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RESEARCH LETTER

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

Luis R. Lopes, MD, PhD; David Murphy, MSc; Enrico Bugiardini, MD; Reem Salem, BSc; Joanna Jager, MSc; Marta Futema, PhD; Mohammed Majid Akhtar, MBBS, BSc; Konstantinos Savvatis, MD, PhD; Cathy Woodward, MSc; Alan M. Pittman, PhD; Michael G. Hanna, MBChB, MD; Petros Syrris, PhD; Robert D.S. Pitceathly, MBChB, PhD*; Perry M. Elliott, MBBS, MD*

Mitochondrial cytopathies caused by mitochondrial DNA (mtDNA) mutations have an estimated prevalence of 1/5000 adults.[1] Cardiac manifestations are common (up to 40%) and include hypertrophic cardiomyopathy (HCM). Some mtDNA mutations (eg,m.3243A>G,m.8344A>G,m.4300A>G) are well-recognized causes of cardiomyopathy and may occur as part of a multi-organ syndrome, such as 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 hypothesized 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 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, whole-exome sequencing, variant calling, and annotation were previously reported.[2,3] Mitochondrial variants with read-depth ≥10× 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 fifty-nine samples passed quality control with called mitochondrial variants (mean depth 20.2).

The *MT-TL1* m.3243A>G mutation, a well-recognized cause of HCM, was detected at heteroplasmic levels in 2 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. The patients did not harbor 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. ECG showed left ventricular hypertrophy and T wave inversion; echocardiography revealed symmetrical/concentric left ventricular hypertrophy maximum LV wall thickness 16 mm; cardiac magnetic resonance showed extensive fibrosis with subepicardial distribution (Figure [A]). Cardiopulmonary exercise test revealed a low peak oxygen consumption of 18 mL/min per kg (53% predicted) and

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Correspondence to: Luis R. Lopes, MD, PhD, Barts Heart Centre, St. Bartholomew's Hospital, West Smithfield, London, EC1A 7BE, United Kingdom. Email luis.lopes1@nhs.net

*R.D.S. Pitceathly and P.M. Elliott contributed equally.

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anaerobic threshold of 33%. The m.3243A>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 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(14 mm); relative wall thickness 0.46 (concentric remodeling). Cardiac magnetic resonance showed extensive circumferential mid-myocardial/subepicardial enhancement (Figure [B]) with an LV ejection fraction 33%. Creatine kinase was mildly increased at 340 IU/L. A dual-chamber implantable cardioverter defibrillator 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 left ventricular hypertrophy, inferolateral T-wave inversion(Figure [C]). Cardiac magnetic resonance revealed septal-apical left ventricular hypertrophy with maximum LV wall thickness 26 mm and extensive patchy enhancement, in the anterolateral wall and septum(Figure [C]). Nonsustained ventricular tachycardia was detected, and an implantable cardioverter-defibrillator was implanted. During follow-up, Leber hereditary optic neuropathy 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 harboring the m.3243A>G mutation had features consistent with a nosnsarcomeric cause. Proband 1 had multiple miscarriages, gestational diabetes, and hearing loss, in addition to limited performance on the cardiopulmonary exercise test; 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 one other case series describing cardiac magnetic resonance 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 using whole-genome sequencing detected the pathogenic m.4300A>G variant in 1/46 genotype-negative HCM patients.[5] The coverage achieved with whole-genome sequencing is higher, but most clinical and research cohorts are studied using whole-exome sequencing.

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

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

ARTICLE INFORMATION

Affiliations

Centre for Heart Muscle Disease, Institute of Cardiovascular Science (L.R.L., J.J., M.F., M.M.A., K.S., P.S., P.M.E.), Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology (D.M.), UCL Division of Biosciences (R.S.), University College London. Barts Heart Centre, St. Bartholomew's Hospital, Barts Health National Health Service Trust (L.R.L., M.M.A., K.S., P.M.E.). Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology and The National Hospital for Neurology and Neurosurgery (E.B., M.G.H., R.D.S.P.). William Harvey Research Institute, Queen Mary University London (K.S.). Neurogenetics Unit, The National Hospital for Neurology and Neurosurgery (C.W.). Genetics Research Centre, Molecular and Clinical Sciences, St. Georges University of London, United Kingdom (A.M.P.).

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Disclosures

None.

