

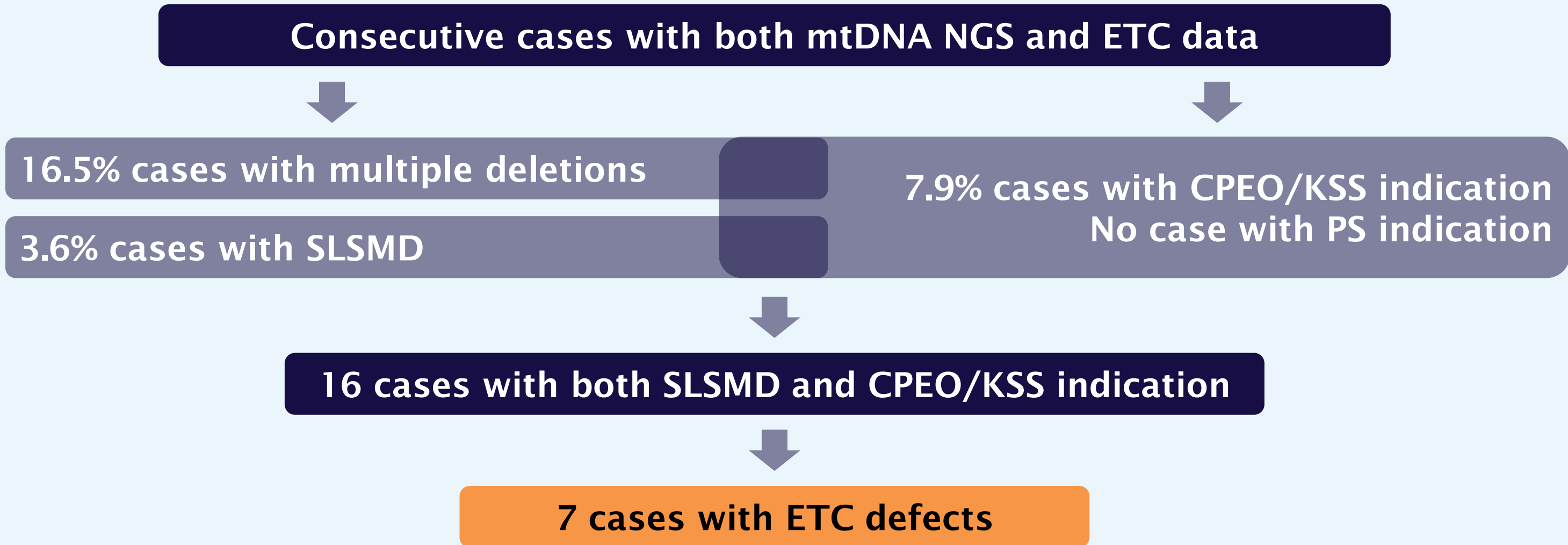
INTRODUCTION

- Large deletions in mitochondrial DNA (mtDNA) are associated with single large-scale mtDNA deletion syndromes (SLSMDSs), including chronic progressive external ophthalmoplegia (CPEO), Kearns-Sayre syndrome (KSS), and Pearson syndrome (PS).
- CPEO and KSS are characterized by a spectrum of symptoms including ptosis, ophthalmoplegia, and mitochondrial myopathy, whereas PS presents with early-onset anemia or pancytopenia in infancy, along with multisystem involvement.
- SLSMDSs often arise from sporadic mtDNA deletions that present in a heteroplasmic state. While the level of heteroplasmy and tissue distribution of the deletions are reported to be determining factors for disease severity, a clear mechanism behind the genotype-phenotype correlation has not been previously documented.

METHODS

- We retrospectively reviewed clinical cases with both electron transport chain (ETC) functional testing and mtDNA genome sequencing results assayed from the same muscle specimen.
- The activities of ETC complexes I through IV were measured using specific electron donors and acceptors. Citrate synthase (CS) activity was also determined and used as a calibrator of mitochondrial copy number or tissue preparation. Respiratory chain enzyme activity was normalized to CS activity.
- The mtDNA deletions were detected by next generation sequencing (NGS) analysis of long-range PCR products of the mitochondrial genome and confirmed using either Southern blot (SB) or multiplex ligation-dependent amplification (MLPA) analysis. The deletion breakpoints were based on NGS results and the heteroplasmy levels were estimated by SB or MLPA.

