Mitochondrial DNA sequence variation and neurodegeneration

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Date received (in revised form): 7th March, 2008

Abstract

Mitochondria, the powerhouse of the cell, play a critical role in several metabolic processes and apoptotic pathways. Many lines of evidence suggest that mitochondria have a central role in ageing-related neurodegenerative diseases. Moreover, there is a long history of investigations on mitochondria aimed at identifying genetic markers relating to ageing and neurodegenerative diseases. In this review, some of the major neurodegenerative disorders are highlighted and the role of mitochondrial haplogroups in the pathogenetic cascade leading to these diseases is discussed.

Keywords: *mitochondria*, *mtDNA*, *mtDNA haplogroups*, *neurodegenerative disorders*

Introduction

Mitochondria and their DNA (mitochondrial DNA [mtDNA]) result from a process of endosymbiosis which occurred about 1.5 billion years ago, when protobacteria populated primordial eukaryotic cells and took permanent residence in the new environment. MtDNA — a relic but not a fossil — has lost much of its independence but keeps functioning under the overarching control of the nuclear genome (nDNA).

Mitochondria are ubiquitous in eukaryotes and are essential for survival. Their primary function is to support aerobic respiration and to provide energy and heat. Mitochondria also play other important roles, including in cell signalling for apoptotic cell death.

By convention, the term 'mitochondrial diseases' refers to disorders of the mitochondrial respiratory chain, thus excluding dysfunction in other metabolic pathways located in the mitochondria (ie pyruvate metabolism, Krebs cycle and fatty acid oxidation). The respiratory chain is the only metabolic pathway in the cell that is under the dual control of mtDNA and nDNA.

There is a long history of investigations on mitochondria, which have been aimed at identifying genetic markers relating to ageing, neuromuscular and neurodegenerative diseases and, more recently, common diseases such as diabetes and cancer.

This paper will include a brief section on mitochondrial biology and genetics, and will then focus on the role of mtDNA haplogroups in selected neurodegenerative disorders.

Mitochondrial compartment

Mitochondria are highly dynamic and pleomorphic organelles. They are composed of a smooth outer membrane surrounding an inner membrane of significantly larger surface area, which, in turn, surrounds a protein-rich core — the matrix.[1] Although mitochondria contain their own genome and protein-synthesising machinery, the majority of mitochondrial polypeptides are encoded in the nuclear genome, synthesised in the cytosol and imported into the mitochondria post-transcriptionally.

The main mitochondrial role is in the synthesis of ATP formed by oxidative phosphorylation. They

are also involved in other metabolic processes, including the biosynthesis of amino acids, vitamin cofactors, fatty acids, iron-sulphur clusters, cell signalling and programmed cell death. ATP molecules are generated via glycolysis or by oxidation of glucose to ethanol or lactic acid. Electrons from oxidative substrates are transferred to oxygen, via a series of redox reactions, to generate water. In the process, protons are pumped from the matrix across the mitochondrial inner membrane via the electron transport chain (ETC), which consists of four multimeric complexes (I to IV) plus two small electron carriers — coenzyme Q (also known as ubiquinone) and cytochrome c. This process creates an electrochemical proton gradient. ATP is produced by the influx of these protons back through the complex V, or ATP synthase (the 'rotary motor').[2]

Mitochondrial genome and common mtDNA haplogroups

Mitochondria contain two to ten molecules of mtDNA. It is a circular molecule of 16.5 kilobases lacking introns, and consisting of a heavy chain (H) and a light chain (L).[1,3] MtDNA carries 37 genes encoding 22 transfer RNAs (tRNAs), two ribosomal RNAs (12S and 16S) and 13 polypeptides (mRNAs). The 13 mtDNA-encoded polypeptides are part of the respiratory chain system and are assembled together with nuclear-encoded subunits. Seven of these belong to complex I or reduced nicotinamide adenine dinucleotide (NADH) dehydrogenase — NADH, ubiquinone oxidoreductase (ND1, ND2, ND3, ND4, ND4L, ND5, ND6). One belongs to complex III or ubiquinol — ferricytochrome c oxidoreductase. Three belong to complex IV or cytochrome c oxidase — COX I, COX II and COX III. The final two belong to complex V or ATP synthase — ATPase6 and ATPase8. The remaining mitochondrial proteins, including all of the complex II subunits, are encoded by nDNA.

