Genetic Testing:

Genome Sequencing A-Z for Mitochondrial Disease

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MitoAction
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Overview

DNA sequencing for mitochondrial disease
Genome sequencing for mitochondrial disease
Clinical case examples

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Background

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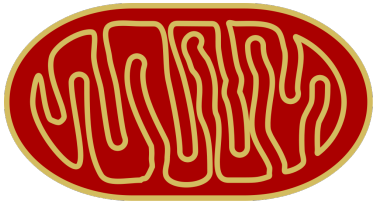
Mitochondria are the “energy factories” of our cells and organs



Mitochondrial disease happens when the mitochondria stop working

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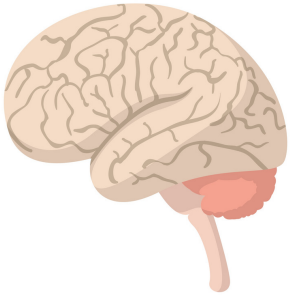
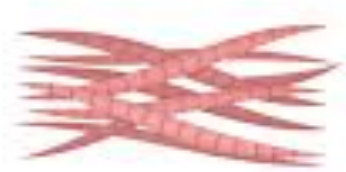
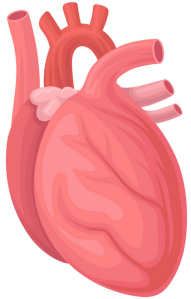
Organs requiring large amounts of energy are usually affected



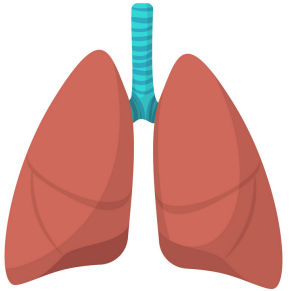
Brain



Muscles



Heart



Lungs

But others can also be affected: kidneys, eyes, liver, pancreas, etc.

Mitochondrial disease is typically considered if 3 or more organs are affected

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Mitochondrial disease is suspected when…

…more than one of the energy demanding organs are affected and result in the following clinical symptoms:

- Developmental delay with other organ involvement
- Cardiomyopathy
- High levels of lactic acid
- Opthalmoplegia or ptosis
- Hearing loss
- Severe gastrointestinal dysmotility
- Severe developmental regression with other illness

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However, some symptoms can be associated with many different disorders, not necessarily mitochondrial disease

- Failure to thrive
- Short stature
- Developmental delay
- Poor muscle tone (hypotonia) and muscle myopathy

Inheritance of Mitochondrial disease

- Mitochondrial genome changes come from the mother (or arise new)
- Nuclear genome changes come from either or both parents (or arise new)

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DNA sequencing for mitochondrial disease

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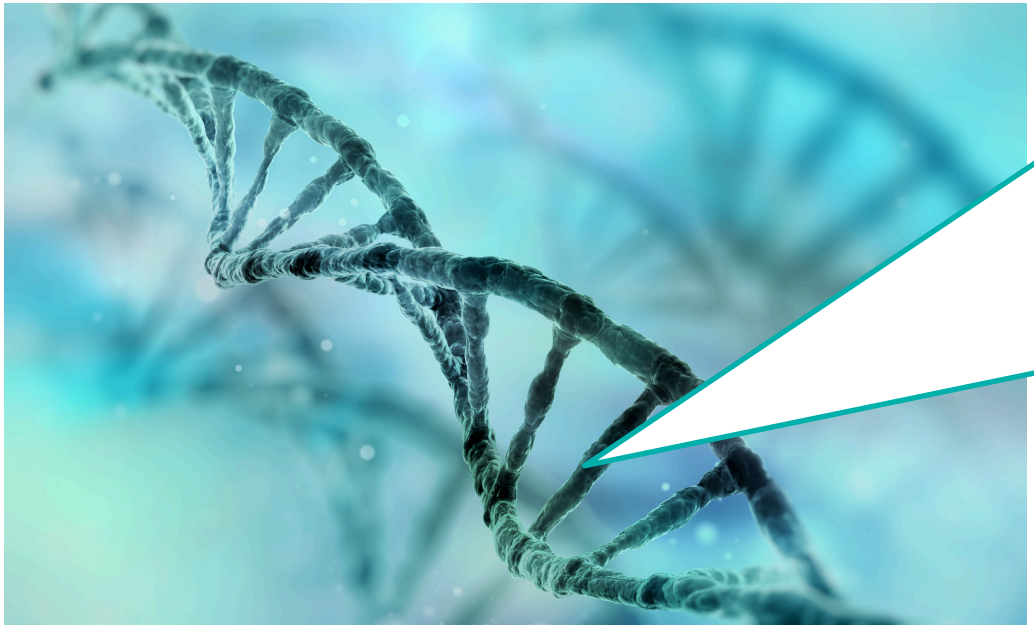
What causes mitochondrial disease?

