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BRIEF COMMUNICATION

GBA mutations are associated with Rapid Eye Movement Sleep Behavior Disorder

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Abstract

Rapid eye movement sleep behavior disorder and *GBA* mutations are both associated with Parkinson's disease. The *GBA* gene was sequenced in idiopathic rapid eye movement sleep behavior disorder patients ($n = 265$), and compared to controls ($n = 2240$). Rapid eye movement sleep behavior disorder questionnaire was performed in an independent Parkinson's disease cohort ($n = 120$). *GBA* mutations carriers had an OR of 6.24 (10.2% in patients vs. 1.8% in controls, $P < 0.0001$) for rapid eye movement sleep behavior disorder, and among Parkinson's disease patients, the OR for mutation carriers to have probable rapid eye movement sleep behavior disorder was 3.13 ($P = 0.039$). These results demonstrate that rapid eye movement sleep behavior disorder is associated with *GBA* mutations, and that combining genetic and prodromal data may assist in identifying individuals susceptible to Parkinson's disease.

Introduction

Mutations in *GBA*, the gene encoding for the enzyme Glucocerebrosidase, are common risk factors for Parkinson's disease (PD)[1] and Lewy Body Dementia (LBD).[2,3] Depending on the origin of the tested population, *GBA* mutations can occur in 2–23% of PD or LBD.[1–4] *GBA* mutations have been associated with earlier age at disease onset,[5] and several studies demonstrated that they are also associated with cognitive decline,[6] dementia and autonomic dysfunction.[7]

Rapid eye movement sleep behavior disorder (RBD) is a prodromal condition for both PD and LBD. Since RBD can occur many years before the onset of PD and LBD, and since more than 80% of RBD patients may convert to these synucleinopathies, it can serve as a clinical marker for PD or LBD development.[8] Postmortem studies demonstrated that patients with RBD, as well as patients who carried *GBA* mutations, have Lewy-Body pathology.[9,10] Interestingly, RBD, just like *GBA* mutations, is also associated with cognitive decline/dementia[11] and with autonomic dysfunction[12] among PD patients.

These similarities raise the hypothesis that *GBA* mutations and RBD may be a part of the same pathway, or at least highly correlated. In this study, we aimed to examine the association between *GBA* mutations and RBD, using cohorts of idiopathic RBD and PD patients screened for RBD.

Methods

Population

An RBD cohort of 265 unrelated idiopathic RBD patients of European ancestry was recruited through the international RBD study group. Patients were diagnosed according to the International Classification of Sleep Disorders criteria (ICSD-2) by neurologists specialized in sleep disorders. The cohort was composed of 79.6% men, with age at enrollment of 67.2 ± 9.8 years (data on gender and age was available for 255 and 264 individuals, respectively), and the *GBA* gene was fully sequenced in all. A total of 2240 controls of European origin, including 189 controls who did not have PD at the time of their recruitment from our laboratory, that were previously sequenced for *GBA* mutations,[13] and 2051 additional controls from similar ethnic origins that were previously published (Table S1). The controls from our lab and from the literature were not examined for RBD, therefore it is possible that some of them (the frequency of RBD is about 1% of the general population) have RBD. Such frequency among the controls would have minimal effect on the results, and it is more likely to weaken the association rather than strengthen it.

An independent PD cohort, including 120 Ashkenazi-Jewish patients from Tel-Aviv, Israel, previously analyzed for founder *GBA* mutations,[5] was screened for RBD using the RBD Screening Questionnaire (RBDSQ).[14] The RBDSQ was filled by a clinical researcher together with the patient, under the supervision of a neurologist, at the time of the visit in the movement disorder clinical at the Tel-Aviv Sourasky Medical Center. Neither the patients nor the interviewers were aware of the genetic status of the patients at the time of performing the RBDSQ, since the status of *GBA* mutation carriage was not disclosed prior to the interview. Therefore, since both the interviewers and the patients had no knowledge of the genetic status, it could not have affected their replies to the RBDSQ.

All participants signed an informed consent form, and the study protocols were approved by the respective institutional review boards or Helsinki committees.

Sequencing of the *GBA* gene

DNA was extracted from blood using a standard salting out method. The exons and exon-intron boundaries of the *GBA* gene were amplified and sequenced using specific primers designed to distinguish it from its pseudogene as was previously detailed.[13] PCRs were performed using the AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA) and the products were sequenced at the Genome Quebec Innovation Centre (Montréal, Quebec, Canada) using a 3730XL DNAnalyzer (Applied Biosystems, CA). Mutations were considered as pathogenic if they were previously associated with PD/LBD, if they are known to cause Gaucher's disease, or if they cause early stop codon.

Statistical analysis

Data are presented as mean (±SD) for continuous variables, and percentages for categorical variables. Logistic regression models, chi-square, or Fisher exact test were used for comparison of categorical variables. SPSS software v. 22 (IBM, Somers, NY) was used for all data analysis.