Case Selection Flowchart

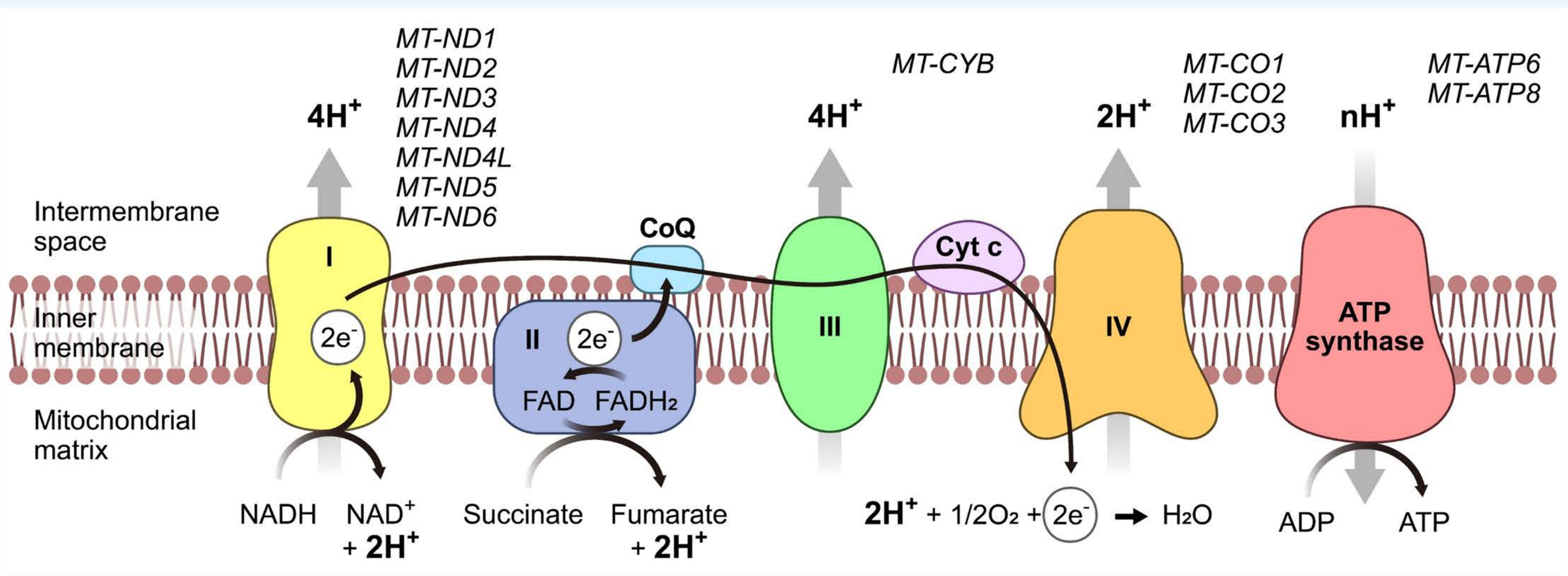


Summary of clinical presentations

Case #	1	2	3	4	5	6	7
Age at test	10	28	31	32	34	39	69
Ptosis	+	+	+	+	+	+	-
External ophthalmoplegia	-	+	+	+	+	+	+
Skeletal myopathy	+	+	+	+	+	-	+
Cardiomyopathy	-	-	-	-	-	-	+
Abnormal muscle biopsy	COX, RRF	NA	NA	NA	+	COX, RRF	COX, type II atrophy
Abnormal brain MRI	+	+	-	-	-	-	-
Pigmentary retinopathy	-	+	+	-	+	-	-
Cardiac block	-	-	-	-	+	-	-

COX: COX deficient fibers; RRF: ragged red fiber, NA: not available

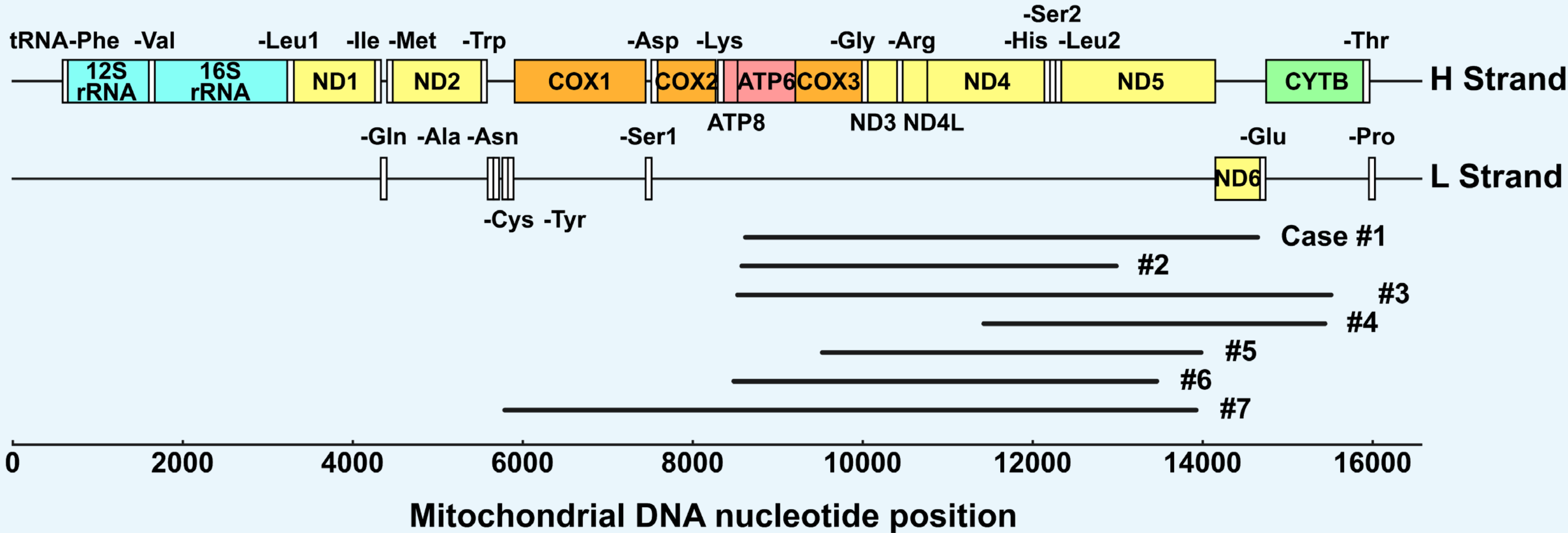
Diagram of Mitochondrial Electron Transport Chain (ETC)