Changes in an individual's DNA



ATGCCTGACGTAAGTCA
ATGCCTGA**G**GTAAGTCA

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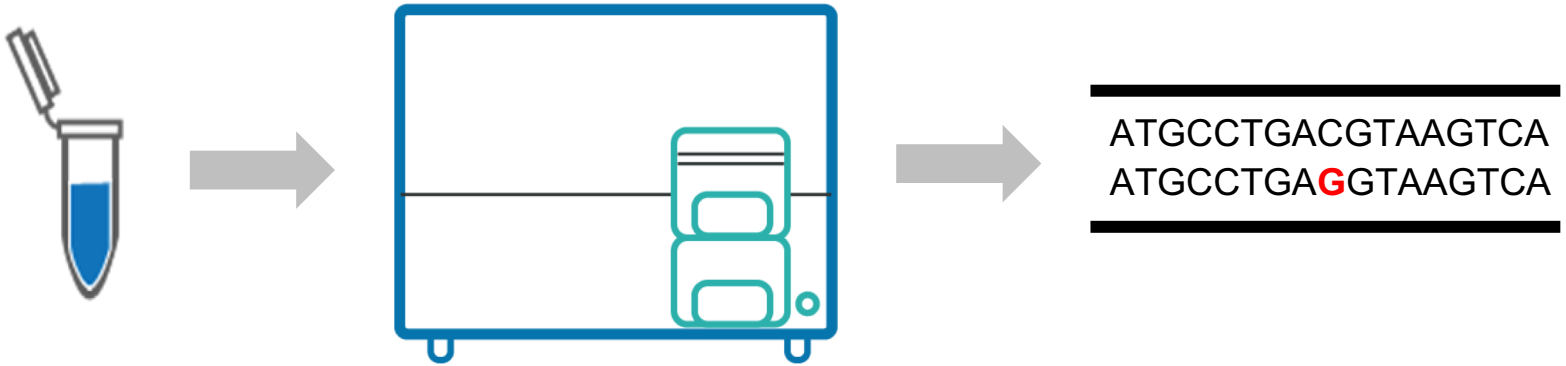
What is sequencing?

The process of reading the letters of an individual's DNA



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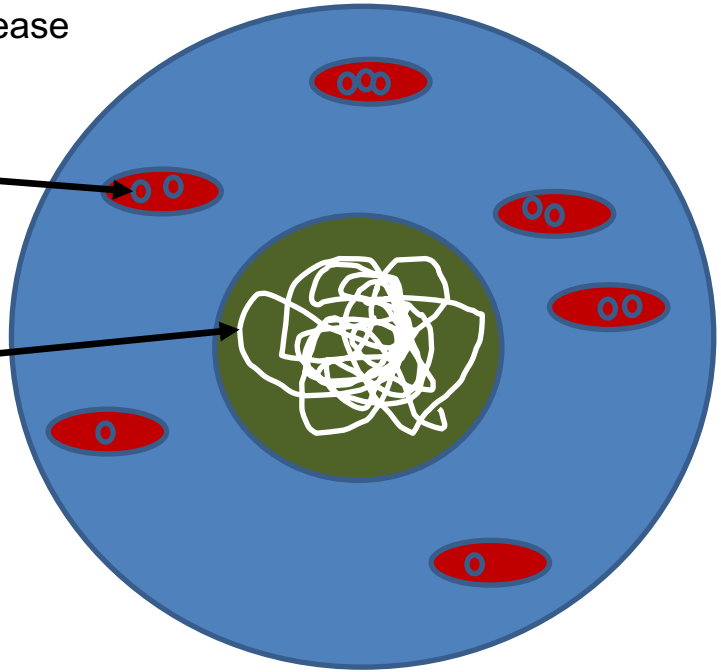
Where is DNA located?

