



TITLE:

# Lower Circulating Lymphocyte Count Predicts ApoE $\epsilon$ 4-Related Cognitive Decline in Parkinson's Disease

AUTHOR(S):

Tsukita, Kazuto; Sakamaki-Tsukita, Haruhi;  
Takahashi, Ryosuke

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CITATION:

Tsukita, Kazuto ...[et al]. Lower Circulating Lymphocyte Count Predicts ApoE  $\epsilon$ 4-Related Cognitive Decline in Parkinson's Disease. *Movement Disorders* 2021, 36(12): 2969-2971

ISSUE DATE:

2021-12

URL:

<http://hdl.handle.net/2433/266630>

RIGHT:



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In conclusion, this study yields first evidence from longitudinal data of individual patients for the potential of iron and ferritin as progression marker in PD. A validation of our findings in a larger cohort, more advanced PD patients and a longer follow-up period is warranted. ■

**Acknowledgments:** We gratefully appreciate the participation of our patients in this study. We thank our Parkinson's and study nurses Gudrun Leyerer and Jennifer Heinemann for their excellent assistance. We also thank Peter Lange for providing helpful advice on assay-related issues. Open Access funding enabled and organized by Projekt DEAL.

### Data Availability Statement

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Fabian Maass, MD,<sup>1\*</sup>  Bernhard Michalke, PhD,<sup>2</sup>  
Desiree Willkommen, (Ms.),<sup>2</sup> Sezgi Canaslan, (Ms.),<sup>1</sup>  
Matthias Schmitz, PhD,<sup>1,3</sup>  Mathias Bähr, MD,<sup>1,4</sup>  
Inga Zerr, MD,<sup>1,3</sup> and Paul Lingor, MD<sup>5,6</sup>

<sup>1</sup>Department of Neurology, University Medical Center Göttingen, Goettingen, Germany, <sup>2</sup>Research Unit Analytical BioGeoChemistry, German Research Center for Environmental Health, Helmholtz Zentrum München, Neuherberg, Germany, <sup>3</sup>DZNE, German Center for Neurodegenerative Diseases, Goettingen, Germany, <sup>4</sup>Center for Biostructural Imaging of Neurodegeneration (BIN), University of Göttingen Medical Center, Göttingen, Germany, <sup>5</sup>Department of Neurology, School of Medicine, Klinikum rechts der Isar, Technical University of Munich, München, Germany, and <sup>6</sup>DZNE, German Center for Neurodegenerative Diseases, Munich, Germany

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### Supporting Data

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

## Lower Circulating Lymphocyte Count Predicts ApoE $\epsilon$ 4-Related Cognitive Decline in Parkinson's Disease

Neuroinflammatory changes in the brain, including infiltration of lymphocytes, particularly T cells, play a critical role in the pathogenesis of Parkinson's disease (PD).<sup>1,2</sup> Interestingly, in the peripheral blood of PD patients, a decrease in circulating lymphocyte counts occurs, mainly due to a decrease in T cells.<sup>1,3</sup> Furthermore, it has recently been reported that lower lymphocyte count might be causally related to the subsequent development of PD.<sup>4</sup> Inspired by these observations, we aimed at assessing whether low lymphocyte count is associated with the subsequent development of the key milestones in PD's disease course, specifically cognitive impairment, with a particular attention to the apolipoprotein E (ApoE)  $\epsilon$ 4 allele, a crucial modifying factor in cognitive impairment.<sup>5,6</sup>

In this retrospective cohort study, using the Parkinson's Progression Markers Initiative data, 167 de novo PD patients were enrolled (Fig. S1) and were followed up for 2 years (Tables S1 and S2; Text S1). R scripts made for the analysis are freely available at <http://dx.doi.org/10.17632/7s8sng9yn8.2> or [https://github.com/KazutoTsukita/Mov\\_Disord\\_2021](https://github.com/KazutoTsukita/Mov_Disord_2021).

