Neuroimaging in Mitochondrial Disorders

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Summary Mutations in either nuclear DNA or mitochondrial DNA can result in disruption of oxidative phosphorylation and lead to mitochondrial dysfunction. Mitochondrial disease manifestations occur predominantly in the central nervous system, peripheral nervous system, and/or involve several organ systems. The consequences range from manifestations of a single organ or tissues, such as muscle fatigue, if confined only to muscle, seizures, intellectual disabilities, dementia, and stroke (if to the central nervous system), leading to disability or even early death. The definitive diagnosis of a mitochondrial disorder can be difficult to establish. Criteria and checklists have been established and are more reflective of adult disease. However, in children, when symptoms suggest a mitochondrial disease, neuroimaging features may have more diagnostic impact and additionally these can be used to follow the course, evolution, and recovery of the disease. This review will demonstrate the common neuroimaging patterns in patients with mitochondrial disorders and point out how various newer neuroimaging modalities may be exploited to glean information as to the different aspects of mitochondrial dysfunction or resulting neurological and cognitive disruption, although reports in the literature using these methods remain sparse.

Keywords Brain injury · diffusion tensor imaging (DTI) · functional MRI (fMRI) · inborn error of metabolism · metabolism · magnetic resonance imaging (MRI) · magnetic

resonance spectroscopy (MRS) · metabolism · mitochondrial disorder.

Introduction

Mitochondria are small organelles measuring 0.5–1.0 microns that are found in the cytoplasm of all eukaryotic cells. Although they are small, they play a very important role in generating cellular energy, in the form of adenosine triphosphate (ATP). Mitochondria produce ATP via oxidative phosphorylation, a process involving a series of reduction-oxidation reactions during which electrons are transferred through several multimeric complexes on the inner mitochondrial membrane [1]. Complex V, the ATP synthase, is responsible for the synthesis of ATP from adenosine diphosphate (ADP) and inorganic phosphate (see Saneto and Sedensky, this supplement).

The mitochondria have a complex and compact structure, consisting of 4 compartments in which distinct biochemical processes occur: 1) the inner membrane, 2) the outer membrane, 3) the intermembrane space, and 4) the matrix. The enzymes of the electron transport chain involved in oxidative phosphorylation are located on the inner membrane. ATP is produced by generating a mitochondrial membrane potential secondary to a proton gradient between the mitochondrial matrix and the inner mitochondrial membrane.

The term “mitochondrial cytopathy” is used clinically and refers to the group of inherited heterogeneous disorders due to the dysfunctional mitochondrial respiratory chain, manifesting in the central nervous system (CNS) plus or minus other organs [2]. With an estimated prevalence of 1:4,000 to 1:5,000, they represent a frequent cause of metabolic disorders [3] in both pediatric and adult patients.

Mitochondrial disorders are unique in that there are 2 genomes that are involved in the production of protein

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subunits that comprise the respiratory chain complexes: the nuclear genome and the mitochondrion DNA (mtDNA). Mitochondrial disorders, therefore, can be due to defects in either genome or signaling and regulatory proteins that govern the interaction between the products of the 2 genomes [1]. Unlike nuclear DNA, mtDNA may be present in multiple copies in varying amounts of wild-type and pathogenic genomes containing mutations, and the percentage of normal and mutated genomes may vary in the tissues. This concept is referred to as heteroplasmy. The degree of heteroplasmy is a factor in determining the severity and number of organ systems involved in disease, but is not the only factor as the pathogenicity of the mutation may play a role as well. For example, symptoms of severe mitochondrial disorders may not appear until adulthood because many cell divisions are required for a cell to receive enough mitochondria containing the mutant alleles to cause symptoms. Furthermore, most mitochondrial proteins are encoded by the nuclear DNA, translated in the cytoplasm, and imported into the mitochondria. There is limited encoding capacity of the mitochondrial genome (mtDNA), which only encodes 13 of 80 protein subunits of the oxidative phosphorylation system [4] (Table 1).

Mitochondria were first observed more than 100 years ago by Altman, who called them “elemental organisms” because they were initially believed to be free living organisms within the cells. They were first linked to human disease in 1962 when a patient with hypermetabolism was found to have loose coupling of electron transport and ATP synthesis. In 1963, mitochondrial DNA was discovered, but the complete sequence was not available until 1981 [4]. The genetic basis of mitochondrial disorders remained obscure until 1988 when the first disease-causing mutations of mitochondrial DNA were found [5]. For the last decade, owing to advancement in sequencing technologies, many genes responsible for nuclear mitochondrial subunits have been identified, establishing their role in disease.

