

MITOCHONDRIAL GENOMICS IN HEREDITARY DISORDERS: A DIAGNOSTIC PERSPECTIVE

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ABSTRACT

Mitochondrial diseases are genetically and clinically heterogeneous disorders arising from defects in oxidative phosphorylation due to mutations in either mitochondrial or nuclear DNA. The advent of next-generation sequencing has significantly improved diagnostic yield, enabling the identification of causative variants with greater accuracy and speed. This review outlines the dual genomic basis of these diseases, the evolving diagnostic algorithms, and the growing role of biochemical and imaging correlates. It also highlights current best practice guidelines, therapeutic advancements, and the future potential of multi-omic and machine learning approaches. Early and accurate diagnosis remains crucial for informed clinical management and genetic counseling.

INTRODUCTION

Mitochondrial diseases are a complex and heterogeneous group of genetic disorders that primarily result from defects in oxidative phosphorylation, the central process through which mitochondria generate adenosine triphosphate (ATP)—the cell's primary energy currency. These disorders may manifest at any age, affect virtually any organ system, and often present with neurological, muscular, cardiac, hepatic, or multisystemic involvement. The clinical variability is largely due to the unique nature of mitochondrial genetics and the interplay between environmental and genetic factors. Mitochondria are the only organelles in human cells that contain their own DNA (mtDNA), separate from the nuclear genome. Mutations in either mtDNA or nuclear DNA (nDNA) that encodes mitochondrial proteins can lead to mitochondrial dysfunction. The mitochondrial genome, while small, plays a disproportionately large role in cellular function, encoding critical components of the respiratory chain. Furthermore, the concept of heteroplasmy—where mutated and wild-type mtDNA coexist within the same cell—adds a layer of diagnostic complexity, as the phenotypic expression depends on the proportion of mutated genomes present in affected tissues.

Over the past decade, there has been a paradigm shift in the diagnosis and understanding of mitochondrial diseases due to the advent of next-generation sequencing (NGS) technologies. These high-throughput genomic platforms have made it possible

to examine both mitochondrial and nuclear genomes simultaneously, identifying mutations with far greater precision and speed. As a result, mitochondrial genomics has become a cornerstone in the diagnosis of hereditary mitochondrial disorders, leading to earlier detection, improved patient stratification, and the emergence of precision medicine strategies tailored to individual genetic profiles.^[1,2]

Mitochondrial DNA and Its Role in Disease

The mitochondrial genome is a 16.6 kb circular DNA molecule located within the mitochondrial matrix. It encodes 13 essential protein subunits involved in complexes I, III, IV, and V of the oxidative phosphorylation system, along with 22 tRNAs and 2 rRNAs necessary for mitochondrial protein synthesis. Unlike the nuclear genome, mtDNA is maternally inherited and lacks introns and protective histones, making it more susceptible to damage from reactive oxygen species (ROS) generated during ATP production. This predisposition to mutations contributes significantly to mitochondrial disease pathogenesis.

MtDNA mutations can be classified into point mutations, large-scale deletions, or rearrangements. These mutations can disrupt ATP production and lead to increased oxidative stress, ultimately causing cell death in energy-demanding tissues such as the brain, muscles, and heart. The phenomenon of heteroplasmy further complicates the clinical picture; even within the same tissue, the proportion of mutated mtDNA can vary from cell to cell, influencing the severity and onset of symptoms.

Furthermore, because of the high mutation rate and unique inheritance pattern, mtDNA disorders can display variable penetrance and expressivity, even among members of the same family. For example, disorders such as MELAS (Mitochondrial Encephalopathy, Lactic Acidosis, and Stroke-like episodes) or LHON (Leber Hereditary Optic Neuropathy) are caused by specific point mutations in mtDNA, but show considerable variability in clinical presentation. This heterogeneity necessitates advanced genomic tools and sophisticated interpretation strategies to diagnose and manage these conditions effectively.^[3,4]

