Lily Family Weekend, 23rd June

Testing for mitochondrial disorders and getting a genetic diagnosis

Dr. Charu Deshpande, London

Carl Fratter, Oxford

Dr. Ines Barbosa, London

NHS Guy's and St Thomas' NHS Foundation Trust

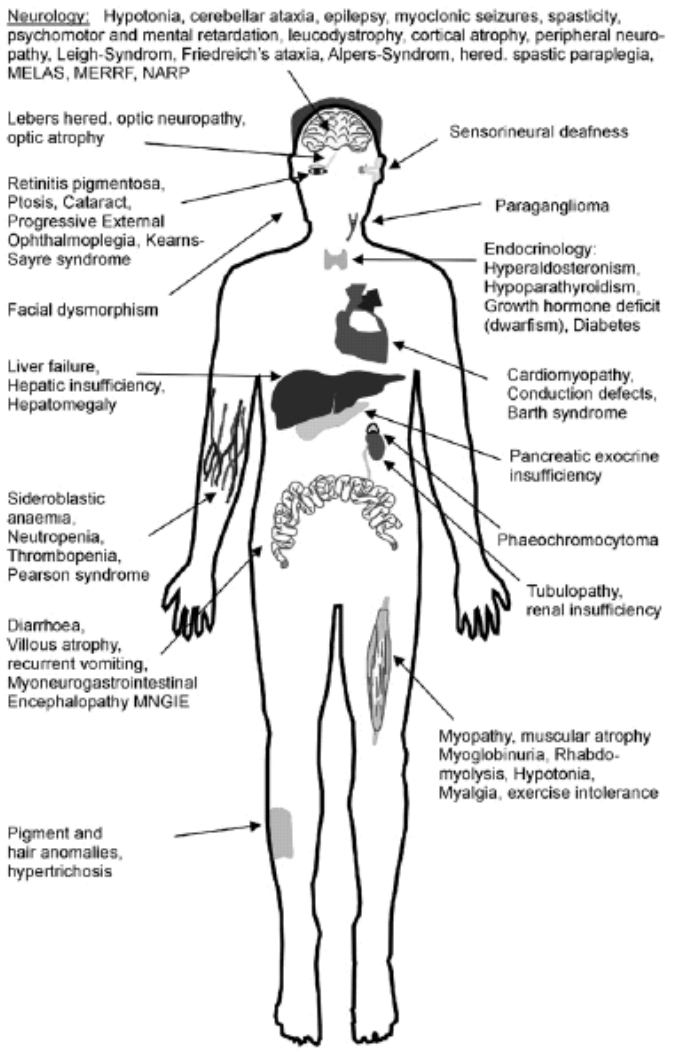
NHS Oxford University Hospitals NHS Foundation Trust

KING'S College LONDON

Two scenarios

Patient

?


Neurology: Hypotonia, cerebellar ataxia, epilepsy, myoclonic seizures, spasticity, psychomotor and mental retardation, leucodystrophy, cortical atrophy, peripheral neuropathy, Leigh-Syndrom, Friedreich's ataxia, Alpers-Syndrom, hered. spastic paraplegia, MELAS, MERRF, NARP
Lebers hered. optic neuropathy, optic atrophy
Sensorineural deafness
Retinitis pigmentosa, Ptosis, Cataract, Progressive External Ophthalmoplegia, Kearns-Sayre syndrome
Paraganglioma
Endocrinology: Hyperaldosteronism, Hypoparathyroidism, Growth hormone deficit (dwarfism), Diabetes
Facial dysmorphism
Liver failure, Hepatic insufficiency, Hepatomegaly
Cardiomyopathy, Conduction defects, Barth syndrome
Pancreatic exocrine insufficiency
Sideroblastic anaemia, Neutropenia, Thrombopenia, Pearson syndrome
Phaeochromocytoma
Tubulopathy, renal insufficiency
Diarrhoea, Villous atrophy, recurrent vomiting, Myoneurogastrointestinal Encephalopathy MNGIE
Myopathy, muscular atrophy Myoglobinuria, Rhabdomyolysis, Hypotonia, Myalgia, exercise intolerance
Pigment and hair anomalies, hypertrichosis

Making a diagnosis of MD

Clinical

Clues from history and examination

Characteristic syndrome/ apparently unrelated symptoms

Family history

Multiple organ involvement

Progressive

Laboratory investigations

Making a diagnosis of MD

Clinical assessment of symptoms

Radiology – brain imaging / MRI

Samples – blood, lumbar puncture, muscle biopsy, liver biopsy, skin biopsy

Biochemistry

- routine blood tests, e.g. lactate levels
- specialist blood tests, e.g. FGF21
- cerebrospinal fluid (CSF) tests form lumbar puncture, e.g. lactate levels
- muscle respiratory chain enzyme analysis

