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Mitochondrial DNA methylation and mitochondria-related epigenetics in neurodegeneration

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Mitochondria are cytoplasmic organelles referred to as the powerhouse of the cell because they are primarily involved in oxidative phosphorylation and energy production. They are particularly abundant in tissues with high energy demands, including muscle, liver, and brain, and mitochondrial dysfunction, oxidative mitochondrial DNA (mtDNA) damage, and impaired mitochondrial dynamics have often been associated with neurodegeneration. The mtDNA is a circular, double-stranded molecule present in two to ten copies per mitochondrion and encodes 13 subunits of the mitochondrial respiratory chain as well as 22 transfer RNAs and two ribosomal RNAs. The existence of mitochondrial epigenetics, and in particular mtDNA methylation, has been largely debated, but a growing body of literature suggests that impaired mtDNA methylation may be involved in neurodegeneration (Coppedè and Stoccoro, 2019). Furthermore, there is increasing evidence for bidirectional crosstalk between the nuclear and mitochondrial genomes to allow coordinated gene expression in response to different cellular stressors. This crosstalk is mainly mediated by epigenetic mechanisms, but is unfortunately still poorly understood in neurodegenerative diseases (Coppedè, 2021). In this perspective, after a brief description of the available literature on impaired mtDNA methylation in neurodegenerative diseases, the author discusses the potential factors contributing to these alterations and their crosstalk with nuclear epigenetics.

Mitochondrial epigenetics in neurodegeneration: The mtDNA lacks histones, so mtDNA methylation and hydroxymethylation are the most studied mitochondrial epigenetic modifications in patients with neurodegenerative diseases. The existence of mtDNA methylation has long been questioned, but is largely supported by recent studies. For example, a recent epigenome-wide study of human brain samples revealed that mtDNA methylation patterns are relatively low but conserved. Indeed, mtDNA methylation averages about 2%, occurs mainly at non-CpG sites, and is likely to be non-biologically relevant at several loci. However, peaks of mtDNA methylation have been observed at several sites, including sites within the regulatory D-loop region, which regulates mtDNA transcription and replication, and within the *MT-ND2*, *MT-ND4*, *MT-ND5*, and *MT-ATP6* genes, which encode subunits of complex I (nicotinamide adenine dinucleotide dehydrogenase) or complex V (adenosine triphosphate synthase), respectively (Devall et al., 2022). This genome-wide study corroborates the results of several previous studies in patients with neurodegenerative diseases that we recently reviewed, which collectively reported very low levels of mtDNA methylation in both blood and postmortem brain regions of patients, but with some peaks at specific loci, including the regulatory D-loop region, that may be biologically relevant and warrant further investigation. For example, an inverse correlation between methylation levels of the D-loop region and mtDNA copy number has often been observed, and given the regulatory function of the D-loop, it has been suggested that DNA methylation levels of this region could regulate both transcription and replication of mtDNA (Coppedè and Stoccoro, 2019). Further support for the existence and biological function of mtDNA methylation comes from a recent study showing that the mtDNA is extensively methylated during the transition from blastocysts to post-implantation embryos, and that the primary function of this wave of *de novo* mtDNA methylation is to protect the mitochondrial genome from oxidative damage (Yue et al., 2022).