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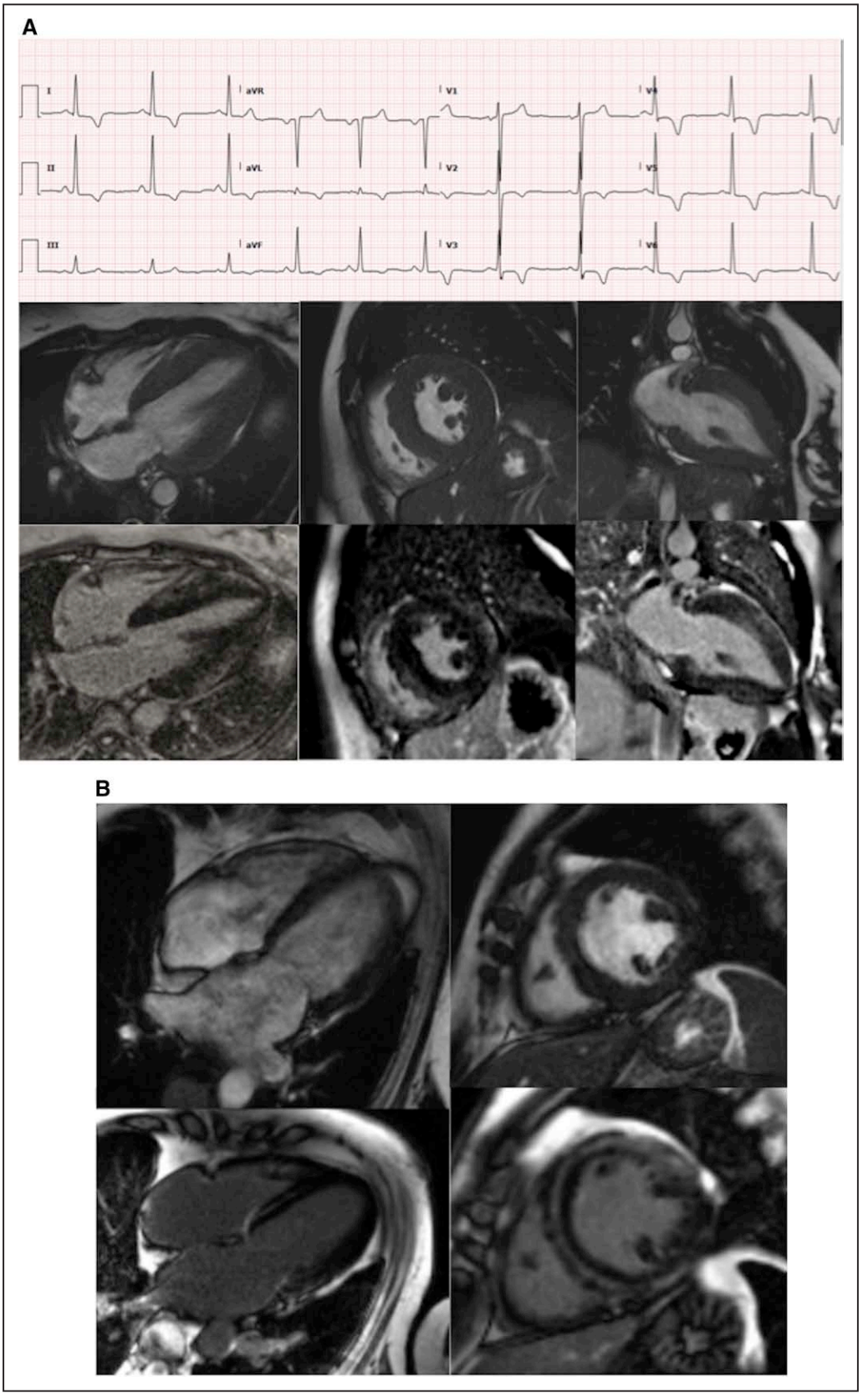

Figure. ECG and cardiac magnetic resonance (CMR) images of the described probands.
A, ECG and cardiac magnetic resonance (CMR) images (from left to right, 4 chamber, short axis and 2 chamber views; upper row end-diastolic cine images, lower row late gadolinium enhancement images) for proband 1, harboring 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 (*Continued*)