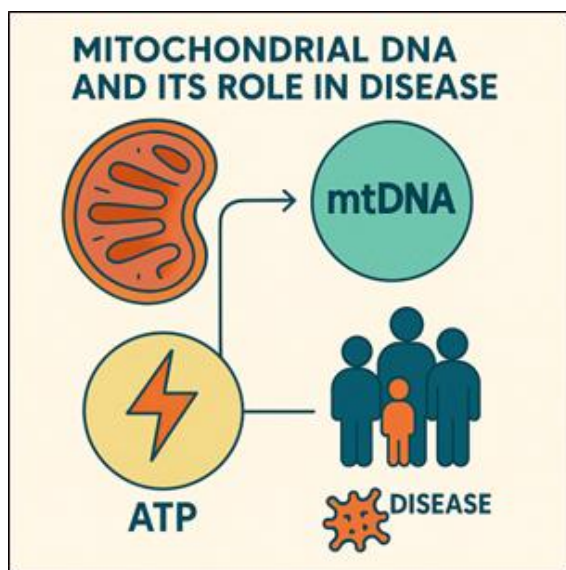


Figure 1: Mitochondrial DNA and Its Role in Disease

Nuclear Gene Involvement and Dual Genetic Control
Although mtDNA mutations are responsible for many mitochondrial disorders, the majority of mitochondrial proteins—over 1000—are encoded by nuclear genes. These proteins are synthesized in the cytosol and subsequently imported into mitochondria, where they perform a range of essential functions including mitochondrial DNA replication, transcription, translation, membrane potential regulation, and respiratory chain assembly. Therefore, mutations in nDNA can affect mitochondrial function indirectly but substantially, giving rise to primary mitochondrial diseases.

Nuclear gene mutations often follow Mendelian inheritance patterns, including autosomal recessive, autosomal dominant, and X-linked transmission. For example, POLG mutations, which affect the nuclear-encoded DNA polymerase gamma responsible for mtDNA replication, are among the most common causes of mitochondrial disease and may lead to progressive external ophthalmoplegia or Alpers-Huttenlocher syndrome. Other nuclear genes implicated in mitochondrial dysfunction include TWNK, SURF1, and RRM2B, each associated with a distinct clinical syndrome.^[5,6]

Because of this dual genomic origin of mitochondrial disease, an integrative approach to diagnosis is

necessary. This includes clinical phenotyping, biochemical assays, and comprehensive genomic testing that encompasses both mtDNA and nDNA. The diagnostic complexity is even more pronounced in children, where nuclear gene mutations are more frequently the underlying cause. Early-onset cases may present with profound encephalopathy, metabolic acidosis, or liver dysfunction, necessitating rapid and accurate genetic diagnosis to guide therapeutic decisions and genetic counseling. Advanced sequencing technologies, including whole-exome sequencing (WES) and whole-genome sequencing (WGS), have made such dual analysis increasingly feasible, reinforcing the need for genomic literacy among clinicians managing suspected mitochondrial disease.^[7,8]

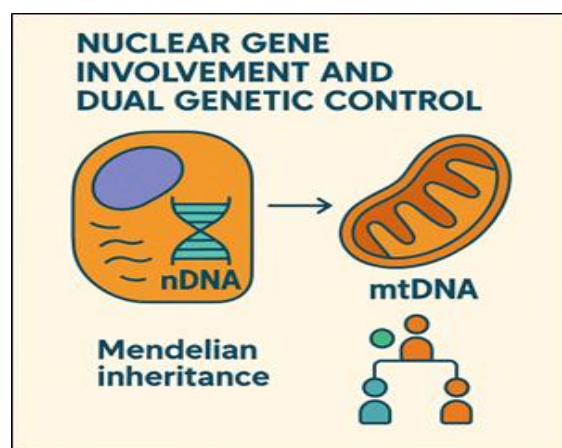


Figure 2: Nuclear Gene Involvement and Dual Genetic Control

Diagnostic Algorithms and Clinical Clues

Clinicians must rely on a combination of clinical signs, biochemical markers, and neuroimaging before advancing to molecular diagnostics. The clinical presentation of mitochondrial disorders is often nonspecific and variable, including symptoms such as lactic acidosis, developmental delay, hypotonia, neuromuscular weakness, fatigue, seizures, cardiomyopathy, liver dysfunction, and progressive neurological deterioration. In neonates and infants, failure to thrive and encephalopathy may be early signs, while older children and adults may present with exercise intolerance or multisystemic symptoms that progress over time.

Given the broad differential diagnosis, it is essential to assess metabolic parameters such as serum and CSF lactate, pyruvate, and amino acid profiles. Neuroimaging, especially MRI, often reveals characteristic patterns such as stroke-like lesions in non-vascular territories (as in MELAS), symmetric basal ganglia involvement (seen in Leigh syndrome), or white matter changes. Magnetic resonance spectroscopy (MRS) can detect elevated lactate peaks in the brain, supporting mitochondrial dysfunction. Biochemical testing of respiratory chain enzyme activity in fresh muscle biopsy or fibroblast cultures has historically been the diagnostic gold standard.

