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GENETIC COUNSELING AND CLINICAL TESTING (B LEROY AND N CALLANAN, SECTION EDITORS)

Mitochondrial Genomics: A Complex Field Now Coming of Age

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Abstract

Purpose of Review The groundwork for mitochondrial medicine was laid 30 years ago with identification of the first disease-causing mitochondrial DNA (mtDNA) mutations in 1988. Three decades later, mutations in nearly 300 genes involving every possible mode of inheritance within both nuclear and mitochondrial genomes are now recognized to collectively comprise the largest class of inherited metabolic disease, affecting at least 1 in 4300 individuals across all ages. Significant progress has been made in recent years to improve understanding of mitochondrial biology and disease pathophysiology.

Recent Findings Markedly improved understanding of the highly diverse molecular etiologies of multisystemic phenotypes in primary mitochondrial disease has resulted from massively parallel genomic sequencing technologies and improved bioinformatic resources that enable identification in individual patients of their disease's precise genetic etiology. Key informatics resources of particular utility to the mitochondrial disease genomics community have been developed, including: (1) Mitocarta 2.0 repository of 1200+ verified mitochondria-localized proteins, (2) MITOMAP Web resource of curated mtDNA genome variants, and (3) Mitochondrial Disease Sequence Data Resource (MSeqDR) that centralizes Web curation and annotation of mitochondrial disease genes and variants in both genomes, ontology-defined phenotypes, and access to many analytic tools to support dual genomic data mining and interpretation. Gene and mutation-based disease categorization has proven particularly useful to identify the full clinical spectrum of disease that may affect a given individual.

Summary Extensive genomic advances, both in technologic platforms and bioinformatics resources, have facilitated dramatic improvement in the accurate recognition and understanding of primary mitochondrial disease.

Keywords Mitochondrial disease · Genomics · Database · Pathophysiology

Introduction

Primary mitochondrial diseases are a heterogeneous group of heritable conditions characterized by energy deficiency [1]. While the function of the mitochondrial electron transport chain (ETC) or oxidative phosphorylation (OXPHOS) system to generate chemical energy in the form of adenosine triphosphate (ATP) has been understood since the 1960s [1, 2], the first demonstrations of its genetic basis in which mtDNA point mutations or deletions can cause human mitochondrial diseases was recognized only 30 years ago [3•, 4, 5]. With the advent and expanded clinical utilization of massively parallel genomic sequencing technologies (primarily whole exome sequencing) in both nuclear and mitochondrial genomes over the past 6 years [6•], affected individuals will often now have a shortened diagnostic odyssey, with estimated 50–70% likelihood of receiving a precise molecular diagnosis from clinical diagnostic genetic testing [7, 8]. The genomics testing revolution has greatly increased understanding of the major pathogenic mechanisms of mitochondrial disease. This review highlights the historical context and impact of recent rapid genomic advancements made in understanding mitochondrial disease.

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Mitochondrial Biology

Mitochondria are subcellular cytoplasmic organelles that arose 2 billion years ago from an ancient symbiont purple sulfur cyanobacteria ancestor that could handle oxygen and allowed multi-cellular life to evolve [9•]. Each cell has many mitochondria on the order of 10 to 100s per cell (and more than 100,000 in oocytes), which form an interconnected network along the cellular cytoskeleton. Mitochondria are highly dynamic organelles, where frequent fission and fusion of mitochondria is essential to their maintenance and function. As schematically depicted in Fig. 1, each mitochondrion is comprised of an outer and inner mitochondrial membrane between which is an intermembrane space. The mitochondrial matrix is encircled by a highly rugated inner mitochondrial membrane that forms cristae microdomains, in which energy is generated by oxidative phosphorylation within the respiratory chain (RC). The outer mitochondrial membrane is relatively permeable and interacts closely with the endoplasmic reticulum to play a key role in buffering intracellular calcium, while the inner mitochondrial membrane is highly impermeable with more than 100 solute carriers and channels that selectively allow the import and export of specific ions and chemicals.

