Comprehensive summary of mitochondrial DNA alterations in the postmortem human brain: A systematic review

Alba Valiente-Palleja,1 Juan Tortajada,1 Bengisu K. Bulduk,1 Elisabet Vilella,1 Gloria Garrabou,1,2 Gerard Muntané,1,2,5 and Lourdes Martorella1,2*

aResearch Department, Hospital Universitari Institut Pere Mata (HUIPM); Institut d’Investigació Sanitària Pere Virgili (IISPV); Faculty of Medicine and Health Sciences, Universitat Rovira i Virgili (URV), 43201 Reus, Catalonia, Spain
bBiomedical Network Research Centre on Mental Health (CIBERSAM), 28029 Madrid, Spain
cLaboratory of Muscle Research and Mitochondrial Function, Department of Internal Medicine-Hospital Clinic of Barcelona (HCB); Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS); Faculty of Medicine and Health Sciences, Universitat de Barcelona (UB), 08036 Barcelona, Catalonia, Spain
dBiomedical Network Research Centre on Rare Diseases (CIBERER), 28029 Madrid, Spain
eInstitute of Evolutionary Biology (IBE), Universitat Pompeu Fabra (UPF), 08003 Barcelona, Catalonia, Spain

Abstract
Background Mitochondrial DNA (mtDNA) encodes 37 genes necessary for synthesizing 13 essential subunits of the oxidative phosphorylation system. mtDNA alterations are known to cause mitochondrial disease (MitD), a clinically heterogeneous group of disorders that often present with neuropsychiatric symptoms. Understanding the nature and frequency of mtDNA alterations in health and disease could be a cornerstone in disentangling the relationship between biochemical findings and clinical symptoms of brain disorders. This systematic review aimed to summarize the mtDNA alterations in human brain tissue reported to date that have implications for further research on the pathophysiological significance of mtDNA alterations in brain functioning.

Methods We searched the PubMed and Embase databases using distinct terms related to postmortem human brain and mtDNA up to June 10, 2021. Reports were eligible if they were empirical studies analysing mtDNA in postmortem human brains.

Findings A total of 158 of 637 studies fulfilled the inclusion criteria and were clustered into the following groups: MitD (48 entries), neurological diseases (NeuD, 55 entries), psychiatric diseases (PsyD, 15 entries), a miscellaneous group with controls and other clinical diseases (5 entries), ageing (20 entries), and technical issues (5 entries). Ten entries were ascribed to more than one group. Pathogenic single nucleotide variants (pSNVs), both homo- or heteroplasmic variants, have been widely reported in MitD, with heteroplasmy levels varying among brain regions; however, pSNVs are rarer in NeuD, PsyD and ageing. A lower mtDNA copy number (CN) in disease was described in most, but not all, of the identified studies. mtDNA deletions were identified in individuals in the four clinical categories and ageing. Notably, brain samples showed significantly more mtDNA deletions and at higher heteroplasmy percentages than blood samples, and several of the deletions present in the brain were not detected in the blood. Finally, mtDNA heteroplasmy, mtDNA CN and the deletion levels varied depending on the brain region studied.

Interpretation mtDNA alterations are well known to affect human tissues, including the brain. In general, we found that studies of MitD, NeuD, PsyD, and ageing were highly variable in terms of the type of disease or ageing process investigated, number of screened individuals, studied brain regions and technology used. In NeuD and PsyD, no particular type of mtDNA alteration could be unequivocally assigned to any specific disease or diagnostic group. However, the presence of mtDNA deletions and mtDNA CN variation imply a role for mtDNA in NeuD and PsyD. Heteroplasmy levels and threshold effects, affected brain regions, and mitotic segregation patterns of mtDNA alterations may be involved in the complex inheritance of NeuD and PsyD and in the ageing process. Therefore, more

Abbreviations: mtDNA, Mitochondrial DNA; MitD, Mitochondrial disease/s; NeuD, Neurological disease/s; PsyD, Psychiatric disease/s; pSNV, Pathogenic single nucleotide variant; CN, copy number; DEL, deletion
*Corresponding author: Research Department, Hospital Universitari Institut Pere Mata, Ctra. de l’Institut Pere Mata, s/n, 43206 Reus, Catalonia, Spain.
E-mail address: martorell@peremata.com (L. Martorell).
1 These authors contributed equally to this work.
information is needed regarding the type of mtDNA alteration, the affected brain regions, the heteroplasmy levels, and their relationship with clinical phenotypes and the ageing process.

Funding Hospital Universitari Institut Pere Mata; Institut d’Investigació Sanitària Pere Virgili; Instituto de Salud Carlos III, Ministerio de Ciencia e Innovación (PI18/00514).

Copyright © 2022 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

Keywords: Mitochondrial DNA; Mitochondrial diseases; Neurological diseases; Psychiatric diseases; Ageing; Postmortem

Research in context

Evidence before this study

The human mitochondrial genome consists of a 16,569 bp molecule present in almost all cell types with some exceptions, the most significant being erythrocytes. On average, there are approximately 1,000 mtDNA molecules in a human cell, but the specific mtDNA CN depends on the energy requirements of each cell. Brain tissue has a high energy requirement, which leads to a large number of mitochondria in the brain. 1 It is known that alterations in mtDNA cause MitD, a clinically heterogeneous group of disorders that arise as a consequence of mitochondrial respiratory chain dysfunction. The clinical characteristics of MitD show enormous variability, and although they can affect a single organ, most of them involve multiple organ systems often presenting with neurological disturbances. Moreover, there is increasing evidence of mitochondrial dysfunction in neurodegeneration, 2 in the development of psychiatric symptoms, 3 and in the brain ageing process. 4, 5 The aims of this study were a) to determine the specific mtDNA CN between affected and nonaffected individuals, with conflicting results, while few studies evaluated mtDNA pSNVs. Similarly, pSNVs and altered mtDNA CNs were not consistently evaluated in PsyD; in contrast, several studies identified mtDNA deletions in some patients. Finally, mtDNA deletions have been recurrently associated with ageing. We also identified some studies that have explored mtDNA gene expression, oxidation and methylation.

Implications of all the available evidence

Among the three types of well-known mtDNA alterations (pSNVs, mtDNA CN and mtDNA rearrangements) that are associated with mitochondrial dysfunction, only one or two of them were investigated in most studies. Most studies reported mtDNA alterations, demonstrating their presence in the postmortem brain of patients with MitD, NeuD and PsyD and the ageing process. With the currently available molecular techniques and bioinformatic tools, it is crucial to further investigate the presence of all types of mtDNA alterations in postmortem brain samples of patients with MitD, NeuD and PsyD in all age groups and in healthy individuals to shed light on the role of mtDNA in brain function, disease development and the ageing process. These studies should be conducted with current validated techniques to obtain unambiguous data regarding mtDNA alterations and associated heteroplasmy levels and are particularly relevant when measuring mtDNA CN. Because mitochondria can be acknowledged as a therapeutic target for ameliorating brain function, it is crucial to decipher the role of all types of mtDNA variations in health and disease.

Introduction

Mitochondrial DNA (mtDNA)

Mitochondria are membrane-bound organelles that generate most of the chemical energy needed to power the cell’s biochemical reactions; this energy is stored as adenosine triphosphate (ATP) molecules. Two distinct bilayer membranes separate the matrix of the mitochondria from the cytosol—the smooth outer membrane and the highly folded inner membrane—forming invaginated structures called cristae. In these cristae, ATP...
synthesis takes place by the oxidative phosphorylation system (OXPHOS) through oxidoreductase complexes I-IV of the electron transport chain (ETC) and the ATP synthase enzyme of complex V. Mitochondria act as a signalling hub regulating cellular processes relevant to cell differentiation, cell proliferation, apoptosis and the immune response.6–10 The mitochondrial proteome is estimated to contain 1,500 proteins, most of which are under nuclear genome control.

Human mtDNA consists of a 16,569 bp circular DNA molecule that is maternally inherited and whose sequence and gene organization were published in 1981.11,12 It encodes 37 genes, including 13 protein subunits of the respiratory chain, 2 ribosomal units (tRNAs 12S and 16S) and 22 transfer (tRNA) genes. mtDNA also contains a noncoding control region of approximately 1,200 bp in length and is known as the displacement loop (D-loop) region, which regulates mtDNA transcription and replication. The 13 resulting proteins are crucial for the proper function of the respiratory chain even though they represent only a small fraction of the ~100 subunits that constitute complexes I-V.13 Another peculiar feature of mtDNA is polyploidy. A uniform collection of mtDNA copies—either completely normal mtDNA or completely mutant mtDNA—is known as homoplasmy, while heteroplasmy refers to different proportions of normal and mutant mtDNA in a mitochondrion, cell, organ or tissue. The amount of mtDNA in a cell, known as the mtDNA content or mtDNA CN, usually varies from hundreds to thousands14 and depends on the cell function and the cell response to endogenous and exogenous agents.15 Tissues with high energy requirements contain large amounts of mitochondria in their cells and, accordingly, a high mtDNA CN. The central nervous system, cardiac and skeletal muscles, endocrine system, and liver and renal systems are among those with the highest energy requirements.16

mtDNA is composed of double-stranded DNA, comprising heavy strands (H) and light strands (L). The H-strand, which is guanine-rich, encodes 28 genes, while the L-strand, which is cytosine-rich, encodes the remaining 9 genes. Several characteristics differ between the nuclear and mitochondrial genomes: mtDNA is circular, small, has no introns, is not enveloped with proteins and is maternally inherited; in contrast, nuclear DNA (nDNA) is linear, has a large number of nucleotides (~3.3 billion bp), has introns, is packaged into chromatin, undergoes recombination and is biparentally inherited. Both mtDNA and nDNA use the same deoxynucleotide triphosphates (dNTPs) for DNA replication, and although mtDNA follows the universal codon usage rules when coding sequences are translated into proteins, there are some specific deviations: UGA codes for tryptophan instead of a stop codon, AGA and AGG are also stop codons, and AUA codes for methionine. Additionally, some nucleotide bases exhibit functional overlap between two genes, as they are the last base of one gene and the first base of the next gene.6 In the mitochondrial matrix, mtDNA forms nucleoids with mitochondrial transcription factor A, which acts to provide structure to the mtDNA genome.

Finally, the mtDNA mutation rate (the speed at which mutations are introduced) is much higher than that of nDNA. In animals, it is estimated that the mutation rate in mtDNA is ~25-fold higher than that in nDNA.17 In humans, based on the appearance of de novo mtDNA variants in human pedigree studies, an ~10-fold higher rate in mtDNA than in nDNA has been suggested.18 The molecular damage to mtDNA is thought to be due to the high levels of reactive oxygen species present in the mitochondrion, the high mtDNA replication levels, and the high coding rate of mtDNA, which is ~93%.19 Additionally, this higher mutation rate suggests that many mtDNA variants may be subjected to poor selection, which can occur at the germline level or at the somatic level throughout life, implying that even though a specific variant is not detected in blood, a tissue commonly used in genetic testing, it cannot be ruled out that the variant is not present in other tissues or organs. Correspondingly, mtDNA alterations can lead to cellular energy impairment that might cause a disease or be implicated in the physiopathology of age-associated diseases or the ageing process itself.20

mtDNA and human ageing

The decline of mitochondrial functioning has been largely implicated in the ageing process and is characterized by a reduced density of mitochondria and reduced mitogenesis.21 In fact, the ageing process is strongly linked to noninherited mtDNA changes, mainly point variants and large deletions, that increase in frequency with age.22–24 Such changes, which originate as replication errors, accumulate in postmitotic tissues during ageing, leading to increased proportions of impaired mitochondria that may differ between cells and tissues.25 In the ageing brain, dysfunctional synaptic mitochondria leading to impaired neurotransmission and cognitive failure26 have been amply demonstrated,27,28 and mtDNA deletions correlate with mitochondrial respiratory chain malfunction.27,28 Thus, elucidating the temporal and spatial distribution of mutated mtDNA in the brain might resolve important questions regarding the importance of mtDNA changes in the ageing process.

mtDNA and disease

Given the nature of the genetic material and the dual genomic control of mitochondria, alterations occurring either in the nDNA or mtDNA sequence can potentially cause mitochondrial functional defects. Indeed, some are known to cause very heterogeneous diseases that together are called primary mitochondrial disease...
(MitD). More than 300 nuclear genes are known to cause MitD, although most adult patients exhibit variants in mtDNA. The mechanisms involved in these nuclear genes involve assembly factors, mitochondrial structure, coenzyme Q biosynthesis, protein synthesis, and mtDNA maintenance. However, this review focuses on MitDs associated with mtDNA pathogenic variants that include pathogenic single nucleotide variants (pSNVs), mtDNA rearrangements (mostly deletions), and altered mtDNA CNs. They can be either maternally inherited or occur de novo, and their pathogenic role can be established by taking advantage of the association between each variant and a specific phenotype. This was investigated by a recent analysis of 265 mtDNA SNVs in 483,626 individuals from the United Kingdom (UK) biobank, which has allowed the identification of 260 new mtDNA-phenotype associations, including type 2 diabetes, multiple sclerosis, adult height, and liver and renal biomarkers. Notably, this study identified a key role for mtDNA common and rare SNV variation (only homoplasmic) in many quantitative human traits and disease risks, with a particular emphasis on cardiometabolic and neurodegenerative diseases. mtDNA pSNVs can be observed in homoplasy or heteroplasy, while mtDNA deletions are always heteroplasmic, since within the deleted region, mtDNA consistently contain one or more tRNAs indispensable for the translation of the protein-associated mtDNA genes, which are essential for life. Known mtDNA pSNVs lead to syndromes such as mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS), myoclonic epilepsy with ragged red fibres (MERRF), neuropathy, ataxia and retinitis pigmentosa (NARP), and Leigh syndrome (LS). mtDNA rearrangements are responsible for Kearns–Sayre syndrome (KSS), progressive external ophthalmoplegia (PEO), and Pearson’s syndrome. These are considered the most typical MitDs associated with mtDNA alterations; however, there are many other diseases and human characteristics related to mtDNA variation, such as diabetes and hypertension, as well as ageing. In fact, it has been estimated that 1 out of 3,500–6,000 individuals are affected by or are at risk of developing a MitD. Human phenotypes associated with mtDNA alterations include extremely severe diseases that can be present from infancy to adulthood and can affect a single organ or multiple tissues, and most of them are included as rare diseases (ORPHANET, https://www.orpha.net/). In June 2021, the Online Mendelian Inheritance in Man (https://omim.org) catalogue included 33 phenotypic descriptions (Supplementary Table 1) in which the molecular basis is known to be associated with mtDNA alterations. The catalogue also included the 37 mtDNA genes (Supplementary Table 2) containing several allelic variants associated with human conditions. In addition, the human mitochondrial genome database (https://www.mitomap.org) includes a large and growing number of variants, some of which are related to a wider constellation of phenotypes. Among the reported mtDNA base substitution disease variants, 431 entries are located in rRNA or tRNA genes, and 481 are located in coding regions or the noncoding (D-loop) control region. These include 52 rRNA/tRNA and 43 coding/D-loop variants that have been confirmed as pathogenic (on March 2021). It is worth mentioning that most of the variants seem to have no effect and have been widely used as haplotype markers in evolutionary anthropology and population history, genetic genealogy, and forensic science in addition to medical genetics. Moreover, a group of phenotypes known as mtDNA maintenance defects or mitochondrial depletion syndromes must be noted; these are characterized by mtDNA depletion and/or the presence of multiple mtDNA deletions, resulting in inadequate energy production. These mtDNA defects are caused by pathogenic variants located in one of the 20 nuclear-encoded genes that are involved in mtDNA maintenance. The involvement of nuclear gene defects causing mitochondrial depletion syndromes is beyond the scope of this review and is discussed elsewhere. Another aspect that must be considered is that an altered mtDNA CN is associated with mitochondrial function and dysfunction, and consequently, several conditions have been associated with either increases or decreases in the mtDNA content.

MitD refers to a heterogeneous group of phenotypes; the common clinical features include ptosis, external ophthalmoplegia, proximal myopathy and exercise intolerance, cardiomyopathy, sensorineural deafness, optic atrophy, pigmentary retinopathy, and diabetes mellitus. Regarding the central nervous system, phenotypes include fluctuating encephalopathy, seizures, dementia, migraine, stroke-like episodes, ataxia, and spasticity. Some MitD types affect only a single organ, while many others involve multiple organ systems, leading to clinical heterogeneity, a hallmark of MitD. The organs/tissues most often affected in MitD are the brain and skeletal muscle, but the heart, liver, peripheral nerves, gastrointestinal tract and endocrine system can also be involved. Therefore, it is relevant to identify the mtDNA defects present in the brain, their nature and prevalence, and the correlation between the genetic defects and the postmortem neuropathologic features to advance our understanding of the underlying mechanisms of mitochondrial function in disease. Within this systematic review, we aim to summarize the main mtDNA alterations reported in postmortem human brain samples and the related phenotypes.
Methods

Search strategy and selection criteria
We examined PubMed and Embase for English language articles published from inception to June 10, 2021 using two search strings combining the following keywords: postmortem/post-mortem, brain, mitochondrial DNA/mtDNA, mutation, variant, deletion, neuron and glia, according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines. The specific search strings in both databases are shown in Figure 1. We examined the retrieved titles and abstracts and selected empirical studies based on the eligibility criteria. The literature search strategy, data collection, data extraction and appraisal were conducted independently by three authors (AV-P, JT and BKB). When there was no agreement, a fourth author (LM) contributed to gain consensus. The abstraction and summary of the main results of the studies were first performed independently by one of the three main authors and revised by the remaining authors.

Inclusion and exclusion criteria. Studies were included only if they reported results of mtDNA analyses in postmortem human brain tissue and were written in English. The exclusion criteria were as follows: i) studies that did not report the results of mtDNA analyses in postmortem human brain tissue, ii) cell models, iii) animal models; iv) review studies; v) studies focused on brain tumours or cancer, vi) studies that reported duplicated data; or vii) forensic studies. No other restrictions were applied.

Review process
The combined search yielded 657 potentially eligible studies. Abstracts or full articles (if the eligibility criteria were not clearly stated in the abstract) were screened to decipher eligibility. Figure 1 shows the PRISMA flow chart depicting the specific information at the different stages of the systematic review, and Supplementary Table 3 provides the PRISMA checklist of items to include when reporting a systematic review. We discarded 479 reports, and a detailed examination was performed on the 158 remaining records and on others obtained from hand-searching references.

Data extraction
From eligible articles, we recorded the name of the first author, PMID number, publication year, number of patients and controls analysed, age, sex, disease or condition, brain region, technique used, main results

Figure 1. PRISMA flow diagram for selecting published articles for review. The two search strategies used, the number of articles with PMID numbers obtained for each of them, and the result of combining them are shown. Those that met the inclusion criteria and those that were excluded and the groups to which they were assigned are indicated. The final number of articles included in each group after manual inclusion of references is indicated in brackets.

Search string Embase
[mutation:ab,t] OR variant:ab,t OR deletion*:ab,t) AND (noncoding DNA:ab,t OR mtDNA:ab,t OR mRNA:ab,t) AND (brain:ab,t OR neuron:ab,t OR glia:ab,t OR postmortem:ab,t OR postmortem:ab,t OR [article in press]/im OR [conference paper]/im OR [data paper]/im OR [letter]/im OR [short survey]/im) AND (english/im AND [humans]/im)

Search string PubMed
[“mutation”[title/abstract]] OR [“variant”[title/abstract]] OR [“deletion”[title/abstract]] AND [“mtDNA”[title/abstract]] AND [“brain”[title/abstract]] OR [“neuron”[title/abstract]] OR [“glia”[title/abstract]] OR [“postmortem”[title/abstract]] OR (“postmortem”[title/abstract]) NOT [“review”[Publication type]]

Filters applied: humans, English

158 entries fulfilled inclusion criteria
48 entries → T1, MIDD
55 entries → T2, NeuO
15 entries → T3, PsyD
5 entries → T4, Miscellaneous
20 entries → T5, Aging
5 entries → Technical issues
1 entry → T2 & T3
8 entries → T2 & T5
1 entry → T4 & T6

497 entries fulfilled exclusion criteria
358 reports → No analysis of mtDNA in postmortem human brain
22 reports → Cell models
43 reports → Animal models/studies
40 reports → Review studies
11 reports → Tumor/cancer studies
2 reports → Duplicated data/studies
3 reports → Forensic studies
Regarding mtDNA variants, mtDNA CN and/or mtDNA rearrangements reported, and additional information.

