Diagnostic Workup of Patients with Mitochondrial Diseases

42

Shamima Rahman and Nicole I. Wolf

Key Facts
- Mitochondrial diseases, also known as respiratory chain disorders, disorders of energy metabolism or mitochondriopathies, are genetic conditions that cause direct or indirect impairment of the oxidative phosphorylation (OXPHOS) system.
- Mitochondrial diseases can involve any organ at any age; most commonly affected are the muscle, brain, retina, extra-ocular muscles, heart, liver, kidney, pancreas, gut and bone marrow.
- The biochemical hallmarks of mitochondrial diseases are lactate elevations in blood, CSF or urine or frank lactic acidosis.

- Diagnosis of mitochondrial diseases requires extensive multidisciplinary investigations which may include examination of blood, urine and CSF as well as tissue biopsies. Genome-wide genetic analyses may become primary diagnostic tests for many patients in the future.

42.1 Background

Within a cell, mitochondria and the mitochondrial respiratory chain are at the centre of all energy-related processes. Over the last 20 years, the term mitochondrial disease has come to be understood to describe a heterogeneous group of diseases with the common underlying pathogenic feature of impairment of the oxidative phosphorylation (OXPHOS) system, either directly or indirectly. As a group mitochondrial disorders are probably the most common metabolic disorders, affecting ~1: 5,000 live births. These disorders can involve any tissue at any age with any degree of severity. Table 42.1 lists some of the most important symptoms associated with mitochondrial dysfunction.

The OXPHOS system is a series of five multimeric enzyme complexes that are embedded in the inner mitochondrial membrane, and whose collective function is to synthesise the majority of cellular ATP from ADP and inorganic

S. Rahman, FRCP, FRCPCH, PhD (✉)
Mitochondrial Research Group, Genetics and Genomic Medicine, UCL Institute of Child Health, 30 Guilford Street, London WC1N 1 EH, UK
e-mail: shamima.rahman@ucl.ac.uk

N.I. Wolf, MD
Department of Child Neurology, VU Medisch Centrum, Postbox 7057, Amsterdam 1007 MB, The Netherlands
e-mail: n.wolf@vumc.nl

Table 42.1 Important symptoms and findings in respiratory chain deficiency

Organ or tissue	Signs and symptoms
Brain and nervous system	Seizures, stroke-like episodes, myoclonus, ataxia, dystonia, developmental delay +/− regression, parkinsonism, migraine, dementia, leukoencephalopathy, peripheral neuropathy
Eye	Optic atrophy, pigmentary retinopathy, cataracts
Extraocular muscles	Ophthalmoplegia, ptosis
Ear	Sensorineural hearing loss
Skeletal muscle	Myopathy, exercise intolerance, rhabdomyolysis
Bone marrow	Pancytopaenia or failure of specific cell lineages (sideroblastic anaemia, neutropaenia, thrombocytopaenia)
Heart	Cardiomyopathy (most commonly hypertrophic), conduction defects
Liver	Hepatic dysfunction, liver failure, cirrhosis, bile stasis
Intestine	Pseudo-obstruction, enteropathy, diarrhoea, exocrine pancreatic insufficiency
Testes, ovaries	Gonadal failure
Kidney	Tubulopathy, Fanconi syndrome, steroid-resistant nephrotic syndrome
Pancreas	Diabetes mellitus, exocrine failure, pancreatitis
Endocrine organs (thyroid, parathyroid, adrenal, pituitary, pancreatic β cells)	Failure of hormone secretion
Skin/hair	Hypertrichosis
Growth	Faltering growth

phosphate (Fig. 42.1). The complexes are generally known by Roman numerals, and their specific functions are as follows: complex I or NADH/ubiquinone oxidoreductase accepts electrons from the Krebs cycle and transfers them to coenzyme Q_{10}, whilst simultaneously pumping protons across the inner mitochondrial membrane into the intermembrane space to generate an electrochemical gradient across the inner mitochondrial membrane. Complex II or succinate/ubiquinone oxidoreductase accepts electrons from the Krebs cycle and also passes them on to coenzyme Q_{10}. Coenzyme Q_{10} also links mitochondrial fatty acid β-oxidation to the respiratory chain by accepting electrons from the electron transfer flavoprotein dehydrogenase. Electrons are transferred from coenzyme Q_{10} to complex III (ubiquinone/cytochrome c oxidoreductase) which passes them on to another mobile electron carrier in the inner mitochondrial membrane, cytochrome c, whilst pumping protons across the inner mitochondrial membrane. Cytochrome c passes electrons to complex IV (cytochrome c oxidase), the final proton pump in the respiratory chain, which then transfers the electrons to molecular oxygen to form water. Complex V (ATP synthase) then harnesses the energy in the electrochemical gradient to synthesise ATP from ADP and inorganic phosphate.

Secondary respiratory chain dysfunction is common in many disease processes, including disorders affecting intermediate metabolism, e.g. organic acidurias or fatty acid oxidation defects, and also many other inherited diseases not primarily affecting metabolic pathways. These will not be discussed further in this chapter.
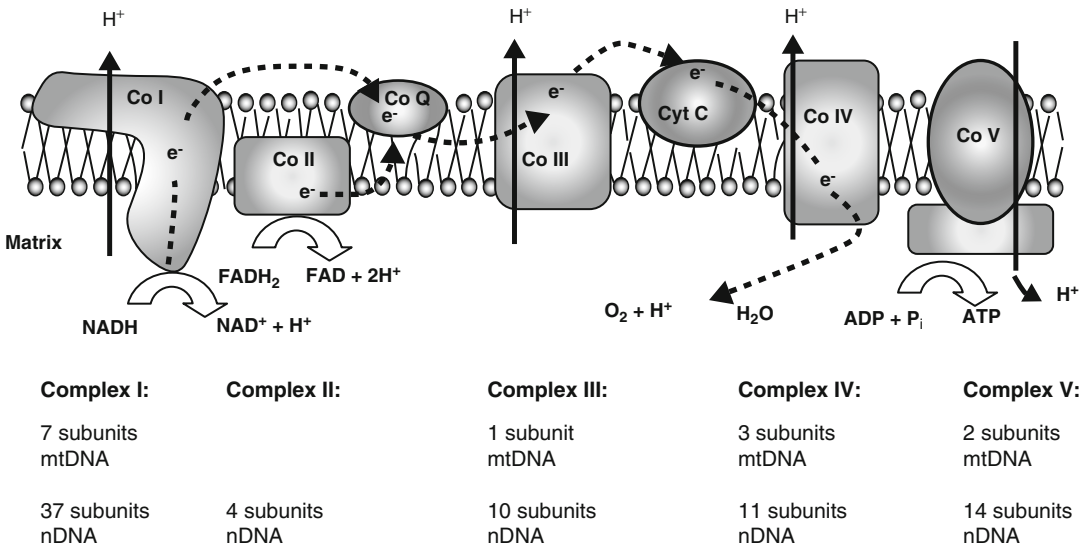
42.2 Mitochondrial Genetics

