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Genetic Testing of Oxidative Phosphorylation Diseases: Comparison of Next Generation Sequencing with Capillary Sequencing Approaches [P03.238]

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Background

Mitochondrial disorders are the most common group of metabolic disorders, with an estimated prevalence of 1 in 5,000. (1) Oxidative phosphorylation (OXPHOS) is critical to cellular function as the primary source for energy (ATP) in most cell types, the control point for cellular redox, and as a control point for essential metabolic and signaling pathways that range from the synthesis of pyrimidines to the regulation of apoptosis. OXPHOS is the only metabolic process that is controlled by two genomes, the mitochondrial DNA (mtDNA) and the nuclear DNA (nDNA). The number of mitochondrial proteins encoded by nuclear genes is estimated to be around 1,500. (2) MtDNA contains 37 genes encoding 13 respiratory chain subunits, 2 rRNAs and 22 tRNAs.

Mitochondrial disease diagnosis should NOT rely on gene sequencing alone in most cases. When gene sequencing is used as the ONLY TOOL for patient diagnosis, the results are often ambiguous as studies have shown. (3) Strict criteria for patient diagnosis requires integration of clinical data, special muscle pathology techniques, OXPHOS enzymology, live tissue for proton gradient and Complex V assessment, protein chemistry of individual OXPHOS enzyme subunits, muscle Coenzyme Q10 quantitation, mtDNA copy number assessment, protein chemistry of monomeric enzyme assembly, protein chemistry of supercomplexes, and nDNA or mtDNA genetic testing. This can only be achieved at centers with specialized expertise in the clinical and laboratory complexities associated with mitochondrial diseases.

In order to properly understand the pathogenesis of OXPHOS dysfunction, assessment of large numbers of genes in a cost effective fashion is necessary. Next Generation sequencing is a cost effective approach for screening a large array of nDNA and mtDNA genes for mutations. Once mutations are identified, an integrated assessment of clinical data and the laboratory approaches described above can be performed. This approach greatly decreases the number of ambiguous interpretations of sequencing data.

MtDNA Point Mutation Detection

Heteroplasmic mtDNA mutation detection is assessed using Next Generation sequencing.

SOLiD System Sequencing (Applied Biosystems)

Coding region heteroplasmic sites assessed: 85

Heteroplasmy assessed across 0-100% range

Heteroplasmic sites scattered across coding regions

Detection of mutations compared with capillary sequencing

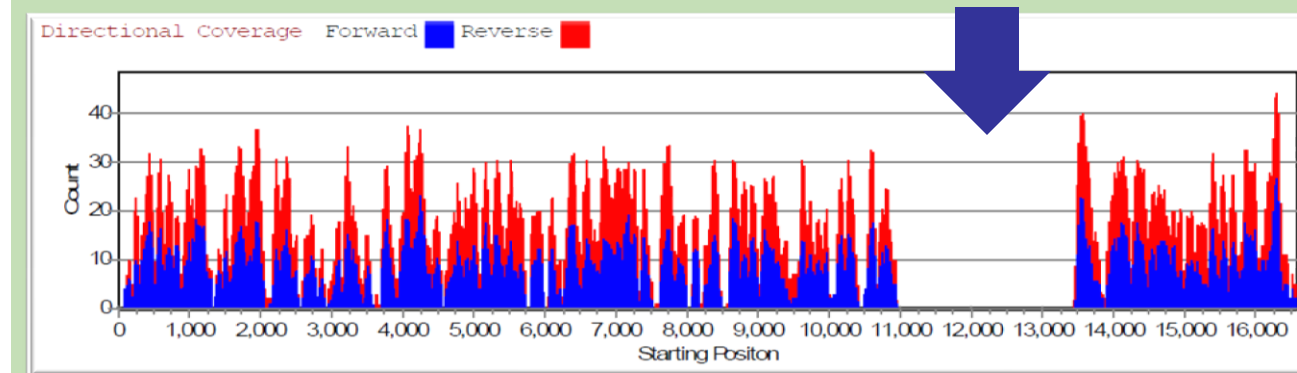
% Heteroplasmy	Total Mutation Detection Across MTDNA
0% and 100%	100%
10% and 90%	99.97%
20% and 80%	99.98%
30% and 70%	99.98%
40% and 60%	99.98%
50% and 50%	99.88%

Next Generation Heteroplasmic Point Mutation Detection

- False positives ranged from 0-3. ALL false positive would be excluded from reporting based on unacceptably low Quality Value scores.
- Next Generation Sequencing exhibited excellent detection of heteroplasmic mtDNA mutations.
- Sequencing coverage of the mtDNA was >99%.