RESULTS

- Of the eligible cases, 16 cases presented with clinical indications consistent with the CPEO/KSS spectrum and exhibited single large mtDNA deletions.
- Of the 16 cases, 7 exhibited significantly deficient ETC complex enzyme activity (defined as <30% based on modified Walker minor criteria) before or after CS activity correction. Notably, all 7 participants had low level heteroplasmy (<10%) for the mtDNA deletions.
- Among them, 4 cases showed defects in a single respiratory chain complex, while the other 3 had defects across multiple complexes. Complex IV was most commonly impacted, being reduced in 4 samples. The activity of nuclear-encoded complex II remained intact in all samples.
- The specific ETC complexes affected in each case are not fully correlated with the mitochondrial genes disrupted by the mtDNA deletions.

SLSMDS observed in the cohort



Summary of ETC complex enzyme activities

Case #	1	2	3	4	5	6	7
%Heteroplasmy	<10%	<10%	<10%	< 10%	<10%	<10%	<10%
Complex I	68, 39	23, 40	30, 37	38, 32	28, 34	37, 106	154, 148
I+III Total	47, 27	43, 76	73, 90	42, 35	53, 65	74, 211	112, 108
I+III Rotenone	32, 18	39, 69	74, 91	26, 22	31, 38	93, 265	71, 68
Complex II	90, 52	49, 86	83, 102	84, 70	89, 109	122, 347	496, 477
Complex II+III	59, 34	69, 121	76, 94	39, 33	47, 57	163, 462	83, 80
Complex IV	82, 47	28, 50	51, 63	37, 31	8, 10	27, 76	19, 18
Citrate Synthase (CS)	173	57	81	119	82	35	104

Major (<20% activity) and minor (20-30% activity) deficiency in ETC complexes. Enzyme activity values are presented as percentages of control values before and after CS normalization.

CONCLUSIONS

- This study demonstrates that the approach of long-range PCR plus NGS can detect low-heteroplasmy mtDNA deletions. Furthermore, muscle functional studies of ETC enzyme activities can provide further support for mtDNA deletion syndromes.
- It has been reported that a biochemical deficiency may not be observed unless the large deletion fraction exceeds 60%. However, within our study cohort, we observed ETC defects in patient muscle tissues with a SLSMD in <10% heteroplasmy.
- The results show that mitochondrial function can be significantly compromised even when mtDNA deletion heteroplasmy level is well below the expected deficient thresholds, emphasizing the need for comprehensive molecular and functional evaluations in making a SLSMDS diagnosis.

References

1. Goldstein A, Falk MJ. Single Large-Scale Mitochondrial DNA Deletion Syndromes. In: GeneReviews®. University of Washington, Seattle, Seattle (WA); 1993.
2. Kirby DM, Thorburn DR, Turnbull DM, Taylor RW. Biochemical assays of respiratory chain complex activity. Methods Cell Biol. 2007; 80:93-119.
3. Trounce JA, Kim, YL, Jun, AS, Wallace, DC. Assessment of mitochondrial oxidative phosphorylation in patients muscle biopsies, lymphoblasts and transmittochondrial cell lines. Methods Enzymol. 1996. 264, 484-509.
4. Walker UA, Collins S, Byrne E. Respiratory chain encephalomyopathies: a diagnostic classification. Eur Neurol. 1996; 36, 260-267.
5. Wong LJ. Comprehensive molecular diagnosis of mitochondrial disorders: qualitative and quantitative approach. Ann N Y Acad Sci. 2004; 1011: 246-258.
6. Wong LJ. Molecular genetics of mitochondrial disorders. Dev Disabil Res Rev. 2010; 16: 154-162.
7. Tang S, et al. Transition to next generation analysis of the whole mitochondrial genome: a summary of molecular defects. Hum Mutat. 2013; 34(6):882-893.
8. Katatana M, et al. Deleted mitochondrial DNA in the skeletal muscle of aged individuals. Biochem Int. 1991; 25:47-56.