Methylation levels of the D-loop regulatory region have been extensively studied in biological samples from humans with neurodegenerative diseases and in animal models of these disorders (**Figure 1**). Blanch et al. (2016) analyzed the entorhinal cortex of human postmortem brains in the early stages of Alzheimer's disease (AD) and observed higher levels of D-loop methylation than in the entorhinal cortex of matched control brains. Furthermore, they observed a dynamic pattern of D-loop methylation levels with disease progression in cortical regions of animal models of AD, namely transgenic amyloid precursor protein/presenilin 1 (APP/PS1) mice. A similar dynamic D-loop methylation pattern was observed by us in blood DNA samples from AD patients. Indeed, D-loop methylation levels were higher than those observed in control blood samples in the prodromal stages of AD, but decreased in advanced stages of AD (Stoccoro et al., 2022). Decreased D-loop methylation levels have been described postmortem in the substantia nigra of PD patients compared to controls, but no differences in methylation levels of this region were observed when comparing PD and control blood samples (Blanch et al., 2016; Stoccoro et al., 2021). In patients with amyotrophic lateral sclerosis, a significant decrease in D-loop methylation, accompanied by an increase in mtDNA copy number, was observed in blood DNA samples, particularly in carriers of superoxide dismutase 1 (*SOD1*) gene mutations (Stoccoro et al., 2018). Regions of mtDNA other than the D-loop have been less frequently examined in human samples and, except for reduced *MT-ND1* methylation levels observed in the entorhinal cortex of patients at early stages of AD-related pathology (Blanch et al., 2016), increases in 12S rRNA gene methylation and in the methylation levels of the mitochondrial cytochrome b (*mt-Cytb*) and cytochrome c oxidase II (*mt-Co2*) genes were observed in the hippocampi of APP/PS1 transgenic mice (Xu et al., 2021).

Taken together, these observations raise two questions that remain unanswered: 1) what causes the mtDNA methylation changes observed to date in neurodegenerative diseases, and 2) what are the nuclear epigenetic changes that accompany, precede, or follow the mtDNA methylation changes.

Regarding what causes mtDNA methylation changes in neurodegeneration, an important question that remains to be addressed is whether the dynamic patterns of D-loop methylation levels observed in brain regions of transgenic AD mice and in the blood of living AD patients are due to a different cellular composition of the examined tissues after disease progression, or whether they rather reflect mitochondrial adaptation to oxidative stress and inflammation, and to the different metabolic demands of patients

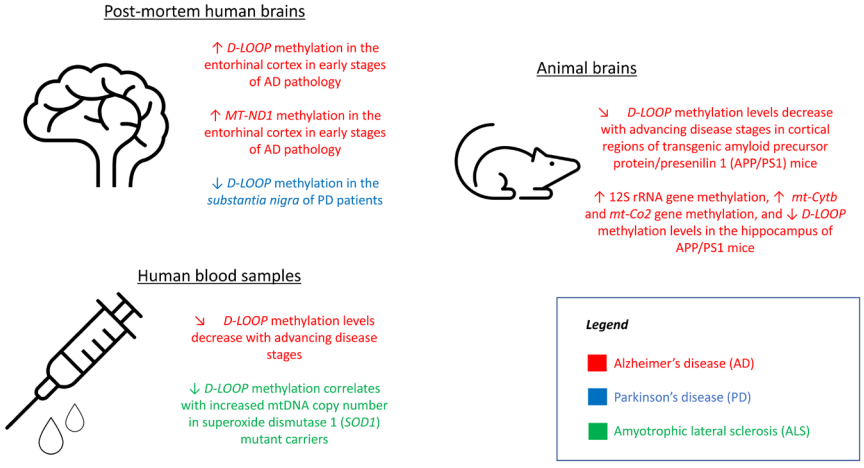

Figure 1 | **Key mitochondrial epigenetic findings in neurodegenerative diseases.**
Studies investigating mtDNA methylation have mainly been performed in post-mortem tissues, blood samples, and in the brain of animal models of neurodegeneration. Studies in Alzheimer's disease tissues and animal models are shown in red, those in Parkinson's disease samples in blue, and those in amyotrophic lateral sclerosis in green. The most studied region is the mitochondrial D-loop regulatory region. Created with Microsoft PowerPoint (version 3.13). AD: Alzheimer's disease; ALS: amyotrophic lateral sclerosis; APP: amyloid precursor protein; D-LOOP: Displacement loop; mt-Co2: mitochondrial cytochrome c oxidase II; mt-Cytb: mitochondrial cytochrome b; MT-ND1: mitochondrially encoded nicotinamide adenine dinucleotide dehydrogenase 1; PD: Parkinson's disease; PS1: presenilin 1; SOD1: superoxide dismutase 1.

