BRIEF REPORT

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

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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.

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

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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).[2,3]

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.[6] RBD occurs in ~50% to 80% of all DLB cases and 30% to 60% of persons with PD.[7,8]

The first genome-wide association study (GWAS) in RBD recently nominated two coding variants at the *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; $P = 1.68E{-}10$).[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 PD risk and age at onset at the *GBA1* locus as the major genetic contributor to PD in this population (risk, rs3115534-G; OR, 1.58; 95% CI, 1.37–1.80; β, 0.4494; standard error [SE], 0.0589; $P = 2.397E{-}14$).[10] This variant exerts substantial risk of PD as compared with common variation identified through GWAS, and it was found to be present in ~55% of the PD cases of Nigerian ancestry and 39% of the PD cases from African admixed ancestry.[10] Importantly, all current *GBA1* knowledge, ranging from clinical to functional, is based on *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 *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.[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.[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.[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).[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.[10] We previously reported a high correlation (96.6%) between short-read whole genome sequencing (WGS) and imputed genotyped data for rs3115534.[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 analyses 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 *GBA1* non-coding rs3115534 risk variant had an effect on pRBD status. Similar to previous observations, the *GBA1* non-coding rs3115534 risk variant was overrepresented in the PD cases versus the

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

Variable	Parkinson's disease (n = 709)			Controls (n = 776)		
Sex distribution (n, %)						
Female	195 (27.5%)			265 (34.2%)		
Male	514 (72.5%)			511 (65.8%)		
Age at study ± SD (range), (y)	63.8 ± 10.1 (23–95)			63.4 ± 9.3 (25–98)		
Age at onset ± SD (range), (y)	59.7 ± 10.3 (17–93)					
RBDQ mean score (SD)	4.19 (3.25)			1.74 (2.05)		
Probable RBD (RBDQ score ≥6 (n, (% of total))	200 (28.2%)			51 (6.6%)		
GBA1 rs3115534 carriers	GG (n = 101)	GT (n = 285)	TT (n = 323)	GG (n = 34)	GT (n = 298)	TT (n = 444)
RBDQ mean score (SD)	4.55 (3.20)	4.44 (3.31)	3.85 (3.18)	1.35 (2.10)	1.87 (2.16)	1.70 (1.97)
Probable RBD (% of total)	31 (30.7%)	90 (31.6%)	79 (24.5%)	1 (2.9%)	24 (8.1%)	26 (5.9%)

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

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%, χ^2 value, 121.95; $P = 2.37E{-}28$). 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 2 *Regression analyses detailing parameter estimates associated with* GBA1 *rs3115534*

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

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.[15] 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 polysomnography-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. ■

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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

1. Sidransky E, Nalls MA, Aasly JO, et al. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. N Engl J Med 2009;361(17):1651–1661. https://doi.org/10.1056/NEJMoa0901281
2. Gan-Or Z, Liong C, Alcalay RN. GBA-associated Parkinson's disease and other Synucleinopathies. Curr Neurol Neurosci Rep 2018;18(8):44. https://doi.org/10.1007/s11910-018-0860-4
3. Petrucci S, Ginevrino M, Trezzi I, et al. GBA-related Parkinson's disease: dissection of genotype-phenotype correlates in a large Italian cohort. Mov Disord 2020;35(11):2106–2111. https://doi.org/10.1002/mds.28195
4. Postuma RB, Iranzo A, Hu M, et al. Risk and predictors of dementia and parkinsonism in idiopathic REM sleep behaviour disorder: a multicentre study. Brain 2019;142(3):744–759. https://doi.org/10.1093/brain/awz030
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
6. Aarsland D, Batzu L, Halliday GM, et al. Parkinson disease-associated cognitive impairment [published correction appears in Nat rev dis primers. 2021 13;7(1):53]. Nat Rev Dis Primers 2021;7(1):47. https://doi.org/10.1038/s41572-021-00280-3
7. McKeith IG, Boeve BF, Dickson DW, et al. Diagnosis and management of dementia with Lewy bodies: fourth consensus report of the DLB consortium. Neurology 2017;89(1):88–100. https://doi.org/10.1212/WNL.0000000000004058
8. Rolinski M, Szewczyk-Krolikowski K, Tomlinson PR, et al. REM sleep behaviour disorder is associated with worse quality of life and other non-motor features in early Parkinson's disease. J Neurol Neurosurg Psychiatry 2014;85(5):560–566. https://doi.org/10.1136/jnnp-2013-306104
9. Krohn L, Heilbron K, Blauwendraat C, et al. Genome-wide association study of REM sleep behavior disorder identifies polygenic risk and brain expression effects. Nat Commun 2022;13(1):7496. https://doi.org/10.1038/s41467-022-34732-5

10. Rizig M, Bandres-Ciga S, Makarious MB, et al. Identification of genetic risk loci and causal insights associated with Parkinson's disease in African and African admixed populations: a genome-wide association study. Lancet Neurol 2023;22(11):1015–1025. https://doi.org/10.1016/S1474-4422(23)00283-1

11. Ojo OO, Abubakar SA, Iwuozo EU, et al. The Nigeria Parkinson disease registry: process, profile, and prospects of a collaborative project. Mov Disord 2020;35(8):1315–1322. https://doi.org/10.1002/mds.28123

12. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry 1992;55(3):181–184. https://doi.org/10.1136/jnnp.55.3.181

13. Halsband C, Zapf A, Sixel-Döring F, Trenkwalder C, et al. The REM sleep behavior disorder screening questionnaire is not valid in de novo Parkinson's disease. Mov Disord Clin Pract 2018;5(2):171–176. https://doi.org/10.1002/mdc3.12591

14. Nomura T, Inoue Y, Kagimura T, Uemura Y, Nakashima K. Utility of the REM sleep behavior disorder screening questionnaire (RBDSQ) in Parkinson's disease patients. Sleep Med 2011;12(7):711–713. https://doi.org/10.1016/j.sleep.2011.01.015

15. Kim Y, Kim YE, Park EO, et al. REM sleep behavior disorder portends poor prognosis in Parkinson's disease: a systematic review. J Clin Neurosci 2018;47:6–13. https://doi.org/10.1016/j.jocn.2017.09.019

16. Perez-Lloret S, Chevalier G, Bordet S, et al. The genetic basis of probable REM sleep behavior disorder in Parkinson's disease. Brain Sci 2023;13(8):1146. https://doi.org/10.3390/brainsci13081146

17. Gan-Or Z, Mirelman A, Postuma RB, et al. GBA mutations are associated with rapid eye movement sleep behavior disorder. Ann Clin Transl Neurol 2015;2(9):941–945. https://doi.org/10.1002/acn3.228

18. Rossetti HC, Lacritz LH, Hynan LS, Cullum CM, van Wright A, Weiner MF. Montreal cognitive assessment performance among community-dwelling African Americans. Arch Clin Neuropsychol 2017;32(2):238–244. https://doi.org/10.1093/arclin/acw095

19. Bramich S, King A, Kuruvilla M, Naismith SL, Noyce A, Alty J. Isolated REM sleep behaviour disorder: current diagnostic procedures and emerging new technologies. J Neurol 2022;269(9):4684–4695.

Supporting Data

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Author Roles

(1) Research project: A. Conception, B. Organization, C. Execution; (2) Statistical Analysis: A. Design, B. Execution, C. Review and Critique; (3) Manuscript: A. Writing of the First Draft, B. Review and Critique.

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

M.M., P.C., D.H., H.H., M.R., A.S., M.N.: 1B, 1C, 2B.