Primary respiratory chain defects are disorders that directly involve OXPHOS and the electron transfer chain. Inheritance can be maternal or Mendelian, and a myriad of genes is involved, so that making a specific genetic diagnosis of a mitochondrial disorder remains challenging.

Mitochondria are unique amongst subcellular organelles in containing their own genetic material: the mitochondrial DNA (mtDNA), a circular structure of 16,569 bp coding for 13 peptide components of the respiratory chain and 2 ribosomal RNAs (rRNAs) and 22 transfer RNAs (tRNAs) needed for protein synthesis within the mitochondria. MtDNA is maternally inherited; the result of maternal inheritance is that familial mitochondrial disorders typically affect all the children of affected women, but not children of affected men.

Despite the importance of mtDNA, most subunits of the five OXPHOS complexes are

Intermembrane space

Complex I:	Complex II:	Complex III:	Complex IV:	Complex V:
7 subunits mtDNA		1 subunit mtDNA	3 subunits mtDNA	2 subunits mtDNA
37 subunits nDNA	4 subunits nDNA	10 subunits nDNA	11 subunits nDNA	14 subunits nDNA

Fig. 42.1 Mitochondrial respiratory chain. Complex I (NADH/ubiquinone oxidoreductase), complex II (succinate/ubiquinone oxidoreductase), complex III (ubiquinol/ cytochrome *c* oxidoreductase), complex IV (cytochrome c oxidase), complex V (ATP synthase)

encoded by nuclear genes. The same is true for all transport proteins, proteins for mtDNA synthesis and maintenance, proteins required for mitochondrial transcription and translation and assembly factors of the five complexes (Fig. 42.1). This means that at least 1,000 nuclear genes are involved in mitochondrial biogenesis, maintenance and functioning. It is therefore not surprising that most mitochondrial disease is caused by mutations in nuclear DNA and follows Mendelian inheritance patterns of inheritance (dominant, recessive, or X-linked), as detailed in Table 42.2.

42.3 Clinical Recognition of Mitochondrial Disease

Because mitochondria are present in all cells except mature red cells, mitochondrial dysfunction can theoretically result in abnormal function of any organ or system of the body in any combination, leading to a multitude of clinical presentations. Some clinical syndromes with characteristic constellations of symptoms are recognised, and these are summarised in Table 42.3.

However, most paediatric patients do not have classical syndromes, and the most common symptoms and signs of mitochondrial disease are highlighted in Table 42.1. Tissues with highest energetic requirements are generally preferentially affected, leading to neurological (encephalopathy, seizures, developmental regression, stroke-like episodes), cardiac (cardiomyopathy, conduction defects), renal (tubulopathy) and liver (acute liver failure) disease. An otherwise unexplained combination of symptoms in different organ systems is the strongest indicator of a mitochondrial disease.

Leigh syndrome is one of the most severe and frequent manifestations of a mitochondrial disorder in infancy and childhood and illustrates the difficulties in the diagnosis of mitochondrial disorders. Affected patients present with developmental delay or a neurodegenerative course including extrapyramidal movement disorder, ataxia, strabismus and swallowing difficulties. MRI reveals symmetric hyperintensities of basal ganglia, mesencephalon and brain stem (Fig. 42.2). Additional symptoms such as cardiomyopathy or renal tubulopathy may occur and may help to pinpoint the genetic

Table 42.2 Examples of nuclear genes involved in mitochondrial disorders

Biochemical defects	Nuclear genes involved	Gene function	Additional abnormalities
Deficiency of single enzymes			
OXPHOS subunit mutations			
Complex I deficiency	*NDUFS1*, *NDUFS2*, *NDUFS3*, *NDUFS4*, *NDUFS6*, *NDUFS7*, *NDUFS8*, *NDUFV1*, *NDUFV2*, *NDUFA1*, *NDUFA2*, *NDUFA9*, *NDUFA10*, *NDUFA11*, *NDUFA12*, *NDUFB3*, *NDUFB9*	Structural subunits of complex I	
Complex II deficiency	*SDHA*, *SDHB*, *SDHC*, *SDHD*	Structural subunits of complex II	Succinate elevated in cerebral ^{1}H-MRS
Complex III deficiency	*CYC1*, *UQCRB*, *UQCRC2*, *UQCRQ*	Structural subunits of complex III	
Complex IV deficiency	*COX4I2*, *COX6A1*, *COX6B1*, *COX7B*, *NDUFA4*	Structural subunits of complex IV	
Complex V deficiency	*ATP5A1*, *ATP5E*	Structural subunits of complex V	
OXPHOS assembly factor mutations			
Complex I deficiency	*NDUFAF1*, *NDUFAF2*, *NDUFAF3*, *NDUFAF4*, *NDUFAF5*, *NDUFAF6*, *ACAD9*, *FOXRED1*, *NUBPL*	Assembly factors of complex I	Typical neuroimaging in NDUFAF2 and NUPBL deficiencies
Complex II deficiency	*SDHAF1*, *SDHAF2*	Assembly factors of complex II	Succinate elevated in cerebral ^{1}H-MRS
Complex III deficiency	*BCS1L*, *HCCS*, *LYRM7*, *TTC19*, *UQCC2*	Assembly factor of complex III	
Complex IV deficiency	*SURF1*, *COA5* (*C2orf64*), *COX14* (*C12orf62*), *COX20* (*FAM36A*), *FASTKD2*, *PET100*, *CEP89*	Assembly factors of complex IV	
	SCO1, *SCO2*, *COA6* (*C1orf31*)	Copper incorporation	
	COX10, *COX15*	Haem synthesis	
	LRPPRC	Complex IV mRNA stabilisation	
	TACO1	Translational co-activator of complex IV subunit I	
Complex V deficiency	*ATP12*, *TMEM70*	Assembly factors of complex V	
Deficiency of multiple enzymesa			

