Disorders of mitochondrial function

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Purpose of review

Mitochondrial diseases are a major category of childhood illness that produce a wide variety of symptoms and multisystemic disorders. This review highlights recent clinically important developments in diagnostic evaluation and treatment of mitochondrial diseases.

Recent findings

Major advances have been made in understanding the genetic bases of mitochondrial diseases. Molecular defects have recently been reported in mitochondrial DNA maintenance, RNA translation and protein import and in mitochondrial fusion and fission, opening new areas of cell disorder. Diagnostic testing is struggling to keep pace with these fundamental discoveries. The diagnostic approach to children suspected of mitochondrial disease is rapidly evolving but few patients have a molecular diagnosis. A better notion of the prognosis of affected children is emerging from studies of long-term outcome. Some therapeutic successes are reported, such as in coenzyme Q deficiency conditions.

Summary

Mitochondrial diseases can present with signs in almost any organ. Well planned clinical evaluation is the key to successful diagnostic work-up of mitochondrial diseases. An approach is presented for further testing in specialized laboratories. Mitochondrial diseases can be caused by mutations in mitochondrial DNA or, more commonly in children, in nuclear genes. Mitochondrial DNA mutations pose special challenges for genetic counseling and prenatal diagnosis. Supportive treatment and avoidance of environmental stresses are important aspects of patient care. Specific treatment of mitochondrial diseases is in its infancy and is a major challenge for pediatric medicine.

Keywords

congenital lactic acidosis, mitochondrial diseases, pediatrics, respiratory chain

Introduction

Mitochondrial diseases are a group of inherited disorders of energy metabolism associated with a vast range of presentations, symptoms, severity and outcome. Combined, they form one of the commonest groups of inherited metabolic diseases, with a minimum birth prevalence estimated at approximately 1/5000 [1•,2•,3]. Because oxidative phosphorylation (OXPHOS) is necessary for nearly all cells, any organ can be affected in mitochondrial diseases. Mitochondrial diseases may present to numerous pediatric subspecialists and are included in the differential diagnoses of a large number of clinical situations. As yet, few affected patients have a definite molecular diagnosis.

The present review concentrates on recent advances of clinical importance in pediatrics. The challenges for the pediatrician are as follows: which patients should be investigated? The answer to this question requires knowledge of the clinical spectrum of mitochondrial diseases. How? Among several specialized, often invasive tests, which is best, when, and in which tissue? How will diagnosis and treatment help the patient?

Mitochondrial genetics and biology

About 10–15% of mitochondrial diseases are caused by mutations in mitochondrial DNA (mtDNA) [1•], a 16 596-base pair circular DNA that contains 13 genes encoding subunits of the respiratory chain and 22 transfer-RNA and two ribosomal-RNA genes for mitochondrial RNA translation. Genetically, mtDNA shows high mutation rate, high copy number (thousands per cell) and exclusively maternal transmission between generations. In some individuals, only a fraction of mtDNA molecules are mutant (heteroplasmy); random distribution at cell division during development and selection for or against cells with high levels of mutant mtDNAs may lead to

different mutation loads in different cells and organs. Mitochondrial dysfunction occurs beyond a certain level of mutant mtDNA; this threshold presumably depends on the energy requirement of each tissue.

Most pediatric mitochondrial diseases are caused by defects of proteins encoded by nuclear genes that are transported into mitochondria [2•]. These mitochondrial diseases are inherited in Mendelian fashion. Proteomic/ bioinformatic analyses predict more than 1 000 such proteins [2•,3]. Although complete inactivation of many of them may be prenatally lethal [4], they form a rich pool of potential candidate genes for mitochondrial diseases.

For this review, we define mitochondrial diseases as genetic conditions of the respiratory chain or preceding steps of pyruvate oxidation and the Krebs cycle (Fig. 1). The main function of mitochondria is ATP synthesis. Proteins of mitochondrial and nuclear origin are assembled in four of the five respiratory chain complexes. Only Complex II contains exclusively nuclear-encoded proteins. The respiratory chain receives energy-rich hydrogen atoms from nicotinamide adenine dinucleotide (NADH) or flavin-adenine dinucleotide (FADH), produced mainly in the Krebs cycle and from fatty acid oxidation. Electrons from the hydrogen are passed between complexes in the chain. Complexes I, III and IV extrude protons from the mitochondrial matrix. Complex IV (cytochrome oxidase) consumes oxygen to form water. Complex V couples ATP synthesis to proton reentry, powered by the electrochemical gradient.

Mitochondria are deeply integrated in cell biology, with roles in urea, porphyrin [5] and steroid hormone synthesis [6], apoptosis [7], calcium homeostasis [8] and free radical production [9,10••]. Changes in mitochondrial shape by active fusion and fission are vital for cell function [11•]. Secondary mitochondrial dysfunction occurs in diverse situations like neurodegeneration [12,13] and aging [14•].

The expanding clinical picture of mitochondrial disease in children

Clinical evaluation is the key to decision making in children with suspected mitochondrial disease. Clinical scoring systems exist [15–17,18•]. Some presentations alone are strong indications for further testing for mitochondrial disease (Tables 1 and 2A), as is the otherwise unexplained coexistence of multiple compatible but less specific findings (Table 2B). The known spectrum of mitochondrial diseases in children, different from that in adults, has regularly expanded in unexpected ways. The maxim 'any tissue, any symptom, any age' [19] is supported by recent pediatric series [20,21•]. A high level of suspicion is necessary in patients with compatible findings, even if

accompanied by signs not previously described in pediatric mitochondrial diseases. Web-based catalogues listing mitochondrial diseases and mutations include Online Mendelian Inheritance in Man (OMIM) (http:// www.ncbi.nlm.nih.gov/sites/entrez?db=omim) and mitomap (http://www.mitomap.org/); the latter has useful links to patient organizations and research laboratories.