Oxidative phosphorylation (OXPHOS) is the process that generates chemical energy in the form of adenosine triphosphate (ATP) through the integrated function of five RC complexes located within the inner mitochondrial membrane (Fig. 1). The RC is comprised of more than 85 protein subunits that are encoded by either the nuclear genes and imported into the mitochondria or by 13 mtDNA genes that encode core subunits of complexes I, III, IV, and V. OXPHOS effectively transfers the electrons generated from intermediary metabolism of cellular nutrients, primarily in the form of reduced nicotinamide dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$), to consume molecular oxygen (thus respiration occurs). In this process, a proton gradient is generated across the inner mitochondrial membrane that represents a potential difference or charge separation (negatively charged matrix and positively charged intermembrane space) that is dissipated through complex V (an enzyme known as ATP synthase) to drive the production of energy by combining adenosine diphosphate (ADP) with inorganic phosphate (P) to form ATP. Well recognized for this essential activity as being the "powerhouse of the cell," mitochondria do have many additional important cellular functions beyond cellular energy production, including regulating calcium homeostasis, initiating programmed cell death (apoptosis), generating and scavenging radical species, and orchestrating diverse aspects of intermediary cellular metabolism including fatty acid oxidation, pyruvate metabolism, tricarboxylic acid cycle function, ureagenesis, purine nucleotide biosynthesis, and steroid biosynthesis, among many others.

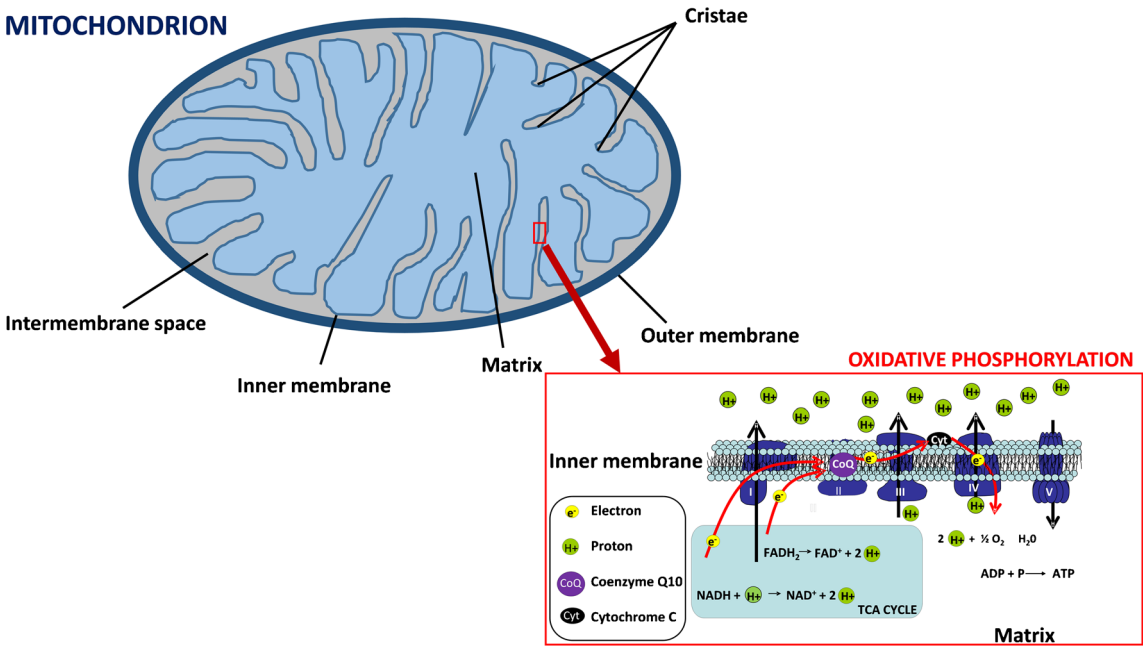

Fig. 1 Schematic overview of mitochondrial structure and oxidative phosphorylation. Each mitochondrion has a double membrane comprised of outer and inner membranes, the latter being folded into cristae structures where the process of oxidative phosphorylation (OXPHOS) occurs. Inset: reducing equivalents generated through intermediary cellular metabolism are shuttled as electrons through the electron transport chain, from complexes I or II to coenzyme Q10 that is embedded in the inner mitochondrial membrane, to complex III, cytochrome C, complex IV, and then molecular oxygen, which is the final electron acceptor. In the process, protons are pumped out of the mitochondrial matrix into the intemembrane space through complexes I, III, and IV, creating a potential difference across the inner mitochondrial membrane and leaving the matrix negatively charged. This electrochemical gradient is used to power complex V (ATP synthase) to generate chemical energy within the mitochondrial matrix from adenosine diphosphate (ADP) and inorganic phosphate (P) as adenosine triphosphate (ATP)

Genomics and Inheritance

Primary mitochondrial disease results from pathogenic variants in either nuclear DNA or mitochondrial DNA (mtDNA) genes [10]. Nuclear DNA genes associated with mitochondrial disease can follow any Mendelian inheritance pattern, including autosomal dominant, autosomal recessive, or X-linked, with both inherited and de novo mutations causing disease. mtDNA pathogenic variants follow maternal inheritance patterns since only oocytes contribute mtDNA to embryos, with both maternally inherited or de novo mutations causing disease.

