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Longitudinal Epigenome-Wide Methylation Study of Cognitive Decline and Motor Progression in Parkinson's Disease

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Abstract

Background: DNA methylation studies in Parkinson's disease (PD) thus far have focused on disease susceptibility but not progression.

Objective: In this epigenome-wide association study (EWAS), we aim to identify methylation markers associated with faster cognitive decline or motor progression in PD.

Methods: We included 232 PD patients from the Parkinson's Environment and Gene follow-up study who provided blood samples at enrolment. Information on cognitive and motor function was collected using the Mini-Mental State Examination (MMSE) and Unified Parkinson's Disease Rating Scale (UPDRS). For EWAS analyses, we used a robust measure of correlation: biweight midcorrelations, *t*-tests, and Cox proportional hazard models. We also conducted weighted correlation network analysis (WGCNA) to identify CpG modules associated with cognitive decline or motor progression in PD.

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AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: BR, SH. Performed the experiments: JB, YB. Analyzed the data: YC, AL, KP, AF. Wrote the first draft: YC. All co-authors contributed to study concept, design, and writing of the manuscript. All authors read and approved the final manuscript.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

The PEG study was approved by the UCLA Institutional Review Board (IRB# 11–001530), and informed consent was obtained from all individuals.

DATA AVAILABILITY

Phenotype and DNA methylation data for PEG participants are available at GEO accession database GSE111629.

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: http://dx.doi.org/10.3233/JPD-181549.

CONFLICT OF INTEREST

The authors have no conflict of interest to report.

Results: Among 197 individuals of European ancestry, with our EWAS approach we identified 7 genome-wide significant CpGs associated with a MMSE 4-point decline and 8 CpGs associated with faster motor progression (i.e., rate of UPDRS increase ≥5-point/year). The most interesting CpGs for cognitive decline include cg17445913 in *KCNB1* (cor = 0.36, $p = 6.85 \times 10^{-7}$) and cg02920897 in *DLEU2* (cor = 0.34, $p = 3.23 \times 10^{-6}$), while for motor progression it was cg01754178 in *PTPRN2* (cor = −0.34, $p = 2.07 \times 10^{-6}$). In WGCNA, motor progression related modules were enriched for genes related to neuronal synaptic functions, Wnt signaling pathway, and mitochondrial apoptosis.

Conclusions: Our study provides the first epigenetic evidence that differential methylation in genes previously identified as being associated with cognitive impairment, neuronal synaptic function, Wnt signaling pathway, and mitochondrial apoptosis is associated with cognitive and motor progression in PD.

Keywords

Parkinson's disease; disease progression; longitudinal studies; cognitive decline; MMSE; UPDRS; DNA methylation

INTRODUCTION

Parkinson's disease (PD) is progressive with decline in both motor function and some non-motor symptoms, importantly cognitive impairment. Both contribute heavily to disability and diminished quality of life in patients. The course of PD is currently unpredictable and treatment addresses symptoms but does not alter disease progression. There is a notable lack of knowledge about factors that contribute to or modify the progression of PD. More than a decade ago, Louis et al. suggested that postural instability/gait dominant motor symptoms, a low 'Activities of Daily Living' score, and dementia early in PD predict faster motor decline [1]. Age at onset has been added to this short list of clinical predictors [2, 3]. Previously, we identified α-synuclein genetic variants (i.e., *SNCA* REP1 263 bp promoter variants and rs356165G allele), as possible contributors to faster motor decline [4]. A recent genome-wide association study (GWAS) of 443 PD patients identified 14 SNPs on 11 genes associated with motor progression and 18 SNPs on 16 genes associated with cognitive decline, with suggestive evidence (p-values $\leq 5 \times 10^{-5}$), but none survived adjustment for multiple comparisons [5]. Recently, we showed that a risk score generated from PD GWAS SNPs predicts faster motor and cognitive decline in patients [6], and we previously showed that exposure to neurotoxic pesticides and motor symptom severity and PD phenotype at baseline predict faster progression to cognitive impairment [7, 8].

Epigenetic (DNA methylation) studies, thus far have focused on PD development but not its progression [9, 10]. However, risk factors for progression might be different from those responsible for the development of PD. Relying on participants in the Parkinson's Environment and Gene (PEG) progression follow-up study, we aim to identify epigenetic methylation markers associated with faster cognitive decline or PD motor progression. They may be useful as new biomarkers for PD progression and for targeting high-risk patients for early treatment or may also serve as new targets for drug development.