## The child with increased lactate

Lactic acidemia, a hallmark of mitochondrial dysfunction, may be absent in proven mitochondrial disease or present only during stress, and is highly variable in individual patients [22•]. Hyperlactatemia can also be caused by hypoxia, hypoperfusion, shock, sepsis, cardiac failure and inborn errors of metabolism, including some organic acidemias, glycogen storage diseases, disorders of gluconeogenesis and of fatty acid oxidation, treatment of which differs from that for mitochondrial diseases. Elevated blood lactate is a common artefact if a tourniquet is used for phlebotomy, causing venous stasis and lactate accumulation from erythrocyte glycolysis, or if the child struggles during sampling. In children with elevated lactate, but otherwise at low clinical risk for mitochondrial disease, we sample on several occasions, if possible before and after meals and through an indwelling catheter, permitting blood sampling at rest and determination of other energy substrates (pyruvate, glucose, amino acids including alanine, ketone bodies). Cerebrospinal fluid (CSF) lactate level is a more reliable diagnostic marker for mitochondrial disease than is blood, especially in patients with brain involvement [23,24]; proton magnetic resonance spectroscopy [25••] allows noninvasive detection of elevated cerebral lactate and other relevant compounds.

Tools for further investigation

In patients strongly suspected of mitochondrial disease, further investigation involves biochemical assays of the respiratory chain [25••] and/or molecular studies.

Direct measures of respiratory chain function include polarographic studies of mitochondrial respiration on fresh tissue and spectrophotometric assays of the respiratory chain complexes, feasible on small samples of frozen tissue. Both mtDNA and nuclear-determined respiratory chain defects may be tissue-specific; heteroplasmy of mtDNA mutations and tissue-specific levels of nuclear gene products [26,27] partially explain this phenomenon. Study of clinically affected tissues provides the highest yield of informative results. Careful attention to rapid freezing or, wherever available, to rapid testing of fresh tissue, reduces artefactual decreases of respiratory chain activities.

In blue native polyacrylamide gel electrophoresis (BN-PAGE) of respiratory chain complexes [28–30], the five