Histology – muscle / liver

Genetics – blood / muscle / liver / urine / saliva

Multidisciplinary approach is essential for diagnosis

Multidisciplinary approach is essential for diagnosis

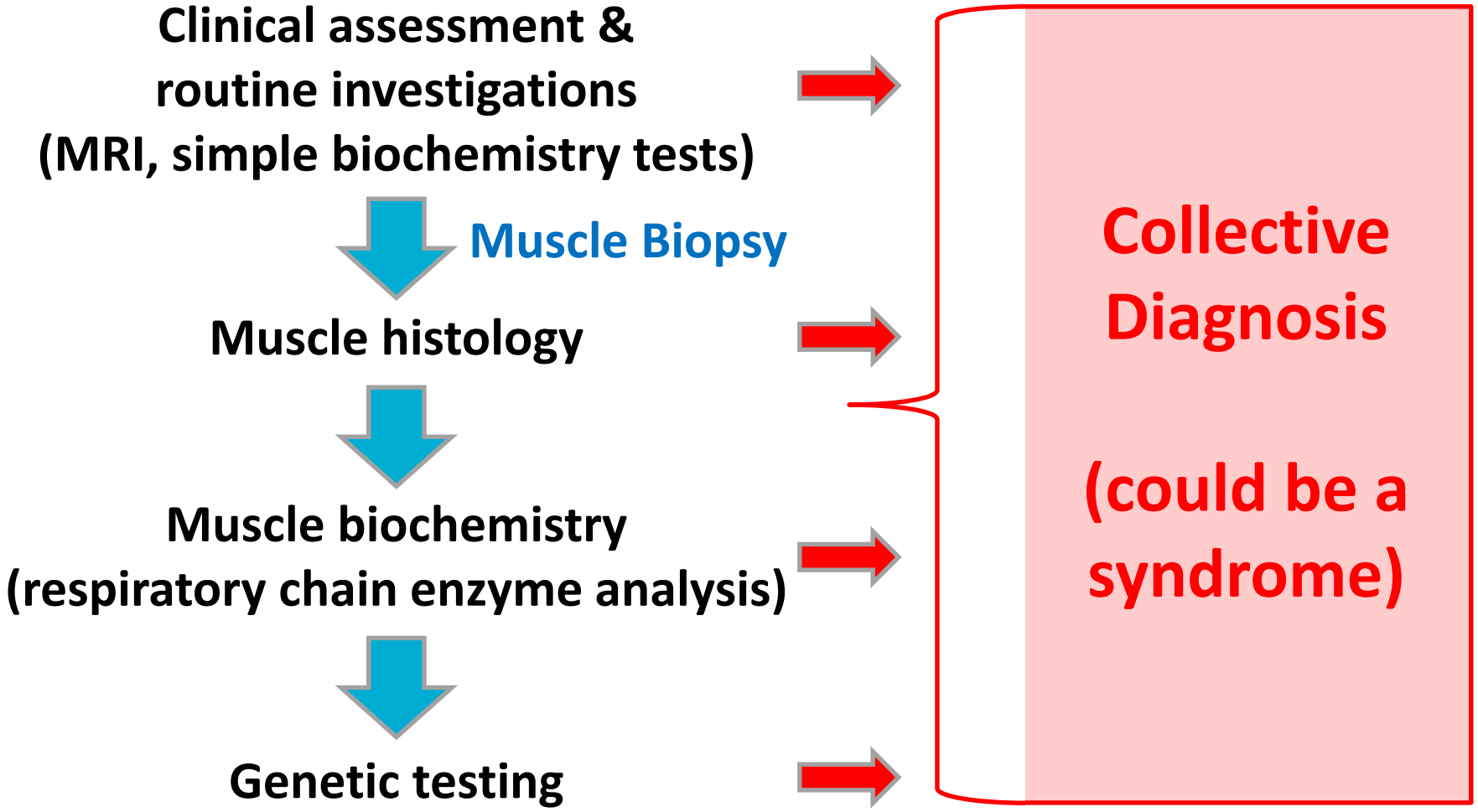
NHS Highly Specialised Services for Rare Mitochondrial Disorders

- Centres in Oxford, London and Newcastle
- These centres bring together clinical, biochemical and genetic expertise.

Website: mitochondrialdisease.nhs.uk

Types of Diagnosis

Oxford University Hospitals NHS
NHS Foundation Trust



Types of Diagnosis

Oxford University Hospitals NHS
NHS Foundation Trust

Clinical assessment & routine investigations (MRI, simple biochemistry tests) → **Clinical Diagnosis**

