Genome-wide brain DNA methylation analysis suggests epigenetic reprogramming in Parkinson disease

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Abstract

Objective
Given the known strong relationship of DNA methylation with environmental exposure, we investigated whether brain regions affected in Parkinson disease (PD) were differentially methylated between PD cases and controls.

Methods
DNA chip arrays were used to perform a genome-wide screen of DNA methylation on the dorsal motor nucleus of the vagus (DMV), substantia nigra (SN), and cingulate gyrus (CG) of pathologically confirmed PD cases and controls selected using the criteria of Beecham et al. Analysis examined differentially methylated regions (DMRs) between cases and controls for each brain area. RNA sequencing and pathway analysis were also performed for each brain area.

Results
Thirty-eight PD cases and 41 controls were included in the analysis. Methylation studies revealed 234 significant DMR in the DMV, 44 in the SN, and 141 in the CG between cases and controls (Sidak p < 0.05). Pathway analysis of these genes showed significant enrichment for the Wnt signaling pathway (FDR < 0.01).

Conclusions
Our data suggest that significant DNA methylation changes exist between cases and controls in PD, especially in the DMV, one of the areas affected earliest in PD. The etiology of these methylation changes is not yet known, but the predominance of methylation changes occurring in the DMV supports the hypothesis that vagus nerve function, perhaps involving the gastrointestinal system, is important in PD pathogenesis. These data also give independent support that genes involved in Wnt signaling are a likely factor in the neurodegenerative processes of PD.
Parkinson disease (PD) is the second most common neurodegenerative disorder affecting older adults. The clinical presentation includes bradykinnesia, resting tremor, and rigidity. Monogenic mutations for PD have been identified that greatly increase the risk of PD. However, 90% or more of PD cases are idiopathic.

Epigenetics is a potentially important factor contributing to PD risk, particularly since environmental factors have been associated with an increased risk of developing PD. However, little work has been done to explore the potential epigenetic contribution to PD. DNA methylation, the mostly studied form of epigenetic modification, has been primarily investigated in PD within select candidate genes. Several studies have found differential methylation in SNCA and MAPT. Furthermore, significant changes in DNA methylation were identified in multiple genes in both blood and brain. Relevant to the current study, dysregulation of Wnt signaling due to methylation was observed in the frontal cortex and midbrain sections of PD brains.

Here, we report an initial analysis of the genome-wide methylation profile in the dorsal motor nucleus of the vagus (DMV), substantia nigra (SN), and cingulate gyrus (CG) of pathology-confirmed PD cases compared with age- and sex-matched, pathology-confirmed controls. Each of these brain regions represents the location of neuropathologic changes in PD at different stages of the disease. We found that patients with PD have significant DNA methylation changes in these 3 brain regions, and find the largest number of significant DNA methylation changes are found in the DMV. Furthermore, pathway analysis in the DMV of patients with PD supports the involvement of the Wnt pathway in the pathophysiology of PD.

### Methods

Brain samples were obtained from the autopsy program of the University of Miami, Morris K. Udall Parkinson Disease Center of Excellence (n = 11), the NIH Neurobiobank (n = 12), and the Pacific Udall Center Neuropathology Core (n = 22). Our initial discovery sample set consisted of 22 PD pathology-confirmed cases and 24 pathology-confirmed controls. The replication data set had 16 PD pathology-confirmed cases and 24 pathology-confirmed controls. The replication data set had 16 PD pathology-confirmed cases and 24 pathology-confirmed controls.

### Profiling of CpG methylation using 450k/850k array

Genomic DNA (500 ng) was bisulfite modified (EZ-96 DNA Methylation Kit; Zymo Research, Orange, CA) as per manufacturers’ instructions. For analysis of CG dinucleotide (CpG) methylation, both the Illumina Infinium HumanMethylation450 BeadChip and the Infinium MethylationEPIC (850K array) Beadchip were used (Illumina, San Diego, CA) because of discontinuation of the 450 BeadChip by the manufacturers.

### Statistical analysis of methylation data

Raw intensity files were processed using the methylation analysis software RnBeads. Because all samples were male, we did not filter sex-specific probes. Beta-Mixture Quantile normalization was used for intra-array normalization of beta values, which are the ratio of the methylated signal intensity to the sum of both methylated and unmethylated signals after background subtraction. The beta values were then logit transformed to attain M values for statistical analysis.
Unsupervised hierarchical clustering of the methylome data revealed 1 outlier from the CG group, which was removed from further analysis.

