Next Generation Sequencing for Genetic Diagnosis of Mitochondrial Diseases

Mitochondrial Diseases

Mitochondrial diseases are a group of heterogeneous diseases caused by dysfunction due to genetic mutations in the mitochondrial genome or mutations in the nuclear genome which has an effect on the structure, function and integrity of the mitochondria.

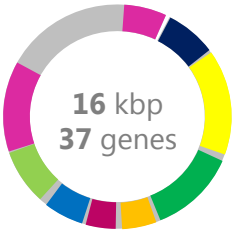
Mitochondrial diseases occur at an estimated frequency of 1:2000 to 1:5000 individuals, making it one of the most commonest genetic diseases.

This high rate is largely contributed by the fact that mitochondria generates a lot of oxidative free radicals during the oxidative phosphorylation step and also lacks a robust proof reading mechanism for DNA replication.

Mitochondria are versatile organelles in the cell. The primary role of mitochondria in the cell is the production of energy. Therefore the mitochondria are popularly known as the powerhouses of the cell.

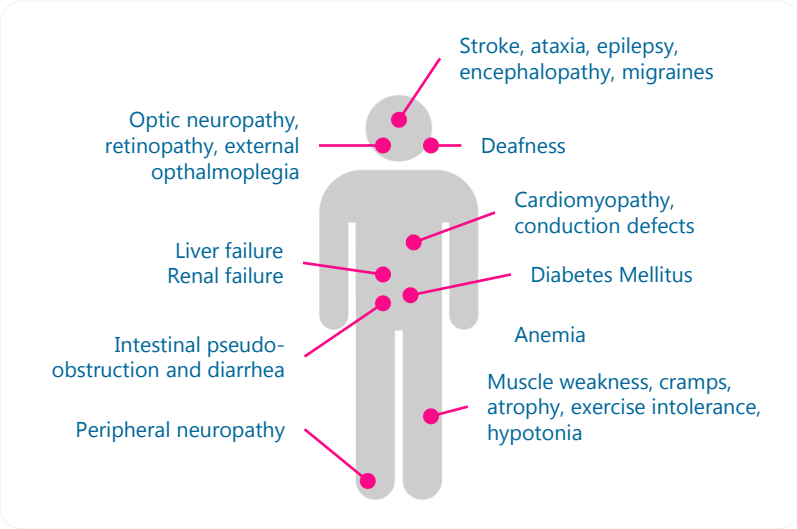
Mitochondrial Genome

The mitochondria has a small genome, approximately over 16 kilobases in length and encodes for a handful of genes. The mitochondrial genome is known to encode for 37 genes.



While the mitochondrial genome only encodes for a small number of genes, the normal functioning of the mitochondria requires a number of gene products to be imported from the cytoplasm.

Manifestation of mitochondrial disease can be heterogeneous



Mitochondrial genetic mutations

Mitochondria has a high mutation rate, approximately 100 times that of the nuclear genome.

Mutations which cause mitochondrial diseases are very well studied and catalogued. This encompass approx. 73 mtDNA functional loci and around 580 disease associations

Mitochondria exhibit a unique property of hetroplasmy, which is defined as the existence of more than one type of organellar genetic material in a single mitochondria or in an individual

Approximately about 90% of the individuals carry at least one heteroplasmic site and ~20% of the individuals harbour heteroplasmies reported to be implicated in disease.

Clinical scoring systems can help diagnose mitochondrial diseases

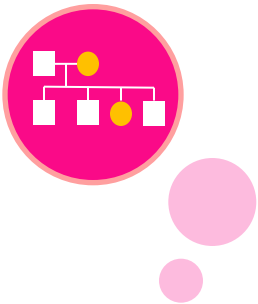
Nijmegen Clinical Scoring for Mitochondrial Diseases

Neuromuscular manifestations	**(Max. of 2 points)**
Progressive external ophthalmoplegia	(2 points)
Ptosis	(1 point)
Exercise intolerance	(1 point)
Muscle weakness	(1 point)
Rhabdomyolysis	(1 point)
Abnormal electromyogram	(1 point)
Central nervous system and other organ involvement	**(Max. of 2 points)**
Isolated central nervous system involvement	(1 point)
Any other isolated organ system	(1 point)
Two or more organ systems	(2 points)
Metabolic and imaging studies	**(Max. of 4 points)**
Elevated blood lactate on 3 occasions	(2 points)
Elevated cerebrospinal fluid lactate	(2 points)
Elevated blood alanine	(2 points)
Elevated cerebrospinal fluid alanine	(2 points)
Elevated urine tricarboxylic acid (Kreb) cycle intermediates	(2 points)
Elevated urine ethylmalonic, 3-methylglutcaonic, or dicarboxylic acids	(1 point)
Abnormal 31P-MRS (magnetic resonance spectroscopy) in muscle with reduced Phosphocreatine/Pi ratio	(2 points)
Abnormal T2 signal in basal ganglia on brain MRI	(2 points)
Decreased resting metabolic rate or abnormal exercise studies (cycle ergometry protocol)	(2 points)
Tissue morphology	**(Max. of 4 points)**
Ragged red fibers on muscle biopsy	(2 points if present, 4 points if >2%)
Diffuse reduction in cytochrome c oxidase histochemical reaction or scattered COX deficient fibers	(4 points)
Strongly succinate dehydrogenase positive vessels by histochemistry	(1 point)

Definite:	**8-12 points**
Probable:	**5-7 points**
Possible:	**2-4 points**
Unlikely:	**1 point**

(Adapted from Wolf NI, Smeitink JA. **Mitochondrial disorders: a proposal for consensus diagnostic criteria in infants and children**. *Neurology*. 2002 Nov 12;59(9):1402-5.)

