

Comparison of Mitochondrial Genome Findings Identified by Genome Sequencing and Focused Mitochondrial Genome Sequencing

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Background

- Clinical detection of mitochondrial DNA (mtDNA) disorders can be performed using genome sequencing (GS) or focused mitochondrial genome sequencing (mtGS).
- GS includes broad coverage of both nuclear and mitochondrial genomes, while focused mtGS offers deeper coverage of the mitochondrial genome.
- The detection of mtDNA variants is influenced by tissue-specific heteroplasmy, interference from nuclear mtDNA segments, platform-dependent sequencing depth, and assay sensitivity.¹
- Here, we identify individuals who underwent both GS and focused mtGS to compare the detection of mtDNA variants.

Results

Figure 1. Cohort characteristics, testing sequence, and specimen types for individuals undergoing GS and focused mtGS.

35 individuals had both GS and focused mtGS testing completed		
GS → mtGS 15/35 (42.9%)	mtGS → GS 12/35 (34.3%)	Concurrent testing 8/35 (22.9%)
Different sample types used for each of the two tests 19/35 (54.3%)		Same sample type used for both tests 16/35 (45.7%)

Figure 2. Frequency of specimen types for GS and focused mtGS. One case had focused mtGS on blood and skeletal muscle. Other specimen types included extracted DNA, liver, tissue, and skin fibroblasts.