For the analysis of individual CpGs, linear regression models were used to test differential methylation between case-control status adjusting for age at death (AAD) and methylation chip effects. To account for additional unmeasured confounding factors such as cellular composition, we included surrogate variables (SVs) estimated from independent surrogate variable analysis (iSVA) as covariates.22 iSVA estimates major independent components (ICs) in genome-wide DNA methylation patterns. We tested each IC with status using a T test. The significant ICs (IC3 for DMV and IC7 for SN), which could be confounders of the association between case-control status and differential methylation, were then included in the linear model: M value ~ PD + AAD + array + IC. We only considered CpGs that showed a difference in group means in methylation M values (|ΔM|) of at least 25% (|ΔM| ≥ 1.5) and false discovery rate (FDR) <0.05 in the 2 group comparison.

Differentially methylated region analysis
The majority of genome-wide methylation studies have focused on single CpG sites. However, modification of single CpG often produces weak correlations with gene expression data.22 Contextualizing the methylation level of an individual CpG by the status of neighboring CpG sites facilitates biological inferences. Clusters of neighboring CpGs with coordinated differential methylation are identified as differentially methylated regions (DMRs). Hypermethylated DMRs in promoters are usually associated with silencing, whereas in the gene body, they associate with upregulation of expression.23 We defined a DMR as a region including (1) 3 or more consecutive significantly differentially methylated (p < 0.05) sites between PD and control groups with the same direction of methylation change; (2) each differentially methylated CpG separated by less than 500 bp; and (3) a multiple-comparison corrected p value (Sidak p) less than 0.05 for the region.

DMR analysis was performed using comb-p.24 Comb-p takes as input unadjusted p values for each probe, identifies regions of enrichment (i.e., series of adjacent low p values), and computes statistical significance of the regions using the Sidak correction.

Levodopa
Levodopa (L-Dopa) has been shown to affect methylation levels25 and thus could be a contributor to the methylation changes observed. To address this, we used 2 approaches: (1) we examined the dose-response relationship with L-Dopa in a line of iPSC-derived dopaminergic neurons generated from a control non-Hispanic white male donor. (2) We also compared methylation changes we found to those reported in a model of 7-day L-Dopa administration to rats rendered hemiparkinsonian through unilateral injections of 6-hydroxydopamine (6-OHDA).25

We selected nontoxic concentrations ranging from 0 to 50 μM of L-Dopa, including treatment with 10 μM, a concentration proven to induce methylation changes in cultured human peripheral blood mononuclear cells.26 iPSC-derived dopaminergic neurons differentiated for 70 days were cultured with varying levels of L-Dopa (0, 5, 10, and 50 μM) in Neurobasal N2/B27 media (Gibco) supplemented with 1 ng/mL of transforming growth factor beta-3, 10 ng/mL of sonic hedgehog signaling molecule, 20 ng/mL of brain-derived neurotrophic factor, and 30 ng/mL of glial cell-derived neurotrophic factor. After 7 days, DNA was extracted and subjected to methylation analysis.

RNA sequencing and statistical analysis
RNA-seq (RNA integrity number ≥ 5) was performed using a paired-end 125 bp protocol on a HiSeq 2500. Reads were aligned to the human reference genome (hg19) using the STAR algorithm and analyzed using the “voom” method in the Limma package.27

A linear model with AAD was fitted to each gene, and empirical Bayes moderated t-statistics and p values were used to assess expression differences between PD and controls. To account for underlying unknown confounding factors, we used SVAseq with default parameters to estimate SVs.28 None of the estimated SVs differed significantly between case-control status, so we did not include them in the linear model: log (cpm) ~ PD + AAD.

Pathway analysis
We used Enrichr (amp.pharm.mssm.edu/Enrichr/), which yields an FDR-adjusted p value for each pathway.29 The binding affinity of most transcription factors to DNA is altered by DNA CpG methylation.30 Thus, we analyzed the presence of transcription factor binding sites (TFBSs) using the R package Goldmine.31

Data availability
Raw data for the primary analyses are available upon reasonable request from the corresponding and senior author.

