Mitochondrial Biogenesis, Telomere Length and Cellular Senescence in Parkinson's Disease and Lewy Body Dementia

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Mitochondrial biogenesis, telomere length and cellular senescence in Parkinson's Disease and Lewy Body Dementia

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Abstract

Background: Progressive age is the single major risk factor for neurodegenerative diseases. Cellular aging markers during the course of Parkinson's disease (PD) have been implicated in previous studies, however majority of these studies have investigated the association of individual cellular aging hallmarks with PD but not jointly.

Method: Here, we have studied the association of PD with three aging hallmarks (telomere attrition, mitochondrial dysfunction, and cellular senescence) in blood and the brain tissue. Telomere length and mitochondrial DNA (*mtDNA*) copy number was assessed by qPCR, while mitochondrial function (*PGC-1α and PGC-1β*) and expression of cyclin-dependent kinase inhibitor 2A (*CDKN2A*), cellular senescence marker was measured by RT-qPCR.

Results: Our results show that patients diagnosed with PD had 20% lower mitochondrial DNA copy number but 26% longer telomeres in blood compared to controls. Moreover, telomere length in blood was positively correlated with medication (Levodopa Equivalent Daily Dose, LEDD). Similar results were found in brain tissue, where patients with Parkinson's disease (PD), Parkinson dementia (PDD) and Dementia with Lewy Bodies (DLB) showed (46-95%) depleted *mtDNA* copy number, but (7-9%) longer telomeres compared to controls. Furthermore, when compared to controls, patients had lower mitochondrial biogenesis (*PGC-1α and PGC-1β*) and higher load of cellular senescent cells in postmortem prefrontal cortex tissue, where DLB showing the highest effect among the patient groups.

Conclusion: Our results show that mitochondrial dysfunction and cellular senescence but not telomere shortening is associated with PD, PDD and DLB. Our findings suggest that mitochondrial copy number and function could be used as viable biomarker in blood as an early indicator for the risk of neurodegenerative diseases.

Background

Parkinson's disease (PD) is the second most common age-related neurodegenerative disease, characterized by motor dysfunctions caused by the progressive death of dopaminergic neurons in the substantia nigra, and is often accompanied by non-motor symptoms such as dementia, mood and sleep disorders[1-3]. Although PD is a complex disease with several causes, including genetic and environmental factors, progressive age remains the single major risk factor for PD[3].

Aging is characterized by a time-dependent progressive deterioration of an organism's functions, caused by the accumulation of deleterious changes throughout its lifetime[4]. Cellular aging markers such as mitochondrial dysfunction and telomere shortening have been associated with age related disorders and neurodegenerative diseases[5-7]. Mitochondria are double-membrane-bound organelles maintaining the functional and structural integrity of post-mitotic tissues, through involvement in cellular bioenergetics and reactive oxygen species (ROS) production[8]. Lower blood mitochondrial DNA (*mtDNA*) copy number has been associated with high mortality, poor health conditions, worse physical performance, and cognitive impairment[9]. Somatic *mtDNA* damage and mutation are part of the natural aging process, however, it has also been linked to age associated diseases and neurodegeneration in humans[7,10-12]. Furthermore, increased accumulation of *mtDNA* mutations and damage has been shown to contribute to impaired mitochondrial respiration[12,13]. Hence, mitochondrial DNA content and function might represent a valuable biomarker to monitor early changes in different physiological and pathological states.

Telomere shortening has been associated with several age-related disorders, infectious diseases and neurodegenerative diseases[14-17]. Telomeres are non-coding, ribonucleotide structures composed of highly conserved repetitive hexamer 5′-TTAGGG-3′ and a core of proteins called

shelterin. Telomeres maintain chromosomes' integrity by capping the ends to prevent end-to-end joining of chromosomes and preventing loss of coding DNA sequences during DNA replication. Telomeres shorten progressively over time until reaching a critical length that leads to cell-cycle arrest, senescence, or apoptosis, respectively[4,18]. Whether telomere shortening also contributes to the pathogenesis of neurodegenerative disorders remains to be understood. Previous studies provide inconclusive findings regarding the association of telomere length and PD, where both shorter and longer telomeres have been identified as a risk factor for PD[17,19].

Furthermore, it has been shown that shorter telomeres and dysfunctional mitochondria in turn lead to cellular senescence[4], a state of irreversible cell cycle arrest, which is associated with age related pathology and phenotypic alternations[20,21]. Expression of cyclin-dependent kinase inhibitor 2A (CDKN2A) gene is positively correlated to cellular senescence and has emerged as a valuable marker of cellular senescence over the last decade [21,22]. CDKN2A is a cell cycle inhibitor gene encoding for p16INK4a and p14arf[20,21]. Expression of CDKN2A is positively correlated with 3-repeat TAU (microtubule-associated protein) transcripts in blood and associated with mild cognitive decline in humans[23].

This study investigated the association of PD with several cellular aging biomarkers and their relationship within the same samples. We investigated the association between PD with cellular aging biomarkers (telomere attrition, mitochondrial copy number) in blood. Furthermore, we investigated the association of PD, Parkinson's Disease Dementia (PDD) and Dementia with Lewy Bodies (DLB) with cellular aging biomarkers (telomere attrition, mitochondrial dysfunction, and cellular senescence) in postmortem prefrontal cortex tissues.