DNA is located in both in the Mitochondria and Nucleus of a cell and both are important to diagnose mitochondrial disease

Mitochondrial DNA
37 genes

Nuclear DNA
~ 20,000 genes total
and > 1000 genes code for mitochondrial protein involved in the mitochondria



CELL

Genes code for proteins needed for the body to function

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BENEFITS OF GENETIC TESTING

- **PROVIDES AN ANSWER**
- **ENDS DIAGNOSTIC TESTING**
- **GUIDES TREATMENT**
- **INFORMS ON AGENTS TO AVOID**
- **EMPOWERS CONNECTIONS**
- **PROVIDES OPTIONS**

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ADDED BENEFITS OF GENOMIC TESTING

- **More comprehensive (detects more variant types)**
  - Sequence variants in exons
  - Structural variants
  - Repeat variants
  - Mitochondrial variants
  - Intronic variants between exons
- **One and done**
  - Reevaluation can be performed in silico, no need for repeat blood draws
  - One turn-around-time rather than sequential or reflexive testing with long cumulative TATs
- **Reusable**
  - Pre-symptomatic (cancer)
  - Carrier (reproductive risk)
  - PGx (drug treatments effectiveness)

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Genome sequencing for mitochondrial disease

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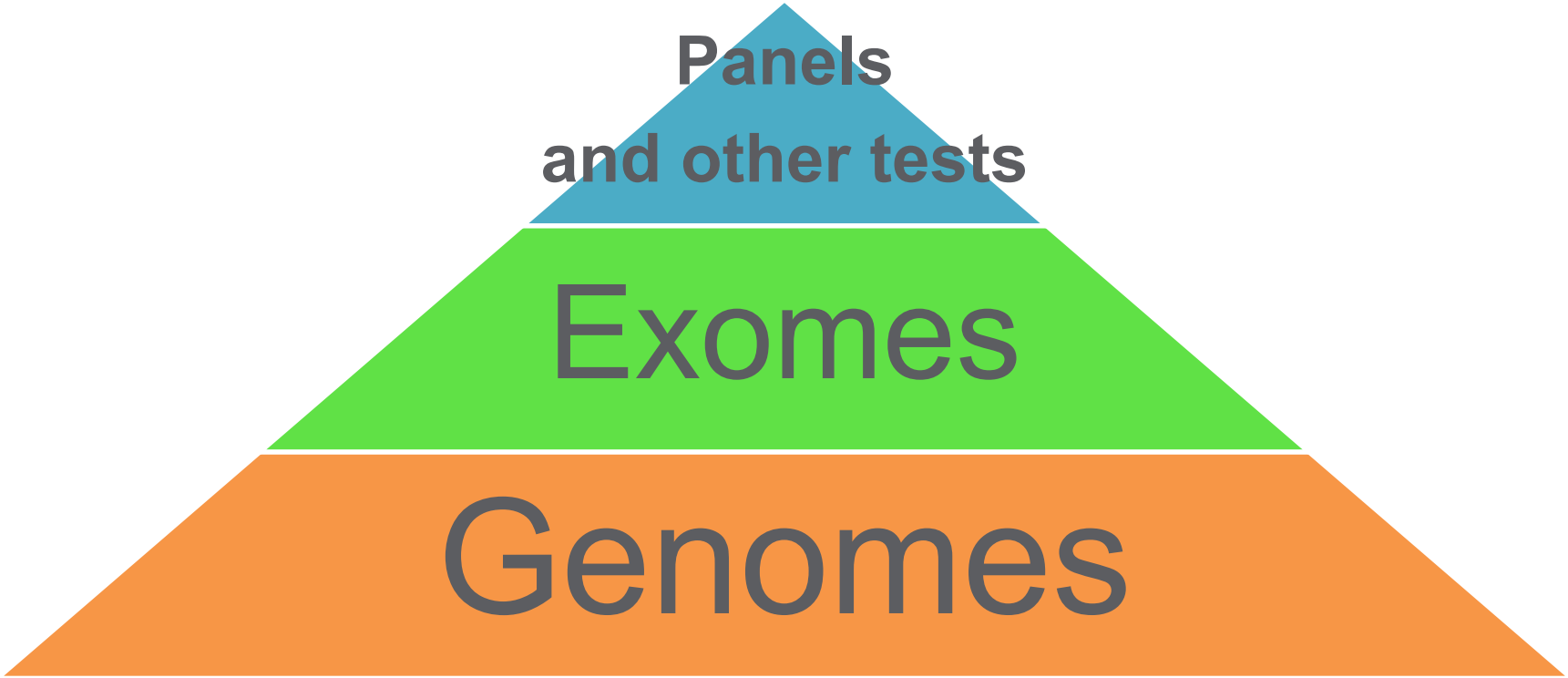

