Iterative Reanalysis of Hypertrophic Cardiomyopathy Exome Data Reveals Causative Pathogenic Mitochondrial DNA Variants

Running title: Lopes et al.; Mitochondrial DNA mutations in HCM exome data

Luis R. Lopes, MD, PhD1,2; David Murphy, MSc3; Enrico Bugiardini, MD4; Reem Salem, BSc5;
Joanna Jager, MSc1; Marta Futema, PhD1; Mohammed Majid Akhtar, MBBS, BSc1,2;
Konstantinos Savvatis, MD, PhD1,2,6; Cathy Woodward, MSc7; Alan M. Pittman, PhD8;
Michael G. Hanna, MBBS, MD4; Petros Syrris, PhD1; Robert D.S. Piteathly, MBChB, PhD4*;
Perry M. Elliott, MBBS, MD, FRCP1,2*

1Centre for Heart Muscle Disease, Inst of Cardiovascular Science, 2Dept of Clinical & Movement Neurosciences, UCL Queen Square Institute of Neurology, 3UCL Division of Biosciences, Univ College London; 4Barts Heart Centre, St. Bartholomew’s Hospital, Barts Health NHS Trust; 5Dept of Neuromuscular Diseases, UCL Queen Square Inst of Neurology & The National Hospital for Neurology & Neurosurgery; 6William Harvey Research Inst, Queen Mary Univ London; 7Neurogenetics Unit, The National Hospital for Neurology & Neurosurgery; 8Genetics Research Centre, Molecular & Clinical Sciences, St. Georges Univ of London, London, United Kingdom

*joint senior authors

Correspondence:
Dr Luis R. Lopes
Barts Heart Centre, St. Bartholomew’s Hospital
West Smithfield
London, EC1A 7BE, United Kingdom
Tel: 00447765109343
E-mail: luis.lopes1@nhs.net

Journal Subject Terms: Cardiomyopathy; Genetics; Computational Biology

Key words: hypertrophic cardiomyopathy; genetics; mitochondrial disease cardiomyopathy; exome
Mitochondrial cytopathies caused by mitochondrial DNA(mtDNA) mutations have an estimated prevalence of 1/5,000 adults\(^1\). Cardiac manifestations are common (up to 40%) and include hypertrophic cardiomyopathy(HCM). Some mtDNA mutations (e.g.m.3243A>G,m.8344A>G,m.4300A>G) are well-recognised causes of cardiomyopathy and may occur as part of a multi-organ syndrome, such as MELAS(Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes), or as the only manifestation.

Up to 60% of HCM probands have no detectable causal mutations, while the prevalence of pathogenic mtDNA variants in large HCM cohorts has not previously been determined. We hypothesised that the presence of mtDNA mutations might account for a proportion of genotype-negative cases and applied a workflow to reliably identify mtDNA variants from whole exome sequencing(WES) data.

The study population and clinical evaluation have been previously described\(^2\). All patients provided written informed consent and the study had ethics committee approval(15/LO/0549). DNA extraction, library preparation, WES, variant calling, and
annotation were previously reported\textsuperscript{2,3}. Mitochondrial variants with read-depth $\geq 10x$ and heteroplasy level $\geq 10\%$ were chosen for validation and confirmation using whole mtDNA next-generation sequencing. Data that support the findings are available upon request.

The cohort comprised 770 unrelated HCM patients (67\% males, age 49.3 $\pm$ 15.9 years at diagnosis); 33\% had candidate variants in sarcomeric genes robustly associated with HCM. Six-hundred-and-fifty-nine samples passed quality control with called mitochondrial variants (mean depth 20.2).

The $MT-TL1$ m.3243A$\rightarrow$G mutation, a well-recognised cause of HCM, was detected at heteroplasmic levels in two probands (0.4\% of sarcomere-negatives) in whom a primary mitochondrial disease diagnosis had not previously been suspected. A third proband was homoplasmic for $MT-ND1$ m.3460G$\rightarrow$A, a pathogenic variant associated with Leber hereditary optic neuropathy (LHON). The patients did not harbour any other candidate variants in nuclear-encoded HCM genes.