Pathogenicity assignment of mtDNA variants
Pathogenicity status was collected based on the information present in the Mitomap and ClinVar databases when available.

Role of the funding source
The funders had no role in the study design, in the collection and interpretation of data, in the report writing, or in the decision to submit the manuscript for publication.

Results

Characteristics of the examined studies
The manuscripts fulfilling the inclusion criteria could be ascribed to the following 6 categories: 1) mitochondrial diseases (MitD), 2) neurological diseases (NeuD), 3) psychiatric diseases (PsyD), 4) other clinical conditions included in a miscellaneous group, 5) ageing, and 6) technical issues. Some reports could be ascribed to two groups, as they presented results from more than one category, and others were manually included after reading specific references of the included studies. A summary of the relevant data, number of evaluated subjects, age, sex, brain region/s studied, techniques used, and mtDNA alterations reported are presented in Tables 1–5, while the technical issues are summarized at the end of this section. We screened the issues for putative pathogenic characteristics, and a selection of presumably pathogenic mtDNA variants is shown in Table 6, with pathogenicity information obtained from public databases.

mtDNA analysis in MitD
We included 50 reports referring to MitD (Table 1). Figure 2 shows the variants reported in a varied number of phenotypes, with the most reported being MELAS (13 reports); MERRF (8 reports); LS (7 reports); KSS (5 reports); mitochondrial encephalomyopathy (ME) (6 reports) and PEO (4 reports); in addition to optic neuropathy, sensoryneuronal hearing loss and diabetes mellitus type I; Leber hereditary optic neuropathy (LHON); early-onset cataracts, ataxia and progressive paraparesis; mitochondrial depletion syndrome; neuropathy, ataxia, retinitis pigmentosa and maternally inherited LS; sideroblastic anaemia; and Alpers-Huttenlocher syndrome (AHS). Among the 50 reports, eight reported variants in the nDNA that ultimately produced mtDNA alterations. Most of the studies were case reports; only 11 were carried out after 2010, and only one study analysed the three different types of mtDNA alterations: pSNV, mtDNA CN variations, and deletions. Seven studies analysed two types of alterations, and 41 investigated just one, mostly pSNV.

mtDNA analyses in MELAS. The m.3243A>G variant in the MT-TL1 gene coding for tRNA-Leu is the most reported variant in postmortem brain samples of patients with MELAS,54–64 although one study also identified the presence of m.13513G>A, p. Asp393Asn, located in the MT-ND5 gene.65 All the studies except one66 reported mutation loads greater than 70% in the brain and similar mutation loads in other evaluated tissues.54,55,57,59,61,66 However, some discrepancies were also observed—while some studies reported that mutation loads did not vary between brain regions,55 others reported high mutation load variability between different cells of the same region.67 None of the studies on MELAS analysed mtDNA CN or the presence of mtDNA deletions in the brain. The most frequent m.3243A>G variant associated with MELAS was also present in the brain of a 4.5-year-old child with a lethal MitD, with a Barth syndrome-like presentation. This child showed the m.3243A>G variant in all of the analysed tissues, including blood, skeletal muscle, cardiac muscle, and liver. Additionally, in the peripheral blood mtDNA of the mother, as well as in four of the 5 siblings, heteroplasmy percentages were not reported.67 m.3243A>G was also detected in a patient with optic neuropathy, sensorineuronal hearing loss and diabetes mellitus type 1, but not MELAS, with a mutation load greater than 75% in white and grey matter, putamen, caudate, pons, visual cortex, among other brain areas and 60% in the biceps muscle.68

mtDNA analyses in MERRF. The m.8344A>G variant in the MT-TK gene coding for tRNA-Lys was reported in all eight studies evaluating postmortem brain samples of patients with MERRF.52,56,60,69–74 Moreover, one of these studies also reported m.8603T>C, p.Phe26Ser, in the MT-ATP6 gene and m.3257A>G in MT-TL1.72 Interestingly, two distinct reports from 1995 and 2010 using distinct molecular techniques that respectively evaluated an 18- and a 16-year-old patient with MERRF syndrome found similar percentages of the m.8344A>G variant that ranged between 93% and 97% across different brain regions. Both patients also showed similar heteroplasmy percentages in other tissues.59,77 Notably, one of the studies reporting the m.8344A>G variant also described a 3.7-fold increased mtDNA CN in brain-affected tissues compared to non-affected tissues.69

mtDNA analyses in LS. Nine studies reported postmortem brain mtDNA data in LS.55,70–82 The m.8993T>G, p.Leu156Arg in the MT-ATP6 gene was reported in four LS studies with mutation load percentages in the brain
| Study reference (PMID) | N | Patient/control characteristics | Brain region | Technique | mtDNA alteration in brain | Rearrangements | Additional information |
|------------------------|---|---------------------------------|--------------|-----------|--------------------------|---------------|------------------------|
| Laine-Menéndez et al. 2021 (34070501) | 1/1 | 43/37 F/F | WM (702, c.323C>T, p. Thr108Met) | qPCR | NR | No differences in mtDNA CN between P and C in brain tissue | Low mtDNA content was observed in skeletal muscle, liver, kidney, small intestine, and particularly in the diaphragm. Heart and brain tissue did not show differences. mtDNA deletions were observed in skeletal muscle and diaphragm |
| Scholle et al. 2020 (32085658) | 1 | 46 F | Optic neuropathy, sensorineural hearing loss and diabetes mellitus type I. Not MELAS | RFLP, qPCR | m.3243A>G, MT-TL1 | Higher heteroplasmy levels significantly correlated with lower mtDNA CN | 60% level of heteroplasmy in the biceps brachii muscle |
| Geffroy et al. 2018 (260944073) | 1 | 32 F | MELAS | Fluorescence RFLP | m.3243A>G, MT-TL1 | Mutation load: 92.1% | - |
| Gramegna et al. 2018 (29348134) | 1/3 | 36/1 Age-matched 1/NA | MNGIE (7188C>G, c.457G>A, p. G153S) | FCtx, SN | NA | mtDNA depletion in frontal GM and WM, and SN | Severe mtDNA depletion in the P, also in smooth muscle and endothelial cells |
| Lax et al. 2016 (25786813) | 5 | 45 4M, 6F | MELAS, MERRF | Pyrosequencing | MELAS, m.3243A>G, MT-TL1, MERRF, m.8344A>G, MT-TK | Mutation load range (%): MELAS 79–94; MERRF 87–93 | Mutation load did not vary between brain regions |
| Tzoulis et al. 2014 (24841123) | 8/15 | Infantile and adult age-matched C | ME (POLG) | qPCR, Long-range PCR, NGS | In homogenate tissue, apparent 20–30% mtDNA depletion was present in infantile P. Microdissected SN neurons showed a 40% lower mtDNA CN | Neurons of both P and C harbored mtDNA DELs in the homogenate. In microdissected neurons mtDNA DELs were more prominent in P with longer disease duration | mtDNA analyses conducted in laser microdissected neurons and homogenate tissue |

Table 1 (Continued)
| Study reference          | Patient/control characteristics | Disease (nuclear gene) | Brain region | Technique                           | mtDNA alteration in brain region | mtDNA CN | Rearrangements | Additional information |
|-------------------------|---------------------------------|------------------------|--------------|-------------------------------------|---------------------------------|----------|----------------|-----------------------|
| Giordano et al. 2014    | 4/8                             | NA                    | LHON         | Optic nerve                         | NA                              | NR       | NR             | Unaffected mutation carriers showed higher mitochondrial DNA CN than their affected relatives and C |
| (24369379)              |                                 |                        |              |                                     |                                 |          |                |                       |
| Lax et al. 2013         | 1/3                             | NA                    | ME (POLG)    | Mesencephalon pons, Th, Str         | NR                              | NR       | NR             |                       |
| (23625061)              |                                 |                        |              |                                     |                                 |          |                |                       |
| Tzoulis et al. 2013     | 2/4                             | NA                    | POLG         | Long-range PCR, nested PCR, qPCR    | NR                              | NR       | NR             |                       |
| Lax et al. 2012         | 3/1                             | M/–                   | KSS and ME   | Long-range PCR, sequencing, qPCR    | NR                              | NR       | NR             |                       |
| (22491194)              |                                 |                        | POLG, p. A467T, p. X1240Q, POLG, p. G8485, p. W7485 |                                 |                                 |          |                |                       |
| Lax et al. 2012         | 14/–                            | 42/–                  | MELAS (7), MERRF (1), MELASS (1), KSS (1), Ataxia-reti-nopath, arPED (3) | qPCR, pyrosequencing            | m.3243A>C; G, m.8344A>G; m.14709T>C, m.13094T>C | NR       | Single and multiple large-scale mtDNA DELs. Mutation load %: Mcer 44, Cbcx 25 – 44 | Neuronal cell loss occurred independently of the level of mutated mtDNA present within surviving neurons |
| (22249460)              |                                 |                        |              |                                     |                                 |          |                |                       |
| Brinckmann et al. 2010  | 1/4                             | F/NA                  | MERRF        | qPCR, pyrosequencing                | m.11657_15636del (3978 bp) and hetro-plasmoy levels in WM were higher than the 60% threshold, thus considered pathogenic. | NR       |                |                       |
| (20975001)              |                                 |                        |              |                                     |                                 |          |                |                       |

Table 1 (Continued)
| Study reference (PMID) | Patient/control characteristics | Disease (nuclear gene) | Brain region | mtDNA alteration in brain | Rearrangements | Additional information |
|------------------------|---------------------------------|------------------------|--------------|--------------------------|----------------|------------------------|
| Sanaker et al. 2010 (19744136) | 1 64 M | mtDNA alteration in brain | | | | |
| Zsurka et al. 2008 (18716558) | 1/30 17/28 M/NA | mtDNA alteration in brain | | | | |
| Götz et al. 2008 (18819985) | 2/2 NA | mtDNA alteration in brain | | | | |
| Rojo et al. 2006 (16525806) | 1 64/- M | mtDNA alteration in brain | | | | |
| Betts et al. 2006 (16866982) | 2 47 F | mtDNA alteration in brain | | | | |

**Table 1 (Continued)**
| Study reference (PMID) | Patient/control characteristics | Disease (nuclear gene) | Brain region | Technique | mtDNA alteration in brain | mtDNA CN | Rearrangements | Additional information |
|------------------------|---------------------------------|-----------------------|--------------|-----------|--------------------------|---------|----------------|------------------------|
| Matthes et al. 2006 (16856911) | 1/2 19/age-matched M/- | AHS (POLG1, c.1399G>A, p.A467T) | NA | Long-range PCR, sequencing, qPCR | NR | NR | Presence of the m.5853_9468del (3614 bp). Mutation load (%): 70 | - |
| Ferrari et al. 2005 (15689359) | 1/2 19/age-matched M/- | AHS (POLG1, c.1399G>A, p.A467T) | NA | NA | NR | 30% mtDNA content reduction | NR | - |
| Pistilli et al. 2003 (14608542) | 1 36/- | F | KSS | PCR-RFLP with radiolabeled nucleotides | NR | NR | Presence of the m.8631_13580del (4949 bp). DEL load (%): FCtx 54; Octx 54; TCtx 58; PCtx 59; basal ganglia 75; Cb 88 | - |
| Uusimaa et al. 2003 (12612282) | 1 7/- | F | AHS-like disease | CSGE + sequencing, PCR-RFLP using radiolabeled nucleotides | m.7706G>A, p. Ala41Thr, MT-CO2. Mutation load: 91% | NR | NR | Mutation load (%): blood 87, heart 87 | - |
| Kirby et al. 2003 (14520659) | 3 31 | 2M, 1F | LS | Medulla oblongata, pons, Cb, basal ganglia, brain stem | PCR-RFLP with radiolabeled nucleotides | m.13513G>A, p. Asp393Asn, MT-ND5 Mutation load (%): 73 | NR | - |
| Jiang et al. 2002 (12174968) | 1 - | - | LS | NA | NA | m.8993T>G, Leu156Arg, MT-ATP6 Mutation load (%): 89 | NR | NR | Mutation load (%): muscles 85, lymphocytes 72 | - |
| De Kremer et al. 2001 (11341464) | 1/20 4/NA | 1/NA | Barth syndrome-like disorder | PCR-RFLP sequencing | m.3243A>G, MT-TL1. The mutation was heteroplasmic in brain but not quantified | NR | NR | The mutation was also heteroplasmic in all the tissues analyzed: blood, skeletal muscle, cardiac muscle, and liver | - |
| - | 1 61 | F | PEO | Long-range PCR, Southern blot | NR | NR | Multiple DELs (25), most of them were less than | - |

Table 1 (Continued)
| Study reference (PMID)                  | Patient/control characteristics | Disease (nuclear gene) | Brain region | Technique | mtDNA alteration in brain | mtDNA CN | Rearrangements | Additional information |
|---------------------------------------|----------------------------------|------------------------|--------------|-----------|---------------------------|----------|----------------|------------------------|
| Moslemi et al. 1999 (10408540)        | N/P/C: 1/43 F                    | WM, FCtx, Th, Pu, Cd, SN, CbCtx | PCR-RFLP     | m.8993T>G, p. Leu156Arg, MT-ATP6 | Mutation load (%): 95% | NR | 8 kb. The CbCtx showed the lowest mutation load %. The common DEL was found in all the specimens | skeletal muscle samples and myocardium |
| Nagaschima et al. 1999 (10208283)     | 1/43 F                           | PCR-RFLP               | m.8344A>G, MT-TK | Mutation load (%): 92% | MT-TK: 84% | NR | Mutation load (%): liver 98, muscle 90, heart 91, kidney 99, pancreas 93 | - |
| Santorelli et al. 1998 (9851442)      | 1/12 F                           | Sequencing with radiolabeled nucleotides | PCR-RFLP     | m.3243A>G, MT-TL1 | Mutation load: 84% | NR | Mutation load (%): liver 79, kidney 86, skeletal muscle 83, cardiac muscle 83 | Analyses of homogenate issue and individual neurons |
| Di Trapani et al. 1997 (9266144)      | 1/27/- M                         | PCR-RFLP               | m.3243A>G, MT-TL1 | Mutation load (%): between 81 and 98. Similar % in soma, neuropil, glia and homogenate tissue. | NR | NR | - | - |
| Zhou et al. 1997 (9315896)            | 1/14 F                           | PCR-RFLP               | m.3243A>G, MT-TL1 | Mutation load: 84% | MT-TL: 84% | NR | Mutation load (%): skeletal muscle 80, kidney 10, liver 10 | - |
| Suomalainen et al. 1997 (9153451)     | 2/67 F                           | Southern blot           | m.3243A>G, MT-TL1 | Mutation load: 84% | MT-TL: 84% | NR | Mutation load (%): muscle 84 | - |
| Santorelli et al. 1997a (9299505)     | 1/45 M                           | PCR-RFLP               | m.3243A>G, MT-TL1 | Mutation load: 84% | MT-TL: 84% | NR | Mutation load (%): muscle 84 | - |
| Santorelli et al. 1997b (9266739)     | 1/14 M                           | Southern blot           | m.3243A>G, MT-TL1 | Mutation load: 84% | MT-TL: 84% | NR | Mutation load (%): muscle 84 | - |
| Kado et al. 1996 (8870835)            | 1/53/- M                         | PCR-RFLP               | m.3243A>G, MT-TL1 | Mutation load: 84% | MT-TL: 84% | NR | - | - |
| Zhou et al. 1997 (9315896)            | 1/14/- F                         | PCR-RFLP               | m.3243A>G, MT-TL1 | Mutation load: 84% | MT-TL: 84% | NR | - | - |

*Table 1 (Continued)*
| Study reference (PMID) | Patient/control characteristics | Disease (nuclear gene) | Brain region | Technique | mtDNA alteration in brain | mtDNA CN | Rearrangements | Additional information |
|------------------------|---------------------------------|------------------------|--------------|-----------|--------------------------|-----------|-----------------|------------------------|
| Sanger et al. 1996 (8652018) | 1 20 M | MELAS-like syndrome | FCtx | PCR-RFLP using radiolabeled-nucleotides | CbCtx 97, FCtx 88, Cd 88, Pu 88 | No presence of these two variants | NR | NR | 86 – 93, spinal cord 83, medulla 87 - |
| Melberg et al. 1996 (8937533) | 1 14 F | MM, lactic acidosis and complex I deficiency | NA | Southern blot for variants m.3243A>G and m.8344A>G | m.3251A>G, MT-TL. Mutation load (%): 90 | NR | NR | Mutation load (%): muscle 94, fibroblast 93, heart 79, liver 80 |
| Houshmand et al. 1996 (8786060) | 1 18/- M | MERRF | FCtx, PCtx, TCtx, OCtx, frontal WM, Pu, pallidum, Th, pons, ID, CbCtx, Dt | PCR-RFLP using radiolabeled-primer | m.8344A>G, MT-TK. Mutation load (%): FCtx 95, PCtx 95, TCtx 95, OCtx 95, Pu 96, pallidum 96, Th 94, pons 94, ID 94, low Brain stem 94, CbCtx 95, Dt 93 | NR | NR | skeletal muscle 97, myocardium 97, aorta 97, subcutaneous adipose tissue 95, liver 95, pancreas 91, spleen 95, lymph node 93, bone marrow 94, testis 99, adrenal gland 97, thyroid gland 98 |
| Oldfors et al. 1995 (8525809) | 1 53/- M | ME | Ctx, Cb | Southern blot | m.5549G>A, MT-7W | Mutation load (%): Ctx 87, Cb 88 | NR | NR | Mutation load (%): blood 40, muscle 83 – 86, myocardium 93, kidney 93, lung 79, liver 77, optic nerve 51 |
| Oldfors et al. 1995 (8525809) | 1 14 F | MELAS | PCR-RFLP | NR | Presence of the common DEL. DEL load (%): TCtx 62, OCtx 70, Cb 17 | NR | NR | Mutation load (%): quadriceps 91, psoas 72, diaphragm 75, cardiac muscle 25, kidney 21, liver 84, lung 18, spleen 0, testis 0, blood 0 |
| Sweeney et al. 1994 (8133313) | 1 18 M | LS | Cb, CbCtx | PCR | m.8993T>G, p. Leu156Arg, MT-ATP6 | Mutation load (%): Cb 97, CbCtx 97 | NR | NR | Mutation load (%): blood 81, quadriceps 99, extraocular muscle 97, cardiac muscle 97, liver 99, kidney 98, blood 72 |

Table 1 (Continued)
| Study reference (PMID) | Patient/control characteristics | Disease (nuclear gene) | Brain region | Technique | mtDNA alteration in brain | mtDNA CN | Rearrangements | Additional information |
|------------------------|---------------------------------|------------------------|--------------|-----------|---------------------------|----------|----------------|-----------------------|
| MacMillan et al. 1993 (8351017) | | | PCtx GM, Octx GM, PCtx WM, Cd, Cb, pons, pons | | m.3243A>G, MT-FL | | | Mutation load (%): psoas muscle 82, oculomotor muscle 59, cardiac asarium 86, myocardium 67, oesophagus 84 |
| Love et al. 1993 (8326463) | 2 23, 16 F | MELAS | FCtx, TCtx, pons, PCtx, OCTx | PCR-RFLP, sequencing with radiola-beled primers | Case 1: m.3243A>G, MT-FL1. Mutation load (%): FCtx 46, TCtx 40, PCtx 33, pons 30. Not detected in OCTx. Case 2: neither m.3243A>G nor m.3271T>C, MT-FL1, was present. 8344A>G, MT-FL1. Mutation load (%): FCtx 97, TCtx 98, OCTx 97 | NR | NR | |
| Tanno et al. 1993 (8170566) | 2 29, 30 M, F | MERRF | FCtx, TCtx, CBCTX | PCR-RFLP | | | | Mutation load (%): heart 96, kidney 96, adrenal gland 93, liver 94, muscle 96–99, leukocytes 93 |
| Shiraiwa et al. 1993 (8138807) | 1 27 F | MELAS | FCtx, TCtx, PCtx, OCTx, Cd, Pu, pallidum, Th, frontal WM, Pit, CBCTX, DT | PCR | m.3243A>G, MT-FL1. Mutation load (%): Pit 95, FCtx, TCtx, PCtx and OCTx 85–88, Cd, Pu, pallidum and WM 73–79 | NR | NR | |
| Tatuch et al. 1992 (1550128) | 1 0.6 F | LS | NA | Southern blot PCR-RFLP | m.8993T>G, p. Leu156Arg, MT-ATP6 | NR | NR | Mutation load (%): >95 in fibroblasts, kidney and liver |
| Suomalainen et al. 1992 (1634620) | 1 60 F | PEO and MIDD | FCtx, basal ganglia | Southern blot PCR | | | | Presence of several DELs. Most of the DEL breakpoints were between ~m.11,900 and m.12,600. DEL sizes between ~2.0 to 10 kb |
| Lombes et al. 1991 (1849240) | 3 5, 2, 0.5 M | LS and COX deficiency | NA | Southern blot Northern blot | | | | No mtDNA DEL detected |

Table 1 (Continued)
| Study reference (PMID) | N | Age (y) | Sex | Disease (nuclear gene) | Brain region | Technique | mtDNA alteration in brain | mtDNA CN | Rearrangements | Additional information |
|-----------------------|---|---------|-----|------------------------|-------------|-----------|--------------------------|----------|----------------|-----------------------|
| Ciafoloni et al. 1991 (1922812) | 1 | 26 | M | MELAS | NA | Southern blot | PCR-RFLP | m.3243A>G, MT-TL1 | Mutation load (%): 84 | NR | NR | nDNA encoded subunits |
| Enter et al. 1991 (1684568) | 1 | 12 | F | MELAS | NA | Southern blot | PCR-RFLP | m.3243A>G, MT-TL1 | Mutation load (%): 80 | NR | NR | Mutation load (%): |
| Bordarier et al. 1990 (2359483) | 1 | 17 | F | KSS | NA | Southern blot | NR | NR | DEL located between positions ~ m.8,200 and m.13,000 (±400 bps) | The DEL was also found in muscle and spinal cord |

Table 1: Studies reporting the results of mtDNA analyses in postmortem brain samples of patients with mitochondrial diseases (MitD).

