Chapter 2
Neurodegeneration in Mitochondrial Disorders

Jonathan Phillips, Hannah Hayhurst, and Nichola Zoe Lax

Abstract Mitochondria are critically responsible for the generation of energy in the form of adenine triphosphate (ATP) through the mitochondrial respiratory chain (see Chap. 1). The central nervous system (CNS) performs highly energy-intensive tasks and is therefore particularly dependent on ATP. Defects residing within the complexes of the respiratory chain can affect the synthesis of ATP and consequently severely compromise neuronal function. It is unsurprising then that mitochondrial DNA (mtDNA) defects are an important cause of neurological disease. The clinical presentation is often heterogeneous in terms of age of onset and different neurological signs and symptoms which might include ataxia, seizures, cognitive decline, blindness and stroke-like episodes. The clinical course can vary considerably, but in many patients there are progressive neurological decline and marked neurodegeneration. Our understanding of the mechanisms underpinning neurodegenerative changes due to mitochondrial DNA defects is limited due to the availability of appropriate animal models of disease. However, studies on human post-mortem CNS tissues have provided an invaluable insight into the distribution and severity of neuronal degeneration in patients harbouring mitochondrial DNA defects. In this chapter, we describe the neuropathological changes occurring in the CNS associated with different mutations of the mitochondrial genome and discuss the mechanisms which might contribute to neural dysfunction and cell death.

Keywords Mitochondria • Mitochondrial DNA • Respiratory chain deficiency • Neurodegeneration • Neuropathology

2.1 Introduction

Mitochondrial disease was first described in 1962, when Luft and colleagues described a patient with non-thyroidal hypermetabolism [1]. Since this discovery, major advances in our understanding of mitochondrial biology and genetics have

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permitted the recognition of a number of mitochondrial disorders [2]. Mitochondrial diseases are heterogeneous disorders that arise from dysfunction of the mitochondrial respiratory chain due to mutations in either the mitochondrial or nuclear genome. Mitochondrial diseases might be described as primary disorders arising from mtDNA defects, either in the form of point mutations or rearrangements, or secondary disorders due to intergenomic signalling failure resulting in accumulation of mtDNA deletions or mtDNA depletion. MtDNA defects and respiratory chain abnormalities are increasingly linked with the pathogenesis of other neurodegenerative disorders, such as Parkinson's disease. As discussed in Chap. 1, mitochondria contain their own circular double-stranded DNA encoding for 13 polypeptides of the mitochondrial respiratory chain, 22 transfer RNAs (tRNA) and 2 ribosomal RNAs (rRNA) [3]. This genome is under dual genetic control, with many mtDNA maintenance genes encoded by the nuclear genome.

Mitochondrial DNA diseases present an important social and economic burden with an estimated prevalence of 1 in 10,000 people with a clinical manifestation of the disease and a further 1 in 6000 at risk of developing mitochondrial disease [4].

Neurological deficits are consistently reported in patients with mitochondrial disease and are often the most disabling [5]. Neurological symptoms are wide ranging and may include seizures, dementia, peripheral neuropathy, sensorineural deafness, stroke-like episodes and cerebellar ataxia. It is important to recognise that patients with mitochondrial disease do not always conform to the exact clinical criteria/presentation for a specific syndrome and many only manifest with a few features (Table 2.1).

There are a number of reasons why the central nervous system (CNS) is particularly vulnerable to mitochondrial dysfunction. Firstly, the brain is highly metabolically active and therefore particularly susceptible to bioenergetic failure [6]. Secondly, it has considerably fewer antioxidant defences than other tissues, so it is less able to protect against excessive reactive oxygen species (ROS) production [7]. Thirdly, with the exception of the subventricular zone, olfactory epithelium and the hippocampus, most neurons within the brain are post-mitotic and therefore irreplaceable [8]. Consequently, any particular neuronal insult will prove fatal to the cell if not alleviated in some way. In addition, there is increasing evidence linking mitochondrial dysfunction to neuronal cell loss in age-related neurodegenerative disorders, including Alzheimer's disease and Parkinson's disease.

2.2 Neurodegeneration in Mitochondrial Diseases

2.2.1 Brain Atrophy

Post-mortem brain atrophy is a common observation in patients with mitochondrial disease though quantitative data are sparse. In Newcastle, a series of 18 brains dissected at post-mortem with genetically and clinically diverse mitochondrial disease showed evidence of atrophy in 16. The average fresh brain weight was reduced by

Table 2.1 Clinical features of mitochondrial syndromes

Disorder	Primary feature	Causative mutations
Alpers' syndrome	Childhood onset characterised by severe developmental delay, intractable epilepsy and liver failure	The most common mutations are autosomal recessive mutations to the gene, *POLG*, which encodes the mitochondrial DNA polymerase
Chronic progressive external ophthalmoplegia (CPEO)	Bilateral ptosis and external ophthalmoplegia	CPEO can be caused by point mutations in the *MT-TL1* gene or mtDNA rearrangements resulting from mutations in nuclear-encoded mitochondrial maintenance genes such as *POLG*
Leigh syndrome (LS)	Presentation of nystagmus, hypotonia and respiratory dysfunction due brainstem dysfunction in infancy	The mtDNA point mutation, m.8993T>G, accounts for 10 % of affected individuals. Approximately 30 % of patients have a mutation to the nuclear-encoded gene *SURF1* which is involved in the assembly and maintenance of complex IV
Leber hereditary optic neuropathy (LHON)	Degeneration of the retinal ganglion cell layer and optic nerve leading to bilateral subacute loss of central vision with a predilection to males	95 % of LHON cases are due to the following mtDNA point mutations: m.3460G>A (*MT-ND1*), m.11778G>A (*MT-ND4*) and m.14484T>C (*MT-ND6*)
Kearns-Sayre syndrome (KSS)	Presentation of retinitis pigmentosa and progressive external ophthalmoplegia before the age of 20 years	KSS results from a sporadic large-scale deletion in the mtDNA. The most common deletion size is 4779 bp
Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS)	The phenotype is characterised by lactic acidosis, stroke-like episodes and seizures	80 % of MELAS cases are caused by the point mutation m.3243A>G (*MT-TL*)
Myoclonic epilepsy with ragged red fibres (MERRF)	Presentation of cerebellar ataxia, myoclonic epilepsy, myoclonus and myopathy in childhood and adolescence	Over 80 % of MERRF cases are caused by the point mutation, m.8344A>G (*MT-TK*)
Polymerase gamma encephalopathies	The typical syndromes associated with *POLG* mutations are ataxia neuropathy spectrum (ANS) and myoclonic epilepsy myopathy sensory ataxia (MEMSA). Characteristic symptoms associated with these syndromes are ataxia, myoclonic seizures, neuropathy and myopathy	Depletion, multiple deletion and multiple point mutations to mtDNA due to either autosomal dominant or autosomal recessive mutations to the gene, *POLG*, which encodes the mitochondrial DNA polymerase

about 16 % (range: 2.4–36.7 %; Table 2.2) compared to control individuals matched for age and gender. Of the 16 patients with atrophic brains, over half of the patients were aged 20–60 years and harboured a m.3243A>G mutation, three patients were aged between 18 and 55 years and harboured autosomal recessive polymerase gamma (*POLG*) mutations, two patients aged 42 and 58 harboured the m.8344A>G mutation, one patient harboured the m.13094T>C mutation associated with a MELAS (mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes)/Leigh overlap phenotype, one patient aged 50 harboured the m.14709T>C mutation and one patient aged 40 years harboured a single large-scale mtDNA deletion and Kearns-Sayre syndrome (KSS). Brain weights were within the normal limits in two patients aged 59 and 79 years harbouring autosomal recessive *POLG* mutations.

