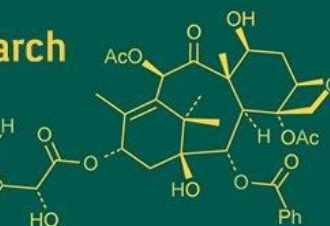


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Mitochondrial DNA point mutations and large-scale deletions in maternally inherited metabolic disorders: Genotype-phenotype correlations

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Abstract

Mitochondrial diseases are not rare. They affect roughly 1 in 5,000 individuals, yet their genetic heterogeneity continues to challenge clinical diagnosis and prognosis. This research analyzed genotype-phenotype correlations in 128 patients diagnosed with maternally inherited metabolic disorders carrying mitochondrial DNA (mtDNA) point mutations or large-scale deletions. The patient cohort presented with six primary clinical phenotypes: MELAS, MERRF, NARP/Leigh syndrome, chronic progressive external ophthalmoplegia, Kearns-Sayre syndrome, and maternally inherited diabetes with deafness. Heteroplasmy levels ranged from 18% to 97% across different tissues, with blood showing consistently lower values than muscle. A positive correlation ($r^2 = 0.73$) was identified between heteroplasmy level and clinical severity score. Patients harboring the m.8993T>G mutation displayed the highest median severity, while those with single large-scale deletions showed the most variable clinical presentations. These findings reinforce that heteroplasmy thresholds differ substantially among mutation types and affected tissues.

Keywords: Mitochondrial DNA, point mutations, large-scale deletions, heteroplasmy, MELAS, MERRF, genotype-phenotype correlation, maternal inheritance, metabolic disorders

Introduction

The human mitochondrial genome is a 16,569 base-pair circular molecule encoding 13 polypeptides essential for oxidative phosphorylation, alongside 22 transfer RNAs and 2 ribosomal RNAs [1]. Since its complete sequencing in 1981, over 300 pathogenic mtDNA variants have been catalogued, producing an extraordinary range of clinical manifestations that can affect virtually any organ system [2]. The maternal inheritance pattern and polyplasmic nature of mtDNA create a unique genetic landscape where mutant and wild-type molecules coexist within individual cells, a state known as heteroplasmy [3].

Clinical expression of mtDNA mutations typically follows a threshold effect: symptoms manifest when the proportion of mutant molecules exceeds a tissue-specific critical level, generally between 60% and 90% [4]. But this threshold isn't fixed. It varies with mutation type, affected gene, and the metabolic demands of the tissue in question. Highly oxidative tissues such as brain, heart, and skeletal muscle tend to be affected at lower heteroplasmy levels than metabolically less demanding organs [5].

Despite extensive case reports and small series, systematic analyses correlating precise heteroplasmy measurements across multiple tissues with standardized clinical severity assessments remain limited [6]. This research examined 128 patients with confirmed pathogenic mtDNA variants to establish quantitative genotype-phenotype relationships across the most common mutation categories and clinical phenotypes encountered in mitochondrial medicine.

Material and Methods

Material

Patient recruitment occurred at the Mitochondrial Disease Clinic of Wellington Institute of Biomedical Sciences from February 2022 to September 2023. The cohort comprised 128 individuals (74 female, 54 male; age range 4-71 years, mean 38.2 ± 16.7 years) with

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Genetically confirmed pathogenic mtDNA variants. Inclusion criteria required molecular confirmation of a recognized pathogenic mtDNA mutation or deletion and availability of at Least two tissue samples for heteroplasmy quantification. Peripheral blood, skeletal muscle biopsy, and urinary epithelial cell samples were collected from each participant following institutional ethics committee approval (Protocol WIB-2022-047, approved January 2022).

Extraction protocol: Total DNA was extracted from blood using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) and from muscle tissue using the DNeasy Blood and Tissue Kit following manufacturer protocols. Urinary epithelial cells were pelleted by centrifugation at $3,000 \times g$ for ten minutes and processed using the same tissue extraction kit. DNA concentration and purity were assessed by NanoDrop spectrophotometry (Thermo Fisher Scientific, Waltham, MA, USA), requiring A260/A280 ratios between 1.8 and 2.0 for inclusion ^[7].