C: control; DEL: deletion; F: female; GM: gray matter; M: male; mtDNA CN: mitochondrial DNA copy number; N: number of subjects; NA: information not available; NR: not reported; P: patient; y: years; WM: white matter.
### Table 2 (Continued)

| Study reference (PMID) | Patient/control characteristics | Disease (N) | Brain region | Technique | mDNA alteration | Additional findings |
|------------------------|---------------------------------|-------------|--------------|------------|----------------|---------------------|
| Castora et al. 2020 (31951357) | 40/40 NA NA AD (40) P/PC PCr, T0x, Gt, H | 6 AD P showed the m.9861T>C variant compared to a normal C mutation load % between 11 and 95 | PCR/RELP | NR | NR | m.9861T>C was found in multiple samples, with the highest mutation load % occurring in PCs and TCtx |
| Chen et al. 2020 (31689514) | 20/15 49-81/75 21/4 AD (12) PD (8) C (5) SN qPCR | In AD P showed the m.9861T>C variant compared to none in C. Mutation load %: between 11 and 95 | PCR/RELP | NR | NR | - |
| Kim et al. 2020 (32005289) | 34/25 NA NA ALS (34) AD (10) C (5) Ox Aldehyde reactive probe-based assay, ELISA | NT NR NR Apurinic/apyrimidinic sites (abasic sites) did not differ between ALS and C. Levels of oxidized mDNA did not differ between ALS and C. mDNA analyses were conducted in laser-microdissected neurons | PCR/RELP | NR | NR | Apurinic/apyrimidinic sites (abasic sites) did not differ between ALS and C. Levels of oxidized mDNA did not differ between ALS and C. mDNA analyses were conducted in laser-microdissected neurons |
| Thibron et al. 2019 (31388037) | 44/30 76/77 36/38 AD (34) PD (10) NO (30) PCr, T0x, Cb | In healthy aged TH-positive neurons of C, despite showing similar DEL levels as P with PD, mDNA ON was significantly lower in neurons of P with NO showing mDNA point mutations or multiple deletions, and in P with PD. Data showed evidence of mDNA depletion in PD neurons, with large individual diversity in cases. | PCR/RELP | NR | NR | - |
| Alvare-Mera et al. 2019 (30801640) | 2/3 93,97/73-83 NA FIX1 (3) FIX2 (3) Ver, P0x, T0x, Gt, H | mDNA ON in Ver, P0x and T0x was decreased in FMR1 mutation carriers with FXTAS than in C but no differences were detected in Th, Gt and H. P with FXTAS showed lower mDNA ON than C. No mDNA ON depletion observed in T0x of AD P vs. HP0x or C. No mDNA ON depletion in Cb between all three groups | PCR/RELP | NR | NR | - |
| Soltys et al. 2019 (30359878) | 10/20 82/80 11/19 AD (10) Hpc (10) C (9) Ob, T0x | mDNA ON depletion observed in T0x of AD P vs. HP0x or C. No mDNA ON depletion in Cb between all three groups | PCR/RELP | NR | NR | - |
| Strobel et al. 2019 (30475765) | 22/10 75/70 16/16 AD (22) C (10) H, Cb | mDNA ON depletion observed in astrocytes and microglia of the H compared to brain stem and Cb | PCR/RELP | NR | NR | - |
| Reference | Study Design | Study Population | Brain Regions | Technique | mtDNA Alteration | Additional Findings |
|-----------|--------------|------------------|---------------|-----------|------------------|---------------------|
| Bury et al. 2017 (29149768) | PD (6) C (6) | PD from PPN | qPCR | mtDNA CN | Significant mtDNA CN increase in PPN cholinergic neurons from PD P (10.7%) vs. those from C (7.0%) | mtDNA DEL levels were significantly increased in PPN cholinergic neurons from PD P (21.6%) vs. those from C (17.2%). mtDNA DELs correlated with Braak staging in PD. |
| Wei et al. 2017 (28153046) | AD (282) CJD (181) DLB-PD (89) FTD-ALS (236) Other (114) | frontal cortex, caudate, other | exome sequencing | No evidence of disease association with homoplasmic or heteroplasmic rare variants | mtDNA CN was significantly lower in AD and CJD P. Positive correlation between age and mtDNA CN in CJD P. NR No correlation between mean levels of heteroplasmy, total number of heteroplasmic variants or variant pathogenicity score with age or disease group. |
| Nido et al. 2018 (29257976) | PD (2) SN | Sequence heteroplasmy | NGS | mtDNA CN was significantly different between deleted and nondeleted mtDNA populations for 4/55 of the DELs. 11.3% of the analysed positions had a heteroplasmic frequency significantly different between deleted and nondeleted mtDNA populations. | 29 single laser-microdissected neurons showed 373 unique DELs (only 31 previously annotated). Each neuron contained on average 38.2 ± 29.8 distinct DELs. Mean size deletion was 5080 ± 2367 bp. The common deletion was detected in 15/17 neurons and was the most prevalent deletion in 6 neurons. |
| Flones et al. 2017 (29270838) | PD (18) C (11) | F, P, Cb, Ctx, Hi, Pu | qPCR | NR | There was no significant difference between C and PD formtDNA CN in Pu or Hi. Only dopaminergic neurons from the SN harboured significantly higher mtDNA DEL levels. 1189 single laser-microdissected neurons were evaluated. Neuronal complex I deficiency did not correlate with mtDNA damage. |
| Dölle et al. 2016 (27874000) | PD (10) C (22) | SN, FCtx, Cb | qPCR, NGS-(4,767 bp) | Mean load of heteroplasmic SNVs was 33.14%/1,000bp per neuron, and most of these clustered in the low-frequency spectrum in both PD and C. The overall burden of heteroplasmic SNVs was similar in PD and C. The proportion of G:C to T:A transversions was also similar in the two groups. mtDNA CN was similar in PD and C in SN; however, the subset of neurons with high mtDNA depletion (<10,000 copies/cell) was 14% in PD and 2.7% in C (p=0.01). No differences were observed in FCtx or Cb. SN neurons from PD contained significantly higher mtDNA DEL levels than C. The proportion of neurons with DEL levels exceeding 60% was 21.4% in PD and 10.8% in C. mtDNA DEL levels were generally low in frontal neurons and Cb of both PD and C. 871 single laser-microdissected neurons were evaluated. In SN but not in FCtx or Cb, DELs and mtDNA CN showed a positive correlation, with DEL being a predictor of mtDNA CN. |
| Chen et al. 2016 (27299301) | AD (13) C (12) | FCtx | NGS, qPCR | NR | Similar heteroplasmy levels were observed in some mtDNA positions in AD P and C. DELs were increased in AD P (9%) vs. C (2%). Rearrangement rate was higher in AD P (18%) than in C (7%). The common deletion was detected in most samples but at low % (1.5%). Different numbers and types of mtDNA rearrangement fragments were detected depending on the sequencing coverage depth. |

Table 2 (Continued)
| Study reference | Patient/control characteristics | Disease (N) | Brain region | mtDNA alteration | mtDNA CN | Rearrangements | Additional findings |
|-----------------|--------------------------------|-------------|--------------|-----------------|----------|---------------|-------------------|
| (PMID)          |                                |             |              |                 |          |               |                   |
| Gatt et al. 2016 (26853899) | 26/18 | PD (41) | Ent, SN | PD2, qPCR | NR | NR | Non-significant decrease was observed in PD. |
| Blanch et al. 2016 (26776077) | 26/18 | PD (10) | SN, F0x | qPCR | NR | NR | Increased 5-methylcytosine levels observed in the D-loop in Ent of PD P vs. C. |
| Coshed et al. 2016 (26639157) | 180/40 | AD (10) | NGS | NR | NR | NR | No significant correlation of heteroplasmy with age. |
| Granwech et al. 2016 (26505748) | 10/10 | PD (10) | SN, F0x | qPCR | NR | NR | Increased heteroplasmic variation was observed in COX genes. |
| Rice et al. 2014 (24448779) | 10/9 | HI (6 regions) | Pyrosequencing | NR | NR | HI | HI contained more heteroplasmic variants than blood. |
| Muller et al. 2013 (23566333) | 14/14 | NA | SN, HI | qPCR | NR | NR | 2–6 single laser-microdissected neurons were evaluated per patient/control. |
| Krishnan et al. 2012 (21925769) | 1/0 | NA | HI (6 regions) | qPCR, Long-range PCR + sequencing | NR | NR | mtDNA DEL levels were higher in COX-deficient neurons (57%) than in COX-normal neurons (9%) in AD P and in C (48% and 24%, respectively). No differences were observed in COX-deficient neurons. |

**Table 2 (Continued)**
| Study reference (PMID) | Patient/control characteristics | Disease (N) | Brain region | Technique | mtDNA alteration | Additional findings |
|------------------------|---------------------------------|-------------|--------------|-----------|----------------|---------------------|
| Keeney et al. 2010 (20540367) | 10/7 NA NA ALS (10) AD (11) C (6) | Neurons from AD P and C. mtDNA DELs ranged in size from 3670 to 6088 bp. mtDNA DELs accumulated with age. | Claims, Ctx qPCR NR | There were no differences in ND2 CN between P and C. | Single laser-microdissected neurons from cervical spinal cord, anterior gray matter and Cb were evaluated. |
| Naydenov et al. 2010 (20740286) | 32/31 75/71 NA PD (32) Pu, Cb | Dopamine led to a 25% downregulation of mtDNA levels and L-Dopa caused an increase in mtDNA levels. In dyskinetic-PD, lower mtDNA levels in early disease processes were found. Pu showed a 50% reduction and a significant negative correlation with L-DOPA/year. Pu from dyskinetic-PD showed a higher % of deletions and a correlation with mtDNA levels (mtDNA deletions in PD and dyskinetic-PD). | qPCR NR | Dopamine led to a 25% downregulation of mtDNA levels and L-Dopa caused an increase in mtDNA levels. | Dopamine led to a 25% downregulation of mtDNA levels and L-Dopa caused an increase in mtDNA levels. In dyskinetic-PD, lower mtDNA levels in early disease processes were found. Pu showed a 50% reduction and a significant negative correlation with L-DOPA/year. Pu from dyskinetic-PD showed a higher % of deletions and a correlation with mtDNA levels (mtDNA deletions in PD and dyskinetic-PD). |
| Gordon et al. 2010 (20463402) | 38/25 NA NA AD (13) DS (11) DSAD (14) C (25) | The frequency of mtDNA control region mutations was significantly higher in AD than in C. m.414T > G was found in 65% of AD but not in C. Additionally, m.309delC, m.309insC, m.408T > C, m.414T > C, m.418C > T, m.466.2insCC. | PCR-Cox PNA-sampling PCR, qPCR, sequencing (1,15 bp) | There were no differences in ND2 CN between P and C. | AD-like neuropathology was present in AD and DS/AD but not in DS. The frequency of somatic mutations in the regulatory control region increased with age in the normal brain (p=0.0029). The MT-NO2 to MT-ND2 transcript ratio did not change with age but was significantly lower in AD, DSAD and DS compared to C. Thus, reduced mtDNA L-strand transcription level was associated with intellectual disability and dementia. |
| 13/10 55/74 NA NA MS (13) C (0) | Ox | Dels were evident in 66% of normal appearing gray matter regions and in 53% of lesions regions of MS.P and in 11% of C. Multiple Dels were observed in respiratory-deficient laser | Long-range PCR, qPCR, sequencing NR | Dels were evident in 66% of normal appearing gray matter regions and in 53% of lesions regions of MS-P and in 11% of C. Multiple Dels were observed in respiratory-deficient laser. | Single laser-microdissected neurons from cervical spinal cord, anterior gray matter and Cb were evaluated. |

Table 2 (Continued)
| Study reference (PMID) | Patient/control characteristics | Disease (N) | Brain region | Technique | mtDNA alteration | Additional findings |
|-----------------------|--------------------------------|-------------|--------------|------------|-----------------|---------------------|
| **Patient/control characteristics** | **N** | **P/C** | **Age (y) in P/C** | **Sex (M/F)** | **Variant** | **mtDNA CN** | **Rearrangements** |
| Arthur et al. 2009 (19775436) | 8/10 | 70/67 | 12/6 | spD (6) C (10) | FGx and SN | Surveyor nuclease assay, qPCR | No differences in mtDNA CN between spD and C | microdissected pooled neurons showed decreased complex IV activity in P compared to C, suggesting mtDNA depletion. Heteroplasmy levels were increased in respiratory-deficient neurons. |
| Alev et al. 2008 (18827923) | NA | NA | NA | AD | Ox, H, Endothelial cells | Cytological in situ hybridization | NR | NR | 5 kb mtDNA DEL was localized in lysosomes of P but not in neurons. |
| Bender et al. 2008 (18604467) | 9/8 | 76/71 | 10/7 | AD (9) C (10) | Pn, F, Ox, SN | qPCR | NR | NR | DEL levels were higher in AD (SN 32%) than in C (13%) and Pn (14%) but did not differ from that in Ox (35%) in neuronal cell compartments. |
| Hakonen et al. 2008 (18775955) | 4/9 | 23/44 | 9/6 | IOSCA (4) C (9) | Ox, Cb | Long-range PCR, qPCR, Southern blot, sequencing | IOSCA P did not show increased mtDNA point mutation load in affected tissues | mtDNA depletion was present in C and Cb of IOSCA P. |
| Reeve et al. 2008 (18179904) | 6/5 | 77/78 | NA | P (1) C (9) | SN | Long-range PCR | NR | NR | Various DELs were found in P and C, there was neither an increase in the distribution nor in the types of DEL break points detected between groups. |
| Blokhin et al. 2008a (18286391) | 5/9 | 38-53/34-80 | 8/6 | MS (5) C (9) | FGx, P, Ox | qPCR | NR | NR | Various single laser-microdissected neurons were evaluated. There was no difference in the rate of mtDNA DELs between MS and C. |
| Blokhin et al. 2008b (18566918) | 5/12 | 38-53/34-80 | 8/6 | MS (5) C (12) | FGx, P, Ox | qPCR | NR | NR | Various single laser-microdissected neurons were evaluated. There was no difference in the rate of mtDNA DELs between MS and C. |

Table 2 (Continued)
| Study reference | Patient/control characteristics | Disease (N) | Brain region | Technique | mtDNA alteration | Rearrangements | Additional findings |
|-----------------|--------------------------------|-------------|--------------|-----------|-----------------|---------------|-------------------|
| Borthwick et al. 2006 (16358336) | 1/0 73 M | MND (1) | FCtx, Pons, spinal cord | Sequencing PCR-RFLP with radiolabelled nucleotides | m.4274T>C in mt-TF: Mutation load % F Ox 4.5%, Pons 3.7% | NR | was higher in COX- than in COX+ mtDNA |
| Bender et al. 2006 (16604074) | 15/8+8 76/77 NA | PD (15) C (8-): Hi, SN | Hi, SN | Long-range qPCR, sequencing | Non-pathogenic: m.18164A>G, m.16184T>C and m.9633T>C variants were detected | NR | In SN, mtDNA DEL levels did not differ between PD (2.2%) and aged C (4.3%), however, they differed in the Hi (17.8% in PD and 14.2% in C); p<0.0001. DEL levels correlated with age |
| Wang et al. 2006 (16405502) | 8/6 90/81 NA | MCI (6) C (6) | F OX, TCO, Pcox, OB | GC/MS-SIMA | NR | NR | Levels of multiple oxidised bases were significantly higher in F OX, TCO and Pcox of AD P than in C. Levels of multiple oxidised bases were significantly higher in F OX, TCO and Pcox of AD P than in C. mtDNA had ~10-fold higher levels of oxidised bases than nDNA |
| Wang et al. 2005 (15857398) | 8/8 85/4 8/8 | AD (6) C (6) | AD (6) C (6) | F OX, TCO, Pcox, OB | GC/MS-SIMA | NR | NR | 5 kb mtDNA DELs were mostly localized in lysosomal structures but not in neuronal cell bodies. There was a 3-4 fold increase of mtDNA DEL in AD compared to C |
| Aliyev et al. 2005 (15760652) | NA NA NA | AD | Hi | Cytological in situ hybridization | NR | NR | S + mtDNA DELs were mostly localized in lysosomal structures but not in neuronal cell bodies. There was a 3-4 fold increase of mtDNA DEL in AD compared to C |
| Coskun et al. 2004 (15247418) | 23/40 NA NA | AD Q (3) C (40) | qPCR | mtDNA control region showed a 63% increase in P with AD compared to C | Frequency of heteroplasmic variants in the mtDNA control region showed a 63% increase in P with AD compared to C (p<0.05). In P 80 years and older this increase was 130%. m.414T>G proved to be specific for AD brains: m.1460T>C, m.195T>C and m.477T>C showed heteroplasmic levels up to 70-80% in P with AD aged between 74 and 83 years | NR | There was a significant 50% mtDNA CN reduction in AD compared to C |
| | 4/4 61/57 3/5 | qPCR | NR | NR | | Paper focused on studying the mtDNA control region. Variants identified in brains of P with AD were preferentially located in known functional transcription and replication elements and were also frequently present at exceptionally high proportions |