Materials and Methods

Blood Samples from Swedish cohort

The blood samples were obtained from PD patients included in the Swedish BIOPARK cohort (approved by the Swedish Ethical Review Authority, reference number 2019-04967)[24]. Patients were recruited in clinics within Stockholm region, Sweden, and from the Sunderby Hospital in Luleå, Sweden. Both verbal and written consent were obtained at the time of inclusion. Blood was drawn by venepuncture by trained personnel and collected in EDTA tubes. DNA was extracted using QIAmp DNA Blood Maxi Kit (Cat# 51994, QIAGEN) according to manufacturer's instructions. DNA concentration was measured using a Nanodrop (Marshall Scientific). The *mt*DNA copy number and the telomere length were measured in the whole blood of n=112 individuals including 100 PD patients and 12 controls. Age range of patients diagnosed with PD was between 47-97 years and male/female ratio was 1.5, while controls had an age range between 54-73 and male/female ratio was 0.3.

Clinical data was collected from all PD patients including, Movement Disorder Society Unified Parkinson's Disease Rating Scale part 3 (MDS-UPDRS III) for motor symptoms, Hoehn and Yahr for disease severity, Montgomery-Åsberg Depression Rating Scale (MADRS) for depression, Hospital Anxiety and Depression Scale subscores for anxiety (HADS-Anxiety) and depression (HADS-Depression), Montreal Cognitive assessment (MoCA) for cognitive assessment, and Levodopa Equivalent Daily Dose (LEDD) as a standard measure for patients' dopaminergic medication.

Brain tissue samples from UK cohort

Postmortem human prefrontal cortex brain tissues were obtained from the MRC London Neurodegenerative Diseases Brain Bank, King's College London, United Kingdom. The permission to collect human brain tissue included participants consent for research purposes and ethical approval was obtained from the UK National Research Ethics Service (08/H1010/4 and KI IRB)[25]. Total 58 brain tissues were used including 13 PD patients, 8 PDD patients, 19 DLB patients and 16 healthy controls. An overview of demographic characteristics of donors used in this study are shown in Table 4. 30 mg of frozen human brain tissues were used to extract total RNA using RNeasy Plus Mini Kit (Qiagen) according to manufacturer's protocol. RNA concentration was measured and evaluated for purity (260/280 nm ratio) using a Nanodrop (Marshall Scientific).

Telomere and mitochondria copy number Assay

Telomere length and mitochondria copy number was measured using ScinceCell kit (cat# 8958) from blood and brain tissues DNA. Each 15 ul reaction contained 7.5ul QuantiNova Syber green (cat # 208054, Qiagen), 0.5 ul telomere or single copy (SCR) or mitochondria primers, 0.1 ul ROX (passive reference dye), 1.9ul DNA/RNA free water and 5 ul (1ng/ul) template DNA. For telomere qPCR, the thermal cycle profile included incubation at 50°C for 2 min and 95°C for 10 min before running 30 thermal cycles (95°C for 15 s, 56°C for 45 s, and 72°C for 45 s). For single-copy gene and mitochondrial copy number qPCR, the thermal profile included incubation at 50 °C for 2 min and 95°C for 10 min before running 40 thermal cycles (95°C for 15 s, 54°C for 45 s, and 72 °C for 45 s). Each assay was run on a separate plate, with each plate containing a serially diluted DNA sample to calculate the PCR efficiency. PCR acceptance value was set to 100 ± 15 %, any plate producing the PCR efficiency outside this range was

repeated. Samples were run in triplicate, and mean C_T value was used for final calculation after carefully checking the melt curve for each sample.

A reference genomic DNA of known telomere length (369 ± 11 kb) and mitochondria copy number (1200 ± 9 copies) was added on each plate. ΔC_T for both telomere length and mitochondrial copy number was calculated using the formula C_T target sample - C_T reference sample after adjusting the PCR efficiency using Pfaffl method[26]. We then calculated the $\Delta\Delta C_T$ for both telomere length and mitochondrial copy number was calculated using the formula (TELΔC_T - SCRΔC_T). Relative telomere length of target sample to reference sample was calculated as 2^- $\Delta\Delta C_T$ and the ratio was then multiplied with 369 Kb to get telomere length per diploid cell. Telomere length of the diploid cell was divided by number of chromosomes ends (92) to get average telomere length of each chromosome end (2^- $\Delta\Delta C_T$ x 369/92). Mitochondria copy number per diploid cell of target sample to reference sample was calculated as 2^- $\Delta\Delta C_T$ and the ratio was then multiplied with 1200 mtDNA copy number for each sample (2^- $\Delta\Delta C_T$ x 1200), as described elswhere[27].