Table 42.2 (continued)

Biochemical defects	Nuclear genes involved	Gene function	Additional abnormalities
Defects of mtDNA maintenance			
Impaired intergenomic communication (activity of respiratory chain enzymes may be normal; depletion or multiple deletions of mtDNA common)	*POLG*, *POLG2*, *C10orf2*, *MGME1*, *DNA2*	mtDNA replication	
	SLC25A4	ADP/ATP translocator	
	TK2	Nucleotide salvage	CK strongly elevated
	DGUOK	Nucleotide salvage	Elevated plasma tyrosine
	SUCLA2, *SUCLG1*	Succinate-coenzyme A ligase	Elevated excretion of methylmalonic acid
	TYMP	Thymidine phosphorylase	Elevation of thymidine and deoxyuridine in plasma and urine
	RRM2B	p53-controlled ribonucleotide reductase subunit	
	MPV17, *FBXL4*, *ABAT*	Mitochondrial proteins with unknown function in mtDNA maintenance	
Defects of mitochondrial gene expression			
Mitochondrial ribosomal defects	*MRPS7*, *MRPS16*, *MRPS22*, *MRPL3*, *MRPL12*, *MRPL44*	Mitochondrial ribosomal proteins	
Impaired mitochondrial RNA modification	*PUS1*	Pseudouridine synthase 1	Sideroblastic anaemia
	ELAC2, *MTO1*, *TRMU*, *GTPBP3*, *TRIT1*, *TRNT1* *MTFMT*, *MTPAP*, *PNPT1*, *HSD17B10*	Enzymes required for mitochondrial RNA metabolism	
Impaired mitochondrial tRNA aminoacylation	*DARS2*	Mitochondrial aspartyl-tRNA synthetase	Lactate elevated in ^{1}H-MRS, typical neuroimaging (LBSL)
	EARS2	Mitochondrial glutamyl-tRNA synthetase	Lactate elevated in ^{1}H-MRS, typical neuroimaging (LTBL)
	RARS2	Mitochondrial arginyl-tRNA synthetase	Pontocerebellar hypoplasia type 6
	SARS2	Mitochondrial seryl-tRNA synthetase	Hyperuricaemia
	YARS2	Mitochondrial tyrosyl-tRNA synthetase	Sideroblastic anaemia
	AARS2, *CARS2*, *FARS2*, *HARS2*, *IARS2*, *LARS2*, *MARS2*, *NARS2*, *PARS2*, *TARS2*, *VARS2 GARS*, *KARS*	Other mitochondrial and cytosolic aminoacyl tRNA synthetases	
Impaired translation elongation	*GFM1*, *TSFM*, *TUFM*	Mitochondrial translation elongation	
Other mitochondrial translation factors	*C12orf65*, *RMND1*	Other proteins required for mitochondrial gene expression	

Table 42.2 (continued)

Biochemical defects	Nuclear genes involved	Gene function	Additional abnormalities
Defects of coenzyme Q_{10} biosynthesis			
Impairment of coenzyme Q_{10} biosynthesis (decreased activity of complex I + III and II + III)	*COQ2, COQ4, COQ6, COQ9, PDSS1, PDSS2, ADCK3, ADCK4*	Coenzyme Q_{10} biosynthesis	Decreased ubiquinone content in muscle
Defects of lipoic acid and/or iron sulphur cluster biosynthesis			
Lipoic acid biosynthesis	*LIAS*	Lipoic acid biosynthesis	Elevated glycine concentration in plasma and urine
	LIPT1	Required for lipoylation and activation of 2-ketoacid dehydrogenases	
Iron sulphur cluster biosynthesis	*BOLA3, GLRX5, NFU1*	Iron sulphur cluster biosynthesis	Hyperglycinaemia
	FDX1L, FXN, IBA57, ISCU, LYRM4, ABCB7, NFS1	Iron sulphur metabolism	
Toxic damage to respiratory chain enzymes			
	ETHE1	Sulphur detoxification	Ethylmalonic aciduria
	HIBCH, ECHS1	Valine degradation	Elevated 4-hydroxybutyryl carnitine, characteristic urinary metabolites
Normal activity of respiratory chain complexes			
Defects of mitochondrial import			
Components of the mitochondrial protein import apparatus	*TIMM8A, GFER*	Mitochondrial protein import	
	DNAJC19	Mitochondrial inner membrane translocase subunit TIM14	Elevated excretion of 3-methylglutaconic acid
Mitochondrial solute translocases	*SLC25A3, SLC25A12, SLC25A13 SLC25A19, SLC25A22*	Mitochondrial solute carriers	
	SLC25A38	Mitochondrial solute carrier required for haem biosynthesis	Sideroblastic anaemia
Defects of mitochondrial membrane lipids			
	TAZ	Tafazzin	Elevated excretion of 3-methylglutaconic acid, cardiolipin deficiency
	AGK, SERAC1	Enzymes involved in mitochondrial lipid biosynthesis and/or remodelling	Elevated excretion of 3-methylglutaconic acid
Defects of mitochondrial membrane dynamics			
Mitochondrial fusion proteins	*OPA1, MFN2*	Required for mitochondrial fusion	
Mitochondrial fission factors	*DNM1L*	Dynamin-like protein, deficient fission of mitochondria and peroxisomes	Lactic acidosis and mild elevation of VLCFA
	MFF, STAT2	Required for mitochondrial fission	

Table 42.2 (continued)