2.2.2 Neuroradiological Imaging

Neuroradiological imaging in patients with mitochondrial diseases often demonstrates focal lesions in cerebral cortex, white matter, basal ganglia and brainstem and particularly in patients with longer disease duration, generalised cerebral and cerebellar atrophy, all suggestive of CNS neuron loss. Enlargement of the fourth ventricle is often profound in younger patients and could be a predictor of cerebellar atrophy [9, 10].

Most of the imaging findings are diverse and nonspecific showing little correlation with genotype or biochemical phenotype, but in some of the mitochondrial diseases, lesions show a tendency for particular anatomical areas of the brain. For instance, in patients with MELAS, there is often prominent dilation of occipital horns with focal stroke-like cortical lesions in a non-vascular distribution generally in the posterior of the brain consistent with stroke-like episodes. At early stages of the disease, the lesions show features of vasogenic oedema, rather than ischaemic-like lesions [11], and often exhibit rapid resolution associated with clinical improvement. However, presence of cerebral atrophy in long-standing disease suggests association with gradual attrition of neuronal cells [9, 10]. In patients with MERRF (myoclonic epilepsy with ragged red fibres) due to the m.8344A>G mutation, neuroradiological imaging most often shows atrophy in the cerebral cortex, cerebellum, superior cerebellar peduncle and brainstem [12, 13]. More recently, MRI showed evidence of thalamic demyelination in a young male harbouring m.8344A>G but without the typical clinical presentation [14]. In patients with Leigh syndrome necrotic-like lesions distributed along the brainstem, thalamus and basal ganglia are considered characteristic [9]. Patients harbouring *POLG* mutations demonstrate a combination of thalamic and cortical lesions without basal ganglia involvement but with lesions in deep cerebellar nuclei and inferior olivary nuclei which can be indicative of mitochondrial spinocerebellar ataxia and epilepsy (MSCAE). In contrast, the main finding in patients with *POLG* mutations and Alpers' syndrome with paediatric onset is stroke-like lesions in occipital cortex [15], although thalamic changes have also been described, particularly in patients presenting as young adults [16, 17].

Table 2.2 Post-mortem brain weights

Patients with mitochondrial disease						Normal cases (Dekaban, Sadowsky. *Ann Neurol*. 1978;4:345–356)		
Age (years)	Sex	Genetic defect	Wt (g)	Wt (% normal)	Wt (% reduction)	Age range (years)	Sex	Wt (g) mean ± SD
47	M	m.3243A>G	1355	94.1 %	5.9 %	41–50	M	1440 ± 20
45	M	m.3243A > G	1200	83.3 %	16.7 %	41–50	M	1440 ± 20
20	F	m.3243A>G	829	63.3 %	36.7 %	19–22	F	1310 ± 10
30	M	m.3243A>G	1174	81.5 %	18.5 %	22–30	M	1440 ± 20
60	F	m.3243A>G	984	78.7 %	21.3 %	56–60	F	1250 ± 20
37	F	m.3243A > G	930	72	28 %	31–40	F	1290 ± 30
42	F	m.3243A>G	1070	82.9 %	17.1 %	41–50	F	1290 ± 20
57	F	m.3243A>G	1150	92 %	8 %	56–60	F	1250 ± 20
42	F	m.8344A>G	1075	83.3 %	16.7 %	41–50	F	1290 ± 30
58	M	m.8344A>G	1121	79.5 %	20.5 %	56–60	M	1410 ± 10
34	F	m.13094T>C	1260	97.6 %	2.4 %	31–40	F	1290 ± 30
55	M	m.14709T>C	1325	94 %	6 %	56–60	M	1410 ± 10
40	F	Single mtDNA deletion	1040	80.6 %	19.4 %	31–40	F	1290 ± 30
18	F	*POLG*	1098	81.9 %	18.1 %	16–18	F	1340 ± 30
24	F	*POLG*	1081	83.2 %	16.8 %	22–30	F	1300 ± 10
55	M	*POLG*	1292	91.6 %	8.4 %	56–60	M	1410 ± 10
59	M	*POLG*	1480	105 %	0 %	56–60	M	1410 ± 10
79	M	*POLG*	1421	105 %	0 %	76–81	M	1350 ± 10

In some mitochondrial diseases imaging shows predominantly white matter lesions. In patients with KSS symmetrical lesions in the white matter of the cerebrum, cerebellum, globus pallidus, dorsal midbrain and thalamus are consistently reported [9]. In mitochondrial neurogastrointestinal encephalopathy (MNGIE) patients, there are diffuse white matter lesions in cerebral hemispheres, brainstem and cerebellar peduncle in addition to demyelinating peripheral neuropathy [18].

Intracerebral calcification has been commonly reported in MELAS, KSS and Leigh syndrome. A neuroimaging study from 1998 revealed that basal ganglia (BG) calcification, involving the caudate, putamen, thalamus and global pallidus, was the most common finding in 54% of patients harbouring the m.3243A>G mutation [10]. A similar distribution pattern is seen in ageing suggesting that BG calcification in mitochondrial disease, and particularly in MELAS, may occur due to accelerated ageing [19]. This particular feature is also seen in a range of insults to the brain including anoxia [20], exposure to radiation [21], and parathyroid [22] and hypothyroid disorders [23], suggesting that this brain region responds to damage by laying down minerals. Although BG calcification in mitochondrial diseases is often severe, it has not been reported in association with BG atrophy. It is also unclear if there are any functional symptoms related to this pathology in the patients with mitochondrial diseases, and while there is evidence of BG calcification in other diseases, there does appear to be a lack of correlation between the degree of pathology and emergence of clinical symptoms [24, 25]. It is intriguing that in the very old and also in younger patients with psychiatric illnesses, psychotic symptoms are strongly associated with basal ganglia calcification [19].

2.3 Neurological Features, Genetics and Neuropathology of Mitochondrial Diseases

Microscopic analyses of post-mortem brain tissue from patients with mitochondrial disease typically show profound neuronal cell loss, evidence of grey matter cortical lesions and accompanying astrogliosis and microgliosis. There is also evidence of mitochondrial respiratory chain impairments, predominantly affecting complexes I and IV in remaining cells, which can be defined using either a sequential cytochrome *c* oxidase and succinate dehydrogenase (COX/SDH) assay or the use of monoclonal antibodies raised against various subunits of the respiratory chain complexes.