Figure 1 Schematic diagrams of mitochondrial morphology and energy metabolism
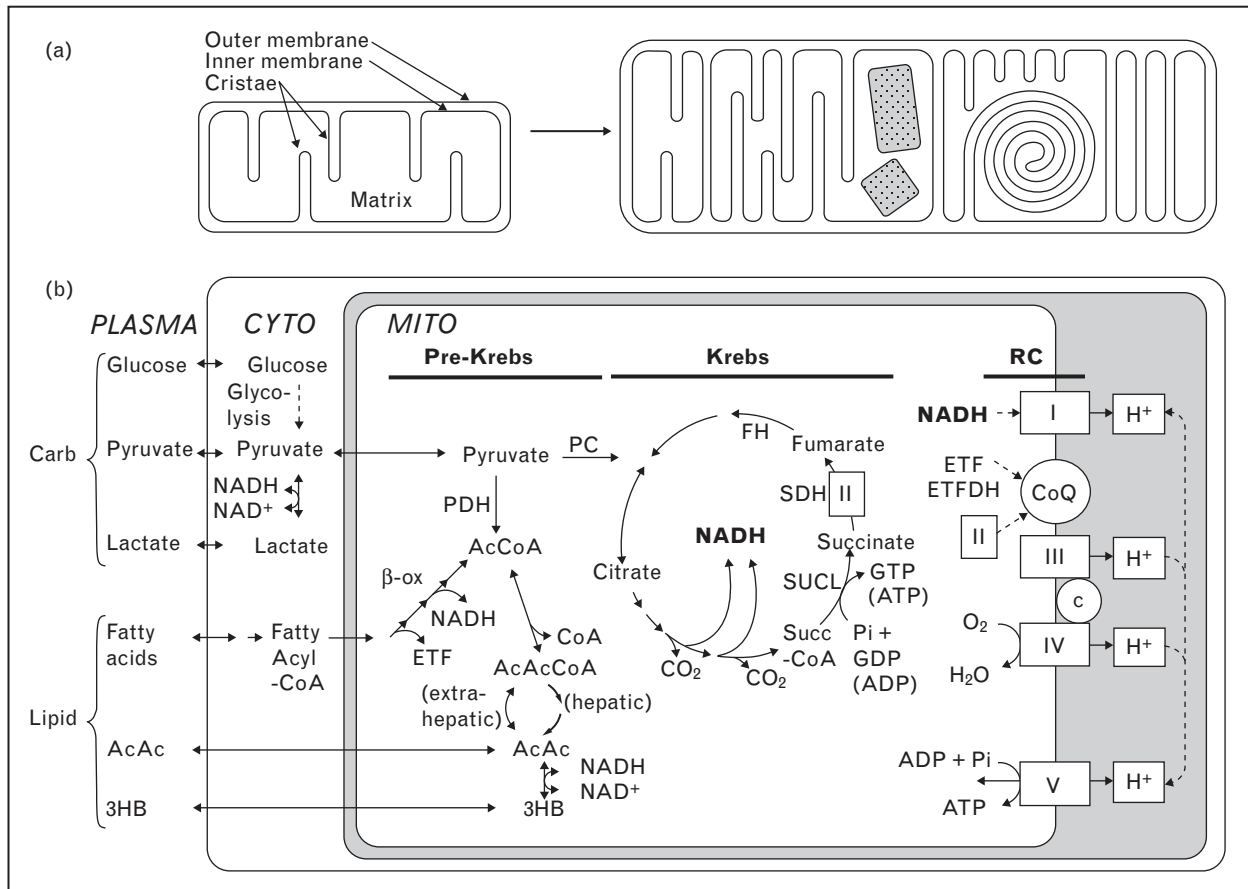
(a) Mitochondrial morphology. The left side of the figure shows the structural features of normal mitochondria, including the two membranes and an inner matrix. The outer membrane is quite permeable. The inner mitochondrial membrane has a distinct chemical composition. Its folds (cristae) protrude into the matrix, increasing the surface area. This membrane is less permeable but contains many metabolite transporters (not shown) and houses the respiratory chain. In mitochondrial disease (right), the changes are highly variable and are shown in composite. Mitochondria may proliferate, change in size (megamitochondria) or shape (elongated, branched), have hypertrophied or bizarrely-shaped cristae, or may contain crystal-like (paracrystalline) inclusions within the matrix. (b) Energy metabolism can be divided into circulating, cytoplasmic and mitochondrial compartments; mitochondrial metabolism is further divided into pre-Krebs, Krebs and respiratory chain components. The degradation products of fatty acids and of glucose enter the Krebs cycle at different sites, which are used therapeutically in some pre-Krebs cycle mitochondrial diseases [e.g. a high-fat, ketogenic diet in some cases of pyruvate dehydrogenase (PDH) deficiency]. For simplicity, amino acids are not shown; different amino acids enter the Krebs cycle at various points. In mitochondrial diseases, there is a paradoxical increase of energy metabolites and of reduced nucleotides [(nicotinamide adenine dinucleotide (NADH), flavin-adenine dinucleotide (FADH)] but a deficiency of ATP production, despite normal oxygen availability. The cytoplasmic NADH/NAD ratio, a measure of redox potential, can be roughly estimated from the ratio of circulating lactate to pyruvate; intramitochondrial redox potential, from the plasma 3HB/AcAc ratio. High ratios are frequent in mitochondrial diseases. Circulating ketone bodies are derived mainly from liver, by unidirectional hepato-specific ketogenesis, but may also arise from other tissues which have a high concentration of AcCoA, by reversal of the enzyme that normally activates AcAc for intracellular metabolism; this may be substantial in some mitochondrial diseases. Some NADH and FADH are produced from glycolysis (not shown) and beta oxidation (β-ox), but the Krebs cycle is the cell's main source of these compounds. The three Krebs cycle steps discussed in the text are shown. Distinct isozymes of succinate-CoA ligase (SUCL) catalyze the synthesis of either guanosine triphosphate (GTP) or ATP. The respiratory chain is composed of five multisubunit complexes as shown and is the main site of cellular oxygen consumption, ATP synthesis and (not shown) oxygen radical production. Electrons from NADH are donated to Complex I, which passes them to coenzyme Q. FADH-containing molecules include succinate dehydrogenase (SDH, that is Complex II) and electron transfer flavoprotein (ETF) and ETF dehydrogenase (ETFDH). They donate electrons directly to Coenzyme Q (CoQ). Electrons pass to Complex III, cytochrome c (c) and finally to Complex IV (cytochrome oxidase), the main site of cellular oxygen consumption, forming water. Electron transport is accompanied by the extrusion of protons (H^+) from the matrix by complexes I, III and IV, creating an electrochemical gradient that is used by Complex V to catalyze most of cellular ATP synthesis. The direct relationship between oxidation, proton gradient formation and ATP synthesis is termed 'coupling'. AcAc, acetoacetate; BNG, blue native gel; Dx, diagnosis; MD, mitochondrial disease; RRF, ragged red fibers.

mitochondrial respiratory chain complexes are resolved electrophoretically but remain intact and catalytically active. Histochemical staining and immunodetection of respiratory chain subunit proteins allows detection of both defective enzyme function and reduced amounts of respiratory chain complexes. BNG-PAGE may be less influenced than functional studies by suboptimal tissue conservation and has substantial diagnostic yield [21•,31].

