

Mitochondrial DNA Mutations in Disease, Aging, and Neurodegeneration

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Patients with disorders from mutations in the mitochondrial genome have variable phenotypes, but common to many of these disorders are underlying changes in postmitotic cells, particularly neurons and muscle fibers. The mitochondrial dysfunction caused by these mutations has been shown to be associated with signs of apoptosis and to cause cell loss. Mutations of the mitochondrial genome have also been shown to accumulate with age and in common neurodegenerative diseases, such as Parkinson's disease. This review presents recent data to show that the information gained from studying patients with mitochondrial disorders can help our understanding of the role of mitochondrial DNA mutations in brain aging and neurodegeneration.

Key words: mitochondrial; DNA; mutations

Introduction

The production of energy, in the form of ATP, is a process which is essential for all cells. The generation of this energy occurs through a number of protein complexes, the electron transport chain, or oxidative phosphorylation (OXPHOS) system. These protein complexes are situated within the inner mitochondrial membrane. The transfer of electrons through the protein complexes to molecular oxygen and the translocation of protons generates a proton gradient across the inner mitochondrial membrane. This gradient is then used by the final complex in the chain, ATP synthase, to generate ATP. Mitochondria are dynamic organelles that, via the processes of fission and fusion, are capable of forming networks within individual cells.¹ They have a number of other important roles in cell function, including maintenance

and storage of intracellular calcium levels and regulation of apoptosis.^{2,3} Mitochondria are also the main site of reactive oxygen species (ROS) generation within the cell. Such radicals are produced as a natural by-product during OXPHOS. These ROS are capable of causing damage to DNA, proteins, and lipids. It has been previously proposed that this may initiate a vicious cycle whereby ROS damages mitochondrial DNA (mtDNA), which leads to an inefficient OXPHOS system, which in turn leads to a further production of ROS—the proposal of a mitochondrial theory of aging.^{4,5}

Mitochondria are thought to be descended from free-living bacteria that became trapped to form eukaryotic cells.⁶ During evolution, mitochondria have transferred the majority of their genetic material to the nucleus,⁶ but some DNA remains within the mitochondria. MtDNA is a double-stranded circular genome of approximately 16.5 kb in length, and, with the exception of approximately an 1 kb noncoding region, the rest of the genome is entirely transcribed. MtDNA can be replicated independently of the cell cycle and independent

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of replication of the nuclear genome. MtDNA contains 37 genes that encode for 13 proteins, all of which are subunits of the respiratory chain complexes, 22 tRNAs, and 2 rRNAs, which are required for intramitochondrial protein synthesis. Those proteins required for mitochondrial function that are not encoded by mtDNA are encoded by the nucleus, translated within the cytoplasm, and then transported into the mitochondria.

Mitochondrial DNA Genetics

There are multiple copies of the mitochondrial genome within cells, which are organized within the mitochondria as nucleoids. Nucleoids consist of several copies of the mitochondrial genome as well as key proteins involved in mitochondrial replication and transcription, such as single-stranded binding (SSB) protein and mitochondrial transcription factor A (TFAM).⁷ The multicopy nature of the mitochondrial genome means that mutant mitochondrial genomes can exist among wild-type molecules, a situation known as heteroplasmy. Heteroplasmy is important at the single cell level where the level of mutated mtDNA within the cell is critical as, once it reaches a certain threshold, a biochemical defect can be observed. Heteroplasmy is a key finding in some patients with pathogenic mtDNA mutations, and the higher the amount of mutated mtDNA, the more likely a patient is to have severe symptoms.⁸

MtDNA is reported to have a higher mutation rate than nuclear DNA because of a number of factors including the lack of protective histones and the close proximity of the mitochondrial genome to the inner membrane where ROS are continually produced.⁹ However, mitochondria contain a number of antioxidant enzymes to counteract this damage and also DNA repair enzymes, which include MUTYH and OGG1.¹⁰ If a mutation in the mitochondrial genome does arise they are usually either point mutations or rearrangements,

with the majority of the latter being large-scale deletions.