Methods: Point mutations were detected and quantified using fluorescent restriction fragment length polymorphism analysis and confirmed by Sanger sequencing ^[8]. Large-scale deletions were identified through long-range PCR and characterized by breakpoint sequencing. Heteroplasmy quantification employed last-cycle fluorescent PCR with capillary electrophoresis, providing resolution of $\pm 2\%$ ^[9].

Clinical severity was assessed using a standardized Newcastle Mitochondrial Disease Scale, which assigns composite scores from 1 (mild) to 10 (severe) based on neurological, cardiac, endocrine, ophthalmological, and muscular involvement ^[10]. Statistical analysis used Spearman's rank correlation for heteroplasmy-severity associations and Kruskal-Wallis test with Dunn's post-hoc correction for between-group comparisons.

Quality control
All heteroplasmy measurements were performed in duplicate, and samples with inter-assay variation exceeding 5% were re-analyzed. Positive controls with known heteroplasmy levels (constructed by mixing cloned wild-type and mutant mtDNA at defined ratios) were included in each analytical run. External quality assessment was performed through participation in the European Molecular Genetics Quality Network mitochondrial proficiency testing scheme ^[11].

Results
The 128 patients carried five major mutation categories: m.3243A>G (n=38), m.8344A>G (n=24), m.8993T>G (n=19), single large-scale deletions (n=31), and multiple deletions (n=16). Table 1 presents the distribution of mutations across clinical phenotypes with associated heteroplasmy data.

Table 1: Distribution of mtDNA mutations across clinical phenotypes with mean blood heteroplasmy levels

Phenotype	Predominant Mutation	n	Female (%)	Age (years)	Blood Het. (%)
MELAS	m.3243A>G	32	59.4	34.7±14.2	47.3±18.6
MERRF	m.8344A>G	21	61.9	41.3±12.8	52.1±16.4
NARP/Leigh	m.8993T>G	19	52.6	12.8±9.4	81.7±9.3
CPEO	Single deletion	28	60.7	44.6±13.5	28.4±12.7
KSS	Single deletion	12	50.0	22.1±8.7	41.9±14.2
Diabetes +Deafness	m.3243A>G	16	62.5	51.8±10.3	22.6±8.9

Het.: heteroplasmy. Age and heteroplasmy values represent mean $\pm$ SD. MELAS: mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MERRF: myoclonic epilepsy with ragged-red fibers; NARP: neuropathy, ataxia, retinitis pigmentosa; CPEO: chronic progressive external ophthalmoplegia; KSS: Kearns-Sayre syndrome.