2.3.1 Adult-Onset Disorders

2.3.1.1 Kearns-Sayre Syndrome

Symptoms and Cause

Kearns-Sayre syndrome (KSS) is characterised by triad of symptoms including retinitis pigmentosa, progressive external ophthalmoplegia and an onset before the age of 20 years [26]. Other neurological features include deafness, cerebellar ataxia,

raised cerebrospinal fluid (CSF) protein levels, subclinical neuropathy and cognitive impairments (impairments in visuospatial attention and executive function) that are commonly associated with KSS. As KSS is a multisystem disorder, symptoms are not restricted to the nervous system; patients also often present with endocrinopathies, short stature, complete heart block, proximal myopathy and dysphagia [27].

KSS is caused by a single large-scale deletion or complex rearrangements of mtDNA that typically arise sporadically. MtDNA deletions range in size from 2.0 to 7.0 kb with about one third of patients harbouring a 'common deletion' of 4977 bp [28]. These deletions are typically located in the major arc between the between the two proposed origins of replication (O_H and O_L).

Neuropathology

Neuropathologically, KSS is characterised by spongiform encephalopathy in the white matter tracts of the cerebrum, cerebellum (Fig. 2.1a), basal ganglia, thalamus and spinal cord [26, 29, 30]. The spongiform encephalopathy varies in severity from myelin splitting to vacuolation of the tissue [30]. The susceptibility of the white matter in KSS is confirmed by the observation of preferential loss of myelin-associated glycoprotein (MAG), and 2′,3′-cyclic nucleotide phosphodiesterase (CNPase) the reduction in oligodendrocyte lineage cells (see Fig. 2.1b, c) and respiratory chain deficiency in mature oligodendrocytes (see Fig. 2.1d, e) which contribute to distal-dying back of oligodendrocytes in the cerebellum [31]. The pathology is not only restricted to the white matter, with neuronal cell loss observed in the cerebellum and brainstem. In the cerebellum, there is moderate loss of Purkinje cells accompanied by spongiform degeneration, capillary proliferation and reduced mitochondrial respiratory chain protein expression. The brainstem reveals neuronal cell loss and gliosis plus iron deposits, specifically in the globus pallidus [26].

2.3.1.2 Mitochondrial Encephalomyopathy Lactic Acidosis and Stroke-Like Episodes

Symptoms and Cause

The most common mutation associated with mitochondrial encephalomyopathy lactic acidosis and stroke-like episodes (MELAS) is the m.3243A>G mutation in the $tRNA^{Leu}$ gene. Central to the phenotype of MELAS are a triad of symptoms: lactic acidosis, stroke-like episodes and seizures. In addition, individuals can also suffer with hemianopia, hemiparesis, maternally inherited diabetes, deafness and ataxia. m.3243A>G is not the sole mutation to cause the MELAS phenotype; point mutations at positions m.3251A>G [32], m.3271T>C [33] and m.3291T>C [34] within the $tRNA^{Leu}$ gene can also produce a MELAS phenotype. Additionally to the $tRNA^{Leu}$ gene mutations, point mutations within $tRNA^{Val}$ (m.1642G>A) [35] and subunit III of cytochrome *c* oxidase (m.9957T>C) have been shown to be causative of a MELAS phenotype [36].

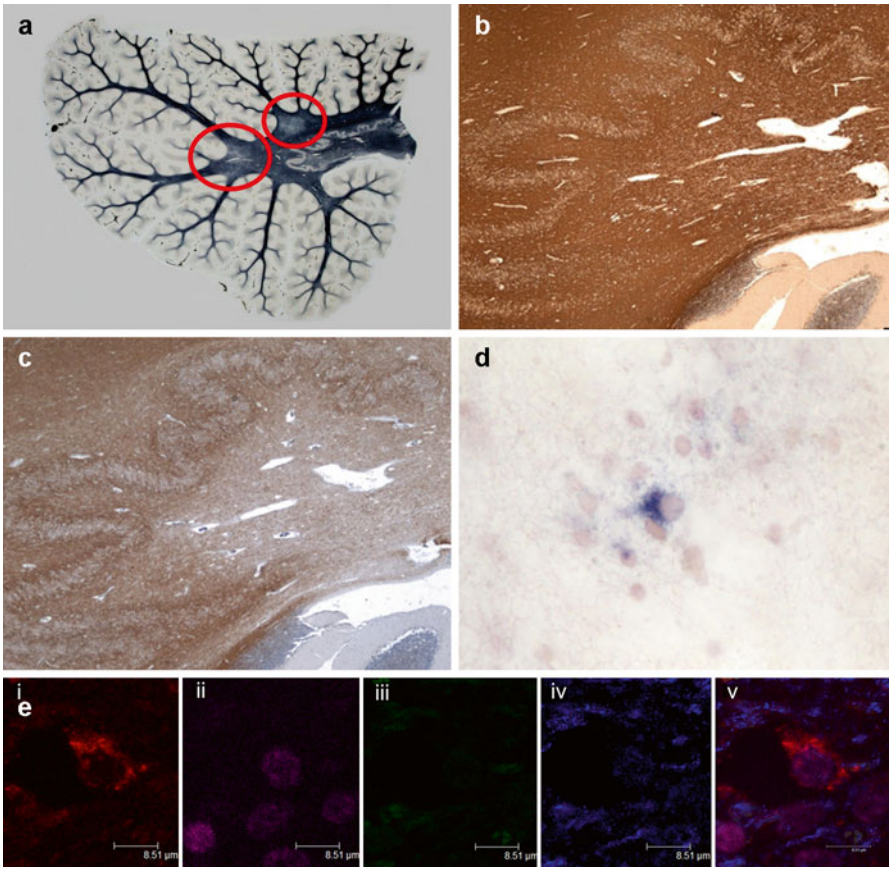


Fig. 2.1 White matter pathology in patients harbouring a single large-scale mtDNA deletion and KSS. Multiple foci of mild spongiform degeneration and myelin pallor are observed in the cerebellar deep white matter of a patient with KSS harbouring a single large-scale mtDNA deletion (**a** *circled*, Loyez myelin stain). High magnification of the white matter neighbouring the dentate nucleus reveals high and uniform expression of myelin basic protein (**b** anti-myelin basic protein immunohistochemistry), while there is a selective loss of CNPase expression in the corresponding region indicating a loss of oligodendrocytes in the dentate nucleus (**c** anti-CNPase immunohistochemistry). Sequential COX/SDH histochemistry reveals COX-deficient cells in the white matter (**d** COX/SDH), while immunofluorescent studies confirm the presence of oligodendrocytes and their nuclei (**e**i and ii anti-Olig2, *red*, and nuclei, *magenta*) which demonstrate a loss of complex IV expression (**e**iii anti-COX1 – *green*) despite high density of mitochondria (**e**iv anti-porin – *green*) which can be seen as a lack of colocalisation in the merged image (**e**v). A loss of complex IV expression is indicative of respiratory chain deficiency in those cells