Results
Samples
The average AAD for PD cases was 78.1 years (range 67–90 years) in the discovery data set and 79.8 years (range 66–89 years) in the replication data set. For controls, it was 77.7 years (range 64–91 years) in the discovery and 79.6 years (range 67–95 years) in the replication data set. In multiple samples, we were unable to isolate all 3 regions because of insufficient material or excessive degeneration. This is shown in tables 1 and 2, with age at onset, AAD, and the regions isolated from each donor.

DMR analysis
Discovery data set
Analysis in the discovery data set identified 85 DMRs in the DMV, 65 in the CG, and 27 in the SN samples with Sidak p < 0.05 (table e-1, links.lww.com/NXG/A164). These DMRs were associated with 108, 84, and 31 genes in the DMV, CG, and SN, respectively. Within the discovery data set, comparison
of the DMRs identified in the 3 tissues revealed that a region spanning the promoter region of dual-specificity phosphatase 22 (DUSP22) was hypomethylated in PD brains in the 3 tissues. Furthermore, another 7 genomic regions were differentially hypermethylated in both the DMV and the SN (RNF5, AGPAT1, LANCL2, LMTK3, SCAND3, SLFN12, and ZDHHC14).

Replication data set
We tested whether any of the significant DMRs identified in the discovery data set were also differentially methylated in the replication data set (Sidak $p < 0.05$). We found that 7 of the discovery DMRs were reproduced in the replication data set in the CG, 4 in the DMV, and none in the SN (figure and table 3). We therefore performed a gene-based analysis that revealed genes that contained DMRs in both the DMV (FRMD4A and GPT) and the CG (HOXA3 and PRDM16) of patients with PD in which the location of the DMR is not the same in the discovery and the replication data set (figure). This analysis identified several genes, including ARFGAPI1, a reported regulator of LRRK2 toxicity in PD.32,33

Joint data set
As both the discovery and replication autopsy data sets were limited in sample numbers and showed replication between them, to increase the power of detecting disease-associated

| Table 1 Samples investigated in the discovery methylation and RNA-seq study |
|---------------------------|---------------------------|---------------------------|
| Control | AAD | Methylation | RNA-seq | PD | AAO | Methylation | RNA-seq | Additional neuropath diagnosis |
| DMV | CG | SN | DMV | CG | SN | DMV | CG | SN | DMV | CG | SN |
| C-001 | 71 | Y | Y | Y | Y | P-395 | 65 | Y | Y | 74 | Y | Y | Acute hemorrhage |
| C-002 | 75 | Y | Y | P-346 | 73 | 81 | Y | Y | Y | AD |
| C-005 | 76 | Y | Y | Y | P-548 | 70 | 76 | Y | Y | 76 | Y | Y |
| C-007 | 78 | Y | Y | Y | P-784 | 48 | 67 | Y | Y | Y | Y |
| C-008 | 79 | Y | Y | Y | P-002 | 63 | 68 | Y | Y | Y | Y |
| C-009 | 80 | Y | Y | Y | P-755 | 65 | 81 | Y | Y | 76 | Y | Y |
| C-010 | 65 | Y | Y | Y | P-225 | 62 | 72 | Y | Y | 72 | Y | Y |
| C-011 | 66 | Y | Y | Y | P-545 | 62 | 72 | Y | Y | Y | Y |
| C-012 | 68 | Y | Y | Y | P-547 | 73 | 86 | Y | Y | Y | Y |
| C-013 | 67 | Y | Y | Y | P-549 | 68 | 83 | Y | Y | Y | Y |
| C-336 | 64 | Y | Y | Y | P-554 | 64 | 80 | Y | Y | Y | Y |
| C-132 | 77 | Y | P-812 | 62 | 76 | Y | Y | Y | Y |
| C-738 | 78 | Y | Y | Y | Y | P-447 | 70 | Y | Y | Y | Y |
| C-598 | 73 | Y | Y | Y | Y | P-438 | 84 | Y | Y | Y | Y | AD |
| C-632 | 78 | Y | Y | Y | Y | P-748 | 88 | 90 | Y | Y | Y | Y | Subdural hematoma |
| C-790 | 83 | Y | Y | Y | Y | P-524 | 75 | 78 | Y | Y | Y | Y | AD |
| C-331 | 81 | Y | Y | Y | Y | P-533 | 73 | Y | Y | Y | Y | AD |
| C-535 | 86 | Y | Y | Y | Y | P-080 | 76 | Y | Y | 76 | Y | Y |
| C-642 | 84 | Y | Y | Y | Y | P-376 | 84 | 88 | Y | Y | Y | 78 | AD |
| C-353 | 91 | Y | Y | Y | Y | P-610 | 81 | 81 | Y | Y | Y | Y | AD |
| C-511 | 87 | Y | Y | Y | P-206 | 77 | 78 | Y | Y | 78 | Y | Y | AD |
| C-434 | 84 | Y | Y | Y | P-698 | 71 | 85 | Y | Y | Y | Y | AD |
| C-752 | 87 | Y | Y | Y | Y | 87 | Y | Y | Y | Y | Y | Y | AD |
| C-492 | 87 | Y | Y | Y | Y | 87 | Y | Y | Y | Y | Y | Y |
| Total | 16 | 11 | 20 | 6 | 5 | 12 | 13 | 11 | 19 | 5 | 9 | 13 |