MATERIALS AND METHODS

The Parkinson's Environment and Gene (PEG) study

Study population—The PEG study is a population-based case control study in central California that first examined PD patients between 2001–2007. 1,167 PD patients were initially identified by neurologist, large medical groups, or public service announcements, and 563 were eligible to participate based on the following criteria: a PD diagnosis within 3 years, being a resident of Fresno, Kern, or Tulare counties, living in California for at least 5 years, and at least 35 years old [11,12]. Of that, 36 were too ill to participate and 54 chose to withdraw from the study, leaving 473 to be invited for a visit with a UCLA movement disorder specialist (JB, YB) for clinical evaluations using UK Brain Bank and Gelb diagnostic criteria [13–15]. 379 were confirmed to have probable, possible, or definite PD, and 342 (90%) completed the baseline interview and provided blood samples for DNA extraction. Our study included 232 PD patients who were successfully followed up between early 2008 and January 2018 with Mini Mental State Examination (MMSE) and Unified Parkinson's Disease Rating Scale (UPDRS) performed at one or two follow-up examinations (for detail see Ritz et al. [4]).

DNA methylation profiling—DNA methylation data containing 486k CpGs were obtained from Illumina Infinium HumanMethylation450 BeadChip using DNA samples extracted from peripheral whole blood. The raw DNA methylation data (beta value) was preprocessed using the background normalization method from the Genome Studio software. Sex concordance was confirmed and no outliers were identified.

Outcome assessment—At baseline, PD patients were screened for cognitive function using the MMSE test (≥26 scores, referring to no dementia) and interviewed to obtain lifestyle-related and medical information including medication use. PD patients were also assessed for motor symptoms according to the UPDRS exam while in a functional 'off' state for PD medications (overnight withdrawal) [4]. If a patient was unable or unwilling to come for physical examination with our movement disorder specialists without having taken PD medications (18%), we imputed the 'off' exam score by adding to the patient's 'on' exam score the mean difference of the study population's off- and on-scores (for detail see Ritz et al. [4]). We also calculated levodopa equivalent doses at time of blood draw based on the reported PD specific medications [16].

For both MMSE and UPDRS, annual rates of change were calculated as the difference of baseline and last follow-up scores divided by duration of follow-up. Faster cognitive decline was defined as an MMSE score reduction greater than 0.6-point/year i.e. the third quartile of the annual MMSE reduction rate, and compared with slow and non-progressors as the reference group. Alternatively, in time to event analyses, we defined cognitive decline as a 4-point decline (a suggestive reliable change indices for the MMSE for longer term follow-up) between baseline and the follow-up exam when a 4-point decline was first seen (for detail see Paul et al. [6]). Fast motor progressors were those whose motor function impairment was greater than a clinically relevant change of 5-points/year [17, 18]. In time to event analyses,

we defined motor progression as the first occurrence of a 20-point increase in the motor score.

Statistical analysis—We focused analyses on 197 individuals of European ancestry to account for confounding by ethnicity, but sensitivity analyses that include all 232 subjects and further adjusted for European ancestry were also conducted. For our epigenome-wide association analysis (EWAS) approach, we related 486k CpGs separately to outcomes of interest adjusting for age, gender, blood cell counts, and L-dopa use using the R function "standardScreening" in the WGCNA R package. Specifically, this program applies biweight midcorrelations (bicor), a robust measure of correlation, to numeric traits, and *t*-tests to binary traits. Cox proportional hazards models were used for time to event analyses and produced a measure of corDeviance. A positive corDeviance value indicates that the hazard ratio (HR) is >1 in support of a shorter than expected time to event, whereas a negative value (HR <1) indicates that the observed event time is longer than expected. Blood cell types were imputed based on the Houseman algorithm in the minfi R package and the epigenetic clock software [19–21]. We used a modified Bonferroni corrected threshold of 5×10^{-6} to adjust for multiple comparisons since the threshold of 1×10^{-7} was termed too stringent (for detail see Chuang et al. [9]).

We also implemented a system biology approach based on weighted correlation network analysis (WGCNA) [22, 23], focusing on the 250k CpGs with the highest variance across individuals to identify co-methylation modules in an unsupervised manner. Blockwise module function and biweight midcorrelation were used to construct CpG networks; module eigengenes (ME) that represent a weighted average of methylation levels were then related to outcomes. We then applied functional enrichment analysis on gene modules to identify their biological function using the online bioinformatics tool DAVID v.6.7.

Lastly, we validated our EWAS findings for cognitive decline in PD using the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort study under the assumption that progression of the dysfunction in all of these neurodegenerative disorders may share biologic pathways.

The Alzheimer's Disease Neuroimaging Initiative—The ADNI (http://adni.loni.usc.edu) is a large-scale longitudinal cohort started in 2004, designed to develop biomarkers for the early detection and tracking progression of Alzheimer's disease (AD). The ADNI cohort recruited participants with AD, with mild cognitive impairment (MCI), and with normal cognition and gathered brain scans, genetic profiles and biomarkers in blood and cerebrospinal fluid of the participants. Whole-genome DNA methylation profiling was done from blood sample of 653 participants at baseline or later phase, with ~2 to 3 longitudinal measures. The Illumina Infinium HumanMethylationEPIC BeadChip Array (www.illumina.com), which covers ~866,000 CpGs, was used for methylation profiling. Samples were randomized using a modified incomplete balanced block design, whereby all samples from a subject were placed on the same chip, with remaining chip space occupied by age- and sex-matched samples. Subjects from different diagnosis groups were placed on the same chip to avoid confounding. Unused chip space was leveraged for technical reproducibility assessment via replicated DNA samples. Methylation beta values were generated using the Bioconductor *minfi* package with Noob background correction [24].