In humans, mtDNA is transmitted through the maternal lineage.[1,3] MtDNA is commonly used to complement information provided by Y chromosome studies on the evolution of modern humans. We can theoretically follow the transmission of mtDNA from the original 'ancestor mother' by identifying common polymorphisms that have accumulated with time. Specific and unique sets of those common polymorphisms define groups of mtDNA, named haplogroups, that have evolved from the same ancestor. Phylogeographic studies identified the first common ancestor, named 'mitochondrial Eve',[4] postulated to have lived in Africa about 200,000 years ago. Direct sequencing and restriction fragment length polymorphism analysis enabled the identification of the mtDNA haplogroup tree and the mtDNA migration route (Figure 1).

The basal branching structure of mtDNA variation in most parts of the world is now well understood.[5] African haplogroups fall into seven major families (L0, L1, L2, L3, L4, L5 and L6). About 85,000 years ago, probably in the Horn of Africa, the root of haplogroup L3 gave rise to many descendant haplogroups (probably because of some colonisation event or local population growth). Non-African mtDNA descends from L3 and belongs either to the M or N superclades (obviously excluding the descendants of migrations from Africa within the past few thousand years). In the Indian subcontinent and in South-East Asia, there is the richest basal variation in the tree originated by haplogroups M and N, and this suggests a rapid colonisation along the southern coast of Asia about 60,000 years ago.[5] The expansions northward occurred later, about 45,000 years ago. Over 30 subclades of haplogroup M are present in Asia. Haplogroups A, B, C, D and X have been found in the Americas, although come mainly from Asia.

In Europeans and Near Easterners (who share a rather recent common ancestor), nine different mitochondrial haplogroups have been identified (H, I, J, K, T, U, V, W and X). The variation in the basal European mtDNA haplogroups dates to about 45,000 years ago.[5] Complete mtDNA sequencing and the increasing number of samples analysed allow haplogroups to be subdivided into smaller groups, identifying younger branches on the mtDNA evolutionary tree. Therefore, subhaplogroup classification is continuously evolving.[5]