Table 1 Typical mitochondrial syndromes in children and adults

Syndrome	Typical clinical features		Molecular findings
	Children	Adults	
Pearson syndrome	Infantile onset, exocrine pancreatic insufficiency, sideroblastic anemia and bone marrow failure, tubular and liver dysfunction, encephalopathy. Survivors develop Kearns–Sayre syndrome.		Large mtDNA deletion[a]
Alpers syndrome	Infantile onset, progressive poliodystrophy with liver failure, seizures, severe encephalopathy		mtDNA depletion (of nDNA origin)
Leigh syndrome (subacute necrotizing encephalopathy)	Infantile onset, recurrent attacks of psychomotor regression in infancy, clinical and radiological signs of brainstem and/or basal ganglia disease, lactic acid increased in blood or CSF, typical neuropathology.	(Occasional adults described)	Highly heterogeneous, most cases of nDNA origin, up to 10% linked to mtDNA (see NARP and MILS, below)
Fulminant neonatal lactic acidosis	Neonatal primary lactic acidosis, cardiac and/or liver involvement, encephalopathy		As for Leigh syndrome
MELAS[b]	Onset in childhood (>50% before the age of 10 years)	Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes. Intermittent migraine headaches, proximal limb weakness, recurrent neurological deficit resembling strokes (hemiparesis, cortical blindness, hemianopsia, etc.) with neuroradiological features (stroke-like lesions), lactic acidosis, hearing loss, diabetes, cardiomyopathy.	mtDNA A3243G or T3271C (Leucine tRNA) are the commonest mutations
MERRF[b]	Onset usually in childhood	Myoclonic epilepsy and ragged red fibers. Encephalomyopathy, myoclonic seizures, ataxia, muscle weakness, hearing loss, lactic acidosis, cardiomyopathy.	mtDNA A8344G in 80–90% (Lysine tRNA)
MNGIE[b]	Possible onset in adolescence	Myoneurogastrointestinal encephalomyopathy with intestinal pseudoobstruction, demyelinating peripheral neuropathy, mitochondrial myopathy, leukoencephalopathy; high circulating thymidine and deoxyuridine levels	Thymidine phosphorylase (nDNA)
Kearns–Sayre syndrome	Possible onset in adolescence or childhood	CPEO with mitochondrial myopathy, pigmentary retinopathy, cerebellar ataxia, heart block, elevated CSF protein >1000 mg/l; onset < 20 years.	Large mtDNA rearrangement[a]
CPEO[b]		Chronic progressive external ophthalmoplegia, ptosis	Large mtDNA rearrangement[a]; some tRNA point mutations.
MILS & NARP[b]	Maternally inherited Leigh syndrome	Neurogenic muscle weakness, ataxia and retinitis pigmentosa	mtDNA T8993G or C in Complex V[c]
LHON[b]	Possible adolescent onset	Leber's hereditary optic neuropathy: rapidly progressive optic atrophy with blindness; young adults; men > women	mtDNA G3460A, G11778A, T14484C in Complex I subunits
Mitochondrial myopathy		Onset at any age. Myopathy often with exercise intolerance, sometimes with ragged red fibers, occasionally with episodic rhabdomyolyis.	Various mt-tRNA mutations

Typical pediatric presentations of mitochondrial disease are listed in bold. CPEO, chronic progressive external ophthalmoplegia; CSF, cerebrospinal fluid; LHON, Leber's hereditary optic neuropathy; MELAS, mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke; MNGIE, mitochondrial neurogastrointestinal encephalomyopathy; MILS, maternal inherited Leigh syndrome; NARP, neurogenic muscle weakness, ataxia and retinitis pigmentosa.
[a] A 4.9 kb deletion is the most frequently observed large mtDNA rearrangement in Pearson, Kearns–Sayre, and CPEO (~ 1/3 of cases), but other deletions and duplications occur. The phenotype is determined by tissue distribution and fraction of mutant genomes, not by the specific deletion. Such mtDNA rearrangements disrupt translation of mtDNA-encoded respiratory chain subunits and cause multiple respiratory chain deficiencies.
[b] These abbreviations are widely used and useful mnemonics for frequent symptoms, but other typical mitochondrial disease signs can occur in each condition.
[c] T8993G or C in families with NARP syndrome and/or MILS. A mutation load >90% confers an increased risk of MILS.

Table 2 Clinical and biochemical findings of mitochondrial disease in children

(A) Signs and symptoms highly suggestive of a mitochondrial disease[a,b]	(B) Signs and symptoms compatible with mitochondrial disease[b]

Neurologic
  Episodic or progressive mental regression
  Episodic neurological symptoms of unknown cause
  Cerebral stroke-like episode with nonvascular distribution of lesions
  Unexplained brainstem dysfunction (rapid onset, hypoventilation or hyperventilation, drooling, oculomotor changes, altered level of consciousness, hypothermia or hyperthermia, hypotension or hypertension)
  Brainstem (± basal ganglia) involvement in MRI (Leigh syndrome-like)
Muscular
  Myopathy with presence of ragged red fibers
Cardiovascular
  Unexplained hypertrophic cardiomyopathy
  Arrhythmia of unknown cause: heart block, Wolff–Parkinson–White, and others
Ophthalmologic
  External ophthalmoplegia with or without ptosis
  Sudden or insidious optic neuropathy
Gastroenterologic
  Unexplained liver failure (especially if valproate-related), Reye-like syndrome
  Severe intestinal dysmotility, chronic pseudoobstruction
Clinical biochemistry
  Persistent elevation of blood lactate
  Episodes of acidosis, ketosis or hyperlactatemia, exceeding the expected physiological concentration; postprandial ketosis
  Characteristic MRS spectra (lactate); e.g. succinate in succinate dehydrogenase deficiency
	General
	  Failure to thrive; short stature
	  Fatigue
	Neurologic
	  Progressive or static developmental delay; encephalopathy
	  Cerebral atrophy
	  Seizures, especially myoclonic
	  Peripheral (usually axonal) neuropathy; unexplained spinal muscular atrophy
	  Cerebellar ataxia
	  Extrapyrimidal movement disorders
	  Hypotonia or progressive spasticity
	  Leukodystrophy
	  Exercise intolerance with or without rhabdomyolyis
	  Migraine
	Other
	  Ophthalmologic (optic atrophy, cataracts), pigmentary retinal degeneration
	  Sensorineural hearing loss; aminoglycoside-induced deafness
	  Sideroblastic anemia
	  Dermatological (hypertrichosis, pili torti, subcutaneous lipomas)
	  Endocrine (hypoparathyroidism, glucose intolerance, diabetes)
	  Dilated cardiomyopathy
	  Recurrent vomiting
	  Unexplained liver disease (fatty liver, hepatocellular lysis, cirrhosis)
	  Pancreatic insufficiency
	  Renal (tubular acidosis; renal Fanconi syndrome; unexplained glomerulopathy; nephrotic syndrome)

MRS, magnetic resonance spectroscopy.
[a] The unexplained occurrence of one or more of these signs places mitochondrial disease high on the list of possible diagnoses.
[b] The suspicion of a mitochondrial disease increases with the presence of each additional unexplained sign from Table 2B, especially if they arise from different organs (multisystemic), or if one or more signs from Table 2B occur with sign(s) from Table 2A.

