Cardiac Outcomes in Adults with Mitochondrial Diseases

Brief title: Mitochondrial disease and cardiac outcomes

Konstantinos Savvatis, MD, PhD[1,2,3], Christoffer Rasmus Vissing, MD[4,5], Lori Klouvi[6], Anca Florian, MD, PhD[7], Mehjabin Rahman, MD[3], Anthony Béhin, MD[8], Abdallah Fayssoil, MD, PhD[9,10], Marion Masingue, MD[8], Tanya Stojkovic, MD[8], Henri Marc Bécane, MD[8], Nawal Berber[8], Fanny Mochel, MD, PhD[11,12], Denis Duboc, MD, PhD[8,13,14], Bertrand Fontaine, MD, PhD[15], Bjørg Krett, MD[4], Caroline Stalens, PhD[6], Julie Lejeune, PhD[6], Robert D S Pitceathly, MD, PhD[16], Luis Lopes, MD, PhD[1,3], Malika Saadi, MD[13], Thomas Gossios, MD, PhD[17], Vincent Procaccio, MD PhD[18,19], Marco Spinazzi, MD[19,20], Céline Tard, MD, PhD[21], Pascal De Groote, MD[22], Claire-Marie Dhaenens, MD[23], Claire Douillard, MD[24], Andoni Echaniz-Laguna, MD, MhD[25], Ros Quinlivan, MBBS, MD[16], Michael G Hanna, MBChB, MD[16], Ali Yilmaz, MD, PhD[7], John Vissing, MD, PhD[4], Pascal Laforêt, MD, PhD[24,28,29], Perry Elliott, MD, PhD[1,3], Karim Wahbi, MD, PhD[8,13,14,30]

[1] Inherited Cardiac Conditions Unit, Barts Heart Centre, St. Bartholomew's Hospital, London, United Kingdom.

[2] William Harvey Research Institute, Queen Mary University London, London, United Kingdom.

[3] Centre for Heart Muscle Disease, Institute for Cardiovascular Science, University College London, London, United Kingdom.

[4] Copenhagen Neuromuscular Centre, Department of Neurology, Rigshospitalet, University of Copenhagen, Denmark.

[5] The Capital Region's Unit for Inherited Cardiac Diseases, Department of Cardiology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark.

[6] AFM Telethon, 1 rue de l'Internationale, 91002 Evry, France.

[7] Department of Cardiology I, Division of Cardiovascular Imaging, University Hospital Münster, Münster, Germany.

[8] AP-HP, Pitié-Salpêtrière Hospital, Reference Center for Muscle Diseases Paris-Est, Myology Institute, 75651 Paris 13, France.

[9] AP-HP, Raymond Poincare University Hospital, Garches, France.

[10] Université de Versailles-Saint Quentin, Boulogne-Billancourt, France.

[11] AP-HP, Pitié-Salpêtrière Hospital, Genetics Department, Inserm UMR S975, CNRS UMR7225, ICM, 75651 Paris 13, France.

[12] Pierre et Marie Curie-Paris 6 University, Myology Institute, Pitié-Salpêtrière Hospital, 75013 Paris, France.

[13] AP-HP, Cochin Hospital, Cardiology Department, Paris Cedex 14, France.

[14] Université de Paris, Paris, France.

[15] Sorbonne-Université, INSERM, Assistance Publique-Hôpitaux de Paris (AP-HP), Centre de Recherche en Myologie-UMR 974, Service de Neuro-Myologie, Institut de Myologie, Hôpital Universitaire Pitié-Salpêtrière, Paris

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

[17] Cardiomyopathies Laboratory, 1st Aristotle University of Thessaloniki Cardiology Department, AHEPA University Hospital, Thessaloniki, Greece.

[18] Equipe Mitolab, Unité Mixte de Recherche MITOVASC, CNRS 6015, INSERM U1083, Université d'Angers, Angers, France.

[19] Département de Biochimie et Génétique, Centre Hospitalier Universitaire, Angers, France.

[20] Neuromuscular Reference Center, Department of Neurology, CHU Angers, 49933, Angers, France.

[21] Université de Lille, INSERMU1172, Lille, France; Centre de référence des maladies neuromusculaires Nord Est Ile de France, CHU de Lille, 2,Avenue Oscar-Lambret, 59000 Lille, France.

[22] Service de cardiologie, Pôle cardio-vasculaire et Pulmonaire, CHRU de Lille, Lille, France Inserm U1167, Institut Pasteur de Lille, Université de Lille 2, Lille, France.

[23] Univ. Lille, Inserm, CHU Lille, U1172-LilNCog-Lille Neuroscience & Cognition, 59045 Lille, France.

[24] CHU de Lille, Département d'Endocrinologie et Métabolisme, Centre de Référence des Maladies Héréditaires du Métabolisme, Hôpital Huriez, Lille, France.

[25] Department of Neurology, APHP, CHU de Bicêtre, Le Kremlin-Bicêtre, France.

[26] French National Reference Center for Rare Neuropathies (NNERF), Le Kremlin-Bicêtre, France.

[27] INSERM U1195, Paris-Saclay University, Le Kremlin-Bicêtre, France.

[28] Nord/Est/Île-de-France Neuromuscular Reference Center, Neurology Department, Raymond-Poincaré Teaching Hospital, AP-HP, Garches, France.

[29] INSERM U1179, END-ICAP, Versailles-Saint-Quentin-en-Yvelines University, Université Paris Saclay, Montigny-le-Bretonneux, France.

[30] Paris Cardiovascular Research Center (PARCC), INSERM Unit 970, Paris, France.

