Telomere Length Variation in the Lewy Body Diseases and its Relationship to Epigenetic Age

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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). As both the LBDs and the shortening of telomeres are well established with aging it is reasonable to hypothesise some involvement of telomere shortening in the LBDs. There are few studies addressing telomere length changes in the LBDs and as these studies are often conflicting, we aim to rectify this disparity and establish whether there is a relationship between disease diagnosis, LB Braak staging and epigenetic age.

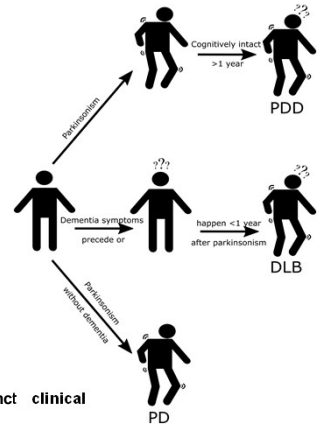

Figure 1. Diagram illustrating the distinct clinical symptoms of the Lewy Body diseases.

Methods

Sample Collection and DNA Extraction

921 brain tissue samples were obtained from 474 elderly individuals from four brain banks at Imperial College London, Oxford, Newcastle and the University College London Queens Square. Where possible we requested two brain regions from each individual, the prefrontal cortex (PFC) and cingulate gyrus (CNG). After cutting and weighing each brain sample was milled into a fine powder on liquid nitrogen and DNA and RNA were subsequently extracted using the Qiagen Universal kit.

Infinium Human Methylation EPIC BeadChip Array

After DNA extraction, the DNA was 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 [1]. To ensure the data was of sufficient quality the bisulfite conversion efficiency, median sample intensities, p-filter, reported/predicted genders, outliers were checked.

Epigenetic Age Calculation

Epigenetic (predicted) age was calculated using the cortical DNAm age clock developed by Shireby *et al.* [2]. In brief the QC'd and normalised data and predicted ages were calculated using a weighted sum of the cortical clock coefficients plus the intercept. Information about the calculation and code to run it are available at: https://github.com/gemmashireby/CorticalClock.

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.

Quantitative Real Time PCR

Methodology used is an adaptation of the methods developed by O'Callaghan and Fenech [3] and Cawthon [4]. In brief, we performed qRT-PCR in triplicate in 384 well optically clear plates in a total reaction volume of 5µl. On each plate we also included 8 standard DNA samples in triplicate of known copy number. Samples were run on a QuantStudio 6K qPCR machine.

Linear modelling of Telomere Length

Data was analysed using linear regression models controlling for the co-variates of age, sex, plate, brain bank, brain region, post mortem interval and neurofibrillary tangle Braak stage. Individual telomere lengths were compared to (a) biological age (b) clinical diagnosis, (c) LB Braak stage and (d) epigenetic age [2].

Primer/Standard design

Telomere primers and standards were taken from Cawthon, R., 2002 [4] as here the primers had been specifically designed to prevent primer dimer derived amplification products. GAPDH primers were designed to map to the transcription start site and the GAPDH standard was used as this is the predicted PCR amplicon.

Primer sequences	GAPDH Forward	GCCCCCGGTTTCTATAAATT
	GAPDH Reverse	GAACAGGAGGAGCAGAGAG
	Telomere Forward	GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT
	Telomere Reverse	TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA
Standard sequences	GAPDH	GCCCCCGGTTTCTATAAATTGAGCCCGCAGCCTCCCGCTTCGCTCTCTGCTCCTCCTGTTC
	Telomere	(TTAGGG)14

Table 2. Primer and standard sequences. Table showing the primer and standard sequences to determine telomere length, where GAPDH is used as the single copy gene to determine absolute telomere length. The telomere standard is compromised of the same hexanucleotide sequence repeated 14 times.

Results

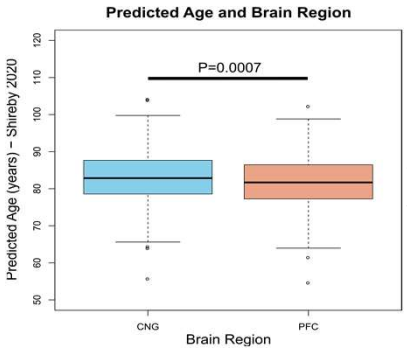

Figure 2. Relationship between predicted age and brain region. Using the predicted cortical age from Shireby *et al.* [2] we explored the relationship between predicted epigenetic age and brain region. From this analysis we determined that the epigenetic age of the PFC samples was significantly lower than the cingulate samples. This could be the result of the CNG being affected earlier in the disease course than the PFC.