Most patients with mitochondrial disease do not currently have a precise molecular diagnosis. Molecular testing as an initial step has low yield because of the large number of different mitochondrial diseases; this may change as molecular tests incorporate large numbers of genes. If there is clinical suspicion of a disease frequently caused by readily testable mutations [mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke (MELAS), maternal inherited Leigh syndrome (MILS), etc.] or if the patient is from an ethnic group with a strong founder effect and has suggestive signs, molecular testing is indicated. Testing for common mutations and complete mtDNA sequencing are clinically available. For mtDNA mutations not previously known as pathogenic, it may be difficult to distinguish between true causal mutations, functionally neutral variants and technical artefacts [32•].

Fasting and dietary loading tests have limited diagnostic usefulness and can precipitate crises in some patients. They are useful in selected patients to determine and monitor diet and other treatments. Exercise testing in patients with myopathic mitochondrial disease characteristically reveals low endurance and low oxygen utilization [33••], but requires a level of collaboration rare in children with mitochondrial disease.

Current protocols of investigation

Our current approach to further investigation is summarized in Fig. 2. In deciding whether to investigate a patient, we consider clinical phenotype, biochemical findings (mean plasma lactate and pyruvate, urinary organic acids, including lactate and Krebs cycle metabolites, plasma amino acids, etc.). CSF lactate and pyruvate levels and neuroimaging, including magnetic resonance spectroscopy [34•], are obtained in children with neurological symptoms and suspected mitochondrial disease.

Clinical suspicion of a specific mitochondrial syndrome calls for testing in an appropriate sample obtained by the least invasive means. For example, common MELAS, Leber's Hereditary Optic Neuropathy (LHON) and neurogenic weakness, ataxia and retinitis pigmentosa (NARP)/MILS mtDNA mutations are detectable in leukocyte DNA. mtDNA deletions are detectable in blood in Pearson syndrome, but otherwise are tissue-specific, for example, muscle in Kearns–Sayre syndrome. Infantile liver failure suggests hepatic mtDNA depletion; liver mtDNA quantification is a logical first step.

Muscle biopsy is performed in myopathic patients. The presence of ragged red fibers in a mosaic pattern,

Figure 2 Diagnostic evaluation in children suspected of mitochondrial disease is governed by clinical parameters
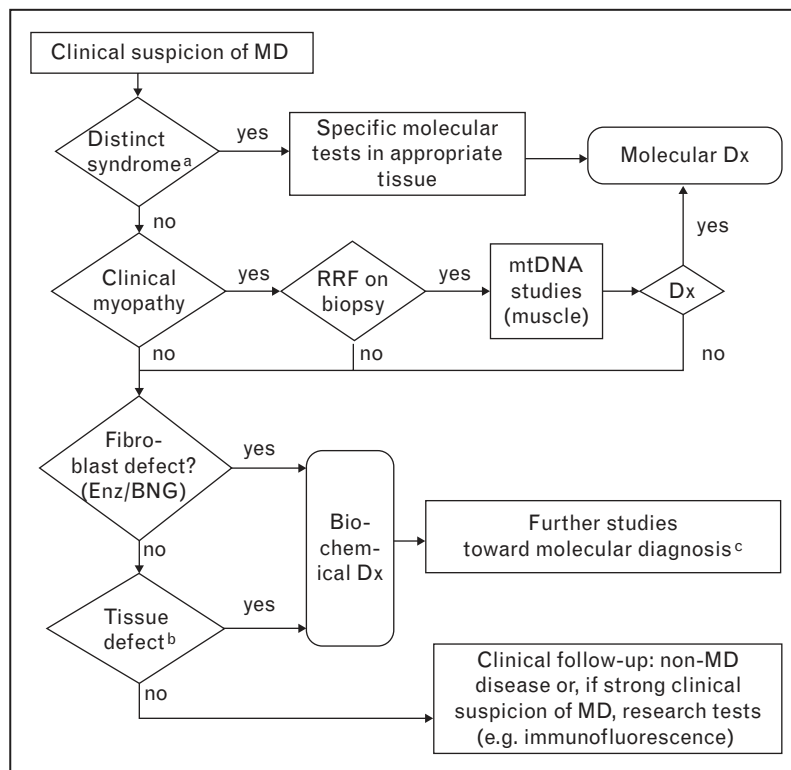
It is impossible to summarize the investigation of the entire spectrum of patients with mitochondrial disease in a single algorithm, but the approach to most patients with mitochondrial disease who are not acutely ill resembles this. (a) Clinically distinct syndromes (see Table 1) or compatible signs in patients from ethnic groups with strong founder effects and known mutations [e.g. Leigh disease in French Canadians (LRPPRC), dilated cardiomyopathy and cerebellar ataxia in Hutterites (DNAJC19), encephalomyopathy in patients from the Faroe islands (SUCLA2), liver disease and neuropathy in Navajo Indians (MPV17), infantile spinocerebellar ataxia in Finns (Twinkle), etc.]. (b) The most clinically affected tissue (muscle, liver) is chosen for biopsy; studies involve light and electron microscopy, spectrophotometry and blue native gel (BNG), CoQ10 assay (particularly in patients with ataxia or nephrotic syndrome) and possibly mtDNA quantification if clinically suggested or if multiple complex deficiencies are found on spectrophotometry or BNG. (c) Further testing depends on whether single or multiple complex deficiencies are found and is individualized in collaboration with an experienced diagnostic laboratory. 3HB, 3-hydroxybutyrate; AcAc, acetoacetate; β-ox; beta oxidation; ETF, electron transfer flavoprotein; ETFDH, ETF dehydrogenase; GDP, guanosine 5'-diphosphate; GTP, guanosine triphosphate; NADH, nicotinamide adenine dinucleotide; PDH, pyruvate dehydrogenase; SDH, succinate dehydrogenase.

juxtaposed with normal-appearing fibers, strongly suggests mtDNA disease. Frozen tissue obtained at biopsy can be used for mutation detection. If ragged red fibers are not observed, respiratory chain spectrophotometry and BN-PAGE are performed. Coenzyme Q (CoQ) is assayed in both cases.