Neuropathology

The neuropathological hallmarks of MELAS are defined by the presence of 'microinfarct-like' lesions in the occipital, parietal and temporal lobes and cerebellar cortex (Fig. 2.2a, b). The lesions may feature laminar dehiscence and become cystic;

they are associated with necrosis, increased astrogliosis and capillary proliferation. While these lesions are not associated with any major vascular territories [3], there is evidence of respiratory chain impairment in the cells comprising the cerebrovasculature in addition to neurons [37, 38]. Blood vessel wall calcification in the basal ganglia, thalamus and globus pallidus is particularly prominent in these patients; however there is no neuronal involvement in these regions [10]. The cerebellum is almost always affected in these patients with evidence of widespread atrophy of the molecular and granular cell layers accompanied by profound Purkinje cell loss (see Fig. 2.2c, d) and loss of neurons from dentate nucleus. Surviving Purkinje cells undergo a number of morphological changes with the appearance of axonal swellings known as axonal torpedoes in the granular cell layer (see Fig. 2.2g), loss of presynaptic terminals, increased dendritic arborisation and trapped mitochondria within thickened dendritic processes [37, 39]. Respiratory chain deficiencies involving complexes I and IV are particularly prominent in Purkinje cells (see Fig. 2.2e, f) [37, 38] and this has recently been documented in interneurons within the temporal, occipital and frontal lobes [40].

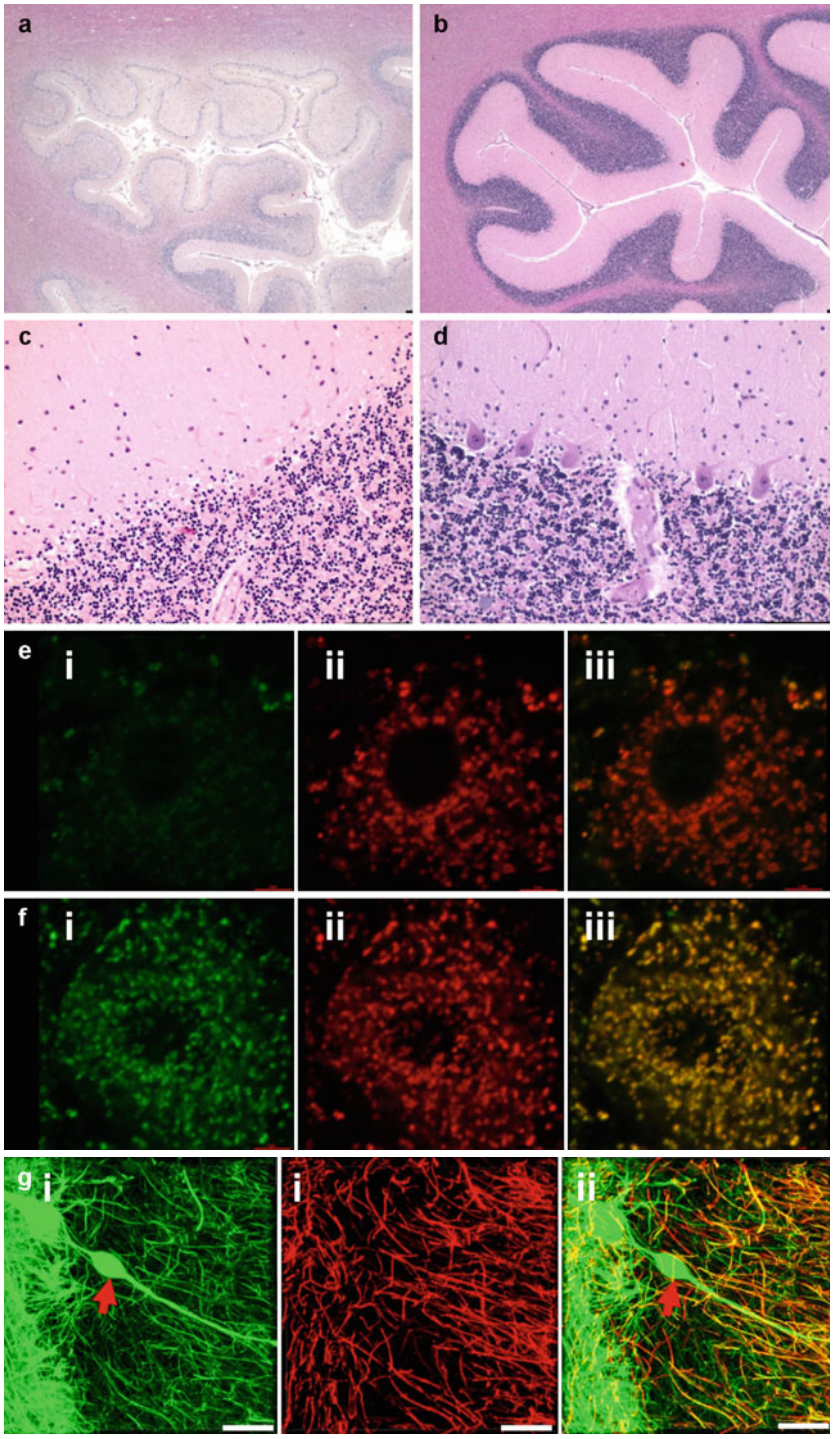
2.3.1.3 Myoclonic Epilepsy with Ragged Red Fibres

Symptoms and Cause

Originally described in 1980, myoclonic epilepsy with ragged red fibres (MERRF) occurs in childhood and early adulthood and is clinically characterised by deafness, seizures, myoclonus, myopathy, ataxia and the presence of ragged red fibres (RRF) in muscle [41]. The RRF may be observed following Gomori trichrome staining to reveal the presence of accumulated mitochondria below the plasma membrane of muscle fibres which might affect the contour of the fibre giving it an irregular or 'ragged' appearance. The typical point mutation associated with MERRF is the m.8344A>G mutation within $tRNA^{lys}$, though a point mutation in the same tRNA, m.8356T>C, produces a phenotype similar to that of MERRF [42].

Neuropathology

The neuropathology of MERRF is characterised by neuronal loss, demyelination and astrocytosis located preferentially within the dentate nucleus and Purkinje cells of the cerebellum and inferior olive, red nuclei and substantia nigra of the brainstem [43]. Pathology is also observed in the spinal cord, with a significant reduction in neurons of Clarke's nucleus as well as mild neuron loss in the anterior and posterior horn [30]. Immunohistochemical analysis of the mitochondrial respiratory chain proteins shows reduced expression of subunits comprising complexes I and IV within the remaining neurons of the cerebellar dentate nucleus, inferior olivary nuclei and frontal cortex [37, 44]. Quantitative immunofluorescent analysis of substantia nigra neurons in a patient with m.8344A>G revealed that 22 % and 23 % of dopaminergic neurons did not contain subunits of complexes I and IV, respectively, despite preserved mitochondrial mass [45]. Another study observed reduced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GABAergic interneuron density in the frontal, temporal and occipital cortices of two patients harbouring the m.8344A>G mutation with remaining interneurons displaying a significant reduction in complex I and complex IV expression [40]. A study looking into the susceptibility of specific neuronal types harbouring the m.8344A>G mutation found that there was no correlation between heteroplasmy level and selective vulnerability of the neurons [46]. This study shows that the pathogenesis of MERRF is likely multifactorial, and neuronal vulnerability is not exclusively determined by high levels of mutated mtDNA.

