RESEARCH ARTICLE

DNA Methylation Age Acceleration Is Not Associated with Age of Onset in Parkinson's Disease

Johannes J. Gaare, MD, PhD,[1,2,3*] Kim Brügger, PhD,[1,3] Gonzalo S. Nido, PhD,[1,2,3] and Charalampos Tzoulis, MD, PhD[1,2,3*]

[1]*Neuro-SysMed Center, Department of Neurology, Haukeland University Hospital, Bergen, Norway*
[2]*K.G. Jebsen Center for Translational Research in Parkinson's Disease, University of Bergen, Bergen, Norway*
[3]*Department of Clinical Medicine, University of Bergen, Bergen, Norway*

ABSTRACT: **Background:** Epigenetic clocks using DNA methylation (DNAm) to estimate biological age have become popular tools in the study of neurodegenerative diseases. Notably, several recent reports have shown a strikingly similar inverse relationship between accelerated biological aging, as measured by DNAm, and the age of onset of several neurodegenerative disorders, including Parkinson's disease (PD). Common to all of these studies is that they were performed without control subjects and using the exact same measure of accelerated aging: DNAm age minus chronological age.
Objective: We aimed to assess the validity of these findings in PD, using the same dataset as in the original study, blood DNAm data from the Parkinson's Progression Markers Initiative cohort, but also including control samples in the analyses.
Methods: We replicated the analyses and findings of the previous study and then reanalyzed the dataset incorporating control samples to account for underlying age-related biases.
Results: Our reanalysis shows that there is no correlation between age of onset and DNAm age acceleration. Conversely, there is a pattern of overestimating DNAm age in younger and underestimating DNAm age in older individuals in the dataset that entirely explains the previously reported association.
Conclusions: Our findings refute the previously reported inverse relationship between DNAm age acceleration and age of onset in PD. We show that these findings are fully accounted for by an expected over/underestimation of DNAm age in younger/older individuals. Furthermore, this effect is likely to be responsible for nearly identical findings reported in other neurodegenerative diseases. © 2023 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: DNA methylation; epigenetic clocks; Parkinson's disease; neurodegenerative disease

*__Correspondence to:__ Dr. Charalampos Tzoulis or Dr. Johannes J. Gaare, Neuro-SysMed Center of Excellence for Clinical Research in Neurological Diseases, Department of Neurology, Haukeland University Hospital, Jonas Lies vei 71, 5021 Bergen, Norway; E-mail: charalampos.tzoulis@helse-bergen.no or E-mail: johannes.jernqvist.gaare@helse-bergen.no

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

Funding agency: This work was supported by the Western Norway Regional Health Authority (grant F-12133).

Received: 20 April 2023; **Revised:** 4 July 2023; **Accepted:** 24 July 2023

Published online in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.29574

Introduction

Epigenetic clocks have become a popular proxy for biological aging, which is being increasingly used in the study of age-related neurodegenerative disorders. These clocks are based on the observation that DNA methylation (DNAm) profiles can be used to predict chronological age.[1] First-generation clocks, like those of Hannum et al[2] and Horvath,[3] use a fairly limited set of cytosine-phospho-guanine (CpG) sites, 71 and 353, respectively, to predict chronological age in healthy individuals. Second-generation clocks, including PhenoAge[4] and GrimAge,[5] incorporate additional DNAm-based estimates (including various plasma proteins and smoking pack-years) to generate biological age estimates that better predict lifespan and healthspan.

Using a measure of accelerated aging defined as the residuals from a linear regression of DNAm age on chronological age, several studies have shown accelerated DNAm aging, compared with chronological aging, for a broad spectrum of diseases and conditions, including Parkinson's disease (PD),[6] Alzheimer's disease,[7] HIV infection,[8] cancer,[9,10] obesity,[11] and Down syndrome.[12] Notably, the reported effect sizes are generally modest. Moreover, recent studies have reported an intriguing inverse relationship between DNAm age acceleration (AA) and age of onset for multiple neurodegenerative diseases, including PD,[13] amyotrophic lateral sclerosis (ALS),[14,15] and spinocerebellar ataxia (SCA).[16] Specifically, a younger age of onset is associated with increased DNAm AA, whereas a later age of onset is associated with decreased DNAm AA. Based on this observation, it has been proposed that DNAm age could be used as a prognostic biomarker for assessing future PD risk (eg, in *LRRK2* mutation carriers) and as a treatment response marker in clinical trials of candidate disease-modifying agents.[13]

The design and results of all of these studies share some conspicuously similar characteristics. First, the effect sizes reported are universally dramatic and strikingly similar across different diseases, ie, every 5-year increase in DNAm age correlates to a 3.2- to 6.4-year earlier age of onset, irrespective of the disease being studied. Second, the studies use a case-only design and do not investigate whether there could be a general association between DNAm AA and chronological age. This is essential to clarify, because the age of onset for a disease is strongly correlated with chronological age. Third, DNAm AA is not defined as originally proposed, ie, the residuals from a linear regression of DNAm age on chronological age, but simply as the difference between the calculated DNAm age and chronological age. Fourth, not only do young-onset individuals appear prematurely biologically aged (positive DNAm AA), but late-onset individuals appear biologically younger (negative DNAm AA) than their chronological age. The latter directly contradicts previous studies showing increased DNAm age in affected individuals compared with healthy control subjects.[6]

Based on the earlier observations, we speculated that the reported association between DNAm AA and age of disease onset may be driven by a general association between DNAm AA and chronological age, rather than a disease-specific process. To test our hypothesis, we reanalyzed a publicly available dataset of blood DNAm data from the Parkinson's Progression Markers Initiative (PPMI), which had been used in a recent study showing a prominent association between DNAm AA and age of disease onset.[13]

First, we attempted to replicate the previously published results[13] using the same methodology. Subsequently, to account for a potential general underlying association between DNAm AA and chronological age, we incorporated the control subjects in our analyses. Finally, we examined differences in DNAm AA between PD and control subjects.

