**GBA1 rs3115534 Is Associated with REM Sleep Behavior Disorder in Parkinson’s Disease in Nigerians**

Oluwadamilola Omolar Ojo, MD,1,2* Sara Bandres-Ciga, PhD,3 Mary B. Makarious, BSc,4,5 Dena G. Hernandez, PhD,4 Peter Wild Crea, BSc,4,5 Dena G. Hernandez, PhD,4 Peter Wild Crea, BSc,4,5 Dena G. Hernandez, PhD,4 Peter Wild Crea, BSc,4,5 Dena G. Hernandez, PhD,4 Peter Wild Crea, BSc,4,5 Dena G. Hernandez, PhD,4 Peter Wild Crea, BSc,4,5 Dena G. Hernandez, PhD,4

**ABSTRACT:** Background: Rapid eye movement (REM) sleep behavior disorder (RBD) is an early feature of Parkinson’s disease (PD) and dementia with Lewy bodies (DLB). Damaging coding variants in Glucocerebrosidase (GBA1) are a genetic risk factor for RBD. Recently, a population-specific non-coding risk variant (rs3115534) was found to be associated with PD risk and earlier onset in individuals of African ancestry.

**Objectives:** We aimed to investigate whether the GBA1 rs3115534 PD risk variant is associated with RBD in persons with PD.

**Methods:** We studied 709 persons with PD and 776 neurologically healthy controls from Nigeria. All DNA samples were genotyped and imputed, and the GBA1 rs3115534 risk variant was extracted. The RBD screening questionnaire (RBDSQ) was used to assess symptoms of possible RBD.

**Results:** RBD was present in 200 PD (28.2%) and 51 (6.6%) controls. We identified that the non-coding GBA1 rs3115534 risk variant is associated with possible RBD in individuals of Nigerian origin (β, 0.3640; standard error [SE], 0.103; P = 4.093e−04), as well as in all samples after adjusting for PD status (β, 0.2542; SE, 0.108; P = 0.019) suggesting that although non-coding, this variant may have the same downstream consequences as GBA1 coding variants.

**Conclusions:** Our results indicate that the non-coding GBA1 rs3115534 risk variant is associated with an increasing number of RBD symptoms in persons with PD of Nigerian origin. Further research is needed to assess if this variant is also associated with polysomnography-defined RBD and with RBD symptoms in DLB. © 2024 The Authors. Movement Disorders published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

**Key Words:** genetics; GBA1; rs3115534; Parkinson’s disease; REM sleep behavior disorder; Nigerians

Glucocerebrosidase (GBA1) variants have been documented as the most significant genetic risk for Parkinson’s disease (PD) globally.1 GBA1-associated PD has an earlier age of disease onset, faster motor progression, and more frequent non-motor symptoms specifically cognitive decline/dementia, visual hallucinations, hyposmia, autonomic features, and rapid eye movement (REM) sleep behavior disorder (RBD).2-4 One of the early clinical symptoms or prodromal features of synucleinopathies is RBD and over 80% of patients with this sleep disorder will ultimately be diagnosed with PD, dementia with Lewy bodies (DLB), or in rare cases, multiple system atrophy (MSA) within 10 to 15 years.4,5 Currently, RBD stands as a powerful...
indicator for the emergence of dementia in individuals with PD, and it is linked to a faster disease progression.\textsuperscript{6} RBD occurs in \textasciitilde 50\% to 80\% of all DLB cases and 30\% to 60\% of persons with PD.\textsuperscript{7,8}

The first genome-wide association study (GWAS) in RBD recently nominated two coding variants at the \textit{GBA1} locus, including p.Glu365Lys (rs2230288) and p.Asn409Ser (rs76763715), as linked to RBD risk in the European ancestry population (p.Glu326Lys; odds ratio [OR], 2.09; 95\% CI, 1.73–2.54; \(P = 4.87E-14\); p.Asn370Ser; OR, 2.84; 95\% CI, 2.06–3.92; \(P = 1.68E-10\)).\textsuperscript{9} To the best of our knowledge, no research aimed at exploring genetic risk factors linked to RBD in the African population has been performed or published to date.

The first GWAS of PD in the African and African admixed populations has identified a novel population-specific intronic variant linked to RBD risk and age at onset at the \textit{GBA1} locus as the major genetic contributor to PD in this population (risk, rs3115534-G; OR, 1.58; 95\% CI, 1.37–1.80; \(\beta, 0.4494; \) standard error [SE], 0.0589; \(P = 2.397E-14\)).\textsuperscript{10} This variant exerts a substantial risk of PD as compared with common variation identified through GWAS, and it was found to be present in \textasciitilde 55\% of the PD cases of Nigerian ancestry and 39\% of the PD cases from African admixed ancestry.\textsuperscript{10} Importantly, all current \textit{GBA1} knowledge, ranging from clinical to functional, is based on \textit{GBA1} coding variants and not much is known about the impact of non-coding variants on disease etiology. Here, we aimed to investigate the role of \textit{GBA1} rs3115534 and the risk of RBD in the African population.