↓ **Blood sample only**

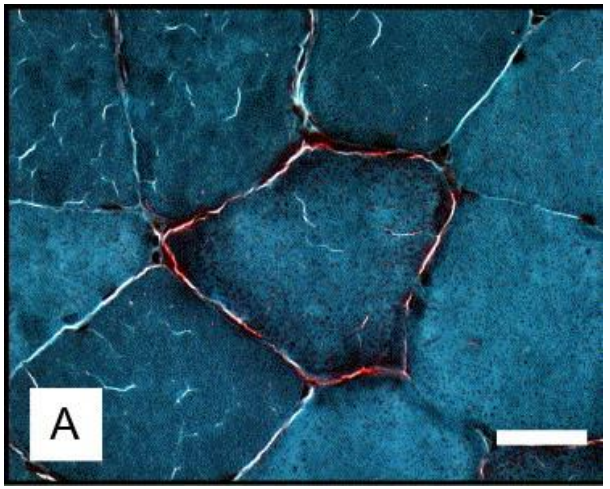
Genetic testing → **Genetic Diagnosis**

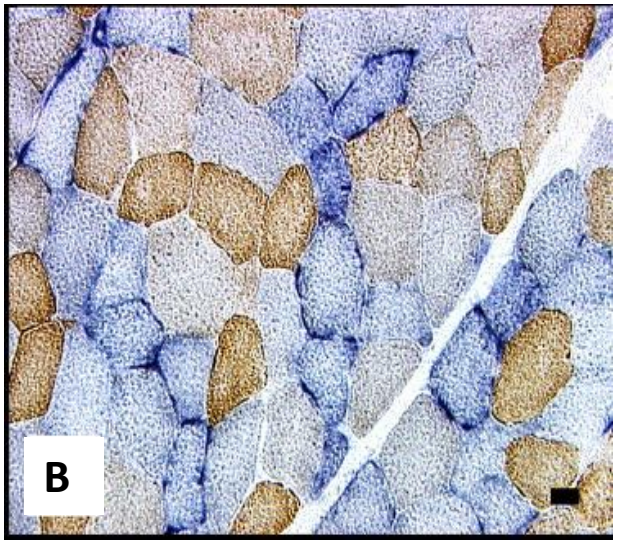
↓

No diagnosis – Muscle biopsy may be required

Histological diagnosis

A. Ragged-red muscle fibre, using modified Gomori trichrome stain.

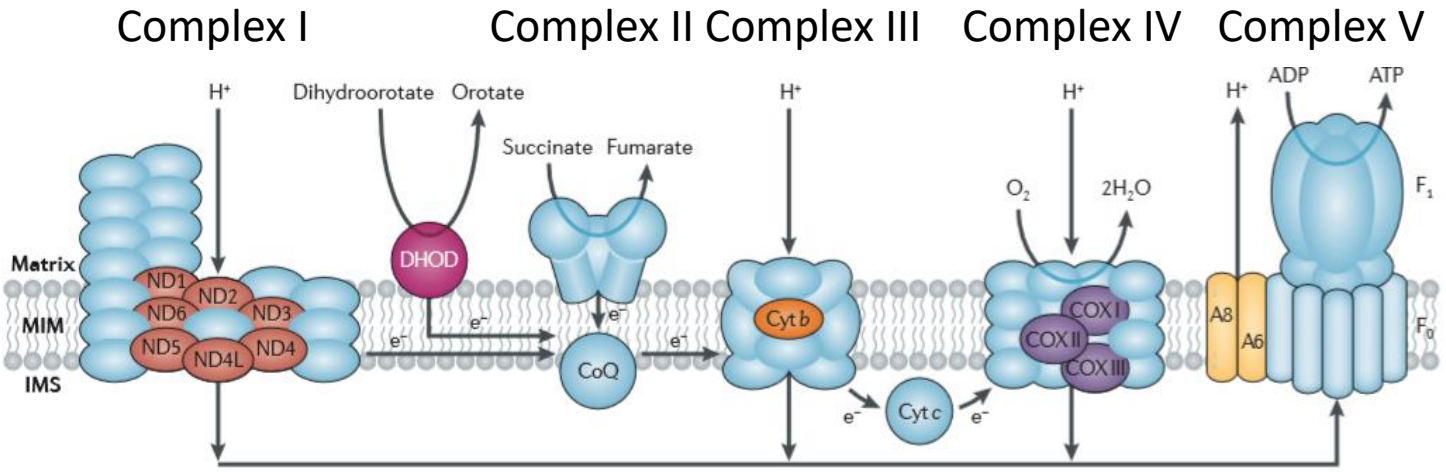




B. COX negative and ragged-blue fibres, using sequential COX (brown) and SDH (blue) histochemistry.

Biochemical diagnosis

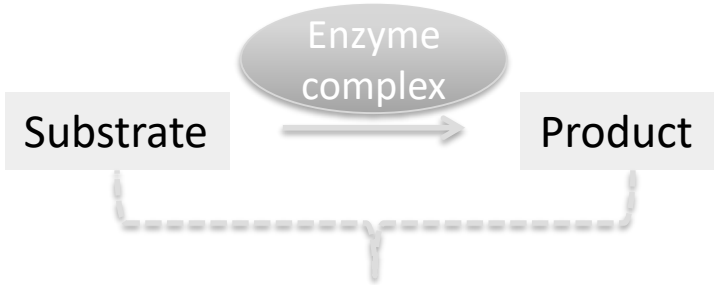
Respiratory Chain Enzyme Pathway



From Schon et al. 2012, Nature Reviews Genetics, 13, 878-890

Respiratory chain enzyme analysis (spectrophotometric assays):

- **Complex I deficiency**
- **Complex II deficiency**
- **Complex III deficiency**
- **Complex IV deficiency**
- **(Complex V deficiency)**
- **Combined deficiencies**

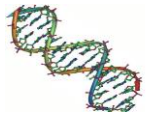


Measure loss of substrate or gain of product

Genetic diagnosis
Genetic testing workflow

Sample: Blood/Muscle biopsy/Liver biopsy/Urine

↓

DNA 

↓

Amplification of DNA

↓

Analysis of the amplified DNA to look for potential DNA changes/mutations

↓

Reporting of results

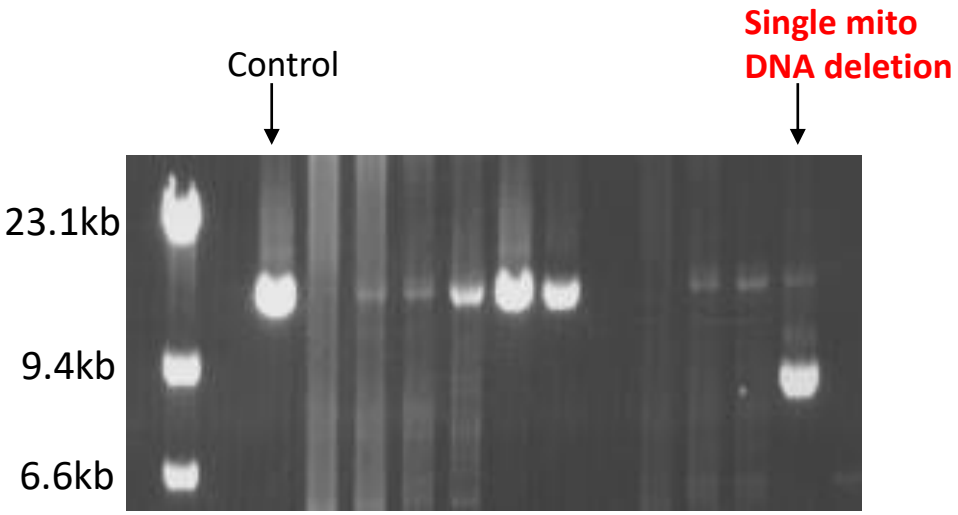
30 years of genetic testing for mitochondrial disease

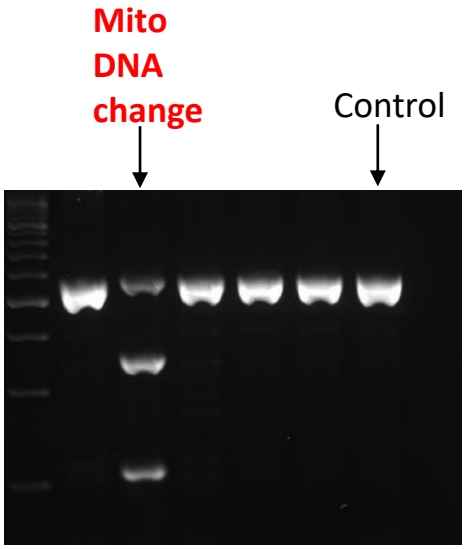
Oxford University Hospitals NHS
NHS Foundation Trust

- 1988 First report of mitochondrial DNA changes causing disease & Prof Jo Poulton established a diagnostic service at Oxford University

- 2018 High throughput and relatively automated DNA sequencing of 100s of genes

Genetic diagnosis
Mitochondrial DNA





Genetic diagnosis

Testing is typically directed by the clinical symptoms and results of other tests.