Subjects and Methods

Subject Cohort and Methylation Analyses

We obtained data from the PPMI cohort (https://www.ppmi-info.org), consisting of baseline clinical characteristics and DNAm data from n = 206 individuals with newly diagnosed PD and n = 81 sex- and age-matched healthy control subjects. Genetic screening for pathogenic mutations in *GBA* and *LRRK2* had been performed for all (PD and healthy control) subjects except for one subject with PD. These and general cohort statistics are available in Table S1.

The DNAm profiles available had been obtained using the Illumina Infinium MethylationEPIC BeadChip Kit. Raw data conversion and quality control were performed using R (v3.6.3) and Bioconductor package *minfi* (v1.28.4).[17] Poor-performing probes, defined as having a detection *P*, computed by *detectionP* in the *minfi* package,[17] of >0.01 in 20% or more of the samples, were removed. The remaining data were SWAN normalized using the *missMethyl*[18] R package (v1.26.1).

Epigenetic Aging Parameters

The Horvath DNAm age calculator (https://dnamage.genetics.ucla.edu) was used to obtain all basic measures of epigenetic aging used in the analyses. The measure of epigenetic used by Tang et al[13] was simply defined as DNAm age (using the Horvath method[3]) minus chronological age. Similar to previously published studies,[12] we defined the parameter AAResidual as the residuals from a linear model regressing DNAm age on chronological age in the control subjects. Specifically, a linear regression of DNAm age on chronological age was fitted for the healthy control subjects, and the resulting regression equation was used to calculate AAResidual for both PD and healthy control subjects (AAResidual = the vertical distance from DNAm age to the regression line fitted for healthy control subjects).

Two other measures of epigenetic aging commonly used are intrinsic epigenetic age acceleration (IEAA) and extrinsic epigenetic age acceleration (EEAA). IEAA is based on the Horvath measure of DNAm age[3] and is designed to be independent of age-related changes in blood cell counts,[3,19] whereas EEAA is based on the Hannum measure of DNAm age[2] and incorporates measures of three blood cell counts (naive cytotoxic T cells, exhausted cytotoxic T cells, and plasmablasts) aiming to capture the aging of the immune system.[2,19]

Statistical Analyses

First, we replicated the analyses by Tang et al[13] and performed a linear regression analysis to determine the

relationship between age of onset and DNAm AA, and adjusting for sex and interval (time between age of onset and sample collection). We additionally used a Cox proportional hazards model for the same purpose, grouping subjects in three groups (AA < −3 [slow], −3 > AA > 3 [normal], and AA > 3 [fast]) and adjusting for sex, interval, and blood cell counts (CDT8, CDT4, B cells, and granulocytes). The covariates were chosen to replicate the exact analyses by Tang et al.[13]

Next, we performed the same analyses using AAResidual as the epigenetic AA parameter, with the same covariates. We also performed similar analyses using IEAA and EEAA, but adjusting for only sex and interval, because blood cell counts are, by definition, used in the calculation of these terms themselves.[6]

We assessed the validity of the DNAm age prediction algorithm by performing linear regression analyses in PD and healthy control subjects separately, adjusting for sex. Finally, linear regression models were used to explore differences in IEAA, EEAA, and AAResidual between PD and healthy control subjects, adjusting for sex and age.

Results

In our replication analyses, DNAm AA was defined as DNAm age minus chronological age (AA), as described by Tang et al.[13] We then used healthy control subjects to account for any underlying age-related biases in the dataset, by fitting a linear model for chronological age on DNAm age in the healthy control subjects and using that model to calculate residuals in the subjects with PD as a measure of DNAm AA (AAResidual), in line with previous studies.[12] Because they have previously been associated with PD,[6] we also performed analyses using two other commonly used measures of DNAm AA, namely, IEAA and EEAA.

Replicating the analysis described by Tang et al[13] by the exact same methodology yielded similar results (Figs. 1A and 2A), demonstrating an inverse relationship between DNAm AA and age of onset. However, when using residuals from the regression of DNAm age on chronological age in the control subjects (AAResidual) as the AA parameter, we observed no significant association between DNAm AA and age of onset (Figs. 1B and 2B). Similarly, no statistically significant effects were observed when using alternative measures of epigenetic aging as DNAm AA parameters (IEAA, linear regression: $P = 0.87$, $B = 0.03$, $R^2 < 0.01$; see Figs. S1 and S2; EEAA, linear regression: $P = 0.83$, $B = -0.04$, $R^2 < 0.01$; see Figs. S3 and S4). Notably, in this dataset, the mean interval between age of onset and sample collection was 2.0 ± 1.93 years), meaning that age of onset and chronological age are almost interchangeable (see Fig. 3). This phenomenon is further emphasized by substituting age of onset with chronological age in the Cox proportional hazards model, showing almost identical results (Fig. 2C,D).