Many mitochondrial disorders present with irreversible brain injury [6, 7]. Mitochondrial-associated brain injury

patterns are often characterized by whether the process primarily involves gray matter, white matter, or both, and beyond that, whether subcortical or cortical gray matter nuclei are involved [8]. Although expected to be global insults due to impaired energy metabolism, some mitochondrial disorders result in selective injury to deep gray matter nuclei or white matter, whereas others have patterns of cerebellar atrophy or strokes in nonvascular territories [9]. Selectivity for particular brain regions or even cell types based on morphology or neurotransmitter systems (astrocytes/neuron: glutamatergic, Gamma-aminobutyric acid (GABA)-ergic) remains poorly understood, as is the case in other inborn errors of metabolism.

The presenting clinical neurological features of a mitochondrial disorder may suggest gray or white matter involvement. Patients with cortical gray matter involvement, as expected, may present with seizures, encephalopathy, or dementia, whereas deep gray matter injury typically manifests with extrapyramidal findings of dystonia, chorea, ataxia, or other involuntary movement disorders. White matter disorders feature pyramidal signs (spasticity, hyperreflexia) and visual findings. Involvement of the cerebellum or its connecting tracts may lead to ataxia. It is typical for more than 1 aspect of the brain to be affected in a single mitochondrial disorder, such as basal ganglia and white matter giving rise to complex neurological symptoms and signs that often mimic more common conditions, such as multiple sclerosis, stroke, or cerebellar ataxias [6].

The clinical presentation in any 1 patient with a mitochondrial cytopathy is influenced by factors that include the specific gene mutation, heteroplasmy within tissues, secondary mutations or polymorphisms in both nuclear and mitochondrial genomes, as well as environmental and immunological factors [10, 11] (Table 2).

Neurological features may reflect dysfunction of any part of the nervous system and encompass a broad range of common neurological symptoms, including dementia, developmental delay, stroke, epilepsy, psychiatric illness,

Table 1 Comparison between mtDNA and Nuclear Encoded U nits

	Mitochondrial	Nuclear
Respiratory chain components	13 subunits	>80 subunits
NADH dehydrogenase	7	>41
Succinate dehydrogenase	0	4
Ubiquinol cytochrome c reductase	1	10
Cytochrome c oxidase	3	10
ATP synthase	2	14
Other proteins	0	DNA/RNA polymerases, and so forth
Protein synthesis components	2 rRNAs 22 tRNAs (13 mRNAs)	All mitochondrial ribosomal proteins (>70)

mRNA = messenger RNA;
mtDNA = mitochondrion DNA;
rRNA = ribosomal RNA;
tRNA = transfer RNA

Table 2 Neurological Markers for Mitochondrial Disorders

Central nervous system	Neuromuscular
Development delay/mental retardation	Peripheral neuropathy
Hypotonia/floppy baby	Exercise intolerance
Autistic features	Muscle weakness
Dementia/encephalopathy	Cramps after exercise
Headaches/migraine	Easy fatigability
Stroke, ischemic episodes	Cardiomyopathy
Ataxia	Heart block
Episodic coma	Arrhythmia
Seizures	Ophthalmoparesis, CPEO
Myoclonus or myoclonic seizures	Abnormal EMG/NCV
Perinatal insult	Ptosis
Pyramidal signs	
Hemiparesis	
Intractable seizure, refractory	
Spasticity	
Dystonia	
Chorea	

CPEO = chronic progressive external ophthalmoplegia; Abnormal EMG/NCV = electromyogram/nerve conduction velocity

neuropathy, and myopathy. Children with mitochondrial disease may present with life-threatening illness in the newborn period; however, the majority of children come to clinical attention for nonspecific problems, including failure to thrive, developmental delay, ataxia, neurosensory loss (vision and/or hearing), seizures, hypotonia, strokes, and loss of developmental milestones.