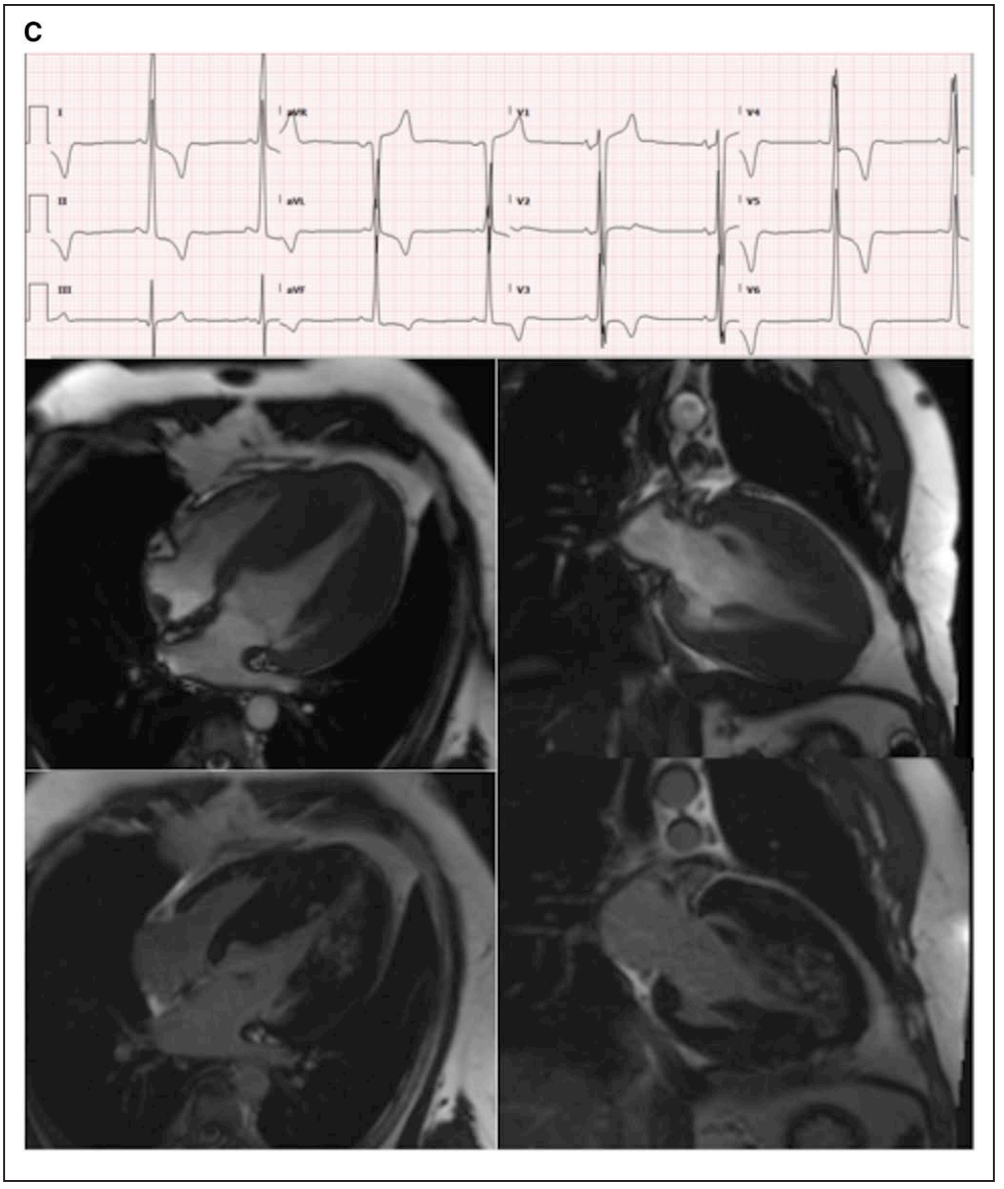

Figure Continued. subepicardial distribution at basal lateral wall and mid-apical anterior, lateral, and inferior walls. **B**, CMR images (from **left** to **right**, 4 chamber and mid short-axis views; upper row end-diastolic cine images, lower row late gadolinium enhancement images), for poband 2 harboring the m.3243A>G mutation in *MT-TL1*, showing extensive circumferential mid-myocardial/subepicardial enhancement and localized inferior septum hypertrophy (14 mm); relative wall thickness was 0.46, indicative of concentric remodeling. **C**, ECG and 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 left ventricular hypertrophy (LVH) and extensive patchy enhancement, mainly in the anterolateral wall and septum.