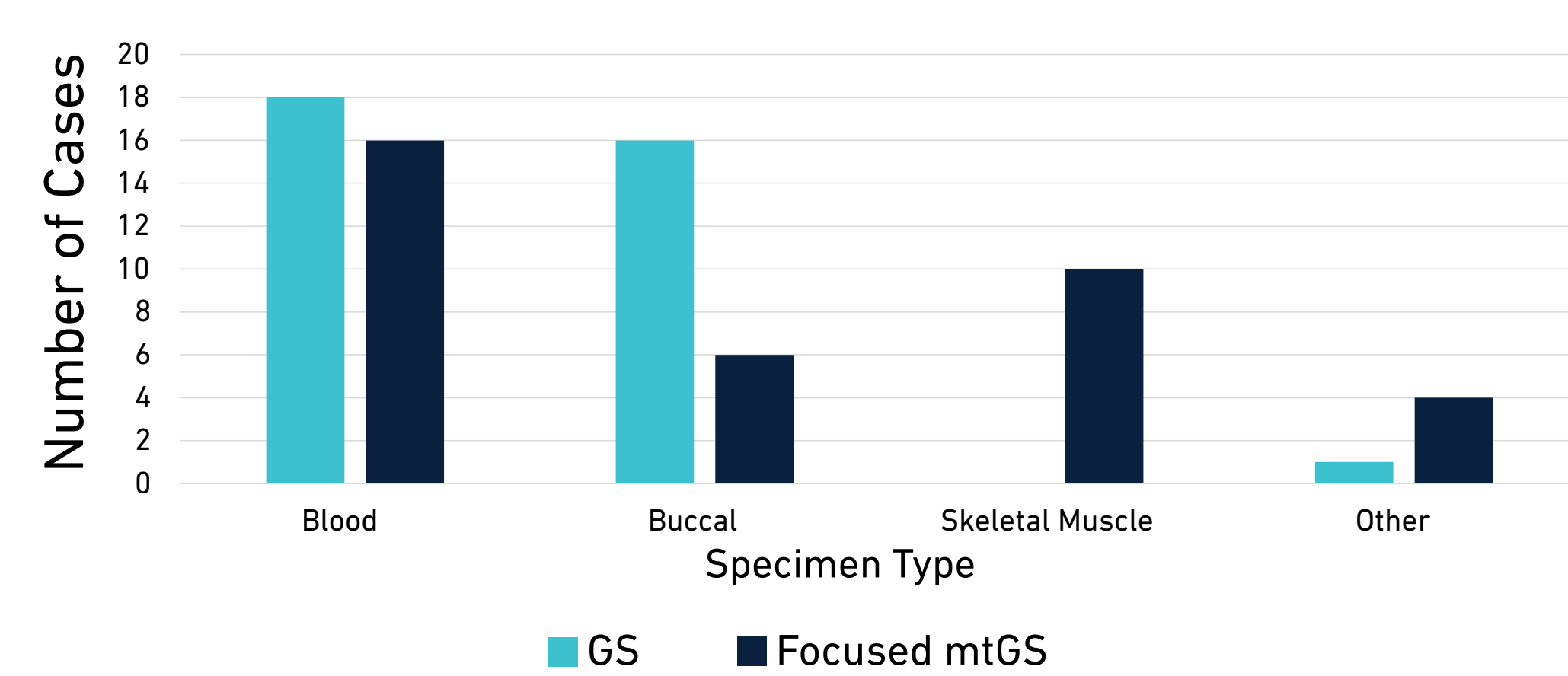
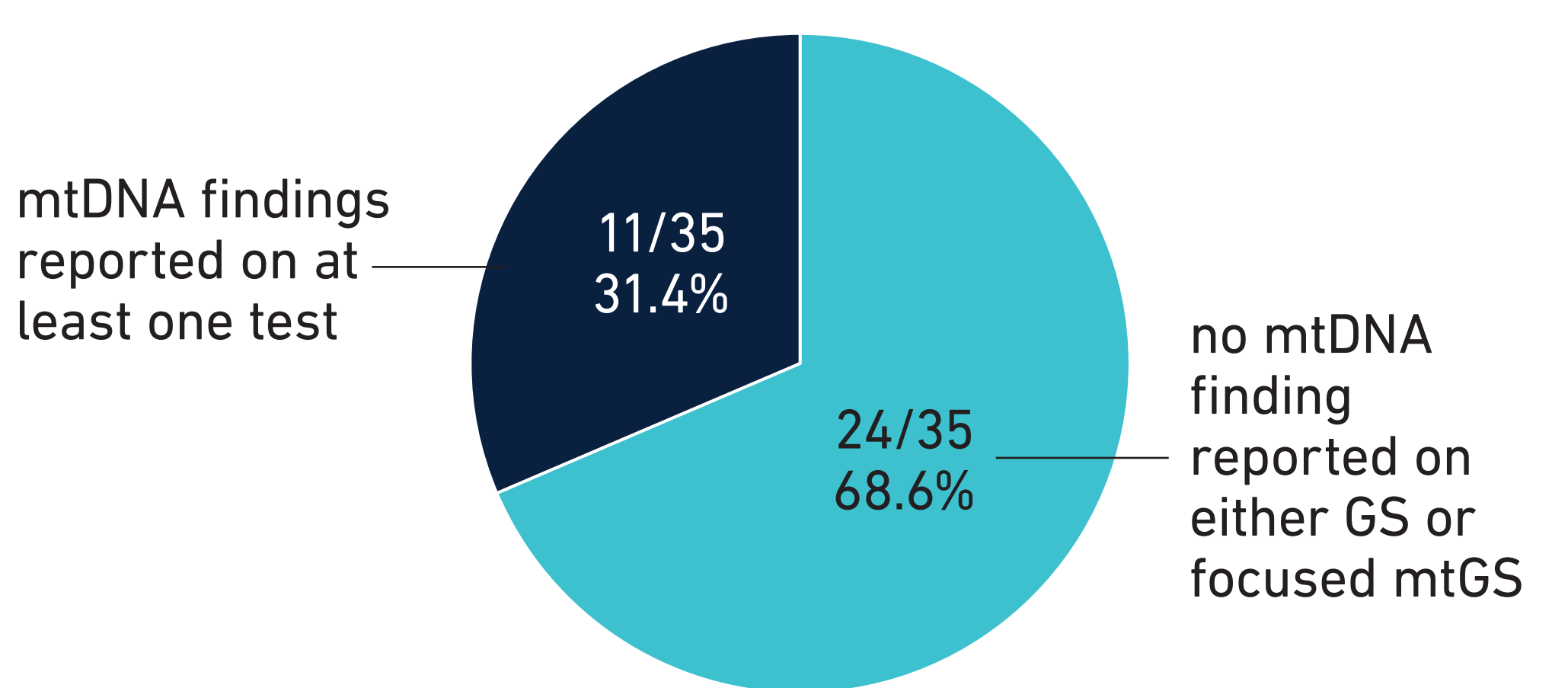


Figure 3. Proportion of cases with concordant negative findings compared to cases with mtDNA findings on at least one test.