Biochemical defects	Nuclear genes involved	Gene function	Additional abnormalities
Miscellaneous defects			
	AIFM1	Apoptosis-inducing factor	
	APOPT1	Mitochondrial apoptogenic protein 1	Cavitating leukodystrophy
	CLPB	AAA ATPase (chaperonin)	Elevated excretion of 3-methylglutaconic acid
	SPG7	Paraplegin (mitochondrial AAA metalloprotease)	
	AFG3L2	AAA ATPase	
	HSPD1	Chaperonin (heat shock protein 60)	
	CLPP	Mitochondrial matrix peptidase proteolytic subunit	
	PMPCA	Mitochondrial processing peptidase alpha	
	SFXN4	Sideroflexin 4	Macrocytic anaemia
	NADK2	Mitochondrial NAD kinase 2	Hyperlysinaemia
	NNT	Nicotinamide nucleotide transhydrogenase	
	TRAP1	TNF receptor-associated protein 1	
	TMEM126A	Mitochondrial membrane protein of unknown function	

[a]Normal respiratory chain enzyme activities reported for some of these defects

Table 42.3 Recognised mitochondrial syndromes

Syndrome	Genes involved	Inheritance	Important clinical features
Alpers-Huttenlocher syndrome	POLG, mtDNA (various mutations), FARS2, other unknown nuclear genes	AR, Mt (rarely)	Neurodegenerative disease with onset in childhood: status epilepticus, epilepsia partialis continua, liver failure (may be triggered by valproic acid), gastrointestinal dysmotility
Barth syndrome	TAZ	XL	Cardiomyopathy, cyclical neutropaenia, myopathy, characteristic facies
Coenzyme Q_{10} biosynthesis defects	COQ2, COQ4, COQ6, COQ9, PDSS1, PDSS2, ADCK3, ADCK4	AR	Heterogeneous presentations, including encephalopathy, cerebellar ataxia, steroid-resistant nephrotic syndrome, rhabdomyolysis
Ethylmalonic encephalopathy	ETHE1	AR	Encephalopathy, diarrhoea, acrocyanosis, petechiae, ethylmalonic aciduria
Growth retardation, aminoaciduria, cholestasis, iron overload, lactic acidosis and early death (GRACILE)	BCS1L	AR	Intrauterine growth retardation, cholestasis, lactic acidosis, aminoaciduria; allelic with *Björnstad syndrome* = congenital sensorineural hearing loss, pili torti

(continued)

Table 42.3 (continued)

Syndrome	Genes involved	Inheritance	Important clinical features
Kearns-Sayre Syndrome (KSS)	Large mtDNA deletions (most common 4,977 bp) of mtDNA	Mt (usually sporadic)	Progressive external ophthalmoplegia, pigmentary retinopathy, progressive ataxia, dementia, cardiac conduction defect, deafness, elevated CSF protein and lactate, typical neuroimaging
Leber hereditary optic neuropathy (LHON)	Missense mutations in different mtDNA genes, especially complex I subunits	Mt, usually homoplasmic	Acute or subacute mid-life painless visual loss, male preponderance, additional mild neurological symptoms possible, also pre-excitation syndromes
Leigh syndrome	>75 genes, especially *MT-ATP6* (m.8993T>G/C mutations) and *SURF1*	Mt, AR, XL	Neurodegenerative disease with onset in infancy or early childhood: movement disorder, swallowing difficulties, strabismus. Variable extra-neurological features. MRI with symmetric abnormalities in basal ganglia and brainstem
Mitochondrial DNA depletion syndrome (MDDS)	Encephalomyopathic: *TK2*, *SUCLA2*, *SUCLG1*, *RRM2B*, *FBXL4*	AR	Encephalomyopathic: myopathy, seizures, developmental delay/regression, variable other features
	Hepatocerebral: *POLG*, *DGUOK*, *MPV17*, *C10orf2*		Hepatocerebral: hepatic failure, seizures, roving eye movements, myopathy
3-Methylglutaconic aciduria, deafness, encephalopathy, and Leigh-like disease (MEGDEL)	*SERAC1*	AR	Neonatal hypoglycaemia, lactic acidosis, hyperammonaemia, hepatic dysfunction, 3-methylglutaconic aciduria; later poor feeding, faltering growth, progressive deafness, dystonia, spasticity, profound developmental delay, behavioural disturbance. MRI: bilateral basal ganglia lesions with a so-called putaminal eye
Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS)	Most common mutation m.3243A>G in *MT-TL1*, other mtDNA mutations, *POLG*	Mt	Stroke-like episodes, epilepsy, migraine, cognitive decline, short stature, diabetes mellitus, deafness
Myoclonic epilepsy, myopathy, sensory ataxia (MEMSA)	*POLG*	AR	Juvenile/young adult onset of ataxia, seizures (often focal, starting in right arm, with subsequent generalisation), myoclonus
Myoclonic epilepsy with ragged-red fibres (MERFF)	Most common mutation m.8344A>G in *MT-TK*, other mtDNA mutations, *POLG*	Mt	Progressive myoclonic epilepsy, ataxia, dementia, encephalopathy, myopathy, deafness, optic atrophy
Mitochondrial neurogastrointestinal encephalopathy (MNGIE)	*TYMP*	AR	Ptosis, progressive external ophthalmoplegia, gastrointestinal dysmotility with pseudo-obstruction, peripheral neuropathy and myopathy. MRI: diffuse leukoencephalopathy

Table 42.3 (continued)

Syndrome	Genes involved	Inheritance	Important clinical features
Myopathy, lactic acidosis, sideroblastic anaemia (MLASA)	*PUS1*, *YARS2*	AR	Myopathy, lactic acidosis, sideroblastic anaemia
NARP (neuropathy, ataxia and retinitis pigmentosa)	*MT-ATP6* (m.8993T>G/C, lower mutant load than in Leigh syndrome)	Mt	Neuropathy, ataxia and retinitis pigmentosa.
Pearson marrow-pancreas syndrome	Large mtDNA deletions (identical to PEO and KSS)	Mt (usually sporadic)	Transfusion-dependent sideroblastic anaemia, exocrine pancreatic insufficiency, faltering growth, enteropathy, evolves into KSS
Progressive external ophthalmoplegia (PEO)	Large mtDNA deletions; *POLG*, *C10ORF2*, *ANT1*, *POLG2*,)	Mt (also sporadic); AD; AR	Progressive external ophthalmoplegia, myopathy and additional symptoms may occur
Reversible infantile respiratory chain deficiency (RIRCD)	*MT-TE* (m.14674T>C/G), *TRMU*	Mt, AR	Severe reversible myopathy or reversible liver failure
Sengers syndrome	AGK	AR	Congenital cataracts, cardiomyopathy, lactic acidosis, 3-methylglutaconic aciduria

AD autosomal dominant, *AR* autosomal recessive, *Mt* mitochondrial, *XL* X-linked

defect. The causes of Leigh syndrome are extremely heterogeneous and include mutations in the mtDNA (especially m.8993T>G/C in *MT-ATP6* coding for one of the complex V subunits) and mutations in more than 50 different nuclear genes, including mutations of subunits and assembly factors of complexes I, II, III and IV as well as mutations in genes required for mtDNA maintenance and expression and mutations in the *PDHA1* gene encoding the E1alpha subunit of the pyruvate dehydrogenase complex. To establish a specific genetic diagnosis of Leigh syndrome, muscle biopsy is often needed in order to guide genetic investigations.

