Mitochondrial Disease in Childhood: mtDNA Encoded

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Abstract Since the first description of a mitochondrial DNA (mtDNA)-associated disease in the late 1980s, there have been more than 275 mutations within the mtDNA genome described causing human disease. The phenotypic expression of these disorders is vast, as disturbances of the unique physiology of mitochondria can create a wide range of clinical heterogeneity. Features of heteroplasmy, threshold effect, genetic bottleneck, mtDNA depletion, mitotic segregation, and maternal inheritance have been identified and described as a result of novel biochemical and genetic controls of mitochondrial function. We hope that as we unfold this fascinating part of clinical medicine, the reader will see how alterations in the tapestry of mitochondrial biochemistry and genetics can give rise to human illness.

Keywords Mitochondrial DNA · Maternal inheritance · Mitochondrial physiology · Genetic diseases · Oxidative phosphorylation

Introduction

Mitochondria are intracellular organelles that produce cellular energy in a series of reactions collectively called oxidative phosphorylation (OXPHOS). Disorders that alter OXPHOS

production of energy, i.e., adenosine triphosphate (ATP), can compromise cellular and organ function leading to disease. The unraveling of how alterations in mitochondrial function can diminish OXPHOS, with resultant low ATP levels, has created a distinct group of diseases, mitochondrial cytopathies. When a nadir of intracellular ATP level compromises cell function, cellular and eventual organ dysfunction and failure can develop. Those organs that rely on high levels of energy for proper functioning are the most vulnerable and have, in part, defined mitochondrial disease. On this background of differential organ involvement, the unique genetic and physiological properties of mitochondria can further alter the clinical presentation of mitochondrial disorders. In addition to the direct effects altering OXPHOS, mitochondrial disorders related to impaired protein importation, defects in mitochondrial dynamics, and alteration of maintenance and expression of mitochondrial DNA (mtDNA) have become evident in disorders seen in infants and children. Consequently, mitochondrial disease can best be viewed as a collection of diseases with variable symptoms and prognoses. Taken together, this group of diseases is a significant cause of human illness [1, 2]. The interplay of energy demand, organ sensitivity, and unique genetics and physiology of mitochondria give rise to a wide variety of clinical manifestations, which range from single organ to multisystem involvement, with age of onset from newborn to the elderly. Phenotypically, disorders can look similar from a wide range of genetic etiologies, yet identical gene mutations can give rise to a variety of phenotypes. Furthermore, environmental factors, such as illness, medications, and toxins, can alter the presentation and course of mitochondrial disease. Most mitochondrial diseases in childhood arise from nuclear gene mutations, but mtDNA-derived diseases still constitute a significant proportion of disease during early life. Our review will highlight mtDNA-induced diseases and try to present the range of disorders during the developmental period from the newborn through adolescence.

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Mitochondrial Physiology

The key to understanding how alterations in mtDNA can induce disease starts with an understanding of mitochondrial physiology. The salient biochemical and genetic factors will be discussed and, hopefully, will demonstrate some of the reasons for the variety and range of mitochondrial disease.

Structure

Mitochondrial internal and external morphology is in a dynamic state and varies between cell type and metabolic state; it can also be altered during disease [3]. Intracellular mobility is regulated by specific proteins involved in mitochondrial fusion and fission, together with organelle motility proteins [4]. Mitochondria (Fig. 1) have outer and inner membranes that encase an aqueous compartment, the mitochondrial matrix. The space between the inner and outer membrane is named the intermembrane space. The inner

Fig. 1 In panel (a), the cartoon represents a mitochondrion with outer membrane, inner membrane (cristae), and matrix (as labeled). The inner mitochondrial space lies between the outer and inner membrane. The morphological shape of a mitochondrion changes with tissue type and energy state, and is dynamic. In panel (b), the electron transport chain is represented. There are 5 complexes as labeled. Electrons produced by the tricarboxylic acid cycle enter via complex I (nicotinamide adenine dinucleotide) and complex II (flavin adenine dinucleotide), and are shuttled via coenzyme Q10 (coQ) to complex III and subsequently to complex IV where molecular oxygen is transformed into water. The proton gradient produced by the shuttling of electrons at complex I, III, and IV are used to generate energy needed to phosphorylate ADP into ATP

membrane provides an effective barrier to ionic diffusion, which is a critical factor in generating the proton electro-magnetic gradient responsible for phosphorylation at complex V in the electron transport chain (ETC) [5]. The inner membrane also makes up the mitochondrial cristae, which is the highly folded inner membrane.

OXPHOS: Structure and Function

The matrix contains multiple enzymes involved in the tricarboxylic acid cycle and beta-oxidation that produce the electron donors, nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH₂). These donors provide electrons for the ETC, which makes up the OXPHOS system. The ETC is composed of 5 transmembrane complexes, made up of over 85 proteins and located within the inner mitochondrial membrane (Fig. 1). Electrons are shuttled through a series of redox reactions, via complexes I, II, III, and IV, liberating energy that is coupled to the flow of hydrogen ions (protons) which are expelled from the matrix into the intermembrane space. The transport of hydrogen ions into the intermembrane space occurs at complex I, III, and IV, and is used to create energy stored as the proton-gradient membrane potential ($\Delta\Psi$). The electrons are fixed at complex IV in the form of molecular oxygen to produce water (oxidative steps). The membrane potential is then utilized at complex V (ATP synthase) by the flow of hydrogen back into the matrix to couple inorganic phosphate and adenosine diphosphate (ADP) into ATP (phosphorylation step). Intrinsic to the ETC are 2 mobile electron carriers, ubiquinone or coenzyme Q10, and cytochrome c, which shuttle electrons between complex I and II to complex III or complex IV respectively. There is a complex of 3 enzymes that shuttle electrons into complex II as FADH₂; succinate-ubiquinone reductase, the electron-transfer flavoprotein-ubiquinone reductase and dihydroorotate dehydrogenase.

The ETC components, complexes I, II, III, IV, and V are made up of gene products derived from both nuclear and mitochondrial genomes. Of the more than 85 proteins within the complexes, only 13 of these proteins are encoded by mtDNA; the rest are encoded in the nucleus and produced outside of the mitochondrion. The 4 subunits of complex II are all encoded in the nucleus. Seven of 46 complex I subunits, 1 of 11 complex III subunits, 3 of 13 complex IV subunits, and 2 of 13 complex V subunits are mtDNA derived. Subunits are assembled with prosthetic groups and metal-containing centers by a set of assembly factors, which are unique to each complex. The complete set of assembly factors remains unknown and probably represents further “unknown” sources for nuclear encoded diseases with hallmarks of mitochondrial cytopathies [6]. All of the assembly factors are nuclear in origin.

Mutations in either nuclear or mitochondrial gene products involved in OXPHOS can give rise to human disease. The unique reliance of the mitochondrial genome on nuclear gene products for DNA replication, expression and maintenance creates multiple and varied avenues for the expression of disease.

MtDNA

The mitochondrial genome is extraordinarily distinctive from nuclear DNA (Table 1). In humans, the mitochondrial genome is a 16,569-base pair double-stranded circular molecule. There are multiple copies within each mitochondrion. Unlike nuclear DNA, mtDNA is densely packed, made up of mostly exons, and lacks histones, as well as repair enzymes. The 2 strands of mtDNA have different masses owing to their nucleotide composition, which alters their buoyant density in a cesium chloride gradient: cytosine-rich strands are light (L), while guanine-rich strands are designated as heavy (H). Twelve of the 13 mtDNA-encoded ETC proteins, together with 2 of the ribosomal RNA and 14 transfer RNA genes, are within the L-strand [7]. The H-strand is limited to genes encoding 1 ETC protein and 8 tRNAs. A number of important regulatory sequences for transcription and replication are located within the only sizeable non-coding region (NCR) within the H-strand. The mitochondrial displacement loop (D-loop) is found within the NCR and recent evidence suggests that this region is responsible for organizing and segregating mtDNA [8].