Results

Table 1 details the *GBA* mutations and variants identified in idiopathic RBD patients ($n = 265$) and our in-house controls ($n = 189$). Since the *GBA* variants c.-15A>G, p.K-27R, p.T369M and p.V460L may be considered as nonpathogenic for PD, the analysis was done with and without these variants. In both analyses, *GBA* mutations were significantly more frequent among RBD patients,

Table 1. *GBA* variants identified in RBD patients and controls.

Variation	RBD (n = 265)	Controls (n = 189)	P value
c.-15A>G[1]	1 (0.4%)	0	NA
p.K-27R[1]	1 (0.4%)	0	NA
p.R131L	1 (0.4%)	0	NA
p.H255Q	1 (0.4%)	0	NA
p.W291X	1 (0.4%)	0	NA
p.E326K	14 (5.3%)	3 (1.6%)	0.046
p.T369M[1]	7 (2.6%)	5 (2.6%)	NS
p.N370S	5 (1.9%)	2 (1.1%)	NS
p.W378G	1 (0.4%)	0	NA
p.V437L	1 (0.4%)	0	NA
p.L444P	1 (0.4%)	1 (0.5%)	NS
p.V460L[1]	1 (0.4%)	0	NA
Compound heterozygous			
p.W179X/P.M361I	1 (0.4%)	0	NA
p.L444P/p.E326K	1 (0.4%)	0	NA
Total	37 (14.0%)	11 (5.8%)	0.0052
Total pathogenic	27 (10.2%)	6 (3.2%)	0.0045

RBD, rapid eye movement sleep behavior disorder; PD, Parkinson's disease; LBD, Lewy Body Dementia.

[1]These variants are with unknown clinical significance, whereas the other variants are causing Gaucher's disease or associated with PD/LBD, or represent a null mutation.

with odds ratio (OR) of 2.63 (95% CI 1.30–5.29, $P = 0.0052$) for all variants, and OR of 3.46 (95% CI 1.40–8.56, $P = 0.0045$) for the pathogenic variants. We further compared the frequency of *GBA* mutations in the RBD cohort to a pooled control population from all studies that performed full sequencing of the *GBA* gene in European controls (Table S1). In this pooled control group, 40/2240 (1.8%) individuals were carriers of *GBA* mutations, resulting in an OR of 6.24 (95% CI 3.76–10.35, $P < 0.0001$). Excluding populations from Spain, Greek and Portugal from this analysis resulted with similar risk estimates (OR = 5.72, 95% CI 3.33–9.84, $P < 0.0001$).

Separate analysis of the *GBA* p.E326K mutation was performed, comparing its frequency among the RBD cohort versus all studies reporting the p.E326K mutation in European control populations (including this study, Table S2, a total of 25 carriers of 2064 controls). The OR for *GBA* p.E326K mutation to have RBD was 4.55 (95% CI 2.33–8.87, $P < 0.0001$).

Among the cohort of 120 PD patients, 19 were carriers of *GBA* mutations. Of these, 9/19 (47%) had an RBDSQ score ≥6, suggestive of RBD, compared to 24/101 (24%) among the noncarriers of *GBA* mutations ($P = 0.026$). To control for the potential effects of disease duration and gender, binary regression with gender and disease duration as covariates was performed, and the OR for a *GBA* mutation carrier to have RBD was 3.13 (95% CI 1.06–9.23, $P = 0.039$). Further exclusion of *LRRK2* p.G2019S mutation carriers from the analysis yielded similar results (OR = 3.10, 95% CI 1.05–9.19, $P = 0.041$). The cut-off of six points was selected instead of five which is usually used, since one of the questions in the RBDSQ is "I have/had a disease of the nervous system", therefore when screening PD patients only, this question can be regarded as redundant. When we performed the same analysis with a cut-off of five points, 11/19 (58%) of the *GBA* mutation carriers had passed the cut-off, and the OR for a *GBA* mutation carrier to have probable RBD, adjusted for gender and disease duration, was 4.06 (95% CI 1.45–11.35, $P = 0.008$). The average RBDSQ questionnaire among *GBA* mutation carriers was 5.63 (±3.73), compared to 3.43 (±2.93) among the rest ($P = 0.005$, Student t-test, $P = 0.013$, Mann–Whitney test). Since the PD patients and the interviewers were not aware of the *GBA* genetic status, it eliminated the possibility of biased response of *GBA* mutation carriers or noncarriers to the RBDSQ.

Discussion

Our findings suggest a strong association between *GBA* mutations and RBD, using two independent cohorts, one with idiopathic RBD patients and the second with PD patients screened for RBD. An association between *GBA* and RBD was previously suggested in a cohort of non-PD *GBA* mutation carriers, in which a deterioration in RBD questionnaire score during follow-up was demonstrated,[15] but this association has never been tested in idiopathic RBD. We recently found that RBD patients share some of the genetic background of PD; single nucleotide polymorphisms in the *SCARB2* and *MAPT* regions, which were previously associated with a reduced risk for PD, were also associated with a reduced risk for RBD.[16] Together with this study, these data suggest that RBD is associated with several PD genetic markers, which may have major importance for future early detection of PD.

