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Brief communication

Lewy body pathology is associated with mitochondrial DNA damage in Parkinson's disease

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ABSTRACT

Mitochondrial dysfunction has been strongly implicated in the pathogenesis of Parkinson's disease (PD) and Alzheimer's disease (AD), but its relation to protein aggregation is unclear. PD is characterized by synuclein aggregation (i.e., Lewy body [LB] formation). In AD, the abnormal accumulation of tau protein forms neurofibrillary tangles. In this study, we laser-dissected LB-positive and -negative neurons from the substantia nigra of postmortem PD brains, and tau-positive and -negative hippocampal neurons from AD brains. We quantified mitochondrial DNA deletions in relation to the cellular phenotype and in comparison with age-matched controls. Deletion levels were highest in LB-positive neurons of PD brains (40.5 ± 16.8%), followed by LB-negative neurons of PD cases (31.8 ± 14.4%) and control subjects (25.6 ± 17.5%; analysis of variance $p < 0.005$). In hippocampal neurons, deletion levels were 25%–30%, independent of disease status and neurofibrillary tangles. The presented findings imply increased mitochondrial DNA damage in LB-positive midbrain neurons, but do not support a direct causative link of respiratory chain dysfunction and protein aggregation.

1. Introduction

Protein misfolding and aggregation are key neuropathological features of Parkinson's disease (PD) and Alzheimer's disease (AD). In PD, deposits of α-synuclein contribute to Lewy body (LB) formation, and in AD, hyperphosphorylated tau protein aggregates to formation of neurofibrillary tangles (NFTs). Despite distinct clinical and pathological characteristics, there are multiple confirmed correlations and pathogenic overlaps (Jellinger, 2012). In particular, the formation of reactive oxygen species (ROS) and the oxidative damage of lipids, proteins, and DNA might unify diverse starting points into a common pathway (i.e., mitochondrial dysfunction, bioenergetic decline, and cell death) (Coskun et al., 2012). Traditionally believed to be catalyzing neuronal loss, LBs and NFTs might rather be formed as a compensatory response to elevated oxidative stress.

Mitochondria are the main source of ROS and mitochondrial DNA (mtDNA) is particularly prone to somatic mutations caused by ROS. Detailed insights into the function of the mitochondrial replication machinery, together with break-point analysis imply that acquired mtDNA deletions most likely occur during repair of oxidatively damaged molecules (Krishnan et al., 2008). Within a single cell, wild type and damaged mtDNA can coexist, a situation referred to as heteroplasmy. When the mutation load exceeds a threshold of approximately 60%, cells start to display a biochemical defect of the mitochondrial respiratory chain (RC) that can be detected histochemically by cytochrome-c oxidase (COX, complex IV) deficiency (Elstner and Turnbull, 2011).

Neuropathological studies have revealed elevated numbers of such COX-deficient neurons in the aging brain, with a further increase in PD and AD patients (Cottrell et al., 2001; Itoh et al., 1997; Kish et al., 1992; Parker et al., 1994). Using laser microdissection (LMD) and real-time quantitative polymerase chain reaction (PCR)

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analysis of single neurons, we previously demonstrated that this COX deficiency is caused by clonally expanded mtDNA deletions (Bender et al., 2006). Melanized dopaminergic neurons of the substantia nigra (SN) are particularly vulnerable (Bender et al., 2008; Elstner et al., 2011). In this study, using LMD and single-neuron quantitative PCR analysis, we investigated the relationship between protein aggregation and mtDNA deletions in human midbrain and hippocampus neurons in PD, AD, and age-matched control individuals.

2. Methods

2.1. Case and control groups

Frozen midbrain tissue was requested from the German brain bank (Brain-Net) and from the Neuropathology Department of the University of Ulm, Germany. Written consent was obtained with verification and/or assent in writing from next of kin who confirmed the wishes at time of death. All procedures were approved by the Local Research Ethics Committees. The study is in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Age-matched control individuals had no previous history of neurological or psychiatric disease ($n = 10$; mean age, 74.0 ± 15.7 years; postmortem interval [PMI], 22.7 ± 10.1 hours). In PD patients, neuropathological examination had demonstrated the presence of LB pathology in the SN with moderate to severe neuronal loss and gliosis ($n = 7$; mean age, 77.5 ± 10.3 years; PMI, 29 ± 11.6 hours). Control individuals for AD cases had no previous history of neurological or psychiatric disease ($n = 4$; mean age, 71.3 ± 8.3 years; PMI, 21.8 ± 6.1 hours). AD patients had a clinical and neuropathological diagnosis ($n = 7$; mean age, 77.3 ± 10.8 years; PMI, 24.8 ± 12.5 hours). Statistical analysis (unpaired parametric t test) did not reveal any significant difference in age or PMI between disease and control groups.