**Patients and Methods**

**Study Design**

Study participants were recruited by neurologists from the Nigeria Parkinson’s Disease Research network.\textsuperscript{11} Research ethics approval for the study was obtained from the institutional health research ethics committees, the National Health Research Ethics Committee in Nigeria and the University College London Institutional Review Board. All participants provided written informed consent. Individuals with PD fulfilled the United Kingdom Parkinson’s Disease Society Brain Bank criteria.\textsuperscript{12} Controls were healthy volunteers of Nigerian origin, with no known family history of PD, no clinically evident neurological condition, and matched for age.

**Sample and Clinical Data Collection**

DNA was extracted either from saliva samples collected using DNA Genotek saliva kits or from venous whole blood samples using standard protocols. The RBD screening questionnaire (RBDSQ), a specific questionnaire for RBD, was used to assess the most prominent clinical features of RBD.\textsuperscript{13} It is a 10-item, patient self-rating instrument with short questions to be answered by either “yes” or “no.” The questionnaire has been shown to be valid and perform with high sensitivity and reasonable specificity in the diagnosis of possible RBD (pRBD).\textsuperscript{14} Briefly, items 1 to 4 address the frequency and content of dreams and their relationship to nocturnal movements and behavior. Item 5 asks about self-injuries and injuries of the bed partner. Item 6 consists of four sub items assessing nocturnal motor behavior more specifically, that is, questions about nocturnal vocalization, sudden limb movements, complex movements, or bedding items that fell down. Items 7 and 8 deal with nocturnal awakenings. Item 9 focuses on disturbed sleep in general and item 10 on the presence of any neurological disorder. The maximum total score of the RBDSQ is 13 points.

**Genotyping and Imputation Procedures**

Genotyping was performed at the National Institutes of Health/Laboratory of Neurogenetics using the NeuroBooster Array (https://github.com/GP2code/Neuro_Booster_Array). Genetic data were QCed and imputed using standard GP2 pipelines and protocols (https://github.com/GP2code/). As previously reported, the GBA1 rs3115534 was reliably imputed in all samples.\textsuperscript{10} We previously reported a high correlation (96.6\%) between short-read whole genome sequencing (WGS) and imputed genotyped data for rs3115534.\textsuperscript{10}

**Statistical Analysis**

All samples with complete data were included in the analyses. Two initial screening models were built to test the association between the quantitative RBD score and a dichotomized pRBD outcome with the outcome positive at RBD score six or higher. For the linear regression model testing RBD score as the outcome and the logistic regression model testing pRBD status as the outcome, female sex and enrollment age were used as covariates with the dosage of the GBA1 rs3115534 variant of interest as the exposure. These models were subsequently repeated with an additional adjustment for PD case status. These same models were then subset to only PD cases and only neurologically normal controls. Although the control-only models maintained the original set of covariates, the PD case-only analysis added case age at onset and disease duration instead of enrollment age.

**Results**

In total, we included 709 persons with PD and 776 neurologically healthy controls of Nigerian descent and assessed whether the novel \textit{GBA1} non-coding rs3115534 risk variant had an effect on pRBD status. Similar to previous observations, the \textit{GBA1} non-coding rs3115534 risk variant was overrepresented in the PD cases versus the...
neurologically healthy controls (minor allele frequency (MAF) cases = 0.34 vs. MAF controls = 0.21). A detailed description of demographics and clinical characteristics of the participants under study can be seen in Table 1. As expected, PD cases had a clear higher frequency of pRBD (score ≥ 6) versus controls (28.2% vs. 6.6%, χ² value, 121.95; P = 2.37E−07). Supplementary Table S1 compares the characteristics of PD with (n = 200) and without possible RBD (n = 509), including the sex distribution, age at study, age at onset, frequency of early onset PD (EOPD; cut off definition ≤ 50 years) (all not significantly different), and mean (standard deviation [SD]) RBDSQ score (P < 0.001). Initial screening models combining cases and controls showed significant associations between the GBA1 non-coding rs3115534-G allele and RBD status (β, 0.3640; SE, 0.103; P = 4.093E−04) as well as the continuous RBD score (β, 0.5509; SE, 0.116; P = 2.043E−06). The association between RBD score and GBA1 non-coding rs3115534-G allele persisted after adjusting for PD case status (β, 0.2542; SE, 0.108; P = 0.019), showing that the association with GBA1 rs3115534-G and severity of pRBD is at least partially independent of PD case status in this dataset. The association with RBD score persists when data are subset to only cases (β, 0.4166; SE, 0.174; P = 0.017). There are no associations between either RBD score and RBD status among neurologically normal controls (P > 0.05). For a detailed description of all statistical tests performed see Table 2.