Next, we assessed the validity of the DNAm age prediction by performing a linear regression analysis in patients with PD and healthy control subjects, adjusting for sex. There was, as expected, a strong correlation between DNAm age and chronological age ($P = 2 \times 10^{-16}$, $B = 0.69$, $R^2 = 0.76$), but no difference between patients with PD and healthy control subjects ($P = 0.22$; Fig. 4A). Both the patients with PD and

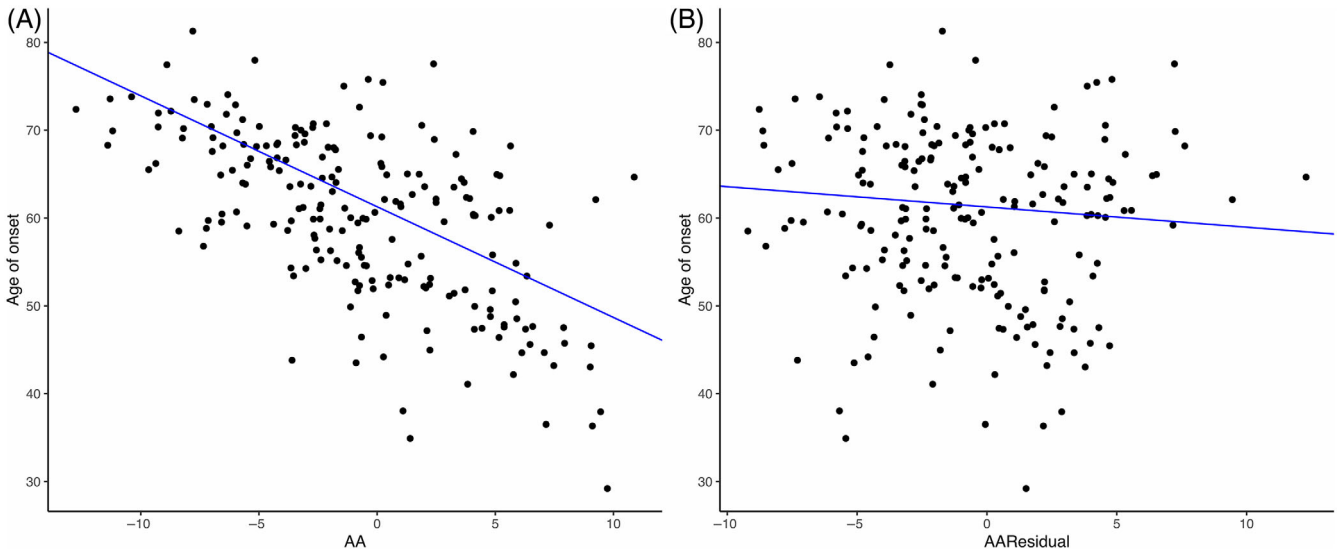

FIG. 1. Scatterplot of DNA methylation (DNAm) age acceleration (AA) and age of onset of Parkinson's disease (PD). (**A**) Replication of the analysis reported by Tang et al[13] using AA (DNAm age − chronological age) as the DNAm AA parameter. Linear regression analysis ($P = 2 \times 10^{-16}$, $B = -1.26$, $R^2 = 0.41$), adjusted for sex and interval (between age of onset and sample collection). (**B**) Same methodology as (**A**) but using AAResidual as the DNAm AA parameter. Linear regression analysis ($P = 0.20$, $B = -0.23$, $R^2 < 0.01$), adjusted for sex and interval (between age of onset and sample collection). All values are measured in years. [Color figure can be viewed at wileyonlinelibrary.com]