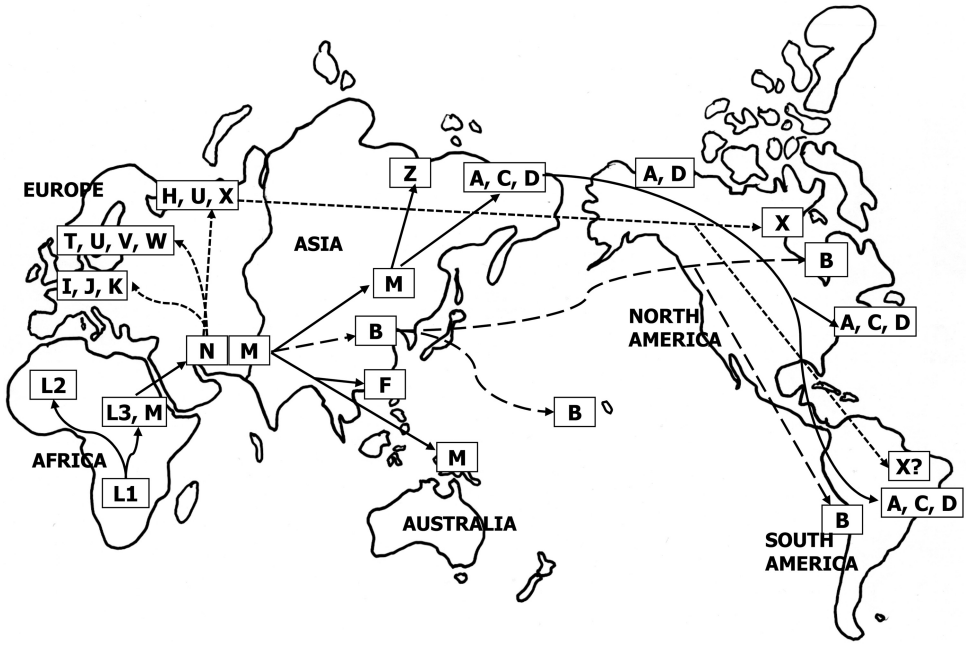

Figure 1. Mitochondrial DNA (MtDNA) haplogroup migration patterns (modified from Shriver and Kittles). The map shows the migration patterns of the main mtDNA haplogroups. The first common ancestor is postulated to have lived in Africa about 200,000 years ago. From Africa, the arrows show the mtDNA migration routes.[62]

MtDNA haplogroups and neurodegeneration

It has been speculated that mtDNA mutations that accumulate with age might lead to impaired energy generation and to increased numbers of reactive oxygen species (ROS), both resulting in cell damage. Polymorphisms in mtDNA may cause subtle differences in the encoded proteins and, thus, minimal changes in mitochondrial respiratory chain activity and free radical overproduction. This could predispose an individual, or a population sharing the same mtDNA genotype, to an earlier onset of apoptotic processes, such as accumulation of somatic mtDNA mutations and mitochondrial impairment. The opposite could be true for different polymorphism(s), which could be beneficial, increasing mitochondrial respiration and/or reducing ROS production.[6,7]

Specific mitochondrial haplogroups have been linked to longevity.[8,9] It therefore follows that the same or other haplogroups could be involved at the other end of the life spectrum, neurodegeneration and, thus, death. Because of the sensitivity of mtDNA as a marker for human migration patterns, all studies of mtDNA haplogroup association with disease must pay rigorous attention to the ethnic matching of cases to controls.

Alzheimer's disease

Alzheimer's disease (AD) is a late-onset progressive neurodegenerative disorder which results in irreversible loss of neurones, particularly in the cortex and hippocampus. It is the most common form of dementia in the elderly and is clinically characterised by an impairment of cognitive function and changes in behaviour and personality. Apart from the neuronal loss, the pathological hallmarks are extracellular senile plaques containing the peptide beta-amyloid (Aβ), and neurofibrillary tangles composed of a hyperphosphorylated form of the microtubular protein, tau.[10] The Aβ cascade hypothesis remains the main pathogenetic model of familial AD with mutations in amyloid precursor protein and presenilin genes,[11] but its role in the majority of sporadic AD cases without mutations in these genes (accounting for 90–95 per cent of AD

cases) is still unclear. There is substantial evidence of morphological, biochemical and genetic abnormalities in mitochondria in various tissues of patients with AD.[12,13] For example, studies have reported a decrease in cyclo-oxygenase (COX) activity in the hippocampus and platelets of sporadic AD patients.[14,15] Further, cybrid models, obtained by transfecting mtDNA from the platelets of AD patients into neuronal cultured cells deprived of their own mtDNA, revealed decreased COX activity, increased ROS generation, increased Aβ production and morphological abnormalities.[16,17] This suggests a mitochondrial genomic contribution to mitochondrial dysfunction in AD.[6,18] Studies attempting to identify mtDNA mutations in the brains of AD patients have had limited success. For example, Elson *et al.*[19] sequenced the complete coding regions of 145 autoptic AD brain samples and 128 normal controls; they observed that for both synonymous and non-silent changes, the overall numbers of nucleotide substitutions were the same for the AD and control sequences.[19]

