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CLINICAL, BIOCHEMICAL, AND GENETIC FINDINGS IN A GROUP OF PATIENTS EVALUATED FOR SUSPECTED MITOCHONDRIAL DISORDERS

Doina SECU, ORCID: 0000-0002-8571-0524
secudoina95@gmail.com
Daniela BLANITA, ORCID: 0000-0001-7736-3406
blanita.daniela@gmail.com
Natalia USURELU, ORCID: 0000-0001-8685-3933
nataliausurelu@yahoo.com
Victoria SACARA, ORCID: 0000-0001-9200-0494
victoriasacara@hotmail.com

Institute of Mother and Child, Chisinau, Republic of Moldova

Abstract. Mitochondrial diseases (MDs) are clinically heterogeneous disorders caused by mutations in either mitochondrial DNA (mtDNA) or nuclear genes that impair oxidative phosphorylation. This study aimed to comprehensively characterize the clinical, biochemical, imaging, and molecular features of 37 patients exhibiting mitochondrial involvement, selected from a larger group of 240 individuals with suspected MD based on a Nijmegen Mitochondrial Disease Score ≥3. All patients underwent a standardized evaluation protocol comprising clinical assessment, targeted biochemical and instrumental investigations, qPCR-HRM screening for common mtDNA mutations, and mtDNA sequencing, with additional nuclear gene analysis performed in selected cases. Multisystemic involvement was prevalent, with neuromuscular and central nervous system manifestations being the most frequent. Elevated lactate levels and abnormal neuroimaging findings further supported the clinical diagnosis. Molecular analyses revealed both common and rare mtDNA variants, while nuclear gene defects were identified in a subset of patients, underscoring the genetic complexity of MDs. The integrated approach enabled accurate diagnosis and highlighted the value of combining clinical and molecular data in MD evaluation.

Keywords: mitochondrial DNA, qPCR-HRM, targeted sequencing, mtDNA mutations, mitochondrial disease.

INTRODUCTION

Mitochondrial diseases (MDs) are among the most frequent inherited metabolic disorders, with an estimated prevalence of approximately 1 in 5000 individuals. They stem from genetic alterations that impair oxidative phosphorylation (OXPHOS), disrupting cellular energy homeostasis [1]. The clinical spectrum is remarkably broad, involving neurological, muscular, cardiac, endocrine, gastrointestinal, and ophthalmological manifestations, which adds

considerable complexity to early diagnosis [2]. Given their clinical heterogeneity, the integration of molecular diagnostics has become essential in confirming MD and guiding treatment. Mutations in both mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) can lead to MDs, necessitating a dual-tiered genetic approach [3].

In this study, we describe the clinical, biochemical, instrumental, and molecular characteristics of 37 patients with genetically confirmed mitochondrial disease, selected from a broader group of 240 individuals with suspected MD based on Nijmegen Mitochondrial Disease Score. Our goal was to characterize this subset in detail, exploring potential associations between clinical presentation, NMDS scores, biochemical abnormalities, and the type of underlying genetic variant.

MATERIALS AND METHODS

This study was conducted between March 2021 and October 2024 at the Institute of Mother and Child, the national reference center for genetic and metabolic disorders in the Republic of Moldova. Patients were included based on a modified Nijmegen Mitochondrial Disease Score [4], ranging from 0 to 8 points, with only individuals scoring ≥3 undergoing further evaluation.

Each selected patient followed a standardized diagnostic protocol, including clinical assessment, targeted biochemical testing, and instrumental investigations. The clinical assessment involved the documentation of neurodevelopmental status, neuromuscular symptoms, growth patterns, and organ-specific dysfunctions. Family history and perinatal data were also recorded when available. Biochemical testing included serum lactate, creatine kinase (CK), lactate dehydrogenase (LDH), electrolytes, ammonia levels, plasma amino acids, acylcarnitine profiles, urinary organic acids, and other parameters tailored to the clinical phenotype. Instrumental investigations comprised electroencephalography (EEG), electromyography (EMG), electrocardiography (ECG), and neuroimaging, which included cranial CT and, when indicated, brain MRI, performed in accordance with clinical necessity.