Funding: This study was funded by grants from the Association Française contre les Myopathies (French Alliance against Myopathies), which was involved neither in the design and conduct of the study, the collection, management, analysis, and interpretation of the data, the preparation, review or approval of the manuscript, nor in the decision to submit the manuscript for publication. LL is supported by a MRC UK clinical academic partnership (CARP) award. RQ was funded by NIHR biomedical research centre, University College

Hospitals Foundation Trust. 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. RDSP is supported by a Medical Research Council (UK) Clinician Scientist Fellowship (MR/S002065/1). RDSP and MGH receive funding from a Medical Research Council (UK) strategic award to establish an International Centre for Genomic Medicine in Neuromuscular Diseases (ICGNMD) (MR/S005021/1).

Disclosures: PE is receiving consultancy fees from Pfizer, Sanofi, Sarepta, DinaQor, Freeline, Novo Nordisk, BMS. The other authors have no potential conflict of interest to disclose.

Corresponding author:

Karim Wahbi, MD

Cardiology Department, Cochin Hospital

27 rue du Faubourg Saint Jacques

75679 PARIS Cedex 14

France

E-mail: <karim.wahbi@cch.aphp.fr>

Tel: +33 1 58 41 16 53

Fax: +33 1 58 41 16 05

Twitter:@KSavvatis @LuisRLopesDr @RobPitceathly @NHSBartshealth

Patients with mitochondrial diseases are at risk of heart failure, arrhythmias and major adverse cardiac events that can be predicted based on their genotype and simple cardiac tests. #ACCEP #ACCHFT

Abstract

Background: Patients with mitochondrial diseases are at risk of heart failure (HF) and arrhythmic major adverse cardiac events (MACEs).

Objectives: We developed prediction models to estimate the risk of HF and arrhythmic MACEs in this population.

Methods: We determined the incidence and searched for predictors of HF and arrhythmic MACEs using Cox regression in 600 adult patients from a multicenter registry with genetically confirmed mitochondrial diseases.

Results: Over a median follow-up time of 6.67 years, 29 patients (4.9%) reached the HF endpoint, including 19 hospitalizations for non-terminal HF, two cardiac transplantations and eight deaths from HF. Thirty others (5.1%) reached the arrhythmic MACE, including 21 with third- or type II second-degree atrioventricular blocks, four with sinus node dysfunction, and five sudden cardiac deaths. Predictors of HF were the m.3243A>G variant (HR, 4.3; 95% CI, 1.8-10.1), conduction defects (HR, 3.0; 95% CI, 1.3-6.9), left ventricular (LV) hypertrophy (HR, 2.6; 95% CI, 1.1-5.8), LV ejection fraction < 50% (HR, 10.2; 95% CI, 4.6-22.3), and premature ventricular beats (HR, 4.1; 95% CI, 1.7-9.9). Independent predictors for arrhythmia were single, large-scale mtDNA deletions (HR, 4.3; 95% CI, 1.7-10.4), conduction defects (HR, 6.8; 95% CI, 3.0-15.4), and LV ejection fraction < 50% (HR 2.7; 95% CI, 1.1-7.1). C-indexes of the Cox regression models were 0.91 (95% CI, 0.88-0.95) and 0.80 (95% CI, 0.70-0.90) for the HF and arrhythmic MACEs, respectively.

Conclusion: We developed the first prediction models for HF and arrhythmic MACEs in patients with mitochondrial diseases using genetic variant type and simple cardiac assessments.

Condensed abstract:

Patients with mitochondrial diseases are at risk of heart failure (HF) and arrhythmic major adverse cardiac events (MACEs). We developed prediction models to estimate these risks in 600 patients with genetically confirmed mitochondrial diseases.

Over a medial follow up period of 6.6 years, 4.9% of the cohort reached the HF and 5.1% the arrhythmic MACEs endpoint. Predictors of HF were the m.3243A>G variant in the mitochondrial DNA, conduction defects, premature ventricular beats, left ventricular hypertrophy (LV) and LV ejection fraction <50% at baseline. Predictors of arrhythmic MACEs were presence of single large-scale deletions, conduction defects and LV ejection fraction <50%.

Keywords: mitochondrial diseases, m3243A>G, single large-scale deletions, heart failure, conduction disease, sudden death

Abbreviations:

EF	Ejections fraction
HF	Heart failure
LV	Left ventricle
MACE	Major Adverse Cardiac Event

Introduction

Mitochondrial diseases are heritable conditions caused by genetic variants in the mitochondrial or nuclear DNA, resulting in dysfunction of the mitochondrial respiratory chain and abnormal ATP production [1-4]. The most common genetic changes are single, large-scale mtDNA deletions and the m.3243A>G point variant in mitochondrial DNA. The prevalence of mitochondrial diseases is estimated to be 1 in 5000 individuals [5,6]. They are characterized by diverse clinical presentations but organs with high energy demand, such as the heart, brain, and skeletal muscle, are preferentially affected. Cardiac involvement is common with left ventricular hypertrophy, conduction disease, Wolff-Parkinson-White syndrome, and dilated cardiomyopathy[7-14] being the most frequently reported manifestations. Patients with mitochondrial diseases have a high risk of HF and arrhythmic major adverse cardiac events (MACEs), which remains extremely challenging to estimate in the absence of specific prediction models [15].

In this international multicenter study, we sought to analyze the frequence of arrhythmic and heart failure (HF) complications in a large cohort of patients with genetically confirmed mitochondrial diseases and to identify risk factors for the development of these two events to guide the implementation of specific diagnostic and therapeutic measures.

Methods

The data, analytic methods, and study materials will not be made publicly available to other researchers for purposes of reproducing the results or replicating the procedure because consent to participate in this study did not include public dissemination of patient data. This study complies with the ethical principles formulated in the Declaration of Helsinki and was approved by the local ethics committees at sites where formal approval was required according to local laws.

Study settings and design

Patients referred to our nine recruiting centres for the management of their mitochondrial disease between January 2000 and December 2019 were retrospectively identified. The inclusion criteria were: 1) age ≥ 18 years at the time of the first cardiac evaluation between January 2000 and December 2019; 2) pathogenic or likely pathogenic variants in mitochondrial or nuclear genes. We reviewed medical records and entered into a dedicated database the information regarding the 1) clinical manifestations of their mitochondrial diseases, 2) results of genetic testing, 3) results of cardiac investigations at the first referral, and 4) long-term major cardiovascular events.