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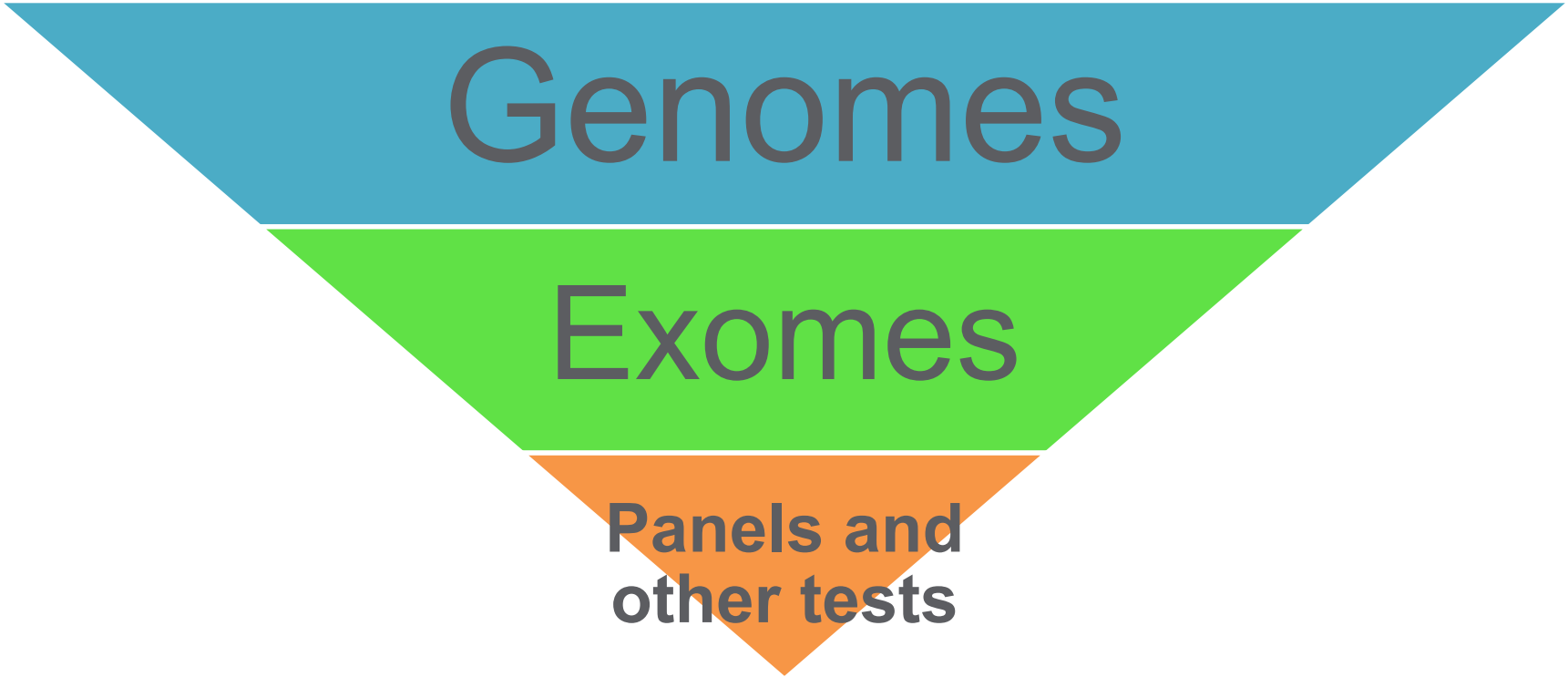