Skin biopsy for fibroblast culture is performed in all patients. We obtain pyruvate dehydrogenase (PDH) and pyruvate carboxylase assays, biochemical respiratory chain studies and BN-PAGE analysis. Skin biopsy is minimally invasive and yields can reach 50% when spectrophotometry and BN-PAGE are combined [21•,30,35]. In some patients without molecular diagnosis, markedly decreased fibroblast activity may provide a marker for prenatal diagnosis (after discussion in advance with an experienced laboratory) and for gene discovery [36]. If fibroblast studies are inconclusive,

muscle and/or liver biopsies are performed according to clinical judgement.

In acute or rapidly progressive illness, the intensity of investigations is accelerated. The potential risk that the stresses associated with imaging or biopsies may occasionally precipitate neurological or acidotic crises is weighed against the need for rapid diagnosis.

Other metabolic diseases, including congenital disorders of glycosylation, overlap clinically with mitochondrial diseases and should be excluded in the absence of a conclusive diagnosis.

Biochemical and clinical phenotypes and molecular causes do not always correlate. For example, Leigh syndrome (MIM 256000) can occur in isolated deficiencies of any of the respiratory chain complexes,

pyruvate carboxylase, PDH or CoQ synthesis. Conversely, complex I-deficient or IV-deficient patients, for instance, can also present with encephalomyopathy, cardiomyopathy, neonatal acidosis or in other fashions. Different mutations in a single gene can cause divergent symptoms (e.g. some BCS1L mutations can cause encephalopathy–tubulopathy [37], neonatal lactic acidosis–liver hemosiderosis [38]; others cause isolated deafness–brittle hair with pili torti (Björnstad syndrome)[39•]). In general, siblings with nuclear-encoded mitochondrial diseases tend to have similar types of symptoms.

Molecular bases of mitochondrial disease in pediatrics: unraveling the genetic complexity of mitochondrial disease

Current rates of discovery are unprecedented in mitochondrial biology and mitochondrial diseases (Table 3). Mitochondrial diseases are now classified not only as nuclear or mtDNA-related, but also by pathophysiology. Respiratory chain deficiency can arise from deficiency of structural respiratory chain proteins or accessory molecules like CoQ, or abnormalities of respiratory chain complex assembly, mtDNA replication or maintenance, mtRNA translation or mitochondrial dynamics (fusion/fission; mobility). Other mechanisms may be discovered.

Deficiencies of respiratory chain components and cofactors

Many mutations in the 13 structural mtDNA-encoded respiratory chain subunits are well known to cause LHON and NARP/MILS (Table 1); other mutations are regularly being identified. At least 74 nuclear genes encode respiratory chain subunits; mutations in only 15 of them are described in mitochondrial diseases. Deficiencies of complex I [40] or complex IV [41] are the commonest isolated defects, perhaps because of the many structural and assembly peptides required by these complexes. Mutations in *NDUFA1*, the first X-linked gene associated with complex I deficiency, were described in boys with Leigh syndrome or myoclonic epilepsy [42•].

CoQ funnels electrons from complexes I and II to complex III. CoQ deficiency, primary or secondary, may respond to replacement therapy. Mutations in *APTX* [43,44•] and *ADCK3* [45,46•] were recently found in CoQ deficiency with ataxia. Mutations in three genes of CoQ biosynthesis, *COQ2* [47], *PDSS2* [48] and *PDSS1* [49] were reported in patients with severe infantile mitochondrial syndromes and tissue CoQ10 deficiency. Nephrotic syndrome has been reported in several patients with COQ2 mutations [50•].

Respiratory chain complex assembly deficiencies

Mutations in assembly factors are the commonest cause of isolated complex I deficiency [51]; three were recently described in patients with infantile encephalopathy and lactic acidosis [51,52,53•]. Assembly factor defects are also the main cause of complex IV deficiency (*SURF1*, *SCO1*, *SCO2*, *COX10*, *COX15*; possibly *LRPPRC*) [31,54]) and are reported for complexes III [37] and V [55].

Disorders of mtDNA replication and maintenance

In these 'disorders of intergenomic communication' [56], nuclear gene defects cause mtDNA abnormalities. These are detected in affected tissues as mtDNA depletion and/or accumulation of multiple different mtDNA deletions, resulting in deficiency of multiple respiratory chain complexes. Mitochondria possess a complete DNA replication/maintenance system, including DNA polymerase gamma (POLG), a helicase (Twinkle) and other enzymes, requiring a continuous supply of deoxynucleotides. mtDNA depletion/multiple deletions syndromes, initially reported in adults with progressive external ophthalmoplegia (PEO) or cerebellar ataxia [57,58], are now recognized as major causes of neonatal/infantile liver failure and infantile encephalomyopathy [59•].