Small amount of data

Simple analysis

Testing for common DNA variants/changes
Mitochondrial DNA and/or nuclear DNA
Some variants aren't detectable in blood

Sequencing of whole mitochondrial DNA (muscle may be necessary)
Sequencing of specific nuclear genes or panels of nuclear genes

Whole Exome Sequencing (WES)
Whole Genome Sequencing (WGS)

Lots of data

Complex analysis

Lily Exome Project - Aims

Diagnosis (and discovery) of pathogenic nuclear DNA variants in mitochondrial disease patients using Exome Sequencing (WES) data

KING'S College LONDON

Why sequence nuclear DNA?

A genome contains all of the information needed to build and maintain an organism.

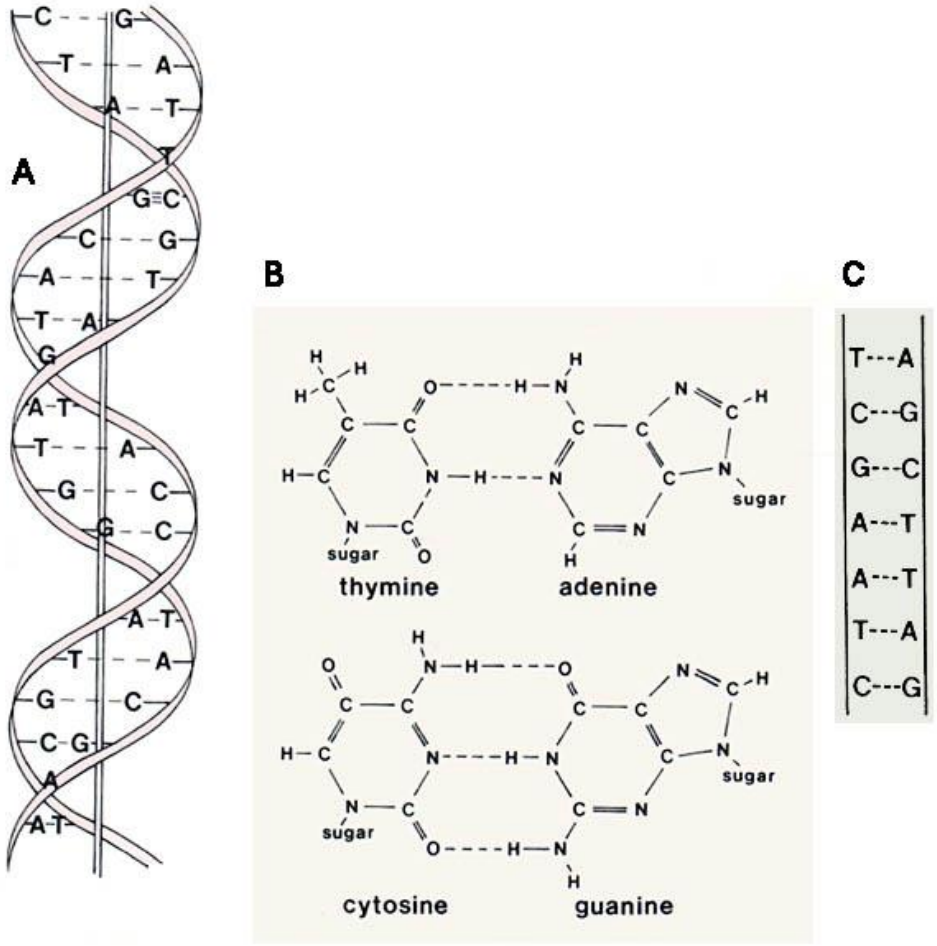
≈ 3 billion DNA base pairs in each of the

≈ 10^{14} cells of the human body

"[A] knowledge of sequences could contribute much to our understanding of living matter" (Frederick Sanger)

What is DNA sequencing?

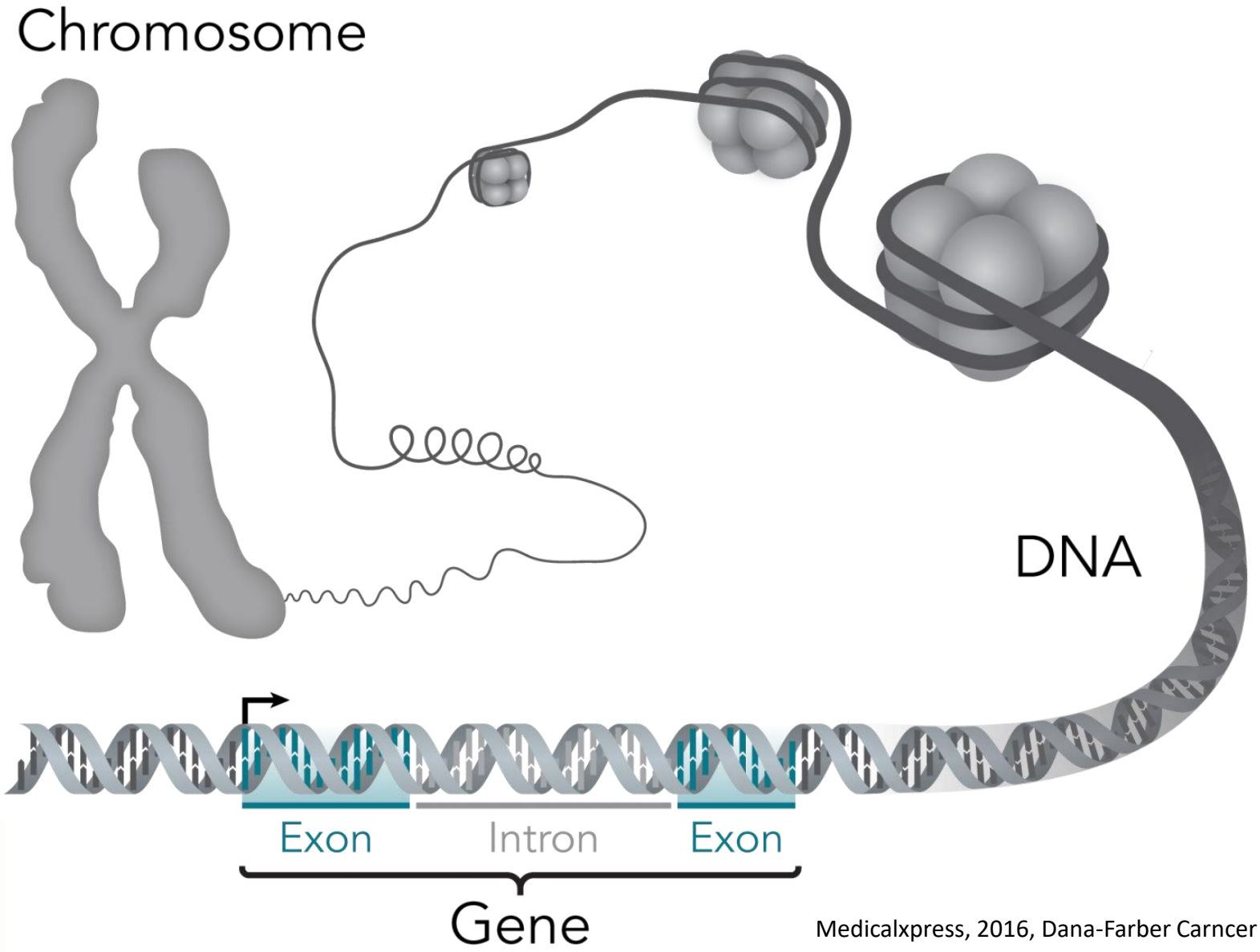
Determination of the order of nucleotide bases in a DNA molecule



