A role for epigenetic mechanisms in the Lewy body dementias

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Background

The Lewy body diseases (LBDs), Dementia with Lewy bodies (DLB), Parkinson's disease (PD) and Parkinson's disease dementia (PDD) are all neurodegenerative diseases classified by the accumulation of alpha-synuclein in neurons, forming Lewy bodies (LBs) [1]. These three diseases all have similar underlying neuropathological profiles, but have distinct staging of clinical symptoms (**figure 1).** In particular, PDD and DLB are often indistinguishable at post-mortem and must be differentiated by the appearance of parkinsonism and dementia symptoms, respectively [1].

Previous studies in other neurodegenerative conditions, such as Alzheimer's disease, have shown that there is disease-associated methylomic variation [2-4]. We hypothesise that disease associated LBs cause DNA methylation changes within neurons and surrounding cells and that these changes can be used to distinguish the different diseases from one another.

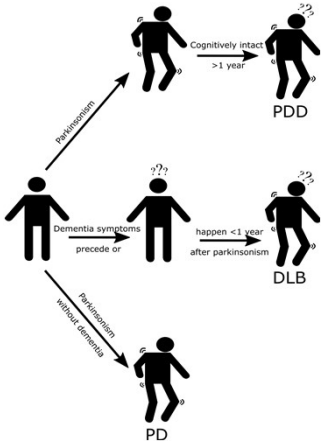
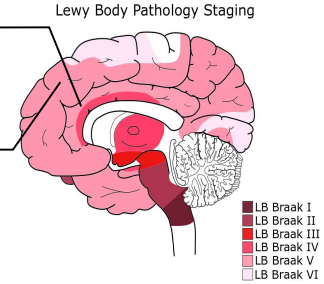


Figure 1. Diagram illustrating the distinct clinical symptoms of the Lewy Body diseases.

Methods

Two cortical regions relevant to clinical phenotype and neuropathology development were selected for bulk tissue analysis. A subset of samples from each of the LB diseases and controls will be used to validate and determine the cellular specificity of the findings.



Anterior Cingulate (BA24/32)

- Effected earlier in Braak PD neuropathology staging.
- Highly associated with cognitive decline in PD [5].
- Susceptible to Lewy body pathology.

Prefrontal Cortex (BA9)

- Effected late in Braak PD neuropathology staging.
- Severity of dementia highly correlated with LB density [6].
- Associated with visual hallucinations [6].

Data Generation

Cortical grey matter from both regions underwent simultaneous DNA/RNA extraction using the Qiagen Universal Kit. DNA was then bisulfite converted before being run on the ***Illumina Infinium HumanMethylationEPIC Array*** generating a quantitative measurement of 5-methyl-Cytosine for more than 850,000 loci sites across the genome [7]. To ensure the data was of sufficient quality the bisulphite conversion efficiency, median sample intensities, p-filter, reported/predicted genders, outliers were checked. Twenty samples failed this pipeline and so were excluded.

Data Analysis

Following quality control (QC) samples were normalised using Dasen (from the watermelon package [7]) and principal components were identified. A cross cortical analysis was performed using linear regression to identify loci and regions associated with LB pathology. Comb-p was used to identify differentially methylated regions (DMRs). An ANOVA followed by a post-hoc Tukey test was performed to identify loci associated with clinical diagnosis.

Results

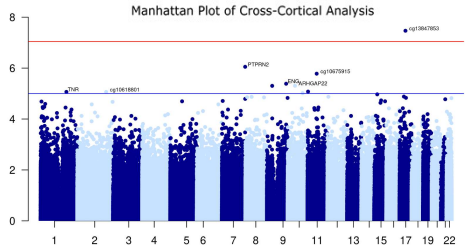


Figure 2. Manhattan plot of cross-cortical analysis. Manhattan plot depicting probe significance against chromosomal location. Where the red line indicates the significance threshold recommended for EPIC array methylation studies [8], $p=9e^{-8}$, and the blue line shows an arbitrary soft threshold at $p=1e^{-5}$. This plot shows that we have one CpG, annotated to *KRT19*, reaching significance and numerous other DMPs reaching the soft threshold significance. Our second most significant probe *PTPRN2* has previously been shown to be differentially methylated in other epigenomic studies of PD [9-11].