Causal mutations are documented in 12 nuclear genes. Three of these genes are directly implicated in mtDNA replication: POLG (both subunits) [58,60] and Twinkle [61]; POLG1 mutations cause Alpers–Huttenlocher syndrome (autosomal recessive infantile hepatic failure, epilepsy and encephalopathy). Seven genes are implicated in regulating mitochondrial deoxynucleotide pools [27,62–65,66•,67,68•,69], and two genes function by unknown mechanisms: MPV17, which causes isolated liver failure [69] and OPA1 (see below).

mtRNA translation defects

The genetic code, tRNAs and rRNAs of mitochondria differ from those of the cytosol. mtDNA tRNA mutations cause mitochondrial disease (Table 1). There are over 50 known nuclear-encoded mitochondrial ribosomal proteins, tRNA maturation enzymes and translation initiation, elongation and termination factors. In seven (three in the last year), mutations have been identified in human mitochondrial diseases: three translation elongation factors [70–72], two ribosomal proteins [73,74•] and two enzymes of tRNA maturation [75,76•]. Clinical presentations include fulminant neonatal lactic acidosis, infantile encephalopathy, hypertrophic cardiomyopathy with encephalomyopathy, and leukoencephalopathy with brain stem and spinal cord involvement and lactic acidosis.

Defects of mitochondrial protein import

Two known mitochondrial diseases are attributable to abnormal protein import. In Mohr–Tranebjaerg (X-linked deafness–dystonia) syndrome, deafness is

Table 3 Genetically defined mitochondrial disorders showing recent discoveries

Pathophysiology	mtDNA	Nuclear genes	Associated clinical features	Reference
Structural subunit proteins and accessory components				
Complex I deficiency	MTND1-6, MTND4L	NDUFS1-4, 6-8, NDUFV1, NDUFV2, **NDUFA1**	LHON, Leigh, other neurological and systemic presentations	
			Partial/complete Leigh syndrome, some with hypertrophic cardiomyopathy, LA	
			Infantile Leigh syndrome, developmental delay and myoclonic epilepsy	[42•]
Complex II deficiency		SDHA, SDHB, SDHC, SDHD	See text	
Complex III deficiency	MTCYB		Isolated exercise intolerance; possible extrapyramidal and other neurological signs	
		UQCRB	Episodic hypoglycemia and LA	
Coenzyme Q10 deficiency		**COQ2**	**Infantile glomerular nephropathy and encephalomyopathy**	[47]
		PDSS1	**Deafness, OA, mental retardation**	[49]
		PDSS2	**Infantile nephrotic syndrome and Leigh disease**	[48]
		ADCK3/CABC1	**Childhood onset progressive ataxia**	[45,46•]
Complex IV deficiency	MTCO1, MTCO2, MTCO3		Wide range of myopathy, OA, retinopathy, LA, epilepsy, mental retardation, ataxia. See OMIM	
Complex V deficiency	MTATP6, MTATP8		MILS/NARP (Table 1); valproate sensitivity	
Multiple complex deficiencies	Large deletions		See Table 1	
RC complex assembly factors				
Complex I deficiency		**B17.2L**	OA, progressive brain stem, basal ganglion, cerebellar, white matter disease	[52]
		NDUFAF1	**Cardioencephalomyopathy**	[53•]
		C6ORF66	**Neonatal lactic acidosis, infantile encephalomyopathy, cardiomyopathy**	[39•]
Complex III deficiency		BCS1L	Encephalopathy, proximal renal tubulopathy, liver disease with iron overload (GRACILE), **pili torti and deafness (Bjornstad syndrome).**	
Complex IV deficiency		SURF1, SCO1, SCO2, COX10, COX15, LRPPRC	Leigh, other	
Complex V deficiency		ATP12	Fatal neonatal LA and cardiomyopathy	
Mitochondrial DNA metabolism		ECGF1, POLG1, TWINKLE, ANT1, POLG2, TK2, DGUOK	See text	
		MPV17	**Hepatic mtDNA depletion syndrome, infantile liver failure**	[66•]
		RRM2B	**Neonatal encephalomyopathy with LA, proximal tubulopathy**	[68•]
		SUCLA2	**Leigh-like encephalomyopathy, deafness, dystonia, methylmalonic aciduria**	[27]
		SUCLG1	**Fatal neonatal LA, muscle and liver mtDNA depletion, methylmalonic aciduria**	[69]
Mitochondrial translation	22 tRNA; 2 rRNA genes	EFG1	Varied. See text, Table 1 and OMIM	
		PUS1	Leigh-like disease, with or without cholestatic liver failure,	
		MRPS16	Myopathy and sideroblastic anemia	
		TSFM,	Severe neonatal LA, corpus callosum agenesis	
		TUFM	**Severe neonatal LA, encephalopathy, cardiomyopathy**	[71]
		DARS2	**Severe neonatal LA, recurrent metabolic crises, encephalopathy**	[72]
		MRPS22	**Leukoencephalopathy with brain stem, spinal cord involvement and LA**	[74•]
			Antenatal skin edema, hypotonia, cardiomyopathy and tubulopathy	[76•]
Mitochondrial protein import		DDP1	X-linked deafness, dystonia, OA (Mohr–Tranebjaerg syndrome)	[79]
		DNAJC19	**Dilated cardiomyopathy and ataxia**	
Mitochondrial fusion/fission		OPA1	OA, ophthalmoplegia, ataxia, deafness, multiple mtDNA deletions	[85]
		DLP1	**Infantile encephalopathy, microcephaly, cerebral gyration defect, LA**	[88••]

Genes identified during the review period to cause mitochondrial disease are shown in bold. Some descriptions are based on a single patient, and clinical spectrum is expected to expand as more patients are described. Clinical summaries are incomplete; see also indicated references and OMIM (http://www.ncbi.nlm.nih.gov/sites/entrez?db=omim). The proposed functional classification of some mitochondrial disease-related genes is tentative because direct functional studies have not been performed for many genes. Deficiencies of general mitochondrial processes (e.g. translation) tend to cause deficiencies of multiple respiratory chain complexes. LA, lactic acidosis; LHON, Leber's Hereditary Optic Neuropathy; MILS, maternal inherited Leigh syndrome; NARP, neurogenic weakness, ataxia and retinitis pigmentosa; OA, optic atrophy; OMIM, Online Mendelian Inheritance in Man; RC, respiratory chain.

followed by progressive neurological troubles, including dystonia and optic atrophy [77,78]. Mutation of *DNAJC19*, encoding a putative mitochondrial import protein, causes autosomal recessive dilated cardiomyopathy with ataxia [79].