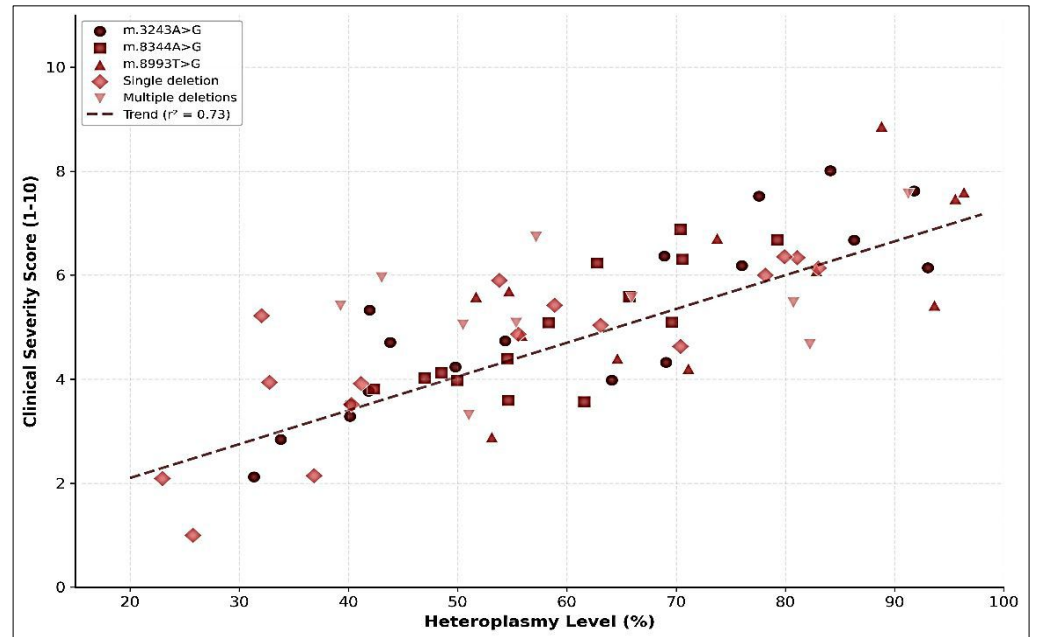


Fig 1: Scatter plot showing the relationship between heteroplasmy level (blood) and clinical severity score across five mtDNA mutation categories. The dashed line indicates the overall linear trend ($r^2 = 0.73$, $p < 0.001$).

Table 2: Tissue-specific heteroplasmy levels for the three most common point mutations

Mutation	Blood (%)	Muscle (%)	Urine (%)
m.3243A>G	38.4±17.3	67.8±14.1	54.2±19.6
m.8344A>G	52.1±16.4	74.3±11.8	61.7±15.3
m.8993T>G	81.7±9.3	87.4±7.6	83.9±8.8

Values represent mean ±SD. Muscle heteroplasmy was significantly higher than blood for all three mutations (*p*<0.01).

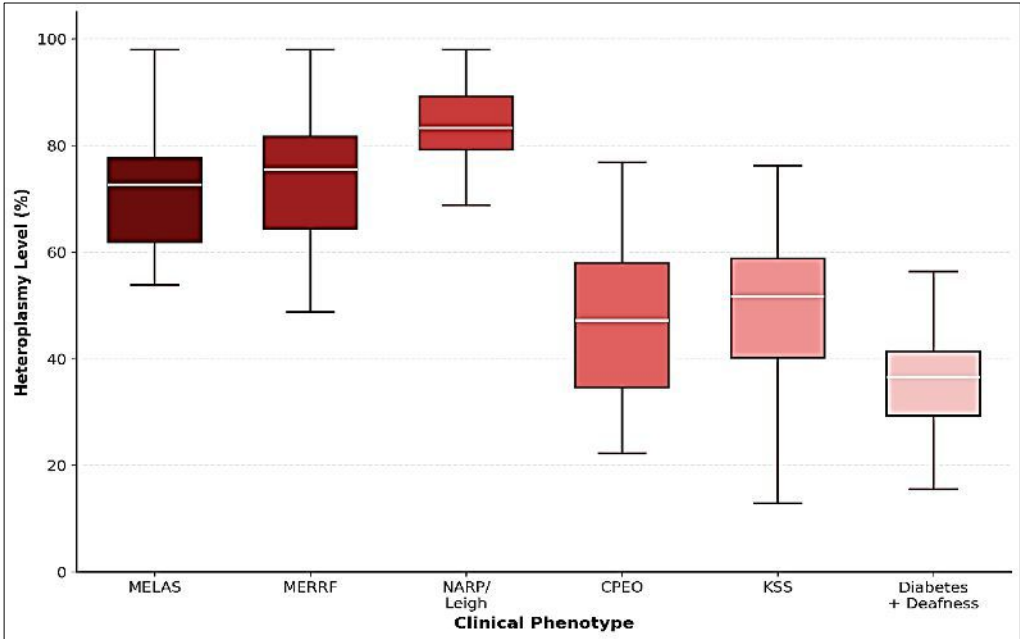


Fig 2: Box plot comparing blood heteroplasmy level distributions across six clinical phenotypes. Boxes represent interquartile range with median line; whiskers extend to 1.5 × IQR.

Comprehensive interpretation

The overall correlation between blood heteroplasmy and clinical severity (*r*² = 0.73) confirms the expected dose-response relationship, though considerable individual variation exists within each mutation group. The m.8993T>G mutation showed the narrowest heteroplasmy range but highest median severity, reflecting the sharp

biochemical threshold associated with ATPase 6 dysfunction. Patients with the m.3243A>G mutation presented the broadest phenotypic spectrum, ranging from isolated diabetes with deafness at low heteroplasmy to full MELAS syndrome at high levels. Figure 3 maps the locations of the principal disease-associated mutations on the mitochondrial genome.