However, limitations such as invasive sampling, lack of specificity, and variability in assay quality have led to its declining role. These assays are now frequently supplemented—or even replaced—by molecular genetic testing, which allows definitive diagnosis with non-invasive methods and enables family screening, prenatal testing, and targeted interventions.^[9-11]

Evolution of Diagnostic Techniques

Historically, the diagnostic process for mitochondrial disease was laborious and dependent on a stepwise approach that involved clinical suspicion, followed by metabolic testing and invasive procedures such as muscle biopsy. Muscle histochemistry, including cytochrome c oxidase (COX) and succinate dehydrogenase (SDH) staining, was used to assess mitochondrial function indirectly, and electron microscopy often revealed abnormal mitochondrial morphology. These methods, while still informative in certain cases, are limited in sensitivity and specificity.

The advent of next-generation sequencing (NGS) marked a turning point. Whole-exome sequencing (WES) and whole-genome sequencing (WGS) now allow clinicians to perform hypothesis-free investigations across the entire coding (or full) genome. These platforms can detect both mtDNA and nDNA mutations in a single test, thereby simplifying the diagnostic workflow. Deep sequencing also enables detection of low-level heteroplasmic variants in mtDNA, which were previously undetectable by traditional methods.

This shift from phenotype-based algorithms to genotype-driven diagnostics has greatly increased diagnostic yields, particularly in pediatric populations with complex presentations. Moreover, the ability to reanalyze sequencing data as new gene-disease associations are discovered adds longitudinal utility to genetic datasets. In many centers, the genetic diagnosis now precedes or completely replaces the need for invasive biopsy, especially when clinical and radiological features strongly suggest a mitochondrial disorder.^[1,5,7,12]

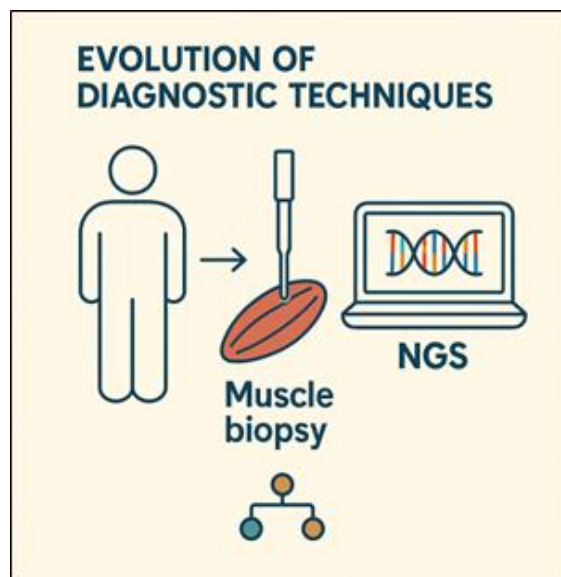


Figure 3: Evolution of Diagnostic Techniques

Targeted vs. Untargeted Genetic Testing

Genetic testing for mitochondrial disorders has evolved along two major pathways: targeted gene panels and untargeted approaches like WES and WGS. Targeted panels include a curated list of genes known to be associated with mitochondrial dysfunction—typically between 100 and 300 nuclear and mitochondrial genes. These panels offer the advantages of high specificity, faster turnaround time, and cost-effectiveness, especially in cases where the phenotype clearly suggests a mitochondrial syndrome or a specific mutation (e.g., POLG-related disorders or mtDNA point mutations such as m.3243A>G in MELAS).

However, targeted panels have limitations, particularly when the clinical presentation is atypical or the causative gene is not yet well-established. In such cases, broader approaches like WES or WGS are recommended. These untargeted strategies allow for the detection of novel or rare variants, copy number variations, and deep intronic mutations that might affect splicing. Moreover, they facilitate the identification of unexpected diagnoses, including disorders that mimic mitochondrial diseases but involve different metabolic pathways.

Comprehensive sequencing also provides a more complete understanding of the genetic architecture of mitochondrial disease in undiagnosed cohorts. With the added benefit of data reanalysis as new knowledge emerges, untargeted approaches have become essential in modern diagnostic pipelines. Given the pleiotropic, overlapping manifestations of mitochondrial disease, combining clinical acumen with broad genomic interrogation often provides the best chance of diagnosis.^[11,13]

Challenges in Variant Interpretation

The interpretation of genomic variants identified through next-generation sequencing remains one of the most significant challenges in the diagnosis of mitochondrial diseases. While sequencing technologies have dramatically increased the volume and scope of data, they have also introduced

complexity in the clinical interpretation of variants. A large proportion of identified variants are classified as variants of uncertain significance (VUS), meaning there is insufficient evidence to determine whether they are pathogenic or benign. These findings can stall the diagnostic process and create uncertainty for both clinicians and patients.

