Mitochondrial DNA variants in genomic data: diagnostic uplifts and predictive implications

William L. Macken[1,2], Anneke M. Lucassen [2,3], Michael G. Hanna [1] and Robert D. S. Pitceathly[1]

[1]Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology and The National Hospital for Neurology and Neurosurgery, London, UK

[2]Wessex Clinical Genetics Service, Princess Anne Hospital, Southampton, UK

[3]Clinical Ethics and Law Group (CELS), Faculty of Medicine, University of Southampton, Southampton, UK

Corresponding author:

Dr Robert D.S. Pitceathly

Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology and The National Hospital for Neurology and Neurosurgery, Queen Square, London WC1N 3BG, UK.

Email r.pitceathly@ucl.ac.uk

Standfirst

A broad spectrum of rare disease presentations can now be investigated by analysing for mitochondrial DNA (mtDNA) variants from whole genome sequencing (WGS) data. However, mtDNA mutations may cause unanticipated, extended phenotypes that have reproductive implications. We recommend these are considered by patients and clinicians before embarking on WGS.

Main text

Primary mitochondrial diseases (PMDs) comprise a diverse group of conditions defined by impaired oxidative phosphorylation or other aspects of mitochondrial function. They are unique among genetic disorders in that they are caused by mutations in either nuclear (nDNA) or mitochondrial (mtDNA) DNA, a small circular molecule, multiple copies of which reside within each mitochondrion, with 100s-1000s of mitochondria in each cell. Mutations can exist in all mtDNA molecules (homoplasmy) or a proportion (heteroplasmy); the level of mtDNA heteroplasmy may vary between tissues and change over time. Among others, PMDs may present as metabolic or developmental syndromes, neurogenetic conditions, cardiomyopathy, monogenic diabetes, deafness, or inherited eye diseases.

PMDs in the genomic medicine era

PMDs have traditionally been diagnosed by evaluating mtDNA in multiple tissues, with nuclear genes that encode mitochondrial proteins being tested separately. PCR-free whole-genome sequencing (WGS) captures both nDNA and mtDNA, and is increasingly being adopted for the diagnosis of rare diseases[1].

mtDNA has a high copy number and is consequently sequenced to a considerable depth (typically >~1,000x) during WGS. Standard variant calling flags mutations present at ~50% and ~100% of reads to identify heterozygous and homozygous nuclear variants, but the application of additional callers to high coverage WGS data now enables heteroplasmic mtDNA variants to be identified at intermediate levels (e.g.~10%)[2]. This technological advance has prompted calls for a WGS-first approach to PMD genetic testing, with the caveat that some mtDNA mutations are not detectable in blood[3].

We agree with the inclusion of pathogenic mtDNA variants in early diagnostic WGS; the clinical overlap that exists between PMDs and other rare disease presentations lends itself this broad approach and is likely to improve diagnostic rates. Establishing a mtDNA diagnosis is essential to 1) facilitate screening of potentially unanticipated disease complications; 2) refine reproductive options; 3) enable cascade testing of family members; and 4) improve understanding of the natural history and pathogenesis of these disorders. However, analysing mtDNA from WGS raises two important ethical considerations. First, PMD diagnoses will potentially be achieved earlier in the disease course and prior to the development of broader phenotypic manifestations; consequently, the findings of such tests are both diagnostic *and predictive* and have major reproductive implications. Second, pathogenic mtDNA variants with no direct contribution to the disease phenotype can be detected incidentally.[2] We consider both implications below.

Predictive implications of mtDNA variants

Once an mtDNA PMD diagnosis has been confirmed, further wide ranging possible clinical manifestations must be considered, but their range and severity are often difficult to predict. One reason for this is that mutant mtDNA level in blood invariably underestimates the levels present in less accessible, clinically manifesting, post-mitotic tissues, such as the brain. For example, the m.3243A>G mutation in *MT-TL1*, a common cause of multisystem PMD, is associated with relatively mild phenotypes (diabetes and deafness) at low mutant levels. However, at higher levels it causes complex disease presentations, including MELAS (Mitochondrial Encephalopathy, Lactic acidosis, and Stroke-like episodes)[4]. A diabetologist may suggest a young person with familial diabetes undergo WGS, with the application of a monogenic diabetes virtual panel (list of genes/ variants linked to inherited diabetes).