Clinical definitions

Criteria for the interpretation of ECG results were in accordance with the recommendations formulated by professional societies [16]. Cardiac involvement due to underlying mitochondrial disease in the cohort was defined as presence of structural and functional myocardial abnormalities (LV hypertrophy, LV dilatation, impaired systolic function, presence of myocardial late enhancement on cardiovascular magnetic resonanse) and/or electrical abnormalities (conduction defects or ventricular premature beats) in the absence of other causes. Conduction disease on a standard 12 lead ECG included complete left or right bundle branch block, left anterior or left posterior fascicular block, and first-degree atrioventricular block. Premature ventricular complexes were defined as >15 premature ventricular events/h. Non-sustained ventricular tachycardia was defined as ≥ three consecutive ventricular complexes at a rate ≥120 bpm on a 24-h ambulatory ECG. Left ventricular (LV) hypertrophy was defined as an LV mass >88 g/m^2 body surface area for women and >102 g/m^2 among men, as measured by echocardiography. LV dilatation was defined as an LV end-diastolic diameter of >31 and >32 mm/m² body surface in men and women, respectively [17].

MACE

The endpoints of this study were 1) HF MACE, 2) arrhythmias as major adverse cardiac events (MACEs), and 3) the total number of MACE corresponding to the one of the first two outcomes, whichever occurred first. HF MACE was defined as 1) death due to HF, 2) cardiac transplantation, or 3) hospitalizations for non-terminal HF. The classification of hospitalizations related to HF was made according to prespecified criteria [18]. Arrhythmias were defined as 1) sudden cardiac death [19], 2) third- or type II second-degree atrioventricular block, 3) sinus node dysfunction, or 4) sustained ventricular tachyarrhythmias. Death was classified as sudden if it occurred unexpectedly within 1 h of onset of cardiac manifestations, in the absence of prior hemodynamic deterioration, during sleep, or within 24 h after the patient was last seen alive and apparently stable clinically [19]. Three of the authors (KW, KS, and CV) reviewed the data and adjudicated MACE according to these prespecified criteria.

Statistical analyses

Continuous variables are presented as mean ± standard deviation or median (interquartile range) values as appropriate. Categorical variables are presented as counts and percentages. Time-to-event curves were constructed using the Kaplan-Meier product limit estimator, and the log rank test was used to compare the event hazard between groups (genetic variant types or number of MACE risk factors). The baseline was defined either as birth for analysis at life scale or as inclusion in the registry for analysis at medical follow-up scale. The observation period ended when <15% of patients were observable. The baseline description was performed on the whole population. For survival analysis from baseline, patients who had experienced an event before the baseline were excluded from the analysis.

Variables with a p-value inferior to 0.15 in univariable analysis were included in a multiple variable Cox regression model, by using a backward selection strategy based on the Wald test [20]. The predictor variables included in the model for HF MACE were genetic variant type (m.3243A>G versus other variants), diabetes, hypertrophy, conduction defect,

premature ventricular contractions, and LV ejection fraction < 50%, whereas the variables included in the model for arrhythmias as MACEs were genetic variant type (single, large-scale mtDNA deletions versus other variants), atrial fibrillation, arterial hypertension, male sex, conduction defect, premature ventricular contractions, and LV ejection fraction < 50%. The assumptions of the Cox model were verified with respect to the proportionality of hazard ratios (HRs) and linear functional forms [21]. HRs are expressed with their 95% confidence intervals (CIs). To gauge model discrimination, we calculated Harrell's concordance (C) index [22]. All tests were two-sided at the 0.05 significance level. Statistical analyses were performed using the Statistical Analysis Software 9.4 (SAS Institute Inc., Cary, North Carolina).

Results

Baseline characteristics

We studied 600 patients (females, 59.2%) with median age of 43.2 years (31.4-54.1 years) with a confirmed genetic diagnosis of mitochondrial disease that included patients from our previous study[15] and 340 additional patients recruited from multiple international centres. Up to date follow up data were collected for all patients. The most common mitochondrial disease manifestations at baseline are presented in Table 1 and Supplemental Table 1. The most prevalent genetic variant was the m.3243A>G variant in *MT-TL1* in 208 (34.7%) patients, whereas 171 patients (28.5%) showed single, large-scale mtDNA deletions, 145 (24.2%) showed other mtDNA point variants, and 76 (12.7%) showed nuclear gene variants (Supplemental Table 2).

Cardiac involvement was present at baseline in 230 patients (38.3%). Left ventricular hypertrophy was present in 135 patients (22.5%). Thirty-seven patients (6.6%) had a left ventricular ejection fraction < 50%, and 51 patients (7.4%) showed symptoms of HF, with most of the patients categorized in NYHA class II. Twenty two patients (3.7%) presented

with LV dilatation, whereas 11 patients (1.8%) had an LVEF<35% (Table 1 and supplement table 1). Fifty-five patients (9.3%) showed evidence of conduction disease at baseline. A short PR interval was present in 31 individuals (5.2%) and Wolff-Parkinson-White syndrome was reported in 20 patients (3.4%). Other common symptoms were palpitations in 49 patients (8.2%), lightheadedness in 29 patients (4.8%), and syncope in 12 patients (2.0%). Eighty-three patients (13.9%) were treated with beta-blockers and 119 (19.9%) with angiotensin-converting enzyme inhibitors. Sixteen (2.7%) patients had a pacemaker and none of the patients had an implantable cardiac defibrillator.