Most cells contain multiple copies of mtDNA, with copy number correlated with cellular respiratory demand; hundreds of copies can be found within highly energetic cells, such as muscle and nerve. There are anywhere between 2 and 10 copies of mtDNA within each mitochondrion. mtDNAs are organized in multigenomic nucleoprotein complexes named nucleoids, and are tightly associated with the inner

mitochondrial membrane [9–11]. Replication of mtDNA is continuous and independent from nuclear DNA replication, and occurs in dividing and non-dividing cells [12, 13]. The replisome nucleoid consists of unique proteins; polymerase gamma 1, which is unique to mitochondria [14–16] and other single-stranded replication factors [17]. The exact mechanism of mtDNA replication remains poorly understood with 2 different mechanisms proposed: a traditional strand-asynchronous method and a leading-lagging strand DNA replication model [18, 19]. There are likely many undiscovered proteins involved in this process [17, 20].

Mitochondrial Transcription, and Translation

Mitochondrial transcription is initiated from 1 of 3 promoters; 2 are found within the H-strand, HSP1 and HSP2, and the other, LSP, is on the L-strand. Transcription from HSP2 and LSP generate almost genome-length polycistronic transcripts containing all the mtDNA proteins and most tRNA molecules [21, 22]. HSP1 produces a polycistronic transcript containing only the 2 rRNAs, 12S and 16S, and 2 mt-tRNAs, tRNA^{Phe} and tRNA^{Val}. Other proteins, such as mt-TFB and mTERF, involved in the transcriptional machinery are all nuclear encoded and are beyond the scope of this article, but reviews are available [23, 24]. The details of mitochondrial translation remain incompletely understood. Unique features include RNA-poor mitoribosomes [25], partial stop codons in some transcripts that are completed by polyadenylation [21, 26], bicistronic transcripts [27], and unique genetic code [28].

Mitochondrial Genetics

The mitochondrial genome has a mutation rate that is much higher than nuclear DNA, but most are neutral polymorphisms. The first pathogenic mutations were identified in 1988; they were found to give rise to Kearns-Sayre syndrome (KSS) [29] and Leber hereditary optic neuropathy (LHON) [30]. In little over 16 years, more than 275 pathological mtDNA mutations (point mutations and rearrangements) have been described [31]. The unique features of mitochondrial genetics have created a wide range of diseases with varying phenotypes and age of onset, as cells can, over time, acquire somatic mutations in mtDNA, creating a possible genetic mosaic for an affected individual. The heterogeneity is compounded by the influence of two genomes, mitochondrial and nuclear, which combine to alter phenotypic expression. The variation in energy demand between tissues alters the energy nadir that induces phenotypic cellular dysfunction. In addition, the more energy-requiring organs have more mtDNA per mitochondrion and more mitochondria per cell. When these factors are taken into account, there is a unique disease heterogeneity that creates a “rheostat”-like disease, with a

Table 1 Human nuclear and mitochondrial genomes

	Mitochondrial Genome	Nuclear Genome
Size	16,569 (base pairs)	~3.3 × 10 ⁹ (base pairs)
Number of genes	37	20,000–30,000
Chromosomes	2–10 per mitochondrion	44 autosomes, 2 sex
Coding DNA	93 % (no introns)	~3 % (introns)
Codons	Unique genetic code	Universal genetic code
Inheritance	Maternal	Autosomal (AR, AD), X-linked
Replication	<i>POLG1</i>	<i>POLG</i> α and δ
Transcription	Large polycistrons	Individual genes
Cell division	Fusion/fission (continuous)	Cell cycle regulated

Modified from [10, 14, 23, 24, 28, 31].

blending of various clinical signs and symptoms depending on mtDNA mutation location, cellular mutational load, environmental stressors, and nuclear and mitochondrial genetics. The key features of mitochondrial genetics are described in the following.

Homoplasmy, Heteroplasmy, and Threshold

There are up to thousands of mtDNA copies per cell (polyploidy). When all the copies of the mtDNA genome are identical, this is termed homoplasmy. When there are 2 or more mtDNA genotypes mixed within a cell, the term heteroplasmy is used. The majority of mtDNA mutations are “loss of function” or recessive mutations. In these types of disease, the clinical phenotype only becomes evident when the percentage of mutant mtDNAs exceeds a critical threshold. This percentage varies for different mutations and tissues, but is usually in the range of >70 % [32]. However, “gain of function” or dominant disease exists, but only a single case has been reported, suggesting that these types of mutations within mtDNA are rare. Low levels of heteroplasmy (<25 %) were found to induce disease in a 3-year-old patient, suggesting a dominant mutation. The mutation m.C5545T in the tRNA^{Trp} induced hypertrophic cardiomyopathy, truncal hypotonia, seizures, microcephaly, lactic acidosis, and progressive developmental delay [33].

Maternal Inheritance

The current model of mtDNA inheritance is that all mtDNA is inherited through the mother [34]. In *Caenorhabditis elegans*, paternal mitochondria are eliminated from the fertilized oocyte by autophagy [35, 36]. Autophagy is a degradation process that removes structures that threaten cell survival via ubiquitination and recruitment of specific proteins involved in cellular degradation [36]. Although autophagy and ubiquitination of paternal mitochondria is unproven in humans, the lack of human paternal mtDNA inheritance is almost universal, and suggests an ancient and highly conserved process. There has been a single report of paternal mtDNA within the muscle of a patient with myopathy, which was proven by the unique haplotype of the father’s mtDNA that was found only in muscle [37]. However, the evidence is overwhelming that if paternal inheritance is present, it is an exceedingly rare event [38–40]. Thus, a mother carrying an mtDNA point mutation may transmit this mutation to all of her offspring, both males and females, but only the daughters are capable of passing the mutation to the next generation. Children of her sons will be free of disease.

Bottleneck Effect

The proportion of heteroplasmic mtDNA mutations can shift dramatically between generations, with a return to homoplasmy

in some individuals. This process has been termed the “bottleneck effect or hypothesis.” Originally described in work with Holstein cows in the 1980s [41], the mechanism(s) remain unclear [42]. Some studies indicate that this bottleneck effect occurs during embryogenesis of the oocyte where the mtDNA content per mitochondrion is reduced in mature oocytes [43]. Other studies demonstrate that mtDNA content is not reduced and the bottleneck effect occurs post-fertilization, without reduction in mtDNA in germ cells [44]. The current hypothesis is that mtDNA replication is limited before blastocyst implantation and therefore expansion of the mtDNA, and the bottleneck effect on heteroplasmic variance occurs after differentiation of primary oocytes [45]. The variation of heteroplasmy levels is likely generated by unequal segregation of mutant and wild type mtDNA genotypes during oocyte maturation in postnatal life [46]. However, the exact mechanism remains unknown.

Clonal Expansion

During cell division, mitochondria are randomly segregated and the proportion of heteroplasmy can shift by random chance. As cell division continues, the mutational threshold is reached with clinical expression of the disease. The opposite can also occur and mutant mtDNA can be lost. In those cells undergoing rapid cell division, mutant mtDNA may be preferentially lost. For example, certain mtDNA mutations are lost in blood during aging [47].

Epidemiology

Mitochondrial diseases are individually rare, but, as a group, they represent one of the most common of the inherited disorders of metabolism [48]. The total minimal prevalence for both mtDNA- and nDNA-derived mitochondrial disease due to OXPHOS dysfunction is estimated to be 1 in 5000 [49]. Unfortunately, the true prevalence of mtDNA mutation-induced disease during childhood remains unknown: the spectrum of clinical presentations, similar symptoms to other diseases diluting ascertainment, varying age of onset, and dual genomic interplay (a nuclear mutation that induces mtDNA mutations) limits accurate identification. For instance, in a prospective study looking at 3000 sequential cord blood samples, 0.54 % of offspring carried 1 of the 10 most common pathological mtDNA mutations, suggesting that 1 in 200 healthy humans harbors a pathological mutation and are at risk for developing disease [50]. The estimate of mtDNA contributions to the prevalence of mitochondrial disease in England is 9.2 in 100,000 [51]. The high occurrence rate of pathological mutations compared to expression of disease suggests that other factors are likely involved in regulation of disease.