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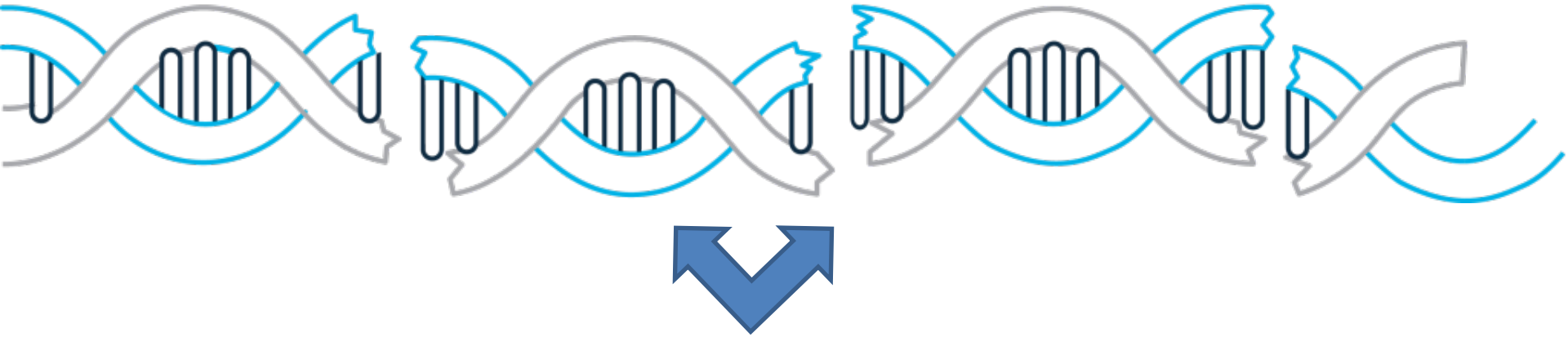
Panels, exomes and genomes



They all begin with fragmented genomic DNA

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PANELS/EXOMES REQUIRE EXON CAPTURE, GENOMES DO NOT

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Variants Identified by the Genome-based Exomes

- Single Nucleotide Variants (SNVs) for nuclear and mitochondrial genes
- Short Tandem Repeats (STRs)**
- Structural Variants (SVs)**
  - –Small insertions/deletions <50bp indels/delins
  - –Small deletions/duplications (50bp-200bp)* typical blind spot
  - –Mid-size Deletions/Duplications (200-1,000) exon level *typical blind spot
  - –Large Deletions/Duplication (1,000-100,000) gene level
  - –Very large Deletions/Duplications (100kb-3Mb)
  - –Gross Structural Variants (>3Mb)

**Using specialized software

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Clinical cases

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CASE 1

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Case 1

- 51 year old woman
- Poor muscle fitness, exercise-induced muscle weakness and myalgia
- Mild dysphagia and choking tendency
- In her 40s, developed ptosis in right eye which was operated on at age 47
- At 49 years, psoriatic skin rash developed
- Clinical neurological exam is normal
- Blood lactate levels are normal
- Muscle histology shows ragged red fibers and COX-negative fibers

Soini et al, 2017. BMC Med Genet 18: 14.