2.2. Histochemistry

A successive COX/SDH succinate dehydrogenase (SDH, complex II) and α-synuclein stain was used for midbrain samples. A combination of an AT8 and Nissl stain was used for hippocampal regions. Protocols are previously published (Elstner et al., 2011) and are detailed in the Supplementary data.

2.3. LMD and DNA extraction

Ultraviolet laser microdissection was performed using a Leica LMD6000 microscope (Leica, Wetzlar, Germany). Single neurons were collected into separate reaction tubes. DNA was extracted using the QIAamp DNA Micro Kit (Qiagen).

2.4. mtDNA deletion analysis and statistical analysis

Quantification of mtDNA levels was determined using real-time PCR and calculated using relative quantification of mitochondrial *ND1* and *ND4* as recently described (Elstner et al., 2011; Krishnan et al., 2007). Deletion levels of 2 to 6 neurons were individually determined for each disease and control specimen; single-cell values were used for comparative statistics using GraphPad Prism 6 (San Diego, CA, USA).

3. Results

In the SN of healthy, aged individuals, no LB formation was seen. mtDNA deletion levels of control SN neurons ($n = 19$) were 25.6 ± 17.5%. In PD, SN neurons without LBs ($n = 29$) had deletion levels that were slightly higher (31.8 ± 14.3%) than in control individuals. As the main finding, neurons with LB pathology ($n = 37$) collected from the same PD specimens had further elevated deletion levels (40.5 ± 16.75%). Significance was verified using analysis of variance ($p < 0.002$; $F = 7.02$); Tukey multiple comparison test showed effects for LB-positive PD neurons versus LB-negative neurons of control individuals (adjusted $p < 0.005$) and PD ($p < 0.05$; Fig. 1). Regression analysis, including all single-neuron deletion values ($n = 85$) revealed a positive association with donor age (linear model; $R^2 = 0.11$; $p = 0.02$), but no association was found for PD neurons alone ($n = 66$; $R^2 = 0.04$; $p = 0.1$). Because LB-negative and LB-positive neurons were collected in equal numbers from the same PD cases, an age-related effect of mtDNA deletion levels can be excluded. mtDNA copy number did not differ between these groups of neurons ($PD^{(LB+)} = 4266 \pm 5299$; $PD^{(LB-)} = 3654 \pm 2211$; $p = 0.77$).