For the reasons described above, different groups have analysed the frequencies of polymorphisms and/or mutations in mtDNA in AD patients with conflicting results. Chagnon and coworkers[20] reported that haplogroup T is under-represented in AD patients, while haplogroup J seems to be over-represented. By studying a sample of Italian subjects, Carrieri *et al.*[21] found that haplogroups K and U were present at a lower frequency in AD patients who were apolipoprotein (*Apo*) E4 carriers than in non-carriers (while in controls there was no association between the *E4* allele and mtDNA haplogroups). This suggests that K and U may act by neutralising the effect of the major AD risk factor — the *E4* allele.[21] The same authors detected a lowering of the *E4* allele odds ratio from statistically significant to non-significant in patients with haplogroups K and U.[21]

Another report showed (independently from *ApoE* genotype) that males classified as haplogroup U had a significantly increased risk of AD, while females demonstrated a significantly decreased risk with the same U haplogroup.[22] Thus, the inheritance of haplogroup U may have a negative effect on ageing in Caucasian males.[22] Two recent studies (including only neuropathologically proven cases of AD in patients of European descent) indicated that mtDNA haplogroups were not associated with AD, either individually or by grouping together closely related haplogroups.[19,23]

In another study, no evidence was found for an aetiological role of haplogroup-associated polymorphisms.[24] In this study, the frequency of European mtDNA haplogroups was investigated in a clinically well defined group of 209 unrelated patients and 191 controls, both of clear Tuscan origin (in order to minimise the risk of false associations between gene markers and disease). The frequency of haplogroups H, I, J, K, T, U, V, W and X was not significantly different between the patients and control groups.[24] Further, there was no significant difference between genders in mtDNA haplogroup distribution in either AD patients or control groups. The *ApoE4* allele was confirmed as a risk factor for AD, as it was found at a significantly higher frequency in patients than in controls (23.3 per cent versus 8.11 per cent), but no association between *ApoE* alleles and mtDNA haplogroups was observed.[24] The data from this study also excluded any association between mtDNA haplogroups, age of onset and mean survival.

In conclusion, although it has been suggested that inherited haplogroups K and U may influence AD risk in Caucasians, this is still an unresolved question. To date, mtDNA haplogroups do not seem to play a major role in AD.

Parkinson's disease

Parkinson's disease (PD) is a common neurodegenerative movement disorder characterised by resting tremor, rigidity and bradykinesia. It is characterised by loss of dopaminergic neurones in the substantia nigra and Lewy bodies in the remaining neurones. PD exists in both familial and idiopathic forms. While the aetiology of sporadic PD remains largely unclear, there is accumulating evidence suggesting that mitochondrial dysfunction occurs in the brain and peripheral tissues of PD patients.[25,26] In

selected cases, mitochondrial genetic abnormalities can directly cause PD.[25,26] Cells fused with mtDNA from PD patients showed 25 per cent decreased complex I activity.[27] Parkinsonism has been associated with large-scale rearrangements in mtDNA.[28,29] Further, the co-existence of parkinsonism and mutations in the mitochondrial polymerase gamma nDNA gene (*POLG1*) was recently described in several families, suggesting that when defective, this gene could be responsible for some of the Mendelian transmission of parkinsonism.[30,31] Clustering of rare variants of the *POLG1* CAG-repeat (encoding a polyglutamine tract) has been found in Finnish patients with idiopathic PD, compared to controls.[32]

By contrast, primary mtDNA point mutations causing parkinsonism have been reported only rarely — typically as one feature of a larger syndrome, as in the case of sensorineural deafness and neuropathy.[33] Very recently, Horvath *et al.*[34] observed the A8344G 'MERRF' (myoclonic epilepsy with ragged red fibres) mutation on the *tRNALys* gene of the mtDNA in a 66-year-old man with dopa-responsive parkinsonism, reduced muscle strength and ragged red fibres. No other clinical signs typical of MERRF were noted.[34] The A8344G mutation was present in a virtually homoplasmic state in the patient's muscle, and 80 per cent mutant mtDNA was detected in blood DNA.[34] In order to evaluate if this mutation can represent a common cause of sporadic PD, 159 Italian patients with PD were analysed;[35] none of the patients carried the A8344 mutation in DNA extracts from peripheral blood lymphocytes. Thus, the screening for this mutation would have been restricted to PD patients with other canonical features of mitochondrial encephalomyopathies (ie myopathy, ophthalmoplegia, neuropathy, cerebellar ataxia), and without *POLG1* mutations.[35]