We established an estimate of cognitive decline on the basis of a growth curve analysis of longitudinal changes in Mini-Mental State Examination (MMSE) tests. Linear mixed models with random intercepts and slopes were regressed on MMSE, adjusted for age, gender and follow-up time (in units of year) as fixed effects in the models. The random slopes reflect subject-specific longitudinal changes not predicted by the fixed effects. We performed the analysis on the control (988 observations on 218 subjects) and MCI group (1718 observations on 332 subjects), respectively, with the diagnosis status aligned with the first profile of DNA methylation. The mean follow-up time was 3.6 years in both groups and the mean age at baseline was around 74 years old. We defined a score of progression in cognitive function as the residuals from regressing random slopes on chronological age and multiplied this by "−1" in terms of progression. Thus, a higher score reflects fast progression in cognitive function. To identify CpG markers tracking cognitive progression, we performed EWAS on the (minus) age-adjusted random slope, using the first profile of the methylation measure. In order to obtain an overall *p*-value across the four subsets, we also conducted a meta-analysis using Stouffer's method to obtain an overall correlation and *p*-value across control and MCI subjects.

RESULTS

Cognitive decline

Among individuals of European ancestry, the mean baseline MMSE score was 28.8 and the mean annual rate of change in PD patients was −0.3 (SD = 0.7), and 18% experienced a MMSE ≥4-point decline during follow-up (Supplementary Table 1).

Conducting an EWAS analysis among individuals of European ancestry adjusting for age, gender, blood cell count and using a modified Bonferroni threshold of $p<5 \times 10^{-6}$ to evaluate genome-wide significance, based on rate of MMSE score decrease ≥0.6-point/year we identified 1 CpG (*TUBGCP3* cg17321915, cor = −0.36, $p = 6.14 \times 10^{-7}$) associated with faster cognitive decline. For the outcome 'having lost ≥4-points in the MMSE' during follow-up, 7 CpGs were associated with cognitive decline (Table 1, Supplementary Table 2). The CpG with the highest genome-wide significance was cg17445913 (cor = 0.36, $p = 6.85 \times 10^{-7}$) located within 1500 bps of the transcription start site of *KCNB1* encoding Potassium Voltage-Gated Channel Subfamily B Member 1. Other CpGs are located in a dementia related gene *DLEU2,* and *SATB1, P4HTM,* and *ABRACL.* These CpGs remained statistically significant in sensitivity analyses that included all subjects while adjusting for European ancestry (Supplementary Table 3). Using all subjects, we identified one additional CpG as associated with a MMSE ≥4-point decline *(SCARNA2* cg11653078, cor = 0.32, $p = 3.24 \times 10^{-6}$).

Using the ADNI data to replicate our EWAS results for cognitive decline (a negative age-adjusted random slope measure of longitudinal MMSE scores from growth curve models), we found that cg07108579 in *SATB1* replicated, i.e., had a *p*-value<0.05 (Supplementary Table 4). Although its association was not significant after Bonferroni correction, the direction of the association was preserved. Study population characteristics of the ADNI cohort are presented in Supplementary Table 5.

WGCNA adjusting for age, gender, and blood cell counts clustered the 250k CpGs into 148 co-methylation modules (Supplementary Figure 1). No module was identified to be significantly associated with faster cognitive decline in both measures using the Bonferroni threshold of $p < 0.05/148$ (approximately $p < 5 \times 10^{-3}$).

Motor symptom progression

Among individuals of European ancestry, the mean baseline UPDRS-III score was 19.2 and the mean annual rate of change in PD patients was 2.4 (SD = 2.7). Fourteen percent of PD patients had an annual rate of UPDRS score increase ≥5 points, and 24% experienced a UPDRS ≥20-points increase during the 5.1 years of mean follow-up (Supplementary Table 1). Seventy percent of patients were ever treated with L-dopa.

Our EWAS analysis of individuals of European ancestry adjusting for age, gender, blood cell count, and L-dopa use, identified 8 CpGs ($p < 5 \times 10^{-6}$) with faster motor progression based on rate of UPDRS score increase ≥5-points/year (Table 2, Supplementary Table 2). Alternatively, using a cutoff of ≥20-points increase in the UPDRS during follow-up, 4 additional CpGs were associated with motor progression. These 12 CpGs are located in genes *PITX2, KCNJ15, PTPRN2, GATA5, MX1, MAD1L1,* and *RGMB.* In sensitivity analyses of all subjects adjusted for European ancestry, all UPDRS-associated CpGs remained statistically significant (Supplementary Table 6). Using all subjects, we identified 6 additional CpGs with rate of UPDRS score increase ≥5-points/year *(SRRM4* cg26649752, intergenic cg00320288, *STT3A* cg05929572, *USP13* cg20568102, intergenic cg06365303, and *SEZ6L2* cg09584855), and one CpG located in the first exon of Amyotrophic Lateral Sclerosis (ALS) gene *ALS2CR11* was associated with a UPDRS≥20 points increase during follow-up.