The 2 most common mtDNA-derived diseases are LHON and mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS). LHON is estimated to have a minimum point prevalence in northern England of 3.13 per 100,000 live births and MELAS is estimated to be 3.65 per 100,000 [51]. In 2 pediatric studies, 1 in western Sweden [52] and the other in Victoria, Australia [2], the minimum birth prevalence of child mitochondrial respiratory chain disease was estimated to be 4.7 per 100,000 and 5.0 per 100,000 respectively. Fifteen percent of children were found to have pathological mtDNA mutations in both studies, making the minimum birth prevalence of mtDNA disease 1 in 100,000. When comparing the birth prevalence from the 2 studies [2, 52] performed in the early 2000s to the estimates from England in 2008 [51], it suggests that as the ascertainment of mtDNA-derived diseases becomes better, a more accurate estimate of the birth prevalence will occur.

MtDNA Mutations

In general, mitochondrial diseases induced by nuclear-encoded genes are more severe and typically have an onset earlier than mtDNA disease, i.e. during infancy or childhood [53–55]. In contrast, mtDNA mutation-related diseases are usually milder and occur later in life, although severe, early-onset mtDNA-related diseases are well described [56, 57]. Pathological mutations within the mtDNA are either point mutations or rearrangements of mtDNA. Although 80 % of the coding regions of the mtDNA genome are protein-coding genes (subunits of complex I, III, IV, and V), a majority of disease-causing mutations involves tRNA genes. Rarely, pathological mutations occur in the rRNA genes. Mutations are divided into 2 groups: mutations affecting mitochondrial protein synthesis (tRNA and rRNA) and those encoding proteins (mRNA) of the ETC.

Pathological mutations in tRNA genes lead to a general impairment of mitochondrial protein synthesis owing to reductions in essential mtDNA-encoded ETC subunits. Mutations in individual encoded ETC subunit proteins alter the function of 1 specific ETC complex. Most disease-causing mutations are heteroplasmic point mutations and manifest in a wide variety of clinical presentations. Homoplasmic mutations are often manifested only in isolated tissues with incomplete penetrance [58–60]. Recently, a developmentally-expressed point mutation in a tRNA gene has also been described—reversible infantile cytochrome c oxidase (COX) deficiency [61]. Specific criteria have been formulated to separate the many mtDNA mutations that are neutral polymorphisms from disease-causing mutations (Table 2) [62]. However, there are a few mutations that do not fulfill “pathological mutation” criteria, but are disease causing, such as the m.8344A > G mutation inducing the myoclonus, epilepsy

with ragged-red fibers (MERRF) syndrome. In addition, there are certain nucleotide changes that are not disease-causing, but may modulate the phenotype of primary pathological mutations [63, 64].

Rearrangements in mtDNA are usually single large nucleotide length rearrangements, which can vary in size from 1.3 to 7.6 kilobases [65]. Large deletions, 5 kilobases in size, have been mapped to identical regions spanning the ATPase8 gene to ND5 gene, typically flanked by a perfect 13-base pair direct nucleotide repeat [66]. In a large referral center, 67 patients with mtDNA deletions and disease were studied. Approximately 40 % of patients had the 5 kilobase deletion, were all older than 6 years, and were mostly only detected from muscle samples [66]. In this same study, in the group <6 year of age, only 15 % had the 5 kilobase deletion. This younger group demonstrated a diffuse pattern of deletion distribution in size and location, with the highest degree of heteroplasmy. Single deletions occurred sporadically early in development, and the identical deletion occurred in all affected tissues [67]. The exact mechanism of producing single deletions is not fully understood, but may occur during repair of damaged mtDNA or during replication [68, 69]. Duplications of mtDNA appear to be benign, but a single case report of a maternally-transmitted tandem duplication giving rise to diabetes mellitus has been described [70].

Multiple deletions within mtDNA have been reported to induce disease, but are due to mutations in nuclear genes involved in maintenance of mtDNA and/or mitochondrial nucleotide pools [71, 72]. There are multiple diseases that have an onset during childhood that are due to multiple deletions and mtDNA depletion, and are reviewed in another article [73].

mtDNA Diseases of Childhood

Neonatal Disease

There have been several large institutional population studies describing patients with neonatal onset of mitochondrial diseases. Three such studies indicated that of the 188 patients described only 3 had disease that was the result of mtDNA mutations [2, 55, 74]. All 3 patients had Leigh syndrome from the m.8993 T>G mutation.

There are physiological reasons that mtDNA mutations do not often cause symptoms in the neonate. The major source of cellular ATP in fetal tissues is anaerobic glycolysis [75]. In addition, the generally heteroplasmic nature of mtDNA mutations tend to be expressed later in life. However, anecdotal experience has suggested that early spontaneous abortions and/or neonatal deaths are common in mtDNA mutations [76]. Larger cohort studies investigating

Table 2 Criteria for pathogenicity of mutations in mtDNA disease [62]

1. Mutation should not occur in normal individuals of the same ethnic group; a minimum of several hundred controls should be screened.
2. The altered nucleotide should be conserved in evolution (indicator of functional importance).
3. Should be associated with OXPHOS deficiency in affected tissues, or the mtDNA when transferred to control cells should manifest an OXPHOS defect.
4. There should be a correlation between clinical and biochemical phenotype and mutant load, where the mutation is heteroplasmic.

mtDNA=mitochondrial DNA; OXPHOS=oxidative phosphorylation

mtDNA mutation contributions to early demise are needed owing to maternal inheritance and possible early intervention counseling.

The ubiquitous distribution of mitochondria suggests that disease manifestations should be clinically heterogeneous and involve multiple organs. The differential vulnerability of specific tissues to defects of energy production can differentially alter disease expression. Therefore, disease symptomatology would be expected to be rather heterogeneous, but there are some stereotypical presentations of “classical” syndromic mitochondrial diseases (Table 3). Three such syndromes are expressed in the neonatal period.

Leigh Syndrome

The most common and genetically diverse syndrome is Leigh syndrome. All neonates described in the large cohort studies above had the mutation within the ATPase6 gene of complex V, m.8993 T > G. Most of the mtDNA mutations inducing Leigh syndrome later in life are found in genes encoding subunits of complex I (ND1, ND2, ND3, ND4, ND5, ND6), complex IV (COXIII), and complex V (ATPase6), with 3 of the tRNA-encoding genes also involved (lysine, valine, and tryptophan). There are at least 26 distinct mtDNA mutations

that have been described in these genes that give rise to Leigh syndrome [77]. Nuclear genes can also give rise to Leigh syndrome and as a group represent a greater percentage of Leigh syndrome patients [77].