It is under debate whether the *GBA* p.E326K mutation is pathogenic, or a benign polymorphism. This variant does not cause Gaucher's disease,[17] and several studies suggested that it is not a risk factor for PD.[1] However, other studies demonstrated a strong association between *GBA* p.E326K mutation and PD,[17,18] and a recent meta-analysis of genome wide association studies (GWAS) data demonstrated association with PD with an OR of 1.71 ($P = 5 \times 10^{-8}$).[18] Our data also support this association, as the *GBA* p.E326K variant was three to four times more frequent among RBD patients.

The current data also raises a possible hypothesis for the "missing heritability" in PD. The heritability of PD has been calculated as at least 27%, yet known genetic factors could only explain 3–7%.[19] The missing heritability, at least in part, can be hidden in subtypes of PD.

That is, genetic factors may selectively contribute to a specific subtype of PD. For example, *GBA* mutations may be associated more with RBD-associated PD, whereas PD patients without RBD may have other genetic associations. This can explain the higher OR for the *GBA* p.E326K mutation in our study (4.55) compared to the PD GWAS (1.71).[18] Pooling such subtypes together in large GWASs may therefore allow only the identification of either strong risk factors or those that are shared between several subtypes. It is possible that subgroup analysis of these GWAS data may identify some of these genetic risk factors responsible for the missing heritability phenomenon.

There are a few limitations to this study. The cohort size is not considered very large, however, to the best of our knowledge it is the largest genetic cohort of idiopathic RBD published to date. The size limitation is especially true for the 19 carriers of *GBA* mutations in the PD cohort, therefore additional studies are required to confirm this association. In addition, the RBDSQ is a screening tool with potential false positive and false negative responses, yet there is no known reason for *GBA* mutation carriers with PD to have a higher false positive rate than noncarriers. Moreover, we used a strict RBDSQ score of 6 or above to suggest probable RBD, which is more appropriate in PD patients, since one of the questions is whether the individual has a neurological disorder, which is redundant when focusing on PD patients. Therefore, the different rates of probable RBD in PD patients with and without *GBA* mutations probably represent a valid support for the initial finding of overrepresentation of *GBA* mutations in RBD. However, it is worth emphasizing that the RBDSQ can only infer probable RBD and not definite RBD, therefore further studies of PD cohorts with polysomnography data are required to ascertain the association between *GBA* mutations and RBD in PD patients.

Perhaps the most important conclusion from our study, is its potential implications toward future neuroprotective trials. The first recommendation of the National Institute of Neurological Disorders and Stroke at the Parkinson's Disease 2014 Conference, was to "define the features and natural history of prodromal PD" and the authors further state that: "genetic risk for PD motor and nonmotor symptoms and their progression is key not only to clinical and translational research, but also to basic research, because the identified genes provide critical clues to the molecules involved."[20] Beginning treatment before advanced irreversible degeneration of the substantia nigra is an important strategy for PD management. It is possible that combining screening tools for RBD with genetic data, as was done in this study, will allow the earlier identification of individuals with high susceptibility for PD, a crucial step toward achieving this goal. This hypothesis needs to be further studied in future prospective studies that will include genetic, clinical, and neuro-imaging data.

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Conflict of Interest

Full disclosures were submitted to Annals of Neurology. R. B. P. reports a travel grant from Teva Neuroscience, speaker fees from Novartis Canada and consultancy fees from Roche and Biotie. I. A. reports consultancy and speaker fees from UCB Pharma. Y. D. is on the advisory board and received travel and consultancy fees from UCB Phrma, bioprojet, and Jazz Pharma. A. D. received research grants from Novartis and GlaxoSmithKline, and lecture fees from UCB Pharma and Paladin labs. S. B. reports consultancy fees from World Meds, and from Bachmann Strauss and Dystonia Medical Research Foundation for attending scientific advisory board meetings. B. H. is on the advisory board and received speaker honoraria from UCB Pharma and Mundipharma. J. Y. M. reports grants from Merck, GlaxoSmithKline, received speaking honoraria from Valeant Pharmaceutical, and Otsuka Pharmaceutical, serves on the advisory boards of Sanofi-Aventis, Servier, Merck, Jazz Pharma, Valeant Pharma, Impax Laboratories, Glaxo-SmithKline, UCB Canada, received consultancy fees from Otsuka Pharma, and Valeant Pharma. N. G. serves as consultant to Teva-Lundbeck, IntecPharma, Neuroderm, Armon Neuromedical Ltd. And Pharma Two B, and received payment for lectures at Teva-Lundbeck, Novartis and UCB.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Studies of European ancestry control populations with full sequencing data of the *GBA*.
Table S2. Studies of European ancestry control populations with data on the *GBA* p.E326K.