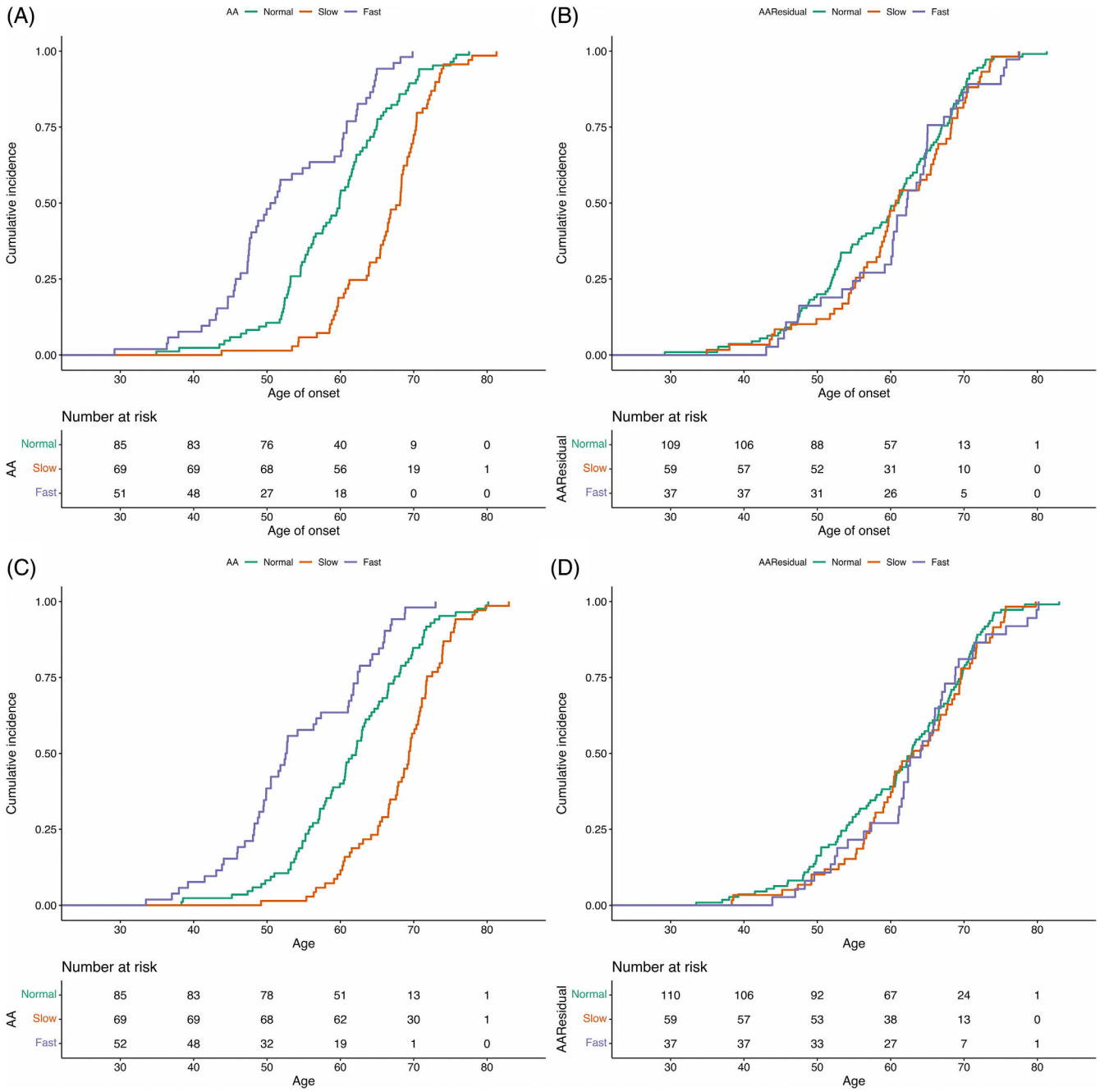

FIG. 2. Kaplan–Meier plot showing the effect of DNA methylation (DNAm) age acceleration (AA) on age of onset. (**A**) Replication of the analysis reported by Tang et al[13] using AA (DNAm age − chronological age) as the DNAm AA parameter. The analysis shows a protective effect of slow (<−3) AA ($P = 2.43 \times 10^{-7}$, hazards ratio [HR] = 0.39 [95% confidence interval (CI): 0.27–0.56]) and detrimental effect of fast (>3) AA ($P = 7.30 \times 10^{-6}$, HR = 2.32 [95% CI: 1.61–3.36]). (**B**) Same methodology as (**A**) but using AAResidual as the DNAm AA parameter. The analysis shows no effect of slow ($P = 0.08$, HR = 0.74 [95% CI: 0.52–1.04]) or fast ($P = 0.41$, HR = 0.84 [95% CI: 0.56–1.26]) DNAm AA on age of onset. (**C**) Same analysis as in (**A**), but substituting age of onset with chronological age shows an almost identical effect of slow (<−3) AA ($P = 2.55 \times 10^{-7}$, HR = 0.39 [95% CI: 0.28–0.56]) and fast (>3) AA ($P = 7.36 \times 10^{-6}$, HR = 2.31 [95% CI: 1.60–3.34]). (**D**) Same analysis as in (**B**), but substituting age of onset with chronological age similarly shows no effect of slow ($P = 0.07$, HR = 0.73 [95% CI: 0.52–1.03]) or fast ($P = 0.39$, HR = 0.84 [95% CI: 0.56–1.25]) DNAm AA. All values are measured in years. [Color figure can be viewed at wileyonlinelibrary.com]

healthy control subjects deviate from a perfect correlation (DNAm age = chronological age) in that younger individuals appear epigenetically slightly older (DNAm age > chronological age) than their chronological age, whereas the inverse is true for older individuals (DNAm age < chronological age). This is further emphasized when fitting a linear model of AA (DNAm age minus chronological age) on chronological age (Fig. 4B), adjusting for sex. In this study, we see a strong inverse relationship between AA and chronological age in both

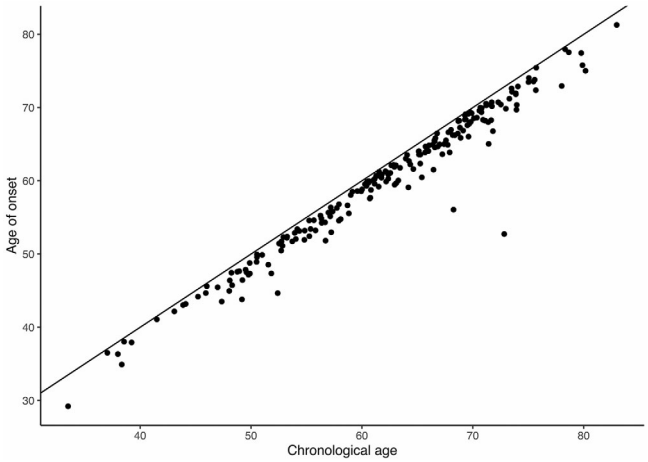

FIG. 3. Relationship between age of onset of Parkinson's disease and age at sample collection. The black line is drawn using the function $y = x$, so the time from age of onset to sample collection can be ascertained by taking the vertical distance between the points and the line.