One of the most intriguing aspects of mitochondrial genetics is how a single mtDNA mutation within an individual cell increases to levels high enough to cause mitochondrial dysfunction and ultimately cell death. In both mitotic and postmitotic cells this process is called clonal expansion and is important in both mtDNA disease and the acquired mutations that are observed in aging and neurodegeneration.¹¹ Although several theories have been proposed for how this process may occur,^{12,13} the most likely seems to be the theory that clonal expansion occurs from relaxed replication of the mitochondrial genome.¹⁴ This theory proposes that, within a population of mitochondrial genomes in a cell, a number will be targeted for replication and a number for destruction. If one of those mitochondrial genomes selected for replication harbors a mutation, then following replication the mutation load will have increased. Mathematical models would support that this is a random process.¹⁴ However, in postmitotic tissues, such as neurons, is there enough mtDNA replication to allow random genetic drift or are there other mechanisms which may explain the clonal expansion? There certainly are instances in which genetic drift does not occur randomly. Diaz *et al.* showed that in cybrids with heteroplasmic levels of deleted mtDNA, levels of deletion decreased with culture after 45 days (10–20% depending on cell line). However, in cells depleted of mtDNA using ethidium bromide, then the mtDNA deletion levels increased immediately after removal of ethidium bromide ($6.2 \pm 4.8\%$).¹⁵ Both the site and rate of mtDNA replication in neurons (and other postmitotic cells) are difficult to estimate because most methods used to study replication, which involve incorporation of labeled nucleotides, cannot distinguish between replication and repair. Similarly, techniques that look at the expression within the cell of the enzymes involved in replication (for example POLG1) do not distinguish between the two processes as

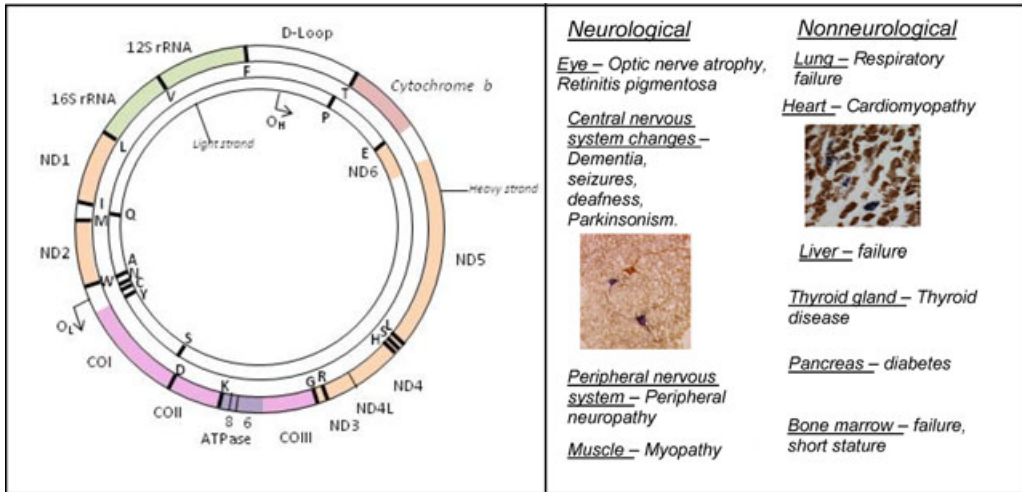


Figure 1. Diagram displaying some of the key phenotypes associated with mtDNA mutations and mutations in the genes encoding the proteins responsible for mtDNA maintenance.

these enzymes are also likely to be involved in repair.

Mitochondrial DNA Mutations in Disease

MtDNA mutations have been shown to cause a number of diseases with varying phenotypes and ages of onset (Fig. 1).⁸ The incidence of these diseases from mtDNA mutations is unknown, but recent epidemiological evidence from the northeast of England suggests that as many as 1 in 10,000 individuals are affected with another 1 in 6000 at risk.¹⁶ Common to many of these disorders is a neurological phenotype ranging from optic neuropathy to dementia.¹⁷ These patients often have other tissue involvement, including the heart and endocrine system.