MACEs

Post-baseline analyses were performed on 589 patients for total MACE, 596 patients for HF MACE and 593 patients for arrhythmic MACE. During a median follow-up time of 6.6 years (3.0-11.2 years), 52 patients (8.8%) reached the total MACE endpoint (Table 2) with a median time to endpoint of 4.3 years (1-8.7). The cumulative incidence of MACEs according to the genetic variant from baseline and from birth is presented in Figure 1 and supplemental Table 3. Within the first 10 years from the first assessment, 11.7% (95% CI 8.5-15.5). of the patients had experienced a MACE endpoint.

Twenty nine patients (4.9%) reached the HF MACE at a median time of 5.1 years (1.9-9.2). Eight patients died from HF, two patients underwent cardiac transplantation, and 19 patients were hospitalized for non-terminal HF. Interestingly, both patients who underwent cardiac transplantation had the m.3243A>G genotype: the first one was transplanted at the age of 35 year and presented with LVH of 15mm wall thickness and LVEF 15%; the second one at the age of 52 years and presented with LVH 12mm and LVEF 25%. The 10-year cumulative incidence of HF MACE was 6.85% (95% CI 4.4-9.9). Figure 1 shows the incidence of HF MACE for the four different genotypes. The risk of HF was highest for the

m.3243A>G genotype and started rising in the early 30s with a relatively stable rate until the mid-50s and a steep rise after that.

Thirty patients (5.1%) reached the arrhythmic MACE endpoint (Table 2) at a median time of 3.5 years (0.7-8.8), with 6.2% reaching the endpoint within the first 10 years of follow-up (95% CI 4.0-9.0). Five of these patients suffered sudden cardiac death, 21 patients progressed to second-degree type II atrioventricular block or third-degree atrioventricular block, and four patients developed significant sinus node dysfunction. Figure 1 shows the incidence of the arrhythmic MACE outcome for the different genotypes. The risk of arrhythmias was the highest for patients with single, large-scale mtDNA deletions with the number of events evenly distributed throughout their life, starting in early adulthood. The number of events was minimal for the other genotypes and occurred later in life.

HF MACE prediction

On univariable analysis, diabetes, conduction disease, left ventricular hypertrophy, ejection fraction < 50%, presence of the m.3243A>G variant, and premature ventricular contractions were significantly associated with the development of the HF MACE (Table 3 and supplementary table 5). On multivariable analysis, conduction disease (HR, 2.63 ; 95% CI, 1.10-6.28), LV hypertrophy (HR, 2.81; 95% CI, 1.23-6.39), LV ejection fraction < 50% (HR, 11.43; 95% CI, 5.17-25.26), m.3243A>G variant (HR, 4.69; 95% CI, 1.97-11.16), and premature ventricular beats (HR, 4.46 95% CI; 1.83-10.88) were independent predictors of the HF MACE (Table 3). The calculated C-index for the HF MACE outcome was 0.92 (95% CI, 0.89-0.95), indicating an excellent risk prediction of the model. Patients with no or one risk factor showed excellent long-term prognosis (Central illustration) with 4 patients (0.8%) reaching the HF MACE at a median time of 7.0 (6.5-9.0), corresponding to a 10-year cumulative incidence of 1.2% (95% CI,0.3- 3.2). Among patients with two or three risk

factors 21 (17.9%) reached the HF MACE at a median time of 3.0 years (0.7-9.2) corresponding to a 10-year cumulative incidence of 23.5% (95% CI, 13.8- 34.6).

Arrhythmic MACE prediction

On univariable analysis, progression to the arrhythmic MACE endpoint was significantly associated with sex, atrial fibrillation, conduction disease at baseline, LV ejection fraction < 50%, and single, large-scale deletion (Table 3 and supplementary table 5). On multivariable analysis, conduction disease (HR, 8.22; 95% CI, 3.67-18.43), LV ejection fraction < 50% (HR 3.25 95% CI1.27-8.34), and single, large-scale mtDNA deletions (HR, 4.92; 95% CI, 2.05-11.79) were independently associated with development of the arrhythmic MACE (Table 3). The calculated C-index for arrhythmic MACE was 0.8 (95% CI, 0.7-0.9), showing a strong risk prediction of the model. Patients with no or one risk factor showed excellent long-term prognosis (Central illustration) with 12 patients (2.1%) reaching the arrhythmic MACE at a median time of 4.9 years (0.7- 9.1), corresponding to a 10-year cumulative incidence of 2.3% (95% CI, 1.1-4.3). Among patients with two or three risk factors 18 (52.9 %) reached the HF MACE at a median time of 2.3 years (0.75-8.84) corresponding to a 10-year cumulative incidence of 58.5% (95% CI, 35.2-76.0).

Occurrence of sudden death in mitochondrial disease

In our cohort, sudden death was rare. The total population of 600 patients included five cases of unexplained sudden death. Two of the patients had single, large-scale mtDNA deletions with advanced conduction disease and progressed rapidly to complete heart block and declined pacemaker insertion. One patient with a m.3243A>G variant and mild left ventricular hypertrophy with a maximum wall thickness of 12mm and experienced a cardiac arrest during hospitalization for anaphylaxis. Two other patients, one with *POLG* and another with nuclear DNA variants, died unwitnessed at home but had normal cardiac investigations (supplementary table 4).

Discussion

In this multicenter study, we show that cardiac involvement is present in approximately 30% of patients and that life-threatening cardiac complications occur in 10% of patients over a 10-year follow-up period. In fact, approximately 14% of deaths of all causes (13 cardiac deaths from total 95 deaths) were directly due to cardiac complications indicating the importance of cardiac surveillance in high-risk patients. We have previously reported on the long-term cardiac prognosis and provided risk factors in 260 patients with mitochondrial diseases[15]. In the present study, we validated our previous results and identified independent risk factors for HF and arrhythmic MACE, that can be used to estimate the long-term risk for adverse outcomes and facilitate the implementation of preventive strategies.