Abbreviations: AAD = age at death; AAO = age at onset; CG = cingulate gyrus; DMR = differentially methylated region; DMV = dorsal motor nucleus of the vagus; PD = Parkinson disease; RIN = RNA integrity number; SN = substantia nigra.

All brains were from non-Hispanic white men, aged >60 years at death with a Braak neurofibrillary tangle stage ≤ IV. In several samples, we were unable to isolate all 3 regions because of insufficient material and/or excessive degeneration. We analyzed the DMV in 67% of control samples and in 59% of PD samples; the CG in 46% of control samples and in 50% of PD cases; and the SN in 63% of control samples and in 86% of PD cases. RNA was used only in those samples that reached a quality cutoff (RNA integrity number, RIN ≥ 5). Samples used are indicated by a “Y.”
methylation changes, we performed a single joint analysis. This provided us a total of 53 DMV (22 PD and 31 controls), 52 CG (26 PD and 26 controls), and 65 SN (29 PD and 36 controls) for the analysis. In the joint analysis, we identified 234 significant DMR in the DMV, 44 in the SN, and 141 in the CG (table e-2, links.lww.com/NXG/A165). These correspond to 266, 53, and 159 genes, respectively. The top 20 DMRs in the joint analysis from each region are shown in table 4. In the joint analysis, a DMR in the promoter area of LOC100420587 is hypermethylated in the 3 brain regions. It is interesting to note that an SNP in this noncoding gene of unknown function was identified as associated with the volume of the CG by neuroimaging and GWASs.34

Pathway analysis identified Wnt signaling as epigenetically affected in the DMV of PD brains

We identified physiologic pathways overrepresented among the genes associated with DMRs identified in the joint analysis. Significant enrichment was observed in the KEGG “Hippo signaling pathway” (FDR = 0.007) and “Wnt signaling pathway” (FDR = 0.01) in the DMV (table e-3, links.lww.com/NXG/A166). No pathway enrichment was observed for CG or SN, even using FDR < 0.25 as the significance cutoff, as previously suggested for pathway analysis.35,36

Integrated analysis of differential methylation and RNA-Seq

We observed that ~80% of the DMRs identified contain TFBS (table e-4, links.lww.com/NXG/A167), suggesting that the identified differences in DNA methylation are likely to have transcriptional consequences. We then performed transcriptome analysis by RNA-seq on a subset of samples (table 1). Analysis of the RNA-seq data identified 515 differentially expressed genes (DEG) in the CG, 390 DEG in the SN, and 3 DEG in the DMV associated with PD (FDR <0.05, adjusted for AAD). An overlap analysis of both methylation data and RNA-seq revealed 6 genes with DMRs that were also differentially expressed (NDRG4, PTPRN2, SYT7, IQSEC1, DLG4, and KCNIP1) in the CG and 1 (NDRG4) in the SN.