42.4 General Approach to a Patient with Suspected Mitochondrial Disorder

It is still the case that there is no gold standard blood biomarker of mitochondrial disease, and so definitive diagnosis continues to require extensive multidisciplinary investigations which may include examination of blood, urine and CSF as well as tissue biopsies, as detailed below. The consideration of mitochondrial disease proceeds along three axes – clinical symptoms, metabolic investigations and functional assays. Rapid advances in genetic diagnostic techniques will likely change this approach, and in the near future, next-generation sequencing of genes involved in mitochondrial disorders (either a panel of genes such as the 'Mito-exome' or whole exome or even whole genome sequencing) may be performed before an invasive muscle biopsy with assessment of respiratory chain function in many cases, although functional confirmation of genetic changes identified by next-generation sequencing will be essential.

In daily clinical work, there are three different clinical scenarios where mitochondrial disorders are suspected (Fig. 42.3). First, clinical symptoms are so suggestive of a specific mitochondrial disease that further investigations are warranted. Second, in the diagnostic workup of a patient with relatively nonspecific symptoms, results of either laboratory or other, e.g. neuroradiological, investigations are suggestive of a mitochondrial disorder. Third, in patients with unexplained symptoms and signs, a mitochondrial
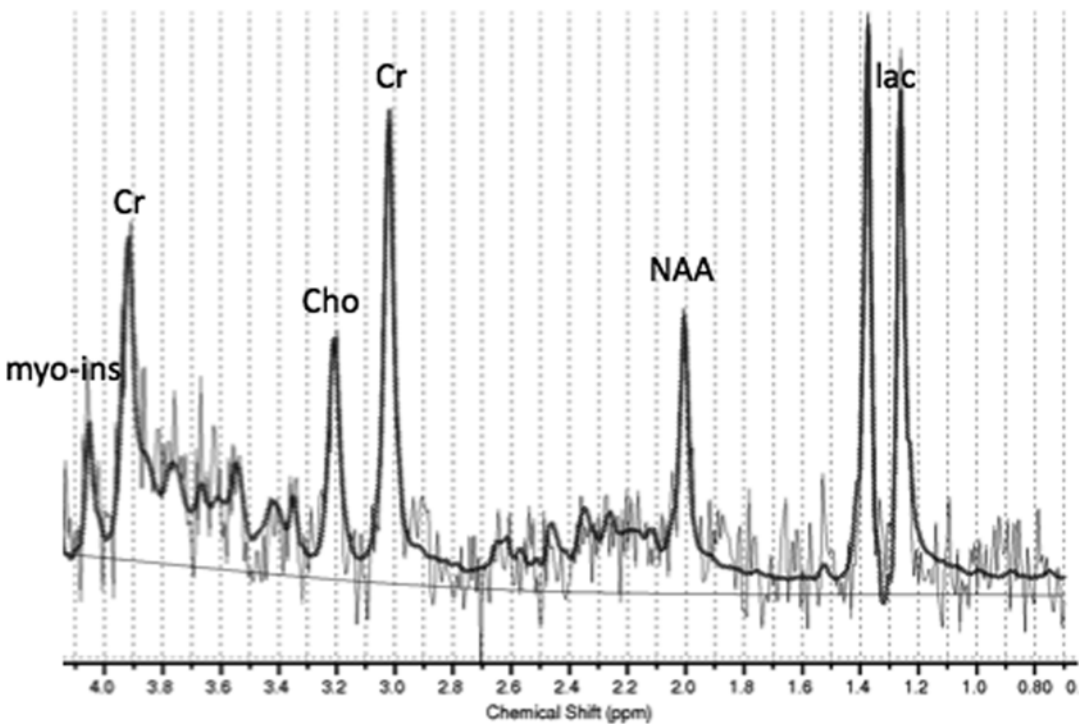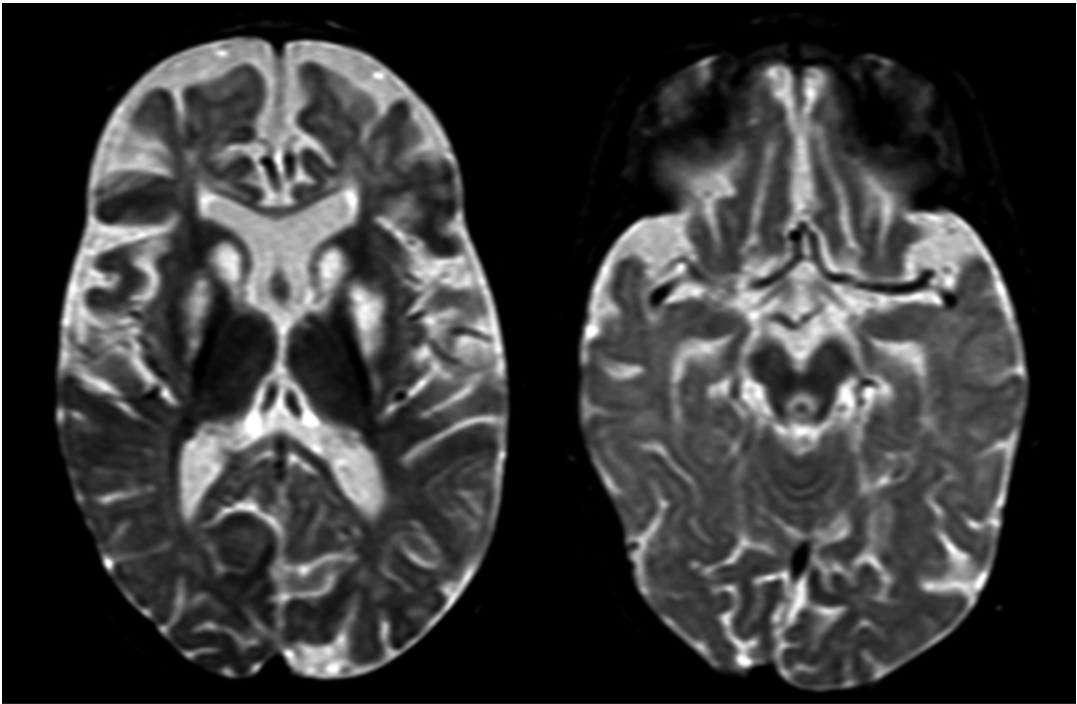
Fig. 42.2 T2w axial images of a child with Leigh syndrome. Nucleus caudatus, pallidum and the periaqueductal area in the mesencephalon show elevated signal. Additionally there is atrophy and white matter changes. ^{1}H-MRS of basal ganglia displays a strongly elevated lactate and decreased NAA (Courtesy of Dr. Inga Harting, Dept. of Neuroradiology, University Hospital Heidelberg)
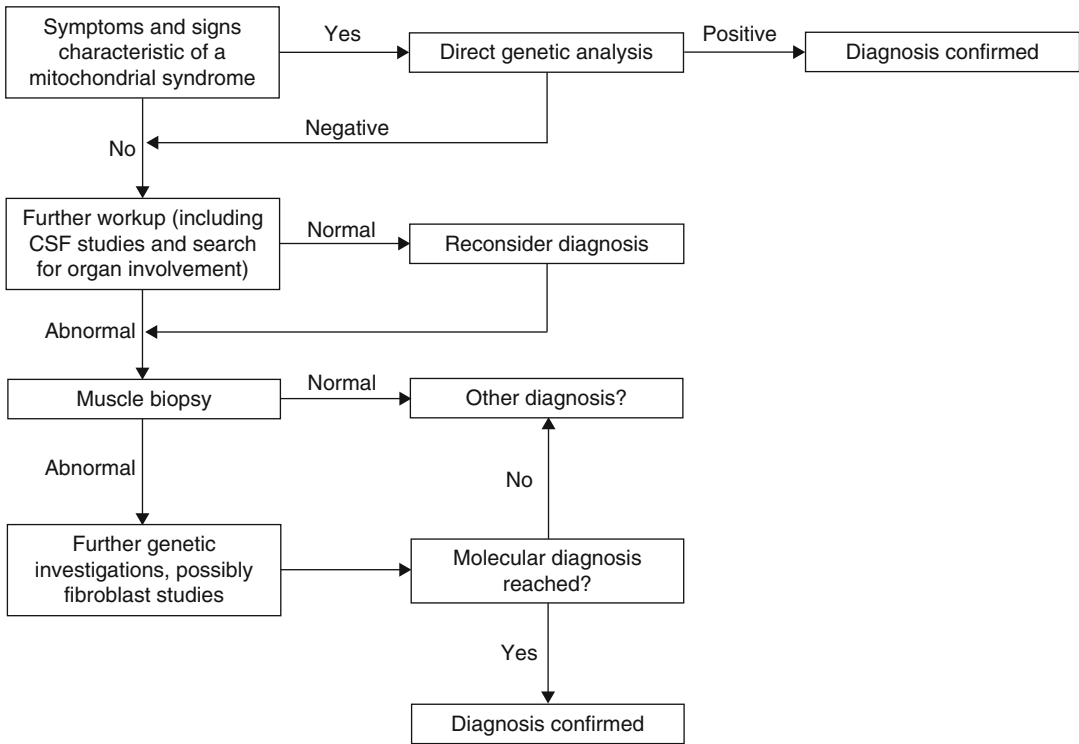
Fig. 42.3 Flowchart for the diagnostic approach in a patient with a suspected mitochondrial disorder