2.3.1.4 Leber Hereditary Optic Neuropathy

Symptoms and Cause

First described by Theodore Leber in 1871, Leber hereditary optic neuropathy (LHON) is clinically characterised by bilateral subacute loss of central vision due to the focal degeneration of the retinal ganglion cell layer and optic nerve [47]. The disease typically manifests between 20 and 40 years of age, with males being more commonly afflicted. The primary cause of 95 % of LHON cases can be attributed one of three mtDNA point mutations, including m.3460G>A, m.11778G>A and m.14484T>C [48]. Each mutation is present in genes that encode for subunits of complex I of the respiratory chain including *MT-ND1*, *MT-ND4* and *MT-ND6*, respectively.

Neuropathology

The neuropathology of LHON is characterised by progressive degeneration of the retinal ganglion cells and axons affecting the optic nerve. In the early stages of the disease, it is the small calibre axons of the papillomacular bundle that are lost before the rest of the fibres leading to optic atrophy [49]. In a number of LHON cases, the

Fig. 2.2 Cerebellar pathology in patients harbouring the m.3243A>G mutation. Infarct-like lesion involving the molecular layer, Purkinje cells, granular cell layer and the white matter of the cerebellar cortex of a patient (**a** haematoxylin and eosin stain; scale bar 100 μm) in contrast to intact morphology of the cerebellar cortex in a control individual (**b** haematoxylin and eosin stain; scale bar 100 μm). Severe Purkinje cell loss and reduced granular cell density in the cerebellar cortex of a patient (**c** haematoxylin and eosin stain; scale bar 100 μm), while preserved Purkinje cell density is seen in the cerebellar cortex of a control individual (**d** haematoxylin and eosin stain; scale bar 100 μm). Purkinje cells from patients typically reveal prominent loss of complex I (**e**i NUDUFA13 subunit) expression despite high expression of complex IV (COX4 subunit **e**ii) which can be seen in the colocalised image (**e**iii). While Purkinje cells from control individuals reveal high expression of both complexes I (**f**i) and IV (**f**ii) and can be seen in the colocalised image (**f**iii). Scale bar 100 μm. The main constituent of axonal torpedoes (*red arrow*) is phosphorylated neurofilament H (**g**i). There is a lack of myelin (myelin basic protein, **g**ii) around the axonal torpedo as seen in the colocalised image (**g**ii). Scale bar 100 μm

involvement of the spinal cord is observed with demyelination, axon loss and macrophage activation in the gracile fasciculus of the posterior column of the spinal cord [50]. As well as the observation of necrotic lesions and gliosis in the brainstem [51].

2.3.1.5 Polymerase Gamma Encephalopathies

Symptoms and Cause

The mitochondrial genome is replicated by mitochondrial polymerase gamma (POLG). The human mtDNA polymerase is a 195 kDa heterotrimer consisting of a 140 kDa catalytic subunit (PolγA) and two identical 55 kDa accessory units (PolγB). The C-terminus of the catalytic subunit of PolγA is responsible for the polymerase function, while the N-terminus is responsible for exonuclease activity and proofreading of mtDNA. The linker mediates a focal contact with the dimeric accessory subunit. PolγA is encoded by the nuclear *POLG* gene. Primary mutations in the *POLG* gene represent one of the mechanisms that cause secondary mutations in the mtDNA, including depletion of mtDNA, multiple mtDNA deletions or multiple point mutations in mtDNA [52].

Recently, numerous mutations have been described in *POLG* in association with a spectrum of clinical/neurodegenerative phenotypes, including autosomal dominant and autosomal recessive progressive external ophthalmoplegia (PEO) [53–55], myoclonic epilepsy myopathy sensory ataxia (MEMSA) [56], ataxia neuropathy spectrum (ANS) [57], parkinsonism [58] and Alpers' syndrome [59].

Neuropathology

The first neuropathological description of a patient harbouring two recessive heterozygous *POLG* mutations, p.Ala467Thr and a novel p.X1240Gln and secondary multiple mtDNA deletions, was in 2000 [60]. In this patient, the inferior medullary olives were the most severely affected region, showing massive neuronal loss. Moderate to extensive neuronal loss was observed throughout the cerebellum and to a lesser extent in the dentate nucleus and the red nuclei, while the pons remained unaffected. This pattern is indicative of cerebello-olivary atrophy and probably underlies the cerebellar ataxia observed in patients with multiple deletions. A later neuropathological study reported that the spinal cord tissues demonstrated evidence of severe myelin and axonal loss within the posterior columns and in dorsal spinal roots. Severe neuronal loss and respiratory chain abnormalities were also present in dorsal root ganglia and in paraspinal sympathetic ganglia (Fig. 2.3). Ventral and lateral spinal tracts, motor roots and motor neurons were intact [61]. There was also severe depletion of neurons from the substantia nigra without Lewy body formation. Throughout the brain of this patient, there was lack of correlation between the distribution of COX-deficient neurons and the degree of neuropathological damage, similar to the findings in patients with MELAS and MERRF.

The neuropathology of a patient harbouring multiple deletions due to two heterozygous *POLG* mutations revealed the loss of pigmented neurons in the substantia nigra (SN) with alpha-synuclein-reactive Lewy body formation. In addition, unlike

in patients with typical Lewy body-Parkinson's disease (PD) but similar to patients with some mitochondrial diseases, there were loss of Purkinje cells, loss of neurons from the cerebellar dentate nucleus, profound loss of myelin and axons from posterior columns of spinal cord and loss of neurons from gracile nucleus. In the patient with the *POLG* mutations, the remaining SN neurons had high levels of mtDNA deletions (~65 %) associated with COX deficiency in 21 % of the neurons; these levels show a manifold increase compared to <3 % of COX-deficient SN neurons observed in PD patients and <1 % of SN neurons observed in ageing controls [62]. High levels of mtDNA deletions in the substantia nigra have recently been shown in