Gene expression

cDNA was synthesized by using QuantiTec Reverse Transcriptase kit (cat# 205311) following the manufacturer guidelines. Thermal profile consisted of 10 minutes incubation at 25°C, followed by 1 hour at 42°C cDNA synthesis and 5 minutes at 85°C to inactivate the enzyme on a QuantStudio5 thermocycler. Relative gene expression of *CDKN2A*, *PGC1 α* and *PGC-1β* was determined using the comparative ΔC_T method by calculating the C_T values of the target genes (*CDKN2a*, *PGC1 α* and *PGC-1β*) against the C_T values of the reference gene (*GAPDH*). Target genes and *GAPDH* were run in triplicates and amplified in the same wells. Respective C_T values

were averaged before performing the ΔC_T calculation ($\Delta C_T = C_{T\ \text{Target}} - C_{T\ \text{GAPDH}}$). Gene expression values were converted into log 2 of ΔC_T (2^- ΔC_T).

Cellular senescence and mitochondrial function

CDKN2A, *PGC1α* and *PGC1b* expression was measured using TaqMan® Gene Expression Assay (cat # HS00923894_m1; cat # Hs00173304_m1, Hs00993805_m1; Applied Biosystem) on a QuantStudio 5 qPCR instrument. The total qPCR reaction of 20 µl contained 3 µl cDNA, 10 µl TaqMan® Multiplex Master Mix (cat # 4461882; Applied Biosystem), 1 µl GAPDH Assay (cat # 4485712; Applied Biosystem), 1 µl of *CDKN2A*, *PGC1α* and *PGC-1β* Assay and ddH2O. TaqMan® *GAPDH* Assay was added to each run as an endogenous control. Thermal profile included 95˚C for 20 s, followed by 45 thermal cycles (95˚C for 1 s and 60˚C for 20 s).

Statistical analysis

Statistical analysis was performed using JMP (version 16). We performed multivariate regression analysis to investigate the correlation of disease with three hallmarks of aging (telomere attrition, mitochondrial dysfunction, and cellular senescence) in blood and brain tissue separately. Age and sex were fitted as fixed factors in all analysis. For further comparison between different groups, we used LS means Student's *t*-test. Pearson correlation was used to access the correlation between different cellular aging markers. Fold change of *PGC1α, PGC-1β* and *CDKN2A* was calculated by diving the individual values with mean value of controls.

Results

Cellular aging biomarkers in whole blood

PD patients had significantly lower number of mitochondria (p = 0.020) but significantly longer telomeres in blood compared to controls (p=0.028), with no effect of age and sex (all p > 0.05,

Figure 1A, B, Table 1). Overall PD patients had 19.7% lower *mtDNA* copy number and 26.3% longer telomeres compared to controls (Figure 1A, B). Mitochondrial DNA copy number and telomere length showed no significant correlation between each other in blood, neither for PD patients nor for controls (all $p > 0.05$, data not shown).