The neurological features in patients with mtDNA disease are often progressive and disabling. Patients may develop a number of symptoms and signs, including ataxia, dystonia, epilepsy, dementia, peripheral neuropathy, optic atrophy, and deafness.¹⁷ While at present there are limited postmortem studies that attempt to correlate clinical features and the neuropathology, it is clear that there is marked cell loss in affected brain regions.¹⁸⁻²⁰ There

is considerable variation between clinical involvement in patients with different mtDNA mutations—for example, in patients with one of the three primary Leber’s hereditary optic neuropathy mutations, a subacute optic neuropathy is the predominant symptom.²¹ In these patients the neuropathology probably relates to an abnormality in the retinal ganglion cells. In patients with the 3243A>G mutation, stroke-like episodes are common and disabling, but similar episodes are unusual in patients with other mtDNA defects. In patients with the 3243A>G mutation, there seems to be marked involvement of the vascular smooth muscle.¹⁹

While there are limited neuropathological studies in patients with mtDNA mutations, there have been extensive studies in skeletal muscle. Muscle is often involved in patients with mtDNA disease with proximal muscle weakness, chronic progressive ophthalmoplegia, and exercise-induced fatigue being prominent symptoms. Muscle is a relatively accessible tissue and important in the diagnosis of mtDNA disease. Muscle cells are postmitotic similar to neurons and therefore are likely to provide a valuable insight into what is happening in the central nervous system. A characteristic observation in muscle from patients with mtDNA disease is the presence of respiratory chain-deficient muscle fibers. These fibers are

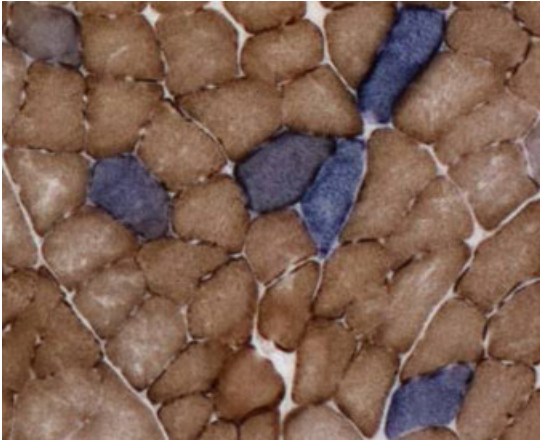


Figure 2. A muscle section stained using cytochrome *c* oxidase/succinate dehydrogenase histochemistry. Respiratory normal fibers are brown and respiratory-deficient fibers are blue. Some show hyper-reactivity, indicating mitochondrial proliferation.

detected by the histochemical measurement of the enzyme activity of two complexes of the electron transport chain, complex II, or succinate dehydrogenase (SDH) and complex IV, or cytochrome *c* oxidase (COX). SDH is entirely encoded by the nuclear genome, and COX contains three catalytic subunits encoded by mtDNA. Respiratory chain deficiency can be visualized as an absence of brown COX staining and the presence of blue SDH staining (Fig. 2).^{22,23}

A recent study by Auré *et al.* investigated mitochondrial changes in the muscle of patients with mitochondrial myopathies. A considerable number of muscle fibers were investigated and a number of features of these fibers studied. This study confirmed previous studies by showing that respiratory chain-deficient muscle fibers harbored higher levels of an mtDNA deletion than respiratory chain normal fibers. This study importantly showed that ragged red muscle fibers have increased proliferation of mitochondria, detected by an intense blue staining following COX/SDH histochemistry. Mitochondrial proliferation was shown to have increased the content of mtDNA within these cells but did not greatly affect the mtDNA

deletion level. Interestingly, those fibers which showed this increased mitochondrial proliferation were associated with the apoptosis markers caspase-3 and Bax.²⁴