patients with PD and control subjects. These results prove that the inverse relationship observed between DNAm AA and age of onset (Figs. 1A and 2A) cannot be attributed to an effect of PD, because the same pattern can be observed in healthy control subjects. This phenomenon is, therefore, driven by a general association between chronological age and DNAm AA, irrespective of disease state.

In further support of these findings, using a linear model and adjusting for age and sex, we did not detect any statistically significant differences in DNAm age between patients with PD and healthy control subjects (Fig. 5), as measured by AAResidual ($P = 0.22$), IEAA ($P = 0.35$), or EEAA ($P = 0.79$).

Discussion

Our results refute the previously reported relationship between DNAm AA and age of onset of PD.[13] Because the age of onset of neurodegenerative diseases such as PD is commonly set relatively close to the time of diagnosis (and thereby inclusion and sample collection in prospective studies such as the PPMI), the relationship between age of onset and DNAm age is virtually the same as between chronological age and DNAm age. It is therefore essential to use a control population, to ensure that there are no general underlying age-related confounding effects present in the dataset. Reliably measuring DNAm AA requires the use of a control population to establish a baseline and calculate residuals in the disease population.[12] The problem with using DNAm age minus chronological age as a measure of DNAm AA is that this approach relies on the assumption that the regression line in a hypothetical control population follows chronological age perfectly ($y = x$). This is, however, not necessarily the case, as we clearly show in Fig. 4 for the PPMI dataset, where the measures of DNAm age deviate from this perfect

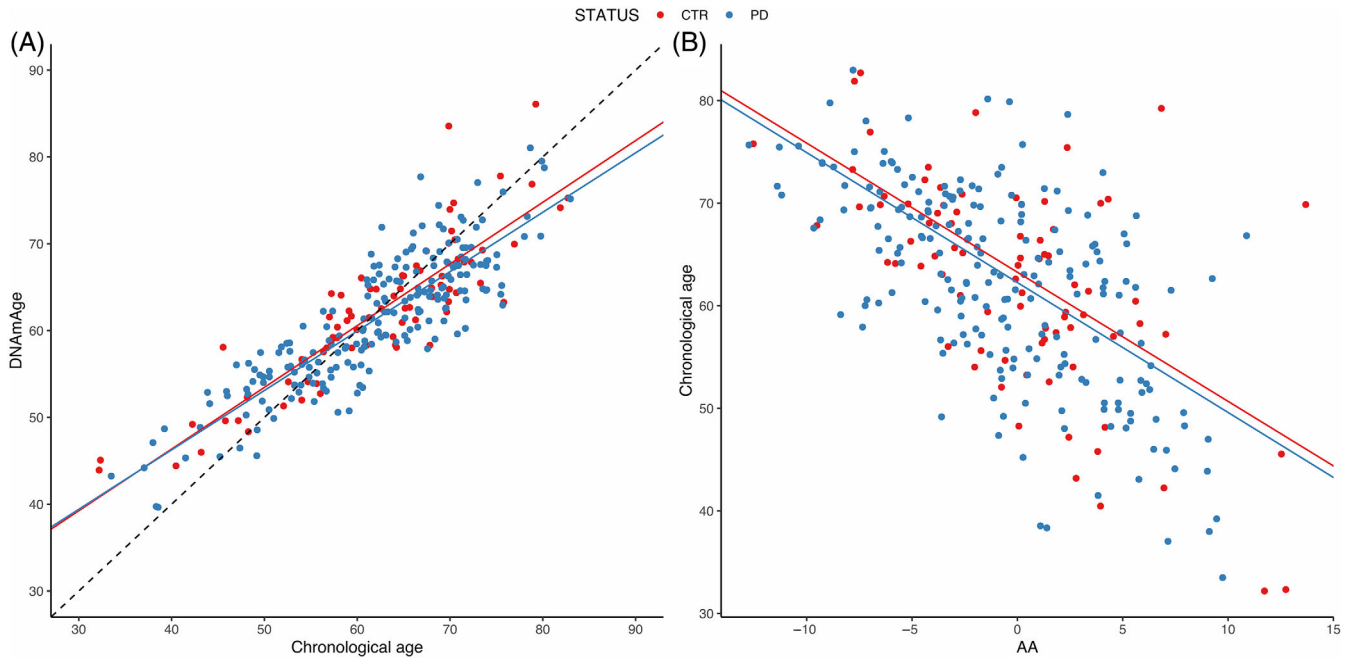

FIG. 4. Relationship between chronological age, DNA methylation (DNAm) age, and DNAm age acceleration (AA) in patients with Parkinson's disease (PD) and healthy control (CTR) subjects. (**A**) Linear regression of chronological age on DNAm age (Horvath) adjusting for sex. The colored lines are the regression lines from a model fitted in PD and control separately, and the black dashed line represents $y = x$, ie, a perfect correlation between DNAm age and chronological age. Patients with PD ($y = 0.68x + 18.9$) and healthy control subjects ($y = 0.71x + 17.9$) deviate from the perfect correlation in a similar fashion. (**B**) Linear regression of DNAm AA on chronological age, adjusting for sex. The colored lines are the regression lines from a model fitted in PD ($P < 2 \times 10^{-16}$, $B = -1.26$) and control ($P = 1.7 \times 10^{-9}$, $B = -1.26$). [Color figure can be viewed at wileyonlinelibrary.com]