Proband 1 was a female who presented at 35 years due to breathlessness and chest pain. Past medical history included well-controlled hypertension diagnosed at 19 years, bilateral deafness attributed to parotiditis, repeat miscarriages (five), and gestational diabetes. Family history was unremarkable. Electrocardiogram showed left ventricular hypertrophy (LVH) and T wave inversion; echocardiography revealed symmetric/concentric LVH - maximum LV wall thickness (MLVWT) 16mm; cardiac magnetic resonance (CMR) showed extensive fibrosis with subepicardial distribution (Figure 1A). Cardiopulmonary exercise test (CPEx) revealed a low peak oxygen consumption of 18ml/min/kg (53\% predicted) and anaerobic threshold of 33\%. The m.3243A$\rightarrow$G mutation was detected at 37\% load in blood.
Proband 2 was a male who presented at 61 years with heart failure and atrial fibrillation. Past medical history included hypertension (well-controlled on medication), diabetes mellitus complicated by retinopathy, and multiple strokes. Family history was uninformative. ECG showed atrial fibrillation and left bundle branch block. Echocardiography revealed severe LV systolic dysfunction and septal hypertrophy (14mm); relative wall thickness 0.46 (concentric remodelling). CMR showed extensive circumferential mid-myocardial/subepicardial enhancement (Figure 1B) with an LV ejection fraction 33%. Creatine kinase was mildly increased at 340 IU/L. A dual chamber implantable cardioverter defibrillator (ICD) with resynchronization therapy was implanted at 72 years and an appropriate shock occurred 3 months thereafter. He died at 74 years due to decompensated heart failure. The m.3243A>G mutation was identified at 11% load.

Proband 3 was a male who presented at 39 years with chest pain and breathlessness. ECG showed marked LVH, inferolateral T wave inversion (Figure 1C). CMR revealed septal-apical LVH with MLVWT 26mm and extensive patchy enhancement, in the anterolateral wall and septum (Figure 1C). Nonsustained ventricular tachycardia was detected and an ICD was implanted. During follow-up, LHON was diagnosed in a maternal aunt and cousin; his mother was known to carry the mutation with no clinical manifestations. He had an ophthalmologic assessment with no features of optic neuropathy. The m.3460G>A was detected at homoplasmic levels in blood.

In retrospect, both patients harbouring the m.3243A>G mutation had features consistent with a non-sarcomeric aetiology. Proband 1 had multiple miscarriages, gestational diabetes, and hearing loss, in addition to limited performance on the CPEx; Proband 2 exhibited systolic dysfunction, diabetes, and multiple strokes. Finally, the distribution and extent of fibrosis was...
unusual for sarcomeric HCM but is consistent with findings in one other case series describing CMR findings in patients with mitochondrial mutations.

Sequencing off-target captured mtDNA from exome data has been described previously and refined in-house. This methodology had never been applied to screen a HCM cohort for pathogenic mtDNA variants. A previous study utilising whole-genome sequencing (WGS) detected the pathogenic m.4300A>G variant in 1/46 genotype-negative HCM patients. The coverage achieved with WGS is higher, but most clinical and research cohorts are studied using WES.

HCM caused by mtDNA mutations is characterised by ventricular arrhythmia, conduction disease and evolution to systolic dysfunction. A thorough assessment for extra-cardiac manifestations is crucial if mitochondrial disease is suspected. The detection of pathogenic mtDNA variants has significant impact for the genetic counselling and management of the proband and their relatives.

Iterative reanalysis of WES data for mtDNA mutations increases the yield of genetic testing in HCM, and should therefore be considered in genetically undiagnosed HCM cohorts.