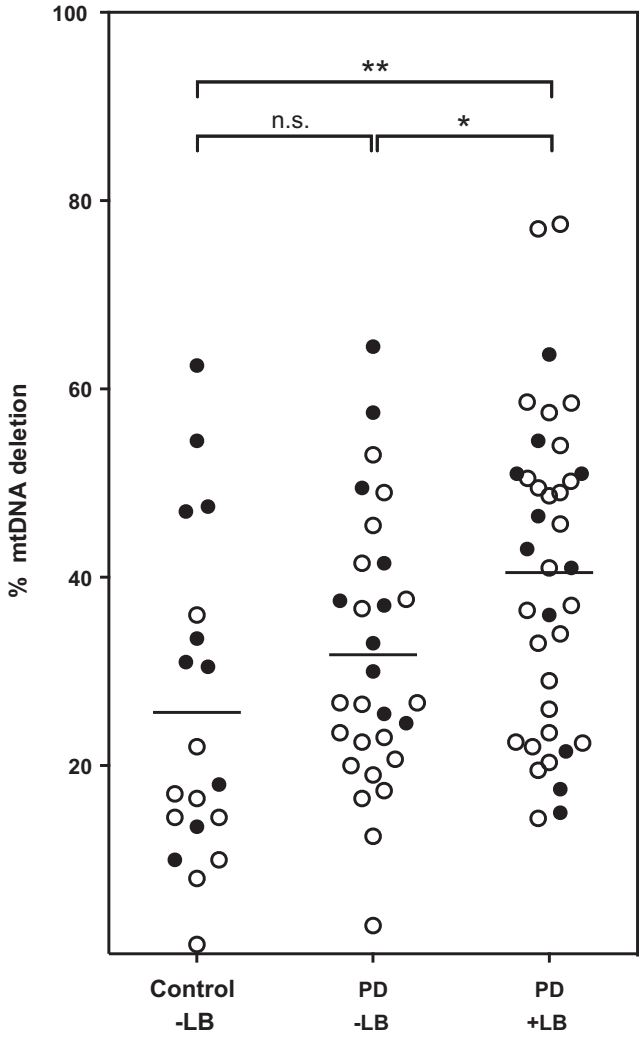

Fig. 1. Relative mitochondrial DNA (mtDNA) deletions of substantia nigra neurons dissected from postmortem midbrains of 10 control individuals and 7 Parkinson's disease cases. Per case and neuronal phenotype, values of 2 to 6 neurons were individually determined and plotted. In Parkinson's disease cases, neurons with (+LB) and without (-LB) Lewy body pathology originated from the same specimens, thus eliminating a potential age-related bias (age of donor <75 years = open circles; age >75 years = filled circles). Abbreviation: n.s., not significant. * $p < 0.05$; ** $p < 0.005$.

NFT-positive neurons were detected in AD hippocampal samples, but were also found in age-matched control samples. Therefore, we were able to collect both, NFT-negative and -positive neurons from control and AD hippocampi. In control samples, NFT-negative

neurons ($n = 54$) had 23.7 ± 15.4% and NFT-positive neurons ($n = 27$) had 25.2 ± 21.7% deletions. In AD, deletion levels of NFT-negative neurons ($n = 100$) were 30.4 ± 17.9% and of NFT-positive neurons ($n = 104$) were 25.5 ± 18.5%. Analysis of variance showed no significant difference between groups or neuronal phenotypes ($p > 0.7$; $F = 0.19$). mtDNA copy number did not differ significantly ($AD^{(NFT+)} = 5270 \pm 9390$; $AD^{(NFT-)} = 2184 \pm 2338$; $p = 0.17$).

4. Discussion

Oxidative stress and mitochondrial dysfunction are increasingly implicated in the promotion of synuclein- and tau-mediated neurodegeneration, but the underlying molecular events are unresolved. As a possible link, we hypothesized that oxidative mtDNA damage and a consecutive reduction of mitochondrial RC activity might be involved. To this end, we investigated the relationship of LB and NFT pathology with mtDNA damage at the single-neuron level.

In neurons, heteroplasmy levels >60% are believed to cause RC dysfunction, determined by COX deficiency (Bender et al., 2006). If protein aggregation was caused by this mechanism, LB- and NFT-positive neurons would be expected to be predominately COX deficient. To evaluate such co-occurrence, we performed successive synuclein-immunohistology and COX histochemistry on midbrain and hippocampus sections, but found no correlation (data not shown). In agreement, Reeve et al. (2012) recently demonstrated that the protein expression of RC complexes are not reduced in LB-positive midbrain neurons. These findings provide evidence against a direct causative sequence of mtDNA deletions > mitochondrial dysfunction > protein aggregation.

Next, we analyzed heteroplasmy levels of phenotypically characterized, laser-dissected, midbrain and hippocampal neurons. Despite considerable variation of deletion levels, synuclein pathology is significantly associated with mtDNA damage in PD neurons. In LB-positive neurons, heteroplasmy reached an average of 40.5% and some of the highest deletions levels (approximately 80%) were seen in these neurons. LB-negative neurons only had an average of 31.8% and deletion levels did not exceed 65%. Nevertheless, most LB-positive neurons had deletion levels in the same range as LB-negative neurons, further arguing against a direct relationship with LB pathology.

As a common caveat of postmortem brain studies, tissue donors predominately have had advanced disease. Lin et al. (2012) recently showed that total and oxidative somatic mtDNA point mutations are dramatically increased in the SN of early PD patients. Surprisingly, the detectable mutation load was lower in remaining neurons of late PD cases, implying a 'clearing' of damaged neurons and the 'survival of the fittest (neurons)'. In analogy, this might serve to explain, why—in this study—mtDNA deletion levels are not significantly elevated in LB-negative PD neurons compared with control neurons and deletions seem to have a 'ceiling effect' at approximately 60% heteroplasmy.

The age-related accumulation of somatic mtDNA mutations in melanized midbrain neurons substantially exceeds that of other brain regions, such as the frontal cortex, putamen, and the locus coeruleus (Bender et al., 2006, 2008; Elstner et al., 2011). The presented data support these findings, because hippocampal neurons have only moderately elevated deletion levels without a measurable effect of NFT pathology. Although elevated numbers of COX-deficient neurons and corresponding mtDNA deletions were reported for AD hippocampus (Cottrell et al., 2001; Krishnan et al., 2011), our findings seem to exclude a larger role of acquired somatic mtDNA deletions in AD.

In conclusion, we show that LB pathology is accompanied by elevated mtDNA damage, and no association is seen for tau pathology. Because somatic mtDNA deletions are believed to arise during the repair of oxidatively damaged molecules, this implies that LB-positive neurons are exposed to increased ROS. However, our available data do not support a direct association of mitochondrial RC dysfunction and LB or NFT pathology.