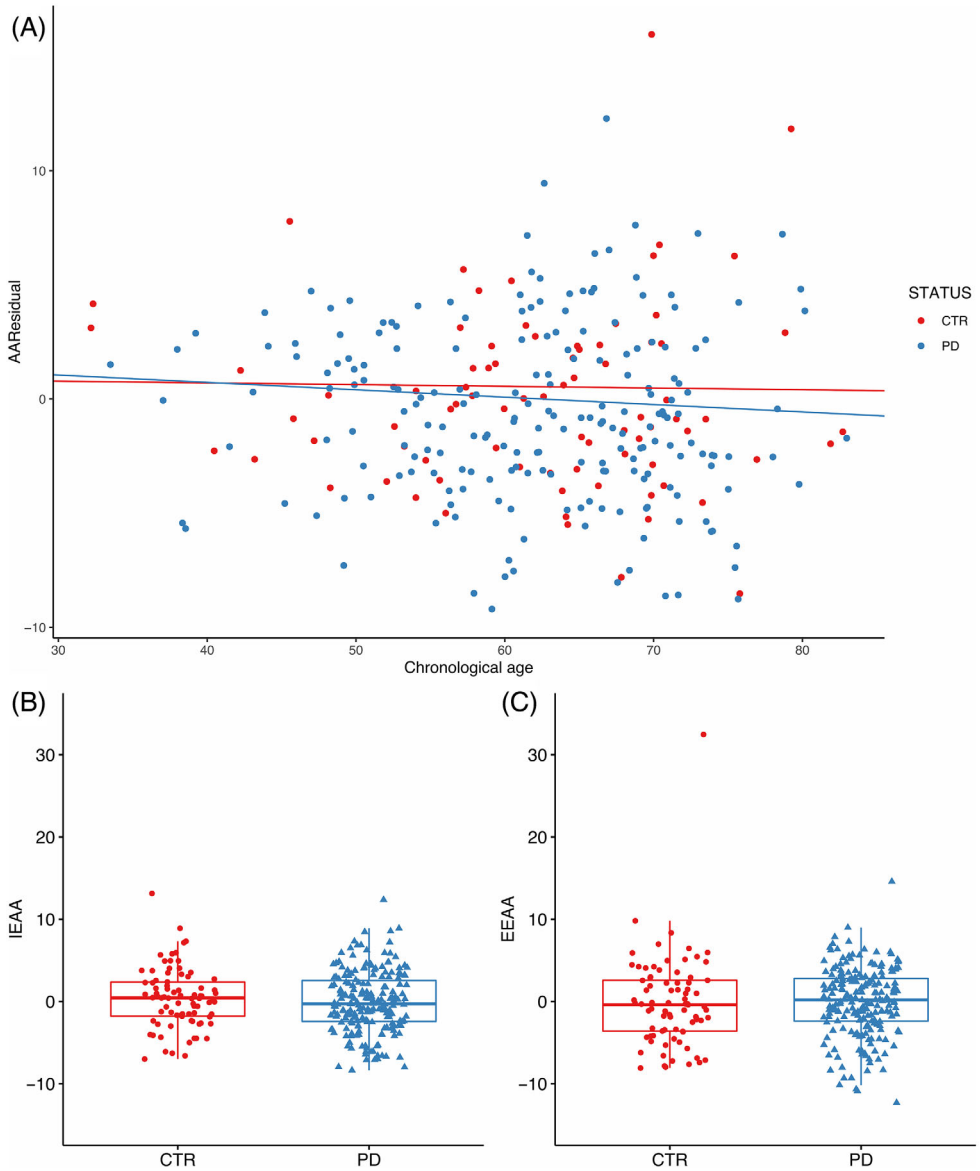

FIG. 5. Differences between Parkinson's disease (PD) and control (CTR) for different measures of DNA methylation (DNAm) age acceleration (AA). (**A**) Scatterplot of DNAm AA (AAResidual) and chronological age. A linear regression adjusting for age and sex showed no statistically significant difference between PD and control ($P = 0.22$, $B = -0.61$). (**B**) Boxplot showing differences in intrinsic epigenetic AA (IEAA) between PD and control. No statistically significant difference as assessed by a linear regression ($P = 0.35$), adjusting for age and sex. (**C**) Boxplot showing differences in extrinsic epigenetic AA (EEAA) between PD and control. No statistically significant difference as assessed by a linear regression ($P = 0.79$), adjusting for age and sex. [Color figure can be viewed at wileyonlinelibrary.com]

correlation. Crucially, this deviation is the same for both patients with PD and healthy control subjects. The effect of DNAm AA on age of onset observed in Fig. 1A can therefore be entirely attributed to this deviation of the prediction algorithm, rather than reflecting a causal relationship with PD.