Molecular testing followed a stepwise approach. All 240 patients underwent qPCR-HRM screening for eight common pathogenic mtDNA point mutations. Based on NMDS >6 and/or positive HRM results, 90 patients were further analyzed by Sanger sequencing of mtDNA. Additional nuclear gene testing was performed in individuals with suggestive clinical features or inconclusive mtDNA results. Variant interpretation followed the American College of Medical Genetics and Genomics (ACMG) guidelines and incorporated in silico tools and public databases (MITOMAP, ClinVar, gnomAD, MitImpact 3D, Ensembl Variant Effect Predictor).

Statistical analyses were performed using IBM SPSS Statistics v27. Categorical variables were expressed as frequencies and percentages, and continuous variables as medians and interquartile ranges. Associations were tested

using Chi-square, and group comparisons with Mann–Whitney U test. A p-value <0.05 was considered statistically significant.

RESULTS AND DISCUSSIONS

Among the 37 individuals exhibiting mitochondrial involvement, the mean age at clinical evaluation was 48.8 ± 10.55 months. At the time of data analysis, 5 patients (13.5%) were deceased, reflecting the severity and progressive nature of the disease spectrum. Perinatal data were available for 33 individuals (89.2%), among whom 6 (16.2%) presented with intrauterine growth restriction, while pregnancy or birth-related complications were documented in 9 cases (24.3%). These findings highlight the potential early onset and systemic burden of mitochondrial dysfunction from the prenatal period. Building upon these early findings, the clinical spectrum observed in this group was remarkably heterogeneous, encompassing multisystemic involvement with variable age at symptom onset and degrees of severity.

Muscular and neuromotor features were among the most prevalent findings. Hypotonia was the most frequent finding (81.1%), followed by exercise intolerance (64.9%), and episodes of rhabdomyolysis (8.1%). Regression or loss of previously acquired motor skills occurred in 27.0% of individuals, typically in the context of global clinical deterioration.

Neurological involvement spanned a broad spectrum of central and peripheral features. Ataxia was present in 24.3% of cases, including both cerebellar ataxia (10.8%) and broader truncal incoordination. Pyramidal signs were observed in 24.3% and extrapyramidal features in 16.2%, while clinical indicators of brainstem dysfunction—such as dysphagia, dysarthria, or abnormal ocular motility—were noted in 43.2% of patients. Seizures were reported in 70.3%, encompassing generalized, focal, and myoclonic types. Stroke-like episodes occurred in 5.4%, and cortical blindness was noted in 8.1% of cases. Intellectual disability or significant developmental delay was a prominent feature in 62.2%, often accompanied by behavioral or neuropsychiatric disturbances. Peripheral neuropathy, most frequently with a sensorimotor distribution, was present in 24.3%.

The multisystemic nature of mitochondrial dysfunction was further underscored by the frequent association of extra-neurological manifestations. Endocrine and growth abnormalities were among the most prevalent, affecting 59.5% of patients. Gastrointestinal symptoms, such as recurrent vomiting, feeding difficulties, and chronic constipation, were reported in 29.7%. Hepatic involvement, including hepatomegaly and mild transaminase elevation, was observed in 27.0% of cases. Cardiac involvement was observed in 35.1% of patients, including conduction abnormalities (18.9%), dilated cardiomyopathy (5.4%), and, less frequently, hypertrophic cardiomyopathy (2.7%). Ophthalmic

involvement included optic nerve atrophy (40.5%), strabismus (24.3%), nystagmus (29.7%), and ophthalmoplegia (16.2%), while sensorineural hearing loss was present in 8.1% of individuals. Hematologic involvement was documented in 32.4% of patients, most frequently manifesting as coagulation abnormalities (27.0%), followed by thrombocytopenia (16.2%), neutropenia (10.8%), and macrocytic anemia (10.8%). Renal impairment was rare, identified in only 2.7% of patients.