Neuroimaging and Mitochondrial Disorders

Neuroimaging has already shown its potential for the investigation and management of patients with mitochondrial disorders allowing clinicians and researchers to study the timing, extent, and potential of reversibility of neural injury [9], and in many cases providing noninvasive and repeatable biomarker inquiry. The term “neuroimaging” now encompasses many investigative modalities beyond routine T1 and T2 imaging, including volumetric analysis, diffusion tensor imaging (DTI), magnetic resonance spectroscopy (MRS), arterial spin labeling, and functional magnetic resonance imaging (fMRI). Each modality can be combined to gain complementary information regarding the brain’s structural, functional, and metabolic dimensions, and how these may be altered in pathologic states (Table 3). With newer

Table 3 Comparison of Imaging Modalities and Potential Role in Mitochondrial Disorders

Modality	Principle	Application
Structural magnetic resonance imaging	Differences in proton spins between tissue types allow this noninvasive modality to capture three-dimensional images of living tissue	In the clinic, MRI is used to detect microstructural abnormalities in the human brain. Structural MRI can be used to investigate volumetric differences throughout the brain between experimental and control populations. Imaging parameters can be adjusted to collect different image contrast qualities, such as T1, T2, and FLAIR
Functional magnetic resonance imaging	Magnetic differences between oxygenated and deoxygenated hemoglobin allow active regions of the brain to be imaged noninvasively in an MRI scanner	fMRI has revolutionized the study of brain function, allowing researchers to administer cognitive tasks concurrently with fMRI scanning to map cognitive functions to individual structures and networks of structures within the brain
Diffusion-weighted and diffusion-tensor imaging	These MR-based methods are used: water diffusion and the degree of ordered anisotropy that define water movement along natural hydrophobic barriers within the brain, such as myelinated axons. DWI and DTI provide an index of brain structure	Diffusion-based imaging techniques are highly sensitive to microstructural abnormalities invisible on traditional MRI, particularly within the brain’s white matter used in clinical research studies to identify differences in white matter integrity between clinical and healthy populations
Magnetic resonance spectroscopy	The characteristic location and height of peaks along the MR spectrum are used to measure the concentration of chemicals and metabolites. The three-dimensional structure and chemical composition of organic molecules determine their spectroscopic resonance frequency and the extent of their “downfield” chemical shift on mass spectrum	Using a three-dimensional voxel, researchers and clinicians can use MRS to determine the chemical profile within a given region of the brain. This can be used as a clinical tool, as inborn error of metabolism that alters the concentration of metabolites within the brain, or by researchers seeking to identify neurochemical abnormalities in disease populations

DTI = diffusion-tensor imaging; DWI = diffusion-weighted imaging; FLAIR = fluid attenuation inversion recovery; fMRI = functional magnetic resonance imaging; MR = magnetic resonance; MRI = magnetic resonance imaging; MRS = magnetic resonance spectroscopy

modalities, such as DTI, MRS, and fMRI, mechanisms of injury are starting to be understood in mitochondrial and other metabolic disorders [12, 13].

What Imaging Tools are Available?

Anatomical Imaging: What can it Tell Us?

Routine magnetic resonance imaging (MRI), using T1- and T2-weighted MRI sequences along with fluid attenuation inversion recovery (FLAIR) and voxel-based morphometry (VBM), can be used to interrogate the macroscopic structure in mitochondrial disorders of the brain. However, much more information that can help inform clinical decisions can be achieved as to the disease state using advanced technologies. Diffusion-weighted imaging (DWI) and DTI, for example, can be used to study microstructural variance in white matter fiber tracts [14, 15], whereas MRS is used to measure brain metabolism in static and dynamic models [16], namely lactate and N-acetylaspartate levels in patients with mitochondrial disorders. Functional MRI can be used to study the neural nodes and networks underlying cognitive operations [17] that may be damaged in patients with mitochondrial disorders and would be done best in the stable state to assess degree of damage and can be repeated after an intervention or to track disease progression, mainly on a research basis. Using these various methods, 1 can probe focal, regional, and global neuropathological sequelae, and monitor disease progression and response to therapies.

Here, we offer an introduction to the principles and applications of each of these neuroimaging modalities, and we review how they have added to our understanding of neurological manifestations in mitochondrial disorders.

Nonspecific MRI Changes When Using Routine T1 and T2 Imaging

Routine T1 and T2 images can be used to characterize gray matter and white matter microstructural and macrostructural changes. When abnormal brain pathology exists, 1 may observe signal abnormalities in T1- and T2-weighted scans that reflect changes in brain tissue water. The limitations of using only routine imaging are that 1 is only able to detect damage at a macroscopic level, typically when the patient is already symptomatic; MRI findings may also lag behind clinical changes. This is particularly a concern in mitochondrial disorders, in which, for example, recognition of the earliest stages of stroke in mitochondrial encephalopathy, lactic acidosis, and stroke syndrome (MELAS) will influence treatment and management. In disorders, in which there is white matter damage, the use of FLAIR imaging has added value in the detection of early white matter injury

[18, 19]. This is a particular concern in a patient with MELAS who may be having symptoms suggestive of an impending stroke, yet no stroke is seen on the routine imaging. In addition, routine MRI detection is limited to macroscopic alterations in brain structure. It lacks the spatial resolution to provide information regarding microstructural neuropathology, and does not capture dynamic processes in space and time related to brain function and metabolism. In addition, many macrostructural neuropathological phenotypes lag behind the presentation of associated clinical manifestations. MRI changes may be nonspecific, such as showing specific regions and general atrophy of cerebrum or cerebellum.