Levodopa

We found 14 DMRs that changed their methylation levels significantly (Sidak p < 0.05; table e-5, links.lww.com/NXG/A168)

Table 2 Samples investigated in the replication methylation study

| Control | AAD | Methylation | PD | AAO | Methylation | Additional neuropath diagnosis |
|---------|-----|-------------|----|-----|-------------|-------------------------------|
|         |     | DMV          |    |     | CG          | SN              |
| C-001   | 84  | Y            | Y  | Y   | P-593       | 57               | 66               |
| C-002   | 92  | Y            | Y  | Y   | P-208       | 70               | 86               |
| C-003   | 80  | Y            | Y  | Y   | P-001       | 82               | 89               |
| C-004   | 67  | Y            | Y  | Y   | P-401       | 53               | 80               |
| C-005   | 70  | Y            | Y  | Y   | P-327       | 44               | 68               |
| C-006   | 70  | Y            | Y  | Y   | P-634       | 68               | 81               |
| C-007   | 70  | Y            | Y  | Y   | P-686       | 80               | 84               |
| C-008   | 85  | Y            | Y  | Y   | P-443       | 79               | 89               |
| C-009   | 82  | Y            | Y  | Y   | P-531       | 48               | 70               |
| C-010   | 84  | Y            | Y  | Y   | P-045       | 62               | 80               |
| C-011   | 80  | Y            | Y  | Y   | P-457       |                 |
| C-012   | 85  | Y            | Y  | Y   | P-678       | 65               | 84               |
| C-013   | 75  | Y            | Y  | Y   | P-679       |                 |
| C-014   | 90  | Y            | Y  | Y   | P-457       | 73               | 86               |
| C-015   | 77  | Y            | Y  | Y   | P-904       | 64               | 78               |
| C-016   | 68  | Y            | Y  | Y   | P-625       | 65               | 77               |
| C-017   | 95  | Y            | Y  | Y   |             |                 |
| Total   |     | 15           | 15 | 16  | 9           | 15               | 10               |

Abbreviations: AAD = age at death; AAO = age at onset; CG = cingulate gyrus; DMV = dorsal motor nucleus of the vagus; PD = Parkinson disease.

All brains were from non-Hispanic white men, aged >60 years at death with a Braak neurofibrillary tangle stage ≤ IV. In several samples, we were unable to isolate all 3 regions because of insufficient material and/or excessive degeneration. We analyzed the DMV in 88% of control samples and in 56% of PD samples; the CG in 88% of control samples and in 93% of PD cases; and the SN in 94% of control samples and in 62% of PD cases. Samples used are indicated by a “Y.”
on L-Dopa treatment of iPSC-derived dopaminergic neurons. Comparison of the DMRs identified in the joint analysis in the different tissues with the methylation changes induced by L-Dopa treatment showed no overlap. Furthermore, comparison of our data (all genes containing DMR irrespective of the brain region) with the genes identified as differentially methylated in the dorsal striatum of 6-OHDA–treated rats receiving L-Dopa with FDR < 0.05 and absolute change of 10% (2703 genes) revealed an overlap of 82 genes. Thus, the rat model–human comparison data suggest that approximately 24.1% of the genes identified as differentially methylated in PD could be related to L-Dopa administration. To attempt to identify methylation changes that could be induced by the presence of cell death or hypofunctioning neurons, we determined which of the DMR-containing genes (from the joint analysis) were also differentially methylated in hemiparkinsonian rats not given L-Dopa. This revealed 60 genes with differential methylation shared between our human data and those responsive to 6-OHDA lesion in the rat striatum as previously identified.

Figure PD-associated DMRs

### Discussion

This initial study of DNA methylation changes in the DMV, SN, and CG supports our hypothesis of an epigenetic contribution to PD risk. Whether the identified changes are inherited, acquired de novo during development, in part due to cellular composition changes or induced by environmental variables is currently unknown. However, it is certainly interesting to speculate that these methylation changes might be due to environmental influences through the vagus nerve. If this were the case, it would suggest that methylation is an early factor in the development of PD, as the DMV is thought to be one of the earliest regions to develop characteristic PD pathologic changes.

Epigenetic patterns are different between cell types, specifically neurons and glia. Unlike the DMV, which does not have extensive degeneration, cell loss in the SN is prominent, and thus, it is possible that a change in cellular composition between controls and patients with PD contributes to some of the changes we have seen despite the use of iSVA to correct for cellular heterogeneity. Furthermore, heterogeneity in the
Table 3 Replicated DMR in the DMV and CG of PD brains