Conclusion

- Results were largely concordant across individuals who underwent both GS and focused mtGS.
- Low-level mtDNA deletions identified by focused mtGS reflect the use of different sample types and higher analytical sensitivity compared to GS.
- GS on blood or buccal samples is an effective first-tier broad genomic test; however, negative results do not exclude mtDNA deletions in clinically suspected cases.
- In patients with significant suspicion of mitochondrial disease, focused mtGS and/or testing of affected tissue may enhance diagnostic yield.
- Focused mtGS can provide supportive functional evidence for nuclear-encoded mitochondrial variants.

Methods

- We conducted a retrospective review of consecutive GS and focused mtGS cases performed by our clinical diagnostic laboratory.
- For individuals who underwent GS and focused mtGS, cross-platform concordance and specimen-dependent differences in variant detection were evaluated.
- Low-level mtDNA findings were defined as variants with a heteroplasmy level below 10%.

Table 1. Comparison of GS and focused mtGS findings in 11 individuals with mtDNA variant detection.

Case	GS		Focused mtGS	
	Specimen	Findings	Specimen	Findings
1	Buccal Swab	Negative	Buccal Swab	Multiple low-level mtDNA deletions
2	Buccal Swab	Pathogenic variant in <i>MT-ATP6</i> , 6%	Blood	Pathogenic variant in <i>MT-ATP6</i> , 6.8%
3	Extracted DNA	VUS in <i>MT-TC</i> , 100%	Skeletal Muscle	VUS in <i>MT-TC</i> , 100%
4	Buccal Swab	Pathogenic variant in <i>MT-TL1</i> , 16%	Skeletal Muscle	Pathogenic variant in <i>MT-TL1</i> , 92%
5	Buccal Swab	Negative	Skeletal Muscle	Multiple low-level mtDNA deletions with a major deletion
6	Blood	Pathogenic variant in <i>MT-TK</i> , 6%	Buccal Swab	Pathogenic variant in <i>MT-TK</i> , 4.1%
7	Buccal Swab	Pathogenic variant in <i>MT-ND2</i> , 34%	Skeletal Muscle	Pathogenic variant in <i>MT-ND2</i> , 95%
8	Buccal Swab	Pathogenic variant in <i>MT-TL1</i> , 7%	Skeletal Muscle	Pathogenic variant in <i>MT-TL1</i> , 11%
9	Buccal Swab	Pathogenic variant in <i>MT-ND3</i> , 9%	Blood	Pathogenic variant in <i>MT-ND3</i> , 4%
10	Blood	Compound heterozygous variants in <i>TOP3A</i>	Tissue	Multiple low-level large mtDNA deletions
11	Buccal Swab	Negative	Skeletal Muscle	Likely pathogenic variant in <i>MT-ND1</i> , 10%

Pediatric Case Example: Combined GS and focused mtGS identified dual nuclear and mitochondrial pathology

Patient presentation:
A female child with dilated cardiomyopathy and seizures.

1. Initial duo GS finding in blood:

± INDETERMINATE FINDINGS						
DISEASE	INHERITANCE PATTERN	GENE/VARIANT	VARIANT TYPE	GENOTYPE	INHERITED FROM	VARIANT CLASSIFICATION
TOP3A-Related Disorders	Autosomal Recessive	TOP3A: c.529C>T, p.R177*	Sequence Variant	Heterozygous	Unknown	Pathogenic
TOP3A-Related Disorders	Autosomal Recessive	TOP3A: c.2154_2159del, p.L718_K719del	Sequence Variant	Heterozygous	Mother	Variant Of Uncertain Significance

2. Subsequent Sanger confirmation in the paternal sample confirmed that these variants are in trans.

3. Follow-up mtGS finding in the corresponding tissue:

mtDNA Large Deletion		
Deletion Position	Deletion Size (bp)	Comments
Multiple deletions		low level

Conclusion:

The *TOP3A* gene encodes a DNA topoisomerase, an enzyme that regulates DNA topology during transcription. *TOP3A* knockdown has been shown to reduce mitochondrial DNA (mtDNA) copy number and lead to the accumulation of high molecular weight mtDNA.²

Multiple low-level large mtDNA deletions in a tissue specimen of this individual consistent with *TOP3A*-related disorder. This helps to reclassify the c.2154_2159del (p.L718_K719del) variant as likely pathogenic.