disorder is considered as a possible differential diagnosis, even without typical clinical or laboratory hallmarks supporting this idea. In the first scenario, it is possible to proceed directly to DNA analysis if the symptoms are typical of a certain syndrome such as MELAS or Alpers-Huttenlocher disease; if this turns out to be negative, muscle biopsy is necessary. In the second case, muscle biopsy is the first step, and, depending on the results, tailored genetic investigations would follow (e.g. in complex I deficiency sequencing of genes coding for its subunits and known assembly factors), followed by research genetic investigations such as whole exome or whole genome next-generation sequencing if the initial candidate gene screen is negative. The third scenario is the most difficult one, since it is virtually impossible to exclude a mitochondrial disorder even with the most sophisticated workup (unless another firm non-mitochondrial diagnosis can be established) and difficult to decide to what extent one should pursue invasive and expensive diagnostic procedures such as muscle biopsy.

42.5   Neuroimaging

Brain magnetic resonance imaging may reveal characteristic lesions in certain mitochondrial syndromes, for example, bilateral symmetrical lesions in the basal ganglia, variably extending into midbrain and brainstem in Leigh syndrome, and parieto-occipital lesions, which do not correspond to vascular territories, in MELAS or Alpers-Huttenlocher syndromes. The lesions in Leigh syndrome appear hyperintense in T2-weighted and FLAIR sequences and often hypointense on T1-weighted images, with appearances of swelling in the acute stage. Cavitating leukoencephalopathies are increasingly recognised in various forms of mitochondrial disease including some types of Leigh syndrome and other subgroups of complex I deficiency. Some patients may be surprisingly stable, with even an improvement of imaging findings during the disease course. Some of the mitochondrial translation defects have characteristic MRI brain 'signatures', e.g. leukoencephalopathy with

brain stem and spinal cord involvement and lactate elevation (LBSL) in patients with *DARS2* mutations, leukoencephalopathy with thalamus and brainstem involvement and high lactate (LTBL) in patients with *EARS2* mutations and pontocerebellar hypoplasia type 6 in patients with *RARS2* mutations. Magnetic resonance spectroscopy may be helpful in revealing a lactate peak. An elevated succinate peak is typical for succinate dehydrogenase (complex II) deficiency.