The role of the mtDNA variants in PD has been extensively studied. Van der Walt and coworkers[36] genotyped ten single nucleotide polymorphisms (SNPs) that define the European mtDNA haplogroups in 609 white patients with PD and 340 unaffected white control subjects. These authors observed that haplotypes J and K reduced the incidence of PD by 50 per cent.[36] Further analysis revealed that the SNP at 9055A of *ATP6* (which defines haplogroup K) reduced the risk in women, and that the SNP at 13708A of the *ND5* gene (haplogroup J) was protective in individuals older than 70 years (probably either through increasing the performance of complex I within the brain and other tissues in individuals derived from J and K haplogroup lineages, or reducing ROS generation).[36] Consistent with the described study, it has been reported that the haplotype cluster UKJT was associated with a 22 per cent reduction in population-attributable risk for PD.[37] Further, Autere *et al.*[38] hypothesised that the risk of PD is conveyed by the total number of non-synonymous substitutions in the complex I genes in various mtDNA lineages. To test this possibility, they determined the number of non-synonymous substitutions of the seven complex I genes in a Finnish population, and observed that the supercluster JTIWX increased the risk of both PD and PD with dementia. This cluster was associated with a twofold increase in non-synonymous substitutions in the mtDNA genes encoding complex I subunits.[38]

Another group evaluated the distribution of the different mtDNA haplogroups in a large cohort of 620 Italian patients with adult-onset idiopathic PD versus two groups of ethnic-matched controls. These authors found that haplogroup K was associated with a lower risk of PD; they also reported that the i0398G polymorphism was protective against PD.[39] This last finding (but not the association between haplogroup K and lower risk) was later confirmed in 271 Spanish PD patients versus 230 healthy controls.[40]

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a motor neurone disease with selective degeneration of the anterior horn cells of the spinal cord and cortical motor neurones. Approximately 90 per cent of cases are sporadic and 10 per cent are familial. About 20 per cent of familial cases result from mutations in the gene encoding for Cu/Zn

superoxide dismutase (*SOD1*). The aetiology and pathogenesis of the sporadic form of the disease are poorly understood; mitochondrial dysfunction and oxidative stress are probably involved.[6,7] ALS cybrids seem to have impaired respiratory chain function, increased ROS production and altered calcium homeostasis, suggesting the presence of a primary mtDNA defect,[41] but these findings have not been confirmed.[42] Further, increased levels of mtDNA single 'common deletion' and multiple deletions have been detected in the brain[43] and skeletal muscle[44] of patients with sporadic ALS.

Three patients with ALS and primary pathogenic mtDNA mutations have been reported. The first was an out-of-frame mutation (leading to premature termination of the translation) of mtDNA encoding for subunit I of COX, discovered during investigation of a severe isolated muscle COX deficiency in a patient with early-onset motor neurone-like degeneration, but without the common features of mitochondrial disorders.[45] The second was a homoplasmic mutation in the mitochondrial *tRNACys*, in a 60-year-old Caucasian male suffering from an asymmetrical pure lower motor neurone variant of ALS and temporal lobe epilepsy.[46] The third was a point mutation in the *tRNAIle* (T4274C), in a patient with pure lower motor neurone disease, diabetes and cardiac involvement.[47]