after disease progression. Other unresolved questions are what is the real function of mtDNA methylation and what is the correlation between mtDNA methylation and mitochondrial dynamics. In addition to a role in the regulation of mtDNA copy number and gene expression, some authors have suggested a protective role for mtDNA methylation against oxidative damage, although these studies are mainly from diseases other than neurodegeneration. A recent study has shown that in utero exposure to pro-oxidants not only induces oxidative mtDNA damage and altered mitochondrial biogenesis, but also induces changes in mtDNA methylation (Mishra et al., 2022). Others suggest that mtDNA methylation in early life may protect against oxidative DNA damage (Yue et al., 2022), and our evidence that among carriers of the major genes causing amyotrophic lateral sclerosis, namely *SOD1*, *FUS*, *TARDBP*, and *C9orf72*, only carriers of *SOD1* mutations showed impaired D-loop methylation levels, all support a link between oxidative stress and mtDNA methylation levels (Stoccoro et al., 2018). In addition, there is substantial evidence for an association between particulate matter exposure and mtDNA oxidative damage, but air pollution is increasingly being linked to changes in mtDNA copy number, mtDNA methylation, and mtDNA mutations. Changes in mtDNA methylation induced by particulate matter exposure may then result in a pro-inflammatory environment and contribute to various human diseases (Rehman et al., 2023). Changes in mtDNA methylation have been observed in different human brain regions, as well as with age and sex (Devall et al., 2022). However, it is still largely unclear to what extent the observed changes in different brain regions reflect hormonal differences between the sexes and differences in metabolic demands, cell tissue composition, oxidative stress and inflammation. Also, the correlation between age-related methylation changes in mtDNA and age-related epigenetic changes in nuclear DNA, which are often used to estimate the biological age of tissues through the application of epigenetic clocks, is still poorly understood.

Regarding the relationship between mtDNA methylation and other epigenetic modifications, it is well known that there is a bidirectional crosstalk between the nuclear and mitochondrial genomes, which is largely mediated by epigenetic mechanisms and allows for the adjustment of gene expression levels according to cell demands and environmental stimuli and stressors. In addition to their role in oxidative phosphorylation and energy production, mitochondria are central to several metabolic pathways, and several intermediates of these pathways, including one-carbon metabolites, acetyl coenzyme A and alpha-ketoglutarate, as well as redox cofactors such as nicotinamide adenine dinucleotide, can regulate the activity of several enzymes that mediate nuclear epigenetic modifications (Coppedè, 2021). Thus, oxidative stress and mitochondrial dysfunction can generate retrograde mitochondrial-to-nuclear signals that, acting through epigenetic mechanisms, can regulate the expression of nuclear genes. Unfortunately, although hundreds of nuclear epigenetic differences have been detected in the comparison of postmortem brain samples from patients with neurodegenerative diseases and matched controls, and several environmental factors have been suggested to contribute to these changes (Migliore and Coppedè, 2022), their correlations with mitochondrial epigenetic modifications have not yet been investigated. In addition, mitochondria-localized microRNAs are recently discovered small non-coding RNA molecules that can be transcribed from the nuclear genome and translocated to the mitochondria or, potentially, from the mitochondrial genome itself. These miRNAs regulate the expression of mtDNA genes and add a new level of complexity to our understanding of the epigenetic mechanisms that regulate nuclear-mitochondrial crosstalk. MicroRNAs are also increasingly implicated as potential contributors to several human diseases. Unfortunately, microRNAs are still poorly understood in neurodegenerative diseases, and their relationship to both mtDNA methylation changes and nuclear epigenetic changes in these conditions remains to be addressed (Rivera et al., 2023).

In summary, there is increasing evidence that mtDNA methylation is disrupted in neurodegenerative diseases and that it may be a promising biomarker of oxidative stress, inflammation, and exposure to pro-oxidant environmental factors. Nevertheless, the field of mitochondrial epigenetics is still in its infancy. The links between oxidative stress, mtDNA damage, and mtDNA methylation, as well as the functional consequences of altered mtDNA methylation in neurons, deserve further investigation. Furthermore, the crosstalk between the nuclear and mitochondrial genomes in neurodegenerative diseases is still poorly understood, so it is not clear which environmental factors and nuclear epigenetic changes can trigger mitochondrial epigenetic changes and vice versa. Overall, mitochondrial epigenetics is a novel, fascinating and still largely unexplored field in neurodegeneration.

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