Sources of Funding: The University College London Hospitals/University College London Queen Square Institute of Neurology sequencing facility receives a proportion of funding from the Department of Health’s National Institute for Health Research Biomedical Research Centres funding scheme. The clinical and diagnostic 'Rare Mitochondrial Disorders' Service in London is funded by the UK NHS Highly Specialised Commissioners. LRL is funded by an MRC UK Clinical Academic Research Partnership (CARP) Award. DM is funded by the National Institute for Health Research University College London Hospitals Biomedical Research Centre. RDSP is supported by a Medical Research Council Clinician Scientist Fellowship (MR/S002065/1). RDSP and MGH receive funding from a Medical Research Council strategic award to establish an International Centre for Genomic Medicine in Neuromuscular Diseases (MR/S005021/1). MF
is funded by the Fondation Leducq Transatlantic Networks of Excellence Program grant (no. 14 CVD03).

**Disclosures:** None

**References:**

1. Gorman GS, Schaefer AM, Ng Y, Gomez N, Blakely EL, Alston CL, Feeney C, Horvath R, Yu-Wai-Man P, Chinnery PF, et al. Prevalence of nuclear and mitochondrial DNA mutations related to adult mitochondrial disease. *Ann Neurol.* 2015;77:753-9.

2. Lopes LR, Futema M, Akhtar MM, Lorenzini M, Pittman A, Syrris P and Elliott PM. Prevalence of TTR variants detected by whole-exome sequencing in hypertrophic cardiomyopathy. *Amyloid.* 2019;26:243-247.

3. Poole OV, Pizzamiglio C, Murphy D, Falabella M, Macken WL, Bugiardini E, Woodward CE, Labrum R, Efthymiou S, Salpietro V, et al. Mitochondrial DNA analysis from exome sequencing data improves the diagnostic yield in neurological diseases. *Ann Neurol.* 2021 Mar 11. doi: 10.1002/ana.26063. Online ahead of print.

4. Florian A, Ludwig A, Stubbe-Drager B, Boentert M, Young P, Waltenerger J, Rosch S, Sechtem U and Yilmaz A. Characteristic cardiac phenotypes are detected by cardiovascular magnetic resonance in patients with different clinical phenotypes and genotypes of mitochondrial myopathy. *J Cardiovasc Magn Reson.* 2015;17:40.

5. Bagnall RD, Ingles J, Dinger ME, Cowley MJ, Ross SB, Minoche AE, Lal S, Turner C, Colley A, Rajagopalan S, et al. Whole Genome Sequencing Improves Outcomes of Genetic Testing in Patients With Hypertrophic Cardiomyopathy. *J Am Coll Cardiol.* 2018;72:419-429.

**Figure Legend**

**Figure 1A.** Electrocardiogram (ECG) and cardiac magnetic resonance (CMR) images (from left to right, 4 chamber, short axis and 2 chamber views; upper row end-diastolic cine images, lower row late gadolinium enhancement images) for Proband 1, harbouring the m.3243A>G mutation in *MT-TL1*. ECG shows T wave inversion V2 to V6, DI, DII and aVL. CMR shows concentric hypertrophy and extensive fibrosis with a subepicardial distribution at basal lateral wall and mid-
apical anterior, lateral and inferior walls. **B.** Cardiac magnetic resonance (CMR) images (from left to right, 4 chamber and mid short axis views; upper row end-diastolic cine images, lower row late gadolinium enhancement images), for Proband 2 harbouring the m.3243A>G mutation in *MT-TL1*, showing extensive circumferential mid-myocardial/subepicardial enhancement and localized inferior septum hypertrophy (14mm); relative wall thickness was 0.46, indicative of concentric remodelling. **C.** Electrocardiogram (ECG) and cardiac magnetic resonance (CMR) images (from left to right, 4 chamber and 2 chamber views; upper row end-diastolic cine images and lower row late gadolinium enhancement images) for Proband 3, homoplasmic for the pathogenic m.3460G>A variant in *ND1*. ECG shows left ventricular hypertrophy and deep T wave inversion V3 to V6, DI, DII, aVL and aVF. CMR shows septal and apical LVH and extensive patchy enhancement, mainly in the anterolateral wall and septum.