Table 2 (Continued)
| Study reference (PMID) | Patient/control characteristics | Disease (N) | Brain region | Technique | mtDNA alteration | Rearrangements | Additional findings |
|------------------------|---------------------------------|-------------|--------------|-----------|-----------------|----------------|-------------------|
| Mawrin et al. 2004 (15036587) | AD (1), PD (1), FTD/AVND (1), DLB (1) C (4) | AD (1) | FCtx, OCtx, TCtx, Hi, SN, Cb, basal ganglia, brain stem | PCR | NR | NR | Levels of the common DEL were markedly raised with increasing age |
| Mawrin et al. 2003 (12924443) | ALS (7), C (3) | AD (6) | Hi, TCtx, Cb, FCtx | In situ hybridization | NR | NR | Single laser-microdissected neurons were evaluated |
| Aliev et al. 2003 (14503022) | ALS (7), C (3) | AD (6) | Hi, Ctx, brain stem | PCR NR NR | Common DEL levels did not differ between ALS and C. Common DEL levels increased with age |
| Gu et al. 2002 (12125742) | PD (6) | PD (8) | SN, Ctx, H, Cb | Long-range PCR + FIGE Southern blot | NR | NR | The number of mtDNA DEL/rearrangements in SN of P with PD was significantly higher than that in other groups. In PD, the number of mtDNA DEL/rearrangements did not differ between other brain regions studied. No significant increase was observed in the total number of DEL/rearrangements in the Hi of AD P. The average number of rearranged forms in patients with PD, AD, MSA, DLB, and C was 1.08, 0.7, 0.48, and 0.06, respectively |
| Zhang et al. 2002 (12039426) | MSA (6) | AD (7), PSP-A (4), AD (7), C (21) | SN, other midbrain regions | PCR-SSCP, sequencing | No presence of the m.414T>G variant. mtDNA variants reported in AD: m.267T>C, m.347G>A, m.380G>A, m.405T>C, m.414T>G. | NR | NR | No further investigation in the pathogenicity of these mutations was made |
| Simon et al. 2001 (1352572) | PD (27), MSA (4), C (44) | AD (7), PD (27), MSA (4), C (44) | FCtx, OCtx, TCtx, FCtx, PCR-RFLP, sequencing | NR | NR | No further investigation in the pathogenicity of these mutations was made |
| Study reference | Patient/control characteristics | Disease (N) | Brain region | Technique | mtDNA alteration | Additional findings |
|----------------|-------------------------------|-------------|--------------|-----------|-----------------|-------------------|
| de la Monte et al. 2000 (10950123) | 37/25 | N/A | AD (37) | T/Ox | m.416T>C, m.456G>T, m.531G>T, m.555A>G, m.564G>T and m.644A>G | NR | mDNA CN levels were significantly lower in ADP than in C although several AD cases showed high levels of mDNA CN |
| Chang et al. 2000 (10873587) | 20/20 | N/A | AD (20) | POx, Hi, Ob | mtDNA CN rearrangements | NR | Common DEL levels of AD did not differ from C. Common DEL frequency was 1.5 to 2.5-fold lower in Ctx than Ctx in both AD and C. Point mutation frequencies did not differ between brain regions |
| Dhaliwal et al. 2000 (10943712) | 6/4 | N/A | ALS (6) | PCR, T/Cx | PCR, RFLP | NR | Common DEL levels were higher in Ctx than in T/Cx in both ALS and C. The relative difference in the two brain regions was >11-fold higher in ALS than in C. The common DEL was also detected in SN and GP |
| Ito et al. 1999 (10561101) | 1/1 | 1/1 | AD (1) | S/L, GP | PCR | NR | Percentage load of the m.3243A>G, MT-TL1 was 0.004% and 0.006% in cybrids obtained from SN and GP, respectively. m.3243A>G, MT-TL1 and the common DEL was not detected in blood |
| Hatanpaa et al. 1998 (9729244) | 5/4 | 83/72 | 2/2 | Northern blot | NR | NR | In blood, point mutation frequencies were not elevated in AD. No correlation was found between age and common DEL frequency in AD and C |
| Haferkamp et al. 1998 (9561330) | 2/0 | 54/0 | 0/2 | PCR, T/Cx | Both patients revealed the same homoplasmic mtDNA variants: m.13708G>A and m.15257G>A. Patient 2 also carried an homoplasmic variant m.15257G>A | NR | - |
| Quesada et al. 1997 (9196054) | 1/1 | 65/65 | 1/1 | Southern blot | NR | NR | Presence of 134 DEL in the P with PD and 98 DEL in the P with ALS. The ALS patient was considered a C individual in this study |
| Hamblin & Castora. 1997 (9357534) | 9/9 | 68/66 | N/A | T/Ox | PCR | NR | Mean % of the Common DEL in AD P and C was 0.09 and 0.08, respectively, a 6-fold significant change |

Table 2 (Continued)
| Study reference (PMID) | Patient/control characteristics | Disease (N) | Brain region | Technique | mtDNA alteration | m/sDNA ON | Rearrangements | Additional findings |
|------------------------|---------------------------------|-------------|--------------|-----------|-------------------|----------|---------------|---------------------|
| Kastel et al. 1997 (9380043) | 4/4 74/73 37/1 PD (6) C (4) | SN, Ctx, Pap/Ctx, Pn, Cb | PCR, PCR-RFLP | m.4336A>G homo-plantic in 1PD
m.5460G>A heteroplasmic (95% mutation load) in 1C
Frequencies of the analysed variants m.3190G>A, m.3397A>G, and m.3627A>G were not detected in P or in C, m.3304A>G was only present in 1.7% of the C, m.5460G>A was present in 31% of P and 24% of C, m.3057C>C was present in 1.5% of AD P but not in C. | NR | DEL levels in CbCtx were 50 to 200-fold lower compared to SN, and 12 to 23-fold greater in Pn than in Cb. |
| Hutchin et al. 1997 (9452573) | 65/76 70/71 NA AD (80) C (76) | NA | PCR-RFLP | NR | NR |
| Jarstby et al. 1996 (9358940) | 48/19 75/71 NA AD (40) C (19) | FOx, Ent, H | Nested allele-specific PCR, sequencing | m.5440G>A, p. Ala131Thr, MTHFD2 was present in 6 AD cases (4 homo- and 2 heteroplasmic, with a mutation load of 9%). Not present in C. | NR | NR |
| Schnepp et al. 1996 (9377822) | 2/2 NA NA PD (2) C (2) | FOx, POx, TCOx, Octx, H, Pn, Th, Cb, CiCOX, CC, SN among others | PCR-RFLP | Ratio of the m.4065G>A, varied between 44% and 98% in the brain regions studied. No differences were obtained when comparing WM and GM, or between PD P and C. | NR | NR |
| Kloot et al. 1996 (8731322) | 45/53 PD (21) C (17) | SN, Ctx, Cb, Pn | PCR-RFLP | m.5440G>A, A heteroplasmic variant was found in 4/21 PD P and in 5/77 C. m.3304A>G homo-plasmic variant was present in 1 PD P | NR | NR |
| Chen et al. 1995 (7599213) | 3/3 27-42/27-42 3/3 HD (3) C (3) | POx, OObx, COx | Competitive PCR | NR | Similar levels of the common DEL in the three regions when comparing HD P and C. Lower levels of the common DEL in Pn and OObx. No correlation between COX activity and the common DEL levels. |
| Cavelier et al. 1995 (8530074) | 33/3 80/72 20/22 AD (33) C (9) | FG, GI, FCox, OCOx, FCox | Competitive PCR | NR | NR |

Table 2 (Continued)
| Study reference | Patient/control characteristics | Disease (N) | Brain region | Technique | Relative mtDNA alteration | Additional findings |
|-----------------|--------------------------------|-------------|--------------|-----------|--------------------------|---------------------|
| Meacci et al. 1994 | 13/12 71/75 15/10 | AD (13) C (12) | FCtx, TCtx, PCtx, Cb | PCR | NR NR | The amount of oxidised mtDNA showed a threshold increase in PCtx of AD P vs. C were higher in Cd than in FG |
| Reichmann et al. 1995 | 7/7 71/75 8/6 | AD (7) C (7) | PCtx, Ent | PCR | NR NR | DELs larger than 500 bp were discarded |
| De Donato et al. 1995 | 1/1 72/62 18/1M | PO (1) C (1) | SN, H, OCtx, Th, FCtx, Pu, GP, CbCtx, S, LC | qPCR | NR NR | The SN showed the highest proportion of the common DEL (6.1%), while CbCtx showed the lowest (6.02%) |
| Blanchard et al. 1993 | 6/6 80/64 7/5 | AD (6) C (6) | FCtx | PCR | NR NR | Similar mtDNA DEL levels were observed in AD (6.14%) and C (6.12%) |
| Lestienne et al. 1995 | 1/1 NA | NA | PO (1) C (1) | PCR | Pu, SN, Ctx | The common DEL was present in PD P and in C |
| Lestienne et al. 1993 | 15/5 60-85/NA | NA | PO (15) C (5) | PCR | Pu, SN, FCtx | No DELs were identified |
| Schapira et al. 1990 | 6/6 NA | NA | PO (6) C (6) | SN | RFLP Hybridization using a radiolabeled probe | No DELs were identified |
| Ikebe et al. 1990 | 5/6 68/55 | PO (3) C (6) | FCtx, Str | PCR | NR NR | Proportion of deleted mtDNA to normal mtDNA was lower in FCtx than in the Str of both PD and C |

Table 2: Studies reporting results of the mtDNA analyses in postmortem brain samples of patients with neurological diseases (NeuD).
| Study reference (PMID) | Patient/Control characteristics | Disease (N) | Brain region | Technique | mtDNA alteration in brain | mtDNA CN | Rearrangements | Additional information |
|------------------------|---------------------------------|-------------|--------------|-----------|--------------------------|---------|---------------|-----------------------|
| Fries et al. 2019 (31746071) | NP/C 32/32 46/47 P: 15/17 C: 19/13 | BD (32) | H | qPCR | NR | Less mtDNA CN in BD vs. C. | NR | mtDNA CN was not correlated with chronological age |
| Hjelm et al. 2019 (30869147) | 39/2 46 33/8 | SZ (12) BD (10) MDD (8) ADO (8) C (2) | ACOS, DLPFCox, H1, Pu, Cd | Long-range PCR and NGS, exome sequencing, splice-break pipeline, qPCR, Sanger sequencing | NR | | |
| Bodenstein et al. 2019 (31793866) | 66/37 H | NA | SZ (35) BD (31) C (37) | H1, BA24, Cb, PFCox | qPCR | | |
| Otsuka et al. 2017 (28600518) | 20/25 SV: 52 C: 58 SV:11/9 C: 11/6 SV (20) C (21) | DLPFCox | qPCR | | | |
| Rollins et al. 2018 (29941335) | 53/41 BD: 46 SV: 41 C: 38 | BD: 19/13 SV: 26/3 | C: 35/6 | qPCR with SYBR green and TaqMan probes | NR | No significant decrease in mtDNA common DEL in PFCox of patients with SZ. | |

Table 3 (Continued)
| Study reference | Patient/Control characteristics | Disease (N) | Brain region | Technique | mtDNA alteration in brain | mDNA CN | Rearrangements | Additional information |
|-----------------|---------------------------------|------------|--------------|----------|--------------------------|--------|--------------|----------------------|
| Mamdani et al. 2014 (25270547) | | NP/C | | | ACOX, Amg, Gl, | qPCR, Sanger sequencing | NR | NR | Female subjects displayed 60% increase in the accumulation of the common DEL. Common DELs in SZ were significantly decreased, mostly in dopaminergic regions, compared to MDD, BD and C. Possible impacts of antipsychotic and antidepressant medications were not quantified. |
| Tomofuji et al. 2013 (23355257) | | SZ: 45 BD: 43 MDD: 47 C: 48 | | | OCox | qPCR | NR | No differences in mDNA ON were observed between study groups. MT-ND1 gene expression was increased in BD P vs. C. |
| Gu et al. 2013 (24002085) | | ASD: 11 C: 11 | | | ASO I-6 C12 | PCR | NR | No differences in mtDNA CN were observed between study groups. MT-ND1 deletion was found in 44% of the ASD group, and 33% of them also had Cy 8 deletion. |
| Tang et al. 2013 (23333625) | | ASD: 20 C: 40 | | | ASO 1/2 C3/2 | TL | MitoChip assay, qPCR | NR | No changes in mDNA ON (situated in 8 ASD P and 8 C). No presence of large-scale DELs or duplications. Certain brain regions accumulated somatic mutations at higher levels than the blood. |
| Sequeira et al. 2012 (22723804) | | Co : 1 | | | Co 1/2 C3/4 | NGS | Affymetrix 6.0 SNP chip, qPCR | NR | 149 homoplasmic novel or rare variants. 7 not previously reported (5 synonymous variants, 1 in the D-loop, 1 in a tRNA). 88% transitions and 11% transversions. Higher number of transitions and transversions in MDD vs. C and vs. SZ or BD. The m.195T>C, D-loop were under-represented in pooled SZ and BD vs. C. The common DEL levels were correlated with age. |
| Ichimura et al. 2012 (20318997) | | SZ | | | FCox | Sanger sequencing, allele specific PCR | NR | m.7184A-C was detected in SZ patients' brain tissue in both a homoplasmic and heteroplasmic state. It was also detected in blood samples of 2 patients and in blood samples of controls as a homoplasmic variant. |
| Study reference | N/P/C | Age(y) in P/C | Sex | Disease (N) | Brain region | Technique | mtDNA alteration in brain | mDNA CN | Rearrangements | Additional information |
|-----------------|-------|---------------|-----|------------|-------------|----------|------------------------|--------|----------------|----------------------|
| Rollins et al.  | 41/36 | BD: 50        | NA  | SZ: 11/3   | DLPFCx      | Affymetrix mtDNA resequencing array, SNaPshot and allele-specific RT-PCR | was heteroplasmic in controls. The rate of synonymous base pair substitutions in the coding regions of the mtDNA was 2.2% higher in P with SZ vs. C. One MDD P carried a homoplasmic mutation in DLPFC at m.10652T>C, Ile61, MT-ND4L, and two P with SZ showed less than 1% heteroplasmy. Low levels of heteroplasmic nonsynonymous mutations were found in the brain. Several mtDNA variants were significantly associated with specific psychiatric disorders: m.11174T>C, m.10939C>T, m.10652T>C and m.19543G>A with BD; m.750A>G, 1438A>G and 4769A>G with SZ; and 16030C>T, 1446G>C and 15043A>G with MDD. | NR | NR | Brain pH was significantly associated with superhypo superhaplogroup U, K and UK. |
| Fuke et al.     | 99/48 | NA            | NA  | SZ: 90     | FCtx        | RT-qPCR with SYBR Green | NR | NR | Age- and sex-dependent accumulation of the common DEL, independent of the diagnosis. One P with SZ showed high levels of the common DEL. No significant difference in mDNA CN level between C and P. Female BD patients had significantly less common DEL (p<0.003) compared with male patients. mtDNA gene expression increased with age and duration. LARS2 was upregulated in cybrids carrying the m.3243A>G, MT-TEF1. | NR | NR | |
| Subuzciyan et al. | 100/44 | ≥60          | NA  | SZ: 45     | PFDx        | qPCR, RT-PCR with TagMan and SYBR Green | NR | No significant difference in the amount of the common DEL between C and P with or without SZ. | NR | No significant difference in the amount of the common DEL between C and P with or without SZ. | |
| Vawter et al.   | 20/20 | BD: 54        | C: 14/6 | BD (96)    | DLPFCx, CBqPCR | NR | No significant difference in the amount of the common DEL between C and P with or without SZ. | NR | No significant difference in the amount of the common DEL between C and P with or without SZ. | |
| Hanakata et al. | 48/14 | BD: 42        | C: 14/6 | BD (96)    | PFDx        | qPCR | NR | Female BD patients had significantly less common DEL (p<0.03) compared with male patients. mtDNA gene expression increased with age and duration. LARS2 was upregulated in cybrids carrying the m.3243A>G, MT-TEF1. | NR | NR | |

Table 3 (Continued)
| Study reference (PMID) | Patient/Control characteristics | N | Age (y) in P/C | Sex M/F | Disease (N) | Brain region | Technique | mtDNA alteration in brain | mtDNA CN | Rearrangements | Additional information |
|------------------------|---------------------------------|---|---------------|---------|-------------|--------------|-----------|--------------------------|----------|----------------|---------------------|
| Marchbanks et al. 2003 (14623372) | 15/9 | 70/69 | SZ: 10/5 C: 5/4 | NA | PCR-RFLP | m.12027T > C, Ile423Thr, MT-ND4 mutation load of 54% in SZ P vs. 58% in C. | NR | NR | No significant difference in the common DEL ratio between 5S and controls. Antidepressant was found in the blood of 5 SVs. |
| Kato et al. 1997 (9359971) | 16/9 | BD: 45 SV: 39 C: 40 | BD: 5/4 SV: 4/5 C: 4/5 | RD (7) | qPCR | NR | NR | The ratio of the common DEL was significantly higher in BD (0.23) compared with that in age-matched C (0.08); p < 0.05. No accumulation of the common DEL with age in C and a decrease in FG. Lack of age-related accumulation of the DEL. Higher DEL levels in Cb compared to FG. |
| Cavelier et al. 1995 (8530074) | 13/9 | 80/73 | SZ: 7/6 C: 5/4 | SZ (15) | Competitive PCR | NR | NR | No correlation between COX activity and levels of common DEL. |

Table 3: Studies reporting the results of mtDNA analyses in postmortem brain samples of patients with psychiatric diseases (PsyD).

C: control; Cb: cohort; DEL: deletion; F: female; M: male; mtDNA CN: mitochondrial DNA copy number; N: number of subjects; NA: information not available; NR: not reported; P: patient; y: years.

ADO: alcohol/drug abuse/other psychiatric symptoms; ASD: autism spectrum disorder; BD: bipolar disorder; MDD: major depressive disorder; SV: suicide victim; SZ: schizophrenia; KSS: Kerns-Sayre syndrome.

Ac: nucleus accumbens; ACCTX: anterior cingulate cortex; Amg: amygdala; BA24: Brodmann area 24; Ce: cerebellum; CbCTX: cerebellar cortex; Cd: caudate nucleus; DLPCXT: dorsolateral prefrontal cortex; FCXT: frontal cortex; FG: frontal gyrus; Hi: hippocampus; OCCTX: occipital cortex; OFCTX: orbitofrontal cortex; PCCTX: prefrontal cortex; Pu: putamen; SN: substantia nigra; Th: thalamus; TL: temporal lobe.

NGS: next-generation sequencing; PNA: peptide nucleic acid; qPCR: quantitative real-time polymerase chain reaction; RFLP: restriction fragment length polymorphism; RT-qPCR: reverse transcription qPCR.

CO1: cytochrome c oxidase I; COX: cytochrome c oxidase; D-loop: displacement loop; HV2: hypervariable segment 2; LARS2: Leucyl-tRNA synthetase 2; MFC: mitochondrially encoded gene; ND5: NADH-ubiquinone oxidoreductase subunit 5; ND4L: NADH-ubiquinone oxidoreductase subunit 4L; TLE: tRNA-Leu s.
| Study reference (PMID) | Patient/control characteristics N P/C | Disease or condition (N) | Brain region (variant) | Technique | mtDNA alteration mtDNA CN | Rearrangements | Additional findings |
|------------------------|----------------------------------------|--------------------------|------------------------|-----------|---------------------------|---------------|---------------------|
| Wnek et al. 2016 (27457581) | 3/5 64/76 5/3 | HSE (3) C (5) | FCtx, Amg, Hi, CiG and ICtx | Microarray, qPCR NR | MT-CO1 exhibited lower abundance in Ctx, Amg and FCtx in P than C | NR | Greater decline in P than C in mtDNA-encoded compared to nDNA-encoded transcripts. |
| Var et al. 2016 (26807965) | 27/30 48/51 57/0 | HIV+METH+ (16) HIV+METH- (11) C (30) | Ctx: Brodmann areas 7, 8, 9, 46 | ddPCR | NR | | Higher abundance of the common DEL was associated with increasing age. Higher proportion of the common DEL was associated with lower neurocognitive function in HIV+METH but higher in HIV+METH- |
| Naue et al. 2014 (25526677) | 0/98 52 67/31 | C (100) | NA | qPCR, sequencing, minisequencing, NGS Heteroplasmies were observed in 37% of the individuals (47 observations). 13 of the 98 samples showed 1 bp deletion between positions 66 and 71 | NR | NR | The highest relative number of heteroplasmies was detected in muscle and liver (79%, 69%), followed by brain, hair, and heart (36.7%–30.2%). Bone (19.8%), blood (18%), lung (17%), and buccal cells (16.2%) showed a comparatively low number of heteroplasmies |
| Lynn et al. 2003 (12627331) | 1/0 46 1/0 | Diabetes and recurrent stroke-like episodes, seizures and cognitive decline | Cb, OCtx | Hot last cycle PCR, radioactive PCR m.3243A→G mutation load %: OCtx 78, Cb 66 | NR | NR | Mutation load %: skeletal muscle 60, liver 60, pancreas 31, kidney 75, myocardium 58, blood 8 |
| Nádasi et al. 2003 (14711030) | 15/8 <4mth/66 13/10 | Deceased neonates, newborns and infants (15), adults (8) | FCtx, TCtx, Ca, Cd, Th, Hi | PCR | NR | NR | The ratio of the common DEL/wild-type mtDNA was lower in blood than in brain |

Table 4: Studies reporting the results of the mtDNA analyses in postmortem brain samples of individuals with a diagnosis not included in Tables 1–3.

C: control; DEL: deletion; F: female; M: male; mtDNA CN: mitochondrial DNA copy number; mth: month; N: number of subjects; NR: not reported; P: patients; y: year; WM: white matter.