MtDNA Deletion Detection

Large mtDNA deletions are easily detected with Next Generation sequencing.

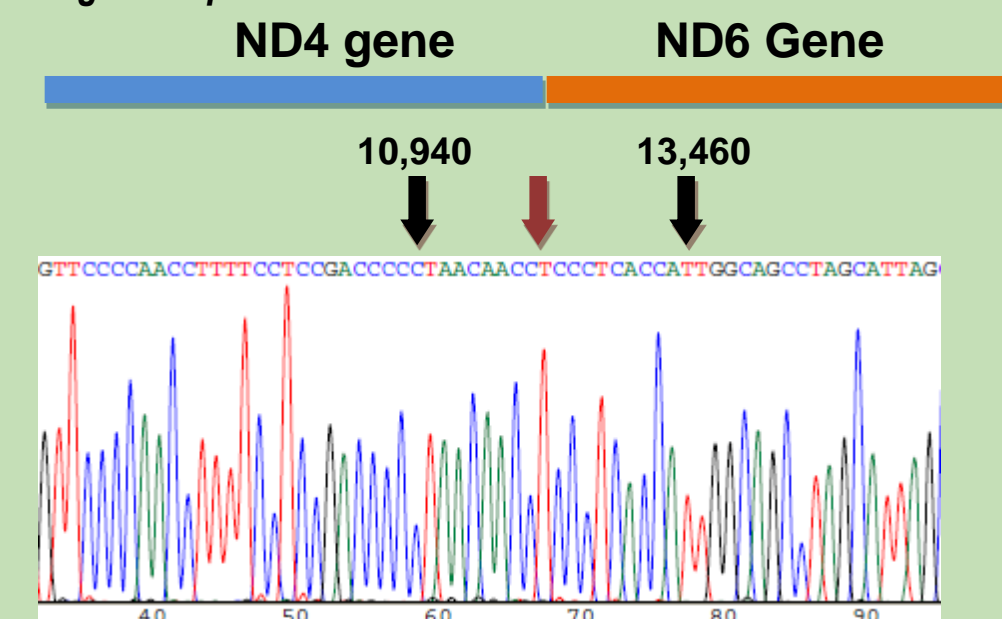


Directional coverage map generated during next generation sequencing clearly shows the 2,502 deleted nucleotide pairs of the mtDNA (arrow).

Numbering: Revised Cambridge Reference Sequence
Deletion breakpoint: 10948:13450 (2502 nucleotides deleted)
Comment: The deletion removes part of the ND4 gene (a complex I subunit), transfer RNAs for histidine, serine (AGY), and Leucine (CUN), as well as part of the ND5 gene. The deletion joins the ND4 gene with the ND5 gene.

The red arrow designates the breakpoint which joins the ND4 and ND6 gene
....CAACC/TCCT....

NOTE: The CAACC sequence is present at the breakpoint in the ND4 and ND6 gene. Numbering of the breakpoint assumed that the CAACC sequence in the ND4 gene is preserved.



Capillary Sequencing identification of deletion breakpoint

Next Generation mtDNA Deletion Detection

- Large mtDNA deletions are easily detected using Next Generation sequencing. We detected deletion breakpoints quickly and easily in other patients harboring mtDNA deletions.
- Deletion breakpoint identification shows 100% correspondence with capillary sequencing assessment of mtDNA deletions break
- Sequencing coverage of the mtDNA was >99%.

Conclusions

- Next Generation sequencing is important for cost effective screening for mtDNA and nDNA mutations.
- Experience with sequencing from many groups demonstrate that gene sequencing is a tool that augments patient diagnosis and management.
- Next Generation sequencing is a tool that is MOST useful when combined with careful clinical, metabolic and biochemical testing.
- Sequencing alone cannot definitively assess whether a mutation is the cause for the patient's phenotype even when careful assessment of the mutation with interspecies homology assessment (evolutionary conservation) or with software tools that predict possible impact of amino acid substitution on the structure and function of human protein (e.g. PolyPhen; <http://genetics.bwh.harvard.edu/pph/>)
- Heteroplasmic mtDNA point mutations are accurately detected across the range of heteroplasmy (0-100%).
- MtDNA deletions are easily detected with Next Generation sequencing and the breakpoints are accurately identified.

Selected References

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- Valeria Vasta, Sarah B Ng, Emily H Turner, Jay Shendure, and Si Houn Hahn Next generation sequence analysis for mitochondrial disorders *Genome Med.* 2009; 1(10): 100