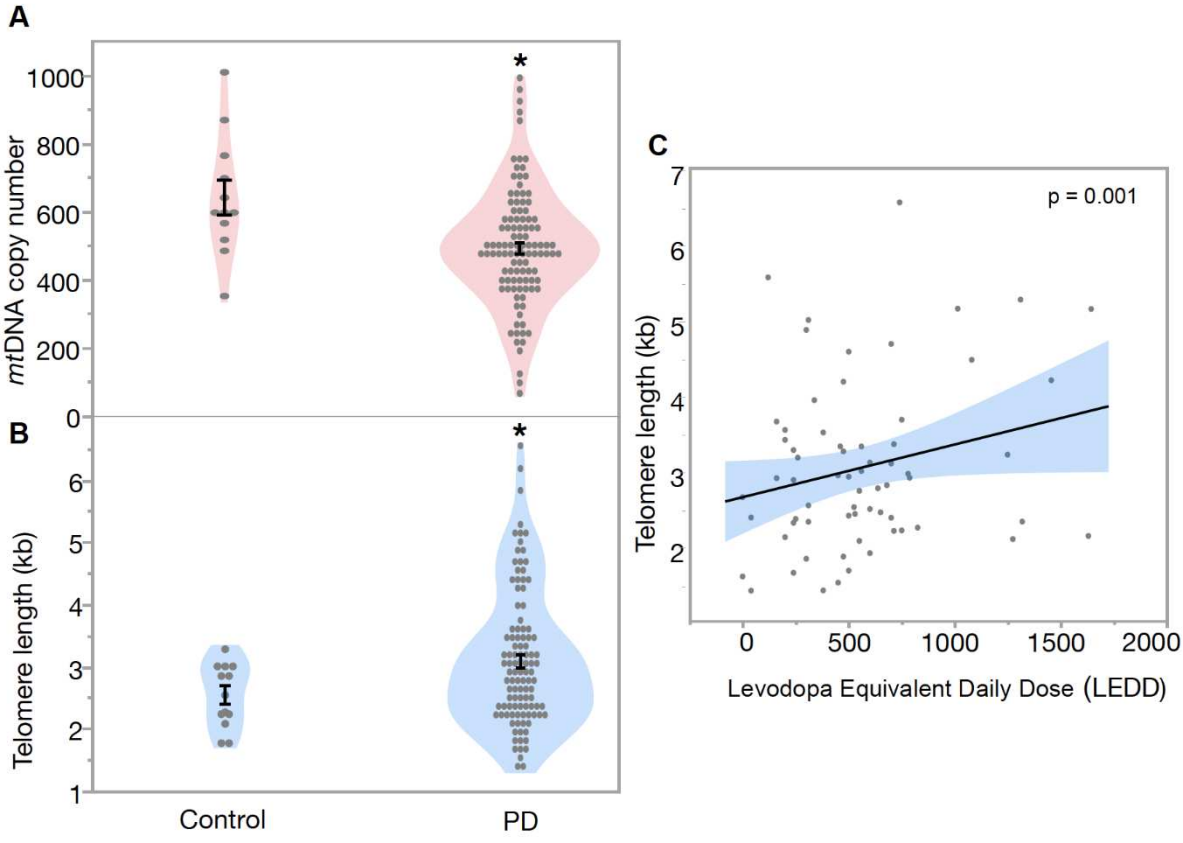

Figure 1. Difference in *mtDNA* copy number (A) and telomere length (B) between Parkinson's Disease (PD) patients and controls in blood. Mean *mtDNA* copy number in PD patients was 505.99 ±17.68, compared to controls 630.48±52.88, while mean telomere length in PD patients was 3.21±1.12 compared to the controls 2.54 ± 0.15 (C) Association between telomere length and Levodopa Equivalent Daily Dose (LEDD) in blood (multivariate regression). Data are presented as mean ± SE. * $p<0.05$ vs control.

Our multivariate regression analysis showed no correlation of *mtDNA* copy number and telomere length in PD with any studied clinical parameter (MDS-UPDRS III, Hoehn and Yahr, MADRS, HADS-Anxiety, HADS-Depression and MoCA; all $p > 0.05$, data not shown).

209 However, telomere length was positively correlated with LEDD in PD patients ($p = 0.015$,
210 Figure 1C) with a significant effect of age and sex (all $p < 0.05$, Table S1), while no such
211 correlation was found for *mtDNA* copy number and LEDD (Table S1).
212
213 ***Cellular aging biomarkers in brain***
214 To assess whether our findings in whole blood DNA are comparable to prefrontal cortex tissue,
215 we studied the *mtDNA* copy number and telomere length in brain tissue from patients with PD,
216 PDD, DLB and healthy controls. Our multivariate regression model show that patients had
217 significantly lower number of *mtDNA* copy number in prefrontal cortex tissue compared to
218 controls ($p < 0.001$ Figure 2A), with no effect of age and sex (Table 2). Comparison of the
219 groups was further explored using LS means Student's *t*-test, which show that all three patients'
220 groups (PD, PDD and DLB) had significantly lower mitochondria copy number (46.4%, 88.9%
221 and 95.6% respectively) compared to controls (all $p < 0.05$, Table S2). Furthermore,
222 mitochondrial *mtDNA* copy number was 91.8% lower in the DLB group than in the PD group
223 ($p = 0.002$, Table S2).
224
225 Telomere length in prefrontal cortex tissue was significantly longer in patients than controls (p
226 < 0.001) with no effect of age and sex (all $p > 0.05$, Figure 2B, Table 2). Further comparison
227 using LS means Student's *t*-test shows that PDD and DLB had significantly longer telomere
228 length (7%, and 9% respectively) when compared to controls (all $p < 0.05$, Table S2). Moreover,
229 the DLB group also shows significantly longer telomeres than PD patients ($p = 0.022$, Table
230 S2). However, there was only a tendency of longer telomeres in PD compared to control ($p =$
231 0.085, Table S2).
232