HSE: Herpes simplex virus type-1 encephalitis; HIV: human immunodeficiency virus infection; METH: methamphetamine use.

Amg: amigdala; Cb: cerebellum; Cd: caudate nucleus; CiG: cingulate gyrus; Ctx: cortex; FCtx: frontal cortex; Hi: hippocampus; ICtx: insular cortex; OCtx: occipital cortex; TCtx: temporal cortex; Th: thalamus.
ddPCR: digital-droplet PCR; NGS: next-generation sequencing; qPCR: quantitative real-time polymerase chain reaction.

MT-CO1: mitochondrially encoded cytochrome c oxidase I gene.
| Study reference     | Patient/Control characteristics | Condition (N) | Brain region | Technique | mtDNA alteration in brain variant | mtDNA CN | Rearrangements | Additional information |
|---------------------|----------------------------------|---------------|--------------|-----------|----------------------------------|----------|----------------|-----------------------|
| Roca-Bayerri et al. 2020(32722761) | NP/C | NA | FWM (G2) HIV-negative C (40) | FCox, FLGM | qPCR, long-range PCR, NGS | Mutations accumulated in the mtDNA non-coding D-loop were significantly associated with age | The mtDNA CN in FCoX decreased with age | An increase of the mutation load of the common DEL was associated with increasing age |
| Dill et al. 2016(27874000) | 21 | 11 - 87 | 1/3/1 | SN, FCox, Qb | dPCR, qPCR, NGS | NR | Total mtDNA CN increased with age (p=0.0016) | Major arc DEL showed a significant positive correlation with age in SN neurons |
| Taylor et al. 2014(23911137) | 21 | 15 - 80 | NA | NA | 3D, ddPCR, NGS | NR | NR | The deletion load increased with age, while the number and diversity of unique deletions remained constant |
| Kennedy et al. 2013(24986148) | 10 | Y1 - 1 M: 79 - 90 | NA | Y1 (C) and AI (5) individuals without known brain pathology | PFCox | qPCR, duplex sequencing | A significant (5-fold) increase in mutatation rate was reported in AI. 79.3% of mutations were nonsynonymous and predicted to be more deleterious. Most of them accumulated in the D-loop during aging. | NR | NR | No significant increase in G-T mutations, considered the hallmark of oxidative damage to DNA with age |
| Coikiet al. 2010 (20-403-002) | 38/25 | DSAD: 46 - 62 | MF ratio equal in both groups | D1AD (14) D2AD (11) AD (13) C (2) | FCox | Mutations in the regulatory coding region of mtDNA increased with age in C and were significantly elevated in AD and D1AD relative to age-matched C and D2 | mtDNA CN decreased in C brains after age 65 in parallel with the increased mtDNA mutation rate | NR | NR | AD D1-D2-3D-4D (H-strand) mtDNA ratio did not change significantly with age in C, while it was significantly lower in both AD and D1AD |
| Meessen et al. 2008 (19-43-9776) | 92 | 0.2 - 10.2 | NA | Acute or peracute cause of death with no known brain pathology | SN, Cd | PCR-CE | NR | NR | A positive correlation between the common DEL amount and ageing, and a strong interindividual variation were detected. Abundance of the common DEL varied with tissue type SN > Cd > PL > Cd |
| 15/16 | NA | SN, H | Long-range PCR, qPCR | NR | NR | NR | High levels of mtDNA DELs were observed | The level of mtDNA DELs accumulated in H |

**Table 5 (Continued)**
| Study reference (PMID) | Patient/Control characteristics | Condition (N) | Brain region | mDNA alteration in brain | mtDNA CN | Rearrangements | Additional information |
|------------------------|---------------------------------|---------------|--------------|--------------------------|----------|---------------|-----------------------|
| Bender et al. 2006 (10004074) | PD 76 | PD (5)| C (3)| 0.2% ± 9% in SN of individuals with PD, and 4.3% ± 9% in AI; p < 0.00. The level of mDNA DELs increased linearly with age. These DELs were characterized as the common DEL and the mycin DEL, 13017, DEL. | NR | NR | The number of mDNA DELs was significantly different between old and young tissues. The was a very high absolute prevalence of mDNA DELs in aged SN neurons was significantly lower than in SN neurons in both groups |
| Kraayenberg et al. 2005 (10004073) | AD 80 | AD (1)| C (0)| SN | Single molecule PCR | NR | NR | The number of mDNA DELs were directly involved in the development of COX defects in aged SN |
| Frohm et al. 2005 (10090018) | 0.2 – 93 | NA | Acute or peracute cause of death with no neurological disease | Cd, FCtx, CbCtx, qPCR | NR | NR | mDNA CN in three age groups (0–30, 31–60 and >60 years) revealed no significant age-dependent increase |
| Cantuti-Castelvetri et al. 2005 (16243605) | 64 | C | without neurological disease | SN | Single-cell, allele-specific PCR, cloning-sequencing | NR | NR | Mean number of somatic point mutations per mitochondrial genome was 3.3 for single neurons and 2.2 for single glia |
| Mawrin et al. 2004 (10036587) | FTD-MND: 33 | FTD-MND: 1 (AD: FBD 94, DUB: 74, PD: 14, C: 35–75) | FL, TL, DL, HI, SN, Qo | FL, TL, DL, HI, SN, Qo | gPCR | NR | NR | The common DEL rate increased with age. In the basal ganglia, it reached the highest level |
| Simon et al. 2004 (16095733) | C Group 1: 10 | C Group 2 (12–24) | FCb, SN | FCb, SN | PCR, sequencing | NR | NR | SN of young individuals had similar accumulation of point mutations as in the SN and FCb of older individuals |

Table 5 (Continued)
| Study reference (PMID) | Patient/Control characteristics | Condition (N) | Brain region | Technique | mDNA alteration in brain | mtDNA CN Rearrangements | Additional information |
|-----------------------|--------------------------------|---------------|--------------|-----------|--------------------------|------------------------|------------------------|
| Storm et al. 2002 (12559408) | Diverse causes of death with no neuropathology | NA | Chb, GSC, FCox, TDox, SN, Pu | Competitive PCR, PNA-directed PCR-clamping | m.3243A>G, m.8344A>G and m.414T>G mutations did not accumulate with age | NR | Distribution of the common DEL levels were not identified, neither between astrocytes and neurons, nor between healthy YI and AI. |
| Murdock et al. 2000 (10313735) | 16 | 23–93 | 7/9 | NA | Chb, GSC, FCox, TDox, SN, Pu | Competitive PCR, PNA-directed PCR-clamping | m.3243A>G, m.8344A>G and m.414T>G mutations did not accumulate with age | NR | The accumulation of m.414T>G mutation was identified in muscle samples of aged individuals. |
| Chang et al. 2000 (11058135) | 20/20 | 62–71 | NA | AD (20) | Chb, GSC, FCox, TDox, SN, Pu | Competitive PCR, PNA-directed PCR-clamping | m.3243A>G, m.8344A>G and m.414T>G mutations did not accumulate with age | NR | The accumulation of m.414T>G mutation was detected with age. |
| Zuttia et al. 1999 (10316991) | 7/6 | 51–79 | AD (7) | Cb, FCox, PCox | Kinetics PCR | NR | The common DEL levels increased with age. The common DEL % in AD was 3-fold lower than in C. DEL levels were much lower in younger AD than in older AD. |
| McDonald et al. 1999 (10001524) | 57/43 | STS-CI: 50 LTS-HI: 56 C: 50 | NA | STS-CI (53) LTS-HI (14) | TL, H4 | PCR, RFLP | NR | The common DEL was found in 54% of C, 57% of SE, and 21% of LS. Additionally, the common DEL was more prevalent in older individuals among C. A trend toward increased prevalence of the m.1989_14366del (12 kb) in older patients in all groups was observed. |
| Melov et al. 1999 (10638530) | 5Y | Y: 23–44 | NA | Chb, FCox, Pu, ED, x 5/11, 6/11 TDox | Long-range PCR, sequencing | NR | An increase in the number and the variety of mtDNA rearrangements in aged brains was detected. |

Table 5 (Continued)
| Study reference | Sex/age | Data region | Technique | Additional information |
|-----------------|---------|-------------|-----------|------------------------|
| Mann et al. 1992 | 6/6 | NA | NA | No significant difference in % of the common DEL among patients and age-matched controls (0.01% vs 0.02%). No correlation between complex I activity and the common DEL levels. |
| Corral-Debrinski et al. 1992 | 7 | 24-94 | NA | No macromolecular complex I deficiency in the common DEL subgroup. |
| Jazin et al. 1996 | 27 | 5/2 | NA | No significant effect on the observed levels of deleted mtDNA by neuroleptic drugs. |
| Kapsa et al. 1996 | 2/2 | C: 83 | Competitive PCR, PNA-directed PCR clamping | The common DEL levels increased with age in C group rather than aging in P group. |
| Soong et al. 1992 | 7 | 2/3 | NA | No macromolecular complex I deficiency in the common DEL subgroup. |
| NR | Several multiple DELs (4.5/C0 7.1 kb) were detected in the SN of both aged P and C groups | 34-70 | Apoptosome assay | Somatic clonal expansion of a single mtDNA polyploid clone could be the signature of aging. |
| NR | NR | NA | NA | NR | NR | A 7.7-fold increase of small insertions and deletions was detected in aged individuals. |
| NR | NA | No history of neurodegenerative disease | PCR, Cd PCR, sequencing | The overall heteroplasmy level in D-loop was 2.2-fold higher in two aged individuals (96 and 99) compared with a 28-year-old individual. |
| NR | NR | NA | NA | NR | NR | A significant increase in the common DEL ratio was detected in Ctx and Pu but not in Cb. In Ctx it ranged from 0.00023 to 0.012 in 67-year-olds and up to 0.034 in those over 80. In Pu: 0.0016 to 0.010 in 66-year-old and up to 0.12 in those over 80. Age-related accumulation of the 7436 bp DEL was also detected. |
| NR | NR | NA | NA | NR | NR | Substitutions did not show any significant increase with age. |
| NR | NR | NA | NA | NR | NR | No correlation between the common DEL levels and cognitive function. |

**Table 5 (Continued)**
| Study reference (PMID) | Patient/Control characteristics | Brain region | Technique | mtDNA alteration in brain | mtDNA CN | Rearrangements | Additional information |
|------------------------|--------------------------------|--------------|-----------|---------------------------|---------|----------------|------------------------|
| Zhang et al. 1992 (1551433) | NP/C | Brain region | Technique | mtDNA alteration in brain | mtDNA CN | Rearrangements | Additional information |
| NP/C | Age (y) | Sex | M/F | In P/C | Condition | Brain region | Technique | mtDNA alteration in brain | mtDNA CN | Rearrangements | Additional information |
| 1 | 69 | F | Patients with primary carcinoma of splenic flexure of bowel | NA | PCR, sequencing | NA | PCR, sequencing | 0.94 correlation efficiency in SN and the 7458 bp DEL were detected in brain tissue | 10–83 times higher than in Cb GM Heart and skeletal muscle samples were examined, and multiple DELs were also detected in these tissues, suggesting that accumulation of multiple DELs is a general phenomenon during normal ageing |
| Lesienn et al. 2011 (2013767) | Adults: 27–104, Stillborn: 32, 40 weeks Spontaneous abortions: 22, 29 weeks Newborn: 4d | Str | PCR, RFLP, nested PCR, dilution PCR | NA | PCR | NA | PCR | Low levels of the common DEL were detected in SN of the aged control without neurological or psychiatric antecedents and the PD P |
| Cortopassi et al. 1990 (2263455) | 22 Adults: 27–104, Stillborn: 32, 40 weeks Spontaneous abortions: 22, 29 weeks Newborn: 4d | Str, FCtx | PCR, sequencing | NA | PCR | NA | PCR | The heart tissue of 7 adults and 5 foetuses were also examined, and the common DEL was only found in aged adults |
| Ikebe et al. 1990 (2390073) | 5/6 | Str, FCtx | PCR, sequencing | NA | PCR | NA | PCR | Accumulation of the common DEL was reported in both PD and aged C. The DEL load % was higher in Str than in Fctx in both PD and aged C |

Table 5: Studies reporting the results of mtDNA analyses in postmortem brain samples in ageing.

AI: aged individuals; C: control; DEL: deletion; E: elderly; F: female; M: male; mtDNA CN: mitochondrial DNA copy number; N: number of subjects; NA: information not available; NR: not reported; P: patient; y: years; YI: young individuals.

ADO: alcohol/drug abuse/other psychiatric symptoms; AD: Alzheimer’s disease; DS: Down’s syndrome; DSAD: Down’s syndrome and dementia; DLR: dementia with Lewy bodies; EAD: early-onset Alzheimer’s disease; FTD-MND: frontotemporal dementia with motor neuron disease-like inclusions; LeAD: late-onset Alzheimer’s disease; LTS-HI: long-term survivor of head injury; PD: Parkinson’s disease; PLWH: people living with HIV; SYJ-CL: short-term survivor of cerebral ischaemia; SZ: schizophrenia.

Cytochrome C oxidase; CYB: cytochrome B; D-loop: displacement loop; MT: mitochondrially encoded gene; ND6: NADH-ubiquinone oxidoreductase core subunit 6; ND5: ADH-ubiquinone oxidoreductase core subunit 2; O1: O1; 8'-hydroxy-2'-deoxyguanosine.
| Locus | Nucleo-tide position | Nucleo-tide change | Variant type | Pathogenicity status/TOOLS | GB Freq (%) | Reported phenotype (Homo-/ heteroplasmy) | Cell or tissue type of reported mtDNA somatic variant (Homo- / heteroplasmy) | Database | Related clinical features in this systematic review |
|-------|----------------------|-------------------|-------------|---------------------------|-------------|------------------------------------------|------------------------------------------------------------------------|----------|-------------------------------------------------|
| MT-HV2, MT-ATT, MT-CR, MT-7S | 68 | G→A | Noncoding | NR | 0.021 | NR | NR (NA) | MITOMAP | Aging brains, POLG/PEO & control muscle, normal tissues (+/-) |
| | 70 | G→A | Noncoding | NR | 0.073 | NR | NR (NA) | MITOMAP | POLG/PEO muscle, bladder tumour back-mutation (+/-) |
| | 72 | T→C | Noncoding | NR/NA | 1.792 | NR | Elderly fibroblasts, elderly/AD brains, POLG/PEO & control muscle, various tumours (+/-) | MITOMAP | Absence of endometriosis (+/-) |
| MT-HV2, MT-OHR, MT-ATT, MT-CR, MT-7S | 114 | C→T | Noncoding | R/NA | 0.442 | BD-associated (+/-) | POLG/PEO muscle, thyroid tumour, glioblastoma (+/-) | MITOMAP | BD |
| | 146 | T→C | Noncoding | R/NA | 19.510 | Absence of endometriosis (+/-) | Elderly fibroblasts, elderly/AD brains, POLG/PEO muscle & fibroblasts, various tumours (+/-) | MITOMAP | AD |
| | 185 | G→A | Noncoding | R/NA | 3.999 | Low VO₂ max response (+/-) | POLG/PEO muscle, thyroid tumour, glioblastoma (+/-) | MITOMAP | AD |
| | 189 | A→G | Noncoding | NR | 5.436 | NR | Elderly fibroblasts, elderly/AD brains, tumours: lung, thyroid, ovarian, prostate, glioblastoma (+/-) | MITOMAP | PD |
| MT-HV2, MT-OHR, MT-ATT, MT-CR | 195 | T→C | Noncoding | R/NA | 19.228 | BD-associated/melanoma (+/-) | Elderly fibroblasts, elderly/AD brains, tumours: lung, thyroid, ovarian, prostate, glioblastoma (+/-) | MITOMAP | AD/BD |
| | 207 | G→A | Noncoding | NR | 4.645 | NR | Oral, prostate & thyroid tumours/OPA1 defect (+/-) | MITOMAP | AD |
| MT-HV2, MT-OHR, MT-CSB1, MT-ATT, MT-CR | 224 | T→C | Noncoding | NR | 0.012 | NR | Low VO₂ max response (+/-) | MITOMAP | BD/MDD |
| | 228 | G→A | Noncoding | R/NA | 2.579 | NR | NR | MITOMAP | AD |
| MT-HV2, MT-OHR, MT-ATT, MT-CR | 267 | T→C | Noncoding | NR | 0.027 | NR | buccal cell, colonic crypt (+/-) | MITOMAP | AD |
| MT-HV2, MT-OHR, MT-CSB2, MT-ATT, MT-CR | 309 | delC | Noncoding | NR | 0.000 | NR | | MITOMAP | AD |
| | insC | Noncoding | R/NA | 1.142 | AD-weakly associated (NR) | NR | | MITOMAP | AD |
| MT-HV2, MT-OHR, MT-CSB3, MT-ATT, MT-CR | 347 | G→A | Noncoding | NR | 0.000 | NR | | MITOMAP | AD |
| | 380 | G→A | Noncoding | NR | 0.004 | NR | | MITOMAP | AD |
| | 405 | T→C | Noncoding | NR | 0.000 | NR | | MITOMAP | AD |

**Table 6 (Continued)**
| Locus                  | Nucleotide position | Nucleotide change | Variant type   | Pathogenicity status/TOOLS | GB Freq (%) | Reported phenotype (Homo-/ heteroplasy) | Cell or tissue type of reported mtDNA somatic variant (Homo-/ heteroplasy) | Database     | Related clinical features in this systematic review |
|-----------------------|---------------------|-------------------|----------------|---------------------------|-------------|----------------------------------------|-----------------------------------------------------------------------------|--------------|----------------------------------------------------|
| MT-OHR, MT-LSP, MT-ATT, MT-CR | 408             | T>C               | Noncoding      | NA                        | 0.004       | NR                                     | AD brains/POLG OPA1 and control samples (+/-)                             | MITOMAP     | AD                                                  |
|                       | 414             | T>C               | Noncoding      | NR/NA                     | 0.002       | NR                                     | Elderly fibroblasts, elderly muscle, POLG/PEO, DS, AD brains, oocytes, normal tissues (+/-) | MITOMAP     | AD                                                  |
|                       | 414             | T>G               | Noncoding      | NR/NA                     | 0.029       | NR                                     | AD brains, ovarian tumour (+/-)                                           | MITOMAP     | AD                                                  |
| MT-OHR, MT-LSP, MT-TFL, MT-ATT, MT-CR | 416           | T>C               | Noncoding      | NR                        | 0.000       | NR                                     | Thyroid tumour (+/-)                                                     | MITOMAP     | AD                                                  |
|                       | 418             | C>T               | Noncoding      | NR                        | 0.114       | NR                                     | AD brains, ovarian tumour (+/-)                                           | MITOMAP     | AD                                                  |
|                       | 436             | C>T               | Noncoding      | NR                        | 0.000       | NR                                     | AD brains, ovarian tumour (+/-)                                           | MITOMAP     | AD                                                  |
| MT-HV3, MT-ATT, MT-CR | 456             | C>T               | Noncoding      | NR/NA                     | 2.450       | NR                                     | AD brains, ovarian tumour (+/-)                                           | MITOMAP     | AD                                                  |
|                       | 466             | 2insCC            | Noncoding      | NR                        | 0.938       | NR                                     | AD brains, ovarian tumour (+/-)                                           | MITOMAP     | AD                                                  |
| MT-HV3, MT-CR         | 477             | T>C               | Noncoding      | NR/NA                     | 0.143       | NR                                     | AD brains, ovarian tumour (+/-)                                           | MITOMAP     | AD                                                  |
| MT-HV3, MT-TFH, MT-CR | 511             | C>T               | Noncoding      | NR                        | 0.191       | NR                                     | AD brains, ovarian tumour (+/-)                                           | MITOMAP     | AD                                                  |
|                       | 523             | delAC             | Noncoding      | NR                        | 0.019       | NR                                     | AD brains, ovarian tumour (+/-)                                           | MITOMAP     | AD                                                  |
| MT-HV3, MT-HSP1, MT-CR | 555           | A>G               | Noncoding      | NR                        | 0.000       | NR                                     | AD brains/POLG OPA1 and control samples (+/-)                             | MITOMAP     | AD                                                  |
|                       | 566             | C>T               | Noncoding      | NR                        | 0.000       | NR                                     | AD brains/POLG OPA1 and control samples (+/-)                             | MITOMAP     | AD                                                  |
| MT-TF                 | 644             | A>G               | (rRNA)          | NR/Likely benign          | 0.050       | NR                                     | AD brains/POLG OPA1 and control samples (+/-)                             | MITOMAP     | AD                                                  |
| MT-RNR1               | 750             | A>G               | (rRNA)          | Benign                    | 98.277      | NR                                     | Juvenile MELAS                                                            | MITOMAP     | AD                                                  |
|                       | 1438            | A>G               | (rRNA)          | R/NA                      | 94.853      | NR                                     | Juvenile MELAS                                                            | MITOMAP     | AD                                                  |
| MT-RNR2, MT-TFL, MT-TL1 | 3196         | G>A                | (rRNA)          | R/NA                      | 0.025       | ADPD (+/-)                             | ADPD (+/-)                                                                | MITOMAP     | AD                                                  |
|                       | 3243            | A>G                | (rRNA)          | Confirmed pathogenic      | 0.019       | MELAS/Leigh syndrome/DMC/DD/MM/NSHL/ CPEO/MM/NSGS/ ASD/cardiac multis- organ dysfunction (+/-) | MITOMAP     | MELAS/BS-LD/AD/PO/ BD/SZ                           |
|                       | 3251            | A>G                | (rRNA)          | R/Possibly benign         | 0.000       | MM/MELAS with chorea-ballism (+/-)     | MM/MELAS with chorea-ballism (+/-)                                       | MITOMAP     | AD                                                  |