Mitochondrial biogenesis, fusion, fission and mobility

Dynamin-type guanosine triphosphatases are essential for mitochondrial mobility and exchange. Mutations in dynamin-like genes were first described in autosomal dominant optic atrophy (OPA1) and Charcot-Marie-Tooth neuropathy types 2A and 4A [11•,80–82]. Mitochondrial fusion defects exert general effects on mitochondrial function. The mitochondrial fusion-related gene OPA1 is implicated in apoptosis [83,84] and oxidative phosphorylation [85], and multiple mtDNA deletions are found in muscle of some patients [86], suggesting that the OPA1 protein also influences mtDNA maintenance [87•].

In an infant with lactic acidosis and increased very long chain fatty acids, Waterham *et al.* [88••] demonstrated a combined defect of mitochondrial and peroxisomal fission, due to mutation in *DLP1*, which encodes a dynamin-like protein. Despite severe lactic acidosis, respiratory chain assays were normal in muscle and fibroblasts. The authors proposed examination of mitochondrial morphology in cultured cells as a new diagnostic tool.

Krebs cycle

Three successive steps of this intramitochondrial pathway provide unexpected phenotypes. Deficiencies of the guanosine 5'-diphosphate (GDP)-specific and ADP-specific succinate-CoA ligase (SUCL) enzymes are clinically distinct (Table 3). Succinate dehydrogenase (Complex II) subunit A deficiency can cause typical autosomal recessive mitochondrial disease, including Leigh disease [89], whereas mutations of subunits B, C and D predispose to pheochromocytoma in a dominant fashion [90–92]. Fumarase deficiency can cause autosomal recessive encephalopathy or autosomal dominant tumors (uterine fibromas, renal cancer) [93], the latter possibly by affecting hypoxia-inducible factor metabolism [94].

Prognosis and management

Prognostic counseling is difficult because of high inter-patient variability. Empirical data clearly show higher risk for symptomatic LHON and Alpers disease in men [95] and for valproate hepatotoxicity in mitochondrial disease in general and Alpers disease in particular [95,96]. Interesting research has identified modifier loci for LHON in mtDNA [97•] and on the X chromosome [98], but as yet does not allow for precise counseling. Reproductive genetic counseling is challenging for heteroplasmic mtDNA disorders because marked, unpredictable differences of mutant mtDNA load occur between mothers and offspring [99•]. Empirical figures are available for transmission and prognosis in some mtDNA-related diseases [100,101]. Preimplantation diagnosis [102] and other reproductive technologies hold promise for the future [10••,33••].

Two recent studies [21•,103] addressed long-term outcome in pediatric mitochondrial diseases, showing that, despite high mortality and morbidity, some patients with mitochondrial disease can become clinically stable and that prognosis is not uniformly poor. A scale for severity of disease course has been proposed [104].

Nonetheless, effective management of mitochondrial diseases is a major challenge in pediatrics. Few controlled therapeutic trials exist [105]. Many pediatric mitochondrial diseases, like Leigh disease, predispose to acute acidotic or neurologic crises that often but not always coincide with periods of physical, nutritional or infectious stress. We have a strong clinical impression that avoidance of extremes of nutrition (fasting, excessive consumption) or physical exertion, and rapid supportive treatment of intercurrent illness can improve outcome. We avoid invasive or stressful testing during and immediately after crises, during which patients seem to be particularly susceptible to further episodes. Many physicians use 'mitochondrial cocktails' of unproven efficiency, containing vitamins and other compounds [10••,33••]. Importantly, primary or secondary [106•] CoQ deficiency can respond to oral CoQ10 supplementation [107••,108], though some patients progress despite treatment [109]. CoQ10 may also scavenge free radicals [10••]. PDH-deficient patients may benefit from thiamine supplementation, a ketogenic diet or dichloroacetate administration [110–112]. The lactic acidosis of biotinidase deficiency is cured by biotin administration. Peripheral neuropathy limits chronic dichloroacetate use [113]. Dichloroacetate can lower lactate level nonspecifically in mitochondrial disease [114] and may be useful for short treatment of severe acidotic episodes. Recent observations in a small number of patients have suggested that defective ATP-dependent cerebral folate transport may result in reduced CSF 5-methyltetrahydrofolate levels in some patients with mitochondrial disease, with detectable clinical improvement with oral folinic acid supplementation [115,116]. Potential future approaches to mitochondrial disease treatment have recently been reviewed [10••,33••].

Conclusion

In the past year, there has been an unprecedented pace of gene discovery for mitochondrial disease. With a high level of clinical alertness and an organized diagnostic

approach, most mitochondrial diseases can be confirmed early in their course and many can benefit from precise molecular diagnosis. Supportive treatment and genetic counseling are important. Few specific treatments for mitochondrial diseases are available and their development is a major challenge for research.

References and recommended reading

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