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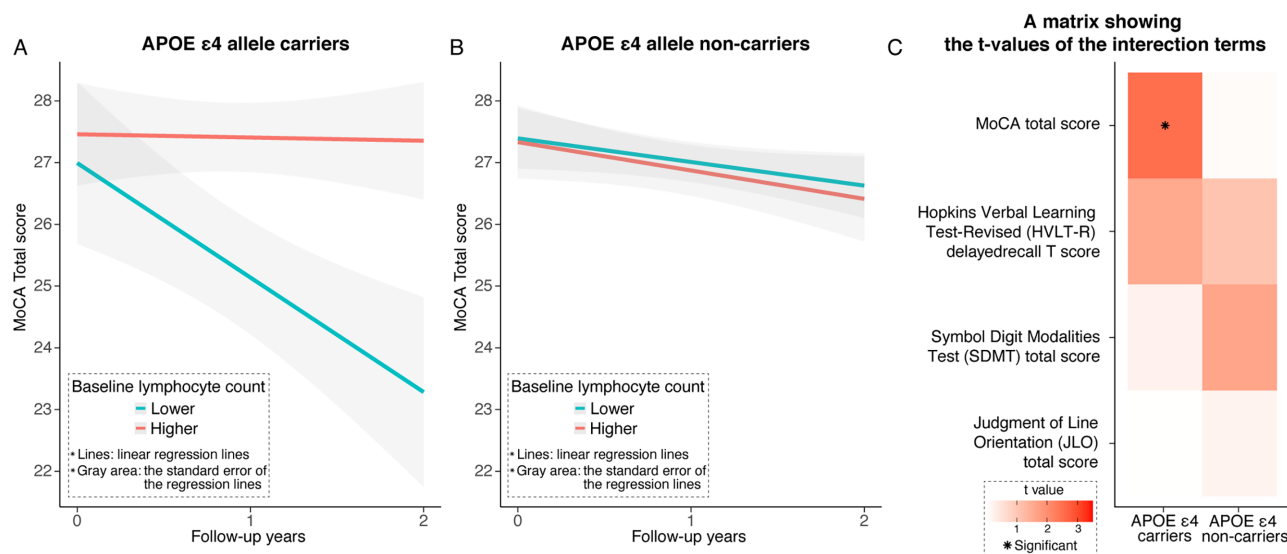
**\*Correspondence to:** Dr Kazuto Tsukita, Department of Neurology, Graduate School of Medicine, Kyoto University, 54 Shogoin-Kawaharacho, Kyoto 606-8507, Japan; E-mail: kazusan@kuhp.kyoto-u.ac.jp

**Relevant conflicts of interest/financial disclosures:** Nothing to report.

**Funding agency:** Nothing to report.

**Received:** 13 August 2021; **Accepted:** 26 August 2021

**Published online 13 October 2021 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.28799**



**FIG. 1.** Evaluations of interaction effects of baseline lymphocyte counts on cognitive decline in patients with PD (A) with or (B) without the ApoE (apolipoprotein E) ε4 allele and (C) those on the progression of specific domains of cognitive impairment. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

We primarily used the multivariate linear mixed-effects model adjusted for various covariates (age, sex, levodopa-equivalent dose, disease duration, and baseline severity of smell deficit and rapid-eye-movement sleep behavior). We observed that only in PD patients carrying ApoE ε4 allele, baseline lymphocyte count had significant interaction effect on the longitudinal decline in the Montreal Cognitive Assessment (MoCA) total score, such that lower baseline lymphocyte count was associated with accelerated MoCA score decline (carrier, the standardized fixed-effects coefficient of the interaction term ( $\beta_{\text{interaction}}$ ) = 0.17 [95% confidence interval, CI: 0.04, 0.30],  $P = 0.01$ ; noncarrier,  $\beta_{\text{interaction}} = -0.00$  [95% CI: -0.10, 0.09],  $P = 0.94$ ). When PD patients, with and without ApoE ε4 allele, were dichotomized using the median of baseline lymphocyte count (carrier,  $1.72 \times 10^3/\mu\text{L}$ ; noncarrier,  $1.74 \times 10^3/\mu\text{L}$ ) (Table S3), the interaction effect was apparent only in PD patients carrying ApoE ε4 allele (carrier,  $\beta_{\text{interaction}} = 0.45$  [95% CI: 0.20, 0.71],  $P < 0.001$ ; noncarrier,  $\beta_{\text{interaction}} = -0.03$  [95% CI: -0.22, 0.15],  $P = 0.72$ ) (Fig. 1A,B). The interaction effects of baseline lymphocyte count on the progression of specific domains of cognitive impairment did not reach statistical significance (Fig. 1C). Sensitivity analyses confirmed the robustness of our result in a range of follow-up periods (Table S4) and even when missing values were imputed (Table S5).