Anatomical FLAIR Imaging

FLAIR imaging is an MRI-based methodology that is sensitive to increases in interstitial water content in CNS diseases [20]; it plays a role in delineating white matter integrity in brain tumors, multiple sclerosis, cerebral infarcts, and metabolic white matter diseases. FLAIR essentially nulls signals from fluids with long longitudinal (T1) relaxation times. Lesions adjacent to cerebrospinal fluid are often more clearly identified on FLAIR. In addition, FLAIR is useful in disorders of myelination (hypomyelinating and demyelination), and as a tool for classifying normal baseline characteristics of myelin in the subcortical U fibers (the arcuate fibers at the cortical-white matter junction), deep white matter, and periventricular white matter. In children, myelination is incomplete at birth, and in healthy children it develops in a predictable, age-specific pattern [21]. Because many mitochondrial disorders present with white matter injuries in the developing brain, FLAIR is expected to be an effective means of comparing subsequent myelination patterns in patients with early metabolic decompensation in mitochondrial disorders, as compared to development in healthy individuals (Fig. 1).

Functional Magnetic Resonance Imaging

Functional MRI is mainly a research tool for imaging the time course of activity associated with neurocognitive processes in the brain. This technology has wide-range applications for both basic research into brain function, and for clinical research into the neurophysiology of neurological and psychiatric illness. Functional MRI investigation of mitochondrial disorders may offer potential insight into the neurocognitive challenges experienced by mitochondrial patients, and may help guide the development of educational support structures. It has not been widely used in this group of disorders.

First described by Roy and Sherrington [22] in 1890, neuronal metabolism and cerebral blood flow are intimately

Fig. 1 Example of white matter lesions as seen on fluid attenuation inversion recovery imaging that may not be seen on T2 imaging

linked by neurovascular coupling. Functional MRI exploits this phenomenon to generate a blood oxygenation level-dependent signal in activated regions of the brain. Through the energy-expensive process of neurotransmission, active neurons consume glucose and oxygen, which are restored by local dilation of neural vasculature and subsequent increases in the flow of oxygenated hemoglobin. MRI pulse sequences are sensitive to the magnetic contrast between oxygenated and deoxygenated hemoglobin, and can be used to map the hemodynamic response of local brain regions to the stimuli of a neurocognitive task presented to the subject in the scanner [23].

The principal drawback of fMRI, however, is that inter-subject variability in the spatial extent and precise location of neurocognitive functions within the brain require relatively large numbers of patients to achieve an acceptable signal-to-noise ratio, which is often impractical in rare disorders. This provides limitations in trying to explain the neurocognitive phenotype at the single subject level.

Voxel-Based Morphometry

VBM is a useful tool for the comparison of brain parameters in a clinical population relative to a group of healthy controls and allows 1 to quantitate volumetric differences in brain structures. VBM relies on the representation of the brain as a three-dimensional matrix of voxels, and each voxel in the VBM matrix provides an index of local brain volume. Imaging is registered to a common three-dimensional template, as in fMRI. VBM templates are commonly an average image comprised of scans from large numbers of healthy subjects. The template image provides morphological landmarks that allow the brain to be segmented into gray matter and white matter structures (Fig. 2). Analysis of VBM data across patient and control groups allows assessment of global differences in whole

Fig. 2 Example of tissue parcellation for a volumetric study

brain, white matter, and grey matter volume throughout the brain, as well as fine differences within particular regions of interest at the voxel level [24].

Diffusion-Weighted and Diffusion Tensor MRI

For the past decade, DTI has emerged as a valuable means of characterizing white matter structure and its alterations in an array of clinical contexts. Its sensitivity to microstructural abnormalities not visible with traditional MRI, and its ability to predict clinical correlates of white matter pathology underscore the value of this imaging platform for multimodal imaging studies of disorders affecting the CNS.