Denis Leigh described the pathological findings of focal bilaterally symmetric, spongiform, necrotic lesions associated with demyelination, vascular proliferation, and gliosis in the brainstem, diencephalon, basal ganglia, and cerebellum that were responsible for an early encephalopathy and death in a young child [78]. More recently, magnetic resonance imaging has replaced pathological descriptions with progressive acquisition of signal abnormalities within the lentiform and caudate nuclei, as well as signal changes in the thalami, periaqueductal gray, tegmentum, red nuclei, and dentate nuclei (Fig. 2) [79]. Typically, onset is within the first 2 years of life, but most patients present in the first month of life [80, 81]. Patients have severe neurological involvements, psychomotor delay, hypotonia, truncal ataxia intention tremor, seizures, ophthalmoparesis, optic atrophy, and dystonia, but a wide range of neurological findings exist [77, 82]. Some patients demonstrate multisystem disease with minimal neurological involvement [83]. Muscle weakness is common [77]. Non-neurological manifestations include cardiomyopathy, severe constipation/

Table 3 Common mitochondrial syndromes caused by mutations in mitochondrial DNA expressed during infancy and childhood

Syndrome	Clinical Phenotype	mtDNA mutation*	Inheritance
Pearson syndrome	Pancytopenia, lactic acidosis, pancreatitis, sideroblastic anemia	Deletion	Sporadic
KSS	Retinitis pigmentosa, ophthalmoplegia, short stature, diabetes	Deletion	Sporadic
LHON	Optic neuropathy	m.3460 G>A m.11778 G>A m.14484 T>C	Maternal
Leigh syndrome	Psychomotor delay, dystonia, seizures, abnormal eye movements, recurrent vomiting, respiratory abnormalities	m.8993 T>G multiple (see text)	Maternal
NARP	Neuropathy, ataxia, retinitis pigmentosa	m.8993 T>G m.8993 T>C	Maternal
MELAS	Myopathy, lactic acidosis, stroke-like episodes, encephalopathy, seizures	m.3243 A>G m.3271 T>C	Maternal
MERRF	Myoclonus, epilepsy, myopathy	m.8344A>G m.8356 T>C	Maternal

*The most common mtDNA pathological mutation(s)

mtDNA=mitochondrial DNA; KSS=Kearns-Sayre syndrome; LHON=Leber hereditary optic neuropathy; NARP=neurogenic weakness, ataxia and retinitis pigmentosa; MELAS=mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes; MERRF=myoclonus, epilepsy with ragged-red fibers

Fig. 2 This is a 3-Tesla magnetic resonance image from a 9-year-old male with Leigh syndrome. The image is a T2-weighted axial slice through the basal ganglia. There is a patchy hyperintensity (prolonged signal) in the caudate (head) and putamen. The patchy hyperintensity represents the pathological findings of microcystic cavitation, vascular proliferation, neuronal loss, and demyelination [78]

gastrointestinal dysmotility to diarrhea, respiratory failure, dysmorphic features, and short stature [77]. Strict criteria for diagnosis have been proposed owing to the range of clinical findings [84].

Pearson Syndrome

The second syndrome that is found in the neonatal period is Pearson syndrome [85, 86]. This syndrome is characterized by infantile onset of refractory sideroblastic anemia with vacuolization of bone marrow precursor cells and pancreatic dysfunction. There are also other severe organ dysfunctions of the exocrine pancreas, liver, and kidney. The clinical course often leads to early death, but those that survive later in childhood develop KSS. The etiology of both disorders is a large deletion in mtDNA. In contrast, patients with adult onset mtDNA deletion syndrome, chronic progressive external ophthalmoplegia, are limited phenotypically to neuromuscular and ophthalmologic symptoms [87]. As described previously, the older patients usually have a 3-fold increase in the common 5-kilobase mtDNA deletion involving 13 perfect repeat regions within the mtDNA [66]. It is tempting to think that disease severity and onset is related to deletion size or genes deletion, but this has not been found. The mechanism is unclear, but one clue is that almost all deletions are somatic and not maternally inherited [86]. One hypothesis is that deletions found in younger patients arise from maternal germ line mutational events or during early embryogenesis, where the deletion enters into all 3 germ layers; in others, deletions occur in later random somatic events [66]. This would be consistent with the

severe multisystemic findings of Pearson syndrome and later-aged onset of KSS, and adult onset of chronic progressive external ophthalmoplegia.

Infantile Reversible Cytochrome c Oxidase Deficiency Myopathy

This third syndrome found in the neonatal period is reversible COX-deficient infantile myopathy. Although first described by DiMauro et al. [88] in the early 1980s, the genetic defect was not elucidated until 2009 [89]. Affected infants present with hypotonia and severe muscle weakness in the first few days to weeks of life. They often require mechanical ventilation and have severe lactic acidosis. The muscle biopsy material demonstrates absent muscle complex IV or COX activity. These patients improve spontaneously between 5 and 20 months of age with reversal to normal COX activity and are phenotypically healthy and without major symptoms [88, 89]. The etiology is a homoplasmic m.14674 T>C mutation in the tRNA^{Glu} gene [89].

Other Neonatal Mitochondrial Diseases

There have been other case series and case reports of neonatal onset diseases. A homoplasmic mutation, m.1624C > T in the tRNA^{Val} gene was found in 1 family that gave rise to 6 children who died with severe lactic acidosis; a seventh had Leigh syndrome [58]. Two siblings having a homoplasmic mutation, m.3303C > T in the tRNA^{Leu} gene died of infantile cardiomyopathy [90].

Infancy and Childhood

There have been at least 4 large-center studies looking at onset of mitochondrial disease during infancy and childhood [52, 54, 91, 92]. There were 262 patients, with approximately 17 % of diseases accounted for by mtDNA mutations. Most mtDNA diseases were “classical” mitochondrial syndromes (Table 3), including MELAS, LHON, KSS, and MERRF. The consistent phenotypes of these disorders may have lead to the potential bias of disease detection.

The most common syndrome caused by mtDNA mutation in this age group is MELAS. The age of onset varies, but usually occurs before the age of 40 years. MELAS presents as a progressive neurodegenerative disorder associated with headache, treatment resistant partial seizures, short stature, exercise intolerance and muscle weakness, diabetes, progressive dementia, and sensorineural hearing loss [93]. Migraine headaches and partial seizures or *epilepsia partialis continua* are common and can precede or accompany stroke-like events [94]. Respiratory depression may be the presenting symptom [95]. On neuroimaging, the stroke-like lesions are often transient, with lesions

predominantly affecting gray matter and not confined to vascular territories; they have lactate peaks demonstrating OXPHOS defects (Fig. 3) [79, 93]. Approximately 80 % of MELAS patients carry the m.3243A > G mutation within the tRNA^{L_{eu}} gene [96]. Other mutations can be found in this same tRNA gene at m.3271 T > C and give rise to MELAS [97]. The tRNA gene for valine may also induce MELAS [98]. The mtDNA-encoded subunits within complex I seem to be a hotspot for mutations causing MELAS, as mutations within ND1 and ND5 have been reported to be associated with it [99, 100]. There are also reports of mutations in the gene encoding a protein in complex IV, COXIII, inducing MELAS [101].

KSS

The second most frequent mtDNA syndrome in the cited studies was KSS [52, 54, 91, 92]. Of historical interest, KSS was one of the first mitochondrial diseases identified with an mtDNA abnormality [29]. This syndrome is due to a large deletion within mtDNA and is a multisystem disorder, with rare patients presenting as a progression of Pearson syndrome [102]. Clinically, KSS is associated with the triad of retinitis pigmentosa, progressive external ophthalmoplegia, and onset before the age of 20 years. In addition, one other clinical symptom is required for diagnosis: cardiac conduction block, elevated cerebrospinal fluid protein concentration (>100 mg/dL), or cerebellar ataxia. Features of endocrinopathies, short stature, and dysphagia are often found.

Fig. 3 This is a magnetic resonance image of a 9-year-old male who has MELAS. The image is from a 3-Tesla magnetic resonance using diffusion weighted image sequence acquisition. The image demonstrates a hyperintensity (bright signal) within the right parietal cortex involving the postcentral gyrus and the medial parietal lobe adjacent to the midline. The hyperintensity represents a metabolic stroke that was described as acute onset of left-sided numbness, unreactive right pupil and gait disturbance

Neuropathy, Ataxia, and Retinitis Pigmentosa

Heteroplasmy can alter phenotype. Neuropathy, ataxia, and retinitis pigmentosa (NARP) and Leigh syndrome have been described with identical mtDNA mutations in ATPase6, m.8993 T > G [103]. In patients with heteroplasmy >90 %, Leigh syndrome is displayed, while percentages between 60 % and 90 % express NARP. Another mutation, m.8993 T > C can give rise to both Leigh syndrome and NARP, with heteroplasmy >90 % required for NARP expression and near homoplasmy required for Leigh syndrome. These data suggest that the degree of ATP production via ATPase6 activity may be related to NARP versus Leigh syndrome expression. However, the degree of heteroplasmy seems to be restricted to the ATPase6 gene as more than 26 mtDNA mutations have been described for Leigh syndrome and none of these other mutations have been found in NARP patients. The reason for the ATPase6 exclusivity remains unknown.

