy. The authors should interpret the data cautiously.

This is of course a very important issue. We have applied the UK Brain Bank criteria to all patients to minimize the possibility of misdiagnosis, but the risk is still there, especially
regarding the patients with short disease duration. Mean disease duration is however nine years. As with all clinical studies, the results need to be confirmed in future publications.

4. In table 2, the authors should state the 95% confidence intervals.

Thank you for pointing out this lack of important information. We have added the standard errors in the table.

Reviewer # 2:

Major

No principal component analysis was performed in neither the Scandinavian nor PPMI samples to exclude samples from other ethnicities. The self-report ethnicity is not enough for genetic studies.

The Norwegian samples were all of Norwegian descent, a population known to be genetically homogenous. RBD or other sleep symptoms were not the primary reason for patients being referred to neither of the recruiting clinics, decreasing the likelihood of any systematic population stratification across the studied groups - unlike the common situation where different approaches are taken to recruit patients and controls. The reported genetic variants in Norwegian samples were, as described in the manuscript, genotyped by single SNP assays. More comprehensive genetic data would be necessary to be able to perform principal component analysis in this dataset.

We are, however, very grateful for the reviewer's suggestion to include principal components in the PPMI data, where full genotype array data was indeed available. We have now performed principal component analysis for the PPMI replication, and the description of this and updated results are included in the revised manuscript.

There is no inclusion of power calculation.

We appreciate this comment and have included our power estimate in the manuscript. Notably, statistical power of case-control studies improves considerably when the studied outcome has a high prevalence, as is the situation when a common symptom is investigated within a patient group, as in our study. This is also an interesting point for future genetic studies of clinical outcomes, which we were happy to include in the article.

There is no inclusion of Multiple test correction even though multiple SNPs were evaluated.

The study is clearly exploratory, being the first genetic study of RBD in PD. The reason for choosing only SNPs previously reported as associated with PD risk was to avoid multiple testing. Multiple test correction for SNCA is not straightforward, as there is some degree of linkage disequilibrium between the studied variants. In this scenario performing Bonferroni correction would be too conservative. However, acknowledging the borderline significance of the results
from the Norwegian sample we performed replication in PPMI, and the results from the meta-analysis is significant also when performing multiple test correction. This has been included in the revised manuscript.

There is no mention of how heterogeneity in both samples for the meta-analysis was evaluated

We thank the reviewer for this comment. We have now reported the heterogeneity index I^2, which indicated no heterogeneity of effects across the two studies.

Minor

Authors should consider the addition of a short paragraph acknowledging the limitations of the study.

We agree that more information on limitations is necessary. We have added a paragraph at the end of “Discussion”

There is no inclusion of the 95% confidence intervals for the OR.

Thank you for pointing out this lack of important information. We have added standard errors in the table.

There is no mention of the statistically significant differences in the duration of the disease and use of SSRIs/SNRIs between the RBD and a non-RBD group which could be confounded factors of the analysis.

Thank you for pointing out these possible confounding factors.

Several studies show that RBD frequency increases with disease duration, while at the same time, idiopathic RBD can precede PD diagnose by several years. The impact of disease duration on RBD symptoms is thus unclear. We have however discussed this in the paper.

As for the use of SSRI/SNRI, this is an obvious possible confounder, and this is now mentioned in the manuscript.

Using a questionnaire in PD patients with an average disease duration of ~11 years, it is worth discussing the prevalence of cognitive impairment in the PD cohort and their ability to answer the questions of the RBDSQ.

We thank the reviewer for this important comment. Patients with clinical symptoms of dementia were excluded. A large proportion of the patients, especially those with long disease duration and advanced disease, were followed by the spouse or another close relative. Spouses were encouraged to participate when patients answered the RBDSQ.

A more detailed description of the imputation protocol from the PPMI samples are needed.
We have expanded this section, including details of pre- and post imputation quality checks.

References:


BACKGROUND AND PURPOSE: Rapid eye movement (REM) sleep behaviour disorder (RBD) is frequently present in patients with Parkinson's disease (PD) and may have prognostic implications. There are few longitudinal studies of RBD in patients with PD. Our aim was to investigate whether RBD was a persistent feature in a follow-up study of 107 patients with PD. METHODS: After a mean follow-up time of 3 years, 96 patients were available for reassessment. Probable RBD (pRBD) was diagnosed by the REM sleep behaviour disorder screening questionnaire. RESULTS: At follow-up, pRBD was found in 49% of the patients, versus 38% at baseline. The pRBD status remained unchanged in three-quarters of the patients, whilst 17% had new pRBD symptoms. Disease duration was longer in the pRBD group, 9.4 vs. 7.6 years (P = 0.02). CONCLUSIONS: Probable RBD is a persistent feature in PD and probably increases over time.


OBJECTIVES: Longitudinal assessment of a Parkinson's disease (PD) cohort, to investigate the evolution or REM sleep behavior symptoms (RBD) over time and to test the relation between RBD at onset and motor dysfunction progression. METHODS: An early stage PD cohort (n = 61) was assessed at two time points, separated by a two years interval. Diagnostic criteria for RBD were: violent behavior during sleep and body movements or vocalization indicative of dream enacting and at least six affirmative answers in the REM sleep behavior disorder screening questionnaire. Motor function assessment was performed with the Unified Parkinson's Disease Scale part II and III (total and partial scores for tremor, bradykinesia, rigidity, gait/postural instability and dysarthria). RESULTS: 25 Patients had RBD at baseline, vs. 35 at follow-up. Three RBD changed to non-RBD at follow-up, while 10 non-RBD patients developed RBD at follow-up (annual incidence of 12.5%). RBD and non-RBD patients did not differ significantly at baseline or follow-up. The presence of RBD at baseline was significantly related to an increase in UPDRS total and bradykinesia scores over time. DISCUSSION: RBD symptoms can vary over time and have a tendency to increase during the early stages of disease. The presence of RBD symptoms could be a risk factor for motor function deterioration and particularly for bradykinesia worsening.


OBJECTIVE: To investigate the frequency, phenomenology, and associated risk factors of REM sleep behavior disorder (RBD) in Parkinson disease (PD). METHODS: An unselected cohort of sleep-disturbed patients with PD (n=457) was investigated with video-supported
polysomnography. We determined the frequency of RBD and analyzed the influence of age, clinical disease features, disease duration, cognitive and physical impairment, medication, comorbidity, and sleep architecture. RESULTS: The overall frequency of RBD was 46%. According to our cohort and modified definition, there was no preferred PD subtype for RBD (p=0.142). There was no gender preference (p=0.770). RBD was associated with older age (p=0.000). Adjusted for age and gender, patients with PD and RBD had longer disease duration (p=0.024), higher Hoehn & Yahr stages (p=0.002), more falls (p=0.018), more fluctuations (p=0.005), more psychiatric comorbidity (p=0.026), and a higher dose of levodopa (p=0.002). The presence of RBD was related to slightly increased sleep efficiency (p=0.007), a higher amount of REM sleep (p=0.000), and more periodic leg movements during sleep (p=0.019). CONCLUSION: RBD is a frequent and clinically relevant nocturnal disturbance for all stages of PD. It increases with age and disease duration and may contribute to the nocturnal problems of patients with PD and their bed partners.