Mitochondrial Proteome

More than 1500 proteins have been variably suggested to be present within mitochondria of different tissues, where all but 13 proteins are encoded by nuclear genes. In 2008, a comprehensive set of experimentally verified genes encoding proteins localizing to mitochondria was cataloged to include 918 genes in humans and 967 genes in mice, a compendium collectively referred to as MitoCarta [11]. This detailed mitochondrial protein inventory of experimentally validated genes was updated in 2016 to include 1158 genes experimentally verified in both mouse and human, referred to as MitoCarta 2.0 [12]. More than 95% of human mitochondrial disease genes have involved genes that encode mitochondria-localized proteins [13].

Nuclear DNA: Inheritance and Expression

More than 300 nuclear genes have now been associated with primary mitochondrial disease [7], with at least one additional gene being associated with primary mitochondrial disease each month [14] and an average exceeding 20 novel gene disorders causing mitochondrial disease published each year since the initiation of exome sequencing. Many of these nuclear-encoded primary mitochondrial disease genes have only been described in the past 10 years, representing a more than four-fold increase over the past decade in causal disease gene recognition that largely coincides with the advent of highly sensitive, massively parallel, next-generation sequencing methods [15]. While the majority of these discoveries have resulted from exome sequencing, additional disease gene recognition is likely from newer sequencing approaches including whole genome sequencing that has the advantage of identifying small and large chromosomal copy number alterations and has improved coverage of a greater proportion of gene-coding regions than does exome-capture based sequencing approaches, as well as RNAseq-based transcriptome profiling that enables identification of splice-site mutations and co-analyses of gene expression with sequence variation [16].

Autosomal Recessive Inheritance

The majority of pediatric-onset primary mitochondrial diseases are inherited in an autosomal recessive fashion, where two pathogenic mutations in a given gene that are biparentally inherited are necessary to cause disease. Asymptomatic carrier parents of individuals with an autosomal recessive disease together have a recurrence risk of 25% (1 in 4) with each pregnancy to have another affected child [17]. Unaffected siblings of affected individuals have a 67% (2 in 3) likelihood of being carriers, who are typically healthy but if proven a carrier then at 50% (1 in 2) risk of passing the mutation on, so that their own children would be carriers. Children of affected individuals with autosomal recessive diseases will definitely be mutation carriers, but unlikely to be symptomatic with the disease; this would require they inherit a second disease-causing mutation in the same gene from their other parent, which is unlikely assuming low background carrier frequency in the population and a non-consanguineous union. Carrier testing for autosomal recessive conditions is typically not performed until adulthood, but is recommended prior to pregnancy to allow for confirmed carriers to have their partners undergo full sequencing of the same gene to accurately assess their risk for transmitting the disease to their children.

Autosomal Dominant Inheritance

Nuclear-encoded gene mutations associated with primary mitochondrial disease can cause disease in an autosomal dominant manner. While these conditions can occur in childhood-onset diseases, particularly in de novo dominant neurodegenerative conditions, autosomal dominant disorders tend to be adult-onset [14]. Pathogenic variants in genes that cause clinical disease in an autosomal dominant manner (meaning a mutation in only one allele of a given gene is sufficient to cause disease) can be inherited from a parent or occur de novo (new) in the affected individual. An affected individual has a 50% (1 in 2) chance of passing the pathogenic variant on to each of their offspring, regardless of gender. Recurrence risk for parents of an affected individual to pass on the disease to their children depends on whether they harbor the same disease gene mutation; if the mutation appears by blood or other non-invasive tissue sequencing in parental samples to be de novo in the affected child, the recurrence risk for asymptomatic parents would be below 1% (1 in 100) but not zero, given the potential for germline mosaicism where a mutation can be present in a proportion of the oocytes or sperm but not in the parent's blood. If an autosomal dominant mutation is confirmed to be inherited from a parent, that parent's recurrence risk for future offspring to inherit the mutation and manifest the disease would be 50% (1 in 2). Autosomal dominant conditions may be subject to variable expressivity, even within individuals of a given family, where some individuals may

have none or only subtle clinical features of primary mitochondrial disease and others may be severely affected.