Table 6 (Continued)
| Locus | Nucleotide position | Nucleotide change | Variant type | Pathogenicity status/TOOLS | GB Freq (%) | Reported phenotype (Homo-/ heteroplasmy) | Cell or tissue type of reported mtDNA somatic variant (Homo-/ heteroplasmy) | Database | Related clinical features in this systematic review |
|-------|---------------------|-------------------|--------------|---------------------------|-------------|-----------------------------------------|-------------------------------------------------|----------|--------------------------------------------------|
| MT-ND1 | 3257 | A->G | (tRNA) | NR | NA | NR | NR | MITOMAP | MERRF |
|        | 3397 | A->G | Met31Val | R/Likely pathogenic | 0.305 | ADPD/possibly LVNC cardiomyopathy-associated/resistance to high altitude pulmonary oedema (+/-) | NR | MITOMAP | AD |
| MT-TI | 4274 | T>C | (tRNA) | Benign | R/Likely pathogenic | 0.000 | PD, LoS, AD | NR | ClinVar | Motor neuron disease |
| MT-TQ | 4336 | A->G | (tRNA) | Unclear/ Possibly benign | Conflicting reports | NA | Sensoineural deafness and migraine/juvenile MELAS | NR | ClinVar | |
| MT-N2D | 4769 | A->G | Met100 | R/NA | 97.604 | R/NA | Not provided | AD/PD/HDH (++) | MITOMAP | SZ |
|        | 5460 | G>A | Ala331Thr | Conflicting reports/ Possibly benign | 6.904 | R/NA | Not provided | AD/PD/HDH (++) | ClinVar | AD/PD |
| MT-TW | 5537 | insT | (tRNA) | Benign | R/NA | 0.000 | LS/ME | NR | ClinVar | LS |
|        | 5549 | G>A | (tRNA) | Pathogenic | R/Likely pathogenic | 0.000 | Dementia and chorea (+/-) | NR | ClinVar | ME |
|        | 5552 | G>C | (tRNA) | Pathogenic | R/ Possibly benign | 0.000 | Encephalomyopathy (+/-) | NR | ClinVar | LoME |
| MT-TN | 5705 | T>C | (tRNA) | NR | NR | NR | NR | MITOMAP | AD |
| MT-CO1 | 6617 | C>T | Phe238 | NR/NA | 0.017 | NR | NR | MITOMAP | AD |
|        | 7064 | T>C | Phe387 | NR/NA | 0.062 | NR | NR | MITOMAP | AD |
| MT-CO2 | 7706 | G>A | Ala41Thr | R/ Possibly benign | 0.015 | AH5-like (+/-) | NR | MITOMAP | ASD |
|        | 7834 | C>T | Ile83 | R/NA | 0.004 | NR | NR | MITOMAP | AHS-like disease |
|        | 8344 | A>G | (tRNA) | Confirmed pathogenic | 0.008 | MERRF, Other-LD/DM1/leukoencephalopathy/HCM (+/-) | Pathogenic | MERRF | |
|        | 8603 | T>C | Phe265er | NR/Possibly benign | 0.336 | LS/MERRF/PD/juvenile MELAS | NR | ClinVar | |

Table 6 (Continued)
| Locus      | Nucleotide position | Nucleotide change | Variant type | Pathogenicity | Status/TOOLS  | GB Freq (%) | Reported phenotype (Homo-/heteroplasmy) | Cell or tissue type of reported mtDNA somatic variant (Homo-/heteroplasmy) | Database       | Related clinical features in this systematic review |
|------------|---------------------|-------------------|--------------|---------------|---------------|-------------|-----------------------------------------|--------------------------------------------------------------------------|----------------|--------------------------------------------------|
| 8881       | T>C                 | Ser119Pro         | Benign       | NR            | LS            | 0.002       | Patient with suspected mitochondrial disease (NR/ NR) | NR                         | ClinVar         | -                                               |
| 8993       | T>G                 | Leu156Arg         | Confirmed/likely pathogenic | NR            | NARP, LS, MELAS/other (+/−) | 0.012       | Mitochondrial complex V (ATP synthase) deficiency, mitochondrial type I/LS/NARP/other | NR                         | MITOMAP         | LS/NARP/MLS                                       |
| 9500       | C>T                 | Phe98             | NR/NA       | NR            | NR            | 0.008       | NR                          | NR                         | MITOMAP         | SZ                                               |
| 9633       | T>C                 | Ser143Pro         | R/ Possibly benign | NR            | NR            | 0.000       | NR                          | NR                         | ClinVar         | PD                                               |
| 9699       | A>G                 | Ile165Val         | NR/ Possibly benign | NR            | NR            | 0.008       | NR                          | NR                         | ClinVar         | SZ                                               |
| 9861       | T>C                 | Phe219Leu         | R/ Possibly benign | NR            | AD (+/−)      | 0.220       | LS                          | NR                         | MITOMAP         | AD                                               |
| 9956       | A>G                 | Leu250            | NR/NA       | NR            | NR            | 0.006       | NR                          | NR                         | MITOMAP         | SZ                                               |
| 10652      | T>C                 | Ile61             | R/NA        | NR            | LD, MDD-associated (−/+)| 0.104     | NR                          | NR                         | MITOMAP         | BD/MDD                                           |
| 10858      | T>C                 | Ile33             | NR/NA       | NR            | NR            | 0.033       | NR                          | NR                         | MITOMAP         | BD                                               |
| 11778      | G>A                 | Arg340His         | Confirmed/ Possibly pathogenic | Pathogenic | LHON/progressive dystonia (+/−) | 0.357     | NR                          | NR                         | MITOMAP         | LHON                                             |
| 12027      | T>C                 | Ile423Thr         | R/ Possibly benign | NR            | LHON | 0.004     | NR                          | NR                         | ClinVar         | SZ                                               |
| 13094      | T>C                 | Val253Ala         | Confirmed/likely pathogenic | NR            | Mitochondrial disease | 0.002     | Ataxia + PEO/MELAS, LD, LHON, myoclonus, fatigue (+/−) | NR                         | MITOMAP         | MELAS                                            |
| 13513      | G>A                 | Asp393Asn         | Pathogenic   | Confirmed/likely pathogenic | NR           | 0.002     | Juvenile MELAS/LS, MELAS/LHON, MELAS overlap syndrome/ negative association with carotid atherosclerosis (+/−) | NR                         | ClinVar         | MELAS/LS                                         |

*Table 6 (Continued)*
| Locus       | Nucleo-tide position | Nucleo-tide change | Variant type | Pathogenicity status/TOOLS | GB Freq (%) | Reported phenotype (Homo-/ heteroplasmy) | Cell or tissue type of reported mtDNA somatic variant (Homo-/ heteroplasmy) | Database               | Related clinical features in this systematic review |
|------------|----------------------|-------------------|--------------|---------------------------|-------------|------------------------------------------|--------------------------------------------------------------------------|------------------------|-------------------------------------------------|
| MT-ND6     | 14668                | C>T               | Met2         | R/NA                      | 3.951       | Depressive disorder-associated (+/-)     | NR                                                                       | MITOMAP                | MDD                                             |
| MT-TE      | 14685                | G>A               | (9RNA)       | R/Likely pathogenic       | 0.000       | Cataracts with spastic paraparesis & ataxia (+/-) | NR                                                                       | MITOMAP                | ECOAPP                                          |
|            | 14709                | T>C               | (9RNA)       | Confirmed pathogenic      | 0.000       | MM+DMDF+encephalomyopathy/ dementia + diabetes + ophthalmoplegia (+/-) | NR                                                                       | MITOMAP                | Ataxia                                          |
| MT-CYB     | 15043                | G>A               | Gly99        | R/NA                      | 23.640      | MDD-associated/possible role in high-altitude sickness (+/-) | NR                                                                       | MITOMAP                | MDD                                             |
| MT-HV1, MT-ATT, MT-CR, MT-7S | 16184 | C>T               | Noncoding    | R/NA                      | 0.735       | Colonic mucosa (+/-)                      | NR                                                                       | MITOMAP                | PD                                              |
|            | 16300                | A>G               | Noncoding    | R/NA                      | 0.536       | BD-associated (+/-)                       | Colonic mucosa (+/-)                      | MITOMAP                | PD                                              |

Table 6: mtDNA disease-related variants with pathogenicity information retrieved from public databases.

NA: not available; NR: not reported; R: reported.

AD: Alzheimer’s disease; ADPD: Alzheimer’s disease and Parkinson’s; AHS: Alpers-Huttenlocher syndrome; ASD: autism spectrum disorder; BD: bipolar disorder; BS-LD: Barth syndrome-like disorder; CPEO: chronic progressive external ophthalmoplegia; CID: combined immunodeficiency; DMD: depressive mood disorder; DMDF: diabetes mellitus + deafness; ECOAPP: early-onset cataracts, ataxia and progressive paraparesis; DS: Down’s syndrome; FSGS: focal segmental glomerulosclerosis; HICM: histiocytoid cardiomyopathy; Lactacid: lactic acidosis and complex I deficiency; LD: learning disabilities; LHON: Leber’s hereditary optic neuropathy; LoME: late-onset mitochondrial encephalomyopathy; LVNC: left ventricular non-compaction; ME: mitochondrial encephalopathy; MELAS: mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MERRF: myoclonic epilepsy with ragged red fibres; MDD: major depressive disorder; MIDD: maternally inherited diabetes and deafness; MILS: maternally inherited Leigh syndrome; MM: mitochondrial myopathy; LoLS: late-onset Leigh syndrome; LoME: late-onset mitochondrial encephalomyopathy; VO2 max: maximum rate of oxygen consumption.

TOOLS: If available, predictive data of pathogenicity are obtained from the tools MitoTIP, HmtVar and/or APOGEE (https://www.mitomap.org/foswik/bin/view////Main/SearchAllele); del: deletion; GB Freq: The frequency data derived from 15836 GenBank sequences with sizes greater than 15.4 kbp. The frequency data and disease-associated phenotypes were retrieved from the MITOMAP and ClinVar databases in June 2021.
Figure 2. Map of human mtDNA with variants (a) and deletions (b) identified in postmortem brain samples of patients with MitD. mtDNA replication initiates within the D-loop region and proceeds from the origin of heavy-strand replication (O_h) until the origin of light-strand replication (O_l). The positions of variants are represented by asterisks, while deletions are represented by circles. MitD diagnoses are indicated in boldface. MELAS: mitochondrial encephalopathy, lactic acidosis and stroke-like episodes; ME: mitochondrial encephalomyopathy; AHS: Alpers-Huttenlocher syndrome; MERRF: myoclonic epilepsy with ragged-red fibres; NARP: neuropathy with ataxia and retinitis pigmentosa; MILS: maternally inherited Leigh’s syndrome; LHON: Leber hereditary optic neuropathy; EOCAPP: early-onset cataracts, ataxia and progressive paraparesis; POLG: DNA polymerase gamma gene; KSS: Kearns-Sayre syndrome. MT-: mitochondrially encoded gene; TL1: tRNA-Leu 1; TW: tRNA-Trp; CO2: cytochrome c oxidase II; TK: tRNA-Lys; ATP6: ATP synthase subunit 6; ND4: NADH-ubiquinone oxidoreductase subunit 4; TE: tRNA-Glu; CO1: cytochrome c oxidase I.
between 89% and 97%, and similar percentages in other tissues were lower than those in blood (72% and 81%).

In addition, m.13513G>A, p. Asp393Asn, MT-ND2 was also reported in LS. Only one study analysed the mtDNA CN, identifying that it was 4.6 times higher in patients with LS than in controls.

**mtDNA analysis in NeuD**

Table 2 includes 67 reports referring to NeuD, and Figure 2 shows the variants reported in neurodegenerative conditions, with the most reported variants being found in Alzheimer’s disease (AD, 33 reports) and Parkinson’s disease (PD, 27 reports). Other reported phenotypes were amyotrophic lateral sclerosis, mild cognitive impairment, Creutzfeldt-Jakob disease, dementia with Lewy bodies, frontotemporal dementia, mesial temporal lobe epilepsy-hippocampal sclerosis, Down syndrome, Down syndrome and dementia; multiple sclerosis, infantile onset spino-cerebellar ataxia, motor neuron disease, multiple system atrophy, diseminated neocortical and subcortical encephalopathy, and Huntington disease. Taking all NeuD studies into account, the alterations most frequently investigated were mtDNA deletions (40 reports), followed by the presence of mtDNA variants (22 reports) and mtDNA CN (19 reports) (Figure 3).

**mtDNA analyses in AD.** Among the AD studies reviewed, deletions were the most frequent mtDNA alterations analysed (17/33), followed by mtDNA variants (10/33) and mtDNA CN (9/33). The most frequently assessed brain tissues were the frontal cortex, hippocampus, cerebellum, temporal cortex and parietal cortex.

The results in relation to the variants are diverse; some studies agreed that the frequency of variants is similar between AD patients and controls regarding their levels of heteroplasmy, while others reported higher levels of heteroplasmy and a higher frequency of variants in the parietal cortex, hippocampus and cerebellum in AD patients. Two studies reported the m.5460G>A variant, which produces the p.Ala331Thr amino acid change in MT-ND2; this is described in MitoMap with conflicting reports regarding its pathogenicity for AD, PD and LHON. This amino acid change was reported in AD and control individuals. Additionally, in six patients with AD, four showed homoplasmy and two showed heteroplasmy, with a mutation load percentage of 5%. In this last study, the variant was not present in the control group. Similarly, the m.3243A>G variant involved in MELAS was identified in a patient with AD, although with a very low percentage (<0.05%). More recent studies, some of them conducted with a large number of individuals and using novel techniques, did not identify that mtDNA variation had a role in AD.

Overall, most of the studies agree that the mtDNA CN levels are lower in AD patients than in controls, although some specific hallmarks should be mentioned: 1) no difference was identified in the mtDNA CN levels between tau-positive and tau-negative neurons; 2) focusing on brain regions, the hippocampus and the temporal cortex showed a significant mtDNA CN reduction in pyramidal neurons compared to other neuronal cells but not in the cerebellum, although a study analysing a large number of samples (282 patients and 461 control subjects) mostly obtained from the cerebellum (87.3%) was able to identify a significant reduction in the mtDNA CN levels in AD. When considering the clinical characteristics of the patients, one study observed that the mtDNA CN was reduced by 48% in nondiabetic patients with AD compared to that in nondiabetic noncognitive-impaired individuals, and this effect occurred in the parietal cortex but not in the frontal cortex or cerebellum; however, compared with nondiabetic patients, diabetic patients showed higher mtDNA CNs in the frontal cortex, parietal cortex and cerebellum; and although a reduced mtDNA CN was reported by most of the studies, some authors highlighted that some patients with AD exhibited a high mtDNA CN.

Regarding mtDNA rearrangements, the first studies carried out in the nineties focused on the analysis of the 4977 bp common deletion (m.8470-13477del). Similar percentages of the deletion have been reported in individuals with AD and in control individuals. However, the deletion was found to be more abundant in the temporal cortex of individuals with AD than in controls, although in both cases the percentage was low (<0.05%). Regarding the mutation load of the common deletion in the distinct brain regions, there are some aspects to note. First, higher percentages were present in the nucleus caudate than in the gyrus frontalis. Second, in a more recent study, a 1.5% mutation load was reported in the frontal cortex samples of patients with AD and in control individuals, although the percentage was still low, it was higher than previously reported. Third, the common deletion was also present in the substantia nigra and the globus pallidus of a single individual with AD but was not detected in the blood. Finally, the mutation load was found to be 15 to 25-fold lower in the cerebellum than in cortices of AD and control individuals. More recent studies have investigated the presence of other rearrangements in addition to the common deletion, with controversial results. Some agree that the number of deletions is higher in AD than in controls, but they differ depending on the region studied. For instance, one of the studies did not find any significant increase in the total number of deletions in the hippocampus of patients with AD, while others observed higher...
Figure 3. Map of human mtDNA with variants (a) and deletions (b) identified in postmortem brain samples of patients with NeuD. mtDNA replication initiates within the D-loop region and proceeds from the origin of heavy-strand replication (OH) until the origin of light-strand replication (OL). The positions of variants are represented by asterisks, while deletions are represented by circles. NeuD diagnoses are indicated in boldface. AD: Alzheimer’s disease; PD: Parkinson’s disease. MT-: mitochondrially encoded gene; TL1: tRNA-Leu 1; ND2: NADH-ubiquinone oxidoreductase subunit 2; NDS: NADH-ubiquinone oxidoreductase subunit 5; ATP8: ATP synthase subunit 8.
Figure 4. Map of human mtDNA with variants (a) and deletions (b) identified in postmortem brain samples of patients with PsyD. mtDNA replication initiates within the D-loop region and proceeds from the origin of heavy-strand replication (O\textsubscript{H}) until the origin of light-strand replication (O\textsubscript{L}). The positions of variants are represented by asterisks, while deletions are represented by circles. PsyD diagnoses are indicated in boldface. SZ: schizophrenia; ASD: autism spectrum disorder; MDD: major depressive disorder; BD: bipolar disorder; ADO: alcohol/drug abuse and other psychiatric symptoms. MT: mitochondrially encoded gene; RNR1: 12S rRNA; RNR2: 16S rRNA; ND4L: NADH-ubiquinone oxidoreductase subunit 4L; D-loop: displacement loop; ND4: NADH-ubiquinone oxidoreductase subunit 4; ND6: NADH-ubiquinone oxidoreductase subunit 6; CYB: cytochrome b; CO1: cytochrome c oxidase 1; TL1: tRNA-Leu 1; ND2: NADH-ubiquinone oxidoreductase subunit 2; CO2: cytochrome c oxidase 2; HSP1: major H-strand promoter 1; ATP8: ATP synthase subunit 8.
deletion rates in both astrocytes and microglia of the hippocampus compared with the brainstem and cerebellum in control individuals. Additionally, a high number of deletions in cytochrome oxidase (COX)-deficient neurons of the hippocampus and in frontal cortices of individuals with AD have been reported, but there were no differences between tau-positive and tau-negative neurons. Reportedly, the average mtDNA deletion size is 5080±2367 bp, the deletion size ranges from 3670 to 6088 bp, accumulation occurs with age, and the number and characteristics of the rearrangements depend on the sequence coverage depth.

### mtDNA analyses in PD.

mtDNA deletions were the most investigated mtDNA alterations (19/27), followed by mtDNA variants (10/27) and the CN (10/27). The brain tissue most frequently assessed was the substantia nigra, followed by the frontal cortex, cerebellum, putamen and hippocampus. mtDNA variants and heteroplasmy levels have been widely discussed regarding PD. The first study investigating mtDNA variants in PD reported no differences between variant heteroplasmy levels in PD and control individuals, this was also found by two additional recent studies. Another recent study investigating a large number of samples and taking advantage of next-generation sequencing found that the mtDNA mutation load in MT-CO1, MT-CO2 and MT-CYB in the substantia nigra pars compacta and in MT-CYB in the frontal cortices of patients with PD was increased compared to those in control individuals. A significant increase in the frequency of heteroplasmy levels in neurons with a higher number of deletions has also been reported. Conversely, no association was found between mtDNA variants, either homoplasmic or heteroplasmic, and PD.