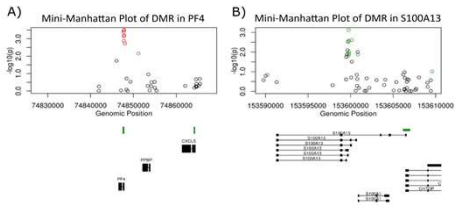


Figure 3. Mini-manhattan plot of DMRs. Mini-manhattan plots depicting probe significance against chromosomal location for **A)** *PF4*, where there are six hypomethylated probes and **B)** *S100A13* where there are nine hyper methylated probes. Red points indicate decreasing methylation with increasing LB Braak stage and green points indicate increasing methylation with increasing Braak LB stage. Under each plot is a UCSC gene track showing nearby genes. Green bars on the gene track indicate CpG islands.

CpG ID	Estimate	Standard Error	T-Value	P-Value	CHR	Position	Annotation
cg13847853	0.002384	0.00042	5.671981	3.41E-08	17	39696336	KRT19
cg10257673	0.001695	0.000337	5.023549	8.88E-07	7	157573278	PTPRN2
cg10675915	-0.00227	0.000465	-4.89214	1.66E-06	11	61801449	FTH1
cg05000435	0.001589	0.000338	4.695445	4.11E-06	9	130610943	ENG
cg00991285	-0.002	0.00043	-4.65422	4.95E-06	10	49710502	ARHGAP22

Table 1. Top five differentially methylated positions (DMPs) in cross-cortical analysis. After QC and normalisation, linear regression was used to determine the top DMPs associated with LB pathology. For each of the top DMPs the CpG ID, estimate, standard error, t-value, p-value, chromosome, position location and gene annotation are given. For annotation the Illumina UCSC reference was used, where this wasn't available GREAT annotation was used in its place, GREAT annotated genes are highlighted in green. The gene *PTPRN2* has been highlighted as this has been previously implicated in other epigenomic studies of PD [9-11].

References

1) Jellinger, K. A., 2018. "Dementia with Lewy Bodies and Parkinson's Disease-Dementia: Current Concepts and Controversies." *Journal of Neural Transmission* 125(4): 615–50.
2) Lunnon, K. *et al.* 2014. "Methylomic profiling implicates cortical deregulation of ANK1 in Alzheimer's disease." *Nature neuroscience*. NIH Public Access, 17(9), pp. 1164–70. doi: 10.1038/nn.3782.
3) De Jager, P. L., *et al.* 2014. "Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci." *Nature neuroscience*. 17(9):1156-63. doi: 10.1038/nn.3786
4) Smith, R. G., *et al.* 2018. "Elevated DNA methylation across a 48-kb region spanning the HOXA gene cluster is associated with Alzheimer's disease neuropathology". *Alzheimer's and Dementia*. 14(12):1580-1588.
5) Prell, Tino. 2018. "Structural and Functional Brain Patterns of Non-Motor Syndromes in Parkinson's Disease." *Frontiers in Neurology* 9(MAR).
6) Sanchez-Castaneda, C. , Rene, R. , Ramirez-Ruiz, B. , Campdelacreu, J. , Gascon, J. , Falcon, C. , Calopa, M. , Jauma, S. , Juncadella, M. and Junque, C. (2010), Frontal and associative visual areas related to visual hallucinations in dementia with Lewy bodies and Parkinson's disease with dementia. Mov. Disord., 25: 615-622.
7) Pidsley, Ruth et al. 2013. "A Data-Driven Approach to Preprocessing Illumina 450K Methylation Array Data." *BMC Genomics* 14(1): 293.
8) Mansell, G., *et al.* 2019. "Guidance for DNA methylation studies: statistical insights from the Illumina EPIC array". *BMC Genomics*.
9) Young, J. I., *et al.* 2019. "Genome-wide brain DNA methylation analysis suggests epigenetic reprogramming in Parkinson disease". *Neurology Genetics*.
10) Chuang, Y., *et al.* "Longitudinal epigenome-wide methylation study of cognitive decline and motor progression in Parkinson' disease". *Journal of Parkinson's disease*.
11) Kochmanski, J., *et al.* "Parkinson's disease associated, sex-specific changes in DNA methylation at PARK7 (DJ-1), ATXN1, SLC17A6, NRAA2, and PTPRN2 in cortical neurons". *BioRxiv*.
12) Bettencourt, C., *et al.* 2020. "White matter DNA methylation profiling reveals deregulation of HIP1, LMAN2, MOBP, and other loci in multiple system atrophy." *Acta neuropathalogica*. 139(1): 135–156.
13) Su, L., *et al.* 2018. "A meta-analysis of public microarray data identifies biological regulatory networks in Parkinson's disease." *BMC Medical Genomics*. 11: 40.
14) Zaltieri, M., *et al.* 2015. "α-synuclein and synapsin III cooperatively regulate synaptic function in dopamine neurons." *Journal of cell science*. 128(13):2231-43.
15) Policicchio, Stephania *et al.* 2020. "Fluorescence-activated nuclei sorting (FANS) on human post-mortem cortex tissue enabling the isolation of distinct neural cell populations for multiple omic profiling". Protocols.io

