https://doi.org/10.14802/jmd.24111 / J Mov Disord 2024;17(4):456-458
pISSN 2005-940X / eISSN 2093-4939

LETTER TO THE EDITOR

NBR and *GBA* Gene Methylation Levels in the Peripheral Blood of Parkinson's Disease Patients

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Dear Editor,

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the accumulation of Lewy bodies (LBs) in the substantia nigra. Properly functioning protein quality control systems, such as the unfolded protein response and autophagy, are crucial for neuronal health. Autophagy dysfunction is primarily implicated in neurodegenerative diseases such as Alzheimer's disease, PD, Huntington's disease, multiple sclerosis, and amyotrophic lateral sclerosis.[1] Autophagy is a well-established pathophysiological mechanism in PD development. While many studies have focused on abnormalities in the central nervous system, recent research has identified peripheral and enteric nervous system anomalies.[2] We hypothesized that changes related to autophagy could also be detected in the peripheral blood of PD patients.

To evaluate autophagy in PD, we aimed to assess a gene known to be significant in PD and associated with autophagy, along with a gene that serves as a primary marker of autophagy but has not been identified in PD patients. Therefore, we selected one of the following genes, *LRRK2*, *DJ-1*, *PINK1*, *Parkin*, or *GBA*, as well as the *NBR1* gene, which encodes the primary receptor protein for autophagy but has not been identified in PD.

Our cross-sectional prospective study was conducted at the Departments of Neurology and Medical Genetics at Inonu University, Malatya, Turkey. We included patients who met the UK PD Society Brain Bank clinical diagnostic criteria.[3] Clinical findings were assessed via the Unified Parkinson's Disease Rating Scale (UPDRS). The exclusion criteria for the study included the presence of diseases associated with autophagy, such as diabetes mellitus, cardiovascular diseases, cancer, fatty liver disease, autoimmune diseases, and kidney disease. Additionally, individuals on PD-related medications or those with an active infection at the time of blood collection were excluded.

DNA was extracted from the peripheral blood of PD patients (n = 20) and controls (n = 20). The extracted DNA samples were digested with methylation-sensitive restriction enzymes (HpaII and Hin6I; Fermentas, Vilnius, Lithuania) for 24 hours, and the digestion efficiency was confirmed by running the samples on 1% agarose gels. We used a methylation-specific quantitative polymerase chain reaction approach to assess the methylation status of the genes. Gene primers (Supplementary Table 1 in the online-only Data Supplement) and protocols are provided in the Supplementary Material (in the online-only Data Supplement).

The mean age of the PD patients was 62.05 ± 7.03 years, while the mean age of the controls was 58.15 ± 4.35 years (p = 0.102). The supplementary material includes a dot plot and a statistical analysis of participants before the removal of outliers (Supplementary Figure 1 and Supplementary Table 2 in the online-only Data Supplement), which revealed outliers in terms of gene methylation levels. After these outliers were excluded, the statistical analyses were repeated, and the results are presented in Table 1. The significance of age and sex remained unchanged following the removal of outliers (after removal of *NBR1* gene methylation participants: PD Group n = 20, control n = 13, age

Received: May 9, 2024 **Revised:** June 13, 2024 **Accepted:** July 19, 2024
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Table 1. Clinical charactaristics and gene methylation levels of participants, compared to controls

Genes	Parkinson's Group (*n* = 20)		Control group (*n* = 13)		*p* value
	Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)	
NBR1	0.21 ± 0.23	0.11 (0.01–0.65)	0.45 ± 0.37	0.27 (0.05–1.24)	0.005
Age (yr)	62.05 ± 7.03	63 (50–75)	58.15 ± 4.35	57 (52–68)	0.102
Sex (F/M)	9/11	N/A	5/8	N/A	0.497
UPDRS	32.40 ± 14.47	30.5 (14–70)	N/A	N/A	N/A
*GBA**	0.45 ± 0.52	0.34 (0.01–1.79)	1.06 ± 1.06	0.78 (0.08–3.23)	0.028
Age (yr)	61.38 ± 7.10	62 (50–75)	58.15 ± 4.35	57 (52–68)	0.196
Sex (F/M)	8/10	N/A	5/8	N/A	0.394
UPDRS	30.66 ± 12.05	30.5 (14–63)	N/A	N/A	N/A

Values are presented as mean ± SD or median (min-max) unless otherwise indicated.
*Among participants with the *GBA* gene variant, 18 have been diagnosed with Parkinson's disease.
NBR1, *BRCA1* neighbor for autophagy 1; UPDRS, Unified Parkinson's Disease Rating Scale; *GBA*, glucocerebrosidase; SD, standard deviation; N/A, not applicable.

significance $p = 0.102$; sex [F/M] for PD: 9/11, for control: 5/8, significance $p = 0.497$; for *GBA*: PD $n = 18$, control $n = 13$, age significance $p = 0.196$; sex [F/M] for PD: 8/10, for control: 5/8, significance $p = 0.394$). The methylation levels of *NBR1* and *GBA* were lower in PD patients than in controls ($p = 0.005$ and $p = 0.028$, respectively). No correlation was found between the methylation levels of the genes and age or UPDRS score. According to Table 1, the *NBR1* and *GBA* genes, particularly the *NBR1* gene, were significantly hypomethylated in PD patients compared with those in controls. These findings suggest that the indirect expression of the autophagy-related genes *NBR1* and *GBA* is elevated in the peripheral blood of PD patients.