Similar to previous reports [11,15,23], we found that a significant proportion of patients presented with cardiac involvement and both structural and electrical abnormalities, which have been associated with a higher risk of cardiac complications in both paediatric and adult populations.[11,15,24-28] We cannot exlude that some of the changes observed such as LVH and AF were secondary to other cardiovascular risk factors, such as hypertension, which are known to be present in patients with mitochondrial diseases with a higher frequency than in the general population.[29] However, LVH is a well described clinical picture and can occur early in paediatric populations with mitochondrial diseases independently.[30] Our data suggest that the cardiac outcomes are worse in patients carrying the m.3243A>G variant and single, large-scale mtDNA deletions in comparison with other variants. The presence of m.3243A>G was an independent risk factor for HF complications with only few arrhythmic events. On the other hand, single, large-scale mtDNA deletions were independently predictive of arrhythmic risk, which mainly drove the cardiac risk in this genotype. The complexity of the clinical presentations in each genetic subgroup is similar to that reported with extracardiac

manifestations of the disease, with incomplete genotype-phenotype correlations and numerous overlapping syndromes. Importantly, our data suggest that irrespective of genotype, patients generally have a good prognosis in the absence of other risk factors.

We have developed the first model to date for HF risk stratification in mitochondrial diseases, which can be easily used after simple cardiac assessments even in non-expert centers. Patients with HF MACE risk factors may benefit from closer follow-up or potentially advanced cardiovascular tests such as cardiac MRI, which may identify early myocardial changes.[26] High-risk patients may also be eligible for early initiation of HF medication to delay myocardial disease progression or novel therapies such as mtDNA elimination or mtDNA gene therapy.[31,32] As shown in other studies as well, our data show that patients with 0 or 1 risk factors have an excellent long-term prognosis and a less frequent follow up in such patients such as every 3-5 years, or less frequently, as is the case in other forms of screening for inherited cardiomyopathies, may be reasonable.[33]

Current guidelines for pacing and sudden death prevention in mitochondrial disease lack clear indications, mainly due to the lack of evidence [34]. In our cohort, the vast majority of arrhythmic events occured in patients with single, large-scale mtDNA deletions, and most of the arrhythmic events were conduction defects. Our observations suggest that prevention of sudden cardiac death in patients with large deletions mtDNA should mainly rely on prophylactic permanent pacing in patients with multiple risk factors. Sudden cardiac death and ventricular arrhythmias were not observed in patients with the m.3243A>G variant from our cohort but have been reported in few cases with extreme left ventricular hypertrophy and fibrosis [35]. We cannot exclude from our study that some patients with the m.3243A>G variant with the most severe hypertrophic patterns may benefit from implantable cardiac if the severity of the mitochondrial disease and life expectancy are compatible with such a decision.

Limitations

One of the primary limitations of the study was its retrospective nature. As a result, some of the conventional cardiovascular risk factors, such as smoking and BMI, were not available. However, standardised protocols were used for all patients with complete follow-up and identification of events. In addition, the study was performed in adult patients, and so may be subject to inclusion bias. Moreover, only tertiary centers with extensive experience in the care of patients with mitochondrial diseases were included, which may have introduced a bias towards referral of the sickest patients. However, most of patients from the geographic areas covered by our centers were referred to them because of the complexity of the management of these conditions.

Conclusions

Our data suggest that adult patients with mitochondrial diseases are at risk of developing severe HF and arrhythmia-related MACE. We developed statistical models with good accurracy for the prediction of these events, which can guide the use of preventive treatments in high-risk patients.

Clinical perspectives:

Competency in Patient Care and Procedural Skills: Patients with mitochondrial disease and cardiac involvement are at risk of heart failure, arrhythmias and major adverse cardiac events that can be predicted based on individual genetic background and clinical features.

Translational Outlook: Future studies should address whether early initiation of medication or device-based therapies for heart failure or arrhythmias can improve clinical outcomes for patients with mitochondrial disease involving the myocardium.

References

1. DiMauro S, Schon EA. Mitochondrial respiratory-chain diseases. N Engl J Med 2003;348:2656-68.
2. Pfeffer G, Chinnery PF. Diagnosis and treatment of mitochondrial myopathies. Annals of medicine 2013;45:4-16.
3. Koopman WJ, Willems PH, Smeitink JA. Monogenic mitochondrial disorders. N Engl J Med 2012;366:1132-41.
4. Hirano M, Davidson M, DiMauro S. Mitochondria and the heart. Curr Opin Cardiol 2001;16:201-10.
5. Elliott HR, Samuels DC, Eden JA, Relton CL, Chinnery PF. Pathogenic mitochondrial DNA mutations are common in the general population. American journal of human genetics 2008;83:254-60.
6. Gorman GS, Schaefer AM, Ng Y et al. Prevalence of nuclear and mitochondrial DNA mutations related to adult mitochondrial disease. Ann Neurol 2015;77:753-9.
7. Limongelli G, Tome-Esteban M, Dejthevaporn C, Rahman S, Hanna MG, Elliott PM. Prevalence and natural history of heart disease in adults with primary mitochondrial respiratory chain disease. Eur J Heart Fail 2010;12:114-21.
8. Anan R, Nakagawa M, Miyata M et al. Cardiac involvement in mitochondrial diseases. A study on 17 patients with documented mitochondrial DNA defects. Circulation 1995;91:955-61.
9. Sorajja P, Sweeney MG, Chalmers R et al. Cardiac abnormalities in patients with Leber's hereditary optic neuropathy. Heart 2003;89:791-2.
10. Roberts NK, Perloff JK, Kark RA. Cardiac conduction in the Kearns-Sayre syndrome (a neuromuscular disorder associated with progressive external ophthalmoplegia and

pigmentary retinopathy). Report of 2 cases and review of 17 published cases. Am J Cardiol 1979;44:1396-400.

11. Wahbi K, Larue S, Jardel C et al. Cardiac involvement is frequent in patients with the m.8344A>G mutation of mitochondrial DNA. Neurology 2010;74:674-7.