Clinically, NARP is characterized by proximal neurogenic muscle weakness, ataxia, and pigmentary retinopathy, but other features are usually present. Patients can have seizures, learning difficulties, dementia, short stature, sensorineural hearing loss, progressive external ophthalmoplegia, cardiac conduction defects, and anxiety disorder [104, 105].

LHON

Historically, the first mtDNA point mutation described to give rise to a mitochondrial disease was the m.11778 G > A mutation inducing LHON [30]. LHON is principally an organ-specific disorder affecting the retinal ganglion cells of the optic system. Patients present with a unilateral acute or subacute central painless vision loss. Visual loss spreads to the other eye within approximately 2 months. Onset is usually between 20 and 40 years of age, and, for unclear reasons, targets males. Males account for more than 50 % of LHON patients, while only 10 % of females are affected in pedigree studies. However, LHON mutations are homoplasmic, which would suggest that all patients with this mutation should express the disorder. The reasons for the incomplete penetrance of LHON mutations, as well as the male predominance of disease expression, remain unclear.

More than 95 % of patients with LHON have 1 of 3 mutations: m.11778 G > A, m.3460 G > A, or m.144484 T > C. Over multiple age ranges, mutations in complex I, more specifically in the gene encoding the ND1 subunit, are the most common, but mutations are spread throughout the mtDNA genes encoding other complex I subunits [106]. Several interesting mechanisms that modify phenotype are also found in LHON. In some patients reversal of vision loss occurs, which is somewhat dependent on which mutation is present. For instance, about 70 % of patients with the m.144484 T > C mutation have some reversal of vision loss

compared with only 25 % of the m.11778 G > A mutation [107]. There are secondary LHON-inducing mutations, which, by themselves, are not disease-causing, but when present with primary LHON pathological mutations may act synergistically to modify disease expression [108]. Evidence also points to non-synonymous polymorphisms found in mitochondrial DNA haplogroups, J1c and J2b, increasing penetrance of LHON mutations, m.11778 G > A and m.1184 T > C [109]. An environmental stressor, such as tobacco use, has also been implicated in LHON expression, especially in males [110]. Finally, there is some evidence that estrogens may modify mitochondrial function within human retinal ganglion cells and therefore reduce female expression of the disease [111]. The above data taken together demonstrate that LHON shows a broad range of epigenetic controls of mitochondrial disease expression.

MERRF

MERRF is one of the progressive myoclonic epilepsies. Clinically, it is characterized by a set of four constant features: myoclonus, generalized epilepsy, ataxia, and ragged-red fibers within muscle tissue [112]. Onset is usually in childhood or early adulthood following normal development. Presentation is often myoclonus. In addition to the canonical features, there is hearing loss, optic atrophy, short stature, progressive myopathy, often in a limb-girdle distribution, and pyramidal signs. Other less common symptoms are multiple lipomas and Wolf-Parkinson-White syndrome.

The most common mtDNA mutation is found in the gene encoding tRNA^{Lys} at m.8344A > G. More than 80 % of patients with MERRF are found to have this mutation [113, 114]. Interestingly, unlike most all other mtDNA mutations, this mutation is not at a conserved site in the protein. Other, less common, disease-encoding mutations are found within the same gene, as well as other tRNA-encoded mtDNA genes [115].

Other Mitochondrial Diseases Expressed During Infancy and Childhood

There are other mitochondrial disorders induced by mtDNA mutations. Most are case/family reports and have been noted to be associated with genes encoding complex I, III, and IV subunits. For a review, the reader is referred to an article by Wong [106].

Diagnosis of mtDNA Disease

There is no single test that is sensitive or specific for the diagnosis of a mtDNA-related disorder. The complexity of mitochondrial genetics with heteroplasmy/homoplasmy and biochemistries, together with the findings from clinical

phenotype, family history, and neuroimaging, must be integrated to guide the diagnosis. A systematic approach to the work-up of patients with mitochondrial disease has been published to help the clinician [116, 117]. The problem of variation of heteroplasmy levels between tissues, as well as changes within particular tissues over time, can make diagnosis difficult [118]. As noted previously, invasively obtained muscle tissue may be superior in consistency level of heteroplasmy compared to blood, which is especially true for KSS compared to Pearson syndrome [66]. The investigation of the m.3243A > G mutation has demonstrated that this mutation decreases in heteroplasmy over time, but clinical symptoms and heteroplasmy levels are most accurately measured in urinary epithelial cells compared to leukocytes and buccal mucosa cells [119].

Possible genotype:phenotype correlations are lacking in many, if not most, mtDNA mutations. There are many reasons for this finding, but most are related to the unique mitochondrial physiology. Owing to polyploidy it is difficult to engineer and deliver mtDNA mutations to alter heteroplasmy. Even if delivery were easy, there is little recombination of mtDNA molecules. Finally, there are few animal models for investigation of specific mtDNA-related disease. Not only have these factors limited elucidation of biochemical mechanisms of phenotypic expression, but, more importantly, it has limited therapeutic approaches.

Treatments

A recent Cochrane review of possible treatments for mitochondrial disorders has concluded that there is no clear evidence of any effective intervention in mitochondrial disease [120]. However, there are small studies indicating that the “mitochondrial cocktail” may be helpful in certain patients [120]. The mitochondrial cocktail consists mostly of antioxidant vitamins/factors (coenzyme Q10, alpha-lipoic acid, vitamin C, and vitamin E), and its formulation and use is based on the hypothesis that increased oxidative damage plays a role in mitochondrial disease. Redox imbalance creating increased production of oxygen radicals and by-products in many mitochondrial diseases substantiate the role of increased oxidative damage in disease [121].

There are some promising avenues for treatment. One is exercise therapy: well-tolerated endurance training has been found to improve OXPHOS function [122]. Resistance training has been found to improve muscular strength and oxidative capacity by decreasing the proportion of COX-negative fibers [123]. Furthermore, there is a novel medication making its way through the clinical trials pathway that may be of some benefit [121]. Hopefully, these new possible therapeutic interventions are only a part of the onset of possible treatments for this devastating group of diseases.

Conclusion

Mitochondrial disorders due to mtDNA mutations can present at any age, although they are more frequent in older age groups. The unique physiology of the mitochondrion can alter expression of the mtDNA with respect to clinical presentation. There can be many influences on phenotypic expression that remain to be explored. Multisystemic disorders are common, but for reasons that are not clear, single organ involvement can also occur. However, within the clinical variability are syndromes that have a degree of constancy of the phenotype that present throughout the neonatal and childhood years. Whether this finding is due to identification bias is unknown. The most consistent organ involvements are the central nervous system and musculoskeletal system, which is likely owing to energy requirements. Neonatal involvement is less common than in later childhood, but can be devastating when present. Future studies will have to define phenotypic-altering external factors, emphasis on earlier detection, and cost-effective ways to detect genetic mutations and heteroplasmy. Understanding of these factors is necessary for our developing of therapeutic interventions to decrease mortality and morbidity of these diseases.