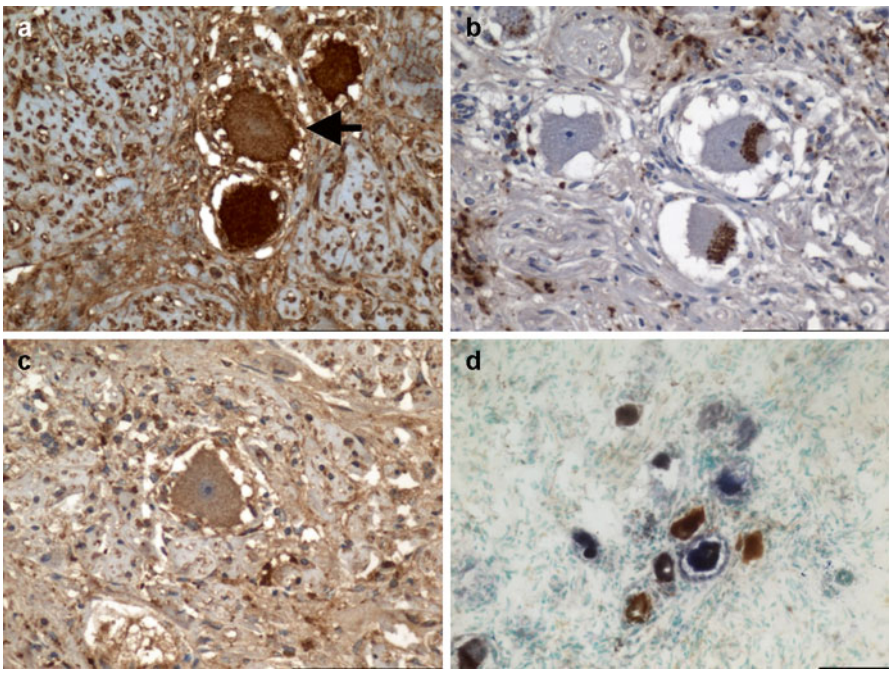

Fig. 2.3 Evidence of prominent deficiencies of mitochondrial respiratory chain proteins within the sensory neurons of the dorsal root ganglia and myelin loss from the dorsal columns of the spinal cord in patients harbouring *POLG* mutations and multiple mtDNA deletions. Unusually reduced mitochondrial density in one neuron (*arrow*) and uneven cellular distribution within sensory neurons (**a** anti-porin immunohistochemistry) which also demonstrate profound neuronal deficiency of the NDUFB4 subunit of complex I with focal cytoplasmic clumping of immunoreactivity (**b** anti-NDUFB4 immunohistochemistry) and reduced expression of the SDHA subunit of complex II within the neurons of the dorsal root ganglia (**c** anti-SDHA immunohistochemistry), similar to the distribution observed with porin. Dual COX/SDH histochemistry reveals a high level of complex IV (COX) deficiency in some neurons (**d** *blue cells*), while in other neurons the COX activity is relatively unaffected (**d** *brown cells*). Further mitochondrial abnormalities can be observed with markedly reduced expression and focal cytoplasmic clumping of subunits I and IV of complex IV in all neurons (**e** anti-COX1 immunohistochemistry and (**f**) anti-COX4 immunohistochemistry – *arrow*). Loss of myelin from the dorsal columns of the spinal cord can be seen in patients (**g** haematoxylin and eosin staining and (**h**) Loyez myelin staining). Scale bar represents 100 μm

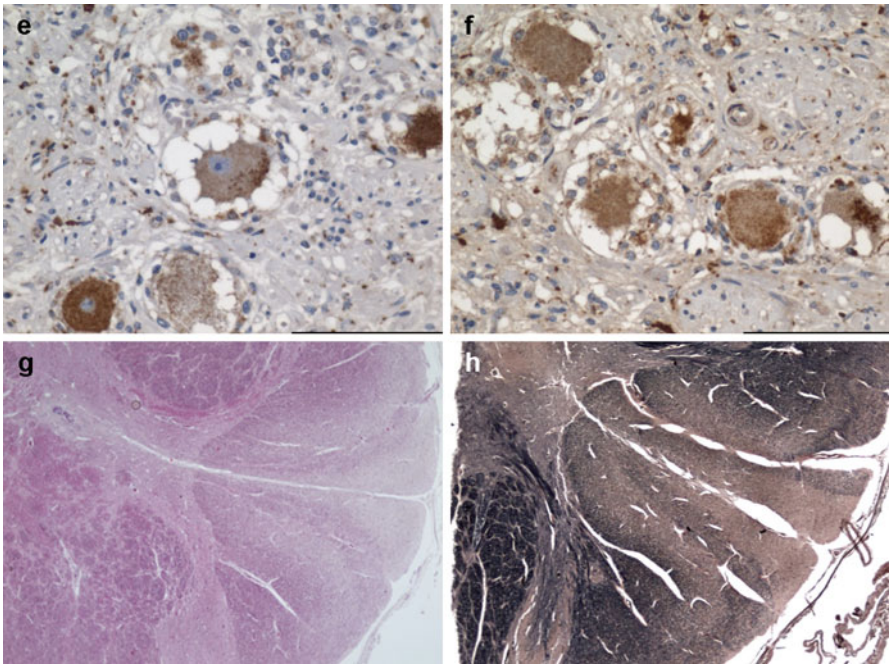

Fig. 2.3 (continued)

a larger cohort of patients with *POLG* mutations. In these patients, severe and time-dependent neuronal cell loss was observed in the cerebellum (affecting both Purkinje cells and dentate nucleus), inferior olivary nucleus and substantia nigra in conjunction with a high percentage of remaining neurons with complex I deficiency. They described foci of necrotic lesions (similar to those observed in patients with the m.3243A>G mutation) in the neocortex, hippocampus, thalamus and cerebellar cortex which they define as 'focal energy-dependent neuronal necrosis' [63].

2.3.2 Childhood-Onset Disorders

2.3.2.1 Leigh Syndrome

Symptoms and Cause

A progressive neurodegenerative disorder has an onset in infants usually before the age of 2 [64]. Leigh syndrome is clinically defined by brainstem dysfunction that results in nystagmus, hypotonia, motor and intellectual retardation and respiratory dysfunctions [65]. In addition to brainstem dysfunction, neurological symptoms including ataxia, dystonia and optic atrophy are common. The symptoms arise due to necrotic lesions to the brainstem, thalamus and basal ganglia, the cause of which

has been attributed to a possible failure in oxidative phosphorylation due to either a mtDNA or nuclear DNA mutation.

Neuropathology

Leigh syndrome can be identified by symmetrical hypointensities on CT scan or hyperintense lesions on T2-weighted MRI in the anterior basal ganglia, medial thalami, periaqueductal region of the midbrain and pons and cerebellar hemispheres [66]. Macroscopically these lesions present with variable vacuolation, astrogliosis, neuron loss, vascular proliferation and demyelination [43]. While in the substantia nigra, necrosis, macrophage infiltration and axonal swelling had also been observed [67]. The lesions that appear in Leigh syndrome are very heterogeneous, likely due to the fact that the mutations associated with the disease are in a multitude of nuclear and mtDNA genes encoding mitochondrial enzymes, including pyruvate dehydrogenase, and respiratory complexes I, II, IV and V; therefore a different mutation will result in a differing phenotype.