First-generation epigenetic clocks are based on the observation that the methylated fraction of certain CpGs is highly correlated with chronological age. However, the CpGs of the clock have themselves no known direct biological relevance and a limited capability in terms of being an accurate measure of biological age.[20] The Horvath methylation clock was trained on data obtained by using the Illumina 27K and 450K platforms,[3] whereas the studies reporting an inverse relationship between DNAm AA and age of onset used data from both the Illumina 450K[14] and EPIC[13,15,16] array. The Illumina EPIC array, released in 2015,[21] has largely replaced the older platforms (27K and 450K), and although it lacks 19 of the 353 CpGs used in the Horvath clock, this does not appear to greatly affect DNAm age predictions.[22] However, because different methods of preprocessing are known to influence and even skew DNAm age estimates, it is generally

advisable to use residuals of a linear regression, rather than simply subtracting chronological age from DNAm age, as a measure of DNAm AA.[22]

In the PPMI dataset, we observed that DNAm age estimates were skewed toward an overestimation in younger individuals and an underestimation in older individuals, irrespective of disease state. The same phenomenon has been observed in multiple studies showing that Horvath DNAm age estimates from both the 450K and the EPIC array are skewed toward an overestimation of DNAm age in younger individuals and an underestimation in older individuals.[23,24] The reported inverse correlation between DNAm AA and age of onset in PD[13] can be entirely explained by this phenomenon and is, therefore, not related to PD. This is likely to be responsible for the remarkably similar correlations and effect sizes reported in ALS[14,15] and SCA.[16] Although data from these studies were not available to us, we would urge the authors to repeat the analyses including healthy control subjects and clarify whether the observed correlation between DNAm AA and age of onset is related to the disease, or simply confounded by the chronological age of the individuals.

In our reanalysis of the PPMI cohort comparing patients with PD with healthy control subjects, we were not able to replicate the results of an earlier study showing increased epigenetic AA in PD.[6] One reason could be that in the previous study, the difference between patients with PD and healthy control subjects across all examined AA parameters (AAResidual, IEAA, and EEAA) was larger in the Hispanic subpopulation than in the non-Hispanic subpopulation. The proportion of individuals with Hispanic ethnicities in the PPMI cohort was only approximately 2.1%, compared with 14.2% in the previous study. Another possible reason for the difference in results could be because of methodological differences, because the previous study used data obtained using the Illumina 450K array, whereas the PPMI cohort methylation data were obtained using the Illumina EPIC array.

Although we believe epigenetic clocks are a powerful and potentially important method in studying biological aging in neurodegenerative diseases, our study emphasizes the need for a careful and considerate implementation to avoid false-positive and misleading results. Crucially, because there appears to be a systematic underestimation in older individuals and, conversely, an overestimation in younger individuals of DNAm age using the Horvath clock, our study emphasizes the importance of control subjects to validate the disease relevance of any findings. At the very least, studies should avoid using DNAm age minus chronological age as a measure of DNAm AA and instead use residuals from a regression model fitted to the dataset in question, even if controls are not present. In conclusion, our findings strongly suggest that the results of previous studies showing an inverse relationship between age of onset and DNAm AA in neurodegenerative diseases can be entirely attributed to this phenomenon and do not reflect disease-specific processes. ■

Acknowledgments: Data used in the preparation of this article were obtained from the PPMI database (https://www.ppmi-info.org/access-data-specimens/download-data). For up-to-date information on the PPMI study, visit https://www.ppmi-info.org. PPMI, a public-private partnership, is funded by The Michael J. Fox Foundation for Parkinson's Research and funding partners, including 4D Pharma; AbbVie Inc.; AcureX Therapeutics; Allergan; Amathus Therapeutics; Aligning Science Across Parkinson's; Avid Radiopharmaceuticals; Bial Biotech; Biogen; BioLegend; Bristol Myers Squibb; Calico Life Sciences LLC; Celgene Corporation; DaCapo Brainscience; Denali Therapeutics; the Edmond J. Safra Foundation; Eli Lilly and Company; GE Healthcare; GlaxoSmithKline; Golub Capital; Handl Therapeutics; Insitro; Janssen Pharmaceuticals; Lundbeck; Merck & Co. Inc.; Meso Scale Diagnostics, LLC; Neurocrine Biosciences; Pfizer Inc.; Piramal Imaging; Prevail Therapeutics; F. Hoffmann-La Roche Ltd and its affiliated company Genentech Inc.; Sanofi Genzyme; Servier; Takeda Pharmaceutical Company; Teva Neuroscience Inc.; UCB; Vanqua Bio; Verily Life Sciences Inc.; Yumanity Therapeutics Inc.; and Voyager Therapeutics.

Data Availability Statement

The data used in this study are available through the Parkinson's Progression Markers Initiative website (https://www.ppmi-info.org). The R code detailing the analyses presented in the study is publicly available in the Neuromics repository (https://git.app.uib.no/neuromics/dna-methylation-age-acceleration).