Disclosure statement

The authors declare that they have no actual or potential conflicts interest.

All procedures were approved by the Local Research Ethics Committees. The study is in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2013.03.016.

References

Bender, A., Krishnan, K.J., Morris, C.M., Taylor, G.A., Reeve, A.K., Perry, R.H., Jaros, E., Hersheson, J.S., Betts, J., Klopstock, T., Taylor, R.W., Turnbull, D.M., 2006. High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. Nat. Genet. 38, 515–517.

Bender, A., Schwarzkopf, R.M., McMillan, A., Krishnan, K.J., Rieder, G., Neumann, M., Elstner, M., Turnbull, D.M., Klopstock, T., 2008. Dopaminergic midbrain neurons are the prime target for mitochondrial DNA deletions. J. Neurol. 255, 1231–1235.

Coskun, P., Wyrembak, J., Schriner, S.E., Chen, H.W., Marciniack, C., LaFerla, F., Wallace, D.C., 2012. A mitochondrial etiology of Alzheimer and Parkinson disease. Biochim. Biophys. Acta 1820, 553–564.

Cottrell, D.A., Blakely, E.L., Johnson, M.A., Ince, P.G., Turnbull, D.M., 2001. Mitochondrial enzyme-deficient hippocampal neurons and choroidal cells in AD. Neurology 57, 260–264.

Elstner, M., Muller, S.K., Leidolt, L., Laub, C., Krieg, L., Schlaudraff, F., Liss, B., Morris, C., Turnbull, D.M., Masliah, E., Prokisch, H., Klopstock, T., Bender, A., 2011. Neuromelanin, neurotransmitter status and brainstem location determine the differential vulnerability of catecholaminergic neurons to mitochondrial DNA deletions. Mol. Brain 4, 43.

Elstner, M., Turnbull, D.M., 2011. Transcriptome analysis in mitochondrial disorders. Brain Res. Bull. 88, 285–293.

Itoh, K., Weis, S., Mehraein, P., Müller-Höcker, J., 1997. Defects of cytochrome c oxidase in the substantia nigra of Parkinson's disease: and immunohistochemical and morphometric study. Mov. Disord. 12, 9–16.

Jellinger, K.A., 2012. The role of α-synuclein in neurodegeneration — an update. Transl. Neurosci. 3, 75–122.

Kish, S.J., Bergeron, C., Rajput, A., Dozic, S., Mastrogiacomo, F., Chang, L.J., Wilson, J.M., DiStefano, L.M., Nobrega, J.N., 1992. Brain cytochrome oxidase in Alzheimer's disease. J. Neurochem. 59, 776–779.

Krishnan, K.J., Bender, A., Taylor, R.W., Turnbull, D.M., 2007. A multiplex real-time PCR method to detect and quantify mitochondrial DNA deletions in individual cells. Anal. Biochem. 370, 127–129.

Krishnan, K.J., Ratnaike, T.E., De Gruyter, H.L., Jaros, E., Turnbull, D.M., 2011. Mitochondrial DNA deletions cause the biochemical defect observed in Alzheimer's disease. Neurobiol. Aging 33, 2210–2214.

Krishnan, K.J., Reeve, A.K., Samuels, D.C., Chinnery, P.F., Blackwood, J.K., Taylor, R.W., Wanrooij, S., Spelbrink, J.N., Lightowlers, R.N., Turnbull, D.M., 2008. What causes mitochondrial DNA deletions in human cells? Nat. Genet. 40, 275–279.

Lin, M.T., Cantuti-Castelvetri, I., Zheng, K., Jackson, K.E., Tan, Y.B., Arzberger, T., Lees, A.J., Betensky, R.A., Beal, M.F., Simon, D.K., 2012. Somatic mitochondrial DNA mutations in early Parkinson and incidental Lewy body disease. Ann. Neurol. 71, 850–854.

Parker, W.D., Parks, J., Filley, C.M., Kleinschmidt-DeMasters, B.K., 1994. Electron transport chain defects in Alzheimer's disease brain. Neurology 44, 1090–1096.

Reeve, A.K., Park, T.K., Jaros, E., Campbell, G.R., Lax, N.Z., Hepplewhite, P.D., Krishnan, K.J., Elson, J.L., Morris, C.M., McKeith, I.G., Turnbull, D.M., 2012. Relationship between mitochondria and α-synuclein: a study of single substantia nigra neurons. Arch. Neurol. 69, 385–393.