Accumulation of Mitochondrial DNA Mutations with Age

MtDNA mutations have been shown to accumulate with age in a number of tissues.^{25–29} In most tissues, the level of these somatic mutations is very low and therefore the significance of these changes has been questioned. However, an important finding in tissues from elderly individuals is the presence of COX deficiency within individual cells. These cells have the same characteristics as the cells seen in patients with inherited mtDNA mutations. Studies on these cells have shown that there is clonal expansion of individual mtDNA mutations within each cell and the level of mutated mtDNA is high enough to cross the threshold to induce a respiratory chain deficiency.²⁸

MtDNA point mutations have also been shown to increase with age.³⁰ Perhaps the most striking example of accumulation of mtDNA point mutations with age is in the human colon.³¹ Human colonic epithelial crypts have stem cells at their base, and the progeny from these stem cells then migrate up the crypt. Thus, it is possible to determine if there are somatic mtDNA mutations in the crypt stem cells by studying the presence of COX deficiency and accumulation of mtDNA mutations in these crypts. Studies have shown that not only are COX-deficient crypts present but there is an exponential increase with age.³¹ In addition, the clonal expansion of mtDNA point mutations in these crypts has been directly linked to the observed respiratory chain deficiency.

In 1999, the role of mtDNA point mutations in aging was brought to the forefront by the report of an “associated” T414G point mutation in the control region of fibroblasts.²⁵ This point mutation was found in over 50% of

fibroblasts taken from elderly subjects. However, subsequent studies have failed to show the presence of this mutation accumulating with age in the brain.^{32,33} This suggests that the mutation may be tissue specific and does not play a role in the aging brain.

In skeletal and cardiac muscle there have been several reports of an increase in the number of mtDNA deletions with age.^{26,34} In a recent paper, Bua *et al.* studied 48 single COX-deficient muscle fibers from normal aging individuals. In all of these fibers they showed the presence of mtDNA deletions. They used a quantitative PCR approach of both wild-type and deleted genomes to confirm that, within the COX-deficient fibers, the mtDNA deletions had reached levels likely to result in a severe biochemical defect (>90% deleted).²⁸ It is clear that if COX-deficient fibers in patients with inherited mtDNA disease result in muscle weakness, then the same can occur in these fibers that occur in aging. The overall contribution of these mtDNA mutations to the aging process in muscle is unknown, but in muscle fibers only short segments are COX deficient. As these segments increase with age, it is likely that the majority (or all) muscle fibers have a segment of COX deficiency, which could result in a decline in muscle function with age.

MtDNA deletions have also been reported to increase with age in the human central nervous system.^{35,36} These studies also suggest the overall level of mtDNA deletions was low in individual brain regions. However, many of the studies had not isolated individual neurons but had concentrated on DNA extracted from brain homogenates.³⁷ We and others recently showed that mtDNA deletions accumulate to high levels with age in neurons from the substantia nigra.^{35,36} These two independent studies looked at the level of deleted mtDNA in isolated neurons using techniques to identify the presence of mtDNA deletions (long-range PCR) and techniques to quantify the level of mtDNA deletions (real-time and single molecule PCR). In these two studies, very high levels of mtDNA deletions were detected in individuals over the age

of 70 (levels of over 40% of total mtDNA). In addition, both studies showed that high levels of mtDNA deletions were associated with a respiratory chain defect. The presence of respiratory chain-deficient cells with high levels of mtDNA mutation is very similar to the situation seen in patients with inherited mtDNA mutations. If there is cell loss in the patients with mtDNA disease associated with a respiratory chain defect, then it seems plausible that a similar cell loss may occur from the mtDNA deletions that accumulate with age in the substantia nigra neurons. Indeed, there are reports of a linear cell loss within the substantia nigra at a rate of approximately 5% per decade.³⁸