12. Malfatti E, Laforet P, Jardel C et al. High risk of severe cardiac adverse events in patients with mitochondrial m.3243A>G mutation. Neurology 2013;80:100-5.

13. Arbustini E, Diegoli M, Fasani R et al. Mitochondrial DNA mutations and mitochondrial abnormalities in dilated cardiomyopathy. Am J Pathol 1998;153:1501-10.

14. Tveskov C, Angelo-Nielsen K. Kearns-Sayre syndrome and dilated cardiomyopathy. Neurology 1990;40:553-4.

15. Wahbi K, Bougouin W, Behin A et al. Long-term cardiac prognosis and risk stratification in 260 adults presenting with mitochondrial diseases. Eur Heart J 2015;36:2886-93.

16. Surawicz B, Childers R, Deal BJ et al. AHA/ACCF/HRS recommendations for the standardization and interpretation of the electrocardiogram: part III: intraventricular conduction disturbances: a scientific statement from the American Heart Association Electrocardiography and Arrhythmias Committee, Council on Clinical Cardiology; the American College of Cardiology Foundation; and the Heart Rhythm Society: endorsed by the International Society for Computerized Electrocardiology. Circulation 2009;119:e235-40.

17. Cheitlin MD, Armstrong WF, Aurigemma GP et al. ACC/AHA/ASE 2003 Guideline Update for the Clinical Application of Echocardiography: summary article. A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (ACC/AHA/ASE Committee to Update the 1997 Guidelines for

the Clinical Application of Echocardiography). Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography 2003;16:1091-110.

18. Hicks KA, Tcheng JE, Bozkurt B et al. 2014 ACC/AHA Key Data Elements and Definitions for Cardiovascular Endpoint Events in Clinical Trials: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Data Standards (Writing Committee to Develop Cardiovascular Endpoints Data Standards). J Am Coll Cardiol 2015;66:403-69.
19. Hinkle LE, Jr., Thaler HT. Clinical classification of cardiac deaths. Circulation 1982;65:457-64.
20. Kalbfleisch JD, Prentice RL. The statistical analysis of failure time data. New York: Wiley, 1980.
21. Lin DY, Wei LJ, Ying Z. Checking the Cox model with cumulative sums of martingale-based residuals. Biometrika 1993;80:557-572.
22. Harrell FE, Jr., Lee KL, Califf RM, Pryor DB, Rosati RA. Regression modelling strategies for improved prognostic prediction. Statistics in medicine 1984;3:143-52.
23. Papadopoulos C, Wahbi K, Behin A et al. Incidence and predictors of total mortality in 267 adults presenting with mitochondrial diseases. Journal of inherited metabolic disease 2020;43:459-466.
24. Quadir A, Pontifex CS, Lee Robertson H, Labos C, Pfeffer G. Systematic review and meta-analysis of cardiac involvement in mitochondrial myopathy. Neurology Genetics 2019;5:e339.
25. Vydt TC, de Coo RF, Soliman OI et al. Cardiac involvement in adults with m.3243A>G MELAS gene mutation. Am J Cardiol 2007;99:264-9.

26. Hollingsworth KG, Gorman GS, Trenell MI et al. Cardiomyopathy is common in patients with the mitochondrial DNA m.3243A>G mutation and correlates with mutation load. Neuromuscul Disord 2012;22:592-6.

27. Imai-Okazaki A, Matsunaga A, Yatsuka Y et al. Long-term prognosis and genetic background of cardiomyopathy in 223 pediatric mitochondrial disease patients. Int J Cardiol 2021.

28. Debray FG, Lambert M, Chevalier I et al. Long-term outcome and clinical spectrum of 73 pediatric patients with mitochondrial diseases. Pediatrics 2007;119:722-33.

29. Chong-Nguyen C, Stalens C, Goursot Y et al. A high prevalence of arterial hypertension in patients with mitochondrial diseases. Journal of inherited metabolic disease 2020;43:478-485.

30. Holmgren D, Wahlander H, Eriksson BO, Oldfors A, Holme E, Tulinius M. Cardiomyopathy in children with mitochondrial disease; clinical course and cardiological findings. Eur Heart J 2003;24:280-8.

31. Nissanka N, Moraes CT. Mitochondrial DNA heteroplasmy in disease and targeted nuclease-based therapeutic approaches. EMBO reports 2020;21:e49612.

32. Mok BY, de Moraes MH, Zeng J et al. A bacterial cytidine deaminase toxin enables CRISPR-free mitochondrial base editing. Nature 2020;583:631-637.

33. Lim AZ, Jones DM, Bates MGD et al. Risk of cardiac manifestations in adult mitochondrial disease caused by nuclear genetic defects. Open Heart 2021;8:e001510.

