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Authors

Fu, Katherine A
Paul, Kimberly C
Lu, Ake T
et al.

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DNA methylation-based surrogates of plasma proteins are associated with Parkinson's disease risk

Katherine A. Fu[1,*], **Kimberly C. Paul**[2], **Ake T. Lu**[3], **Steve Horvath**[3,4], **Adrienne M. Keener**[1,5], **Yvette Bordelon**[1], **Jeff M. Bronstein**[1], **Beate Ritz**[1,2,*]

[1]Department of Neurology, UCLA David Geffen School of Medicine, Los Angeles, California, 90095, USA

[2]Department of Epidemiology, UCLA Fielding School of Public Health, Los Angeles, California, 90095, USA

[3]Department of Human Genetics, UCLA David Geffen School of Medicine, Los Angeles, California, 90095, USA

[4]Department of Biostatistics, UCLA Fielding School of Public Health, University of California Los Angeles, Los Angeles, California, 90095, USA.

[5]Department of Neurology, Veterans Administration Greater Los Angeles Healthcare System, Los Angeles, California, 90073, USA

Abstract

Background: The epigenome may reflect Parkinson's disease (PD) risk, which serves as a point of convergence of genetic and environmental risk factors. Here, we investigate whether blood DNA methylation (DNAm) markers are associated with PD risk.

Methods: We selected 12 plasma proteins known as predictors of cardiovascular conditions and mortality to evaluate their effects on PD risk in a case-control study. In lieu of protein level measures, however, we assessed the influence of their DNAm surrogates. Primary analysis was restricted to 569 PD patients and 238 controls with DNAm data available. Using univariate logistic regression, we evaluated associations between the DNAm markers and PD.

[*]**Corresponding authors:** Katherine A. Fu: kfu@mednet.ucla.edu, Beate Ritz: britz@ucla.edu, **Contact Information:**Katherine A. Fu, Department of Neurology, 710 Westwood Plaza, Room A246, Los Angeles, CA 90095, Telephone number: 949-302-6565.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of the University of California, Los Angeles (IRB#11-001530, date of approval 5/25/2011).

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Results: Of the 12 DNAm surrogates, the most robustly associated were DNAm EFEMP-1 and DNAm CD56, which were associated with PD with and without controlling for blood cell composition. DNAm EFEMP-1 was associated with a decreased risk of PD (OR= 0.83 per SD, 95% CI= 0.70, 0.98) whereas DNAm CD56 was associated with an increased risk of PD (OR =1.41, 95% CI= 1.11, 1.79).

Conclusions: Several DNAm markers, selected as part of a panel to track cardiovascular outcomes and mortality, were associated with PD risk. DNAm markers may inform of factors that are affected differentially in early PD patients compared with controls.

Keywords

Parkinson's disease; epigenetics; DNA methylation; disease risk; cardiovascular disease

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disease classically characterized by motor features including resting tremor, bradykinesia, rigidity, while non-motor features of PD include autonomic dysfunction and cognitive complaints [1]. Some of the complex features of PD risk may be captured by the epigenome, which serves as a reflection of both genetic and environmental risk factors. Methylation patterns in the blood of Parkinson's disease (PD) patients differ between PD cases and controls and may be related to the onset of PD [2]. DNA methylation (DNAm) is a modification in which 5-methylcytosine is the result of a methyl group added to cytosine, a process important for regulating gene expression, cellular differentiation and development [3]. Changes in methylation occur gradually [4], are a strong predictor of aging [5], and also result from long term and lifelong exposures such as diet and smoking [6,7].

There are potential links between cardiovascular disease (CVD) and PD including shared risk factors, such as older age, male sex, and physical inactivity as well as biological processes such as inflammation, disturbances in lipid and glucose metabolism, insulin resistance, and oxidative stress [8]. A more holistic understanding of the contributions of cardiovascular comorbidities in PD may benefit both research and patient treatment. To assess differences related to CVD between PD patients and controls, we use DNAm array derived markers pertaining to levels of plasma proteins measured in blood samples of individuals from the Framingham heart study (FHS) Offspring Cohort; for 12 out of 88 plasma proteins, our lab (Horvath & Lu) built DNAm-based surrogate markers that showed a moderately high correlation ($r>0.35$) between measured blood levels and the respective DNA methylation markers [9]. These 12 identified plasma proteins included adrenomedullin (ADM), beta-2-microglobulin (B2M), CD56, ceruloplasmin, cystatin C (Cystatin C), EGF fibulin-like ECM protein1 (EFEMP1), growth differentiation factor 15 (GDF-15), leptin, myoglobin, plasminogen activator inhibitor 1 (PAI-1), paraoxonase/arylesterase 1 (PON1), and tissue inhibitor metalloproteinase 1 (TIMP-1). The initial 88 plasma protein panel from which these 12 plasma proteins were derived was selected by the FHS to identify protein biomarkers with the potential to predict cardiovascular outcomes and all-cause mortality [10]. In the FHS, the 12 proteins with DNAm surrogates were associated with outcomes including atherosclerotic cardiovascular disease (CVD) (GDF-15, TIMP1, Cystatin-C), heart