Mitochondrial DNA Mutations and Neurodegeneration

There have been a number of reports of mtDNA point mutations associated with neurodegenerative disease.^{37,39-44} However, many of the mtDNA mutations observed have not been confirmed by other studies (for example, the T414G mutation and haplogroups association), therefore the relevance of these findings remains controversial although there seems to be a consistent haplogroup association in Parkinson's disease (PD).^{33,45,46} Other studies have tried to assess the prevalence of mtDNA point mutations in neurodegenerative disease and have reported an increase in mutation load with Alzheimer's disease (AD) and PD.⁴²⁻⁴⁴ However, these studies have used a PCR-cloning-sequencing technique which has been recently challenged for polymerase infidelity and questions the validity of the findings.^{47,48} Some studies have suggested a complex I defect in PD.^{49,50} To correlate with this, an accumulation of point mutations in complex I genes in PD has been reported.^{37,43,44} However, these findings have mainly come from one group, and again, the issue of PCR error comes into question.

Cybrid studies implicate that mtDNA deletions could lead to a complex I deficiency

in PD.⁴⁹ However, these studies use blood platelets to transfer the mitochondrial defect, cells which do not typically display a functional defect; therefore the conclusions of such observations remain uncertain. Also, a study by Aomi *et al.* showed that changes in respiratory chain enzymes occurred in cybrids irrespective of whether the transferred mitochondrial DNA came from a patient with PD or a control.⁵¹

In terms of somatic mtDNA mutations, previous studies have shown there is an increased number of COX-deficient neurons in Alzheimer's disease (AD) compared to age-matched control brains.⁵² A similar increase in COX-deficient neurons was also seen in the substantia nigra from patients with PD compared with age-matched control subjects.³⁶ Studies looking specifically at the level of mtDNA deletions found slightly, but not significantly, higher levels in the neurons from the patients with PD compared to the age-matched controls.³⁶

Thus there appears to be some similarity with the mtDNA changes observed in patients with mtDNA disease, normal aging, and patients with certain neurodegenerative disorders. In all groups there are high levels of mtDNA deletions in neurons from certain brain regions, and in these regions there is the presence of COX-deficient cells. The similarity between patients, normal aging, and neurodegenerative disease was further highlighted by recent studies which explored the characteristics of the mtDNA deletions detected in single substantia nigra neurons in patients with Parkinson's disease, aged-matched controls, and a patient with multiple mtDNA deletions.⁵³ These studies showed there was no difference between the size of mtDNA deletion, length, and nature of the repeat sequence at the deletion breakpoint. This suggests that the mechanism of mtDNA deletion formation is similar (or identical) under a variety of different conditions and favors the hypothesis that the understanding of mtDNA disease will help our understanding of the role of mtDNA mutations in aging and neurodegeneration.

Mouse Models of Mitochondrial Disorders

Mouse models involving alterations to the genes encoding proteins responsible for the maintenance of the mitochondrial genome have provided valuable evidence to suggest that changes to this genome are capable of causing neuronal cell loss and possibly neurodegeneration. By conditional knockout of TFAM in midbrain dopamine (DA) neurons, Ekstrand *et al.* showed that there was decreased mtDNA expression and respiratory chain deficiency in these neurons. The TFAM-knockout mice also developed a progressive impairment of motor function along with protein inclusions and the death of DA neurons.⁵⁴ A similar result was found if TFAM was knocked out in the hippocampus and the neocortex; in this model there was again a progressive neurodegeneration and cell death in these two brain regions.⁵⁵

Mouse models with an "aging" phenotype have been developed using a knockin-deficient, proofreading, mitochondrial polymerase (POLG1) and show an increase in mtDNA point mutations of three to five times.^{56,57} These mice displayed decreased life span and the premature development of a number of age-related phenotypes, such as osteoporosis, alopecia, weight loss, and a reduction in levels of subcutaneous fat.^{56,58} A recent study has questioned the role of the mtDNA point mutations in the aging phenotype as demonstrated by the high point mutation accumulation in the heterozygous POLG mice, which did not display any accelerated aging.⁴⁷ However, the role of mtDNA deletions in the aging process of these mice has not yet been investigated and remains to be established.