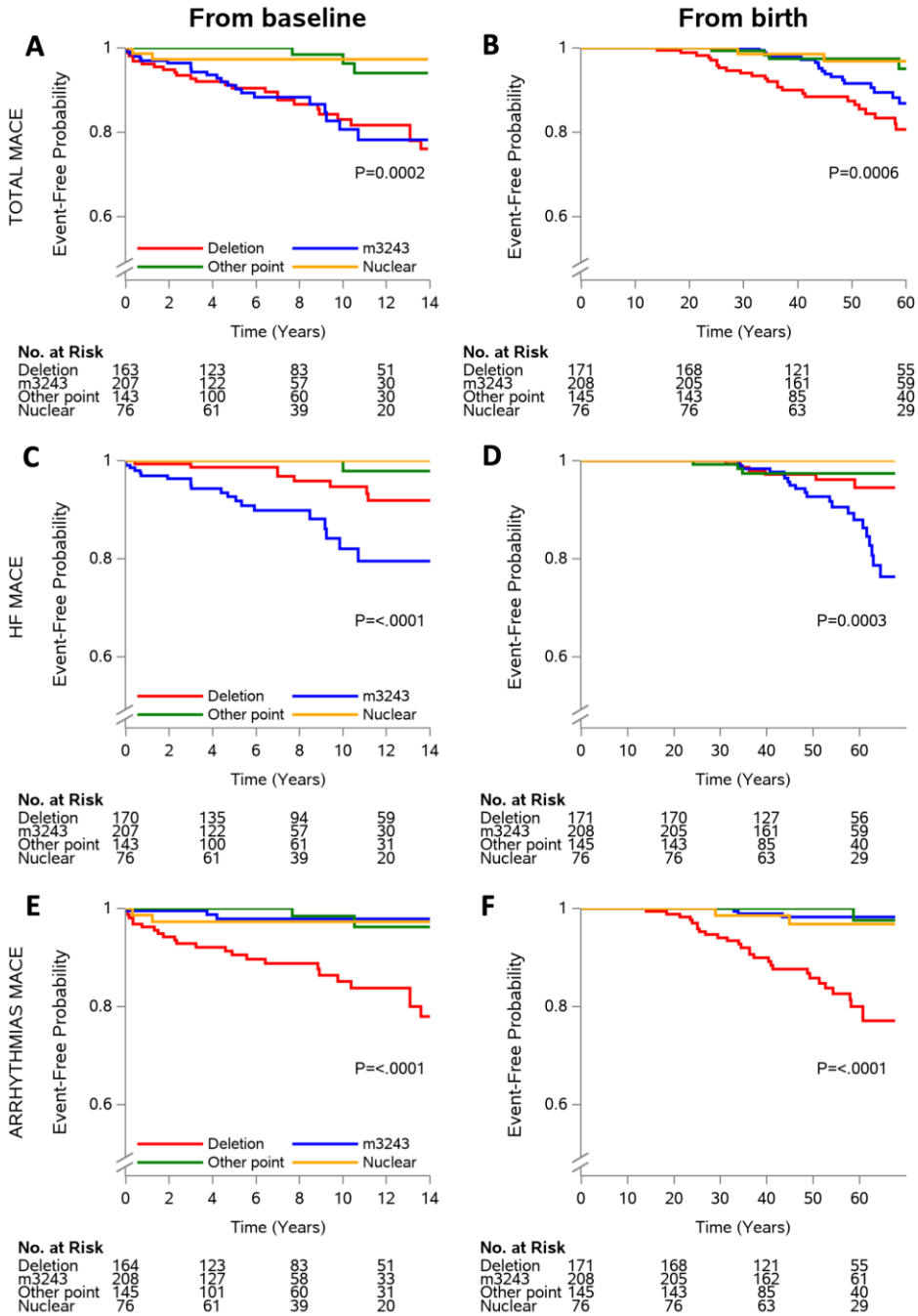
34. Kusumoto FM, Schoenfeld MH, Barrett C et al. 2018 ACC/AHA/HRS Guideline on the Evaluation and Management of Patients With Bradycardia and Cardiac Conduction Delay: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Rhythm Society. Circulation 2019;140:e382-e482.

35. Ng YS, Grady JP, Lax NZ et al. Sudden adult death syndrome in m.3243A>G-related mitochondrial disease: an unrecognized clinical entity in young, asymptomatic adults. Eur Heart J 2016;37:2552-9.

Figure Legends

Figure 1: HF and arrhythmias-MACE free survival by genotype

Total MACE from baseline (A) and from birth (B); HF MACE from baseline (C) and from birth (D); arrhythmias MACE from baseline (E) and from birth (F)


From baseline
From birth
A
B
TOTAL MACE
Event-Free Probability
P=0.0002
P=0.0006
Deletion
m3243
Other point
Nuclear
Time (Years)
No. at Risk
Deletion 163 123 83 51
m3243 207 122 57 30
Other point 143 100 60 30
Nuclear 76 61 39 20
Deletion 171 168 121 55
m3243 208 205 161 59
Other point 145 143 85 40
Nuclear 76 76 63 29
C
D
HF MACE
P=<.0001
P=0.0003
Deletion 170 135 94 59
m3243 207 122 57 30
Other point 143 100 61 31
Nuclear 76 61 39 20
Deletion 171 170 127 56
m3243 208 205 161 59
Other point 145 143 85 40
Nuclear 76 76 63 29
E
F
ARRHYTHMIAS MACE
P=<.0001
P=<.0001
Deletion 164 123 83 51
m3243 208 127 58 33
Other point 145 101 60 31
Nuclear 76 61 39 20
Deletion 171 168 121 55
m3243 208 205 162 61
Other point 145 143 85 40
Nuclear 76 76 63 29

Central Illustration: Predictors of heart failure and arrhythmic MACEs in mitochondrial diseases

HF MACE from baseline according to strata of 0-1, 2-3 and 4-5 risk factors (A); Arrhythmias MACE from baseline according to strata of 0-1, 2-3 risk factors (B). LV, left ventricle; LVEF, left ventricular ejection fraction; PVCs, premature ventricular contractions