Interesting fact

If you stretched the DNA in one cell it would be about 2m long

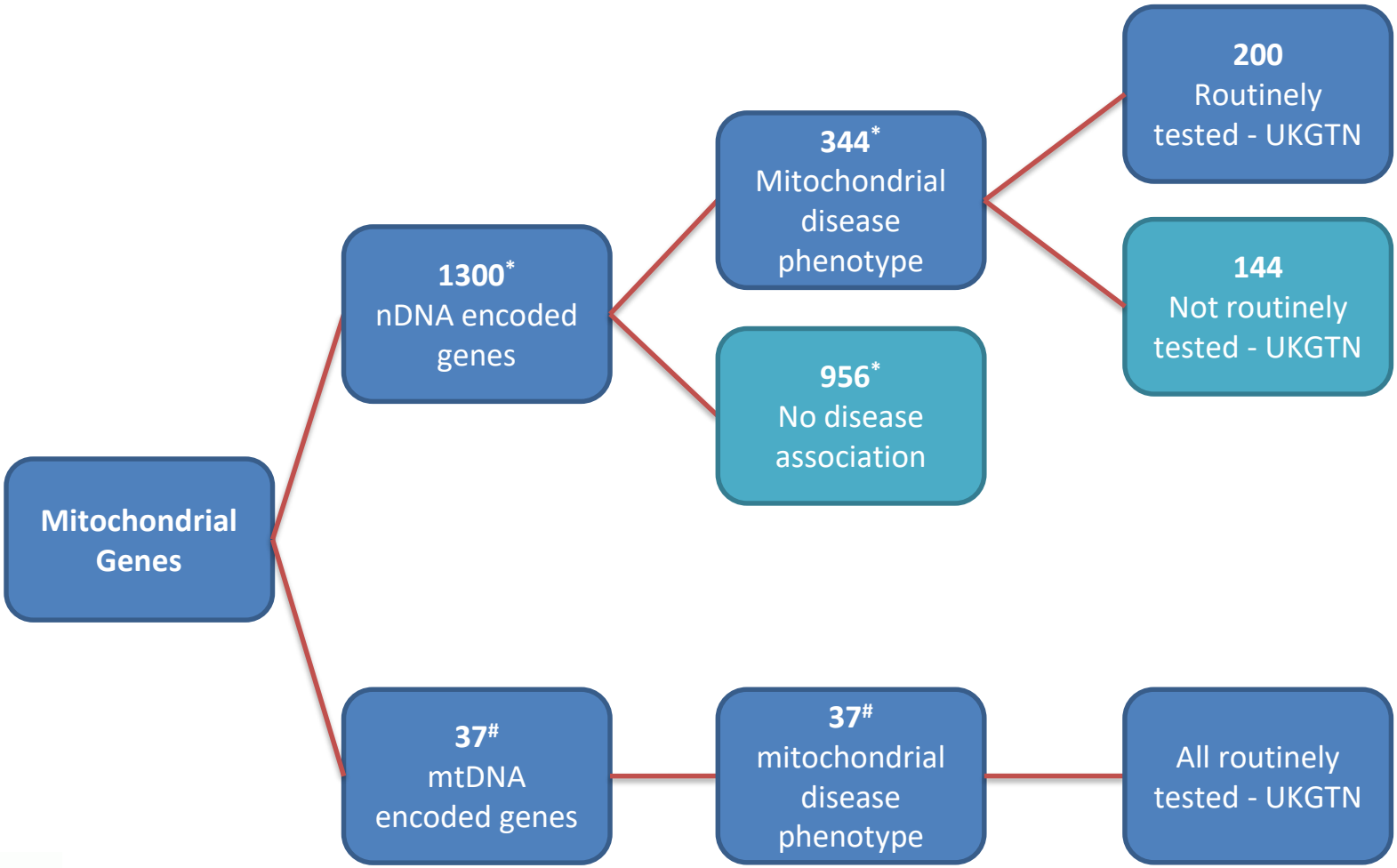
Important concepts



KING'S College LONDON

Medicalxpress, 2016, Dana-Farber Carncer Institute

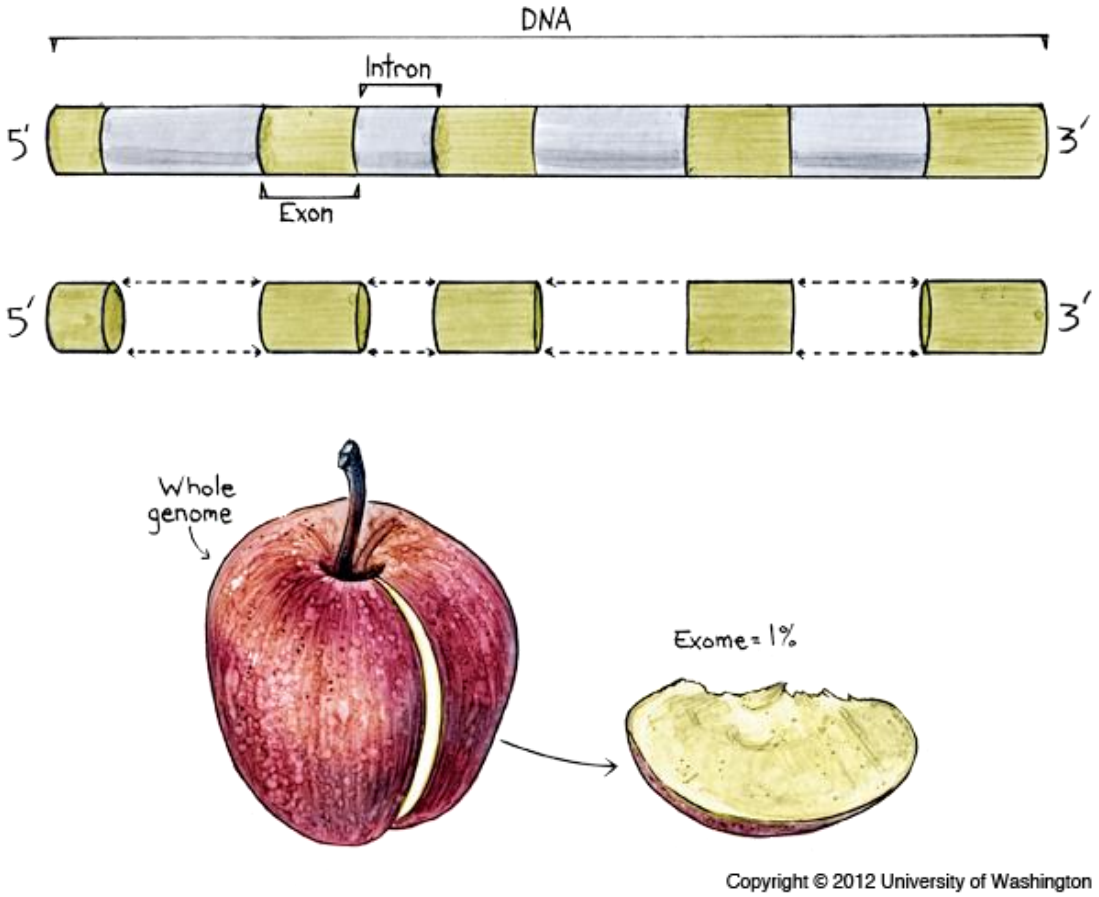
MD Genetic testing



*Information from OMIM and PanelApp
#Information from MITOMAP

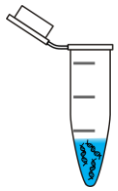
KING'S College LONDON

Whole exome Sequencing (WES)



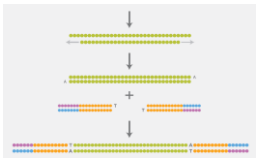
Even though only a very small percentage of the human genome encodes for protein, about 1%, exons **harbor about 85% of the mutations** with large effect on disease development.