2.3.2.2 Alpers' Syndrome

Symptoms and Cause

Alpers' syndrome, also known as Alpers-Huttenlocher syndrome, is an early-onset neurodegenerative disorder characterised by a clinical triad of severe developmental delay, intractable epilepsy and liver failure. Presentation is often sudden with uncontrollable seizures in a previously healthy child. The onset is bimodal: it most commonly affects infants between the ages of 2 and 4 years (range, 3 months to 8 years), but has a second peak of onset between the ages of 17 and 24 years (range, 10–27 years) [68]. Other symptoms include ataxia, hypotonia, cortical blindness, spasticity and dementia. The disorder is progressive and often leads to death from hepatic failure or status epilepticus before age 3 years [69].

Alpers' syndrome is caused by autosomal recessive mutations in *POLG*. Defects in *POLG* compromise replication and repair of mtDNA and can therefore result in mtDNA depletion or accumulation of mtDNA deletions, though depletion is more frequently reported in these patients. This affects mitochondrial respiratory chain function through impaired oxidative phosphorylation, lowered ATP generation leading to cellular dysfunction and death in affected tissues [70].

Neuropathology

In 1931, Bernard Alpers first used the term 'diffuse progressive degeneration of the grey matter of the cerebrum', describing neuropathological findings such as neuronal loss and gliosis [71]. Subsequent studies have also shown degeneration of the cerebral grey matter with loss of neurons, astrogliosis and capillary proliferation

[59, 69]. On macroscopic neuropathological examination, symmetrical atrophy with laminar necrosis and neuronal degeneration is seen in the cerebral cortex (with predominant involvement of the temporal and occipital lobes), hippocampi, olfactory bulbs and cerebellum [72]. The presence of focal ischaemic or necrotic lesions is variable. Neuronal respiratory chain deficiencies, affecting complex I, and lowered mtDNA content correlating with the severity of neurodegeneration have recently been described in a large cohort of patients with *POLG* mutations [63].

2.4 Mechanisms of Neurodegeneration in Mitochondrial Diseases

Understanding the mechanisms contributing to neurodegeneration in patients with mitochondrial disease is complicated, and though post-mortem studies are crucial for documenting the neuropathological changes, it can be difficult to disentangle cause and effect and therefore precisely delineate mechanisms contributing to neuronal cell dysfunction and death.

2.4.1 Mitochondrial Homeostasis

Neurons have a high and constant demand for ATP generated via mitochondrial metabolism which is reflected by high mitochondrial mass within the neuronal cell body, axon, presynaptic terminals and dendritic branches [73]. To support this metabolic demand, mitochondria are highly dynamic and continuously move, fuse and divide. Although mitochondria are essential for ATP generation, mitochondria also play essential roles in the production of iron sulphur clusters and in calcium handling, apoptosis and ROS signalling (see Chap. 1). To some extent, all of these processes have been implicated in neurodegenerative diseases.

2.4.2 Mitochondria and Calcium Handling

Mitochondria play a pivotal role in the tight regulation of intracellular calcium (Ca^{2+}) levels and achieve this via uptake through a membrane potential-driven carrier, known as the mitochondrial calcium uniporter (MCU) [74]. Ca^{2+} homeostasis is particularly important for neuronal function where Ca^{2+} is intricately linked to neurotransmitter vesicle release, synaptic plasticity and mitochondrial transport [75]. Neuronal mitochondria transition between mobile and stationary states in response to intracellular [Ca^{2+}] [76]. It is thought that this mechanism allows

mitochondrial retention in presynaptic terminals and postsynaptic dendritic spines, where calcium influx is dynamic and requires tight regulation to maintain neurotransmission. While there have been a limited number of studies investigating the effect of mtDNA mutations on Ca^{2+} homeostasis, these have often focused on non-neuronal cell lines where physiological differences may be manifold. Fibroblasts derived from patients harbouring the m.3243A>G mutation revealed high basal Ca^{2+} levels and sustained elevation of calcium in response to depolarisation following stimulation with potassium [77]. Furthermore ρ^o osteosarcoma cells derived from a patient with the m.3243A>G mutation found a perturbed intra-mitochondrial calcium homeostasis [78]. A recent study of mature neurons derived from embryonic stem (ES) cells harbouring mtDNA mutations displayed a normal Ca^{2+} baseline, but following repeated glutamate stimulation, there was a progressive deficit in Ca^{2+} transients [79]. These studies show that mtDNA mutations not only impair ATP generation but also the ability of mitochondria to regulate Ca^{2+} homeostasis which could contribute to neurodegeneration since an increase in neuronal calcium concentration is a mechanism of excitotoxicity [80].

2.4.3 Mitochondria and Cell Death

There are three defined modes of cell death which might become induced in energetically compromised neurons: necrosis, apoptosis and autophagy. Mitochondria are intricately involved in the process of apoptosis since disruption of mitochondrial transmembrane potential can cause the release of cytochrome *c* and apoptosis-inducing factor from the mitochondria into the cytoplasm. Although the mechanism of neuronal cell death in patients with mitochondrial disease is not known, there are a few studies which examine apoptosis in muscle fibres in conjunction with respiratory chain deficiency; however the results from these reports are inconsistent and contradictory. One study found increased expression of factors associated with apoptosis (Fas, caspase-3 and p75) and increased TUNEL labelling (indicating double-strand breaks in DNA) in skeletal muscle biopsies from patients with point mutations or mtDNA deletions [81]. However in a different study, TUNEL labelling was not significantly increased in muscle biopsies from patients with mitochondrial disease; however the expression of apoptotic makers (Bax and Apaf-1) and cytochrome *c* release from mitochondria were observed in ragged red fibres, indicating initiation of apoptosis [82]. Another study found that a peptide known as humanin was upregulated in patients with MELAS and MERRF and this was inhibiting apoptosis in muscle fibres [83]. While a study investigating neuropathology of patients with *POLG* encephalopathy observed no neurons that were positive for caspase-3 immunohistochemistry, two patients with Alpers' syndrome demonstrated some TUNEL staining in neurons [63]. The timeframe for capturing neurons undergoing cell death is likely to be narrow, and so it is challenging to identify the exact mode of cell death in post-mortem brain tissue.

2.4.4 Oxidative Stress

Mitochondrial metabolism is also a major source of reactive oxygen species (ROS) production in the cell [84]. Unpaired electrons may leak from complexes I and III and react with oxygen to produce damaging superoxide. The excessive production and release of ROS have been linked with neurodegeneration as ROS can react with and oxidise molecules such as proteins, DNA and lipids resulting in oxidative stress. Oxidative stress occurs when there is an imbalance in the creation and detoxification of reactive oxygen species leading to the oxidisation and damage of proteins, DNA and lipids. However at normal levels, ROS can act as intracellular signals. Dysfunction within mitochondria leading to increased ROS production has been postulated as a pathological mechanism for both disease and the typical ageing phenotype [85]. In a mouse model of LHON where mice harbour a mutation in the *MT-ND6* gene (the human equivalent of the m.14600G>A mutation), decreased complex I activity and increased ROS production were observed in synaptosomes, while ATP homeostasis was preserved which lead the authors to speculate that a chronic increase in ROS production and oxidative damage was the pathological driving force for LHON [86]. Increased ROS production was also observed in primary skin and muscle fibroblasts from a mouse where complex I accessory subunit (*NDUFS4*) was knocked out [87]. However, fibroblasts derived from a French Canadian variant of Leigh syndrome due to a mutation in the *LRPPRC* gene, which encodes for a protein involved in mitochondrial mRNA stability, presented with mitochondrial abnormalities but no increase in ROS production [88]. This was also observed in a different mouse model of mitochondrial disease, where the *POLG* gene is mutated and there was no reported increase in ROS production [89]. As shown by the conflicting results from these studies, it is unlikely that the increase in ROS is solely responsible for neurodegeneration but might act in conjunction with dysfunction of other important mitochondrial pathways to cause neural dysfunction and degeneration.