failure (GDF-15, ADM, B2M, Cystatin-C, TIMP1, EFEMP1), CVD death (GDF-15, B2M, TIMP1, ADM, EFEMP1, Cystatin-C, ceruloplasmin) and all-cause mortality (GDF-15, B2M, TIMP1, ADM, EFEMP1, Cystatin-C, PON1, ceruloplasmin) [10], while the majority were also associated with aging, sex, and other age-related comorbidities, including type 2 diabetes, in three independent studies [9]. Also, a number of these plasma proteins have previously been implicated in PD, including B2M, TIMP1, and Cystatin-C [11–13]. Thus, we assess associations between the 12 DNAm markers and PD status given that these epigenomic markers likely reflect longer-term states or trends in protein levels as they tend to change only gradually, i.e. are less prone to shorter term fluctuations compared with plasma proteins levels.

2. Materials and Methods

Informed consent was obtained from all subjects, and the study protocol was approved by the UCLA institutional review board.

2.1 Study Population

The Parkinson's disease, Environment, and Genes (PEG) study was designed to investigate the interplay between environmental and genetic risk factors in PD [14]. PEG is a population-based study of residents of California's agricultural Central Valley (Kern, Fresno, and Tulare counties), designed first as a case-control study to investigate PD susceptibility (2001–2007 & 2010–2016; n=833 PD patients; n=898 population-based community controls), and second as a longitudinal cohort with prospective follow-up of PD patients for progression (2010–2013 & 2016-present; n=526, 2–4 follow-up exams and a mean follow-up of 4.7 years (SD=2.8)). Local neurologists, clinics, community outreach efforts, and then a pilot PD registry in California starting in 2012 helped to identify eligible patients. Recruitment and participation information has been published [15]. Population controls (community members) were identified for PEG using Medicare lists (prior to HIPAA) and residential tax assessor records. Controls were randomly selected from clusters of five neighboring households, who study staff then contacted in person at their homes. One eligible household member was allowed to enroll.

Movement disorder specialists (co-authors: J.B., Y.B., A.K.) evaluated all patients (n=833) at least once at baseline, many on multiple occasions, and confirmed patients as having probable idiopathic PD based on published criteria. PD patients were enrolled generally within three years of PD diagnosis. Primary analysis was restricted to n=569 PD patients and n=238 controls for whom we had DNA methylation data available. Those with and without methylation data were similar: 62% vs 65% male, 77% vs 76% European ancestry, mean age 70.4 years (SD=11.7) vs 70.5 years (9.8).

2.2 DNA Methylation and DNAm Biomarkers

DNA was extracted from peripheral whole blood, and we profiled and processed DNA samples using the Illumina Infinium 450k platform (486k CpGs) according to the manufacturer's protocol by applying standard settings. The methylation data from the 450k array is publicly available on GEO (accession numbers GSE72774 and GSE72776). The

surrogate DNAm biomarkers used for analysis were previously generated for the following 12 plasma proteins based on CpG patterns: ADM, B2M, cystatin-C, GDF-15, leptin, PAI-1, and TIMP-1, neural cell adhesion molecule (also known as CD56), EFEMP1, myoglobin, PON1, and ceruloplasmin [9]. Our lab correlated the levels of 88 plasma protein variables (measured using immunoassays) with DNAm array data generated from the same blood samples of n=2,356 individuals from the Framingham heart study. The FHS data was divided randomly into a training set (70% of the FHS pedigrees, N= 1731 individuals from 622 pedigrees) and a test data set (30% pedigrees, N=625 individuals from 266 pedigrees). We used the training data to define DNAm-based surrogate markers of 88 plasma protein variables. We restricted the analysis to CpGs that are present on both the Illumina Infinium 450K array and the new Illumina EPIC methylation array in order to ensure future compatibility. Each of the 88 plasma protein variables (dependent variable) was regressed on chronological age, sex, and the CpGs levels in the training data using an elastic net regression model, which automatically selected a subset of CpGs (typically fewer than 200 CpGs) whose linear combination best predicted the corresponding plasma level in the training data. For example, the DNAm levels of 137 CpGs and 211 CpGs allowed us to estimate the plasma levels of GDF15 and PAI-1, respectively. The predicted DNAm values of GDF15 and PAI-1 can then be used as surrogate markers for the measured plasma levels. Only 12 of the 88 plasma proteins exhibited a moderately high correlation coefficient ($r>0.35$) between their measured levels and their respective DNAm-based surrogate marker in the test data set. We calculated age-adjusted levels for each DNAm marker in PEG participants.