In WGCNA with the threshold of 5×10^{-3}, we identified 3 hypermethylated (plum, coral3, light-cyan1; Supplementary Figure 1) and 1 hypomethylated (coral2) modules significantly associated with a UPDRS ≥20 points increase. These modules were enriched for genes involved in transcription, neuronal dendrite and synaptic function, Wnt signaling pathway, mitochondrial apoptosis, and potassium channel activity (Table 3). Moreover, these four modules were not confounded by L-dopa use and disease duration at the baseline.

DISCUSSION

In our population-based cohort followed on average for 5.1 years for an average total PD duration of 7.1 years, we have found methylation patterns associated with PD motor and cognitive progression.

Methylation levels in CpGs located in the genes *KCNB1, DLEU2,* and *SATB1* were associated with faster cognitive decline for the outcome ‘having lost ≥4-points in the MMSE’ during follow-up. A mouse model of traumatic brain injury reported that oxidation of a *KCNB1* channel (a.k.a. Kv2.1) in the brain is toxic and may cause neurodegeneration and cognitive impairment. Moreover, potassium channel dysfunction has been implicated in AD and is important for acquisition of memory in mammals [25, 26]. For the gene *DLEU2,* encoding Deleted In Lymphocytic Leukemia 2, mRNA has been found to be downregulated

both the temporal and frontal cortex of AD patients [27], and has been suggested as a drug target for AD. *SATB1,* encoding SATB Homeobox 1, is a DNA binding protein. A mouse model generated to investigate the translatome-regulatory network in brain cells showed that *SATB1,* as an upstream regulator was specifically expressed more highly in dopaminergic neurons of the substantia nigra pars compacta than in neighboring ventral tegmental area (VTA) neurons [28]. This suggests that *SATB1* may be a potential drug target for PD as *SATB1* may drive dopaminergic stress response related to neuron death in the substantia nigra pars compacta (not VTA).

With respect to motor progression, genome-wide significant CpGs are located in the genes *PTPRN2, GATA5, USP13,* and *ALS2CR11. PTPRN2* encodes Protein Tyrosine Phosphatase Receptor Type N2, and tyrosine is a precursor of dopamine. *PTPRN2* gene expression has previously been found to be downregulated in human substantia nigra from PD patients [29]. On the other hand, an inverse association (upregulation of *PTPRN2*) was reported in purified dopaminergic neurons derived from PD patients carrying *LRRK2* G2019S variants compared with controls [30]. An EWAS study using blood derived DNA identified a CpG in *PTPRN2* (cg15577272) as having lower DNA methylation levels in high pesticide exposed subjects with airway obstruction [31], and many of our PEG subjects have been highly pesticide exposed [7]. We found that hypomethylation of *PTPRN2* cg01754178 was related to faster motor progression in PD patients, suggesting that *PTPRN2* might be a potential therapeutic target. Transcription factors of the GATA family have been found to regulate the expression of the alpha-synuclein gene *(SNCA)* in a PD study [32]. While *GATA1* and *GATA2* are fundamental regulators of hematopoiesis they also act on the *SNCA* intron1 modulating its expression in dopaminergic neurons. However, we found methylation level differences for the transcription factor *GATA5* associated with faster motor progression. *GATA5* has been described as essential for the development of the heart, gastrointestinal and genitourinary tracts and is expressed in normal gastric and colon mucosa [33]; how it relates to motor function in PD is unclear. *USP13* encodes Ubiquitin Specific Peptidase 13. Recently, there is a growing interest in the role of ubiquitin-specific proteases (USPs) in neurodegenerative diseases. In a cell study, *USP5* and *USP13* were found to degrade ubiquitin chains inside stress granules, defined as clumps of protein or RNA created when cells are stressed [34]. This raised the possibility of targeting them for treatment because ubiquitination of alpha-synuclein in Lewy bodies is the pathological hallmark of PD. Finally, *ALS2CR11 (C2CD6),* encoding C2 Calcium Dependent Domain Containing 6, is a protein with calcium binding properties that has been implicated in the pathogenesis of ALS, a motor neuron disease also characterized by the aggregation of ubiquitinated protein in affected neurons [35]. Previously we found the genetic variant rs72939119 in the *ALS2CR11* gene to be significantly associated with better cognitive performance (based on HVLT-R (Hopkins Verbal Learning Test-Revised) delayed recall) in PD patients [36]. In a gene expression study of peripheral blood, higher RNA expression of *ALS2CR11* was associated with cortical thinning of the sensorimotor strip and supplementary motor area in cognitively normal elderly and mild-cognitively impaired subjects [37]. In our current study, lower methylation level of *ALS2CR11* was associated with faster motor progression in PD patients. In a Chinese study of PD patients, shared neurochemical pathways have been suggested for both cognitive and motor decline in PD [38]. The complex interaction between

cognitive and motor functions in patients of motor neuron diseases needs to be studied further. While many DNA methylation studies have implicated SNCA in PD, we did not see evidence for methylation difference in *SNCA* CpGs that are on the Illumina 450K array in PD progression.

