MITOCHONDRIAL DNA COPY NUMBER AND GENOMIC PROFILING IN SUSPECTED MITOCHONDRIAL PHENOTYPES

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Background

Mitochondrial diseases are genetically and clinically heterogeneous disorders that remain challenging to diagnose due to their overlapping manifestations and diverse molecular causes. Beyond pathogenic sequence variants, alterations in mitochondrial DNA (mtDNA) copy number may reflect impaired mitochondrial biogenesis and compensatory cellular responses. Therefore, integrated quantitative and genomic approaches may provide a more comprehensive understanding of suspected mitochondrial phenotypes.

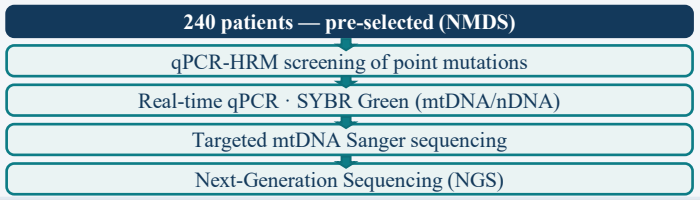
Aim of the Study

This study aimed to investigate quantitative mtDNA copy number dynamics and establish a comprehensive genomic profile in individuals presenting with suspected mitochondrial phenotypes.

Materials & Methods

A cohort of 240 patients was pre-selected using the Nijmegen Mitochondrial Disease Criteria (NMDS). The molecular pipeline integrated qPCR-HRM for rapid screening of common point mutations, real-time qPCR with SYBR Green master mix for relative mtDNA copy number (mtDNA/nDNA ratio), and targeted mtDNA Sanger sequencing for a high-suspicion subgroup. Next-Generation Sequencing (NGS) was applied to a strategic subgroup to identify nuclear-mitochondrial interactions.

Fig. 1 · Molecular diagnostic pipeline



Results

Based on genetic testing, the cohort was stratified into patients with mitochondrial involvement (n = 39; identified via qPCR-HRM, targeted Sanger, and NGS), alternative genetic conditions representing diagnostic mimics (n = 50; resolved via NGS), and undiagnosed cases (n = 151). Concurrently, quantitative analysis across the entire cohort revealed significant deviations from baseline replication states: severe mtDNA depletion was identified in 35 patients (14.6%), a mild increase in 34 individuals (14.2%), and marked overcompensation in 17 patients (7.1%). Notably, these quantitative alterations were not randomly distributed but were strongly and statistically associated with the group presenting mitochondrial involvement ($\chi^2 = 21.0$, df = 3, $p < 0.001$).

Fig. 2 · Diagnostic stratification (n = 240)

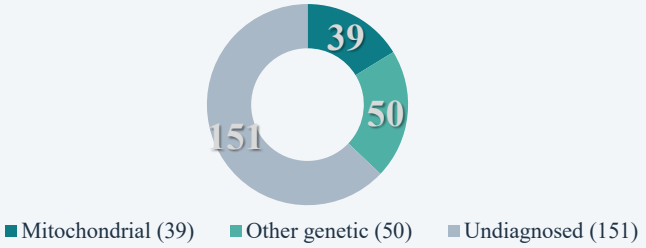


Fig. 3 · mtDNA copy-number distribution (n = 240)

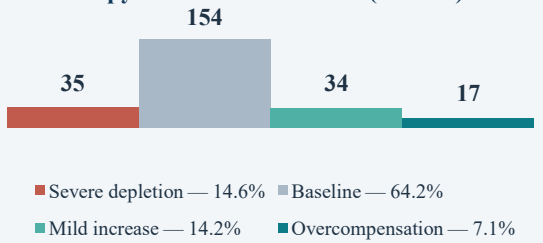
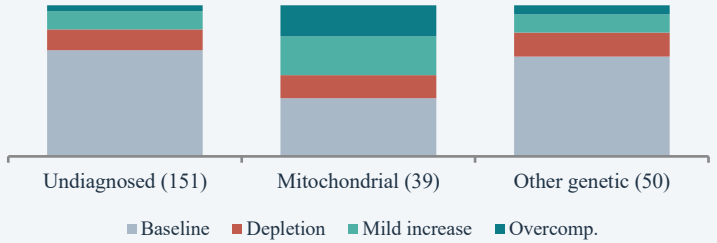


Fig. 4 · Copy-number status across diagnostic groups



$\chi^2 = 21.0$ · df = 3 · $p < 0.001$
copy-number status vs. mitochondrial involvement

% within each diagnostic group.

Conclusions

Quantifying mtDNA copy number provides key insights into depletion and graded metabolic compensatory mechanisms independent of primary mtDNA mutations. Integrating quantitative assays with NGS is essential to accurately differentiate primary mitochondrial defects from nuclear genetic mimics.

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Keywords: *mitochondrial DNA, qPCR-HRM, targeted sequencing, mtDNA copy number.*

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