To investigate if specific genetic polymorphisms within the mtDNA could act as susceptibility factors and contribute to the clinical expression of sporadic ALS, predefined European mtDNA haplogroups were genotyped in 222 patients of clear Italian origin with sporadic ALS and 151 matched controls.[48] Mutations on the entire *SOD1* gene were excluded by single strand conformation polymorphism analysis and/or by direct sequencing. The frequency of haplogroup I was found to be lower in ALS cases than in controls (odds ratio [OR] 0.08, 95% confidence interval [CI] 0.04–0.40, $p < 0.01$).[48] Multiple regression studies which aimed to test the hypothesis that mtDNA haplogroups could influence the age of onset, severity and neurological system involved in the disease did not reach significance. In a comparison developed to investigate how haplogroup I differs from the other haplogroups tested, two nucleotides at positions 16391 and 10034 were identified; only the difference between *16391A* and *10034C* alleles was found to be highly significant ($p < 0.01$).[48] In accordance with the described study, mtDNA polymorphism might contribute to motor neurone degeneration, possibly interacting with unknown genetic or environmental factors.[48] This finding was not confirmed in a recent paper by Chinnery *et al.*,[49] who studied a UK cohort of 504 ALS patients and 493 controls and found no evidence that mtDNA haplogroups contribute to the risk of developing ALS.

Multiple sclerosis

Multiple sclerosis (MS) is a chronic inflammatory autoimmune disease, with myelin loss and gliosis, affecting young people — mostly females. Family-based half-sibling studies suggest that there is a maternal parent-of-origin effect in MS.[50] The involvement of mtDNA in determining susceptibility to MS has been hypothesised based on the detection of Leber's hereditary optic neuropathy (LHON) mutations in MS patients more frequently than expected by chance.[51–55] In a recent study, 21 of the 58 patients (36.2 per cent) tested positive for the T4216C mutation, while only 11.3 per cent of the controls carried this secondary LHON mutation ($p < 0.01$).[56] In a study with 77 Caucasian MS patients versus 84 controls, haplogroups K and J showed an association with MS at a p-value of 0.001.[57]

Two recent papers reported that a SNP (RS660339) in the *UCP2* nuclear gene was a risk factor for MS.[58,59] *UCP2* is a member of the mitochondrial proton transport family, which uncouples proton entry in the mitochondrial matrix from ATP synthesis. The *RS660339* SNP is correlated with lower UCP2 expression levels *in vitro* and *in vivo*.[58] Low levels of UCP2 are a risk factor for MS. In the second study,[59] the authors also found that none of the mitochondrial haplogroups were associated with the disease; however, there seemed to be a protective trend in the 'JT + the protective allele of *UCP2*' combination.[59] These genetic

variables are independently hereditary, but there may be a synergetic effect between them.

Very recently, Yu and co-workers[60] investigated more than 2,500 sporadic cases of MS and a similar number of healthy controls. They studied seven common SNPs in the mtDNA, and found that one of these SNPs (nt13708 G/A) was significantly associated with MS susceptibility (OR = 1.71, 95 per cent CI 1.28–2.26, $p = 0.0002$).[60] The frequency of haplogroup J, a haplogroup comprising nt10398 and nt13708 SNPs, was higher in patients than in controls.[60] Subsequent sequencing of the mtDNA of 50 individuals revealed that the nt13708A variant itself, rather than the SNPs linked to it, was responsible for the association.[60] Thus, this mtDNA variant might represent a susceptibility allele for MS.

Conclusions

In the past 15 years, research has been directed at clarifying the involvement of mitochondria, and defects in mitochondrial oxidative phosphorylation, in late-onset neurodegenerative disorders. A critical role for mitochondrial dysfunction and oxidative damage in neurodegenerative diseases has been greatly strengthened by recent findings. Despite the evidence of morphological and biochemical abnormalities of the mitochondria in various tissues of patients with neurodegenerative disorders, however, the role of the mitochondrial genome and its haplogroups as a risk factor is still controversial. MtDNA haplogroups have been associated with a number of different neurodegenerative diseases, but, to date, the only disease consistently associated with a different mtDNA haplogroup frequency is PD. Because very large cohorts are required reliably to detect an association with complex human diseases,[61] further analysis, using larger samples, will be required to reveal more definitively the contribution of mtDNA mutations or haplogroups, if any, to the pathogenesis of neurodegenerative diseases.

Acknowledgments

The authors are grateful to Dr Anna Choub for her critical reading of the manuscript, and to Dr Lucia Petrozzi for preparing Figure 1.

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