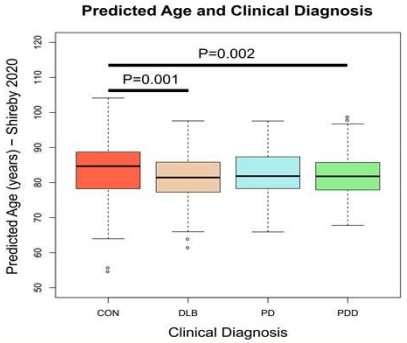

Figure 3. Relationship between predicted age and clinical diagnosis. Using the predicted cortical age from Shireby *et al.* [2] we explored the relationship between predicted epigenetic age and the clinical diagnosis of each individual. Interestingly from this we can see those diagnosed with DLB or PDD are epigenetically younger than controls or those with PD. This suggests that something intrinsic to the dementia aspect of the diagnosis is affecting the epigenetic age prediction and resulting in a younger age. This result seems counterintuitive but may be as a result of the people living with DLB and PDD dying younger.

References

1. Pidsley, R., *et al.* 2013. A Data-Driven Approach to Preprocessing Illumina 450K Methylation Array Data. *BMC Genomics* 14(1): 293.
2. Shireby, G. L., *et al.* 2020. Recalibrating the epigenetic clock: implications for assessing biological age in the human cortex. *Brain.* 143(12), p. 3763-3775.
3. O'Callaghan, N.J. and M. Fenech, M., 2011. A quantitative PCR method for measuring absolute telomere length. *Biological Procedures Online.* 13:3.
4. Cawthon, R. M., 2002. Telomere measurement by quantitative PCR. *Nucleic Acids Research.* 30(10).

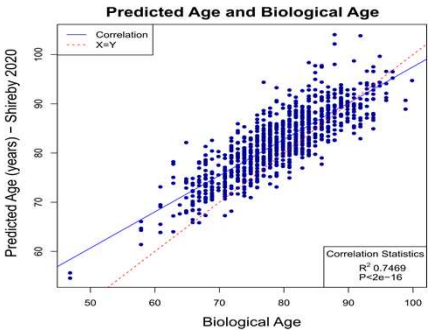

Figure 4. Relationship between predicted age and biological age. Using the predicted cortical age from Shireby *et al.* [2] we explored the relationship between predicted epigenetic age and biological age at death. This analysis demonstrates a very clear and significant correlation between each of the ageing metrics. Also shown is the line X=Y, as a large proportion of samples sit above this line it suggests that they are epigenetically older than their actual age, indicating potential age acceleration.

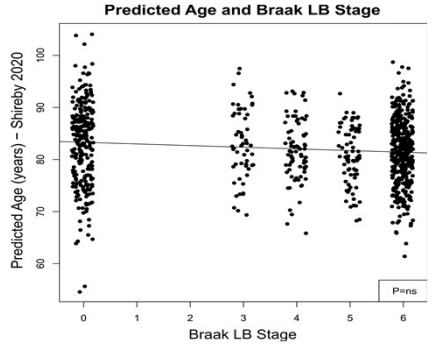

Figure 5. Relationship between predicted age and LB Braak stage. Using the predicted cortical age from Shireby *et al.* [2] we explored the relationship between predicted epigenetic age and Braak LB stage. It is clear from this analysis that there is no significant relationship between epigenetic age and neuropathological stage. This suggests that LBs and their spread have no influence on epigenetic or predicted age. Braak LB stage 1 and 2 are not represented on the graph as we were unable to acquire tissue from people with these pathological stages.

Conclusions

In this study we have used 135 PD, 307 PDD, 176 DLB and 314 control samples in two different brain regions to look at the relationship between epigenetic age, brain region **(figure 2)**, clinical diagnosis **(figure 3)**, biological age **(figure 4)** and neuropathology **(figure 5)**. So far we have determined that there are significant relationships between epigenetic age with brain region, clinical diagnosis and biological age. These relationships could be the result of the differences in progression between the LBDs and how they affect the two brain regions.

Optimisation of the telomere and single copy gene primers have now been completed and so work is under way to measure telomere length in the full cohort of 921 samples. Once completed we shall determine how telomere length relates to epigenetic age, diagnosis, biological age neuropathology.

Funders

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