Diffusion MRI encompasses DWI and DTI, which are both voxel-based modalities that provide indices of axonal integrity *in vivo* by measuring water movement in white matter. DWI measures contrast between brain regions varying with respect to water diffusivity. The diffusion of water within a magnetic field gradient reduces the MRI signal, and as a result, regions in which diffusion is restricted evince lower signal loss and brighter appearance on DWI relative to regions of high diffusivity. This signal is called the apparent diffusion coefficient (ADC) and is used to identify brain regions with abnormally high ADC or low ADC diffusivity, providing a means of identifying pathologic abnormalities in the white matter microstructure of the brain. DTI is also sensitive to the ordered movement of water in the brain, whereas DWI scales each voxel to indicate the degree of water diffusion, and DTI indicates the direction of water diffusion in three-dimensional space. Acquisition of multiple pulse sequences with varying three-dimensional orientation generates a tensor, which indicates the orientation of water diffusion. Ordered water diffusion is said to be anisotropic, due to its lack of randomness, and DTI images render values of fractional anisotropy (FA) to indicate the direction of water diffusion. Decreased FA in the white matter is generally interpreted as a signal of compromised axonal integrity. Taken together, these measures provide a powerful and sensitive basis for inferences about white matter microstructure in healthy and diseased states [14, 15].

Pathological variations in these measures serve as indices of white matter injury, although the complexity of white matter microarchitecture underscores the need to interpret DTI findings in the context of other disease-specific parameters, including additional imaging modalities and relevant clinical parameters. Depending on the nature of the pathology, either ADC or FA values may be increased, decreased, or show a mixed pattern.

Water diffusion in brain tissue is therefore affected by the presence of barriers to its translational motion. The diffusion of water is restricted within a cell and between myelin sheets. The measured water ADC value is therefore frequently anisotropic and its value will change depending on the orientation of restricting barriers. DTI is best used in the imaging of mitochondrial disorders when such information is combined with other MRI modalities to age, grade, and predict ischemic brain injury for its entire temporal evolution.

Proton MRS

MRS is a powerful clinical tool for interrogating the neurochemical environment and characterizing the metabolic features of neurological disease. Although imaging coils have been tailored for sensitivity to a variety of isotopes, such as ^{13}C and ^{31}P , proton ^1H magnetic resonance spectroscopy (^1H MRS) is most commonly used. ^1H MRS produces a spectrum of peaks that contain information regarding the biochemical concentrations in a region of interest (typically $1\text{--}10\text{ cm}^3$) within the brain. These spectra contain information regarding brain metabolite concentrations relevant to a variety of neurological conditions, including brain tumors, traumatic brain injury, white matter disorders, epilepsy, and metabolic disorders [25].

Fig. 3 Example of proton ^1H magnetic resonance spectroscopy showing 2 subjects with peaks labeled

What Does MRS Tell Us About *In Vivo* Chemistry?

For an element to be studied by MRS there are several requirements. First, it should lend itself to excitation by magnetic resonance. The chemical also has to be present in a detectable concentration on the order of milligrams and should produce an acceptable signal-to-noise ratio. These conditions are met by ^{31}P , ^1H , ^{19}F , ^{13}C , ^7Li , and ^{23}Na .

The ability to differentiate chemical compounds on the magnetic resonance (MR) spectrum depends on the differences between compounds in the chemical configuration of protons within the molecule, and how these configurations differentially affect the electromagnetic microenvironment in the region being sampled. Commonly assayed metabolites include the following: N-acetyl aspartate (NAA), creatine, choline, myoinositol, and lactate. Other compounds, such as glutamate and glutamine benefit from short echo time and higher field strength. The relative abundance of metabolites of interest can be determined by comparison of the peak heights between 2 compounds. Successful use of clinical MR spectra requires a radiologist trained to recognize the patterns in which neurochemical abnormalities elicit deviations from normal MR spectra and clinical correlation to determine how these abnormalities may contribute to the diagnosis, course, and management of disease [26] (Fig. 3). Furthermore, MRS includes the requirement for stronger and more homogeneous magnetic fields and is more sensitive to movement artifacts than other modalities of MRI adding to expense in the cost and time.

What Do the Peak Heights of Various Biochemicals Indicate?