Diagnostic workup of patient with suspected mitochondrial disease



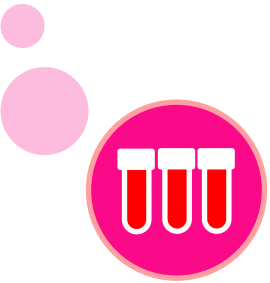
Family history and inheritance

Mitochondrial diseases are typically maternally inherited (with exceptions where the nuclear genes are involved). A detailed family history would provide insights into the inheritance. Specifically ask for signs like deafness, opthalmoplegia, short stature, neurological/ muscular illnesses, sudden cardiac death.

Biochemical workup

Elevated Lactate and Pyruvate levels point to a defect in respiratory chain. The abnormal ratio will point to the type of defect.

Serum Creatine Kinase might not be elevated in all cases, but high elevation would suggest to a mitochondrial depletion syndrome.

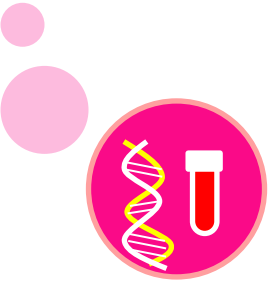


Muscle Biopsy

A variety of techniques including histology, immunohistochemistry and electron microscopy could suggest and even confirm mitochondrial dysfunction/defect.

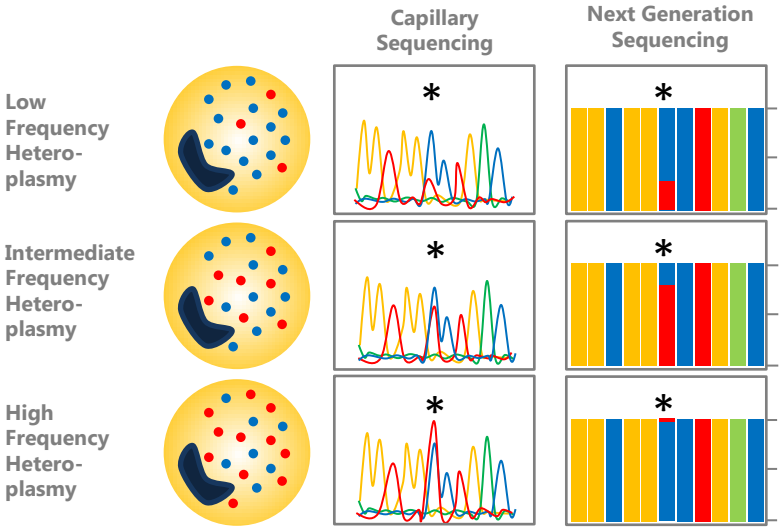
Molecular Genetics

Molecular genetics can confirm the genetic defect and therefore the diagnosis of disease. Understanding the molecular defect is also required for further counselling, carrier detection and pre-natal testing.



Advantages of Next Generation Sequencing for mitochondrial molecular genetics

Traditional capillary sequencing methods for sequencing the mitochondrial genome is time-consuming, tedious and expensive. In a regular setting, this would involve amplification of 24 genomic loci independently followed by sequencing. Additionally, since capillary sequencing measures fluorescence of the pool of amplicons at each position, cannot accurately identify low frequency heteroplasmy.



Next Generation Sequencing (NGS) approaches can obviate these problesm by offering quantitative proportions of nucleotides in the pool, thus making estimation of heteroplasmy accurate. At higher coverages, accurate estimation of even 10% heteroplasmy was possible using Next Generation Sequencing.

In addition, NGS has the advantage of being fast, cost-effective and accurate. It also provides a unique opportunity to offer screening of individuals who have low-copy heteroplasmy.

Vellarikkal SK, *et al.*, **mit-o-matic: a comprehensive computational pipeline for clinical evaluation of mitochondrial variations from next-generation sequencing datasets**. *Human Mutation*. 2015 Apr;36(4):419-24.

How to collect and refer samples for mitochondrial genome sequencing

Informed Consent and Documentation

Use the clinical scoring sheet to evaluate whether the patient has mitochondrial disease. Inform the patient about the test, its benefits and caveats. Get the informed consent and fill in the referral form.

Sample collection

Collect whole blood by venepuncture. It is always best to use Acid Citrate Dextrose (ACD) vacutainers (Yellow cap) to collect blood. Please mark the tubes appropriately.

Alternative body fluids/samples may also be collected, but first confirm with the laboratory.

Packaging

The sample tubes and documents need to be packed into cardboard boxes with appropriate minimal padding.

Transportation

Ship the tubes at room temperature to the designated address of the designated laboratory. Please do not refrigerate.

GUaRDIAN | Genomics for Understanding Rare Diseases India Alliance Network

This information booklet has been designed to increase the awareness on mitochondrial diseases as part of the Genomics for Understanding Rare Diseases - India Alliance Network (GUaRDIAN). To know more about the GUaRDIAN programme, visit our website: http://guardian.meragenome.com

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