In systems biology WGCNA analysis, four motor dysfunction progression modules were significantly enriched for genes related to synaptic function. We previously reported five biological pathways (mitochondrial function, cytoskeleton organization, systemic immune response, the Wnt receptor signaling pathway, and iron handling) to be important for developing PD in an EWAS study using the same subjects from the PEG study (335 PD and 237 controls) [9]. Our findings in this study further suggest that mitochondrial function and the Wnt signaling pathway are not only associated with PD risk but also its motor progressions.

Compared with the previous GWAS of 443 PD patients that reported on SNPs associated with cognitive decline or motor progression which were related to gene expression, we did not find differential methylation levels for these genes such as *C8orf4* (rs10958605, HR= 1.81[1.42–2.31], $p = 1.51 \times 10^{-6}$) related to either outcome.

Strengths of our study are its relatively large size, that PD patients were recruited from a community setting and that we collected both cognitive and motor progression information. In addition, the measures of cognitive decline (MMSE) and motor progression (UPDRS-III) are well validated and widely employed (for detail see Paul et al. [6]). Therefore, it is easy to perform replication studies in independent samples when progression cohorts of PD with methylation data become more available in the future. For instance, we replicated our findings in the ADNI study. The chosen cut-points were based on external data and reliable change indices and represent reliable functional change. Lastly, we used not only an EWAS but also a systems biology approach (WGCNA) that helps to amplify the underlying biological signal in DNA methylation studies.

Our study has some limitations. First, while we have on average 5.1 years of follow-up, at first revisit we had already lost a third ((342–232)/342 = 32%) of our patients mostly due to death. However, this is similar to another PD study where most subjects lost had died during follow-up [39]. Also, selection bias in our study is unlikely because DNA methylation levels were not related to loss to follow-up. Second, the information on medication is self-reported. However, because it is unlikely that motor progression status influenced the accuracy of reporting medication use we would expect non-differential misclassification that tends to bias estimates toward the null. Third, although the measure of cognitive decline used in this study is well validated and widely employed, compared with the Montreal Cognitive Assessment scale it is less sensitive for detecting cognitive changes in domains most commonly impaired early in PD [40, 41]. Forth, in sensitivity analyses that included all subjects, all CpG hits based on individuals of European ancestry were preserved. This may suggest that these hits are not population specific. However, since 85% of our participants are of European ancestry, we cannot rule out they drive our results. Replication in different ethnic groups is needed. Fifth, PD is a disorder that affects dopaminergic neurons in the brain, and DNA methylation levels are tissue specific. It is likely that patterns of DNA

methylation in the brain and the peripheral blood are distinctive. However, blood might also be able to serve as a surrogate for some brain tissue methylation and it is easily obtainable in living subjects [42–45]. Furthermore, inflammation has been suggested as a pathway contributing to PD and peripheral blood cells may be an appropriate target tissue for this reason [9, 46]. Lastly, even though we have genome-wide data, it only covers a small percentage of the CpG sites in the human genome which may lead to an underestimation of the real differences in methylation.

Our study provides the first epigenetic evidence for genes being differentially methylated that also have been previously identified as being associated with cognitive impairment and neuronal synaptic function, and our results suggest that mitochondrial function and the Wnt signaling pathway are strongly associated not only with disease risk but also PD motor symptom progression. Our results based on 197 individuals – although from currently worldwide the largest the population based PD progression studies and with the 5.1 of years of mean follow-up - are preliminary and need to be replicate in independent cohorts of PD patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

List of cognitive decline-associated CpGs with p-value $<5 \times 10^{-6}$ adjusting for age, gender, and blood cell counts based on analyses of individuals of European ancestry ($n = 180$)

	CpG	Gene	Chr.	Position (bp)	Relation to UCSC CpG Island	Gene region	SNPs	SNPs_10	cor	p
MMSE reduction rate ≥0.6-pt/year										
1	cg17321915	TUBGCP3	13	113184845		Body			−0.36	6.14E-07
MMSE ≥4-point decline										
1	cg17445913	KCNB1	20	48100412	N_Shore	TSS1500			0.36	6.85E-07
2	cg22594737		2	241640444	Island				−0.35	1.11E-06
3	cg20813518		1	81929622					0.35	1.30E-06
4	cg02920897	DLEU2	13	50699344	Island	Body			0.34	3.23E-06
5	cg07108579	SATB1	3	18391124		Body			−0.34	3.32E-06
6	cg13432286	P4HTM	3	49027002	N_Shore	TSS1500			0.34	3.44E-06
7	cg00114944	C6orf115 (ABRACL)	6	139350067	Island	5’UTR			0.33	3.77E-06

Chr., Chromosome; bp, base pair; TSS, transcription start site; TSS1500, within 1500 bps of a TSS; TSS200, within 200 bps of a TSS; UTR, untranslated region; SNPs, listing dbSNP entries within a probe; SNPs_10, listing dbSNP entries within 10 bp of the CpG site; Cor, Correlations obtained by Cox proportional hazard models; TUBGCP3, Tubulin Gamma Complex Associated Protein 3; KCNB1, Potassium Voltage-Gated Channel Subfamily B Member 1; DLEU2, Deleted In Lymphocytic Leukemia 2; SATB1, SATB Homeobox 1; P4HTM, Prolyl 4-Hydroxylase, Transmembrane; ABRACL, ABRA C-Terminal Like. Correlations and p-values were obtained by Cox proportional hazard models.