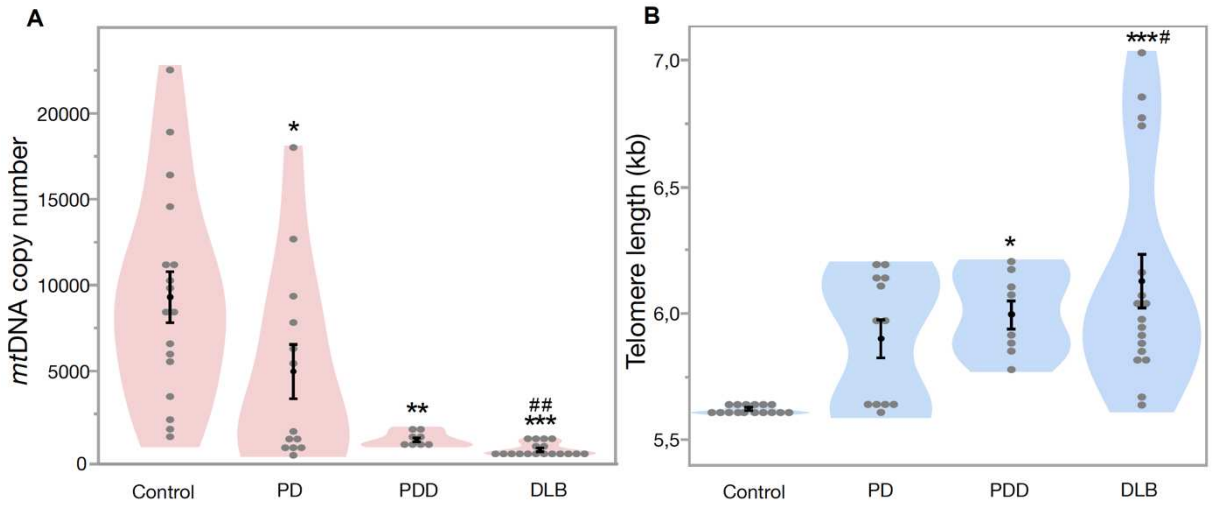

Figure 2. Mitochondrial DNA copy number and telomere length in prefrontal cortex tissue. (A) Mean *MtDNA* copy number in controls (9357.3 ± 1487.4), PD (5015.5 ± 1578.8), PDD (1036.4 ± 119.3) and DLB (410.7 ± 71.3). (B) Mean telomere length in control (5.60 ± 0.001), PD (5.89 ± 0.07), PDD (5.99 ± 0.01) and DLB (6.12 ± 0.10). Data are presented as mean ± SE. Controls vs patients, $p < 0.05$ = *, $p < 0.005$ = **, $p < 0.0005$ = *** and PD vs PDD/DLB, $p < 0.05$ = #, $p < 0.005$ = ##.

Next, we studied mitochondrial biogenesis (*PGC-1α and PGC-1β)* and cellular senescence (*CDKN2A*) in prefrontal cortex tissue. Our multivariate analysis did not show any significant difference between patients and controls for *PGC-1α* gene expression, with no effect of age and sex (Figure 3A, Table 3, Table S2). *PGC-1β* expression was significantly lower in patients ($p = 0.002$), with no effect of age and sex (Figure 3B, Table 3). Further comparison using LS means Student's *t*-test show that controls have higher *PGC-1β* expression when compared to PD ($p = 0.005$), PDD ($p = 0.010$) and DLB ($p = 0.018$), (Table S2).

Overall *CDKN2A* expression was not significantly higher in patients compared to controls (Figure 3C, Table 3), however, the DLB group showed significantly higher *CDKN2A* expression compared to controls, PD and PDD (all $p < 0.05$, Figure 3C, Table S2). Our results show that patients had significantly lower *mtDNA* copy number and *mtDNA* biogenesis gene expression levels but, higher cellular senescence gene expression, where the DLB group

showing the strongest effect among the patient groups (Figure 3D). To further investigate the correlation between different variables, we pooled all the patient data to increase our sample size. In patients' telomere length was negatively correlated with mitochondria copy number (r^2 = 0.167, N = 36, p = 0.012) and positively correlated with *CDKN2A* expression (r^2 = 0.286, N = 18, p = 0.018), while *PGC-1α* and *PGC-1β* were positively correlated with each other (r^2 = 0.543, N = 55, p < 0.001).