| DMR         | Chr | Start          | End            | CpGs | p Value | FDR  | Gene   | Direction |
|-------------|-----|----------------|----------------|------|---------|------|--------|-----------|
| Replicated in DMV | chr8 | 144343915 | 144347945 | 4 | 8.69E-05 | 0.0075 | ZFP41 | -         |
|             | chr5 | 87973439 | 87974548 | 10 | 0.0099715 | 0.0331 | LOC645323 | +         |
|             | chr13 | 112724221 | 112725845 | 3 | 0.001156 | 0.0331 | SOX1 | +         |
|             | chr20 | 61915437 | 61916280 | 5 | 0.001954 | 0.042 | ARFGAP1 | +         |
| Replicated in CG | chr9 | 140171765 | 140175394 | 13 | 0.000116 | 0.0074 | C9orf167 | -         |
|             | chr15 | 81426347 | 81426821 | 9 | 0.00322 | 0.0074 | C15orf26 | -         |
|             | chr19 | 21646006 | 21646782 | 5 | 0.00323 | 0.0074 | -      | -         |
|             | chr17 | 81038827 | 81039991 | 6 | 0.00459 | 0.0079 | METRL | -         |
|             | chr16 | 123246 | 123677 | 5 | 0.01079 | 0.0149 | RHBDF1 | -         |
|             | chr10 | 105420501 | 105421250 | 5 | 0.00333 | 0.0383 | SH3PD2A | -         |
|             | chr3 | 19408257 | 19408499 | 3 | 0.001079 | 0.0149 | SDHD | -         |

Abbreviations: CG = cingulate gyrus; DMR = differentially methylated region; DMV = dorsal motor nucleus of the vagus; PD = Parkinson disease.

A DMR in ADP-ribosylation factor 1 GTPase activating protein 1 (ARFGAP1) is the most significant DMR in the joint analysis of the DMV (table 3). ARFGAP1 and LRRK2 interact and appear to regulate each other’s expression. Thus, the methylation changes would suggest a wider role for ARFGAP1 in PD pathophysiology. Neurexin 3, thought to be involved in synaptic plasticity, has been associated with multiple psychiatric disorders including Alzheimer disease. Of interest, the promoter of DUSP22, associated with the most significant DMR in the SN in this study, was shown to be hypermethylated in the hippocampus, whereas a region upstream of DUSP22 was found to be hypomethylated in the superior temporal gyrus of patients with Alzheimer disease. It has been recently suggested that DUSP22 is involved in both the phosphorylation of tau and CREB signaling, both shown to be involved in Alzheimer disease. Furthermore, hypermethylation of the DUSP22 promoter was associated with schizophrenia. It is interesting that we did not find any DMR in SNCA that has been shown to have methylation changes previously in the cortex, SN, and blood in PD. The reasons for this are not clear, but may reflect tissue degeneration in the earlier affected regions, well as a larger data set reported here, as it is likely to vary between individuals.
Table 4: Top 20 DMRs identified in the joint analysis