We estimated whole blood counts (WBC) using Houseman's estimation method [16] that estimates the proportions of CD8+ T cells, CD4+ T, natural killer, B cells, and granulocytes (mainly neutrophils).

2.3 Statistical Methods

To assess the associations between the DNAm markers with PD onset (n=569 PD patients and n=238 controls), we used logistic regression to estimate odds ratios (ORs) and 95% CIs. Age at blood draw, sex, ancestry estimated from genetic ancestry informative markers, and smoking were controlled for as potential confounders. The DNAm variables were standardized by z-transformation and units correspond to an increase of one-standard deviation.

3. Results

Our analysis included 238 controls and 569 PD patients; characteristics of the study population can be found in Table 1. The patients were slightly older than the controls (70.5 (SD=9.8) vs 67.5 (SD=12.8)) and had a higher proportion of men, Hispanic ethnicity, and never smokers.

The main findings for the 12 DNAm plasma protein markers with PD status can be found in Table 2. In total, five markers showed evidence of association ($p < 0.05$), while another two showed suggestive associations ($0.05 < p < 0.10$). We observed a positive, moderate association between DNAm CD56 and PD risk (OR=1.41 per SD, 95% CI=1.11, 1.79),

which remained after controlling for cell composition (OR=1.34, 95% CI=1.05, 1.70). DNAm EFEMP-1 was negatively associated with PD (OR=0.83, 95% CI=0.70, 0.98), which was also robust to adjustment for cell composition (OR=0.80, 95% CI=0.68, 0.95). Additionally, DNAm B2M (OR=1.20, 95% CI=1.02, 1.41), DNAm TIMP-1 (OR=1.23, 95% CI=1.03, 1.46), and DNAm Cystatin C (OR=1.19, 95% CI=1.00, 1.42) were associated with PD. DNAm ADM (OR=1.21, 95% CI=0.99, 1.47) and DNAm PON1 (OR=0.90, 95% CI=0.77, 1.05) were suggestively associated with PD, however, effect estimates were attenuated towards the null when we controlled for cell composition (Table 2, Model 2).

Analyses stratified by sex did not identify any additional statistically significant associations. Supplemental Figure 1 demonstrates Spearman correlations between the DNAm plasma protein surrogates that were suggestively associated with PD as well as correlations between the DNAm plasma protein surrogates and different lifestyle, co-morbidity, and sex factors among PD patients. Supplemental Figure 2 includes the age correlations for all DNAm plasma protein surrogates.

4. Discussion

This study investigated associations between Parkinson's disease and epigenetic markers for plasma proteins previously identified as contributing to CVD risk and mortality. Of the epigenetic markers identified, DNAm EFEMP-1 was negatively associated with Parkinson's disease, even after correcting for blood cell composition. Epidermal growth factor-containing fibulin-like extracellular matrix protein 1 (EFEMP1), also known as fibulin 3, is part of a family of six secreted extracellular matrix-associated proteins with calcium-binding, epidermal growth factor-like modules followed by a carboxyl-terminal fibulin-type module [17]. It is also a strong interacting protein and binding partner of Tissue Inhibitor of Metalloproteinases-3 (TIMP-3) [17]. The interaction between these proteins has been proposed to lead to degradation-resistant, potentially cross-linked complexes, which result in decreased trafficking of molecules and thereby lead to the accumulation of abnormal protein deposits.

Our results suggest that DNAm EFEMP-1 is associated with a decreased risk for Parkinson's disease or decreases early in PD. While there is limited literature discussing a direct mechanistic role for EFEMP-1 in the pathophysiology of PD, the fibulins are part of the matricellular proteins that help preserve neuronal integrity and play a regulatory role in maintenance of the ECM [19]. Interestingly, fibulin-3 knock-out mice displayed an early onset of aging-associated phenotypes including reduced lifespan, and this protein has been considered to have an important role in regulating aging [20]. Aging has been found to be a significant factor in the clinical course and progression of Parkinson's Disease [21]. Fibulin-3's ability to help maintain the ECM may be related to the decreased risk of developing PD, a mechanism that will need to be better elucidated with further research.