**Discussion**

The strong association between RBD and synucleinopathies and the understanding regarding the linked complex neuropathological basis provides a

### TABLE 1  Demographics and clinical characteristics of the Nigerian cohort under study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Parkinson’s disease (n = 709)</th>
<th>Controls (n = 776)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex distribution (n, %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>195 (27.5%)</td>
<td>265 (34.2%)</td>
</tr>
<tr>
<td>Male</td>
<td>514 (72.5%)</td>
<td>511 (65.8%)</td>
</tr>
<tr>
<td>Age at study ± SD (range), (y)</td>
<td>63.8 ± 10.1 (23–95)</td>
<td>63.4 ± 9.3 (25–98)</td>
</tr>
<tr>
<td>Age at onset ± SD (range), (y)</td>
<td>59.7 ± 10.3 (17–93)</td>
<td></td>
</tr>
<tr>
<td>RBDQ mean score (SD)</td>
<td>4.19 (3.25)</td>
<td>1.74 (2.05)</td>
</tr>
<tr>
<td>Probable RBD (RBDQ score ≥ 6 (n, (% of total))</td>
<td>200 (28.2%)</td>
<td>51 (6.6%)</td>
</tr>
<tr>
<td>GBA1 rs3115534 carriers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG (n = 101)</td>
<td>4.55 (3.20)</td>
<td>4.44 (3.31)</td>
</tr>
<tr>
<td>GT (n = 285)</td>
<td>3.85 (3.18)</td>
<td>1.35 (2.10)</td>
</tr>
<tr>
<td>TT (n = 323)</td>
<td>79 (24.5%)</td>
<td>24 (8.1%)</td>
</tr>
<tr>
<td>Probable RBD (% of total)</td>
<td>30 (30.7%)</td>
<td>90 (31.6%)</td>
</tr>
</tbody>
</table>

**Abbreviations:** SD, standard deviation; RBD, Rapid Eye Movement (REM) Sleep Behavior Disorder; RBDQ, RBD Questionnaire.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Samples (n)</th>
<th>Covariates</th>
<th>β</th>
<th>Standard error</th>
<th>P-value (two-sided)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBDQ score</td>
<td>All (1485)</td>
<td>Standard</td>
<td>0.5509</td>
<td>0.116</td>
<td>2.043E−06</td>
</tr>
<tr>
<td>RBDQ score</td>
<td>All (1485)</td>
<td>Standard with additional adjustment for PD status</td>
<td>0.2542</td>
<td>0.108</td>
<td>0.019</td>
</tr>
<tr>
<td>RBDQ score</td>
<td>PD cases (709)</td>
<td>Modified to adjust for AAO and disease duration</td>
<td>0.4166</td>
<td>0.174</td>
<td>0.017</td>
</tr>
<tr>
<td>RBDQ score</td>
<td>Controls (776)</td>
<td>Standard</td>
<td>0.0258</td>
<td>0.126</td>
<td>0.838</td>
</tr>
<tr>
<td>RBD status</td>
<td>All (1485)</td>
<td>Standard</td>
<td>0.3640</td>
<td>0.103</td>
<td>4.093E−04</td>
</tr>
<tr>
<td>RBD status</td>
<td>All (1485)</td>
<td>Standard with additional adjustment for PD</td>
<td>0.1912</td>
<td>0.106</td>
<td>0.071</td>
</tr>
<tr>
<td>RBD status</td>
<td>PD cases (709)</td>
<td>Modified to adjust for AAO and disease duration</td>
<td>0.2034</td>
<td>0.118</td>
<td>0.084</td>
</tr>
<tr>
<td>RBD status</td>
<td>Controls (776)</td>
<td>Standard</td>
<td>0.1014</td>
<td>0.244</td>
<td>0.678</td>
</tr>
</tbody>
</table>

**Standard covariates included sex and enrollment age. RBD scores ranged from 0 to 13, RBD status = dichotomous trait with 0 being RBD score <6 and 1 being RBD score ≥ 6. P values in bold font are statistically significant.**

