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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*

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Nonstandard Abbreviations and Acronyms
CMR: cardiac magnetic resonance
CPEx: cardiopulmonary exercise test
HCM: hypertrophic cardiomyopathy
ICD: implantable cardioverter defibrillator
LHON: Leber hereditary optic neuropathy
LVH: left ventricular hypertrophy
MLVWT: maximum LV wall thickness
MELAS: Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes
mtDNA: mitochondrial DNA
WES: whole exome sequencing
WGS: whole-genome sequencing

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

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annotation were previously reported[2,3]. Mitochondrial variants with read-depth≥10x and heteroplasmy level≥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±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>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>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>G mutation was detected at 37% load in blood.

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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

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unusual for sarcomeric HCM but is consistent with findings in one other case series describing CMR findings in patients with mitochondrial mutations[4].

Sequencing off-target captured mtDNA from exome data has been described previously and refined in-house[3]. 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[5]. 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.

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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-

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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.

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