2.5 Modelling Neuropathology of Mitochondrial Disease

Recent developments using cell culture models have allowed a more mechanistic approach to understand the impact of mitochondrial dysfunction on neural function. Differentiated neurons derived from mouse embryonic stem cells harbouring mtDNA defects have been generated including wild type parental cells, polymorphic variant cells, mild complex IV mutant cells and severe complex I mutant cells. Those neurons harbouring severe complex I mutations revealed the most dramatic alterations; with reduced ability to undergo neuronal differentiation and delayed development [90]. Despite this, differentiated neurons showed maintenance of high mitochondrial membrane potential with reversal of the ATP synthase and marked ROS production [91] while demonstrating an impaired response to Ca^{2+} transients following repetitive neuronal stimulation [79].

A more promising approach to understand mechanisms of neural dysfunction and degeneration derives from the reprogramming of patient fibroblasts into induced pluripotent stem cells (IPSCs). These cells contain the original nuclear and mitochondrial DNA from patients and are capable of differentiation into any cell type, including post-mitotic neurons. A recent study using neurons derived from patient fibroblasts harbouring the m.3243A>G mutation revealed bimodal segregation of mutated mtDNA with cells containing either 100 % wild type and 100 % mutated mtDNA and prominent complex I deficiency recapitulating the respiratory chain abnormalities detected in post-mortem tissues [92, 93]. Patient-derived IPSCs will not only improve understanding of disease mechanisms but may also play a role in regenerative medicine since cellular homoplasmy for the m.8993T>G mutation was genetically corrected using somatic cell nuclear transfer [94].

The generation of mouse models has helped towards understanding the pathogenesis of mitochondrial disease. The majority of models manipulate nuclear DNA, due to technical challenges associated with modification of the mitochondrial genome and the complexity of the mitochondrial bottleneck, and in recent years many groups have begun to take advantage of Cre-Lox recombination technology which permits the excision of specific genes that are flanked by two LoxP sites through the activity of an enzyme Cre recombinase. Though mouse models are discussed elsewhere in this book (see Chap. 14), there are recent developments using mice where complex I (e.g., *NDUFS4* [95]), complex III (e.g., *UQCRFS1* [96]), complex IV (e.g., *COX10* [96, 97]) and mtDNA maintenance (e.g., *TFAM* [98–100]) genes are floxed by two LoxP sites which induce knockout of the gene when crossed with mice expressing a Cre recombinase under control of neuronal-specific promoter. In these mice, the effects of the gene knockout on neural function and degeneration can be monitored and the mechanisms contributing to neurodegeneration can be further delineated.

2.6 Conclusion

Deciphering the mechanisms of neurodegeneration in patients with mtDNA diseases poses a difficult challenge to neuroscientists because of the diversity in clinical presentations, different types of mtDNA mutations and variable pattern of brain involvement. However, increased understanding of the processes underlying CNS degeneration in patients with mtDNA diseases may be informative for other neurodegenerative disorders where a mitochondrial aetiology is suspected.

There is some evidence that mutations within the mitochondrial genome or mitochondrial dysfunction play a role in a number of neurodegenerative disorders including Parkinson's disease (PD), Huntington's disease (HD), Alzheimer's disease (AD) and multiple sclerosis [101–104]. While the role of these reported mitochondrial abnormalities, whether causative for the onset of neurodegeneration or a consequence of ongoing neurodegenerative processes, it is clear that mitochondrial dysfunction would act to exacerbate disease pathogenesis. However, much like the

mitochondrial encephalomyopathies, our understanding of the pathophysiological mechanisms involved in these diseases remains incomplete.

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Glossary

Anopia A defect to the visual field.

Ataxia Progressive loss of coordination and balance in hands, arms and legs often due to dysfunction of the cerebellum.

Axonal torpedoes Fusiform swellings of the Purkinje cell axons.

Bradykinesia An abnormal slowness of movement, a characteristic clinical symptom seen in Parkinson's disease.

Cardiomyopathy The deterioration of heart muscle resulting in compromised heart function.

Chronic progressive external ophthalmoplegia Slowly progressive paralysis of the extraocular muscle which results in bilateral, symmetrical, progressive ptosis (drooping of the eyelids) followed by ophthalmoparesis (paralysis) months to years later.

Cortical blindness Total loss or partial loss of visual field with normal pupillary responses resulting from damage to the occipital lobe.

Dysarthria A speech disorder characterised by poor articulation. Often present in patients suffering from cerebellar ataxia.

Dysphagia Swallowing problems often present in conjunction dysarthria in patients suffering from cerebellar ataxia.

Dystonia Dystonia is characterised by involuntary and uncontrollable muscle spasms which can force affected parts of the body into abnormal, sometimes painful, movements or postures.

Encephalopathy Any disease that results in the alteration of the structure and function of the brain.

Endocrinopathy A disorder in the function of the endocrine gland which can often result in hormone imbalances.

Epilepsy Recurrent seizures (often described as fits).

Extrapyramidal features May be defined as an inability to initiate movements or an inability to remain motionless.

Hypotonia A state of low muscle tone resulting in reduced muscle strength.

Laminar dehiscence Splitting of the six cortical layers.

Lipoma A benign tumour consisting of brown fat cells that forms under the skin.

Myoclonus Brief involuntary twitching of a muscle or a group of muscles.

Nystagmus Involuntary eye movements that may result in reduced or limited vision.

Oxidative stress The imbalance of reactive oxygen species creation and detoxification leading to the oxidisation of proteins, DNA and lipids.

Peripheral neuropathy Damage to the nerves comprising the peripheral nervous system which can result in a combination of weakness, autonomic dysfunction and sensory abnormalities.

Proximal myopathy Weakness of those muscles located closely (proximal) to the body.

Ptosis A drooping of the upper eyelids.

Retinitis pigmentosa Retinitis pigmentosa is a disorder of the retina which is characterised by dysfunction of the photoreceptors resulting in incurable blindness or tunnel vision.

Stroke-like episodes Neurological deficits often described as resembling stroke however do not conform to a vascular territory. They are often accompanied by cortical blindness, hemianopsia, or hemiparesis.

Wolff-Parkinson-White syndrome This syndrome is a heart condition caused by extra electrical activity and can lead to increased heart rate.

Vasogenic oedema The leakage of fluids from the vascular network into the extracellular space.

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