X-Linked Inheritance

Several X-linked genes have been found to harbor mutations that cause primary mitochondrial disease. In all X-linked conditions, female carriers have a 50% chance (1 in 2) of having an affected son and a 50% chance (1 in 2) of having a carrier daughter, where carrier daughters are usually but not always asymptomatic. However, one third of cases can arise from a de novo (new) X-linked gene mutation in the male that is not present in their mother. An affected male with an X-linked condition will have all carrier daughters, but his sons will not be affected since they inherit his Y rather than X chromosome. Similar to other X-linked conditions, a wide spectrum of variability may be seen in both males and females, as exemplified by pyruvate dehydrogenase complex deficiency caused by mutations in *PDHA1*, where both males and females can present with a severe or mild clinical presentation [18, 19].

Mitochondrial DNA: Inheritance and Biology

Unique Aspects and Inheritance

The human mitochondrial genome is considerably smaller than the nuclear genome. It is comprised of 16,569 base pairs and 37 genes encoding 13 polypeptides that all serve as core respiratory chain (RC) subunits: 7 subunits of complex I, 1 subunit of complex III, 3 subunits of complex IV, and 2 subunits of complex V, as well as 22 mitochondrial tRNAs and 2 mitochondrial rRNAs necessary for the translation of these 13 mtDNA-encoded RC complex protein subunits. Mitochondrial DNA is exclusively maternally inherited [20, 21], with no splicing or homologous recombination, and the genome replicates autonomously [22].

Heteroplasmy

Given the quantitative genetics that follows from the occurrence of many mitochondria per cell and many mtDNA genome copies per mitochondrion, a pathogenic mtDNA variant often is only present in a proportion of total mtDNA genomes within a cell or tissue. This unique biological phenomenon of having two mtDNA genome populations, one healthy (or "wild-type") and one harboring a specific mtDNA mutation, is known as heteroplasmy [4, 5]. Heteroplasmy levels for a given mtDNA mutation may differ greatly both between family members and among tissues of a given individual, due to the random bottleneck phenomenon that dramatically reduces total mitochondrial number from that present in an oocyte and may alter the relative heteroplasmy level within a given embryo or organ progenitor cells (Fig. 2). When a mtDNA variant is identified in a person, it is commonly helpful to obtain its heteroplasmy levels in additional tissues (such as blood, urine, buccal swab, hair bulb, muscle, liver, or fibroblasts) within the affected individual and family members to gain a better understanding of whether heteroplasmy levels follow a pattern that is consistent with it being causal of their clinical disease. mtDNA mutations that are found to be present at homoplasmy in all tissues of both affected and healthy individuals in a given family are likely to be benign, whereas mtDNA mutations present at higher levels in symptomatic tissues and individuals than in asymptomatic tissues and unaffected family members are more concerning for being disease-causing. Identifying the presence of a pathogenic mtDNA variant in even one tissue of a maternally-related family member alters recurrence risk counseling and prenatal testing recommendations for maternal family members.

While assessing mtDNA heteroplasmy levels in easily accessible tissues, such as blood, saliva or buccal cells, and urine is a good starting point, these tissues may not be the best indicator for mutation heteroplasmy levels present in other, more energy demanding, tissues, such as brain and muscle in which clinical problems occur. Further, mtDNA deletions and mitochondrial DNA pathogenic variants may be selected against in rapidly dividing tissues over time [23]. Urine testing to assess heteroplasmy of shed renal epithelial cells has been shown to be a useful indicator of body heteroplasmy levels; however, it is often difficult to obtain sufficient urine DNA resulting in frequent failure of this method. Strategies to resolve this pit fall include collection of first morning urine specimens that are more concentrated and 24-h urine collections that increase the amount of cells present to be tested. Assessing mtDNA heteroplasmy load in skin-derived fibroblast cell lines is another minimally-invasive option. Still, muscle mtDNA heteroplasmy analysis remains the gold standard for exclusion of a mtDNA mutation as the cause for suspected mitochondrial disease, since some individuals may only have mutations present in their muscle and muscle is a commonly affected tissue in mitochondrial disease. mtDNA deletions in particular are often only present in muscle, especially in affected adults, although this phenomenon has also been reported for some mtDNA point mutations. Overall, a mtDNA etiology for a given patient can be firmly excluded only following muscle mtDNA sequencing by a highly sensitive method such as next-generation sequencing, which allows detection of heteroplasmy loads as low as 1%. Similarly, carrier testing in parents of an individual with a disease-causing mtDNA mutation can only reliably exclude disease when a highly sensitive next-generation sequencing methodology is used; classical Sanger sequencing only reliably detects mutations present when heteroplasmy levels in a sample are at least 30–50% [6•]. As Sanger sequencing remains in use to assess parental samples in many CLIA-approved diagnostic