Phenotype-genotype correlations are particularly difficult to establish in mitochondrial disease due to its clinical heterogeneity, variable onset, and tissue-specific expression. Moreover, the same genetic variant may cause different phenotypes in different individuals, or conversely, different variants in the same gene may result in overlapping clinical presentations. To resolve VUS, multidisciplinary approaches are essential. Functional validation through in vitro assays—such as measuring the impact of a variant on mitochondrial respiratory function or protein stability—can provide critical evidence for pathogenicity. Additionally, family segregation analysis can determine whether a variant co-segregates with disease within a pedigree.

Population databases such as gnomAD and ClinVar, along with disease-specific resources like MITOMAP, are increasingly used to assess variant frequency and prior classifications. However, rare or private mutations may lack sufficient data, particularly in underrepresented populations. As a result, collaboration among international genomic consortia and longitudinal re-evaluation of VUS in the light of emerging data are necessary to improve the clinical utility of genetic findings in mitochondrial medicine.^[2,3]

Biochemical and Imaging Correlates

Despite the transformative role of genomic technologies, biochemical and imaging studies continue to play a critical role in the diagnosis and evaluation of mitochondrial diseases. These ancillary investigations provide functional evidence that can support or refute the pathogenicity of specific variants and help identify the most appropriate tissues for genetic analysis. One of the most widely used biochemical markers is blood or CSF lactic acid, which is often elevated due to impaired oxidative phosphorylation and subsequent reliance on anaerobic glycolysis. Other useful metabolites include pyruvate, alanine, and organic acids, which can be analyzed via plasma, urine, or cerebrospinal fluid.

Enzyme assays performed on mitochondria-enriched tissue samples—such as skeletal muscle or cultured fibroblasts—are another important diagnostic tool. These tests measure the activity of individual components of the mitochondrial respiratory chain (complexes I–IV), and their reduction may indicate the presence of an underlying mitochondrial defect. Although such assays are highly informative, they are also technically demanding and require high-quality tissue samples, which can be difficult to obtain non-invasively.

Neuroimaging, especially MRI and MR spectroscopy (MRS), complements biochemical data by providing

structural and metabolic insights. In disorders like Leigh syndrome, symmetric lesions in the basal ganglia, brainstem, or thalamus are highly suggestive. MRS can detect lactate accumulation within brain parenchyma, serving as an indirect marker of mitochondrial dysfunction. Imaging findings, when combined with clinical and laboratory data, can substantially narrow the differential diagnosis and guide the selection of candidate genes for molecular testing. Ultimately, integrating biochemical and imaging data with genetic findings enhances diagnostic accuracy and helps clinicians assess disease severity and progression.^[9,14]

Population Databases and Epidemiological Insights

In recent years, the landscape of mitochondrial disease epidemiology has shifted dramatically due to the availability of large-scale genetic databases and bioinformatics tools. Historically, mitochondrial diseases were considered rare, with early prevalence estimates suggesting around 1 in 5000 individuals. However, new data derived from genomic studies suggest that mitochondrial disorders—particularly those with autosomal recessive inheritance—are more prevalent than previously believed. For example, analyses of carrier frequencies and homozygous pathogenic variants in population-wide databases such as gnomAD have estimated the lifetime risk of certain mitochondrial disorders to be as high as 1 in 4300.^[15]

These findings highlight the substantial underdiagnosis and clinical variability of mitochondrial diseases. Many individuals with mild or non-specific symptoms may go unrecognized, particularly in low-resource settings or where genetic testing is limited. Moreover, mtDNA variants often exhibit incomplete penetrance, meaning that not all carriers develop symptoms, which further complicates epidemiological assessments.

Ethnic and geographic differences also influence variant frequency and expression, emphasizing the need for population-specific reference data. Initiatives such as the Global Mitochondrial Disease Registry and country-level sequencing programs are working to fill these gaps. In parallel, advances in newborn screening and pre-symptomatic genetic testing offer the potential for earlier detection and intervention, potentially altering the natural history of disease. These developments mark a shift toward recognizing mitochondrial diseases not only as a rare disease domain but as a broader spectrum of conditions that deserve more attention in public health and genetic research policy.^[16]

