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Nuclear Mitochondrial DNA disruption in KLHL10 gene causes Spermatogenic Failure – a Case Study

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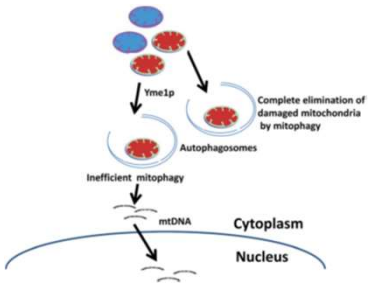
INTRODUCTION

Mitochondrial DNA (mtDNA) translocation into the nuclear genome, also known as Nuclear Mitochondrial DNA insertions (NUMT)[1], is an extremely rare genetic event that may disrupt a protein-coding gene and cause disease. How exactly NUMTs occur is poorly understood. It is suggested that key mechanisms involve a combination of abnormal mitochondria degradation and non-homologous end-joining DNA repair at nuclear double-strand breaks [1,2].

NUMTs have been implicated in human biology and pathology, including aging, cancer, and Mendelian disease. Up to date, only a handful of disease-causing NUMTs have been described. However, recent studies show that NUMTs variation is continuously misreported as mitochondrial mutations in patients [2].

Here we present a rare case of spermatogenic failure in which a NUMT disrupts *KLHL10* through insertion into exon 3 of the gene. Autosomal dominant pathogenic variants in the gene have been reported to cause oligozoospermia.

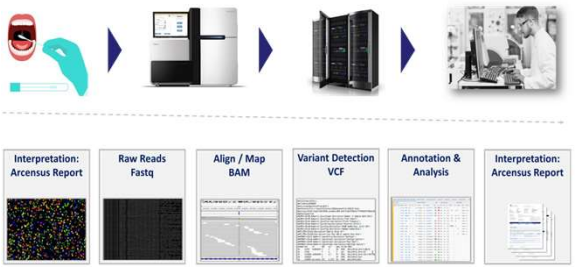


NUMTs Integration into Nuclear DNA: The role of mitochondrial dysfunction and compromised mitophagy[1].

METHOD

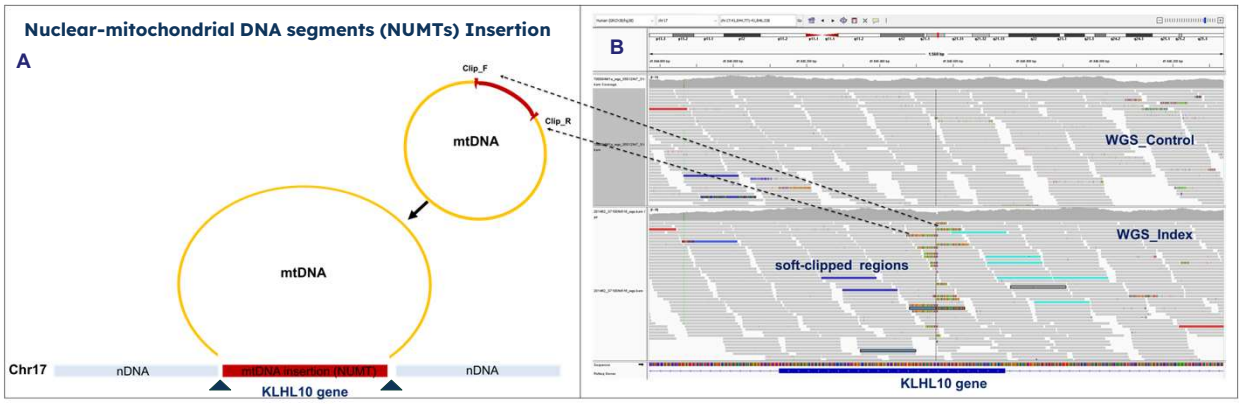
Biological sample was collected after obtaining informed consent from patient. The DNA extracted from the sample was subjected to whole genome sequencing on an Illumina machine using a 150nt pair-end protocol to yield an average coverage depth of 30x for the nuclear genome and over 1000x for the mitochondrial genome. The raw reads were aligned to the reference genome GRCH38, and variant calling, including single nucleotide substitutions (SNVs), small insertions/deletions (Indels), and structural variants (SVs), was performed using DRAGEN with default parameters. Structural variants were annotated with ANNOTSV3.1 and an in-house structural variant database to obtain allele frequencies. Potential disease-causing genetic variants were filtered by considering rare events that affect the coding gene regions supported by phenotypic match between the gene and index' clinical symptoms.

Whole Genome Sequencing and Data Analysis



RESULTS

Identification of Mitochondrial DNA Insertion within the *KLHL10* in a patient with oligoasthenoteratozoospermia (OATS)



A 41-year-old male patient was diagnosed with oligospermia with no familial history of reproductive disorders. Previous examinations ruled out Y-chromosome microdeletions. The patient was sent for whole-genome sequencing (WGS) to identify the potential genetic etiology of the condition. Structural variant analysis of the case revealed an insertion of mitochondrial DNA into exon 3 of KLHL10 (NM_152467.5). This insertion, of which the exact break points could be identified at chr17:41845555 and chrM:3595, is predicted to disrupt the *KLHL10*, in which gene defects have been associated with autosomal dominant spermatogenic failure type 11. Whether the event is inherited or *de novo*, could not be established because DNA from parents was not available.

CONCLUSION

To the best of our knowledge, this is the first reported instance of a NUMT causing a rare form of male infertility. However, additional experimentation is needed to address whether this event is functionally inactive. Single nucleotide variants and small deletions/duplications account for most disease-causing variants. Increasingly, novel next-generation sequencing data analysis methods have revealed the prevalence of non-subtle balanced genetic variants, such as translocations, inversions, and nuclear mitochondrial DNA segments (NUMTs). These types of variants should be routinely evaluated as part of genetic testing, as they can contribute to improving the diagnostic rate.

CONFLICT OF INTEREST STATEMENT: All authors are current employees at Arcensus Diagnostics.

Acknowledgement: This work was made possible by all patients who participated in this study.

References:
[1] Srinivasainagendra ***et al***, Genome Med, 2017
[2] Wei ***et al***, Nature, 2022

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