Low-level Large Deletions in Mitochondrial Genome:
A Potential Diagnosis of Mitochondrial Diseases

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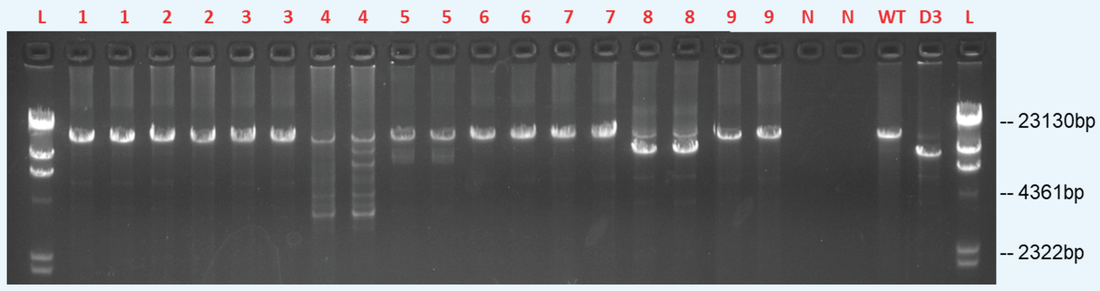
INTRODUCTION

Large deletions in the mitochondrial genome are a significant contributor to mitochondrial diseases. The proportion of mitochondrial DNA (mtDNA) carrying these large deletions is a critical factor in determining phenotype and clinical outcomes. In this retrospective study, we aim to explore low-level large deletions in young patients that may contribute to mitochondrial diseases.

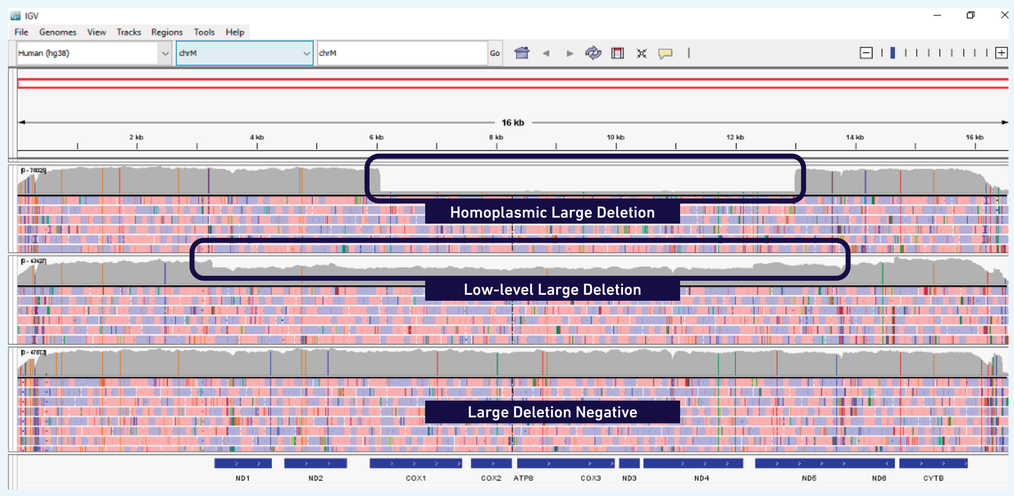
METHODS

A long-term study of mitochondrial genome wide variants was performed using a Next Generation Sequencing (NGS) based platform; long-range PCR was used to specifically amplify and enrich mitochondrial DNA, followed by NGS. NGS data was processed using bioinformatic pipelines to call single nucleotide variants (SNV) and large deletions throughout the mitochondrial genome. SNVs and small indel variants can be confidently called at heteroplasmic levels as low as 1.5%. Copy number variations (CNVs) can be identified simultaneously using NGS read depth analysis. MLPA was used to confirm the large deletion and estimate the fraction of deficient mtDNA.

Example of Long-range PCR

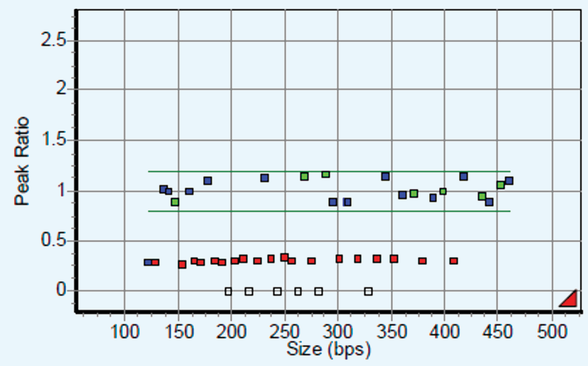


Example of Large Deletion

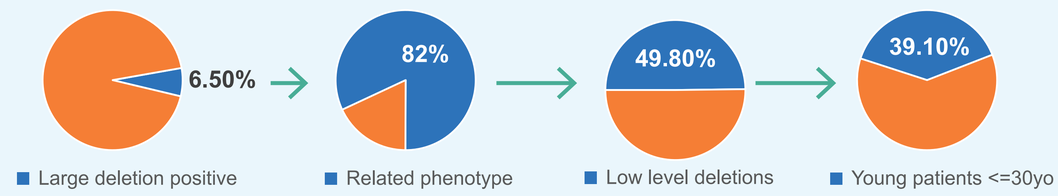


Example of Single Nucleotide Variant

Example of MLPA



Low-level Large Deletions in Young Patients



RESULTS

We conducted mitochondrial whole-genome testing on over 10,000 clinical samples. Among them, 6.5% cases exhibited large deletions in mitochondrial genomic regions, with 4.6% of these cases also testing positive for pathogenic or likely pathogenic SNVs. Out of the cases solely positive for large deletions, 82% displayed phenotypes at least partially consistent with mitochondrial diseases. Among these, ~50% of samples had low-level deletions detected by NGS sequencing and confirmed by MLPA. 39% of patient cases with low-level deletions were younger than 30 years old.

CONCLUSIONS

It has been reported that a biochemical deficiency may not be observed unless the large deletion fraction exceeds 60%, and low-level deletions of muscle mtDNA are known to occur with age in healthy individuals. However, within our study cohort, a significant proportion of young patients exhibit low-level heteroplasmic large deletions. This phenomenon could result from the clonal effect causing biological impact being significantly diluted in the tested sample or may be attributed to tissue specificity. A single large low-level mitochondrial DNA deletion in young patients may indicate an early phase of disease progression; to ensure accurate diagnosis, affected tissues such as muscle instead of blood are recommended for testing.

Considering that mitochondrial disorders may be caused by molecular defects in nuclear genes, sequence analysis of specific nuclear genes may also be indicated. Employing a combination test involving mitochondria-related gene testing with mitochondrial genome-wide testing or utilizing WGS that sequences the entire human nuclear genome and mitochondrial genomes simultaneously could provide opportunities to reveal the etiologies of dual genomes in patients.

References

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Presented at the UMDF Mitochondrial Medicine Conference, June 26-29, 2024, Cleveland, Ohio, USA