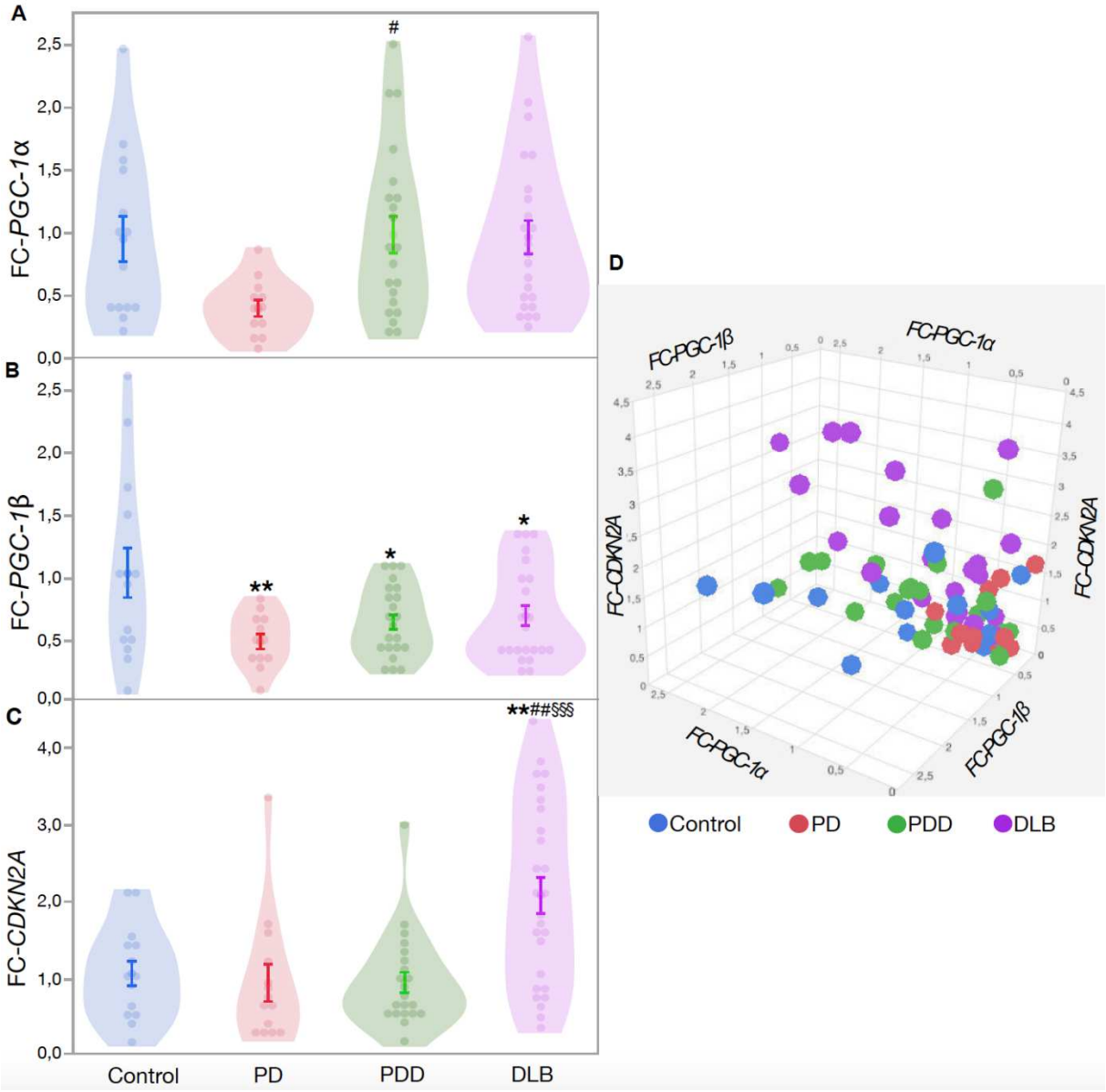

Figure 3. Mitochondrial function and CDKN2A gene expression in brain. (A) Fold change of *PGC-1α* in deceased patients compare to controls, (B) fold change of *PGC-1β* in patients compare to controls, (c) fold change of

CDKN2A in patients compare to controls, (D) 3D graph showing the relation expression of *PGC-1α, PGC-1β* and *CDKN2A* in patients and control. Data are presented as mean ± SE. Controls vs patients $p < 0.05$ = *, $p < 0.005$ = **, $p < 0.0005$ = ***; PD vs PDD/DLB, $p < 0.05$ = #, $p < 0.005$ = ##, $p < 0.0005$ = ###; PDD vs DLB, $p < 0.05$ = §, $p < 0.005$ = §§, $p < 0.0005$ = §§§.

Discussion

Using blood and prefrontal cortex brain tissues from two different cohorts we show that mitochondrial dysfunction (*mtDNA* copy number and *mtDNA* biogenesis gene expression) and cellular senescence, but not telomere shortening is associated with neurodegenerative diseases (PD, PDD and DLB). Our results suggest that mitochondrial dysfunction in blood could be an early indicator for the risk of Parkinson's disease.

A single mitochondrion contains 2-10 copies of *mtDNA*, depending on the type of cell and tissue[28]. Mitochondria include a mutant and a wildtype genome, where the mutant genome is accumulating more aging changes[4]. The number of *mtDNA* copies increases with age, as a compensatory mechanism, which maintains the amount of wild-type *mtDNA* and reverses the effect of defective mitochondria accumulation[29]. However, this compensatory mechanism declines in PD resulting in exhaustion of *mtDNA*, which, in turn, leads to respiratory deficiency in dopaminergic neurons[29]. Here, we report a significant reduction of *mtDNA* copy number in both blood and prefrontal cortex brain tissues of PD, PDD and DLB patients, compared to healthy controls (Figure 1A, 2A). In accordance, previous studies have shown that PD patients have lower *mtDNA* copy number in blood compared to healthy controls[30-32]. We found similar mitochondrial reduction (20%) in whole blood in PD patients compared to 19.6% in PBMC previously reported by Pyle et al.[28]. However, surprisingly, we found lower mtDNA copy numbers (46.4%) in prefrontal cortex tissues, while Pyle et al. showed no significant difference of mtDNA copy numbers between PD patients and controls in frontal cortex[28]. Overall, our

289 results are also in agreement with findings from other neurodegenerative diseases including
290 Alzheimer's disease (AD) and Huntington's disease, where mitochondrial dysfunction is
291 observed[31,33,34].
292
293 Mitochondrial copy number is strongly associated with mitochondrial function, which makes it
294 an important aging marker[35]. *MtDNA* mutation and mitochondrial dysfunction, respectively,
295 have been associated with neurodegenerative diseases such as PD and AD [5,36,37]. Our study also
296 shows lower expression of *PGC-1α* and *PGC-1β* genes (master regulators of mitochondrial
297 biogenesis) in brain tissues of PD, PDD and DLB patients compared to healthy controls (Figure
298 3). In contrast, a recent study by Dölle et al. 2016 showed no difference in *PGC-1α* between
299 PD patients and controls[38]. Inconsistent results of *PGC-1α* correlation could be due to the fact
300 that *PGC-1α* also influences the expression of several other genes involved in metabolic
301 pathways[39], and therefore its expression might be highly regulated to avoid its deleterious side
302 effects. Our study suggests that both lower *mtDNA* copy number and expression of *PGC-1α,*
303 *1β* in PD, PDD and DLB might lead to mitochondrial dysfunction.
304
305 Contrary to the expectation, our results show that PD patients have longer telomeres in blood
306 compared to healthy controls (Figure 1B). we found similar results in brain tissues where PD,
307 PDD and DLB patients show longer telomeres compared to healthy controls (Figure 2B). So
308 far, previous literature has reported no association of telomere length with PD in blood[40-44] and
309 brain tissue[45]. However, a study by Maeda et al., 2012, from Japanese women reported shorter
310 blood telomere length in PD patients[46]. Similarly, DLB patients have been shown to have
311 shorter telomeres compared to controls[47]. However, a recent nested case control study showed
312 a positive association between PD and longer telomere length in leukocytes and PBMCs, where
313 men with shorter telomere length were of lower risk of getting diagnosed with PD[48].