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References

- Chinnery PF, Johnson MA, Wardell TM, et al. The epidemiology of pathogenic mitochondrial DNA mutations. *Ann Neurol* 2000;48:188-193.
- Skladal D, Halliday J, Thorburn DR. Minimum birth prevalence of mitochondrial respiratory chain disorders in children. *Brain* 2003;126:1905-1912.
- Benard G, Rossignol R. Ultrastructure of the mitochondrion and its bearing on function and bioenergetics. *Antioxid Redox Signal* 2008;10:1313-1342.
- Milone M, Benarroch EE. Mitochondrial dynamics: General concepts and clinical implications. *Neurology* 2012;78:1612-1619.
- Saraste, M. Oxidative phosphorylation at the fin de siècle. *Science* 1999;283:579-587.
- Nouws J, Nijtmans LGJ, Smeitink JA, Vogel RO. Assembly factors as a new class of disease genes for mitochondrial complex I deficiency: cause pathology and treatment options. *Brain* 2012;135:12-22.
- Anderson S, Bankier AT, Barrell BG, et al. Sequence and organization of the human mitochondrial genome. *Nature* 1981;290:457-465.
- Di Re M, Sembngi H, He J, et al. The accessory subunit of mitochondrial DNA polymerase gamma determines the DNA content of mitochondrial nucleoids in human cultured cells. *Nucleic Acids Res* 2009;37:5701-5713.
- Holt IJ, He J, Mao CC, et al. Mammalian mitochondrial nucleoids: Organizing an independently minded genome. *Mitochondrion* 2007;7:311-321.
- Wang Y, Bogenhagen DF. Human mitochondrial DNA nucleoids are linked to protein folding machinery and metabolic enzymes at the mitochondrial inner membrane. *J Biol Chem* 2006;281:25791-25802.
- Iborra FJ, Kimura H, Cook PR. The functional organization of mitochondrial genomes in human cells. *BMC Biol* 2004;2:9.
- Bogenhagen D, Clayton DA. Mouse L cell mitochondrial DNA molecules are selected randomly for replication throughout the cell cycle. *Cell* 1977;11:719-727.
- Taanman JW, Muddle JR, Muntau AC. Mitochondrial DNA depletion can be prevented by cGMP and dAMP supplementation in a resting culture of deoxyguanosine kinase-deficient fibroblasts. *Hum Mol Genet* 2003;12:1839-1845.
- Graziewicz MA, Longley MJ, Copeland WC. DNA polymerase gamma in mitochondrial DNA replication and repair. *Chem Rev* 2006;106:383-405.
- Carrodeguass JA, Theis K, Bogenhagen DF, Kisker C. Crystal structure and deletion analysis show that the accessory subunit of mammalian DNA polymerase gamma, Pol gamma B, functions as a homodimer. *Mol Cell* 2001;7:43-54.
- Spelbrink JN, Tolonen JM, Hakkaart GA, et al. In vivo functional analysis of the human mitochondrial DNA polymerase POLG expressed in cultured human cells. *J Biol Chem* 2000;275:24818-24828.
- Copeland WC. Inherited mitochondrial diseases of DNA replication. *Ann Rev Med* 2008;59:131-146.
- Clayton DA. Replication of animal mitochondrial DNA. *Cell* 1982;28:693-705.
- Yasukawa T, Reyes A, Cluett TJ, et al. Replication of vertebrate mitochondrial DNA entails transient ribonucleotide incorporation throughout the lagging strand. *EMBO J* 2006;25:5358-5371.
- Holt IJ. Mitochondrial DNA replication and repair: all a flap. *Trends Biochem Sci* 2009;34:358-365.
- Ojala D, Montoya J, Attardi G. tRNA punctuation model of RNA processing in human mitochondria. *Nature* 1981;290:470-474.
- Clayton DA. Replication and transcription of vertebrate mitochondrial DNA. *Annu Rev Cell Biol* 1991:453-478.
- Scarpulla RC. Transcriptional paradigms in mammalian mitochondrial biogenesis and function. *Physiol Rev* 2008;88:611-638.
- Falkenberg M, Larsson NG, Gustafsson CM. DNA replication and transcription in mammalian mitochondria. *Ann Rev Biochem* 2007;76:679-699.
- O'Brien TW. Properties of human mitochondrial ribosomes. *IUBMB* 2003;55:505-513.
- Nagaike T, Suzuki T, Ueda. Polyadenylation in mammalian mitochondria; insights from recent studies. *Biochim Biophys Acta* 2008;1779:266-269.
- Anderson S, de Bruijn MH, Coulson AR, Eperon IC, Sanger F, Young IG. Complete sequence of bovine mitochondrial DNA. Conserved features of the mammalian mitochondrial genome. *J Mol Biol* 1982;156:683-717.
- Sengupta S, Yang X, Higgs PG. The mechanisms of codon reassignments in mitochondrial genetic codes. *J Mol Evol* 2007;64:662-688.
- Holt IJ, Harding AE, Morgan-Hughes JA. Deletions of muscle mitochondrial DNA in patients with mitochondrial myopathies. *Nature* 1988;331:717-719.
- Wallace DC, Singh G, Lott MT, et al. Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. *Science* 1988;242:1427-1430.
- MITOMAP: A Human Mitochondrial Genome Database: Available at: <http://www.mitomap.org>. Accessed August 1, 2012.