Exome Sequencing workflow

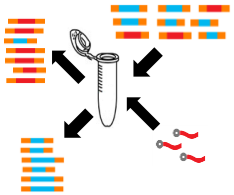


DNA quantification and preparation

DNA fragmentation



Library preparation



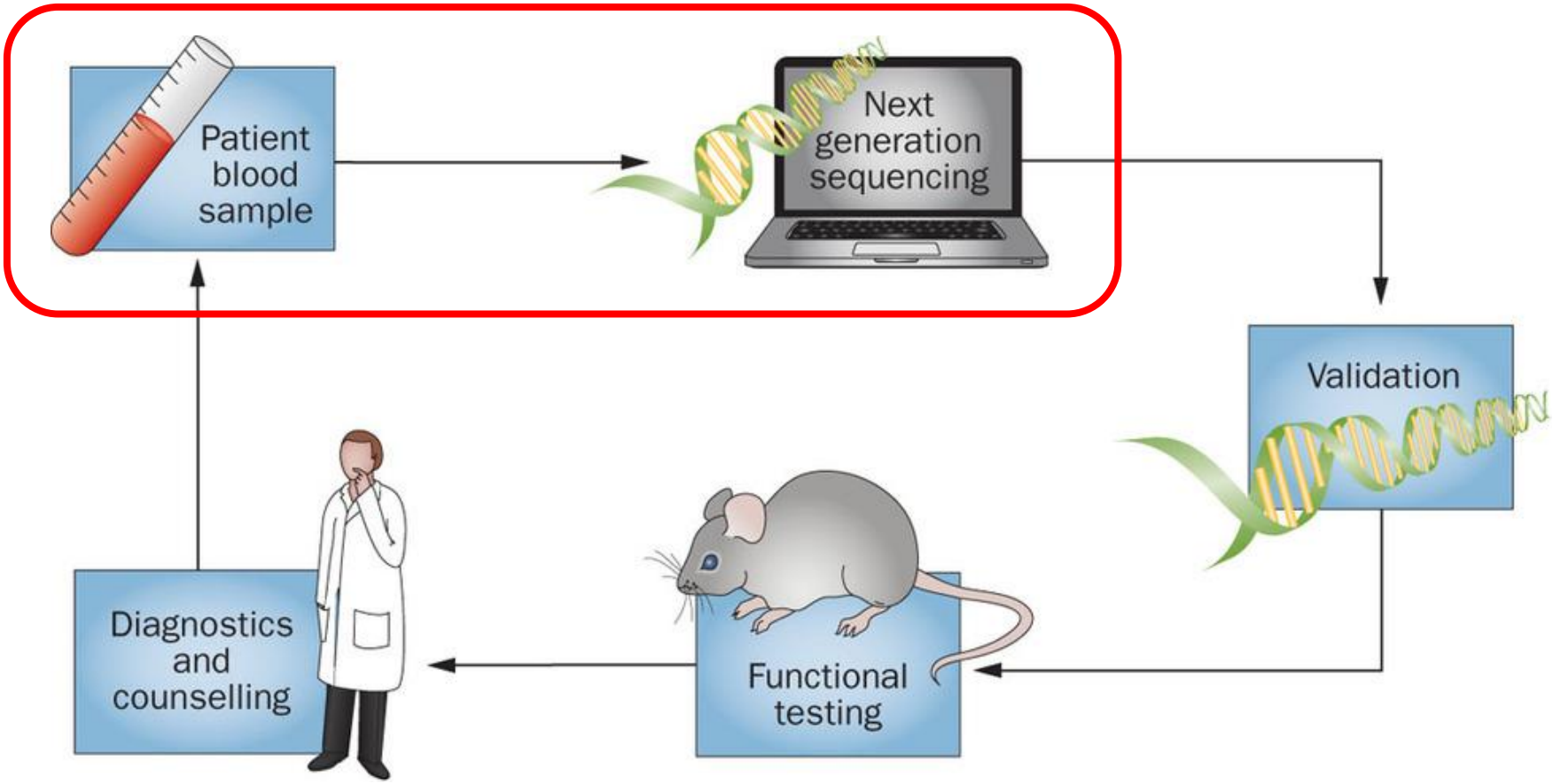
Exome capture

Sequencing

Bioinformatics

KING'S College LONDON

Achieving a Diagnosis



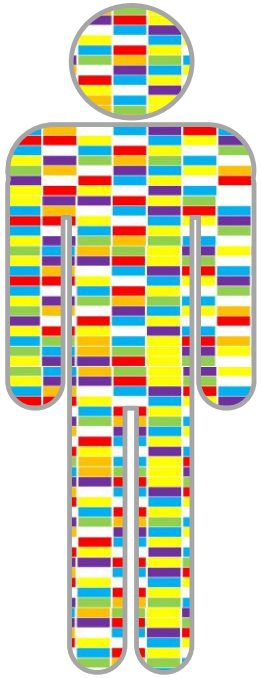
Clinical evaluation
Routine biochemical tests
DNA extraction

Renkema KY; Nat Rev Nephrol. 2014

Exome Sequencing - Bioinformatics

Variant Profile

Affected Individual



~ 22,000 variants

Filtering and Comparison

Exclude Known/Common Variants

Predicted Functionality