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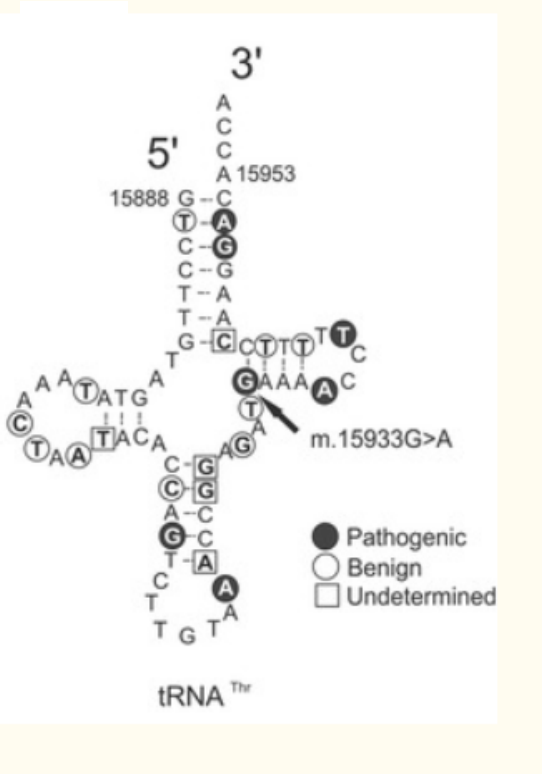
Case 1

Causative variant: m.15933G > A

This is a homoplasmic variant in the MT-TT gene which codes for Threonine transfer RNA (tRNA Thr) that is required to correctly build proteins



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CASE 2

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Case 2

- 25 year old woman
- Dysexecutive syndrome
- Muscle fatigue
- Continuous headache
- Experienced infection-triggered Addison crisis. As progressed, experienced two epileptic seizures and stroke-like episodes with hemiparesis on the right side
- Cerebral MRI showed a substance defect of the parieto-occipital left side exceeding the vascular territories with a lactate peak
- Positive lactic ischemia test
- Muscle biopsy showed single cytochrome c oxidase-negative muscle
- Comorbid autoimmune polyglandular syndrome type 2 with Hashimoto's thyroiditis, Addison's disease, and autoimmune gastritis - consistent with increased antibodies

Endres et al, 2019. Front Immunol 10: 412.

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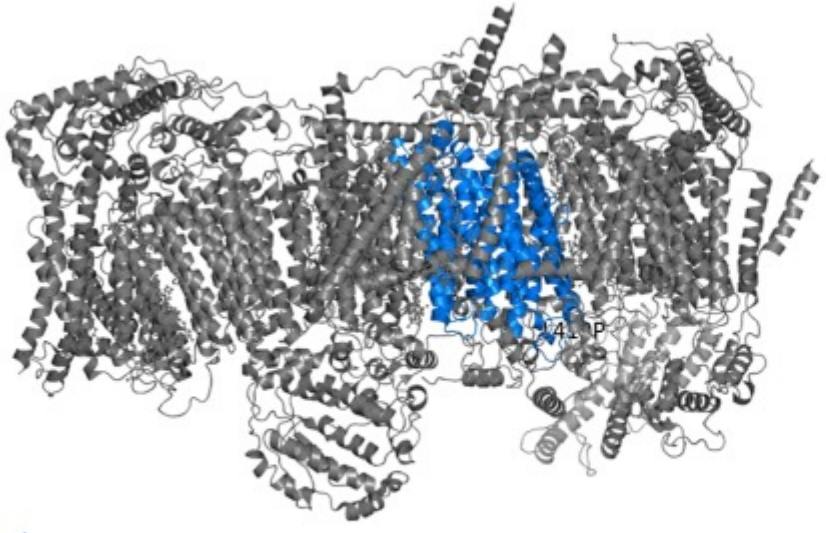
Case 2

Causative variant: m.12015T>C

This is a heteroplasmic variant in the MT-ND4 gene which codes for a component of the respiratory chain Complex I that generates energy for cells



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Take Home Message

- These are both “classic” cases of mitochondrial disease caused by a variant in the mitochondrial genome

CASE 3

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Case 3

- 5 year old girl
- Bilateral club foot, mild facial dysmorphism including macrodontia of the upper central incisors and retrognathia
- Developmental delay
- Truncal hypotonia with brisk extremity reflexes
- Dysarthric and slow speech
- Mitochondrial disease was considered the clinical constellation of failure to thrive, hypotonia, dysarthria, and tremor
- Abnormal biochemical results: elevated plasma lactate, persistent metabolic acidosis, intermittent plasma alanine elevation, elevated urine organic acids

Vernon et al, 2015. Am J Med Genet A 167A(5):1147-51.