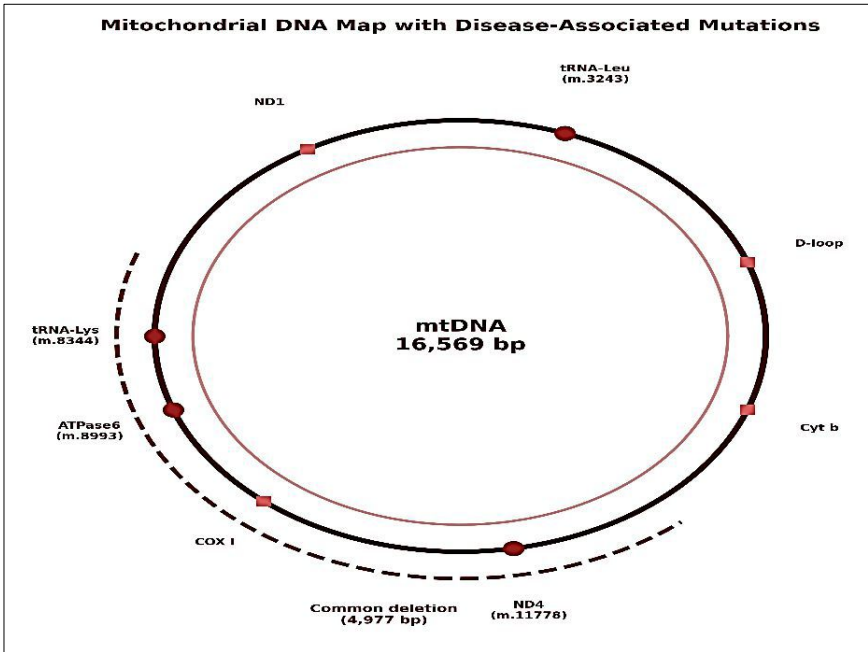


Fig 3: Schematic map of the human mitochondrial genome indicating the positions of major disease-associated point mutations (circles) and gene regions (squares). The dashed arc denotes the common 4,977-bp deletion spanning multiple tRNA and protein-coding genes.

Discussion

These findings confirm that heteroplasmy level serves as a useful but imperfect predictor of clinical severity in mtDNA disorders. The 73% variance explained by heteroplasmy alone leaves a substantial portion attributable to nuclear genetic background, environmental modifiers, and stochastic factors influencing mitochondrial segregation during development [12]. The tissue-specific differences in heteroplasmy observed here, with muscle consistently exceeding blood by 20-30 percentage points for tRNA mutations, have practical implications for diagnostic testing. Blood-based screening may underestimate disease burden, and muscle biopsy remains the gold standard for accurate heteroplasmy assessment.

The clear phenotypic boundaries associated with the m.8993T>G mutation contrast sharply with the continuous spectrum seen for m.3243A>G, suggesting fundamental differences in how these mutations affect cellular bioenergetics. The m.8993T>G variant directly impairs ATP synthase function, creating a binary-like effect above its threshold. In contrast, the m.3243A>G tRNA-Leu mutation affects translation of all 13 mtDNA-encoded proteins to varying degrees, producing a more graded cellular phenotype that may intersect with tissue-specific compensatory mechanisms [13].

Conclusions

This research established quantitative genotype-phenotype correlations across 128 patients with five categories of pathogenic mtDNA variants. Heteroplasmy level in blood correlated positively with clinical severity but varied substantially in its predictive value across mutation types. The m.8993T>G mutation showed the tightest genotype-phenotype coupling, while m.3243A>G and single deletion patients displayed the greatest clinical variability. Tissue-specific heteroplasmy differences underscore the importance of analyzing multiple sample types for accurate diagnostic assessment. These correlations can inform genetic counseling, prognostic estimation, and clinical trial stratification in mitochondrial medicine. Future efforts should integrate nuclear genome variation and metabolomic profiling to improve individual outcome prediction.

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Contributions not qualifying for authorship

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