Prior Biological Knowledge
Candidate genes and pathways

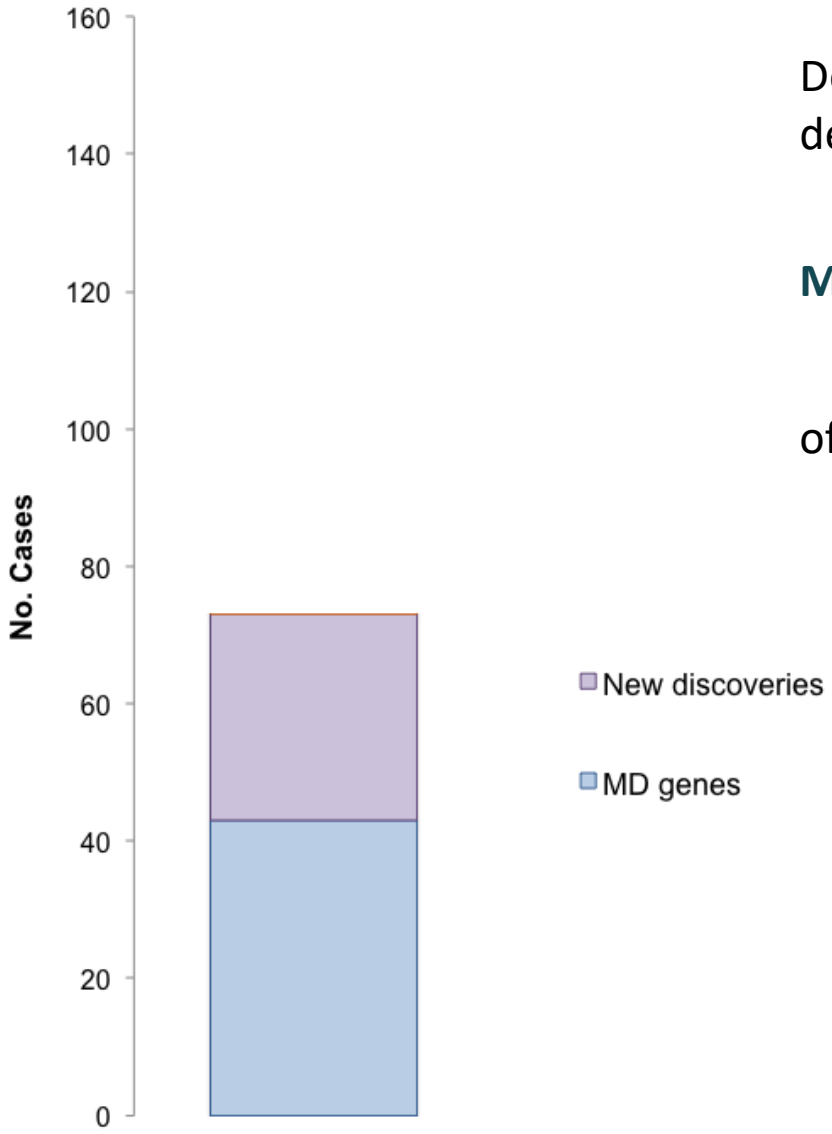
Linkage
Variant profiles of parents

Association of an Allelic Series
Variant profiles of unrelated cases

Causative Variant

CCTCGGGGGCGGGGAGTGG
TTTGCAAGGGAACCGCGCG
ATGTGTTATTCTCGGTGAC
CGTCGCGCCTCCCCGCGCC
GCTCCCGCTTCTTCCCACC
AATCAGAACTATTGACATT
CTGCGCCAGGGCCGCCCGC
GGCGCGAACCGGGTTAT
CCGGCAGCGCCGGATGG
CTCAGCTCAGCCCAGGCCC
CGGCGGCGCTGACATGGAG
GGCAGCAGAAAGCGGCTCC
TGCTTGTTGGAAAAGGAGA
CCATTCCTTCTCATATCAC
TAGCGGGCGGAGGTGACGG
GGGTGTCTGTTGATTTTAC
GAAAAGAGGGAGACTTTAC
GCACGGCTCCCCCGGAGTT