An interesting aspect of the present result is that baseline lymphocyte count was clearly associated with subsequent cognitive decline only in PD patients carrying ApoE ε4 allele. Given the importance of ApoE ε4 allele in blood-brain barrier (BBB) dysfunction and the role of circulating T cells in PD pathogenesis (Text S2),<sup>1,7</sup> our result might indicate the cooperative pathological role of BBB dysfunction and circulating lymphocytes in PD. Alternatively, the brain cortex of patients carrying ApoE ε4 allele may be particularly vulnerable to lymphocyte infiltration. Admittedly, this study has some limitations (Text S3); however, because many covariates were adjusted for, we believe that our result indicates that biological phenomenon reflected by the decrease in the lymphocyte count might

actively exacerbate the pathology driving cognitive dysfunction in synergy with the ApoE ε4 allele, thereby providing important clinical and pathophysiological implications. ●

**Acknowledgments:** This work was supported by JST [Moonshot R&D] [Grant Number JPMJMS2024]. PPMI—a public-private partnership—is funded by the Michael J. Fox Foundation for Parkinson’s Research funding partners 4D Pharma, AbbVie, Acurex Therapeutics, Allergan, Amathus Therapeutics, ASAP, Avid Radiopharmaceuticals, Bial Biotech, Biogen, BioLegend, Bristol-Myers Squibb, Calico, Celgene, Dacapo Brain Science, Denali, the Edmond J. Safra Foundation, GE Healthcare, Genentech, GlaxoSmithKline, Golub Capital, Handl Therapeutics, Instiro, Janssen Neuroscience, Lilly, Lundbeck, Merck, Meso Scale Discovery, Neurocrine Biosciences, Pfizer, Piramal, Prevail, Roche, Sanofi Zynzyme, Servier, Takeda, Teva, UCB, Verily, and Voyager Therapeutics. We thank Dr. Takahiro Kamada for inspiring us to do this study. He died in January 2019, and we wish to dedicate this article in his memory.

## Data Availability Statement

Data used in this retrospective cohort study were obtained from the Parkinson’s Progression Markers Initiative (PPMI) database ([www.ppmi-info.org/data](http://www.ppmi-info.org/data)) on July 28, 2021. For up-to-date information on the study, visit [www.ppmi-info.org](http://www.ppmi-info.org). R scripts made for the analysis are freely available at <http://dx.doi.org/10.17632/7s8sng9yn8.2>. or [https://github.com/KazutoTsukita/Mov\\_Disord\\_2021](https://github.com/KazutoTsukita/Mov_Disord_2021).

Kazuto Tsukita, MD,<sup>1,2,3\*</sup> Haruhi Sakamaki-Tsukita, MD,<sup>1</sup> and Ryosuke Takahashi, MD, PhD<sup>1</sup>

<sup>1</sup>Department of Neurology, Graduate School of Medicine, Kyoto University, Kyoto, Japan, <sup>2</sup>Advanced Comprehensive Research Organization, Teikyo University, Tokyo, Japan, and <sup>3</sup>Division of Sleep Medicine, Kansai Electric Power Medical Research Institute, Osaka, Japan

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## Supporting Data

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

# Screening of *GBA* Mutations in Nigerian Patients with Parkinson's Disease

Heterozygous mutations in the  $\beta$ -glucocerebrosidase (*GBA*) gene are reported in 5% to 30% of patients with Parkinson's disease (PD) across White and Asian populations with a relative absence of studies in other populations.<sup>1,2</sup> Nigeria is the most populated African country and has more than 5 million people who are aged older than 65 years.<sup>3</sup> To date, the only *GBA* screening reported in Sub-Saharan Africa populations was performed in Black South African patients<sup>4</sup>; two novel missense variants (p.F216L and p.G478R) and three previously described (p.K(-27) R, p.T36del, and p.Q497\*) variants were identified in 30 patients with PD.<sup>4</sup> The aim of this study was to assess the frequency of *GBA* mutations in a series of Nigerian patients with PD and controls by gene sequencing.