Conflicting results have also been reported regarding mtDNA CN in PD, such as 1) decreased levels in the prefrontal cortices of patients with PD and dementia and in neurons of the substantia nigra in patients with idiopathic PD; 2) increased levels in cholinergic neurons of the pedunculopontine nucleus in PD patients; and 3) no significant differences between patients and controls.

In general, patients with PD showed higher mtDNA deletion levels in the substantia nigra than in other brain regions, although some studies found a significant increase in other regions, such as the putamen or hippocampus, but not in the substantia nigra. Additionally, although most reports showed a trend or reported increased mtDNA deletion levels in PD, others did not. Notably, mtDNA deletion levels have been reported to be significantly lower in the cerebellum than in other brain regions. One of the most recent studies found that patients with PD tended to exhibit deletion levels greater than 60% in the substantia nigra, while markedly lower levels were found in the frontal cortex, cerebellum and putamen. The authors also reported that in the substantia nigra, deletion levels were a good predictor of mtDNA CN variation. This is in line with a reported correlation between the percentage of deletions and mtDNA CN in the putamen; the lower the CN, the higher the deletion levels. Some specificities regarding mtDNA deletions in PD should also be mentioned. First, increased mtDNA deletion levels were found to correlate with Braak staging, although these changes were not explored in other studies. Second, a few studies using laser microdissection techniques to focus on specific cell types identified 1) a significant increase in deletion levels in cholinergic neurons of the substantia nigra; 2) increased deletions in the dopaminergic neurons of the substantia nigra compared with those in Lewy-negative neurons or neurons from control individuals; and 4) the common deletion was also identified as the most frequent deletion in the substantia nigra of microdissected neurons.

### mtDNA analysis in PsyD

Table 3 includes 19 reports referring to PsyD, and Figure 4 shows the variants reported in various phenotypes, with the most reported being schizophrenia (SZ, N=13) and bipolar disorder (BD, N=13), followed by major depressive disorder (MDD, N=8). Other phenotypes assessed included subjects with alcohol/drug abuse and other psychiatric symptoms (ADO), suicide victims, and autism spectrum disorder (ASD). The most recurrent brain region explored was the dorsolateral prefrontal cortex (DLPFC), but many others were included in some studies. Eleven studies investigated the presence of mtDNA rearrangements, eight studies examined mtDNA CN and seven studies focused on mtDNA variants.

### mtDNA analyses in SZ

A study that focused on mtDNA variants identified a total of 142 rare variants, with a minor allele frequency less than 1% based on mtDB and PhyloTree, but a relevant role in SZ was not identified. Similarly, in the DLPFC, a 22% higher rate of synonymous variants and an increased number of mtDNA substitutions were found in SZ patients compared with control individuals, but again, there was no major involvement in the diagnosis. Other rare variants (frequency <0.02%), such as m.6617C>T p. Phe238 (MT-CO1), m.8881T>C p. Ser119Pro (MT-ATP6), and m.9500C>T p.Phe88, m.9699A>G p. Ile365Val and m.9956A>G p.Leu230 (MT-CO1) were identified only in patients with SZ. Finally, two
studies reported results regarding specific variants. The m.3243A>G variant was detected in one patient with SZ with a 60% mutation load,\textsuperscript{122} and the m.12027T>C, p.Ile423Thr (MT-ND4) was found to have no prominent effect on SZ.\textsuperscript{123}

mtDNA deletions were detected in two patients with SZ, m.2973_15573del (MT-RNR2–MT-CYB) and m.1148_15607del (MT-RNR1–MT-CYB), with deletion read percentages of 31.8% and 16.8%, respectively.\textsuperscript{124} Regarding the common deletion, most studies did not observe differences between SZ patients and control individuals,\textsuperscript{95,119,125} but it has also been reported that the deletion levels highly differed among the 11 brain regions analysed, increased with age, and showed little change in blood samples.\textsuperscript{119} Additionally, one study showed a significant decrease in the accumulation of the common deletion in patients with SZ compared to MDD, BD and control subjects, mostly in dopaminergic regions.\textsuperscript{126}

mtDNA analyses in BD. The synonymous variant m.10858T>C in the MT-ND4 gene was identified in a patient with BD,\textsuperscript{119} and the m.3243A>G variant was present in two patients with a low variant frequency of 0.90%-1%.\textsuperscript{122}

The mtDNA CN was lower in 32 patients with BD than in 32 control individuals, and no differences were observed between subjects who died from suicide and those who did not,\textsuperscript{127} which was not observed in a previous study.\textsuperscript{128} On the other hand, high mtDNA CNs in postmortem hippocampal tissue from 47 BD patients have been described.\textsuperscript{129}

Two of 10 patients showed m.5897_15840del (MT-TY/MT-CO1–MT-CYB) and m.467_14122del (D-LOOP–MT-ND5) with deletion percentages of 23.0% and 5.0%, respectively. The authors reported that patients with SZ and BD had a higher cumulative deletion ratio in the DLPFC/anterior cingulate cortex (ACCtx) than those in the MDD and ADO diagnostic groups.\textsuperscript{124} The ratio of the common deletion was significantly higher in patients with BD than in control individuals in the DLPFC\textsuperscript{119} and in the cerebellum,\textsuperscript{129} with a significant association between increased levels and advanced age.\textsuperscript{119} This age association was previously observed but was unrelated to the BD diagnosis.\textsuperscript{119} On the other hand, no significant accumulation of the common mtDNA deletion across distinct brain regions of patients with BD compared to control subjects has been reported.\textsuperscript{125}

mtDNA analyses in MDD. The m.224T>C variant in the H-strand replication origin (O\textsubscript{H}) located in the D-loop region was present in one of five evaluated patients with MDD, and the m.16692T>C homoplasmic variant (which does not alter the amino acid Ile661) in the MT-ND4L gene was previously reported in another patient.\textsuperscript{120} Finally, the m.3243A>G MELAS variant identified in patients with BD was not present in 15 patients with MDD.\textsuperscript{123}

Interestingly, the m.1243_15340del (MT-RNR1–MT-CYB) deletion with a high mutation load of 90.1% in the DLPFC and 85% in the ACCtx was suggested to considerably impact a 75-year-old male who had MDD and diabetes mellitus.\textsuperscript{124} Furthermore, another 46-year-old patient with MDD who committed suicide also exhibited four high-impact deletions: m.7863_15677del (MT-CO2–MT-CYB), m.7816_14807del (MT-CO2–MT-CYB), m.8790_14774del (MT-RNR1–MT-CYB), and m.6468_15600del (MT-CO1–MT-CYB), with read percentages of 52.4%, 26.5%, 10.2% and 8.5%, respectively. Even though deletion mutation loads were lower than those of the former patient, the cumulative mutation load was very high. In the latter patient, five brain regions and blood samples were explored, and deletions were detected only in the caudate nucleus. Interestingly, some deletions were clonally expanded to some brain regions, while they were absent in others.\textsuperscript{124} Additionally, this study 1) did not find significant differences in the cumulative mtDNA deletion mutation load in the DLPFC or ACCtx across disorders; 2) stated that only 12 of the 30 most frequent deletions identified were previously described, 14 of which were detected only in the brain and not in other tissues from the same subjects; and 3) found that the brain contained significantly more deletions than the blood.\textsuperscript{124}

mtDNA in other clinical conditions

Table 4 shows the mtDNA alterations explored in a few studies regarding other phenotypes: 1) herpes simplex virus type-1 encephalitis, 2) human immunodeficiency virus infection with or without methamphetamine use, 3) diabetes and recurrent stroke-like episodes, seizures and cognitive decline and 4) deceased neonates, newborns and infants and 5) control individuals. The most notable finding is that the common deletion was present in brain samples from stillborn individuals.\textsuperscript{127}

mtDNA analyses in ageing

Table 5 presents 29 studies that analysed mtDNA in postmortem brain samples; these studies support mtDNA involvement in ageing.

mtDNA variations. Variants in the D-loop have been significantly associated with age, although when they were weighted by their heteroplasmic levels, the association was lost.\textsuperscript{134} Additionally, the overall heteroplasm level in the D-loop was found to be higher in older individuals than in younger individuals.\textsuperscript{133} It has been reported that 78% of the accumulated variants were nonsynonymous and more deleterious in older
individuals. In addition, a high aggregation of somatic point variants in the tRNAs for Thr and Pro, portions of the MT-CYB gene, and the D-loop region were detected in neurons of the elderly. The ageing process is inextricably associated with neurodegeneration. In this sense, variants in the regulatory control region were found to be increased with age in AD and Down syndrome and dementia. Similarly, 23 misense variants (8 of them causing nonconservative amino acid replacements at evolutionarily constrained sites), 2 tRNAs and one nonsense polymorphism were detected in the substantia nigra of elderly nonparkinsonian and idiopathic PD patients. Moreover, the accumulation of G>C to T>A and T>A to G>C transversions was found to increase with age in the frontal cortex of patients with PD.

**mtDNA CN.** Most of the studies found that the mtDNA content in the frontal cortex decreased with age. However, in the substantia nigra, it was recently reported to be increased with age, allegedly to maintain the pool of wild-type mtDNA despite accumulating deletions. Additionally, no significant age-dependent increase in mtDNA CN among three age groups (0–30, 31–59 and >60 years) was identified.

**mtDNA rearrangements.** Most of the studies on ageing focused on the common deletion in the brain, and they reported that the accumulation of the common deletion was associated with increasing age. According to Cortopassi et al., deletions in foetal tissues were estimated to be 1/100 to 1/100,000 times less than those in adults, while Soong et al. reported that the common deletion level was detected in neonatal brain regions as 4/10,000. The distribution of the common deletion varies among different parts of the brain, with the highest and the lowest levels reported in the substantia nigra and cerebellum, respectively. Similarly, the common deletion ratio has been reported to reach 10 times more deleted mtDNA to obtain the ratio of deleted mtDNA to normal mtDNA. According to their serial dilution PCR method (in which total DNA is diluted and amplified by primers spanning the common deletion) and in AD, the common deletion levels were much lower in younger patients than in older patients. Similarly, small insertions and deletions were found to be significantly increased in aged individuals among controls, early- and late-onset AD patients, and SZ patients. On the other hand, no significant increase in common deletion with age in AD has been reported. Major arc deletions showed a significant positive correlation with age in nigral neurons, while the level of mtDNA deletions was commonly detected at low levels and did not increase with age in frontal neurons and Purkinje cells.

Other mtDNA deletions have been investigated in relation to age. The accumulation of the 7436 bp deletion and a unique 12 kb deletion were also age-related in brain samples. In addition, several multiple deletions (4.5–7.1 kb), including m.7409_13687del, have also been reported in the substantia nigra of both aged patients with PD and controls. Apart from these findings, one study showed that mtDNA deletions were associated with chronic hypoxia conditions rather than ageing in the samples of patients with PsyD. mtDNA technical issues

Historically, studies exploring mtDNA variants often used radiolabelled nucleotides, primers or probes for PCR, sequencing or Southern blot techniques, while most recent studies have used exome or genome sequencing. The mtDNA CN can be assessed by quantitative real-time PCR (qPCR). Some studies explored just one region, while others investigated mtDNA alterations in distinct brain regions, in homogenate tissues, or in laser-captured single cells. Although most of the studies used molecular techniques focused on mtDNA sequences, others were based on obtaining mtDNA. In this case, the first and crucial step of mtDNA analysis is effective extraction. Phenol-chloroform DNA extraction, which isolates both nDNA and mtDNA, is a widely used method. Devall et al. performed the first systematic comparison of the effectiveness of five different mtDNA isolation protocols from frozen postmortem brain tissue. They reported that linear DNA digestion that leaves circular DNA (mtDNA) intact gave the lowest purity (mtDNA/nDNA), while the magnetic isolation of mitochondria using anti-human TOM22-labeled microbeads to isolate mitochondria gave the highest mtDNA enrichment.

Although PCR-based technologies have accelerated the analysis of mtDNA deletions, their effectiveness can vary. Distinct results were obtained when comparing the serial dilution PCR method (in which total DNA is diluted and amplified by primers spanning the common deletion) and the kinetic PCR method (based on removing reaction tubes from 10 to 20 cycles for undetected mtDNA and stopping reactions from 22 to 32 cycles for deleted mtDNA to obtain the ratio of deleted mtDNA to normal mtDNA). According to their serial dilution PCR results, the caudate had 10 times more deleted mtDNA than the parietal cortex, while kinetic PCR resulted in a lowering difference. Taylor et al. used a digital deletion detection (3D) assay for absolute quantification and
characterization of rare mtDNA deletions in aged human brain samples. This technique involves an enrichment step for deleted molecules by wild-type targeted endonucleolytic digestion, the amplification of intact mutant molecules by target-specific TaqMan probes in water-in-oil droplets, and, finally, a quantification step for chambers carrying many droplets representing thousands of single-molecule reactions.

As an alternative to standard PCR techniques, Marquis et al. developed a novel sensitive mtDNA assay that used rolling circle amplification and sequencing (MitoRS) to detect mtDNA variants and their heteroplasmy level with high accuracy. In the first step, they used Phiz9 polymerase (with a low error rate and strong strand displacement activity) to generate several individual mtDNA copies (mtDNA enrichment) that were not species-specific and were insensitive to nuclear mtDNA sequences and to mtDNA polymorphism priming events. Combined with high-throughput tagmentation-based library generation for next-generation sequencing (NGS), they could quantify mtDNA SNVs at the minimum 1% frequency level.

Some additional difficulties have been reported in mtDNA analyses. The chromogen 3,3′-diaminobenzidine, a standard stain for COX activity, has a strong inhibitory effect on qPCR, thus causing significant bias in the estimation of mtDNA CN and deletion levels between COX-positive and COX-negative neurons. Regarding methylation, Devall et al. developed an assay to identify differentially methylated regions in mtDNA among different regions of the cortex and cerebellum by using pre-existing methylated DNA immunoprecipitation sequencing data. Interestingly, they identified 74 nominally differentially methylated regions in the mtDNA and 8 differentially methylated regions between the total cortex and cerebellum.

Discussion

Biological processes are defined not only by cell structures but also by energy status. The brain represents between 2 and 3% of the weight of our body while consuming 20% of the total energy, which is mainly generated in the mitochondria. Unlike muscle, the brain is always highly metabolically active and thus is highly sensitive to mitochondrial functioning. For this reason, the mitochondria operate under several control mechanisms, such as mitochondrial fusion and fission, the removal of damaged proteins, and mitophagy. Recently, it has been suggested that if the available energy is limited, remaining below the bioenergetic threshold, neurological symptoms may appear; however, if the bioenergetic defect is subtler, the lack of energy can lead to the appearance of psychiatric symptoms.

We collected mtDNA variants, CN and/or deletions reported in postmortem human brain samples. mtDNA variants and deletions can be inherited or occur at the germline or somatic level and have been associated with clinical and nonclinical conditions, while mtDNA CN is a proxy measure for mitochondrial function that has been associated with ageing-related diseases. Multiple mtDNA deletions and duplications can arise due to the accumulation of multiple errors in postmitotic tissues, often with clonal expansion of one particular mtDNA form, or be attributed to variants in nuclear genes involved in mtDNA maintenance and repair. Some MitD syndromes arise as a result of a sporadic large-scale single deletion that is the only deletion present. This single deletion can be of any size but there is a common deletion of 4.9kb. Notably, none of the studies in this review reported mtDNA duplications.

This review identified that many pSNVs are present in high heteroplasmy levels (generally >80%) in the postmortem brains of patients with MitD, although with variable heteroplasmy levels across brain regions and nonbrain tissues. However, the clinical condition characterized by early-onset cataracts, ataxia and progressive paraparesis showed mutation loads less than 60% in the affected tissues. Some pSNVs may impact the mtDNA CN in the brain, either decreasing or increasing its levels, but few studies have analysed both mtDNA alterations. A low mtDNA CN was reported in most of the studies, but not all. mtDNA deletions have only been investigated in KSS and DNA polymerase gamma gene (POLG) encephalopathy. Only one study investigated the three types of mtDNA alterations in patients affected by POLG encephalomyopathy, and interestingly, the three types were present. All-encompassing mtDNA analyses were not performed in most of the reviewed studies and should be encouraged in prospective studies.

The current data from postmortem human brain samples indicate that pSNVs do not have a prominent role in NeuD or PsyD, and a reduced mtDNA CN has been extensively observed in NeuD but not PsyD. Conversely, mtDNA deletions may have a more prominent role in PsyD. This would be in accordance with the hypothesis that MitD may be underdiagnosed in some metabolic disorders that are often not considered because they are not the target of the studies. Regarding this issue, the parietal cortices of diabetic individuals showed a 47% reduction in the mtDNA CN compared with those of nondiabetic individuals. In nondiabetic AD subjects, the loss of mtDNA could lead to the loss of mitochondrial mass and bioenergetic capacity, whereas in diabetic AD subjects, an increased nutrient supply...
due to insulin resistance and hyperglycaemia could result in reduced oxidative phosphorylation and increased glycolysis, which would also lead to an energy deficit. In line with this idea, in one of the evaluated studies, a high-impact deletion was present in a patient with MDD who also had diabetes.\textsuperscript{144} Future studies should include all the phenotypic characteristics of the assessed individuals to shed light on the role of mtDNA alterations in other comorbid traits. Notably, in NeuD and PsyD, many of the early studies on human post-mortem brain samples were based on the comparison of frequencies and heteroplasmy levels of specific SNV between patients and controls, and even though some reported significant differences, these results have not been confirmed in a more recent and larger dataset, which advocates that mtDNA CN rather than mtDNA SNV would have more clinical relevance in NeuD.\textsuperscript{84} However, the association of homoplasmic common and rare SNV on longevity and NeuD such as multiple sclerosis has been recently confirmed by analysing a large number of blood samples.\textsuperscript{12} An interesting study that also investigated blood samples demonstrates the involvement of the nuclear female genome in the evolution of human mtDNA variation and also suggests the different values of heteroplasmy that an individual cell, tissue or organism may exhibit during embryonic development of human germ cells.\textsuperscript{103} The different expansion of heteroplasmic variants during the ageing process could have consequences for age-associated diseases such as NeuD but also PsyD, which present in many cases within certain age ranges. However, the biological processes associated with ageing have mostly been studied in relation to NeuD.\textsuperscript{5}

Different methodological issues may have interfered with the results reported in this systematic review. These include, among others, the origin of the samples and the way the DNA was obtained and stored, as some reagents and degraded samples considerably limit the PCR/qPCR technique.\textsuperscript{104} In addition, PCR-based techniques may favour the amplification of short (deleted) versus long (nondeleted) fragments resulting in inadequate detection of mtDNA fragments. NGS methodology based on previous long-range amplification is the most powerful tool to detect mtDNA alterations. It provides high and uniform coverage of each of the 16,569 bases, allowing the detection of nucleotide changes, as well as heteroplasmic levels. Moreover, it also allows the detection of small insertions/deletions (indels) and large deletions and the mapping of exact deletion breakpoints.\textsuperscript{165} Several bioinformatic tools allow a reliable analysis of large mtDNA sequences with high coverage levels for detecting mtDNA alterations.\textsuperscript{124,166–169} Additionally, current genome and exome sequencing techniques can detect heteroplasmic levels at less than 5%, and it has been reported that heteroplasmic levels depend on the coverage and the number of sequence reads. Moreover, these techniques have also been reported to be more accurate for mtDNA CN quantification than the gold standard qPCR technique.\textsuperscript{169} In any case, even with the NGS technique, it is necessary to take into account the initial enrichment strategy used and the reference used in the downstream bioinformatic analysis, as both may influence the accurate detection and quantification of mtDNA heteroplasmy levels.\textsuperscript{170}

A broad understanding of brain regional variation regarding heteroplasmy levels of pSNVs, mtDNA CN and mtDNA deletions is necessary for understanding the metabolic requirements of different regions of the brain, which can improve our understanding of region-specific cell type changes and/or vulnerability to metabolic insults and related neuropathological processes. Highly automated and sensitive tools to evaluate mtDNA alterations from data obtained through exome or genome sequencing are currently available.\textsuperscript{124,166,167,171,172} and larger datasets should be assessed for mtDNA analyses, along with a full phenotypic characterization to better associate molecular mtDNA defects or variations with biochemical, metabolic and clinical or health aspects. This is supported by the recent success in identifying associations between a large number of phenotypes and homoplasmic mtDNA variants by analysing a large number of blood samples from the UK Biobank.\textsuperscript{32} In summary, most studies that explored mtDNA alterations in neurological diseases, mental illnesses and the natural ageing process have reported conflicting results. Some reasons for this are that different studies have investigated different mtDNA alterations, different diagnoses and/or different brain regions. This has made it difficult to draw conclusions. In addition, methodological issues mainly related to the techniques used but also to sample collection have led to difficulties in data interpretation. More studies are needed to identify the specific mtDNA alterations associated with health and disease. Nevertheless, the findings discussed in this systematic review argue for the involvement of mtDNA in brain disorders.