Table 2

List of motor progression-associated CpGs with p-value $<5 \times 10^{-6}$ adjusting for age, gender, blood cell counts, and L-dopa use based on analyses of individuals of European ancestry ($n = 187$)

	CpG	Gene	Chr.	Position (bp)	Relation to UCSC CpG Island	Gene region	SNPs	SNPs_10	cor	p
UPDRS rate of increase ≥5-point/year[a]										
1	cg13891220	PITX2	4	111549953	Island	Body		rs80199976	0.36	2.86E-07
2	cg18248112	KCNJ15	21	39629123		5'UTR			0.36	3.50E-07
3	cg04304333		17	59474038	Island				0.34	1.44E-06
4	cg05759421		8	10447962					0.34	1.52E-06
5	cg01754178	PTPRN2	7	157507610	S_Shelf	Body			−0.34	2.07E-06
6	cg12664464	GATA5	20	61051021	Island	1stExon;5'UTR			0.34	2.39E-06
7	cg02017634	MX1	21	42791359	N_Shore	TSS1500			0.33	3.23E-06
8	cg26570165		1	119541833	N_Shore		rs10923720		0.33	3.26E-06
UPDRS ≥20-point increase[b]										
1	cg25744355	MAD1L1	7	1889299	N_Shore	Body		rs35185954	−0.35	7.38E-07
2	cg25414463		7	152564066					−0.33	3.08E-06
3	cg08036399		7	32336585	N_Shore			rs7790355	−0.33	3.38E-06
4	cg05214151	RGMB	5	98106564	S_Shore	Body			0.33	3.38E-06

Chr., Chromosome; bp, base pair; TSS, transcription start site; TSS1500, within 1500 bps of a TSS; TSS200, within 200 bps of a TSS; UTR, untranslated region; SNPs, listing dbSNP entries within a probe; SNPs_10, listing dbSNP entries within 10 bp of the CpG site. PITX2, Paired Like Homeodomain2; KCNJ15, Potassium Voltage-Gated Channel Subfamily J Member 15; PTPRN2, Protein Tyrosine Phosphatase, Receptor Type N2; GATA5, GATA Binding Protein 5; MX1, MX Dynamin Like GTPase 1; MAD1L1, Mitotic Arrest Deficient 1 Like 1; RGMB, Repulsive Guidance Molecule Family Member B. Correlations and p-values were obtained by

[a] t-tests and

[b] Cox proportional hazard models.

Table 3.

Functional enrichment analysis for CpGs in 4 motor progression-associated modules in individuals of European ancestry adjusting for age, gender, and blood cell counts (module p-value cutoff = 5×10^{-3})