32. Rossignol R, Faustin B, Rocher C, Malgat M, Mazat JP, Letellier T. Mitochondrial threshold effects. *Biochem J* 2003;370:751-762.
33. Sacconi S, Salviati L, Nishigaki Y, et al. A functionally dominant mitochondrial DNA mutation. *Hum Mol Genet* 2008;17:1814-1820.
34. Giles RE, Blanc H, Cann HM, Wallace DC. Maternal inheritance of human mitochondrial DNA. *Proc Natl Acad Sci* 1980;77:6715-6719.
35. Sato M, Sato K. Degradation of paternal mitochondria by fertilization-triggered autophagy in *C. elegans* embryos. *Science* 2011;334:1141-1144.
36. Al Rawi S, Louvet-Vallee S, Djeddi A, et al. Postfertilization autophagy of sperm organelles prevents paternal mitochondrial DNA transmission. *Science* 2011;334:1144-1147.
37. Schwartz M, Vissing J. Paternal inheritance of mitochondrial DNA. *New Engl J Med* 2002;347:576-580.
38. Schwartz M, Vissing J. No evidence for paternal inheritance of mtDNA in patients with sporadic mtDNA mutations. *J Neurol Sci* 2004;218:99-101.
39. Filosto M, Mancuso M, Vives-Bauza C, et al. Lack of paternal inheritance of muscle mitochondrial DNA in sporadic mitochondrial myopathies. *Ann Neurol* 2003;54:524-526.
40. Taylor RW, McDonnell MT, Blakely EL, et al. Genotypes from patients indicates no paternal mitochondrial DNA contribution. *Ann Neurol* 2003;54:521-524.
41. Hauswirth WW, Paipis PJ. Mitochondrial DNA polymorphism in a maternal lineage of Holstein cows. *Proc Natl Acad Sci U S A* 1982;79:4686-4690.
42. Carling PJ, Cree LM, Chinnery PF. The implications of mitochondrial DNA copy number regulation during embryogenesis. *Mitochondrion* 2011;11:686-692.
43. Piko I, Matsumoto L. Number of mitochondria and some properties of mitochondrial DNA in the mouse egg. *Dev Biol* 1976;49:1-10.
44. Cao L, Shitara H, Sugimoto M, Hayashi J-I, Abe K, Yonekawa H. New evidence confirms that mitochondrial bottleneck is generated without reduction of mitochondrial DNA content in early primordial germ cells of mice. *PLoS Genet* 2009;5:e1000756. doi:10.1371/journal.pgen.1000756.
45. Jenuth FP, Peterson AC, Fu K, Shoubridge EA. Random genetic drift in the female germline explains the rapid segregation of mammalian mitochondrial DNA. *Nat Genet* 1996;14:146-151.
46. Wai T, Teoli D, Shoubridge EA. The mitochondrial DNA genetic bottleneck results from replication of a subpopulation of genomes. *Nat Genet* 2008;40:1484-1488.
47. Rahman S, Poulton J, Marchington D, Suomalainen A. Decrease of 3243 A>G mtDNA mutation from blood in MELAS syndrome: a longitudinal study. *Am J Hum Genet* 2001;68:238-240.
48. Schaefer AM, Taylor RW, Turnbull DM, Chinnery PF. The epidemiology of mitochondrial disorders-past, present and future. *Biochim Biophys Acta* 2004;1659:115-120.
49. Smeitink JA, Zeviani M, Turnbull DM, Jackobs HT. Mitochondrial medicine: a metabolic perspective on the pathology of oxidative phosphorylation disorders. *Cell Metab* 2006;3:1-13.
50. Elliott HR, Samuels DC, Eden JA, Reltoin CL, Chinnery PF. Pathogenic mitochondrial DNA mutations are common in the general population. *Am J Hum Genet* 2008;83:254-260.
51. Schaefer AM, McFarland R, Blakely EL, et al. Prevalence of mitochondrial DNA disease in adults. *Ann Neurol* 2008;63:35-39.
52. Darin N, Oldfors A, Moslemi Ar, Holme E, Tulinius M. The incidence of mitochondrial encephalomyopathies in childhood: clinical features and morphological, biochemical and DNA abnormalities. *Ann Neurol* 2001;49:377-383.
53. Ferrari G, Lamantea E, Donati A, et al. Infantile hepatocerebral syndromes associated with mutations in the mitochondrial DNA-polymerase- γ A. *Brain* 2005;128:723-731.
54. Debray F-G, Lambert M, Chevalier I, et al. Long-term outcome and clinical spectrum of 73 patients with mitochondrial disease. *Pediatrics* 2007;119:722-733.
55. Gibson K, Halliday JL, Kirby DM, Yapfite-Lee J, Thorburn DR. Mitochondrial oxidative phosphorylation disorders presenting in neonates: clinical manifestations and enzymatic and molecular diagnosis. *Pediatrics* 2008;122:1003-1008.
56. Rubio-Gozalbo ME, Dijkman KP, van den Heuvel LP, Sengers RC, Wendel U, Smeitink JA. Clinical differences in patients with mitochondrial cytopathies due to nuclear versus mitochondrial DNA mutations. *Hum Mutat* 2000;15:522-532.
57. Sue CM, Bruno C, Andreu AL, et al. Infantile encephalopathy associated with the MELAS A3243G mutation. *J Pediatr* 1999;134:696-700.
58. McFarland R, Clark AA, Morris AA, et al. Multiple neonatal deaths due to a homoplasmic mitochondrial DNA mutation. *Nat Genet* 2002;30:145-146.
59. Taylor RW, Giordano C, Davidson MM, et al. A homoplasmic mitochondrial transfer ribonucleic acid mutation as a cause of maternally inherited hypertrophic cardiomyopathy. *J Am Coll Cardiol* 2003;41:1786-1796.
60. McFarland R, Schaefer AM, Gardner JL, et al. Familial myopathy: new insights into the T14709C mitochondrial tRNA mutation. *Ann Neurol* 2004;55:478-484.
61. Horvath R, Kemp JP, Tuppen HA, et al. Molecular basis of infantile reversible cytochrome c oxidase deficiency myopathy. *Brain* 2009;132:3165-3174.
62. Mitchell AL, Elson JL, Howell N, Taylor RW, Turnbull DM. Sequence variation in mitochondrial complex I genes; mutation or polymorphism? *J Med Genet* 2006;43:175-179.
63. Swalwell H, Blakely EL, Sutton R, et al. A homoplasmic mtDNA variant can influence the phenotype of the pathogenic m7472CinsMTTTSI mutation: are two mutations better than one? *Eur J Hum Genet* 2008;16:1265-1274.
64. Cai W, Fu Q, Zhou X, Qu J, Tong Y, Guan MX. Mitochondrial variants may influence the phenotype manifestation of Leber's hereditary optic neuropathy-associated ND4 G11778A mutation. *J Genet Genomics* 2008;35:649-655.
65. Schon EA, Rizzuto R, Moraes CT, Nakase H, Zeviani M, DiMauro S. A direct repeat is a hotspot for large-scale deletion of human mitochondrial DNA. *Science* 1989;144:346-349.
66. Sadikovic B, Wang J, El-Hattab A, et al. Sequence homology at the breakpoint and clinical phenotype of mitochondrial DNA deletion syndromes. *PLoS One* 2010;5:e15687. doi:10.1371/journal.pone.0015687.
67. Chen X, Prosser R, Simonetti S, Sadlock J, Jagiello G, Schon EA. Rearranged mitochondrial genomes are present in human oocytes. *Am J Hum Genet* 1995;57:239-247.
68. Shoffner FM, Lott MT, Voljavec AS, Soueidan SA, Costigan DA, Wallace DC. Spontaneous Kearns-Sayre/chronic external ophthalmoplegia plus syndrome associated with mitochondrial DNA deletion: a slip-replication model and metabolic therapy. *Proc Natl Acad Sci U S A*, 1989;86:7952-7956.
69. Krishnan KJ, Reeve AK, Samuels DC, et al. What causes mitochondrial DNA deletions in human cells? *Nat Genet* 2008;40:275-279.
70. Dunbar DR, Moonie PA, Swingle RJ, Davidson D, Roberts R, Holt IJ. Maternally transmitted partial direct tandem duplication of mitochondrial DNA associated with diabetes mellitus. *Hum Molec Genet* 1993;2:1619-1624.
71. Saneto RP, Naviaux RK. Polymerase gamma disease through the ages. *Dev Disabil Res Rev* 2010;16:163-174.