Best Practice Guidelines and Global Frameworks

Recognizing the complexity and heterogeneity of mitochondrial disease diagnosis, several international professional bodies have developed best practice guidelines aimed at standardizing diagnostic workflows and enhancing clinical decision-making. Among the most comprehensive are the United Kingdom best practice guidelines, which recommend a tiered approach beginning with detailed

phenotyping, followed by appropriate biochemical and molecular testing. These guidelines advocate for early use of genetic testing—particularly in pediatric cases or those with suggestive clinical signs—to avoid unnecessary invasive procedures and to expedite accurate diagnosis.^[13]

The guidelines further emphasize the need for a multidisciplinary team approach involving neurologists, metabolic specialists, geneticists, radiologists, and laboratory scientists to interpret complex clinical and molecular findings. Moreover, they include recommendations on the classification and reporting of genomic variants, including standardized terminology (e.g., ACMG criteria), and call for periodic re-evaluation of variants of uncertain significance as new evidence emerges.

Global harmonization of diagnostic protocols is essential, especially as genetic testing becomes more accessible through national health systems and research initiatives. Shared frameworks can ensure consistency in variant interpretation, data sharing, and follow-up care. Furthermore, efforts like the Mitochondrial Disease Sequence Data Resource and other open-access platforms are helping to compile genotype-phenotype correlations across diverse populations, enhancing both clinical utility and equity in care delivery.^[13]

Therapeutic Implications of Genetic Diagnosis

Accurate molecular diagnosis plays a pivotal role not only in confirming the presence of mitochondrial disease but also in shaping clinical management and future therapeutic options. For many years, treatment of mitochondrial diseases was primarily supportive, focusing on symptom relief, nutritional supplementation (e.g., CoQ10, riboflavin), and metabolic crisis prevention. However, the expanding understanding of the genetic basis of these disorders has paved the way for precision medicine approaches.^[10]

For instance, gene therapy trials targeting nuclear-encoded mitochondrial genes—such as those for TK2 or SURF1 deficiency—are showing promise in preclinical and early clinical stages. In cases of mtDNA-related disorders, innovative strategies like mitochondrial replacement therapy (MRT) have been developed to prevent maternal transmission of pathogenic variants, particularly in families with a history of severe, recurrent mitochondrial disease. Although MRT remains controversial in some countries due to ethical and regulatory concerns, it represents a transformative step in reproductive genetics.

Additionally, pharmacological agents that target specific metabolic pathways or enhance mitochondrial biogenesis are under investigation. Drugs like elamipretide (SS-31) and KH176, which aim to stabilize mitochondrial membranes or modulate redox status, are currently in clinical trials. Beyond direct patient care, molecular diagnosis also has profound implications for genetic counseling, carrier detection, and prenatal or preimplantation genetic diagnosis, thereby empowering affected

families to make informed reproductive decisions.^[10,17]

Future Perspectives

The future of mitochondrial disease diagnostics and therapeutics lies in the convergence of genomic innovation, systems biology, and global collaboration. Multi-omic integration—combining genomics, transcriptomics, proteomics, and metabolomics—has the potential to illuminate disease mechanisms beyond the limitations of DNA alone. This approach could help decipher the functional consequences of variants of uncertain significance and identify novel therapeutic targets through pathway analysis and biomarker discovery. Advancements in single-cell sequencing offer granular insights into mitochondrial heteroplasmy and its impact on individual cell function, which is particularly relevant given the tissue-specific manifestations of these disorders. Meanwhile, machine learning algorithms are being trained to recognize pathogenic variant patterns, predict phenotypic outcomes, and prioritize candidate genes for testing, enhancing the interpretive power of sequencing data.^[10]

In parallel, global rare disease networks and patient registries are playing an increasingly important role. Initiatives such as the International Mito Patients Network and RD-Connect provide platforms for data sharing, cross-border clinical trial recruitment, and long-term outcome tracking. As more therapies enter the pipeline, the role of real-world evidence from these collaborative databases will be crucial in guiding clinical practice and policy. Ultimately, the trajectory of mitochondrial genomics is one of growing precision, personalization, and inclusivity in tackling one of medicine's most enigmatic and diverse groups of disorders.^[10,18]

CONCLUSION

Mitochondrial genomics has transformed the diagnostic landscape of hereditary mitochondrial disorders by enabling precise identification of pathogenic variants across both mitochondrial and nuclear genomes. The integration of clinical, biochemical, and genomic data enhances diagnostic accuracy and facilitates early intervention. Emerging therapies tailored to specific genetic defects highlight the importance of molecular diagnosis in guiding management. Continued advancements in sequencing technologies, data interpretation, and global collaboration are essential to address current challenges and improve outcomes for affected individuals and families.

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