In the FHS, EFEMP-1 was associated with greater risk of heart failure, all-cause mortality, and CVD death [10]. Our analyses found that DNAm EFEMP-1 was associated with a decreased risk of PD. EFEMP-1 may therefore be another factor that exhibits an inverse association with PD, akin to blood cholesterol [8]. Studies have found that fibulin-3

appears to inhibit oxidative stress and reduce vascular calcification [22]. Others have demonstrated the role that oxidative stress plays in the degeneration of dopaminergic neurons in Parkinson's disease [23]. Thus, fibulin-3's ability to inhibit oxidative stress is a potential mechanism to explain that DNA EFEMP-1 seems to decrease the risk of PD in our study.

We also found that DNAm CD56 levels may increase PD risk. CD 56 lymphocytes, or natural killer (NK) cells, are integral to innate immunity. Prior research has demonstrated that NK cells are elevated in Parkinson's Disease patients compared to patients with other neurologic conditions [24]. Other studies have found that target-cell-dependent NK activation could be augmented in PD patients compared to non-PD individuals, implicating a role for innate immunity in PD [25]. NK cells have more recently been implicated in the pathogenesis of Parkinson's Disease, and human NK cells have been found to be able to internalize and degrade α-syn aggregates via the endosomal/lysosomal pathway. Earls et al. found in their preclinical mouse PD model that in vivo depletion of NK cells resulted in exacerbated motor deficits and increased phosphorylated α-syn deposits [26]. Other mechanisms of NK function in the CNS have also been proposed, including scavenging α-syn aggregates via receptor-mediated endocytosis, modulation of CNS inflammation through interactions with neurons or glia, or by elevating blood plasma levels of IFN-γ, which may promote the lysosomal digestion of excess α-syn aggregates [27]. This may be related to increased expression of genes associated with protein degradation (Ubiquitin D) and proteasome degradation (proteasome subunit β9) in IFN-γ response genes in astrocytes [28].

As seen in Table 2, Model 2, some of the estimated effects for the DNAm markers with suggestive associations were diminished after factoring in cell composition. Cell composition has been previously demonstrated to differ in Parkinson's patients, with epigenetic age of the immune system noted to be increased in PD patients [29]. PD patients were found to have more granulocytes, but fewer T helper cells and B-cells [29]; i.e. our prior work on DNA methylation levels in blood and saliva samples of PD patients similarly found correlations with changes in cell composition making it necessary to control for cell composition in our analyses. We considered how our statistical power may be impacted by using only surrogate measures of the plasma proteins versus directly measuring and analyzing whole blood protein levels. There is likely some level of "exposure" misclassification when using surrogate measures versus actual levels. Measurement error, when not dependent on the outcome (nondifferential), generally attenuates risk estimates toward the null. However, as the DNAm surrogate measures were created in one and validated in other external populations (i.e., not in our PD study), we do not expect the measurement error of the DNAm surrogates to depend on PD status. Thus, our results would most likely be biased toward the null leading to underestimating true associations. The presence of measurement error can also reduce the precision of the estimates and our statistical power.

Limitations of our study include our focus on blood-based proteins and DNAm. While Horvath et al. demonstrated that blood appears to be a promising surrogate for brain tissue when studying the effects of age on DNA methylation profiles [30], extension to brain tissues would greatly enhance insights and would be potential avenues for future work. An

advantage of epigenetic markers is their likely relative temporal stability, given that the epigenome changes only gradually [4]. While we do not know how well they correlate with actual plasma protein levels in our population, we validated the DNAm biomarkers in two independent studies that showed adequate correlations between the plasma protein levels and DNAm surrogate levels. However, a strength of the approach is that we and others will be able to rely on epigenetic arrays and previously validated DNAm-based surrogate markers to investigate plasma proteins without the availability of samples that would allow for plasma level measurements. This opens the new realm of research possibility to all studies with methylation data, versus to only to those with sufficient amounts and adequately stored biological samples. While it is often not feasible for epidemiologic studies to measure each newly suggested protein or factor of interest as they are introduced over time, it might instead be feasible to assess DNAm measured with Illumina arrays in existing studies. Thus, studies that assessed methylation can be revisited to study many different biologic factors for which DNAm biomarkers exist or are newly developed in the future. This, in our opinion, will allow for much more systematic research approaches and replication of results across studies. With the increasing use of genome-wide methylation arrays, future studies may aim to replicate these findings in other PD cohorts. Finally, our PD patients were evaluated within approximately three years of diagnosis; therefore, these DNAm-based surrogate markers may reflect either a response to what occurs in early PD or their contribution to PD risk, but this will need to be examined longitudinally with whole blood taken prior to the onset of PD to distinguish risk factors from disease-influenced changes.