Furthermore, Degerman et al. 2014 reported that PD patients who developed dementia within three years after diagnosis had longer telomere length at diagnosis compared to the other PD patients without early development of dementia[40].

Contradictory results of telomere association with PD could be due to the heterogeneity of the study setup (cross sectional vs nested case control), sample heterogeneity and quality, or differential methods for assessing telomere length. An alternative explanation could be the effect of PD medication on telomere length. Interestingly, our results show that blood telomere length was significantly positively correlated with Levodopa Equivalent Daily Dose (LEDD) medication in PD patients. Furthermore, we found a positive correlation of telomere length with age in PD patients, which may further reflect the cumulative effect of LEDD on telomere length, as older individuals might be on the treatment for a longer time compare to younger PD patients. Nevertheless, to elucidate the relationship between neurodegenerative diseases and telomere length, and to pinpoint whether short/long telomeres are the cause or consequence of neurodegenerative diseases, a longitudinal study set-up is needed.

Here we show a significantly higher expression of cyclin dependent kinase inhibitor 2A (CDKN2A) in prefrontal cortex brain tissue of DLB patients compared to healthy controls. CDKN2A reflects the increased load of cellular senescence and has been shown to be negatively associated with telomere length[15,21,22]. A previous study showed that expression of CDKN2A has been associated with mild cognitive decline in aging humans, where CDKN2A expression was positively associated with 3 repeat TAU (microtubule-associated protein) in blood[23]. However, contrary to previous finding we find a positive correlation between telomere length and increased CDKN2A expression in PD patients. Mechanisms behind such association are yet to be investigated, and we speculate that this could be due to medication (LEDD) effect. A

positive correlation between LEDD treatment and telomere length in this study, suggests that medication might be influencing telomere length by either activating telomerase (a holoenzymes capable of elongating telomere length) or inducing mutation in telomeres, resulting into longer, but dysfunctional telomeres and higher CDKN2A expression.

Conclusion

In conclusion, our results show that mitochondrial dysfunction and cellular senescence but not telomere shortening is associated with neurodegenerative diseases (PD, PDD, DLB). The identification of biomarkers in neurodegenerative diseases in blood would potentially facilitate the drug development process as utility of measuring such markers in brain is limited. Our findings further extend our knowledge that mitochondrial copy number and function could be a viable biomarker in blood as an early indicator for the risk of PD.

Abbreviation

PD: Parkinson's disease

PDD: Parkinson dementia

DLB: Dementia with Lewy Bodies

mtDNA: mitochondrial DNA

CDKN2A: cyclin-dependent kinase inhibitor 2A

LEDD: Levodopa Equivalent Daily Dose

TAU: microtubule-associated protein

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Author contribution

Conceptualization and study design: M.A. and P.S. designed the study. Methodology and Lab analysis: A.J.F., A.E.H. and M.A. Clinical interpretation: S.K. and P.S. Statistical analysis and visualization: M.A. Writing (original draft): A.O., M.A. Writing (review and editing): A.O., A.M., S.K., P.S. and M.A. Funding acquisition and supervision: M.A. and P.S. All authors discussed the results, commented, and approved the final version of the manuscript.

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Data availability

All data are available upon reasonable request to corresponding author, Muhammad Asghar (asghar.muhammad@ki.se).

Declarations

Ethical approval and consent for participate

Swedish BIOPARK cohort (approved by the Swedish Ethical Review Authority, reference number 2019-04967) and UK National Research Ethics Service (08/H1010/4 and KI IRB). The permission to collect samples for research purposes was publications were obtained (for detail, see method section).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing of interests.

Table 1. Difference in *mtDNA* and telomere length between Parkinson's Disease patients and controls in blood (multivariate regression).

	MtDNA copy number					*Telomere length*				
	Est.	*SE*	*df*	*t ratio*	*p*	*Est.*	*SE*	*df*	*t ratio*	*p*
Sex	-9.237	16.42	1	-0.56	0.576	0.188	0.107	1	1.75	0.083
Age	-1.506	1.764	115	-0.857	0.395	0.009	0.011	115	0.84	0.400
Condition (control)	62.74	27.02	1	2.36	**0.020**	-0.393	0.176	1	-2.23	**0.028**

Table 2. Difference of *mtDNA* and telomere length between patients (PD, PDD and DLB) and controls in prefrontal cortex tissue (multivariate regression).

	MtDNA copy number					*Telomere length*				
	Est.	*SE*	*df*	*t ratio*	*p*	*Est.*	*SE*	*df*	*t ratio*	*p*
Sex	155.95	622.2	1	0.25	0.803	0.022	0.043	1	0.51	0.611
Age	125.71	94.54	50	1.33	0.189	-0.007	0.006	47	-1.09	0.280
Condition (control)	5075.89	1016.04	3	5.00	**<0.001**	-0.264	0.071	3	-3.68	**<0.001**

Table 3. Difference of *PGC-1α*, *PGC-1β* and *CDKN2A* between patients and controls in prefrontal cortex tissues (multivariate regression).

