Epigenome-Wide DNA Methylation Changes Associated with Levodopa-Induced Dyskinesia in Parkinson's Disease – A Pilot Study

Nathan Dunton, **Rayian Higzi, Oliver Rushton, Nurul Sarifah, Mark Kristiansen**.

UCL Genomics, UCL Great Ormond Street Institute of Child Health, University College London, London, WC1N 1DX, United Kingdom

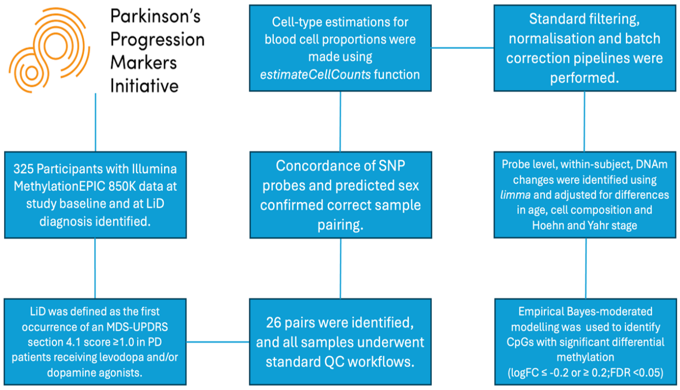
Abstract

Levodopa remains the gold-standard therapy for Parkinson's disease (PD). However, chronic exposure often results in **levodopa-induced dyskinesia (LiD)**, a debilitating side effect with a poorly characterised aetiology. Using Illumina MethylationEPIC 850K array data from the Parkinson's Progression Markers Initiative (PPMI), we performed a paired epigenome-wide association study (EWAS) comparing individuals at the pre-LiD stage of PD and at LiD onset. After adjustment for blood cell composition, age, and PD progression, 566 CpG loci showed significant differential methylation (logFC ≤ −0.2 or ≥ 0.2; FDR < 0.05) and will inform future, larger-scale epigenetic studies in LiD. However, no significant differentially methylated regions (DMRs) or GO/KEGG pathway enrichment were detected, likely reflecting the low statistical power of this pilot study. These findings represent, to our knowledge, the first attempt to characterise the human LiD methylome. Our results indicate subtle yet widespread changes to DNA methylation, with affected loci linked to genes associated with neuronal stress, plasticity and dopaminergic signalling.

Introduction

- Approximately 40% of PD patients develop LiD within five years of starting levodopa therapy[1]
- LiD is characterised by a range of involuntary movements including chorea, dystonia, and athetosis[2]
- There is a strong relationship between increasing levodopa dosages and the onset of more frequent and severe dyskinesias[3]
- In rat models, levodopa induces genome wide methylation changes in striatal tissue[4]
- Little is known about the role DNA methylation plays in the development of LiD. We hypothesise that chronic exposure to levodopa induces DNA methylation changes

Methods



Parkinson's Progression Markers Initiative

325 Participants with Illumina MethylationEPIC 850K data at study baseline and at LiD diagnosis identified.

LiD was defined as the first occurrence of an MDS-UPDRS section 4.1 score ≥1.0 in PD patients receiving levodopa and/or dopamine agonists.

26 pairs were identified, and all samples underwent standard QC workflows.

Concordance of SNP probes and predicted sex confirmed correct sample pairing.

Cell-type estimations for blood cell proportions were made using *estimateCellCounts* function

Standard filtering, normalisation and batch correction pipelines were performed.

Probe level, within-subject, DNAm changes were identified using *limma* and adjusted for differences in age, cell composition and Hoehn and Yahr stage

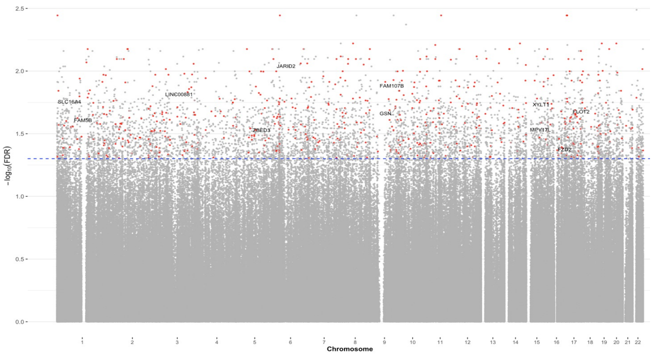
Empirical Bayes-moderated modelling was used to identify CpGs with significant differential methylation (logFC ≤ -0.2 or ≥ 0.2;FDR <0.05)

Results

1) Summary of EWAS findings

Measure	Finding
Number of CpGs analysed	865,859
Significant CpGs (LogFC ≤ −0.2 or ≥ 0.2; FDR < 0.05)	566
Top 10 significant associated genes by LogFC	**FAM107B, DNM1L, NCKAP1, SORBS2, KIAA0513, RPH3A, NEFM, C10orf11, TMEM132C, TSPAN2**
Methylation shift of 566 significant CpGs	71% hypomethylated 29% hypermethylated
GO/KEGG enrichment	None significant
Differentially methylated regions	None Significant
Study power (paired t-test model)	~8%
Samples required for 80% power	~65 paired samples

2) Manhattan plot showing CpGs with the greatest LogFC and associated genes



Conclusions and Limitations

- Although statistically underpowered, this pilot study broadly supports our hypothesis that LiD is associated with changes in DNA methylation.
- Subtle and widespread changes in DNA methylation are observed in LiD including within genes that play a role in dopaminergic and GABAergic signalling, neuronal stress and plasticity
- A small proportion of DMPs are also linked with PD progression and different metrics used to adjust for this co-variate should be explored
- This work shows the continuing power of methylation microarrays in biomarker discovery

1.Martinez-Carrasco A et al. Genetic meta-analysis of levodopa induced dyskinesia in Parkinson's disease. NPJ Parkinsons. 2023.
2. Gerlach M et al. Mechanisms underlying and medical management of l-Dopa-associated motor complications. J Neural Transm. 2011
3. Servillo F et al. Molecular and cellular determinants of L-Dopa-induced dyskinesia in Parkinson's Disease. NPJ Parkinsons Dis. 2024
4. Figge DA et al. Methylation Regulates Levodopa-Induced Dyskinesia. J Neurosci. 2016.