**Limitations**

Many of the sample sizes used to study mtDNA CN were underpowered. As an example, differences in mtDNA CN between patients with AD and control individuals were not often identified in the cerebellum\textsuperscript{18,49} until a larger number of patients and controls were screened.\textsuperscript{62} The techniques used in early mitochondrial genetics studies are not comparable to more recent technologies. It would therefore be interesting if some of the phenotypes that were analysed at the time of identifying the first mtDNA alterations could be analysed with the new technologies, especially to confirm the levels of heteroplasmy that were indicated at the time.
Conclusions
This study provides a comprehensive summary of the mtDNA alterations reported in human brain samples. The results identified in relation to MitD provide a clear idea of which genetic alteration is involved in each disorder, despite the great heterogeneity of the alterations described. Unfortunately, this is not the picture for NeuD and PsyD, where the findings are often contradictory. While mtDNA alterations have pathological implications in MitD, for most of the alterations identified in NeuD and PsyD an association has been suggested, but the pathological consequences are not yet proven. Low mtDNA CN is the most reported mtDNA alteration in NeuD, and specific mtDNA deletions may have prominent consequences for PsyD. There is also strong and abundant evidence that the ageing process is related to neurodegeneration and the loss of mtDNA integrity. Future research directions should include mtDNA analyses in larger samples and concurrent analyses of all types of mtDNA alterations and in several brain regions. Additionally, genotype-phenotype correlation studies will help to advance our understanding of the underlying molecular mechanisms and the implications of mtDNA variability in health and disease.

Declaration of interests
The authors declare that they have no conflicts of interest.

Contributors
Conceptualization and design, L.M.; Methodology, writing and editing, A.V.-P., J.T., B.K.B., and L.M.; Writing and editing, E.V., G.G., and G.M.; Acquisition and verification of reported data, A.V.-P., J.T., B. K.B. and L.M. All authors contributed to the interpretation of the findings, read and approved the final version of the manuscript, had full access to all the data and final responsibility for the decision to submit for publication.

Acknowledgments
This work was supported by the Instituto de Salud Carlos III fellowship PI18/00514 to Lourdes Martorell and Gerard Muntané and by the IISPV-Cerca Programme of the Generalitat de Catalunya, cofunded by FEDER. Alba Valiente-Pallejà was the recipient of a Talent-IISPV fellowship from the Diputació de Tarragona. Juan Tortajada and Bengisu Buldük were the recipients of an industrial doctoral (DI-21) and a grant for the recruitment of new research staff (FIDGR-2020) respectively, from the Generalitat de Catalunya. Figures showing mtDNA variants and deletions in the human mtDNA map were created with BioRender.com.

Data sharing statement
The data collected for this study can be provided upon reasonable request.

Supplementary materials
Supplementary material associated with this article can be found in the online version at doi:10.1016/j.cebmi.2022.1035815.

References
1. Attwell D, Laughlin SB. An energy budget for signaling in the grey matter of the brain. J Cereb Blood Flow Metab. 2001;21:1133–1145.
2. Lux NZ, Gorman GS, Turnbull DM. Review: Central nervous system involvement in mitochondrial disease. Neuropathol Appl Neurobiol. 2017;43:112–118.
3. Pei L, Wallace DC. Mitochondrial etiology of neuropsychiatric disorders. Biol Psychiatry. 2018;85:722–730.
4. Lujan SA, Longley MJ, Humbale MH, et al. Ultrasensitive deletion detection links mitochondrial DNA replication, disease, and aging. Genome Biol. 2020;21:248.
5. Hou Y, Dan X, Babbar M, et al. Ageing as a risk factor for neurodegenerative disease. Nat Rev Neuro. 2019;17:565–581.
6. Spinelli JB, Haigis MC. The multifaceted contributions of mitochondria to cellular metabolism. Nat Cell Biol. 2018;20:741–754.
7. Formosa LE, Ryan MT. Mitochondrial OXPHOS complex assembly lines. Nat Cell Biol. 2019;20:711–713.
8. Meyer A, Laveryn G, Bernardi L, et al. Mitochondria: An organelle of bacterial origin controlling inflammation. Front Immunol. 2018;9:516.
9. El-Hattab AW, Scaglia F. Mitochondrial cytopathies. Cell Calcium. 2016;60:199–206.
10. El-Hattab AW, Craigen WJ, Scaglia F. Mitochondrial DNA maintenance defects. Biochim Biophys Acta Mol Basis Dis. 2017;1861:1539–1555.
11. Giles RE, Blanc H, Cann HM, Wallace DC. Maternal inheritance of human mitochondrial DNA. Proc Natl Acad Sci U S A. 1980;77:6715–6719.
12. Anderson S, Bankier AT, Barrell BG, et al. Sequence and organization of the human mitochondrial genome: There is a difference between phylogenetic and pedigree rates. J Mol Biol. 1980;141:195–212.
13. Schapira AH. Mitochondrial disease. Lancet. 2006;368:79–82.
14. Keogh MJ, Chinnery PF. Mitochondrial DNA mutations in neurodegeneration. Biochim Biophys Acta. 2015;1847:1401–1411.
15. Wang L. Mitochondrial purine and pyrimidine metabolism and beyond. Vol. 35. Nucleotides Nucleic Acids. 2016;35:578–594.
16. Wallace DC. A mitochondrial etiology of neuropsychiatric disorders. JAMA Psychiatry. 2017;74:863–864.
17. Lynch M, Koskella B, Schaack S. Mutation pressure and the evolution of organellar genomic architecture. Science. 2006;311:1727–1730.
18. Howell N, Smejkal CB, Mackey DA, Chinnery PF, Turnbull DM, Herrnstadt C. The pedigree rate of sequence divergence in the human mitochondrial genome: There is a difference between phylogenetic and pedigree rates. Am J Hum Genet. 2003;72:659–670.
19. Lawless C, Greaves L, Reeve AK, Turnbull DM, Vincent AE. The rise and rise of mitochondrial DNA mutations. Open Biol. 2016;6:100056.
20. Kaupilla TES, Kaupilla JHK, Larsson NG. Mammalian mitochondrial aging: an update. Cell Metab. 2017;25:37–71.
21. Sun N, Yohe RJ, Finkel T. The mitochondrial basis of aging. Mol Cell. 2016;61:654–666.
22. Corral-Debrinski M, Horton T, Lott MT, Shoffner JM, Beal MF, Wallace DC. Mitochondrial DNA deletions in human brain: Regional variability and increase with advanced age. Nat Genet. 1992;2:324–329.
23. Taylor SD, Ericson NG, Burton JN, et al. Targeted enrichment and high-resolution digital profiling of mitochondrial DNA deletions in human brain. Aging Cell. 2014;13:269–278.
24. Kennedy SR, Salk JJ, Schmitt MW, Loeb LA. Ultra-sensitive sequencing reveals an age-related increase in somatic mitochondrial mutations that are inconsistent with oxidative damage. PLoS Genet. 2013;9:1003794.
92 Rice AC, Keeney PM, Algarzae NK, Ladd AC, Thomas RR, Bennett de la Monte SM, Luong T, Neely TR, Robinson D, Wands JR. Mito-
77 Tanno Y, Yoneda M, Tanaka K, et al. Uniform tissue distribution of functional integrity of mitochon-
90 Ito S, Ohta S, Nishimaki K, et al. Maternally inherited enceph-
79 Santorelli FM, Tanji K, Sano M, et al. Maternally inherited enceph-
87 Chang SW, Zhang D, Chung HD, Zassenhaus HP. The frequency of point mutations in mitochondrial DNA is elevated in the brains of Parkinson's disease. 
73 Nagashima T, Mori M, Katayama K, et al. Adult Leigh syndrome caused by 8993 T—G mitochondrial DNA mutation in mitochondrial DNA in MERRF patients. 
92 Arthur CR, Morton SL, Dunham LD, Keeney PM, Bennett JP. Mitochondrial DNA mutation underlying Leigh's syndrome: clinical, pathologi-
1992;50:852–858.
1997;12:639–645.
1999;241:221–225.
1993;43:2262–2268.
1991;144:710–715.
1999;17:450–452.
1997;17:450–452.
1994;147:437–446.
1995;29:217–224.
1995;4:37.
2016;11:e0154582.
2002;17:450–452.
2019;73:161–170.
2016;31:352–359.
2019;9:11386.
2000;80:1323–1335.
2000;135:409–425.
1992;50:852–858.
2002;17:450–452.
2003;54:473–478.
1997;12:639–645.
1994;91:1038–1043.
1994;147:437–446.
1995;29:217–224.
1996;36:149–153.
1996;36:149–153.
1994;147:437–446.
1995;29:217–224.
1997;17:450–452.
1995;4:37.
2019;9:11386.
2000;135:409–425.
1992;50:852–858.
1994;147:437–446.
1995;4:37.
2019;9:11386.
2000;135:409–425.
1992;50:852–858.
1994;147:437–446.
1995;4:37.
2019;9:11386.
2000;135:409–425.
1992;50:852–858.
1994;147:437–446.
1995;4:37.
2019;9:11386.
2000;135:409–425.
1992;50:852–858.
1994;147:437–446.
1995;4:37.
2019;9:11386.
2000;135:409–425.
1992;50:852–858.
1994;147:437–446.
1995;4:37.
2019;9:11386.
Marchbanks RM, Ryan M, Day IN, Owen M, McGuffin P, Whatley Kapsa RM, Jean-Francois MJ, Lertrit P, et al. Mitochondrial DNA Mawrin C, Kirches E, Krause G, et al. Region-specific analysis of Ichikawa T, Arai M, Miyashita M, et al. Mitochondrial DNA Munakata K, Iwamoto K, Bundo M, Kato T. Mitochondrial DNA Hjelm BE, Rollins B, Morgan L, et al. Splice-Break: Exploiting an Ichikawa T, Arai M, Miyashita M, et al. Schizophrenia: maternal B o d e n s t e i D F , K i m H K , B r o w n N C , N a v a i d B , Y o u n g L T , A n d r e a z z a Rubino F, Piredda R, Calabrese FM, et al. HmtDB, a genomic Bodenstein DF, Kim HK, Brown NC, Navid B, Young LT, Andreuza AC. Mitochondrial DNA content and oxidation in bipolar disorder and its role across brain regions. NPJ Schizophr. 2019;5:1–8.

Mandani F, Rolls B, Morgan L, Sequeira PA, Vawter MP. The somatic common deletion in mitochondrial DNA is decreased in schizophrenia. Schizophr Res. 2014;159:370–375.

Friso GR, Bauer HE, Scaini G, et al. Accelerated hippocampal biological aging in bipolar disorder. Bipolar Disord. 2020;22:498–507.

Torrell H, Montanà E, Abasolo N, et al. Mitochondrial DNA (mtDNA) in brain samples from patients with major psychiatric disorders: gene expression profiles, mtDNA content and presence of the mtDNA common deletion. Am J Med Genet B. 2015;162B:213–223.

Kato T, Stine OC, McMahan FJ, Crowe RR. Increased levels of a mitochondrial DNA deletion in the brain of patients with bipolar disorder. Biol Psychiatry. 1997;44:871–875.

Fuks S, Kametani M, Kato T. Quantitative analysis of the 4977 bp common deletion of mitochondrial DNA in postmortem frontal cortex samples from patients with bipolar disorder and schizophrenia. Neuropadiet Lett. 2008;43:171–177.

Nadati EA, Melegh B, Seres I, Kosztolányi G. Mitochondrial DNA 4977 deletion in brain of newborns died after intensive care. Acta Bioi Hung. 2005;54:253–262.

Roca-Bayyar C, Robertson F, Pyle A, Hudson G, Payne BAI. Mitochondrial DNA damage and brain aging in human immunodeficiency virus. Clin Infect Dis. 2015;61:142–149.

Jain EE, Cavaleri L, Eriksson I, Orelan L, Gylensten U. Human brain contains high levels of heteroplasy in the noncoding regions of mitochondrial DNA: evidence for adaptive selective forces. Proc Natl Acad Sci U S A. 1995;92:1328–1332.

Cantuti-Castelvetri I, Lin MT, Zheng K, et al. Somatic mitochondrial DNA mutations in single neurons and glia. Neurobiol Aging. 2005;26:1343–1355.

Kapa RM, Jean-Francois MJ, Lertrit P, et al. Mitochondrial DNA polymorphism in substantia nigra. J Neurol Sci. 1996;144:204–211.

Simon DK, Lin MT, Zheng L, et al. Somatic mitochondrial DNA mutations in cortex and substantia nigra in aging and Parkinson’s disease. Neurobiol Aging. 2004;25:351–361.

Frahm T, Mohamed SA, Pazzaglia C, Oehmichen M, Meissner C. Lack of age-related increase of mitochondrial DNA amount in brain, skeletal muscle and human heart. Mech Ageing Dev. 2005;126:1192–1200.

Meissner C, Bruse P, Mohamed SA, et al. The 4977 bp deletion of mitochondrial DNA for human in skeletal muscle, heart and different areas of the brain: a useful biomarker or more? Exp Gerontol. 2008;43:645–652.

Mawrin C, Kirches E, Krause G, et al. Region-specific analysis of mitochondrial DNA deletions in neurodegenerative diseases in humans. Neuropedi et Lett. 2004;137:111–114.

McDonald RPA, Horsburgh RJ, Graham DI, Nicoll JAR. Mitochondrial DNA deletions in acute brain injury. NeuroReport. 1999;10:387–393.

Kravtsovsky Y, Kudryavtseva E, Mcke AC, Geula C, Kowall NW, Khrapko K. Mitochondrial DNA deletions are abundant and cause functional impairment in aged human substantia nigra neurons. Nat Genet. 2003;38:418–420.

Zhang C, Bauer A, Maxwell RJ, Linnane AW, Nagle P. Multiple mitochondrial DNA deletions in an elderly human individual. FEBS Lett. 1992;307:34–38.

Cortopassi GA, Arbeeni N. Detection of a specific mitochondrial DNA deletion in tissues of older humans. Nucleic Acids Res. 1990;18:6927–6933.

Soong NW, Hinton DR, Cortopassi G, Arbeeni N. Mosaicism for a specific somatic mitochondrial DNA mutation in adult human brain. Nat Genet. 1992;2:318–324.

Bender A, Krishnan KJ, Morris CM, et al. High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. Nat Genet. 2006;38:515–517.

Mann VM, Cooper MJ, Schapira AHV. Quantification of a mitochondrial DNA deletion in Parkinson’s disease. FEBS Lett. 1992;299:218–222.

Lezza AM, Mezetti P, Corgio A, et al. Mitochondrial DNA 4977 bp deletion and OH 8 DQ levels correlate in the brain of aged subjects but not Alzheimer’s disease patients. FASEB J. 1999;13:1863–1888.

Melos S, Schneider JA, Coskun PE, Bennett DA, Wallace DC. Mitochondrial DNA rearrangements in aging human brain and in situ PCR of mtDNA. Neurobiol Aging. 1999;20:665–751.

Merril CR, Zullo S, Gianlance H, et al. Possible relationship between conditions associated with chronic hypoxia and brain mitochondrial DNA deletions. Arch Biochem Biophys. 1996;328:172–177.

Deval M, Burrage J, Caswell R, et al. A comparison of mitochondrial DNA deletion analysis: a practical comparison of PCR quantitative methods. Biochem Biophys Res Comm. 1995;209:842–847.

Hamblet NS, Castorri FJ. Mitochondrial DNA deletion analysis: a comparison of PCR quantitative methods. Biochem Biophys Res Comm. 1995;209:842–847.

Marquis J, Lefebvre G, Kourmpetis YAI, et al. MitORS, a method for high throughput, sensitive, and accurate detection of mitochondrial DNA heteroplasmy. BMC Genomics. 2017;18:316.

Dölle C, Bindolf LA. Tzoulis C, 153 Diaminobenzidine staining interferes with PCR-based DNA analysis. Sci Rep. 2018;8:13722.

Deval M, Smith RG, Jeffries A, et al. Regional differences in mitochondrial DNA methylation in human post-mortem brain tissue. Clin Epigenetics. 2017;9:47.

Sharma P, Sampath H. Mitochondrial DNA integrity: role in health and disease. Int J Mol Sci. 2017;18:1037.

Yang SY, Castellani CA, Longchamps RJ, et al. Blood-derived mitochondrial DNA copy number is associated with gene expression across multiple tissues and is predictive for incident neurodegenerative disease. Elife. 2019;8:e4773.

Tzoulis C, Tran GT, Coxhead J, et al. Molecular pathogenesis of polymeerase gamma-related neurodegeneration. Ann Neurol. 2014;76:86–91.

Tzoulis C, Tran GT, Schwarzmüller T, et al. Severe nigrostriatal degeneration without clinical parkinsonism in patients with polymeerase gamma mutations. Brain. 2013;136:3295–3304.

Grammegna LL, De Luca G, et al. Cerebral mitochondrial DNA mutational profiling in postmortem frontal cortex of patients with Parkinson’s disease. BMC Genomics. 2016;17:51.

Hamblet NS, Castorri FJ. Mitochondrial DNA deletion analysis: a comparison of PCR quantitative methods. Biochem Biophys Res Comm. 1995;209:842–847.

Marquis J, Lefebvre G, Kourmpetis YAI, et al. MitORS, a method for high throughput, sensitive, and accurate detection of mitochondrial DNA heteroplasmy. BMC Genomics. 2017;18:316.

Dölle C, Bindolf LA. Tzoulis C, 153 Diaminobenzidine staining interferes with PCR-based DNA analysis. Sci Rep. 2018;8:13722.

Deval M, Smith RG, Jeffries A, et al. Regional differences in mitochondrial DNA methylation in human post-mortem brain tissue. Clin Epigenetics. 2017;9:47.

Sharma P, Sampath H. Mitochondrial DNA integrity: role in health and disease. Int J Mol Sci. 2017;18:1037.

Yang SY, Castellani CA, Longchamps RJ, et al. Blood-derived mitochondrial DNA copy number is associated with gene expression across multiple tissues and is predictive for incident neurodegenerative disease. Elife. 2019;8:e4773.

Tzoulis C, Tran GT, Coxhead J, et al. Molecular pathogenesis of polymeerase gamma-related neurodegeneration. Ann Neurol. 2014;76:86–91.

Tzoulis C, Tran GT, Schwarzmüller T, et al. Severe nigrostriatal degeneration without clinical parkinsonism in patients with polymeerase gamma mutations. Brain. 2013;136:3295–3304.
prioritization of mitochondrial DNA variants of clinical interest. *Hum Genet*. 2016;135:121–136.

167 Calabrese C, Simone D, Diroma MA, et al. MToolBox: a highly automated pipeline for heteroplasmy annotation and prioritization analysis of human mitochondrial variants in high-throughput sequencing. *Bioinformatics*. 2014;30:3165–3177.

168 Damas J, Carneiro J, Amorim A, Pereira F. MitoBreak: The mitochondrial DNA breakpoints database. *Nucleic Acids Res*. 2014;42:D1261–D1268.

169 Longchamps RJ, Castellani CA, Yang SY, et al. Evaluation of mitochondrial DNA copy number estimation techniques. *PLoS ONE*. 2020;15:e0228166.

170 Santibanez-Koref M, Griffin H, Turnbull DM, Chinnery PF, Herbert M, Hudson G. Assessing mitochondrial heteroplasmy using next generation sequencing: A note of caution. *Mitochondrion*. 2019;46:302–306.

171 Goudenège D, Bris C, Hoffmann V, et al. eKLIPse: a sensitive tool for the detection and quantification of mitochondrial DNA deletions from next-generation sequencing data. *Genet Med*. 2019;21:1407–1416.

172 Basu S, Xie X, Uhler JP, et al. Accurate mapping of mitochondrial DNA deletions and duplications using deep sequencing. *PLoS Genet*. 2020;16:e1009344.