Biochemical testing revealed increased serum lactate levels in 91.9% of patients, with levels exceeding 4 mmol/L in 21.6% of cases. Hyperlactatemia weas significantly associated with developmental impairment ($\chi^2 = 6.11$, $p = 0.013$), highlighting the relevance of metabolic disturbance in early neurodevelopmental impairment. Elevated LDH and ammonia levels were observed in 43.2% and 8.1% of patients, respectively, while increased creatine kinase was detected in 21.6%. Electrolyte disturbances were less frequent and primarily consisted of mild hypocalcemia (21.6%), hyponatremia (18.9%), or hypokalemia (10.8%), without consistent correlation to clinical or imaging findings. Elevated plasma alanine was observed in 32.4% of patients, while altered acylcarnitine profiles and urinary organic acid abnormalities were identified in 13.5% and 10.8%, respectively – findings consistent with metabolic signatures of mitochondrial dysfunction.

Instrumental investigations demonstrated abnormal electroencephalographic activity in 56.8% of individuals, with predominant patterns including hypsarrhythmia (16.2%), polymorphic slow waves (10.8%), frequent epileptiform discharges (5.4%), and atypical complexes potentially indicative of epileptic phenomena (21.6%). Electromyographic studies revealed a myopathic pattern in 21.6% of the assessed patients. Neuroimaging abnormalities were documented in 75.7% of cases, most frequently encompassing cerebral atrophy (40.5%), white matter signal alterations (18.9%), basal ganglia involvement (16.2%), and stroke-like lesions (5.4%). Additionally, bilateral symmetric basal ganglia lesions compatible with Leigh syndrome were observed in 8.1%. Notably, individuals with abnormal neuroimaging exhibited significantly higher rates of psychomotor delay ($\chi^2 = 7.38$, $p = 0.025$) and elevated Nijmegen scores (median 6.82 vs. 6.0; $p = 0.012$, Mann–Whitney U test), supporting an association between structural cerebral involvement and overall disease severity.

Molecular investigations revealed variants in mitochondrial or nuclear genes consistent with the observed phenotypes. Eight patients carried one of the common pathogenic point mutations identified via qPCR-HRM screening: m.3243A>G (n=3), m.8993T>G (n=2), m.11778G>A (n=1), and m.3460G>A (n=2). In the remaining cases, Sanger sequencing of the mitochondrial genome—focused primarily on protein-coding genes—uncovered additional variants, most of which were homoplasmic. Genes most commonly affected were those encoding Complex I subunits (*MT-ND* family) in 25.0% of cases, followed by mitochondrial

RNA genes (21.9%), Complex V subunits (*MT-ATP6/8*) in 15.6%, and Complex III (*MT-CYB*) and Complex IV (*MT-CO2*), each accounting for 3.1%. Mutations spanning multiple respiratory chain complexes were observed in 31.3% of cases, indicating diffuse mitochondrial dysfunction, though not significantly associated with clinical severity ($\chi^2 = 3.44$, $p = 0.064$).

Out of the total, 19 individuals (59.4%) had pathogenic or likely pathogenic variants, while 13 (40.6%) presented VUS with a high likelihood of phenotypic relevance. These VUS were predominantly identified in Complex I-related genes (34.1%), followed by Complex V (29.2%) and Complex IV (17.0%). Patients carrying pathogenic or likely pathogenic variants had significantly higher NMDS scores compared to those with only VUS ($\chi^2 = 6.82$, $p = 0.033$), indicating greater clinical burden in genetically confirmed cases. These distributions are further illustrated in Figure 1, which depicts gene-level frequency, and in Figure 2, which details the classification categories of variants detected exclusively within mtDNA.