Rank	Category	Term	*p*	Bonferroni	Benjamini	FDR	Fold Enrichment	Overlap Genes (N)
UPDRS 20-pt increase								
*plum module (cor = 0.22, p=2*10^{-3}, 133 CpGs in 202 genes)*								
	Annotation Cluster 1 Enrichment Score: 5.84							
1	GOTERM_MF	GO:0000978~RNA polymerase II core promoter proximal region sequence-specific DNA binding	1.07E-07	2.46E-05	8.21E-06	1.37E-04	6.26	15
2	GOTERM_MF	GO:0001077~transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding	5.25E-07	1.21E-04	3.02E-05	6.73E-04	7.53	12
3	GOTERM_BP	GO:0006366~transcription from RNA polymerase II promoter	5.34E-07	4.75E-04	4.75E-04	8.31E-04	4.72	17
4	GOTERM_BP	GO:0045944~positive regulation of transcription from RNA polymerase II promoter	1.47E-04	1.23E-01	6.35E-02	2.29E-01	2.76	19
	Annotation Cluster 2 Enrichment Score: 4.04							
1	GOTERM_MF	GO:0003700~transcription factor activity, sequence-specific DNA binding	3.55E-09	8.16E-07	8.16E-07	4.55E-06	4.01	26
2	GOTERM_MF	GO:0003677~DNA binding	2.51E-04	5.61E-02	1.15E-02	3.21E-01	2.21	25
3	GOTERM_BP	GO:0006351~transcription, DNA-templated	3.91E-03	9.69E-01	2.35E-01	5.91E+00	1.82	25
4	GOTERM_CC	GO:0005634~nucleus	1.97E-02	9.51E-01	6.35E-01	2.12E+01	1.31	50
	Annotation Cluster 3 Enrichment Score: 1.10							
1	GOTERM_CC	GO:0045211~postsynaptic membrane	1.62E-02	9.16E-01	7.10E-01	1.77E+01	4.05	6
2	GOTERM_CC	GO:0030054~cell junction	1.02E-01	1.00E+00	8.37E-01	7.24E+01	2.17	7
3	GOTERM_CC	GO:0014069~postsynaptic density	1.37E-01	1.00E+00	8.22E-01	8.30E+01	3.10	4
4	GOTERM_CC	GO:0043025~neuronal cell body	1.78E-01	1.00E+00	7.91E-01	9.04E+01	2.26	5
coral2 module (cor = −0.22, p = 2 × 10^{-3}, 293 CpGs in 147 genes)								
1	GOTERM_BP	GO:0042759~long-chain fatty acid biosynthetic process	7.09E-04	4.31E-01	4.31E-01	1.08E+00	71.15	3
2	GOTERM_MF	0046872~metal ion binding	2.85E-03	5.14E-01	5.14E-01	3.65E+00	1.81	27
3	KEGG_PATHWAY	hsa05412:Arrhythmogenic right ventricular cardiomyopathy (ARVC)	1.29E-02	8.25E-01	8.25E-01	1.41E+01	7.94	4
4	GOTERM_BP	GO:0006465~signal peptide processing	1.30E-02	1.00E+00	9.95E-01	1.82E+01	17.08	3
5	KEGG_PATHWAY	hsa04310:Wnt signaling pathway	1.50E-02	8.68E-01	6.37E-01	1.62E+01	5.11	5
6	GOTERM_MF	GO:0019904~protein domain specific binding	1.70E-02	9.87E-01	8.86E-01	2.00E+01	3.99	6
7	GOTERM_BP	GO:1900740~positive regulation of protein insertion into mitochondrial membrane involved in apoptotic signaling pathway	1.84E-02	1.00E+00	9.93E-01	2.48E+01	14.23	3

Rank	Category	Term	*p*	Bonferroni	Benjamini	FDR	Fold Enrichment	Overlap Genes (N)
8	GOTERM_MF	GO:0003700~transcription factor activity, sequence-specific DNA binding	2.03E-02	9.94E-01	8.22E-01	2.34E+01	2.02	14
9	GOTERM_BP	GO:0007160~cell-matrix adhesion	2.50E-02	1.00E+00	9.93E-01	3.22E+01	6.32	4
10	GOTERM_BP	GO:2001137~positive regulation of endocytic recycling	2.76E-02	1.00E+00	9.88E-01	3.49E+01	71.15	2
11	GOTERM_BP	GO:0006605~protein targeting	3.02E-02	1.00E+00	9.83E-01	3.75E+01	10.95	3
12	KEGG_PATHWAY	hsa04151:PI3K-Akt signaling pathway	3.10E-02	9.85E-01	7.55E-01	3.08E+01	2.86	7
13	GOTERM_CC	GO:0042470~melanosome	3.23E-02	9.98E-01	9.98E-01	3.36E+01	5.73	4
14	GOTERM_CC	GO:0070062~extracellular exosome	4.08E-02	1.00E+00	9.82E-01	4.05E+01	1.44	28
15	GOTERM_CC	GO:0005788~endoplasmic reticulum lumen	4.33E-02	1.00E+00	9.43E-01	4.24E+01	3.77	5
16	GOTERM_BP	GO:1901017~negative regulation of potassium ion transmembrane transporter activity	4.78E-02	1.00E+00	9.96E-01	5.28E+01	40.66	2
17	GOTERM_BP	GO:0045822~negative regulation of heart contraction	4.78E-02	1.00E+00	9.96E-01	5.28E+01	40.66	2
coral3 module (cor=0.22, p=2 × 10^{-3}, 84 CpGs in 54 genes)								
	Annotation Cluster 1 Enrichment Score: 3.15							
1	GOTERM_BP	GO:0006366~transcription from RNA polymerase II promoter	2.96E-04	1.30E-01	1.30E-01	4.22E-01	5.95	8
2	GOTERM_MF	GO:0001077~transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding	3.96E-04	5.21E-02	5.21E-02	4.64E-01	9.33	6
3	GOTERM_BP	GO:0045944~positive regulation of transcription from RNA polymerase II promoter	3.07E-03	7.66E-01	3.84E-01	4.30E+00	3.50	9
	Annotation Cluster 2 Enrichment Score: 2.26							
1	GOTERM_CC	GO:0030425~dendrite	2.00E-03	1.85E-01	1.85E-01	2.21E+00	6.53	6
2	GOTERM_BP	GO:0071805~potassium ion transmembrane transport	3.65E-03	8.22E-01	3.51E-01	5.09E+00	12.62	4
3	GOTERM_CC	GO:0008076~voltage-gated potassium channel complex	2.34E-02	9.11E-01	2.92E-01	2.32E+01	12.43	3
	Annotation Cluster 3 Enrichment Score: 1.92							
1	GOTERM_CC	GO:0030425~dendrite	2.00E-03	1.85E-01	1.85E-01	2.21E+00	6.53	6
2	GOTERM_CC	GO:0030054~cell junction	7.61E-03	5.41E-01	3.23E-01	8.15E+00	4.76	6
3	GOTERM_CC	GO:0045211~postsynaptic membrane	1.10E-01	1.00E+00	6.61E-01	7.28E+01	5.18	3
	Annotation Cluster 4 Enrichment Score: 1.79							
1	GOTERM_CC	GO:0030425~dendrite	2.00E-03	1.85E-01	1.85E-01	2.21E+00	6.53	6
2	GOTERM_CC	GO:0045202~synapse	1.27E-02	7.29E-01	2.30E-01	1.33E+01	8.05	4
3	GOTERM_CC	GO:0043204~perikaryon	3.30E-02	9.67E-01	3.48E-01	3.12E+01	10.32	3
4	GOTERM_CC	GO:0005887~integral component of plasma membrane	8.27E-02	1.00E+00	6.24E-01	6.18E+01	2.06	8
lightcyan1 module (cor=0.20, p=5 × 10^{-3}, 1026 CpGs in 704 genes)								