42.6   Metabolic Investigations

There are no universally abnormal blood metabolites in patients with mitochondrial disease, but several molecules have been suggested as potential blood biomarkers. These include lactate and the lactate/pyruvate ratio, but it has become clear that normal blood lactate levels do not exclude the possibility of mitochondrial disease. Furthermore lactate levels may fluctuate between being elevated and normal in the same individual at different times. For this reason it may be worth measuring the blood lactate levels on several occasions, and some clinicians advocate determination of postprandial lactate levels (see Sects. 41.2.2 and 41.2.3). Lactate/pyruvate ratios are classically low in patients with pyruvate dehydrogenase deficiency and elevated in those with respiratory chain defects, but can be normal in both groups. A final caveat is that lactate can be elevated for a multitude of other reasons, including artefactual elevation caused by squeezing and/or struggling during venepuncture, hypoxia, hypovolaemia, sepsis and other metabolic disorders (e.g. glycogen storage diseases, Krebs cycle defects, long-chain fat acid oxidation disorders and organic acidurias). Plasma amino acid analysis may be helpful, particularly if alanine is elevated; this suggests persistent lactic acidosis, since pyruvate is transaminated to alanine. Other abnormalities which may be seen in the plasma amino acid profile include high proline or low citrulline or arginine in some mitochondrial disorders and elevated glycine in certain defects of lipoid acid

biosynthesis (Table 42.2). More recently fibroblast growth factor 21 (FGF21), also known as a 'starvation response hormone', has been suggested as a better blood biomarker for mitochondrial disease, particularly in patients with muscle involvement. However other disease processes, which may lead to elevation of FGF21 levels, include diabetes, obesity and non-alcoholic fatty liver disease. More specific blood biomarkers are clearly needed in order to identify patients with mitochondrial disease without the need for invasive tissue biopsy.

42.7   Muscle Biopsy

When a muscle biopsy is performed, care must be taken that all necessary investigations are conducted. Every muscle biopsy in a patient with suspected mitochondrial disease should be examined morphologically (if possible including electron microscopy to look at mitochondrial ultrastructure) as well as functionally. Histopathological examination may reveal strongly suggestive abnormalities such as ragged-red or cytochrome oxidase negative fibres, whilst electron microscopy may demonstrate abnormal mitochondrial size, number or shape or crystalline inclusions within the mitochondrial matrix, but these appearances are not sufficient of themselves to establish a specific genetic diagnosis of mitochondrial disease. Biochemical and genetic investigations are also needed.

Complete biochemical assessment of mitochondrial function (including measurement of global OXPHOS function and activity of individual respiratory chain enzyme complexes) can only be performed wholly in fresh muscle. However, for the sake of expediency, many centres measure respiratory chain enzyme complex activities in snap-frozen muscle tissue, with the caveat that some defects may be missed by this approach. Patients may have a defect of an individual respiratory chain complex (suggesting a defect in a structural subunit or assembly factor) or a more global defect affecting several complexes (suggesting a defect in mtDNA maintenance or expression).

Biochemical techniques measuring other aspects of mitochondrial function include measurement of CO_2 production from ^{14}C-labelled substrates and polarographic measurement of oxygen consumption using different substrates. Traditionally the latter was determined using an oxygen electrode which required up to 1 g of fresh muscle, but in recent years, new techniques have allowed oxygraphy to be performed on a miniaturised scale, for example, using the Oroboros oxygraph or the Seahorse bioanalyser. Blue native gel electrophoresis can also be used to measure in gel activity and assess assembly of the OXPHOS complexes. Other parameters of mitochondrial function which can be measured include coenzyme Q_{10} levels, reactive oxygen species, glutathione levels and mitochondrial ATP synthesis. These biochemical assays are complex and difficult to interpret and should ideally be performed in a specialist centre.

42.8   Genetic Investigations

Genetic diagnosis of mitochondrial disease is complicated since >150 mtDNA mutations and defects in >200 different nuclear genes have already been linked to mitochondrial disease (Table 42.2). Molecular defects can be present in the mitochondrially encoded tRNAs, mitochondrial and nuclear-encoded subunits of the OXPHOS complexes and many other proteins including factors needed for mtDNA maintenance and expression, cofactor biosynthesis, mitochondrial import and mitochondrial membrane function and dynamics (Table 42.2). There are many areas of overlap – the same mutation can give rise to different syndromes, and the same syndrome can be caused by different functional impairments or mutations in different genes, so investigations are necessarily wide ranging. It is therefore difficult, if not impossible, to provide guidelines which can be applied to all patients in all settings. Functional deficiencies of a single respiratory chain complex may be due to a mutation involving one of the subunits or assembly factors for that

enzyme, whilst mutations in genes required for mtDNA maintenance or expression usually give rise to multiple complex deficiencies. However, respiratory chain activity can also be normal in some of these defects (see Table 42.2).

As discussed above, in a few rare instances, initial genetic investigations can be directed by the clinical phenotype, e.g. screening for the m.3243A>G mutation in *MT-TL1* in MELAS, for *POLG* mutations in Alpers-Huttenlocher syndrome and for *PUS1* or *YARS2* mutations in MLASA. However, for most other cases, a broader approach will be needed, encompassing mtDNA and nuclear gene testing.

Unless there is obvious recessive inheritance (e.g. suggested by parental consanguinity), initial genetic investigations should target the mtDNA, ideally in a muscle sample. Three types of analyses may be performed to assess the integrity of the mitochondrial genome: long-range PCR to screen for large-scale rearrangements of the mtDNA, sequence analysis to determine the presence of sequence variants and real-time PCR to quantitate the amount of mtDNA.

In infants and children, nuclear gene defects are much more common than mtDNA mutations which cause only 10–20 % of respiratory chain disorders in this age group. Next-generation sequencing methods have revolutionised nuclear gene testing for mitochondrial disorders in recent years. Strategies for nuclear gene testing include using a candidate gene approach (e.g. *SURF1* analysis in complex IV-deficient Leigh syndrome, complex I subunits and known assembly factors in cases with isolated complex I deficiency, etc.), usually in the form of a targeted gene panel or a genome-wide approach such as whole exome or whole genome sequencing (Fig. 42.3). It is probable that genome-wide approaches will become first-line tests in the future, as discussed above, leading to ambiguous results in some patients and also to the discoveries of the mildest (and most severe) ends of a spectrum, at least for some conditions. Functional assays will still be necessary in patients with negative or ambiguous results of genetic testing.