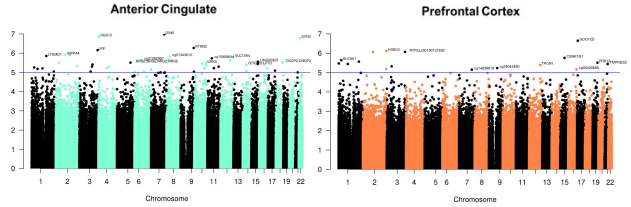


Figure 2. Manhattan plots of clinical analysis. Manhattan plots depicting probe significance against chromosomal location for clinical analysis undertaken in **A)** the anterior cingulate and **B)** the prefrontal cortex. Where the blue line shows an arbitrary soft threshold at $p=1e^{-5}$. From this analysis there were no genome wide significant genes but in each there were numerous nominally significant loci many of which are disease relevant and have been previously implicated in synucleinopathies [12-13].

CpG ID	ANOVA P-value	DLB vs CON	PDD vs. CON	DLB vs. PDD	CHR	Position	Annotation
cg01720013	1.05E-07	1.24E-07	0.663115	2.62E-05	7	137529320	DGKI
cg16895261	1.39E-07	1.87E-07	0.441748	0.000101	4	3391305	RGS12
cg06116251	1.65E-07	0.009757	0.004301	5.18E-08	22	33359846	SYN3
cg17374726	5.32E-07	0.002479	3.51E-07	0.210596	9	135068203	NTNG2
cg04441577	7.03E-07	8.76E-06	4.22E-05	0.849012	3	188429875	LPP

Table 2. Top five differentially methylated positions (DMPs) in clinical analysis. After QC and normalisation an ANOVA followed by post-hoc Tukey test was used to identify loci that are differentially methylated between diseases. For each of the top DMPs the CpG ID, ANOVA p-value, post-hoc p-values for each comparison, chromosome, position location and gene annotation are given. For annotation the Illumina UCSC reference was used, post-hoc p-values that are significant have been highlighted in green. Our top two genes, *DGKI and TGS12,* have both been previously implicated in synucleinopathies [12-13]. Alongside this *SYN3* (synapsin III) has been shown to interact with alpha-synuclein to regulate dopaminergic neuronal synaptic function [14].

Conclusions

By assessing genome wide levels of DNA methylation we have been able to look at methylomic variation and its association with LB pathology and diagnosis in two LBD relevant brain regions. Loci highlighted through our analyses, such as *PTPRN2* **(table 1 and figure 2)**, have previously been implicated in methylomic studies of PD [9-11] suggesting these findings are robust. We have also identified two DMRs associated with pathology, these are *PF4,* which shows decreased methylation, and *S100A13,* which shows increased methylation **(figure 3)**. Further to this findings in the clinical analyses, such as *DGKI,* which has been previously associated with other synucleinopathies [12-13], and *SYN3* which is known to interact with alpha-synuclein [14] add additional robustness **(table 2 and figure 4)**.

Work is also currently underway to determine the cell type specificity of our findings using laser capture microdissection and fluorescence activated nuclei sorting [15].

Funders

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