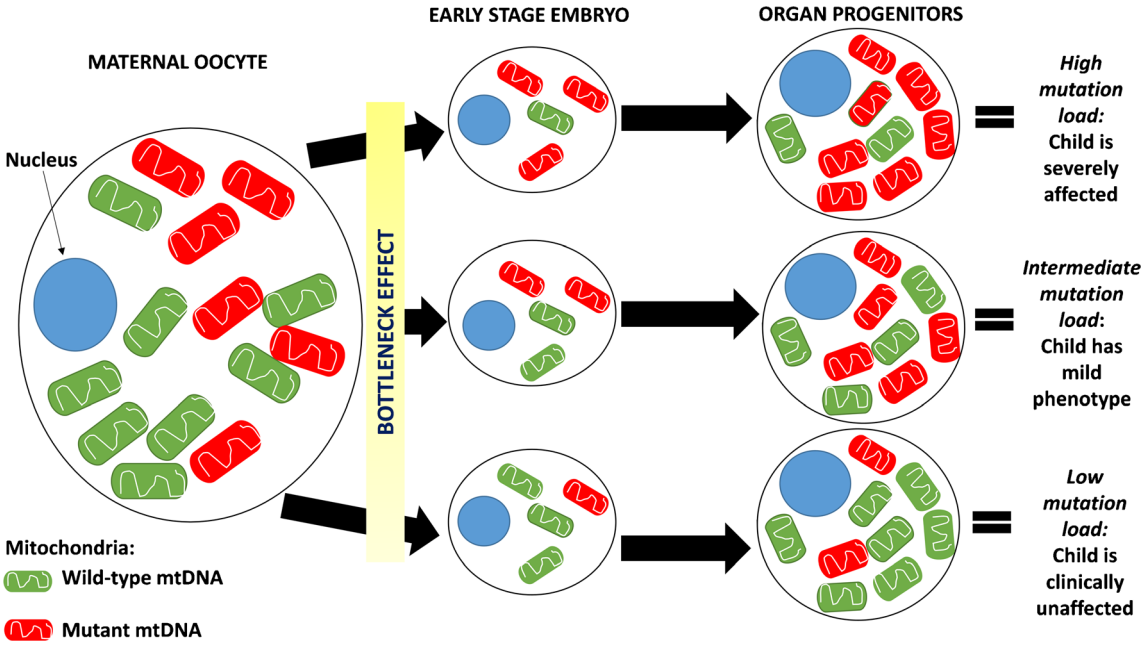

Fig. 2 Schematic representation of variable mitochondrial DNA heteroplasmy transmission in mitochondrial disease. Mitochondrial DNA (mtDNA) transmission to offspring occurs through the maternal germline. A mixture of wild-type (healthy) and mutant mtDNA genomes may be present among the more than 100,000 mitochondrial copies within an oocyte. The bottleneck that occurs in the early embryo effectively reduces the total number of mitochondria to several hundred copies and the total mtDNA quantity to several thousand copies. Through this process, the percentage of mutant to wild-type mtDNA (heteroplasmy level) in the cells of the early embryo that emerges from the bottleneck may vary substantially from that in either the oocyte or the mother's somatic cells. As fetal development progresses, stochastic variation may lead to widely different mutant heteroplasmy levels within different organs of the same individual. The higher the mutation heteroplasmy level in given organ(s), the more likely the resulting child will develop a severe and early onset clinical phenotype. These factors underlie both the wide phenotypic variability characteristic of mtDNA diseases, as well as the complex recurrence risk for future children of mothers who carry pathogenic mtDNA mutations

laboratories, understanding such limitations of specific testing used is especially important. Most mtDNA variants are assessed by a common mtDNA genome sequencing approach, rather than custom tests for each rare variant.

Threshold Effect

Many mtDNA variants only cause clinical manifestations when present in a given tissue at a certain heteroplasmy level, a phenomenon known as "threshold effect." The precise threshold for symptom occurence is not clear for every potential disease-causing mtDNA mutation; this must be considered for each mutation independently, as clinical symptoms may be present for some mtDNA mutations but not others even when present at relatively low levels. A general rule-of-thumb is that mutation levels above 70% are typically more likely to manifest with mitochondrial disease symptoms, although some mutations manifest with severe disease even at much lower mtDNA mutation levels. The difference likely relates to the severity of the mutation in altering function of the encoded protein or RNA molecule, and the cumulative impact on the ability of the mtDNA genome to encode functional RC subunits that support energy needs of a given tissue.