5. Conclusions

Our study demonstrates the potential utility of epigenetic arrays in identifying DNAm markers that are relevant in assessing PD risk. Defining additional epigenetic-based markers and identifying risk factors for PD is of significant clinical interest, with the hopes of eventually creating DNA methylation-related diagnostic tools for PD, similar to what has been done for certain cancers, including some DNA methylation tests which are already commercially available. We found that certain blood DNAm markers are associated with PD disease risk, even after adjusting for cell composition. DNAm EFEMP-1 was associated with decreased risk of PD, whereas CD56 was associated with a higher risk of PD. These DNAm markers have the potential to inform on factors affected differentially in early PD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Declarations of Interest:

S.H. is a founder of the non-profit Epigenetic Clock Development Foundation which plans to license several patents from his employer UC Regents. These patents list S.H. as inventor. The other authors declare no declarations of interest.

Data Availability Statement:

Availability of data and materials; The methylation data analyzed during the current study is available in the GEO repository, accession numbers GSE72774 and GSE72776.

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Highlights

- Epigenome reflects genetic and environmental risk factors of Parkinson's disease
- Blood DNA methylation markers may be associated with PD risk
- DNAm EFEMP-1 and DNAm CD56 were associated with PD risk

Table 1.

Study characteristics (n=807)

n (%) or Mean (SD)		PD Patients n=569	Controls n=238
Age at PD diagnosis (SD)		67.7 (10.1)	NA
UPDRS-III (mean (SD))		21.6 (11.4)	NA
HY Stage (mean (SD))		2.1 (0.8)	NA
0–2.5 (n (%))	452 (82.0)		
3–5 (n (%))	99 (18.0)		
Age at Blood Draw (SD)		70.5 (9.8)	67.5 (12.8)
Male Sex (%)		356 (62.6)	127 (53.4)
Ancestry (%):			
White		468 (82.4)	228 (95.8)
Hispanic		84 (14.8)	9 (3.8)
Smoking Status:			
Never Smoker (%)		301 (53.2)	96 (40.3)
Ex-Smoker (%)		240 (42.4)	125 (52.5)
Current Smoker (%)		25 (4.4)	17 (7.1)
Co-Morbidities (%)			
Type 2 diabetes		87 (15.4)	33 (13.9)
High blood pressure		292 (51.6)	98 (41.4)

Table 2.

Relationship between DNAm estimated plasma proteins and Parkinson's disease

	Model 1: Age, Sex, Ancestry, Smoking		Model 2: Age, Sex, Ancestry, Smoking, Cell Comp	
Predictor:	**OR**	**95%CI**	**OR**	**95%CI**
DNAm CD56	1.41	1.11, 1.79*	1.34	1.05, 1.70*
DNAm EFEMP-1	0.83	0.70, 0.98*	0.80	0.68, 0.95*
DNAm B2M	1.20	1.02, 1.41*	1.14	0.97, 1.34
DNAm TIMP-1	1.23	1.03, 1.46*	1.06	0.88, 1.29
DNAm Cystatin-C	1.19	1.00, 1.42*	1.11	0.93, 1.32
DNAm ADM	1.21	0.99, 1.47	1.13	0.92, 1.38
DNAm PON1	0.90	0.77, 1.05	0.91	0.77, 1.07
DNAm Leptin	0.84	0.62, 1.13	0.89	0.65, 1.20
DNAm PAI-1	0.93	0.80, 1.09	0.93	0.79, 1.09
DNAm GDF-15	1.07	0.90, 1.26	1.04	0.88, 1.23
DNAm Ceruloplasmin	1.16	0.68, 1.99	1.31	0.76, 2.27
DNAm Myoglobin	1.07	0.83, 1.38	1.04	0.81, 1.33

* = significant associations ($p < 0.05$)

Each DNAm marker is modeled in a univariate logistic regression with all covariates and the DNAm marker included in the same model. Odds Ratio per 1 SD of DNAm plasma protein levels.