| DMR | Chr   | Start   | End     | CpGs | SLK p value | Sidak p value | Associated genes       | Direction | Ref. |
|-----|-------|---------|---------|------|-------------|---------------|------------------------|-----------|------|
| DMV | chr20 | 61915437| 61916280| 5    | 5.01E-12    | 2.70E-09       | ARFGAP1               | +         | 39 (PD) |
|     | chr14 | 79744991| 79746781| 12   | 8.80E-11    | 2.23E-08       | NRXN3                 | +         | 43 (AD) |
|     | chr5  | 87973439| 87974548| 10   | 7.76E-11    | 3.17E-08       | LOC65323              | +         |       |
|     | chr8  | 144343915| 144344794| 4    | 6.88E-11    | 3.54E-08       | ZFP41                 | −         |       |
|     | chr6  | 99278991| 99280514| 9    | 4.43E-10    | 1.32E-07       | POU3F2                | +         |       |
|     | chr10 | 118892211| 118894181| 13   | 7.21E-10    | 1.66E-07       | VAX1                  | +         |       |
|     | chr2  | 27529325| 27531536| 18   | 5.73E-09    | 1.18E-06       | TRIM54, UCN           | +         |       |
|     | chr16 | 3017495 | 3018471 | 7    | 2.95E-09    | 1.37E-06       | KREMT2, PAQR4         | +         |       |
|     | chr7  | 79315848| 79317340| 7    | 6.05E-09    | 1.84E-06       | ENSG0000171282, TMEM105| +         |       |
|     | chr1  | 221053409| 221055965| 15   | 1.35E-08    | 2.40E-06       | HLX                   | +         |       |
|     | chr11 | 73356316| 73357397| 11   | 1.03E-08    | 4.33E-06       | PLEKH1                | +         |       |
|     | chr6  | 33279563| 33284498| 99   | 9.04E-08    | 8.30E-06       | TAPBP, ZBTB22         | +         |       |
|     | chr2  | 233924713| 233925276| 11   | 1.37E-08    | 1.10E-05       | INPP5D                | +         | 59 (AD) |
|     | chr11 | 68919873| 68920772| 9    | 3.46E-08    | 1.74E-05       | CCND1, TPCN2          | +         | 60 (PD) |
|     | chr2  | 54785178| 54786149| 9    | 4.41E-08    | 2.06E-05       | SPTBN1                | +         | 61 (PD) |
|     | chr17 | 79372242| 79374742| 16   | 1.71E-07    | 3.09E-05       | BAHCC1                | +         |       |
|     | chr11 | 2889602 | 2891496 | 41   | 1.44E-07    | 3.45E-05       | KCNQ1DN               | −         |       |
|     | chr7  | 1120465 | 1121930 | 9    | 1.36E-07    | 4.21E-05       | C7orf50               | +         |       |
| CG  | chr15 | 96868857| 96869221| 8    | 1.73E-09    | 2.14E-06       | NR2F2                 | −         |       |
|     | chr16 | 123246 | 123677  | 5    | 1.96E-09    | 2.05E-06       | RBHDF1                | −         |       |
|     | chr14 | 24640947| 24641707| 11   | 1.12E-08    | 6.63E-06       | REC8                  | −         |       |
|     | chr17 | 33825172| 33825375| 3    | 1.27E-08    | 2.82E-05       | SLFN12L               | −         |       |
|     | chr1  | 156610966| 156612437| 8   | 3.55E-08    | 1.09E-05       | BCAN                  | −         |       |
|     | chr19 | 29217858| 29218775| 7    | 5.77E-08    | 2.84E-05       | LOC100420587         | +         |       |
|     | chr3  | 46506104| 46506865| 11   | 6.44E-08    | 3.81E-05       | LTF                   | −         |       |
|     | chr6  | 33560953| 3361450 | 8    | 7.62E-08    | 6.91E-05       | C6orf227              | −         |       |
|     | chr1  | 167682648| 167683014| 5   | 7.81E-08    | 9.62E-05       | MPZL1, RCSD1          | −         |       |
|     | chr17 | 81038827| 81039991| 6    | 1.42E-07    | 5.50E-05       | METRNL                | −         |       |
|     | chr1  | 221060360| 221061255| 6   | 1.54E-07    | 7.75E-05       | HLX, DUSP10           | −         |       |
|     | chr19 | 2041905 | 2042593 | 6    | 1.64E-07    | 0.000107       | MKNK2                 | −         |       |
|     | chr20 | 821854 | 822789  | 4    | 1.76E-07    | 8.48E-05       | FAM110A               | −         |       |
|     | chr19 | 58907184| 58907510| 3    | 2.09E-07    | 0.000289       | ENSG000002522355     | −         |       |
|     | chr13 | 36048892| 36051074| 15   | 2.37E-07    | 4.90E-05       | MAB21L1, MIR548G5, NBEA| −         |       |
|     | chr3  | 138655775| 138656629| 6   | 3.02E-07    | 0.000159       | PIK3CB, FOXL2         | −         |       |
|     | chr17 | 38465281| 38465511| 7    | 3.45E-07    | 0.000676       | RARA                  | −         |       |
|     | chr9  | 140171097| 140175394| 16  | 3.55E-07    | 3.72E-05       | C9orf167              | −         |       |

Continued
Thus, our data support an epigenetic component to the development of PD and fit well within the growing body of evidence involving the DMV and the vagus nerve in PD. Furthermore, our data support the previous studies suggesting deregulated Wnt signaling contributing to the pathogeneses of PD.