^1H MRS is useful as one can measure: NAA, the neuronal marker and CNS lactate, the end product of nonoxidative metabolism (glycolysis). Lactate is often referenced to internal water or NAA, a putative neuronal/axonal marker. Marked elevations of lactate can be observed in patients with mitochondrial disease ranging from 3 to 11 mM [27, 28], especially in those with confirmed defects in electron transport chain. Lactate elevation is a consistent feature observed in Leigh syndrome, and the majority of patients with MELAS (Fig. 6). It is important to note that cerebral lactic acidosis occurs in the absence of plasma lactic acidemia in many patients, thus CNS lactate determination is often a more specific marker of cerebral metabolism than plasma lactate levels [28]. Elevated lactate shown by MRS often correlates well with other markers of mitochondrial disease, but ^1H MRS does not depict elevated lactate in all cases. In an instructive study by Lin et al. [29], this group found abnormal CNS concentrations of lactate may be undetected by ^1H MRS, according to differences in the type of mitochondrial disorder, timing, severity, or location of the affected tissues and the site of interrogation. Often a voxel in the ventricles proved more diagnostic than a voxel placed in the parenchyma. Appearance of a lactate peak on MRS may be the first biomarker seen in a metabolic stroke and occurs even prior to changes that occur on DWIs [30–32].

Decreased NAA can indicate neuronal damage with the level of reduction taken as a marker of the extent of neuronal or axonal loss. In epilepsy, decreased NAA may be indicative of neuronal metabolic dysfunction. Clinicians working with pediatric populations should note that NAA levels are lower in the developing brain and typically peak at 10 to 15 years of age [33].

Choline is an important molecule to consider in mitochondrial disorders. Choline containing compounds or total Choline is seen as a peak at 3.22 ppm. It is comprised of several compounds, mainly phosphorylcholine, phosphorylated cholines, and glycerophosphorylcholine. These are membrane and myelin markers, and can increase with pathological changes to membrane turnover [34]. Fluctuations in total Choline compounds occur with diffuse axonal injury [25] among other findings. The major resonance of creatine is present at 3.00 ppm, and in a healthy brain it is comprised of equal proportions of free creatine and phosphocreatine, with creatine and phosphocreatine in constant enzymatic exchange. Phosphocreatine is a bioenergetic marker because of its large role in the synthesis of ATP.

What are the Imaging Patterns in Mitochondrial Disorders?

The main imaging patterns seen in mitochondrial disorders include the more nonspecific findings of cortical and cerebellar atrophy to the more specific T2 hyperintensities of deep gray matter nuclei, leukodystrophic changes, and strokes in nonvascular territories [8, 35, 36]. Nonetheless, in children, imaging can be used in combination with the clinical, neurological, and biochemical data, as well as pedigree to consider pursuing molecular diagnosis.

Patterns of Neural Injury in Mitochondrial Disorders That Can Be followed by MRI

Focal Lesions in Deep Gray Matter Structures

Among child neurologists, bilateral lesions in the putamen and basal ganglia nuclei are recognized as being the *sin qua non* and most prevalent feature of many mitochondrial syndromes [8, 9, 27] (Fig. 4). Focal, bilateral, symmetric brain lesions involving basal ganglia and periaqueductal gray matter are typical of Leigh syndrome (subacute necrotizing encephalomyelopathy) [27–37], which can be caused by mutations in mtDNA and nuclear DNA encoded mitochondrial proteins. This disorder classically presents in infancy with brainstem dysfunction leading to abnormal cranial nerve findings, respiration, and oral motor dysfunction interfering with feeding, long tract signs leading to spasticity, multiple organ involvement, and global developmental delay.

Structural pathology of the brain lesions shows vascular proliferation, demyelination, gliosis, and necrosis [38–42]. The typical anatomic distribution of the lesions is seen in brainstem, diencephalon, basal ganglia, and cerebellum. Clinical severity is typically dependent on mutation type and state of heteroplasmy in mtDNA subtypes.

Genotype predictions have been observed clinically, despite similar imaging features. Patients with Leigh disease due to the nuclear mutations, surfeit protein-1 (SURF-1) mutations, and other cytochrome oxidase (COX) assembly genes have an imaging pattern on MRI showing prominent T2-signal abnormalities in the deep brainstem nuclei, rather than the caudate and putamen lesions that are most characteristic in patients with Leigh disease due to mtDNA mutations [33, 34].

Strokes in Nonvascular Territories

Patients with MELAS have a characteristic imaging pattern that should prompt further evaluation for a mitochondrial disorder (Fig. 5). MELAS is a progressive neurodegenerative disorder associated with migraine-like headaches, refractory partial or generalized seizures, short stature, muscle weakness, exercise intolerance, sensorineural deafness,

Fig. 6 This proton ^1H magnetic resonance spectroscopy shows a very large lactate peak. The characteristics of the lactate peak are doublet with a 7 Hz split between the peaks (yellow arrow). The height of the left side of the doublet is usually slightly higher than the right, giving an asymmetric appearance. These characteristics are required to differentiate this signal from noise in the background of the spectrum. The concentration must be at least 1 mmol/kg tissue for a signal to be seen. Although this spectrum was acquired at a long TE, a shorter TE will also demonstrate a lactate peak, as well as other metabolites of interest

MRI in patients with MELAS may be higher than those of normal subjects. Pathological changes take place in the non-affected areas of the patients with MELAS and are best detected by these newer imaging methods [43].