Rank	Category	Term	*p*	Bonferroni	Benjamini	FDR	Fold Enrichment	Overlap Genes (N)
	Annotation Cluster 1 Enrichment Score: 1.69							
1	GOTERM_MF	GO:0004674~protein serine/threonine kinase activity	1.34E-02	1.00E+00	6.20E-01	1.87E+01	1.72	24
2	GOTERM_MF	GO:0004672~protein kinase activity	1.53E-02	1.00E+00	6.36E-01	2.10E+01	1.72	23
3	GOTERM_BP	GO:0006468~protein phosphorylation	4.19E-02	1.00E+00	9.19E-01	5.29E+01	1.50	26
	Annotation Cluster 2 Enrichment Score: 1.56							
1	GOTERM_BP	GO:0023019~signal transduction involved in regulation of gene expression	5.13E-05	1.19E-01	6.12E-02	9.03E-02	9.71	7
2	GOTERM_BP	GO:0060070~canonical Wnt signaling pathway	6.14E-01	1.00E+00	1.00E+00	1.00E+02	1.27	4
3	GOTERM_BP	GO:0001947~heart looping	6.78E-01	1.00E+00	1.00E+00	1.00E+02	1.30	3
	Annotation Cluster 3 Enrichment Score: 1.50							
1	GOTERM_CC	GO:0005913~cell-cell adherens junction	2.56E-02	1.00E+00	4.76E-01	3.09E+01	1.71	20
2	GOTERM_BP	GO:0098609~cell-cell adhesion	2.93E-02	1.00E+00	8.99E-01	4.08E+01	1.75	18
3	GOTERM_MF	GO:0098641~cadherin binding involved in cell-cell adhesion	4.35E-02	1.00E+00	7.82E-01	4.94E+01	1.67	18
	Annotation Cluster 4 Enrichment Score: 1.28							
1	GOTERM_BP	GO:0006338~chromatin remodeling	1.61E-02	1.00E+00	8.38E-01	2.49E+01	2.76	9
2	GOTERM_CC	GO:0071565~nBAF complex	8.89E-02	1.00E+00	6.51E-01	7.36E+01	5.93	3
3	GOTERM_CC	GO:0016514~SWI/SNF complex	1.00E-01	1.00E+00	6.80E-01	7.79E+01	5.53	3
	Annotation Cluster 5 Enrichment Score: 1.25							
1	GOTERM_BP	GO:0035023~regulation of Rho protein signal transduction	1.15E-02	1.00E+00	7.95E-01	1.84E+01	2.93	9
2	GOTERM_MF	GO:0005089~Rho guanyl-nucleotide exchange factor activity	2.41E-02	1.00E+00	7.22E-01	3.12E+01	2.79	8
3	GOTERM_MF	GO:0005085~guanyl-nucleotide exchange factor activity	6.43E-01	1.00E+00	9.99E-01	1.00E+02	1.14	5
	Annotation Cluster 6 Enrichment Score: 1.11							
1	KEGG_PATHWAY	hsa04070:Phosphatidylinositol signaling system	2.35E-02	9.96E-01	7.50E-01	2.64E+01	2.56	9
2	KEGG_PATHWAY	hsa00562:Inositol phosphate metabolism	4.07E-02	1.00E+00	8.00E-01	4.13E+01	2.75	7
3	GOTERM_BP	GO:0006661~phosphatidylinositol biosynthetic process	6.80E-02	1.00E+00	9.65E-01	7.11E+01	2.73	6
4	GOTERM_BP	GO:0043647~inositol phosphate metabolic process	5.36E-01	1.00E+00	1.00E+00	1.00E+02	1.68	3

GOTERM_BP, Biological Process; GOTERM_MF, Molecular Function; GOTERM_CC, Cellular Component; KEGG, Kyoto Encyclopedia of Genes and Genomes.