References

1. McCrory C, Fiorito G, Hernandez B, et al. GrimAge outperforms other epigenetic clocks in the prediction of age-related clinical phenotypes and all-cause mortality. J Gerontol: Series A 2021;76(5):741–749.
2. Hannum G, Guinney J, Zhao L, et al. Genome-wide methylation profiles reveal quantitative views of human aging rates. Mol Cell 2013;49(2):359–367.
3. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol 2013;14(10):3156.
4. Levine ME, Lu AT, Quach A, et al. An epigenetic biomarker of aging for lifespan and healthspan. Aging 2018;10(4):573–591.
5. Lu AT, Quach A, Wilson JG, et al. DNA methylation GrimAge strongly predicts lifespan and healthspan. Aging 2019;11(2):303–327.
6. Horvath S, Ritz BR. Increased epigenetic age and granulocyte counts in the blood of Parkinson's disease patients. Aging 2015;7(12):1130–1142.
7. Levine ME, Lu AT, Bennett DA, Horvath S. Epigenetic age of the pre-frontal cortex is associated with neuritic plaques, amyloid load, and Alzheimer's disease related cognitive functioning. Aging 2015;7(12):1198–1211.
8. Horvath S, Levine AJ. HIV-1 infection accelerates age according to the epigenetic clock. J Infect Dis 2015;212(10):1563–1573.
9. Dugué P-A, Bassett JK, Joo JE, et al. DNA methylation-based biological aging and cancer risk and survival: Pooled analysis of seven prospective studies. Int J Cancer 2018;142(8):1611–1619.
10. Levine ME, Hosgood HD, Chen B, Absher D, Assimes T, Horvath S. DNA methylation age of blood predicts future onset of lung cancer in the women's health initiative. Aging 2015;7(9):690–700.
11. Horvath S, Erhart W, Brosch M, et al. Obesity accelerates epigenetic aging of human liver. Proc Natl Acad Sci U S A 2014;111(43):15538–15543.

12. Horvath S, Garagnani P, Bacalini MG, et al. Accelerated epigenetic aging in Down syndrome. Aging Cell 2015;14(3):491–495.

13. Tang X, Gonzalez-Latapi P, Marras C, et al. Epigenetic clock acceleration is linked to age at onset of Parkinson's disease. Mov Disord 2022;37(9):1831–1840.

14. Zhang M, Tartaglia MC, Moreno D, et al. DNA methylation age-acceleration is associated with disease duration and age at onset in C9orf72 patients. Acta Neuropathol 2017;134(2):271–279.

15. Zhang M, McKeever PM, Xi Z, et al. DNA methylation age acceleration is associated with ALS age of onset and survival. Acta Neuropathol 2020;139(5):943–946.

16. Li J, Shu A, Sun Y, et al. DNA methylation age acceleration is associated with age of onset in Chinese spinocerebellar ataxia type 3 patients. Neurobiol Aging 2022;113:1–6.

17. Fortin J-P, Triche TJ Jr, Hansen KD. Preprocessing, normalization and integration of the Illumina HumanMethylationEPIC array with minfi. Bioinformatics 2017;33(4):558–560.

18. Phipson B, Maksimovic J, Oshlack A. missMethyl: an R package for analyzing data from Illumina's HumanMethylation450 platform. Bioinformatics 2016;32(2):286–288.

19. Smith JA, Raisky J, Ratliff SM, et al. Intrinsic and extrinsic epigenetic age acceleration are associated with hypertensive target organ damage in older African Americans. BMC Med Genomics 2019; 12(1):141.

20. Bernabeu E, McCartney DL, Gadd DA, et al. Refining epigenetic prediction of chronological and biological age. Genome Med 2023; 15(1):12.

21. Logue MW, Smith AK, Wolf EJ, et al. The correlation of methylation levels measured using Illumina 450K and EPIC BeadChips in blood samples. Epigenomics 2017;9(11):1363–1371.

22. McEwen LM, Jones MJ, Lin DTS, et al. Systematic evaluation of DNA methylation age estimation with common preprocessing methods and the Infinium MethylationEPIC BeadChip array. Clin Epigenetics 2018;10(1):123.

23. Dhingra R, Kwee LC, Diaz-Sanchez D, et al. Evaluating DNA methylation age on the Illumina MethylationEPIC bead Chip. PloS One 2019;14(4):e0207834.

24. El Khoury LY, Gorrie-Stone T, Smart M, et al. Systematic underestimation of the epigenetic clock and age acceleration in older subjects. Genome Biol 2019;20(1):283.

Supporting Data

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

SGML and CITI Use Only
DO NOT PRINT

Author Roles

Johannes J. Gaare conceptualized and designed the study, analyzed and interpreted the data, and wrote the first draft of the manuscript. Kim Brügger performed preprocessing and quality control of the DNA methylation dataset, as well as revision of the manuscript. Gonzalo S. Nido was a contributor to data analysis and interpretation, as well as revision of the manuscript. Charalampos Tzoulis was responsible for acquisition of the data and was a contributor to study design, data interpretation, and critical revision of the manuscript.

Financial Disclosures

J.J.G. has received grants from the Regional Health Authority of Western Norway and is employed at the Department of Neurology at Haukeland University Hospital. K.B. and G.S.N. are employed at the Department of Medicine, University of Bergen. C.T. has received consultancies and honoraria from Biogen, Desitin, and Merz; has submitted patent applications pertaining to new therapies for Parkinson's disease; and has received grants from The Michael J. Fox Foundation, The Research Council of Norway, and the Regional Health Authority of Western Norway.