Case 3

Causative variant: c.1255C>T

This is a heterozygous variant in the nuclear FARS2 gene which codes for an enzyme that regulates mitochondrial Phenylalanine transfer RNA (tRNA Phe). It was inherited from the patient's father.

Causative variant: 116kb deletion

This is a heterozygous deletion in the same FARS2 gene that removes exon 6 as well as parts of intron 5 and intron 6. It was inherited from the patient's mother.

The two FARS2 variants are compound heterozygous and together impact the function of the enzyme.

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Take Home Message

- The causal variants would not have been identified if only the mitochondrial genome was considered
- Two different tests were needed to identify the two different types of variants if performing traditional testing
- If whole genome sequencing had been used, the variants would have been identified by a single test

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CASE 4

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Case 4

- 24 year old woman
- Presented with clinical symptoms of MELAS from age 15 onwards, including
  - Stroke like episodes
  - Seizures
  - High lactate levels
- Genetic testing identified m.1630A>G variant which could be consistent with MELAS, however her mother also carries the variant at higher heteroplasmy (93% vs 75% in blood) and is asymptomatic
- Mild ataxia and unsteady gait
- Occasional headaches and tinnitus
- Normal truncal tone with mild bilateral weakness in the upper and lower extremities in proximal and distal muscles
- Positive tremor that worsens with movement, otherwise normal fine motor skills

Uittenbogaard et al, 2019. Mol Genet Metab 126(4):429-438.

Case 4 continued

- Positive ankle clonus and mild contracture to the lower right extremity
- Loss of peripheral vision, decreased upgaze, nystagmus on extreme right gaze
- Speech articulation problems
- Underwent kidney transplant for chronic renal failure

Uittenbogaard et al, 2019. Mol Genet Metab 126(4):429-438.

Case 4

Causative variant: c.1000C>T

This is a heterozygous variant in the nuclear VARS2 gene which codes for an enzyme that regulates mitochondrial Valine transfer RNA (tRNA Val)

Causative variant: m.1630A > G

This is a variant in the mitochondrial MT-TV gene which codes for Valine transfer RNA (tRNA Val)

Reduced function of VARS2 due to the nuclear variant exacerbates the effect of the mitochondrial MT-TV variant, which causes the patient's symptoms.

Take Home Message

- Analyzing the mitochondrial gene alone was not sufficient to explain the patient's symptoms, given that her mother carried the same mitochondrial variant

- The connection between the nuclear and mitochondrial variants could not have been identified by exome analysis only, additional analysis of the mitochondrial genome was required

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Summary and Conclusions

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Mitochondrial Disease Summary

- Mitochondrial disease is very challenging to diagnose
- Mitochondrial disease is caused by changes in either the mitochondrial DNA or the nuclear DNA or BOTH
- Genetic testing is the recommended first step in diagnosis and comprehensive genomic testing the shortest path to a diagnosis
- Genomic testing can also identify a cause that is not mitochondrial in nature but has overlapping clinical symptoms
- Genomic testing can identify more than one disorder in an individual

Ordering testing

- Legally your physician is the only one who can order genetic testing
- Your physician will review a consent form with you and you will both sign the test requisition form
- Your insurance company may or may not cover genetic testing and therefore you will need preauthorization
- If your result is negative or inconclusive, you may request a repeat analysis of the data, usually in a year.
- You can receive copies of your results from the ordering clinician, but you also have the right to receive your test results directly from the diagnostic lab upon request
- If you order testing from another lab ask them if they can detect all variant types including deletions/duplications and short tandem repeats and if they sequence both the mitochondrial and nuclear genomes

Thanks for listening!

Interested in learning more about whole genome sequencing and its role in diagnosis of mitochondrial disease?

Read the information on our website at www.variantyx.com/mitochondrial-analysis/ .

Or contact us at info@variantyx.com

Have you been diagnosed with a large mitochondrial deletion? Variantyx is recruiting patients like you for whole genome sequencing. Contact us.

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