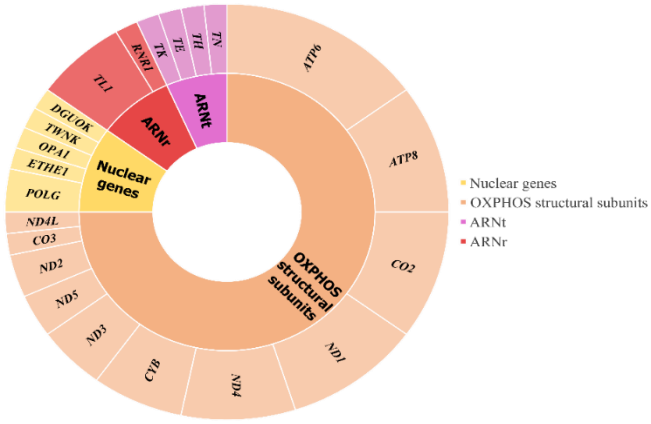

Figure 1. Gene-level distribution of identified variants across mtDNA and nDNA

A subgroup of five individuals was diagnosed with nuclear gene mutations affecting mitochondrial dynamics and replication: *POLG* (n=2), *DGUOK* (n=1), *TWNK* (n=1), and *ETHE1* (n=1), each associated with distinct syndromic forms of mitochondrial dysfunction. Notably, one patient harboring the m.3243A>G variant also carried a pathogenic variant in *OPA1*, a gene implicated in optic atrophy and mitochondrial membrane remodeling.

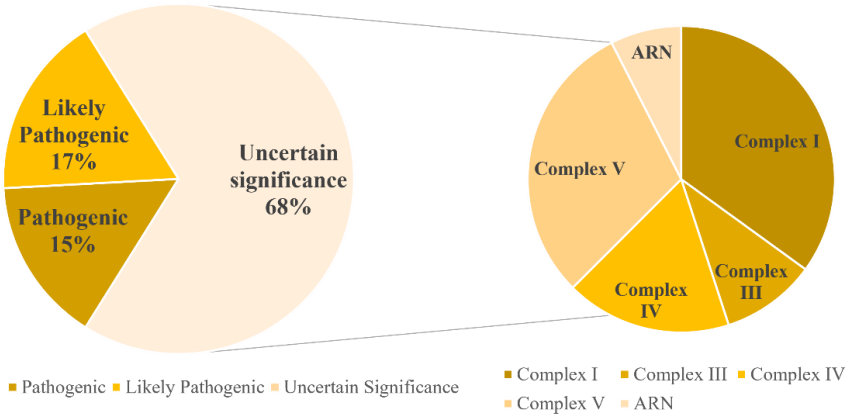

Figure 2. Variant pathogenicity and VUS subdistribution by OXPHOS complexes and RNA genes

Taken together, these findings highlight the heterogeneity and complexity of mitochondrial disorders. The high prevalence of neuromuscular and multisystemic involvement aligns with existing literature [5,6] and reinforces the need for an integrated diagnostic approach combining clinical, biochemical, imaging, and molecular data [7]. The significant associations observed between clinical severity and genetic findings (particularly Nijmegen score and variant classification), as well as with neuroimaging and EEG abnormalities, support the utility of these tools in guiding diagnostic suspicion and stratifying patients for genetic testing [8]. Furthermore, the distribution of mutations across respiratory chain complexes – most notably Complex I – emphasizes its central role in mitochondrial pathology [9]. Despite the presence of VUS in a substantial proportion of patients, their phenotypic relevance and co-segregation with core clinical features suggest their potential pathogenic contribution, warranting future functional validation.

CONCLUSIONS

This study underscores the clinical and molecular heterogeneity of mitochondrial diseases, revealing consistent patterns of early-onset, multisystemic involvement, with a predominance of neuromuscular and neurodevelopmental features. The Nijmegen score proved valuable not only as a selection tool but also as a proxy for disease severity, correlating significantly with key clinical and imaging parameters. Elevated serum lactate and neuroimaging abnormalities were associated with more severe phenotypes, supporting their relevance as accessible diagnostic markers. Molecular analysis identified diverse mtDNA and nDNA variants, notably in Complex I subunits and mtRNA genes, with pathogenic variants

associating with higher clinical scores. The integration of clinical, biochemical, imaging, and genetic data enabled a refined stratification of patients and highlighted the diagnostic yield of a tiered genetic approach. These findings support the implementation of early and comprehensive evaluation strategies to improve diagnostic accuracy and inform management in suspected MDs.

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