	Factors	*Est.*	*SE*	*df*	*t ratio*	*p*
PGC-1α						
	Sex	0.041	0.082	1	0.50	0.620
	Age	0.012	0.011	64	1.01	0.3167
	Condition (control)	0.104	0.141	3	0.74	0.464
PGC-1β						
	Sex	0.035	0.057	1	0.62	0.536
	Age	0.002	0.008	65	0.34	0.735
	Condition (control)	0.320	0.100	3	3.19	**0.002**
CDKN2A						
	Sex	-0.008	0.121	1	-0.07	0.944
	Age	0.023	0.017	68	1.35	0.180
	Condition (control)	-0.249	0.209	3	-1.19	0.239

Table 4. Demographic characteristics of UK brain tissues samples.

	DNA			*RNA*		
	Number	*Age range (years)*	*M/F ratio*	*Number*	*Age range (years)*	*M/F ratio*
Control	16	68-96	1.6	13	66–96	1.6
Parkinson's disease PD	13	69–89	1.1	13	59–89	1.1
Parkinson's disease dementia PDD	8	68-81	1.6	22	68–89	1.2
Dementia with Lewy Bodies DLB	19	74–92	0.9	27	65–92	1.1
Total	58	86–91	1.2	82	059–96	1.2

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Supplementary Tables

Table S1. Association between LEDD and *mtDNA* in blood in Parkinson's patients.

	MtDNA copy number					*Telomere length*				
	Est.	*SE*	*df*	*t ratio*	*p*	*Est.*	*SE*	*df*	*t ratio*	*p*
Sex	-23.61	18.91	1	-1.25	0.216	0.332	0.135	1	2.64	**0.016**
Age	1.094	1.925	63	0.57	0.572	0.032	0.013	63	2.35	**0.020**
LEDD	-0.001	0.046	63	-0.12	0.905	0.001	0.0003	63	3.45	**0.001**

Table S2. LS means Student's *t* test comparison of *mtDNA* copy number, telomere length, *PGC1a*, *PGC1b* and *CDKN2A* between patient and controls.

Condition	*Condition*	*Mean diff*	*SE*	*t ratio*	*p value*	*lower 95*	*upper 95*
			***mtDNA* copy number**				
Control	PD	3584.95	1732.17	2.07	**0.043**	105.79	7064.12
Control	PDD	7471.8	1980.2	3.77	**<0.001**	3494.46	11449.16
Control	DLB	9246.82	1498.03	6.17	**<0.001**	6237.92	12255.73
PD	PDD	3886.85	1962.35	1.98	0.053	-54.64	7828.35
PD	DLB	5661.87	1754.23	3.23	**0.002**	2138.39	9185.35
PDD	DLB	1775.01	2012.81	0.88	0.382	-2267.84	5817.87
			Telomere length				
Control	PD	-0.22	0.125	-1.76	0.085	-0.473	0.032
Control	PDD	-0.322	0.136	-2.37	**0.022**	-0.596	-0.048
Control	DLB	-0.514	0.101	-5.06	**<0.001**	-0.718	-0.309
PD	PDD	-0.101	0.131	-0.77	0.443	-0.365	0.162
PD	DLB	-0.293	0.124	-2.35	**0.022**	-0.544	-0.042
PDD	DLB	-0.191	0.135	-1.42	0.163	-0.464	0.08
			PGC1 α				
Control	PD	0.463	0.257	1.8	0.076	-0.05	0.976
Control	PDD	-0.053	0.213	-0.25	0.803	-0.479	0.372
Control	DLB	0.007	0.206	0.04	0.971	-0.405	0.42
PD	PDD	-0.516	0.226	-2.28	**0.026**	-0.969	-0.063
PD	DLB	-0.455	0.234	-1.94	0.056	-0.924	0.013
PDD	DLB	0.06	0.186	0.33	0.744	-0.311	0.432
			PGC-1β				
Control	PD	0.531	0.182	2.91	**0.005**	0.166	0.895
Control	PDD	0.395	0.149	2.65	**0.010**	0.097	0.694
Control	DLB	0.355	0.147	2.41	**0.018**	0.060	0.649
PD	PDD	-0.135	0.161	-0.84	0.403	-0.457	0.186
PD	DLB	-0.176	0.166	-1.06	0.294	-0.509	0.156
PDD	DLB	-0.04	0.13	-0.31	0.756	-0.30	0.219
			CDKN2A				
Control	PD	-0.041	0.373	-0.11	0.911	-0.786	0.702
Control	PDD	0.057	0.318	0.18	0.856	-0.577	0.692
Control	DLB	-1.012	0.301	-3.36	**0.001**	-1.613	-0.411
PD	PDD	0.099	0.327	0.3	0.762	-0.554	0.752
PD	DLB	-0.97	0.33	-2.94	**0.004**	-1.630	-0.311
PDD	DLB	-1.069	0.269	-3.97	**<0.001**	-1.607	-0.532

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