Haplogroups

Haplogroups are sets of single nucleotide polymorphisms (SNPs) that arise over time and become fixed homoplasmic variants present in all mtDNA copies of an individual and their maternal ancestry. Indeed, fixed variant sets that define mtDNA haplogroups are used to effectively track migrations of aboriginal human populations and ancestral lineages, and are thought to define the inherent efficiency of an individual's mitochondrial RC to convert nutrient-derived reducing equivalents to generate energy versus heat. An individual's mtDNA haplogroup background is important to consider when assessing the pathogenicity of mtDNA variants. Some ethnic backgrounds do not exhibit symptoms of mtDNA disease mutations seen in other ethnic backgrounds, which have led to speculation that mtDNA haplogroups may play a role in modulating variant pathogenicity [24–27].

mtDNA Variant Pathogenicity Assessment

Given the unique biology of mtDNA discussed above, mtDNA variant pathogenicity interpretation may be challenging, with inconsistencies seen between individuals and diagnostic laboratories; a given mtDNA variant may

be reported by one laboratory as a variant of uncertain significance (VUS) but not reported at all by another laboratory that considers it a benign variant. This may be especially problematic to clinicians when it is unclear if the variant was detected and classified as benign or not detected by a laboratory's sequencing methology used in a given tissue. Whereas benign variants are by definition irrelevant to disease, one that is present at variable heteroplasmy levels in different tissues raises suspicion for its pathogenicity. Clinicians must directly contact diagnostic laboratories to obtain clarification when such conflicts arise. Guidelines for mtDNA variant pathogenicity classification have been proposed [28], but currently none are widely used.

Several online Web resources have become valuable to aid in the clinical assessment of mtDNA variants in individuals with suspected mitochondrial disease. MITOMAP is an online database of mitochondrial DNA variants reported to date [29–31]. The Mitochondrial Disease Sequence Data Resource (MSeqDR), is an online bioinformatic resource that provides central access to public and expert-curated knowledge of mtDNA variants, mitochondrial disease population genomic sequence datasets, interactive tools for mtDNA and nuclear genome annotation and interpretation, and a series of tools to enable ready deposition and determination of mtDNA variant nomenclature and annotation [32•, 33–35].

Overview of Major Groupings and Numbers of Genetic Diseases Now Recognized

Pathogenic variants in nearly 300 nuclear genes [7, 13] and all 37 mtDNA genes, including over 300 pathogenic mtDNA mutations identified to date [36], have been found to cause primary mitochondrial diseases, revealing many different molecular mechanisms underlying their pathogenicity (see Table 1).

Mitochondrial Disease Classification by Gene and Mutation

Prior to precise recognition of their molecular etiology, mitochondrial disease manifestations were commonly grouped into several clinical syndromes. These discrete clinical syndromes are now recognized to better represent varying degrees of multisystem involvement along a wider and often difficult to classify spectrum of mitochondrial disease.

mtDNA pathogenic variants may be present at different levels of heteroplasmy in different tissues of family members. While they all have the same mtDNA mutation, different individuals may manifest with classical mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS), late-onset maternally-inherited diabetes and deafness (MIDD), or no clear diagnosis because they do not fit

Table 1 Major molecular categories of mitochondrial disease genes

Component	Causal genome	Gene mutation effects	Disease examples
Electron transport chain enzyme subunits	Nuclear or mtDNA	Decreased functioning of electron transport chain complex	-Complex I deficiency -Complex II deficiency -Complex III deficiency -Complex IV deficiency -Complex V deficiency
Electron transport chain assembly factors	Nuclear	Decreased assembly of electron transport chain enzyme complex	
Electron transport chain cofactors	Nuclear	Decreased functioning of electron transport chain	-Coenzyme Q10 deficiency -Iron sulfur cluster defect -Lipoyltransferase deficiency
mtDNA translation	Nuclear or mtDNA	Decreased translation of protein-coding mitochondrial DNA genes leading to decreased functioning of electron transport chain enzymes	-Combined oxidative phosphorylation complexes deficiency
mtDNA maintenance	Nuclear	Increased errors in mitochondrial DNA leading to increased presence of point mutations and deletions, resulting in decreased translation of electron transport chain subunits	-Mitochondrial DNA depletion syndromes -Mitochondrial DNA multiple deletion disorders
Mitochondrial membrane fission and fusion	Nuclear	Increased mtDNA point mutations and deletions; clumped and fragmented mitochondria	-*OPA1*-related conditions -*MFN2*-related conditions

neatly into one of these predefined categories. Rather, they may have additional symptoms such as ranging from Leigh syndrome to isolated diabetes mellitus, headaches, exercise intolerance, or fatigue. Confirming the genetic etiology and monitoring for development of multisystem problems that may present over time is increasingly recognized as the most important diagnostic goal, rather than simply defining a classical syndrome [37]. Furthermore, emerging clinical trials now base inclusion criteria on having a confirmed molecular diagnosis rather than classical syndromic presentation.