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\*Correspondence to: Dr. Shamsideen Abayomi Ogun, Division of Neurology, Lagos State University College of Medicine/Teaching Hospital, PMB 21266 Ikeja, Lagos, Nigeria, E-mail: yomiogun2002@yahoo.com; Dr. Owen A. Ross, Department of Neuroscience, Mayo Clinic Jacksonville, 4500 San Pablo Road, Jacksonville, FL 32224, USA, E-mail: ross.owen@mayo.edu

†These authors contributed equally.

**Relevant conflicts of interest/financial disclosures:** Mayo Clinic is an American Parkinson Disease Association (APDA) Mayo Clinic Information and Referral Center, an APDA Center for Advanced Research, and the Mayo Clinic Lewy Body Dementia Association Research Center of Excellence. L.M.M. is supported by the Polish National Agency for Academic Exchange Iwanowska's Fellowship PPN/IWA/2018/1/00006/U/00001/01. Z.K.W. is partially supported by the Mayo Clinic Center for

Blood specimens were collected and participants were clinically characterized by the movement disorder specialists (O.O. and S.A.O.) in the Division of Neurology at Lagos State University Teaching Hospital, Lagos, Nigeria. The patients were diagnosed according to the UK Brain Bank criteria.<sup>5</sup> The study protocol was approved by the Institutional Review Board of Lagos State University Teaching Hospital and Mayo Clinic Florida. For 92 patients and 51 controls, the 11 exons of *GBA* were polymerase chain reaction amplified using previously described primers.<sup>6</sup> The identified variants were labeled according to *GBA* reference sequence (NM\_001005742). The pathogenicity of non-synonymous variants was assessed with in silico tools (Combined Annotation Dependent Depletion, PolyPhen-2, Sorting Intolerant From Tolerant, ClinVar); the Genome Aggregation Database (gnomAD) was used to assess the published variant frequencies.

The demographic characteristics of the studied population are in Table 1. In the PD group, there were 10 variants (5 missense, 4 synonymous, and 1 loss of function) and 4 in controls (3 missense and 1 intronic) (Table 1). The most frequently observed variant in both groups was K(-27) R (6.0% in cases and 4.0% in controls). Using in silico pathogenicity prediction tools, the potential disease-related variants (p.W184R, n = 1; p.L383PfsX3, n = 2; and p.L444P, n = 3) were observed in six PD cases (6.5%), and no likely pathogenic variants were seen in controls.

A limitation of our study is the relatively low number of samples from the Nigerian population; however, studies in Sub-Saharan Africa can be challenging due to access and availability of healthcare. In addition, although our case control study is the first *GBA* screening of patients with PD from Nigeria, we only have limited clinical details. Further genetic studies are needed in Sub-Saharan Africa, and there are still many populations in this region in which no genetic analysis has been performed. The recognition of population-specific mutations responsible for PD may also lead to new biomarker discoveries and support clinical genetic advances in Sub-Saharan populations.<sup>7</sup> The expansion of current international consortia (eg, Human Heredity and Health in Africa, International Parkinson's Disease Genomics Consortium Africa, and Genetic Epidemiology of Parkinson's Disease) and the Global Parkinson's Genetics Program efforts should help to create large, deeply phenotyped clinical cohorts for future studies. ■

Regenerative Medicine, Mayo Clinic in Florida Focused Research Team Program, the gifts from The Sol Goldman Charitable Trust, the Donald G. and Jodi P. Heeringa Family, the Haworth Family Professorship in Neurodegenerative Diseases fund, and The Albertson Parkinson's Research Foundation. He serves as principal investigator or co-principal investigator on Biohaven Pharmaceuticals, Inc. (BHV4157-206 and BHV3241-301) and Neuraly, Inc. (NLY01-PD-1) grants and as external advisory board member for the Vigil Neuroscience, Inc. He serves as co-principal investigator of the Mayo Clinic APDA Center for Advanced Research.

**Received:** 10 August 2021; **Revised:** 27 August 2021; **Accepted:** 30 August 2021

Published online 29 September 2021 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.28803