These animal models are of particular interest as they provide evidence that disruption of mitochondrial maintenance resulting in secondary changes to the mitochondrial genome are capable of affecting neurons. These mtDNA mutations can then lead to respiratory chain deficiency and neuronal cell death. In

previous studies the level of mtDNA deletion in substantia nigra neurons has been shown to reach almost 90%;³⁵ however, levels of mtDNA deletions above this have not been observed. This may suggest that cells which harbor very high mtDNA deletion levels (>90%) may have already died; this seems especially plausible given the results of Auré *et al.*²⁴ and that the tissue used in these studies is postmortem and so at the end stage of a process that has taken decades.

Conclusions

By studying neuropathological changes in the brains of patients with inherited mtDNA disorders, it has been shown that neurodegeneration is a prominent feature with evidence of cell loss in many brain regions. These studies highlight the importance of the level of specific mtDNA mutations to cross the threshold for the cell to become respiratory chain deficient and compromise the cell's function. Similar studies in muscle from both aging and diseased muscle also find a close association of high levels of mtDNA mutations within individual muscle fibers and changes likely to result in cell loss or death. In both brain aging and neurodegenerative diseases we found high levels of mtDNA mutations, particularly in substantia nigra neurons compared to hippocampal neurons.³⁶ It remains uncertain as to why such high levels are observed specifically in these neurons, although it is tempting to speculate that this is because of the high levels of oxidative stress seen in these neurons. How these mtDNA molecules harboring a large deletion clonally expand within these postmitotic cells is still unknown. This area of research is difficult to tackle since the rate of mtDNA replication in neurons remains unknown. Also, the role of these mutations in neuronal cell loss is not clear.

Although some authors have attempted to link primary mtDNA mutations directly with common neurodegenerative diseases, such as AD and PD, there is no clear evidence that they are the primary cause of the disease. There is

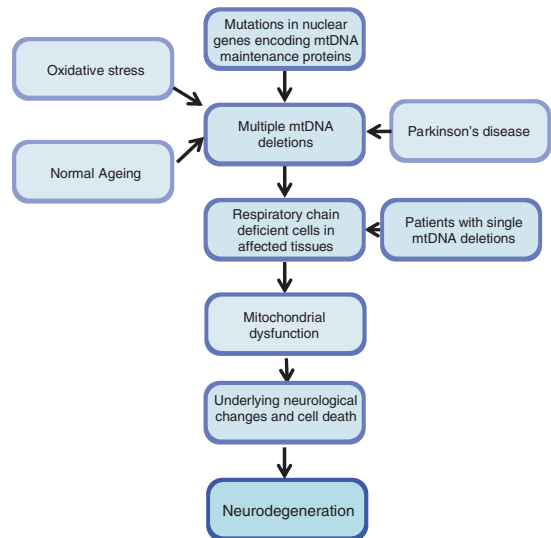


Figure 3. Schematic diagram to demonstrate how mtDNA deletions from patients with mitochondrial disease and also from normal aging and patients with neurodegenerative disease could lead to mitochondrial dysfunction and ultimately neurodegeneration, especially within the substantia nigra.

some evidence that certain mitochondrial haplogroups influence either incidence or expression of PD.⁴⁶ Perhaps a more plausible hypothesis is that somatic mtDNA mutations occur as a result of stress within cells (for example, oxidative stress) and, once formed, these often clonally expand. If this is the case, then cells that are affected by AD or PD are more likely to have mtDNA mutations as a result of the stress these cells clearly undergo. Thus, the mtDNA mutations are secondary to the primary disease process but, once clonally expanded to high levels, can contribute to neuronal cell death similar to that seen in primary mitochondrial diseases (Fig. 3).

Conflicts of Interest

The authors declare no conflicts of interest.

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