**Abbreviations:** AAO, Age at onset; RBDQ, RBD Questionnaire; PD, Parkinson’s disease; RBD, Rapid Eye Movement (REM) Sleep Behavior Disorder.
premise for exploring the genetic basis of RBD in PD. Additionally, the PD-RBD phenotype appears to confer a more aggressive PD progression, with a higher burden of motor and non-motor (predominantly cognitive/behavioral) manifestations.13 Data from the Parkinson Progression Markers Initiative (PPMI) study, using similar diagnostic criteria as applied in our study identified several single nucleotide polymorphisms (SNPs) associated with an increased risk of pRBD and a distinctive genetic signature for pRBD in PD.16 Specifically, GBA_N370S_rs76763715 (OR, 95% CI, 3.38, 1.45–7.93), SNCA_A53T_RS104893877 (8.21, 2.26–36.34), ANK2. CAMK2D_rs78738012 (2.12, 1.08–4.10), and ZNF184_Rs9468199 (1.89, 1.08–3.33) all increased the risk of pRBD albeit with different effect sizes.16 The PPMI cohort is, however, predominantly comprised of persons of Caucasian (North American White) in whom the genetic underpinnings of disease may vary from persons of Black African ancestry.

In this study, we performed the first genetic assessment of RBD risk in PD in the Nigerian population. Our results demonstrate the association between the novel GBA1 rs3115534 PD risk variant and an increased risk of RBD symptoms overall and in PD, and show that risk carriers are ~1.43 times more likely to have RBD symptoms than non-carriers. Our results are in line with those from previous PD studies, including other ethnicities, which clearly demonstrated similar relationships between GBA1 coding variants and the risk for RBD.17 The fact that RBD serves as a potent predictor for the onset of dementia suggests that this variant may also be implicated in PD-dementia and DLB risk. In the current study, we were unable to explore whether this variant is associated with dementia risk in the African population given that we did not have access to dementia data. Additionally, the most commonly used cognitive screening tests such as the Montreal Cognitive Assessment and the Mini Mental State Examination do not seem to be well suited for this population.18

Our study has some limitations. First, questionnaires are subject to recall bias and inaccuracies. The RBDSQ has been found to have high diagnostic accuracy in healthy controls, with 96% sensitivity and 96% specificity. However, when including patients with other sleep disorders, the specificity drops to only 56%.19 Validation of current scales are necessary to ensure reproducibility, reliability, and accuracy as polysonography-defined RBD data become available.

In the present study, we were not able to confirm that RBD symptoms preceded PD diagnosis because of the lack of longitudinal data in this cohort. Further studies are needed to explore whether GBA1 rs3115534 affects the rate of phenoconversion from RBD to PD and other synucleinopathies such as DLB and MSA.

In summary, we show here that the non-coding GBA1 rs3115534 is associated with symptoms of RBD defined by a commonly used screening questionnaire. Identifying individuals at risk of RBD represent an appealing target for clinical trials focused on preventing neurodegeneration in conditions such as PD and DLB. As neuroprotective treatments emerge in the future, an early identification of RBD will be key to treat these conditions.

Acknowledgments: This work was supported in part by the Intramural Research Program of the National Institute on Aging (NIA), and the Center for Alzheimer’s and Related Dementias, within the Intramural Research Program of the NIA and the National Institute of Neurological Disorders and Stroke. Data used in the preparation of this article were obtained from Global Parkinson’s Genetics Program (GP2). GP2 is funded by the Aligning Science Across Parkinson’s initiative and implemented by The Michael J. Fox Foundation for Parkinson’s Research (https://gp2.org). This work used the computational resources of the National Institutes of Health high performance computing Biowulf cluster (https://biowulf.nih.gov). M.A.N.’s participation in this project was part of a competitive contract awarded to DataTecnia LLC by the National Institutes of Health to support open science research. M.A.N. also currently serves on the scientific advisory board at Clover Therapeutics and is an advisor and scientific founder at Neuron23.

Data Availability Statement
The data used and that support the findings in this study are available via GP2 at the https://www.amp-pd.org/ portal. Code availability: All code is available as follows: https://github.com/GP2code/GBA1_RBD_NIGERIA and Zenodo: https://zenodo.org/record/8399986.

References
5. Schenck CH, Boeve BF, Mahowald MW. Delayed emergence of a parkinsonian disorder or dementia in 81% of older men initially diagnosed with idiopathic rapid eye movement sleep behavior disorder: a 16-year update on a previously reported series. Sleep Med 2013;14(8):744–748. https://doi.org/10.1016/j.sleep.2012.10.009


Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site.
Author Roles


O.O., S.B.-C., A.N., C.B., N.O.: 1A, 1B, 1C, 2A, 2B.