Many of the well-known pathogenic variants in both mtDNA and nDNA genes result in a wide spectrum of features ranging from asymptomatic throughout the lifespan to childhood-onset Leigh syndrome, with considerable phenotypic overlap seen with each mutation. Here, we explore common mtDNA variants and a common nuclear gene disorder (*POLG*), focusing on the range of clinical features that may be associated with a given molecularly-defined etiology.

m.3243A>G (*MT-TL1*)

Classically associated with MELAS, the m.3243A>G variant in the mtDNA tRNA-leucine gene (*MT-TL1*) can result in a wide spectrum of features. MELAS is a clinical syndrome classically defined as having stroke-like episodes before age 40 years, seizures, dementia, and myopathy, with biochemical evidence of lactic acidosis and ragged red fibers on muscle biopsy, headaches, recurrent vomiting, and normal early development also considered common in MELAS. This classical syndrome is only manifest in approximately 10% of individuals who carry the m.3243A>G variant [38, 39]. While most individuals with the m.3243 A>G variant do not meet clinical criteria for MELAS, many have some clinical features of this disorder. Additional multisystem involvement including hearing loss, diabetes mellitus, cardiomyopathy, exercise intolerance, and renal involvemen can also be seen in those with the m.3243A>G variant. The m.3243A>G variant at high heteroplasmy levels (typically above 90%) has also been associated with early-onset severe manifestations of Leigh syndrome [40–42].

m.8344A>G (*MT-TK*)

The m.8344A>G mutation in the mtDNA tRNA-lysine gene (*MT-TK*) is classically associated with the clinical syndrome of myoclonic epilepsy with ragged red fibers (MERRF), which encompasses four clinical features: myoclonus, epilepsy, ataxia, and ragged red fibers (indicating mitochondrial proliferation) on muscle biopsy histology. As with all mtDNA variants, however, the m.8344A>G mutation can cause medical concerns along a spectrum ranging from asymptomatic adults to early-onset Leigh syndrome [43]. Additional features commonly seen in m.8344A>G mutation carriers include cardiac involvement, sensorineural hearing loss, lipomas, neuropathy, and exercise intolerance [44].

m.8993T>G and m.8993T>C (*MT-ATP6*)

The m.8993T>G and m.8993T>C mutations in the mtDNA-encoded complex V subunit gene, *MT-ATP6,* are classically associated at high heteroplasmy levels with early-onset Leigh syndrome [45] and at lower heteroplasmy levels with the clinical syndrome of neurogenic muscle weakness, ataxia, and retinitis pigmentosa (NARP). Patients with NARP also can have seizures, optic atrophy, neuropathy, learning difficulty, cerebral and cerebellar atrophy on brain MRI, and periods of relative medical stability [46, 47]. Onset of symptoms associated with these variants may range from childhood (classically associated with NARP) to adult-onset [46, 48].

The m.8993T>G mutation has been shown to cause a more severe phenotype. Typically, those with mutation levels below 60% are mildly affected (headaches and mild pigmentary retinopathy) or unaffected, those with 70–90% mutation levels have symptoms consistent with NARP, and those above 90% heteroplasmy load have more severe features typically falling in the Leigh syndrome spectrum [49, 50]. The m.8993T>C mutation has been shown to be less severe, where only those with mutation loads greater than 90% are affected [48].

m.3460G>A (*MT-ND1*), m.11778G>A (*MT-ND4*), and m.14484T>C (*MT-ND6*)

The m.3460G>A, m.11778G>A, and m.14484T>C mutations in the mtDNA-encoded complex I subunit genes, *MT-ND1, MT-ND4,* and *MT-ND6*, respectively, cause Leber's hereditary optic neuropathy (LHON). Many individuals with LHON have very high heteroplasmy or homoplasmy for one of these three common mutations. Typically, a person with LHON experiences a sudden vision loss in one eye followed weeks to months later by vision loss in the other eye. Vision loss is characterized by a central scotoma. Spontaneous recovery has been shown to occur, most commonly in those with the m.14484T>C mutation in *MT-ND6.* The m.3460G>A mutation in *MT-ND1* causes the most severe phenotype. Remarkably, full penetrance for LHON is not even seen when one of these potentially causal mutations is present at homoplasmy. Overall, penetrance for visual loss is 50% in males and 15% in females. This difference is thought to stem in part from the protective effects of estrogen in females and influenced by environmental factors, such as smoking or alcohol that increase disease penetrance in mutation carriers [51].