NBR1 has been shown to have a neuroprotective effect by enabling LB aggregates to enter the autophagosome in synucleinopathies.[1] However, there are no data in the literature concerning epigenetic regulation affecting *NBR1* gene expression in PD patients. In a mouse model of LB disease, the protein and mRNA levels of *NBR1* were increased compared with those in controls.[4] Our study demonstrated that autophagic pathways are upregulated by decreased methylation levels of *NBR1* in the peripheral blood of PD patients and that autophagy is active in the peripheral system of PD patients, similar to the central system. Further studies should investigate *NBR1*-related molecules as potential biomarkers in the peripheral system of PD patients.

GBA is one of the genes most significantly associated with PD. Gaucher disease, a lysosomal storage disease, is caused by a deficiency in the enzyme *GBA*, which interacts bidirectionally with alpha-synuclein to generate positive feedback. Alpha-synuclein aggregation is increased due to *GBA* mutations.[5] None of the PD patients in our study had a history of Gaucher disease. One study reported greater *GBA* enzyme activity in PD patients without *GBA* mutations than in those with mutations and in controls.[6] To the best of our knowledge, there are no data on *GBA* methylation levels in PD patients. In our study, the *GBA* methylation level was also decreased in PD patients, similar to *NBR1* methylation. *GBA*-related molecules could be useful biomarkers in the peripheral blood.

The role of DNA methylation in neurodegenerative diseases is an area of intense research. Studies suggest that DNA methylation may contribute to the pathogenesis of PD.[7] In our study, we selected genes relevant to the pathophysiology of PD and found significant differences between the groups. However, no correlation was found between gene methylation levels and age or the UPDRS.

Recent studies on DNA methylation in the blood cells of PD patients have highlighted significant epigenetic changes that may serve as potential biomarkers for early detection and understanding of the disease.[7] Additionally, the integration of gene expression and DNA methylation data revealed dysregulation of genes associated with neurotransmission and protein degradation pathways, suggesting that DNA methylation patterns could reflect environmental exposures and genetic predispositions, offering a new approach for PD diagnosis and progression monitoring.[8]

In conclusion, the methylation levels of the *NBR1* and *GBA* genes are lower in the peripheral blood of PD patients than in that of controls. These findings suggest that autophagy-related changes are also present peripherally. *NBR1* and *GBA*-related molecules should be investigated in future studies as potential PD biomarkers.

Ethics Statement

The study was approved by the Ethics Committee of the Institute (Ethics number: 2020/05). Informed consent was obtained from the patient.

Supplementary Materials

The online-only Data Supplement is available with this article at https://doi.org/10.14802/jmd.24111.

Conflicts of Interest

The authors have no financial conflicts of interest.

Funding Statement

This study was supported by the Inonu University BAP unit, project number TDK-2020-2263.

Acknowledgments

None

Author Contributions

Conceptualization: Yagmur Inalkac Gemici, Ahmet Koc. Data curation: Yagmur Inalkac Gemici. Formal analysis: Yagmur Inalkac Gemici, Ahmet Koc. Funding acquisition: Ahmet Koc. Investigation: Yagmur Inalkac Gemici. Methodology: Yagmur Inalkac Gemici. Project administration: Ahmet Koc. Supervision: Ahmet Koc. Validation: Ahmet Koc. Visualization: Ahmet Koc. Writing—original draft: Yagmur Inalkac Gemici. Writing—review & editing: Ahmet Koc.

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REFERENCES

1. Filfan M, Sandu RE, Zăvăleanu AD, GreşiŢă A, Glăvan DG, Olaru DG, et al. Autophagy in aging and disease. Rom J Morphol Embryol 2017;58:27-31.
2. Comi C, Magistrelli L, Oggioni GD, Carecchio M, Fleetwood T, Cantello R, et al. Peripheral nervous system involvement in Parkinson's disease: evidence and controversies. Parkinsonism Relat Disord 2014;20:1329-1334.
3. Fereshtehnejad SM, Zeighami Y, Dagher A, Postuma RB. Clinical criteria for subtyping Parkinson's disease: biomarkers and longitudinal progression. Brain 2017;140:1959-1976.
4. Tanji K, Odagiri S, Miki Y, Maruyama A, Nikaido Y, Mimura J, et al. p62 deficiency enhances α-synuclein pathology in mice. Brain Pathol 2015;25:552-564.
5. Behl T, Kaur G, Fratila O, Buhas C, Judea-Pusta CT, Negrut N, et al. Cross-talks among GBA mutations, glucocerebrosidase, and α-synuclein in GBA-associated Parkinson's disease and their targeted therapeutic approaches: a comprehensive review. Transl Neurodegener 2021;10:4.
6. Sawangareetrakul P, Ngiwsara L, Champattanachai V, Chokchaichamnankit D, Saharat K, Ketudat Cairns JR, et al. Aberrant proteins expressed in skin fibroblasts of Parkinson's disease patients carrying heterozygous variants of glucocerebrosidase and parkin genes. Biomed Rep 2021;14:36.
7. Chuang YH, Paul KC, Bronstein JM, Bordelon Y, Horvath S, Ritz B. Parkinson's disease is associated with DNA methylation levels in human blood and saliva. Genome Med 2017;9:76.
8. Wang C, Chen L, Yang Y, Zhang M, Wong G. Identification of potential blood biomarkers for Parkinson's disease by gene expression and DNA methylation data integration analysis. Clin Epigenetics 2019;11:24.