A role for epigenetic mechanisms in the Lewy body dementias

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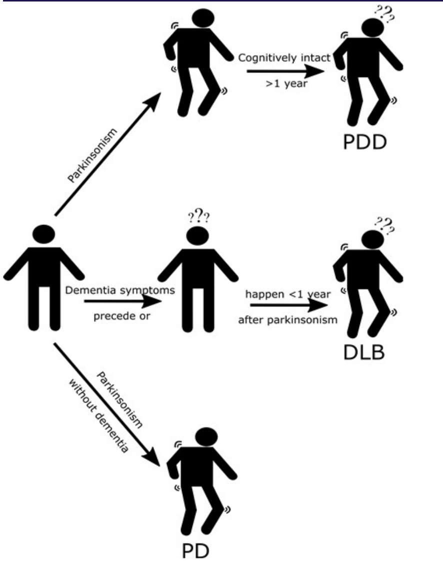
Background



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 (LB)[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].

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

Study Aims

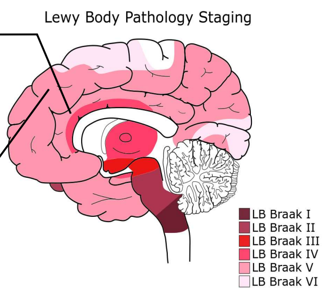
DNA methylation is a reversible epigenetic modification with robust effects on gene expression. Previous work from our group has shown an association between DNA methylation status and Alzheimer's disease[2]. We now aim to design a study to assess its relation to the spectrum of Lewy Body dementias:

1. Profile methylation at a genome wide resolution for cohorts of Lewy Body dementia and assess profile relationship to:
   a. Neuropathology
   b. Clinical phenotype
2. Assess the cell type specific affects of Lewy Body Dementia using cell sorting.

Figure 2. Diagram of project workflow.

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[3].
- Susceptible to Lewy Body Pathology.

Prefrontal Cortex (BA9)

- Effected late in Braak PD neuropathology staging.
- Severity of dementia highly correlated with LB density[4].
- Associated with visual hallucinations[4].

Data Generation

Cortical grey matter from both regions underwent simultaneous DNA/RNA extraction using the Qiagen Universal Kit. DNA was then bisulphite 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[5]. 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[5]) 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).

Cohort Demographics

Braak LB	0	3	4	5	6
n	197	56	76	57	197
CNG / PFC	99 / 98	28 / 28	38 / 38	28 / 29	99 / 98
Mean Age (SD)	80.64 (9.8)	80.32 (7.9)	78.26 (6.1)	76.87 (6.7)	77.82 (6.9)
Female/Male	92 / 105	26 / 30	24 / 52	23 / 34	62 / 135

Table 1. Cohort Demographics. Table outlining the number and characteristics of samples at each Braak LB stage. This includes number (n), number of CNG and PFC samples, mean age, and number of male and female samples per group.

Results

CpG ID	Estimate	Standard Error	T-Value	P-Value	CHR	MAPINFO	Annotation
cg13847853	0.002391	0.000421	5.684756	3.19E-08	17	39696336	KRT19
cg10257673	0.001698	0.000338	5.022656	8.92E-07	7	157573278	PTPRN2
cg10675915	-0.00227	0.000465	-4.88538	1.71E-06	11	61801449	FTH1
cg05000435	0.001598	0.000339	4.720291	3.67E-06	9	130610943	ENG
cg25282776	0.001746	0.000373	4.687395	4.26E-06	9	35798844	NPR2
cg00991285	-0.002	0.00043	-4.65802	4.87E-06	10	49710502	ARHGAP22
cg10618801	-0.00287	0.000631	-4.54102	8.22E-06	2	196397607	SLC39A10
cg20571958	0.001547	0.000342	4.523485	8.88E-06	1	175469299	TNR
cg15608402	0.000581	0.000129	4.517311	9.12E-06	11	3122933	OSBPL5
cg17828015	0.00199	0.000443	4.495595	1.00E-05	10	101677862	DNMBP

Table 2. Top ten differentially methylated positions (DMPs). After QC and normalisation linear regression was used to determine the top DMPs associated with LB pathology. For each of the top ten DMPs the CpG ID, estimate, standard error, t-value, p-value, chromosome, MAPINFO location and 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.

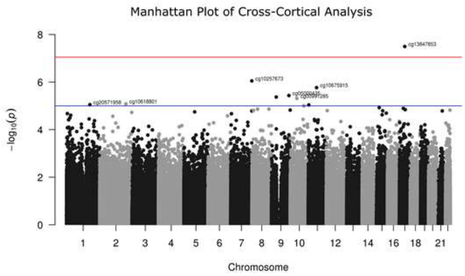


Figure 3. Manhattan plot of cross-cortical analysis. Manhattan plot depicting probe significance against chromosomal location. Where the red line indicates the significance threshold recommended for EPIC array methylation studies[6], $p=9e^{-8}$, and the blue line shows an arbitrary soft threshold at $p=1e^{-5}$. 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[7-9].

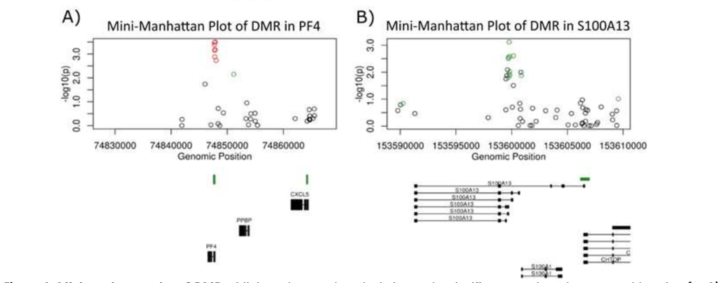


Figure 4. Mini-manhattan plot of DMRs. Mini-manhattan plots depicting probe significance against chromosomal location for **A)** *PF4*, where there are six probes showing decreased methylation and **B)** *S100A13* where there are nine probes showing increased methylation. Red points indicate decreasing 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.

Conclusions

By assessing genome wide levels of DNA methylation we have been able to look at methylomic variation and its association with LB pathology in two LBD relevant brain regions. Loci highlighted through our analyses, such as *PTPRN2* **(table 2 and figure 3)**, have previously been implicated in methylomic studies of PD[6-8] 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 4)**.

In the future we would like to take these analyses further by assessing the brain regions separately, looking at the association between methylation and both clinical diagnosis and neuropathology. Work is also currently underway to determine the cell type specificity of our findings using laser capture microdissection and fluorescence activated nuclei sorting[10].

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