mtDNA Deletions

While mitochondrial DNA deletions accumulate at low levels with age in muscle of all individuals, large heteroplasmic deletions are a common cause of mitochondrial disease that may be inherited in a maternal pattern, occur de novo in the embryo, or result over time from inherited mutations in nuclear genes involved in mitochondrial genome maintenance. mtDNA deletion

precise size may be very variable [52], although a common 5 kilobase (Kb) deletion is most often seen. mtDNA deletions are associated with a spectrum of medical problems that have been grouped into three clinical syndromes based on the tissues involved and predominant clinical features: Pearson syndrome, Kearns Sayre syndrome, and chronic progressive external ophthalmoplegia (CPEO). As these large-scale mtDNA genome deletions are selected against in blood overtime, testing the proper tissues is crucial to correctly diagnose affected individuals with a mtDNA deletion disorder.

Pearson syndrome is defined as infantile or early childhood-onset sideroblastic anemia and exocrine pancreatic dysfunction, often requiring transfusions and having a high rate of mortality [53]. Kearns Sayre syndrome (KSS) is classically defined by the presence of CPEO and retinal dystrophy onset before 20 years old, along with at least one of the following features: cardiac conduction block, ataxia, and/or cerebral spinal fluid protein concentration above 100 mg/dL. Additional clinical features commonly seen in mtDNA deletion disorders are sensorineural hearing loss, muscle weakness, diabetes, and other endocrine manifestations including growth hormone deficiency, exocrine pancreatic insufficiency, and hypoparathyroidism [54, 55]. It is commonly recognized that Pearson survivors are at high risk of later developing multisystem manifestations typical of KSS.

POLG

POLG is a nuclear gene that encodes polymerase gamma, the sole mtDNA polymerase with central roles in both mtDNA replication and repair. *POLG* has three domains: exonuclease domain, linker region, and polymerase domain that corresponds with its functions, where pathogenic variants present in each domain are associated with common clinical syndromes. Pathogenic *POLG* mutations in the exonuclease domain result in accumulation of mtDNA point mutations and deletions due to its decreased proofreading ability. Pathogenic *POLG* mutations in the linker region have been associated with mtDNA deletions. Pathogenic mutations in the polymerase domain result in deficient mtDNA replication, resulting in mtDNA depletion over time. More than 300 disease-causing *POLG* mutations have been identified [56] and are expertly cataloged in a public web resource (https://tools.niehs.nih.gov//polg/). Well defined *POLG* variant clusters having distinct biochemical roles have recently been identified, where individual or compound heterozygous mutations in these major clusters lead to distinct clinical consequences that can be predicted with a user-friendly *POLG* variant server prediction tool (http://polg.bmb.msu.edu/) [57].

POLG-related diseases can be inherited in either an autosomal dominant and recessive manner, depending on the exact pathogenic variant, although different variants tend to cause these different classes of *POLG* disease. It has been rare that a child with a childhood-onset *POLG* recessive condition has a parent affected with an adult-onset *POLG* dominant condition.

Pathogenic *POLG* mutations have been associated with numerous discrete clinical syndromes [58], although individual patients may manifest variable clinical features that overlap the distinct categories. Pathogenic *POLG* mutations inherited in an autosomal recessive fashion (where biallelic *POLG* mutations are necessary to cause disease) may cause severe medical problems onset in the first months to years of life that range from Leigh syndrome to myocerebrohepatopathy to Alpers-Huttenlocher syndrome that was classically defined as seizures with valproate-induced liver failure [59]. Adult-onset *POLG* diseases are more commonly inherited in an autosomal dominant fashion, with symptoms ranging from isolated PEO to a host of clinical neurologic syndromes involving variable and progressive symptom combinations of PEO, myopathy, ataxia, neuropathy, epilepsy (particularly epilepsia partialis continua, EPC), sensorineural hearing loss, hypogonadism, infertility, and Parkinsonism.

Conclusion

Understanding primary mitochondrial disease has markedly improved over the past decade, coinciding with the advent and increased utilization of advanced sequencing technologies and robust bioinformatics resources that enable evaluation and interrogation of complex genomic data.

Compliance with Ethical Standards

Conflict of Interest Marni J. Falk reports other from REATA Pharmaceuticals, grants, personal fees and other from Stealth Pharmaceuticals, other from United Mitochondrial Disease Foundation, other from GENESIS, grants and other from Raptor Pharmaceuticals, outside the submitted work. The other authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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