**First-line genomic diagnosis of mitochondrial disorders**

**F. Lucy Raymond1,2,3, Rita Horvath4 and Patrick F. Chinnery1,5\***

1NIHR Translational BioResource in Common and Rare Diseases, University of Cambridge, Cambridge, CB2 0XY, UK.

2Cambridge Institute for Medical Research, University of Cambridge, Cambridge, CB2 0XY, UK.

3Department of Medical Genetics, University of Cambridge, Cambridge, CB2 0XY, UK.

4Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne, NE2 4HH, UK.

5Department of Clinical Neurosciences and MRC Mitochondrial Biology Unit, University of Cambridge, Cambridge, CB2 0XY, UK.

\*e-mail: pfc25@cam.ac.uk

**Current approaches for diagnosing mitochondrial disorders involve specialist clinical assessment, biochemical analyses and targeted molecular genetic testing. There is now a strong rationale for undertaking first-line genome-wide sequencing, accelerating the speed of diagnosis and avoiding the need for expensive and invasive investigations.**

The clinical and genetic heterogeneity of mitochondrial disorders have shaped the diagnostic approach, with most clinicians advocating a systematic clinical and laboratory algorithm1, building evidence to support the diagnosis. In all but the rarest cases, where pattern recognition indicates a specific molecular cause, investigations involve a biopsy followed by histochemical or biochemical analysis of an affected tissue. The results of these investigations guide targeted molecular genetic investigations, usually starting with mitochondrial DNA (mtDNA) and ending with a growing panel of nuclear genes previously shown to explain the clinical or biochemical phenotype. However, the pace of recent technological advances and tumbling sequencing costs have brought this approach into question as it is now possible to rapidly screen all relevant nuclear genes and mtDNA simultaneously. Here, we argue that it is time for change: diagnostic investigations should begin with whole-genome sequencing.

**Clinical and genetic heterogeneity**

To the non-specialist, mitochondrial disorders are complicated and confusing. Often presenting with a variety of seemingly disconnected clinical features affecting multiple organ systems, different family members presenting with very different phenotypes can obscure the overall diagnosis1. Although there are a number of well-recognized clinical syndromes strongly suggestive of a mitochondrial etiology, many patients do not fit neatly into established diagnostic categories. The situation is compounded by the clinical overlap with other neurogenetic, metabolic and non-genetic diseases. The old adage that mitochondrial disorders can present at any age and affecting any organ is not too far from the truth.

To make matters worse, mitochondrial biogenesis requires the concerted interaction of over ~1,500 proteins derived from two distinct genomes: the majority are synthesized from nuclear genes, but 13 are derived from maternally inherited mtDNA2. Each cell contains multiple copies of mtDNA (typically ~1,000, depending on the cell type). If a mutation is present, it can affect any number of these molecules — a situation termed heteroplasmy. The ratio of mutant to wild type mtDNA is important in determining whether a biochemical defect is expressed, and the proportion can vary from cell to cell, organ to organ, and individual to individual within the same family. This genetic heterogeneity explains some of the clinical variability seen in families with mtDNA disorders.

Since the first molecular diagnosis of a mitochondrial disorder in 1988, hundreds of different point mutations and rearrangements of mitochondrial DNA (mtDNA) have been associated with different mitochondrial diseases3. With the advent of next-generation sequencing, the exploration of nuclear genetic mitochondrial disorders has become more tractable, leading to an almost exponential rate of mitochondrial disease gene discovery, which continues to the present day.

**Barriers to a single diagnostic test**

An increasing number of mitochondrial disorders are known to be caused by mutations in nuclear genes (Supplementary figure S1). Making a molecular diagnosis in this context is no different to other Mendelian disorders and hinges on the identification of a putative pathogenic variant in an affected individual that is detected in the germline, segregates with the clinical phenotype in the family, and is extremely rare or absent in a control population4. The molecular diagnosis is secure when the same mutation has been identified previously in a different family with the same disorder, and functional analysis of the variant confirms pathogenicity.

In contrast to Mendelian variants causing mitochondrial disorders, pathogenic mutations of mtDNA can either be present in 100% of the mtDNA (homoplasmy) or be mosaic (heteroplasmy)3. The most common mtDNA mutations are homoplasmic, that is, all molecules are mutated and can easily be detected in blood (for example, m.11778A>G causing Leber’s hereditary optic neuropathy, LHON). However, for heteroplasmic mutations, the percentage level of mutant mtDNA varies from tissue to tissue, and can also vary over time in the same individual. For example, for the most common pathogenic heteroplasmic mutation, m.3243A>G (first described in mitochondrial encephalopathy with lactic acidosis and stroke-like episodes, MELAS), the heteroplasmy level decreases exponentially over time in blood and may fall below the detection threshold for some diagnostic assays. To confidently exclude this mutation as the cause of disease, it is necessary to analyse other tissues, typically uroepithelium or skeletal muscle. Importantly, for some mutations, such as large-scale single deletions of mtDNA, the causative mutation may not be detectable in blood at all. Nevertheless, the vast majority of heteroplasmic pathogenic mtDNA mutations can be detected in blood samples provided the detection method is sufficiently sensitive. For novel mtDNA mutations, the same clinical and laboratory approach is taken as for nuclear-genetic disorders (discussed in Ref.4). Only a minority (~11.5%) of patients with established mitochondrial disease have causative mtDNA mutations that are not likely to be detectable in blood (Supplementary figure S2).