Identification of a m.3243A>G variant would constitute a primary finding and provide a diagnosis, but also imply a risk of developing additional future phenotypes, for which screening may ameliorate the clinical course of the disease (for example, hearing loss, cardiac involvement, and renal dysfunction). However, screening for other potential manifestations, such as stroke-like episodes (SLEs), are of no proven benefit and are therefore not considered 'actionable'. This is despite these complications resulting in devastating cerebral injury, severe cognitive decline, and early death. Unfortunately, the predictive value of extrapolating m.3243A>G levels in blood to brain tissue is limited, and a risk of SLEs exists in any person harbouring the m.3243A>G mutation. It is therefore important that the poorly defined likelihoods of certain complications for which there are no effective treatments is explicitly considered prior to WGS, since some may not wish to pursue a molecular diagnosis in the face of such uncertainties.

mtDNA single nucleotide variants are inevitably passed on by women to their children. However, as a random sample of wild-type and mutant mtDNA are 'bottlenecked' into each individual ovum, the mutant load can vary widely between mother and offspring[5]. There is therefore potential for a child to manifest more severe disease than their mother. A woman found to carry the m.3243A>G variant may choose to proceed with a pregnancy, have prenatal testing with the option of terminating a pregnancy with high variant levels, or (in some jurisdictions) undergo mitochondrial donation *in vitro* fertilisation[6]. Each option may result in considerable anxiety, and the woman may already have offspring who have been *de facto* tested for what is typically an adult-onset condition. We recommend potential reproductive issues are discussed at the time of diagnosis, including the possibility that the child may be more severely affected than the parent; that predictions about severity are weak

and that despite many technical advances, difficult reproductive decisions may follow on from a diagnosis.

The wide-ranging predictions following the diagnosis of a PMD are a distinct consideration to the identification of PMD variants as incidental findings in patients without PMD symptoms. The American College of Medical Genetics (ACMG) has developed a list of 59 genes suggested to be analysed for such incidental, or secondary, findings following WGS, since their detection may result in early (preclinical) screening and/or health interventions[7]. This list does not include pathogenic mtDNA variants and we consider this appropriate until there is further evidence of benefit. At present we cannot predict whether a pathogenic mtDNA variant in an unaffected person will ever manifest in clinical disease or what the emotional burden of such uncertainty will be on the unaffected carrier.

Knowledge limitations

Much of the public discourse surrounding genomic medicine suggests that results will provide clear cut diagnoses or predictions, yet the reality is often far more complex. This is particularly relevant for PMDs, where multiple determinants (for example, unmeasurable rates of heteroplasmy in "at risk" organs) are likely to modulate a person's health risks. At present, predictions for individual patients remain unclear even after molecular confirmation of a PMD. Consequently, the evidence base for screening is weak. While patients may be relieved to receive a diagnosis, future health risks can be distressing and a [prospective] parent may face considerable anguish especially regarding the possibility of a more severe disease in their offspring.

Future directions

WGS enables early molecular diagnosis of PMDs, yet the communication of, and surveillance for, attendant extended phenotypes, require further societal debate. In the first instance, we suggest that these issues are flagged for any virtual panel that contains mtDNA genes. Patients and their physicians should be aware that discovery of pathogenic mtDNA variants may predict additional medical problems with varying degrees of certainty. Long term screening may be recommended (e.g., echocardiograms for cardiomyopathy), while for other complications there may be no reliable intervention (e.g., SLEs). Given that many patients decide not to be tested for untreatable genetic conditions (e.g., Huntington disease), how can, and should, we communicate multiple possible phenotypes, some of which will be treatable, while others are not? Furthermore, how can we help facilitate reproductive decision making regarding the risks to offspring? Such decisions must rest with the patient. However, placing all the emphasis on whether a patient has consented to testing potentially obfuscates the critical need for support in complex decision making through pre-test counselling so that the available options are fully considered.

We welcome the improved diagnostic yield WGS confers in PMDs but call for a wider debate on the communication of attendant uncertainties concerning extended phenotypes and the benefits of screening for these. While further research in larger patient cohorts will help, we consider that the prediction of extended phenotypes is likely to remain a significant challenge for the foreseeable future. This highlights the need for open discussion of uncertainty in the pre-test setting, with a need for both clinicians and patient to avoid overly deterministic promises from genomic medicine.

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Competing interests

The authors declare the following competing interests: A.M.L is a member of Ethics Advisory Committee for Genomics England.