Only single case reports have described the use of serial MRI in patients with MELAS. Brain MRIs, obtained at symptom onset, at 3 weeks, and at 1 year, in a patient with stroke due to MELAS revealed migrating T2-weighted hyperintensities in the temporal/parietal and occipital cortices and later revealed atrophy [52]. MRS at 1 year poststroke revealed increased lactate in both the occipital and temporal lobes [53]. At autopsy, there was gliosis found that mainly consisted of deep white matter. Typically there is sparing of U-fibers.

In another study, MRI was performed 2 days after the sudden onset of cortical blindness in a 25-year-old patient with MELAS. FLAIR images demonstrated multifocal cortical and subcortical hyperintensities located bilaterally in the frontobasal and temporo-occipital distributions. Diffusion-weighted images showed normal-to-increased apparent diffusion coefficient values in the acute left temporo-occipital lesion and increased values in the older stroke-like lesions. Based on these findings, I might concur that metabolic rather than the ischemic mechanisms account for the stroke-like episodes occurring in MELAS. The authors suggest that normal or increased apparent diffusion coefficient values within 48 h of a neurological deficit of abrupt onset should raise the possibility of MELAS, particularly when the infarcts are in the characteristic areas of cortex [52].

Newer imaging modalities will allow classification and elucidation of the pathomechanism. Currently, the 2 main hypotheses that have been presented to explain these cerebral lesions invoke a vascular *versus* metabolic etiology. In the vascular hypothesis, metabolic damage of the endothelium leads to small vessel occlusion and secondary neuronal death. Using routine imaging, it is supported to some extent by disease course and the computed tomographic and MRI appearance of the lesions and some pathological reports of endothelium alterations in the brains of patients with MELAS [54]. On the other hand, the metabolism hypothesis states that mitochondrial dysfunction results in anaerobic metabolism and neuronal death owing to lactic acidosis. To confirm or refute this would require demonstration of hyperperfusion and dissociation between glucose and oxygen consumption, which would rely on positron emission tomographic and single photon emission computed tomographic studies.

Cortical and Cerebellar Atrophy

Cerebral atrophy is a common, but nonspecific finding in mitochondrial disease that may occur in multiple mitochondrial subgroups [55]. Although tissue atrophy may be slowly progressing with time, rapid progression may be seen in association with the clinical decline [56] and may prompt evaluation for Alpers hepatorenal presentation. Cerebellar atrophy might be the primary neuroimaging feature in some patients with mitochondrial disorders [57–60], particularly children, and should figure into the differential diagnosis (Fig. 7). Mutations in polymerase gamma-1 (POLG1) (the mitochondrial DNA replicase), can present with volume loss predominantly in cerebellar and occipital cortices, reflecting specific loss of Purkinje cells and neurons in the dentate

Fig. 7 Cerebellar atrophy as a sign of a mitochondrial disorder. The yellow arrow demonstrates cerebellar atrophy

Fig. 8 Abnormal, bilateral T2-hyperintense signal is again identified throughout the supratentorial and infratentorial white matter in these images of children with mitochondrial disorders

nucleus [61]. Cerebellar atrophy should be differentiated from cerebellar hypoplasia, as a major metabolic disorder that can mimic mitochondrial disorders with intellectual delay, seizures, hypotonia, vision defects, and other organ findings are the congenital disorders of glycosylation.

White Matter/Leukodystrophy

White matter changes are often considered core features of distinct leukodystrophies. White matter changes are

frequently observed in association with many syndromes related to idiopathic developmental delay [62, 63]. Clinically, leukoencephalopathies are clinically characterized by the predominance of motor disturbances, with slow neurological deterioration and a low (but not absent) incidence of seizures. Leukoencephalopathies are generally classified according to either features on histopathology or location of the myelin involvement, or alternatively by the causative enzyme defect. Cerebral white matter involvement is commonly seen in childhood onset mitochondrial disorders. However, when