Lily Exome Project - Results



Developed pipeline prioritizes genes previously described to cause MD

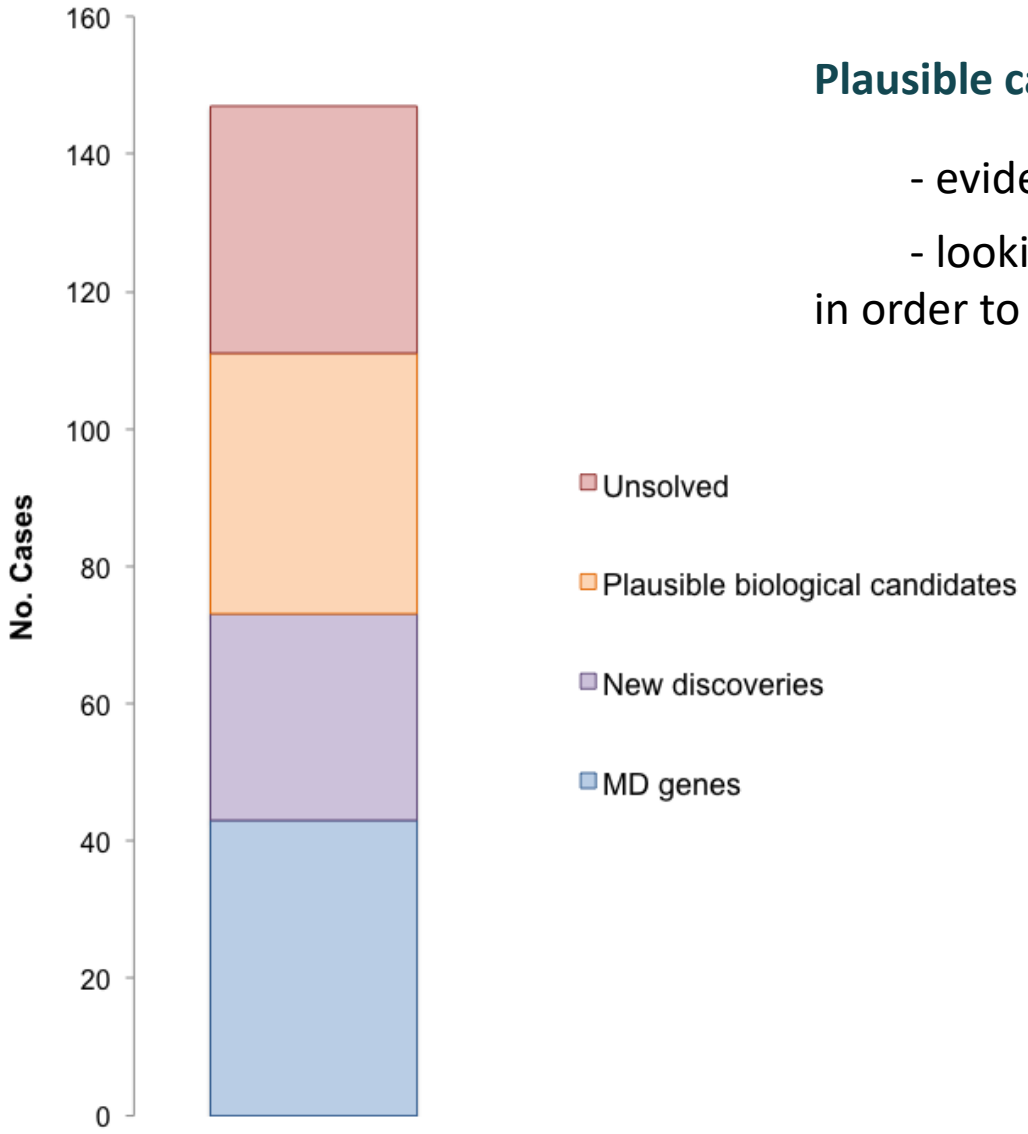
MD genes

- strong clinical and biochemical evidence of disease-gene association

New Discoveries

- evidence of mitochondrial gene
- reports of unrelated similarly affected cases from collaborators around the world

Lily Exome Project - Results



Plausible candidates

- evidence of mitochondrial gene
- looking forward for more evidences in order to pursue

Overview

Total analysed cases – 147

MD genes – 43
New discoveries – 30
Plausible candidates – 38
Unsolved – 36

Whole Exome Sequencing & Whole Genome Sequencing

NHS Diagnostic Services for genes known to be associated with mitochondrial disease:

- Specialist labs – Whole Exome Sequencing with analysis of known mito disease genes only
- Genomics England 100,000 Genomes Project – initial analysis of known mito disease genes only
- New NHS genomics services being introduced late 2018 / 2019

Diagnostic testing in a research setting:

- Genomics England 100,000 Genomes Project – follow on analysis by researchers
- Lily Foundation Mitochondrial Exome Sequencing Project

We have a genetic diagnosis – what next?

Explanation and some closure

Look to the future

Prenatal Diagnosis

One couple referred for PGD

Questions

- Older sibs who appear healthy – should we worry
- When should we test?
- What do/ should we tell our families?

De novo (New event in the affected individual) – no risk to sibs or extended family

Autosomal recessive inheritance – affected child has two faulty copies of the gene. Discussion with parents re testing

mtDNA - implications?

We do not have a genetic diagnosis

Feel forgotten/ overlooked

Further methods of analysis being tried

Thank you for your support

Important concepts

DNA – Deoxyribonucleic acid. Long molecule made up of nucleotides – adenine (A), thymine (T), cytosine (C), and guanine (G).DNA holds the instructions telling our bodies how to develop and function.

Chromosome – made up of DNA tightly coiled many times around proteins called histones that support its structure

Genome – organism's complete set of DNA. Contains the instructions for making and maintaining you.

Gene – basic physical and functional unit of heredity. Genes, which are made up of DNA, act as instructions to make molecules called proteins.

Exome – part of the genome consisting of exons that code information for protein synthesis