**A genome-based approach**

Circulating leukocytes typically contain >200 copies of mtDNA compared to the two copies detected of nuclear DNA3. As a consequence, even with the most stringent enrichment techniques, exome sequencing protocols generate multiple copies of the mtDNA sequence, reliably delivering >100-fold coverage across the mitochondrial genome. Non-PCR amplified whole-genome sequencing (WGS) is even more promising, with stable ~1,200-fold coverage across the entire mtDNA at a 14-fold coverage of the nuclear genome5. A linked bioinformatic pipeline enables the simultaneous analysis of both the nuclear and mitochondrial genomes, reliably detecting heteroplasmy levels >2%. Mitochondrial disorders unlikely to be diagnosed using this approach are sporadic cases with no family history: they include chronic progressive external ophthalmoplegia, which has a very characteristic phenotype1, and patients with a pure myopathy, who are likely to have had a muscle biopsy in any case, often being investigated for suspected muscular dystrophy.

 The challenges presented by mtDNA analysis are conceptually no different to the nuclear genome, where *de novo* mutations can show tissue-specific mosaicism4. In our view there is no longer a need for ‘mitochondrial exceptionalism’ when it comes to rare disease diagnostics. WGS of blood DNA allows the near comprehensive investigation of both genomes. Using the ‘genome first’ approach, the minority of patients will require invasive tests and biochemical investigations to substantiate the diagnosis. The genomic approach has several additional advantages, allowing the genetic diagnosis of other rare treatable diseases, mitochondrial disease phenocopies and, even more importantly, ensuring that mitochondrial disorders are diagnosed when they have been overlooked clinically. By sharing data globally, the approach will accelerate new disease gene discovery, reducing the proportion of nuclear-mitochondrial disorders that cannot be diagnosed (currently ~40% and falling). Together, this will accelerate the rate of diagnosis and shorten the diagnostic odyssey that is so distressing to patients and families. With WGS results delivered within days or weeks, there will be no need to send either the patient, or frozen tissue biopsy samples, over long distances.

Naturally, targeted genetic analysis is appropriate if there is a highly specific phenotype (for example, in the case of LHON), or if the aim is to confirm a molecular diagnosis already known in the family. If WGS is inconclusive, biochemical approaches are invaluable to distinguish real, sustained mitochondrial disorders from the acutely ill and from transiently deranged mitochondrial dysfunction, especially in the critical care setting. Transcriptomic, metabolomic and proteomic approaches may help resolve difficult cases, and biochemistry remains invaluable in understanding the clinical significance of variants of uncertain significance in WGS data.

For mitochondrial disorders, our view is to start with the genome, reserving invasive, expensive and time-consuming tests for the cases that cannot be solved by a first-line genomic approach. It is likely that many extremely rare genetic disorders are yet to be discovered. Adopting a first-line sequencing approach will accelerate this gain of knowledge. Now is the time to reshape the mitochondrial diagnostic approach for the future.

**References**

1. Gorman, G.S. *et al.* Mitochondrial diseases. *Nat Rev Dis Primers* **2**, 16080 (2016).

2. Vafai, S.B. & Mootha, V.K. Mitochondrial disorders as windows into an ancient organelle. *Nature* **491**, 374-83 (2012).

3. Stewart, J.B. & Chinnery, P.F. The dynamics of mitochondrial DNA heteroplasmy: implications for human health and disease. *Nature Reviews Genetics* **16**, 530-42 (2015).

4. Wright, C.F., FitzPatrick, D.R. & Firth, H.V. Paediatric genomics: diagnosing rare disease in children. *Nat Rev Genet* **19**, 325 (2018).

5. Li, M. *et al.* Transmission of human mtDNA heteroplasmy in the Genome of the Netherlands families: support for a variable-size bottleneck. *Genome Res* **26**, 417-26 (2016).

**Acknowledgements**

FLR receives support from the MRC, Rosetree Foundation, National Institute for Health Research (NIHR) Biomedical Research Centre based at Cambridge University Hospitals NHS Foundation Trust. RH is a Wellcome Investigator (109915/Z/15/Z), who receives support from the Wellcome Centre for Mitochondrial Research (203105/Z/16/Z), Medical Research Council (UK) (MR/N025431/1), the European Research Council (309548), the Wellcome Trust Pathfinder Scheme (201064/Z/16/Z) and the Newton Fund (UK/Turkey, MR/N027302/1). PFC is a Wellcome Trust Senior Fellow in Clinical Science (101876/Z/13/Z), and a UK NIHR Senior Investigator, who receives support from the Medical Research Council Mitochondrial Biology Unit (MC\_UP\_1501/2), the Medical Research Council (UK) Centre for Translational Muscle Disease (G0601943), the Evelyn Trust, and the National Institute for Health Research (NIHR) Biomedical Research Centre based at Cambridge University Hospitals NHS Foundation Trust and the University of Cambridge. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

**Competing interests**

The authors declare no competing interests.