Fig. 9 Two examples of delayed myelination

Table 4 Diagnostic Criteria in adults and Children*

Major criteria	Minor Criteria
Clinical presentation (↑lactate)	Clinical presentation (+/-)
Histology	Histology
>2 % RRF	<2 % RRF (age, 30–50 years)
2–5 % COX-negative fibers	>2 % SSMA (<16y)
Enzymology	Abnormal mitochondria (EM)
<20 % RC in a tissue or	Enzymology
<30 % RC ≥2 tissues	20–30 % RC in a tissue or 30–40 % RC ≥2 tissues
<30 % RC in a cell line	30–40 % RC in a cell line
Functional	Functional
Fibroblast ATP synthesis rates >3 SD below normal	Fibroblast ATP synthesis rates 2–3 SD below normal
Molecular	Molecular
Nuclear or mtDNA mutation of undisputed pathogenicity	Nuclear or mtDNA mutation of probable pathogenicity

COX = cytochrome oxidase;
mtDNA = mitochondrion DNA;
RC = respiratory chain;
RRF = ragged red fibers;
SD = succinate dehydrogenase;
SSMA = subsarcolemmal mitochondrial aggregates

*[80]

MRI denotes a leukodystrophy pattern, respiratory chain defects are not often considered in the differential diagnosis. Genetic defects of oxidative phosphorylation should always be considered when the MRI is suggestive of leukoencephalopathy in childhood. Although the literature on neuroimaging in mitochondrial disorders is not vast, there are many individual case reports and case series of patients with primary electron transport chain (ETC) deficits who have white matter changes [64–66] on MRI with clinical features that predict and confirm this finding (Fig. 8).

Diffusion-weighted imaging and DTI are especially useful measures of axonal integrity *in vivo* by its ability to measure water movement and would be predicted to be useful in the evaluation of patients with mitochondrial disorders. As previously reviewed, diffusion of water within a magnetic field gradient reduces the MR signal. Therefore, regions in which diffusion is restricted demonstrate lower signal loss and brighter appearance on DWI, relative to regions of high diffusivity. Cytotoxic edema can be demonstrated on diffusion-weighted imaging, is an early change that precedes T2 signal changes, and maybe a helpful discriminator of mitochondrial disease. The complexity of white matter microarchitecture in mitochondrial disorders underscores the importance of DTI as a tool; however, the findings need to be interpreted in the context of clinical parameters and other imaging findings. Depending on the nature of the pathology, either ADC or FA values may be increased, decreased, or show a mixed pattern in mitochondrial disorders.

Several of the mitochondrial disorders present with widespread leukodystrophy in a pattern that should suggest the underlying etiology. Patients with mitochondrial neurogastrointestinal encephalopathy due to mutations in the thymidine phosphorylase gene present with severe gastrointestinal dysmotility, ophthalmoplegia, ptosis, cachexia,

and leukodystrophy, as well as peripheral neuropathy due to involvement of peripheral nervous system myelin [64, 67]. A mutation in the RRM2B gene that leads to neurogastrointestinal encephalopathy due to severe depletion of mtDNA copy number also produces a leukodystrophy pattern [68].

Another common radiological finding is that of “delayed myelination” (Fig. 9). This pertains to an MRI appearance that is less mature than that expected by the chronological age of the child, which is corrected for any prematurity [21, 69]. Although common teaching is that myelin delay due to a metabolic condition does not “catch up,” as it would when the delay is due to nutritional factors, as evidenced from clinical cases suggests that myelination delay may “catch up” with a period of time [28, 70]. White matter changes may be present at disease presentation or as a consequence of the disease progression [65, 71].

Conclusions

Mitochondrial respiratory chain disorders can present at any age with vast heterogeneity, and any organ or tissue may be affected, although the CNS and the peripheral nervous system are most commonly involved [72]. As a group, they represent a challenge to clinicians, especially in children (ages, birth to 16 years), in whom clinical presentation and disease course show great variability [73].

In an attempt to navigate the complexities of mitochondrial disorders, various consensus diagnostic criteria have been proposed, which mainly addresses adult disease [74, 75] (Table 4). A modification to accommodate diagnosis of pediatric cases has also been proposed [76]. These classifications, in addition to clinical, genetic, histopathological, and metabolic criteria, include a scoring criterion for the

results of biochemical studies for which no gold standard is generally accepted. In children, neuroimaging patterns are an important aspect of scoring systems and the recognition of patterns seen in the developing brain, which may change in time, are necessary for familiarity with neurologists [77–79].

Common patterns of brain MR imaging can be identified in patients with mitochondrial disorders. However, using the new modalities of DTI, volumetrics, MRS, and fMRI allows an expansion of the field of knowledge in terms of combining clinical and biochemical data with brain imaging to guide genetic studies.

Required Author Forms Disclosure forms provided by the authors are available with the online version of this article.

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