72. Spinazzola A, Zeviani M. Disorders of nuclear-mitochondrial intergenomic signaling. *Gene* 2005;354:162-168.
73. Koopman WJH, Willems PHGM, Smeitink JAM. Mongenic mitochondrial disorders. *N Engl J Med* 2012;366:1132-1141.
74. Garcia-Cazorla A, De Lonlay P, Nossogne MC, Rustin P, Touati G, Saudubray JM. Long-term follow-up of neonatal mitochondrial cytopathies: a study of 57 patients. *Pediatrics* 2005;116:1170-1177.
75. Papa S. Mitochondrial oxidative phosphorylation changes in the life span. *Molecular aspects and physiopathological implications*. *Biochim Biophys Acta* 1996;1276:87-105.
76. McFarland R, Clark KM, Morris AA, et al. Multiple neonatal deaths due to homoplasmic mitochondrial DNA mutation. *Hum Genet* 2002;30:145-146.
77. Finsterer J. Leigh and Leigh-like syndrome in children and adults. *Pediatric Neurology* 2008;39:223-235.
78. Leigh D. Subacute necrotizing encephalomyelopathy in an infant. *J Neurol Neurosurg Psych* 1951;14:216-221.
79. Saneto RP, Friedman SD, Shaw DWW. Neuroimaging of mitochondrial disease. *Mitochondrion* 2008;8:396-413.
80. Ostergaard E, Hansen FJ, Sorensen N, et al. Mitochondrial encephalomyopathy with elevated methylmalonic acid is caused by SUCLA2 mutations. *Brain* 2007;130:853-861.
81. Piao YS, Tang GC, Yand H, Lu DH. Clinico-neuropathological study of a Chinese case of familial adult Leigh syndrome. *Neuropathology* 2006;26:218-221.
82. Bugiani M, Tiranti V, Farina L, Uziel G, Zeviani M. Novel mutations in COX15 in a long surviving Leigh syndrome patient with cytochrome c oxidase deficiency. *J Med Genet* 2005;42:e28.
83. Martin E, Burger R, Wiestler OD, Caduff R, Boltshauser E, Boesch C. Brainstem lesion revealed by MRI in a case of Leigh's disease with respiratory failure. *Ped Radiol* 1990;20:349-350.
84. Rahman S, Blok RB, Dahl HHM, et al. Leigh syndrome: clinical features and biochemical and DNA abnormalities. *Ann Neurol* 1996;39:343-351.
85. Pearson HA, Lobel JS, Kocoshis SA, et al. A new syndrome of refractory sideroblastic anemia with vacuolization of marrow precursors and exocrine pancreatic dysfunction. *J Pediatr* 1979;95:976-984.
86. Rotig A, Cormier V, Blanche S, et al. Pearson's marrow-pancreas syndrome: a multisystem mitochondrial disorder in infancy. *J Clin Invest* 1990;86:1601-1608.
87. Moraes CT, DiMauro S, Zeviani M, et al. Mitochondrial DNA deletions in progressive external ophthalmoplegia and Kearns-Sayre syndrome. *N Engl J Med* 1989;320:1293-1299.
88. DiMauro S, Nicholson JF, Hays AP, Eastwood AB, Koenigsberger R, DeVivo DC. Benign infantile mitochondrial myopathy due to reversible cytochrome c oxidase activity. *Trans Am Neurol Assoc* 1981;106:205-207.
89. Horvath R, Kemp JP, Tuppen HAL, et al. Molecular basis of infantile reversible cytochrome c oxidase deficiency myopathy. *Brain* 2009;132:3165-3174.
90. Silvestri G, Santorelli FM, Shanske S, et al. A new mtDNA mutation in the tRNA (Leu(UUR)) gene associated with maternally inherited cardiomyopathy. *Hum Mut* 1994;3:37-43.
91. Skladal D, Sudmeier C, Konstantopoulou V, et al. The clinical spectrum of mitochondrial disease in 75 pediatric patients. *Clin Pediatr* 2001;42:703-710.
92. Scaglia F, Towbin JA, Craigen WJ, et al. Clinical spectrum, morbidity, and mortality in 113 pediatric patients with mitochondrial disease. *Pediatrics* 2004;114:925-931.
93. Hirano M, Pavlakis SG. Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS): current concepts. *J Child Neurol* 1994;9:4-13.
94. Ribacoba R, Salas-Puig J, Gonzalez C, Astudillo A. Characteristics of status epilepticus in MELAS. Analysis of four cases. *Neurology* 2006;21:1-11.
95. Saneto RP, Bouldin A. A boy with muscle weakness, hypercarbia, and the mitochondrial DNA A3243G mutation. *J Child Neurol* 2006;21:77-79.
96. Groto Y, Nonaka I, Horai S. A mutation in tRNA^{Leu(UUR)} gene associated with the MELAS subgroup of mitochondrial encephalopathies. *Nature* 1990;348:651-653.
97. Groto Y, Nonaka I, Horai S. A new mtDNA mutation associated with mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS). *Biochim Biophys Acta* 1991;1097:238-240.
98. Taylor RW, Chinnery PF, Haldane F, et al. MELAS associated with a mutation in the valine transfer RNA gene of mitochondrial DNA. *Ann Neurol* 1996;40:459-462.
99. Shanske S, Coku J, Lu J, et al. The G13513A mutation in the ND5 gene of mitochondrial DNA as a common cause of MELAS or Leigh syndrome: evidence from 12 cases. *Arch Neurol* 2008;65:368-372.
100. Liolitsa D, Rahman S, Benton S, Carr LJ, Hanna MG. Is the mitochondrial complex I ND5 gene a hot-spot for MELAS causing mutations? *Ann Neurol* 2003;53:128-132.
101. Manfredi G, Schon EA, Moraes CT, et al. A new mutation associated with MELAS is located in a mitochondrial DNA polypeptide-coding gene. *Neuromuscul Disord* 1995;5:391-398.
102. Maceluch JA, Niedziela M. The clinical diagnosis and molecular genetics of Kearns-Sayre syndrome: a complex mitochondrial encephalomyopathy. *Pediatr Endocrinol Rev* 2006;4:117-137.
103. Holt IJ, Harding AE, Petty RK, Morgan-Hughes JA. A new mitochondrial disease associated with mitochondrial DNA heteroplasmy. *Am J Hum Genet* 1990;46:428-433.
104. Sembrano E, Barthlen GM, Wallace S, Lamm C. Polysomnographic findings in a patient with the mitochondrial encephalomyopathy NARP. *Neurology* 1997;49:1714-1717.
105. Santorelli FM, Tanji K, Shanske S, DiMauro S. Heterogeneous clinical presentation of the mtDNA NARP/T8993G mutation. *Neurology* 1997;49:270-273.
106. Wong L-JC. Pathogenic mitochondrial DNA mutations in protein-coding genes. *Muscle Nerve* 2007;36:279-293.
107. Riordan-Eva P, Sanders MD, Govan GG, Sweeney MG, Da Costa J, Harding AE. The clinical features of Leber's hereditary optic neuropathy defined by the presence of a pathogenic mitochondrial DNA mutation. *Brain* 1995;118:319-337.
108. Wallace DC, Lott MT, Brown MD, Kerstann K. Mitochondria and neuro-ophthalmologic diseases. In: Scriver CR, Beaudet AL, Sly WA, Valle D (eds). *The metabolic and molecular bases of inherited disease*, 8th ed. Vol II. McGraw-Hill, New York, 2001, pp. 2425-2509.
109. Carelli V, Achilli A, Valentino ML, et al. Haplogroup effects and recombination of mitochondrial DNA: novel clues from the analysis of Leber hereditary optic neuropathy pedigrees. *Am J Hum Genet* 2006;78:564-574.
110. Kirkman MA, Yu-Wai-Man P, Korsten A, et al. Gene-environment interactions in Leber hereditary optic neuropathy. *Brain* 2009;132:2317-2326.
111. Giordano C, Monopoli M, Perli E, et al. Oestrogens ameliorate mitochondrial dysfunction in Leber's hereditary optic neuropathy. *Brain* 2011;134:220-234.
112. Hirano M, DiMauro S. Clinical features of mitochondrial myopathies and encephalomyopathies. In: Lane RJM (ed.), *Handbook of Muscle Disease*, Vol 1. Marcel Dekker Inc., New York, pp. 479-504.
113. Shoffner JM, Lott MT, Lezza AM, Seibel P, Ballinger SW, Wallace DC. Myoclonic epilepsy and ragged-red fibers (MERRF) is associated with mitochondrial DNA tRNA(lys) mutation. *Cell* 1990;61:931-937.
114. Yoneda M, Tanno Y, Horai S, Ozawa T, Miyatake T, Tsuji S. A common mitochondrial mutation DNA mutation in the tRNA

- (Lys) of patients with myoclonus epilepsy associated with ragged-red fibers. *Biochem Int* 1990;21:789-796.
115. DiMauro S, Hirano M. MERRF. In: Pagon RA, Bird TD, Dolan CR et al. (eds) *GeneReviews* [Internet]. University of Washington, Seattle, WA, 1993. Available at: <http://www.ncbi.nlm.nih.gov/books/NDB1520>.
116. Haas RH, Parikh S, Falk MJ, et al. Mitochondrial disease: a practical approach for primary care physicians. *Pediatrics* 2007;120:1326-1333.
117. Haas RH, Parikh S, Falk JM, et al. The in-depth evaluation of suspected mitochondrial disease. *Mol Genet and Metab* 2008;94:16-37.
118. Frederiksen AL, Andersen PH, Kyvik KO, Jeppesen TD, Vissing J, Schwartz M. Tissue specific distribution of the 3243A->G mtDNA mutation. *J Med Genet* 2006;43:671-677.
119. de Laat P, Koene S, van den Heuvel LPWJ, Rodenburg RJT, Jensen MCH, Smeitink JAM. Clinical features and heteroplasmy in blood, urine and saliva in 34 Dutch families carrying the m.3243A>G mutation. *J Inherit Metab Dis* 2012 Mar 9 (Epub ahead of print).
120. Pfeffer G, Majamaa K, Turnbull DM, Thorburn D, Chinnery PF. Treatment for mitochondrial disorders. *Cochrane Database Syst Rev* 2012;4:CD004426.
121. Enns GM, Kinsman SL, Perlman SL, et al. Initial experience in the treatment of inherited mitochondrial disease with EPI-743. *Mol Genet Metab* 2012;105:91-102.
122. Taivassalo T, Gardner JL, Taylor RW, et al. Endurance training and detraining in mitochondrial myopathies due to single large-scale mtDNA deletions. *Brain* 2006;129:3391-3401.
123. Taivassalo T, Haller RG. Exercise and training in mitochondrial myopathies. *Med Sci Sports Exerc* 2005;37:2094-2101.