In every patient diagnosed with or suspected to have a mitochondrial disorder, organs must be systematically screened for possible involvement. This includes cerebral MRI (and, if possible, ^{1}H-MRS to assess intracerebral lactate); CSF studies for lactate, alanine, protein, 5-methyltetrahydrofolate and neurotransmitter levels; ECG and echocardiography to detect rhythm abnormalities and cardiomyopathy; urine studies to screen for renal tubulopathy and pathological elevations of organic acids; liver function tests; and assessment of the retina, optic nerve and hearing. Diabetes mellitus should be excluded and thyroid function and cortisol levels measured. If these investigations remain normal and no other final diagnosis has been reached, they need to be repeated at regular intervals.

In patients with a clinical presentation suggesting a well-defined mitochondrial syndrome, direct genetic testing is possible. Next-generation sequencing techniques might also become the test of choice in patients with less well-defined clinical entities, but a muscle biopsy is necessary for functional validation of mutations of uncertain pathogenicity identified by next-generation sequencing to confirm the diagnosis of a mitochondrial disorder and to help guide further genetic investigations in some patients. Functional analysis of respiratory chain function in fresh muscle tissue remains the gold standard in diagnosis. Morphological studies alone are not sufficient.

42.9 Prenatal and Preimplantation Genetic Diagnosis and Novel Reproductive Options

Prenatal diagnosis in mitochondrial disorders is straightforward if inheritance is Mendelian and the genetic defect is known. For mtDNA mutations, prenatal diagnosis and prediction of likely disease severity in the offspring are challenging, owing to the random segregation of mtDNA, but have been reliably performed for some mutations known to have highly skewed mutation loads in oocytes, notably the m.8993T>G/C mutations associated with maternally inherited Leigh syndrome. Preimplantation genetic diagnosis may be available for some mtDNA mutations and nuclear-encoded mitochondrial defects, subject to local regulations and expertise. These investigations are performed only in a few highly specialised laboratories. Recently the mitochondrial 'donation' techniques of pronuclear transfer and maternal spindle cell transfer have been approved for further development in the United Kingdom, as a potential method for preventing transmission of mutated mtDNA from the mother to the embryo.

Prenatal diagnosis of mitochondrial disorders is straightforward if nuclear genes are affected and the mutation is known. With mtDNA mutations, prenatal diagnosis and risk assessment remain difficult and are very much dependent on the specific mutation and the mutation load in the mother.

42.10 Differential Diagnosis

There are many possible differential diagnoses for primary respiratory chain disorders. Metabolites accumulating in propionic and methylmalonic acidurias directly affect respiratory chain function and can thereby give rise to 'mitochondrial' symptoms. The clinical presentation of other inherited metabolic disorders, including congenital disorders of glycosylation, the glycogenoses and fatty acid oxidation defects, can also mimic mitochondrial disorders. Biotinidase deficiency causes lactic acidosis, deafness and optic atrophy and must be sought for in every child with elevated lactic acid, since it is treatable. Deficiency of pyridox(am)ine 5-phosphate oxidase (encoded by the *PNPO* gene) may lead to markedly increased CSF lactate. Thiamine deficiency also results in severe lactic acidosis, as does thiamine transporter deficiency due to *SLC19A3* mutations, which is treatable except for its most severe forms. Hypoxia, sepsis and low cardiac output are systemic causes of lactate

elevation. In several neurological disorders of childhood, lactate has been found to be intermittently increased in some patients, including patients with Rett and Angelman syndromes. Respiratory chain enzyme deficiencies and/or decreased ATP production has been demonstrated in patients with variable other non-mitochondrial diagnoses. Since correct diagnosis in these cases is important for prognosis and accurate genetic counselling, clinical suspicion of other disorders must be high, and these should be actively investigated prior to making a definitive diagnosis of mitochondrial disease. Again, increased use of whole exome or whole genome sequencing will facilitate alternative diagnoses in patients with metabolic abnormalities pointing to a possible mitochondrial disorder.

Remember

Besides respiratory chain defects, there are many differential diagnoses for lactate elevation/lactic acidosis including non-metabolic inherited and acquired disorders. The most common situation in which the concentration of lactic acid in blood is elevated is factitious, the result of improper technique, the use of a tourniquet or difficulty in drawing the blood. Depending on the clinical setting, treatable disorders such as biotinidase, PNPO and thiamine deficiency must be considered first.

42.11 Treatment

The vast majority of mitochondrial disorders lack curative therapies. The exceptions are disorders of CoQ_{10} biosynthesis (most cases respond to pharmacological doses of CoQ_{10}), complex I assembly defect caused by *ACAD9* mutations (respond to riboflavin) and reversible infantile respiratory chain disorders caused by *MTTE* or *TRMU* mutations (recover spontaneously with supportive therapy which may include the need for artificial ventilation for several months). Some patients (particularly those with Kearns-Sayre syndrome) have cerebral folate deficiency and clinical benefit from folinic acid supplementation has been documented in some cases. There is some evidence

for efficacy of L-arginine or L-citrulline in prevention and amelioration of stroke-like episodes in MELAS syndrome. The mainstay of treatment for most patients with mitochondrial disorders, however, is symptom management, for example, with gastrostomy feeding, antiepileptic drugs, hormone replacement (insulin, growth hormone, cortisol, thyroxine), surgery for ptosis, hearing aids or cochlear implantation for SNHL, pacemakers for heart block, medical management of cardiomyopathy, blood transfusions for sideroblastic anaemia in Pearson syndrome and fluid and electrolyte replacement for renal tubulopathy. Experimental therapies currently at a preclinical stage include various antioxidants aimed to counteract the effects of reactive oxidative species accumulation (some are currently being evaluated in clinical trials), methods to stimulate mitochondrial biogenesis and innovative gene therapy strategies to tackle defects of the mitochondrial genome.

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