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**Disclosure**
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### Table 4 Top 20 DMRs identified in the joint analysis (continued)

| DMR | Chr | Start | End | CpGs* | SLK p value | Sidak p value | Associated genes | Direction | Ref. |
|-----|-----|-------|-----|-------|-------------|--------------|------------------|-----------|------|
| SN  | chr6| 291687| 293332| 10    | 5.18E-13    | 1.42E-10      | DUSP22          |          | 45 (AD) |
| chr17| 33759512| 33760528| 12 | 3.25E-10 | 1.44E-07 | SLFN12 | + |
| chr19| 29217858| 29218775| 7 | 3.75E-10 | 1.84E-07 | LOC100420587 | + |
| chr7 | 64348740| 64350151| 9 | 1.08E-08 | 3.46E-06 | ZNF273, ZNF138 | – |
| chr11| 50257256| 50258751| 10 | 1.24E-07 | 3.73E-05 | LOC441601 | + |
| chr19| 55972504| 55973779| 11 | 4.47E-07 | 0.000158 | ISOC2 | – |
| chr4 | 40858965| 40859345| 7 | 2.79E-07 | 0.00033 | APBB2 | – |
| chr22| 47081634| 47082261| 5 | 1.89E-06 | 0.001352 | CERK | + |
| chr1 | 2138442| 2139658| 7 | 4.09E-06 | 0.001513 | C1orf86 | + |
| chr2 | 223164459| 223167618| 20 | 1.65E-05 | 0.00235 | PAX3, CCDC140, CCDC140 | – |
| chr7 | 55430948| 55431277| 3 | 1.91E-06 | 0.002611 | LANCL2 | + |
| chr17| 7757148| 7759141| 20 | 1.35E-05 | 0.003041 | KDM6B, TMEM88, TMEM88 | + |
| chr6 | 158013621| 158014656| 6 | 8.81E-06 | 0.003821 | ZDHHC14 | + |
| chr5 | 174158195| 174159904| 8 | 1.46E-05 | 0.003838 | MSX2, DRD1 | – |
| chr3 | 46792023| 46792463| 5 | 5.95E-06 | 0.006069 | PRSS45, PRSS50 | + |
| chr9 | 13095135| 130955437| 3 | 4.17E-06 | 0.006197 | CIZ1 | + |
| chr6 | 28601271| 28601520| 12 | 3.58E-06 | 0.006444 | SCAND3, TRIM27 | + |
| chr16| 58534681| 58535557| 7 | 1.28E-05 | 0.006526 | NDRG4 | – |

Abbreviations: AD = Alzheimer disease; CG = cingulate gyrus; DMR = differentially methylated region; DMV = dorsal motor nucleus of the vagus; NRXN3 = neurexin 3; PD = Parkinson disease; SN = substantia nigra. Direction refers to hypermethylation (+) or hypomethylation (−) in PD.

* CpGs refers to the number of CpGs included in the DMR.

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**Appendix Authors**

| Name            | Location                | Role               | Contribution                                                                 |
|-----------------|-------------------------|--------------------|------------------------------------------------------------------------------|
| Juan I. Young,  | University of Miami, FL | Author             | Designed and conceptualized the study; major role in the acquisition of data; |
| PhD             |                         |                    | analyzed data; and drafted the manuscript                                  |
| Sathesh K.      | University of Miami, FL | Author             | Analyzed data and drafted the manuscript                                     |
| Sivasankaran,   | University of Miami, FL |                    |                                                                               |
| PhD             |                         |                    |                                                                               |
| Lily Wang, PhD  | University of Miami, FL | Author             | Analyzed data and drafted the manuscript                                     |
| Deborah C.      | University of Miami, FL | Author             | Provided samples, performed pathologic evaluations, and conceptualized the   |
| Mash, PhD       |                         |                    | study                                                                         |
| Aleena Ali, BSc | University of Miami, FL | Author             | Major role in the acquisition of data                                         |
| William K.      | University of Miami, FL | Author             | Performed biostatistical review of results                                   |
| Scott, PhD      |                         |                    |                                                                               |
| Thomas J.       | Stanford University,    | Author             | Provided samples, performed pathologic evaluations, and conceptualized the   |
| Montine, MD,    | Stanford, CA            |                    | study                                                                         |
| PhD             |                         |                    |                                                                               |

Continued
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CORRECTION

**Genome-wide Brain DNA methylation analysis suggests epigenetic reprogramming in Parkinson disease**

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In the article “Genome-wide Brain DNA methylation analysis suggests epigenetic reprogramming in Parkinson disease” by Young et al.,1 first published online June 24, 2019, Dr. Sathesh K. Sivasankaran should have been listed as co-first author. The authors regret the error.

**Reference**

1. Young JI, Sivasankaran SK, Wang L, et al. Genome-wide brain DNA methylation analysis suggests epigenetic reprogramming in Parkinson disease. Neurol Genet 2019;5:e342.