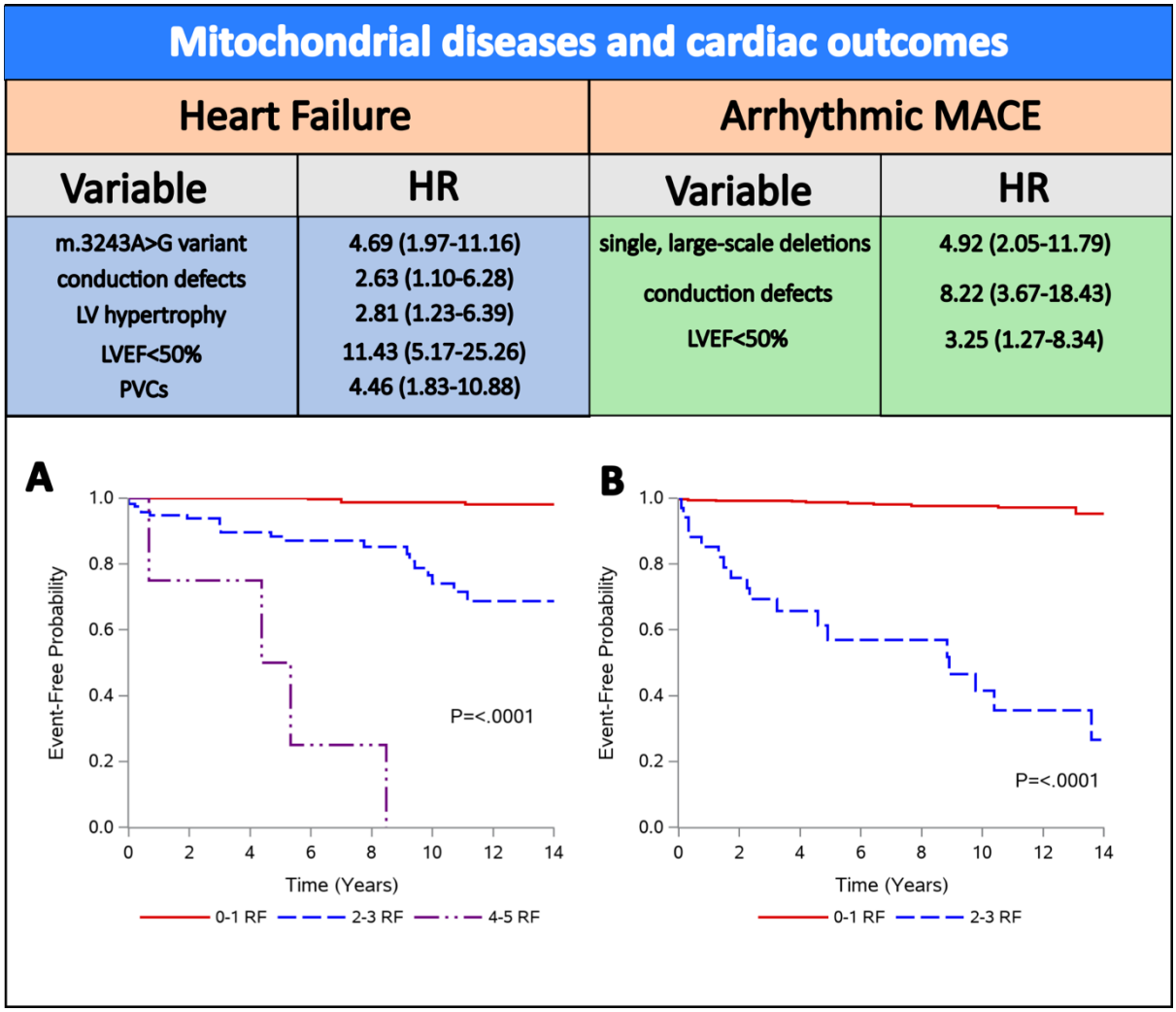


Table 1: Baseline characteristics of the 600 study participants

Patient characteristics	Total population
Age at baseline, years	43.2 (31.4-54.1)
Male	245 (40.8)
Genetic variant type	
m.3243A>G	208 (34.7)
Single, large-scale mtDNA deletions	171 (28.5)
Other mtDNA point variants	145 (24.2)
Nuclear gene variants	76 (12.7)

Patient characteristics	Total population
Mitochondrial disease manifestations	
Myopathy	300 (50.1)
Chronic progressive external ophthalmoplegia	285 (47.7)
Epilepsy	83 (13.9)
Dysphagia	129 (21.5)
Respiratory failure	57 (9.5)
Renal failure	56 (9.3)
Diabetes	160 (26.7)
Retinopathy	68 (11.4)
None	53 (8.8)

Patient characteristics	Total population
Cardiac manifestations	
Myocardial involvement	
NYHA II	44 (7.3)
NYHA III-IV	7 (0.1)
Left ventricular hypertrophy	135 (22.5)
Left ventricular dilatation	22 (3.7)
Left ventricular ejection fraction <50%	37 (6.6)
Left ventricular ejection fraction <35%	11 (1.8)
Conduction defect	55 (9.3)
First-degree atrioventricular block	19 (3.2)
Left bundle branch block	11 (1.9)
Right bundle branch block	24 (4.0)
Atrial fibrillation	22 (3.7)
Premature ventricular contractions	21 (3.5)
Non-sustained ventricular tachycardia	4 (0.7)
Systemic hypertension	167 (27.8)

mtDNA = mitochondrial DNA; NYHA = New York Heart Association. Values are medians (IQR) or numbers (%) of observations.

Table 2: Major adverse cardiac events in the entire study sample

All-cause mortality	95 (15.8)
Total MACE	52 (8.8)
Cumulative incidence at 10 years	11.7 (8.5- 15.5)
Time to event, years	4.3 (1.0- 8.7)
HF MACE	29 (4.9)
Cumulative incidence at 10 years	6.8 (4.4; 10.0)
Time to event, years	5.1 (1.9; 9.2)
Type of event	
Death due to heart failure	8
Cardiac transplantation	2
Other heart failure hospitalization	19
Arrhythmias MACE	30 (5.1)
Cumulative incidence at 10 years	6.2 (4.0; 9.0)
Time to event, years	3.5 (0.7; 8.8)
Type of event	
Sudden death	5
Third- or type II second-degree atrioventricular block	21
Sinus node dysfunction	4
Ventricular tachyarrhythmias	0

MACE = major adverse cardiac event. Values are medians (IQR) or numbers (%) of observations.

Table 3: Relationship between patient characteristics and major adverse cardiac events

	HF MACE				Arrhythmias MACE			
	Univariable analysis		Multivariable analysis		Univariable analysis		Multivariable analysis	
Parameters	**HR (95% CI)**	**P**	**HR (95% CI)**	**P**	**HR (95% CI)**	**P**	**HR (95% CI)**	**P**
Male	1.42 (0.69-2.95)	0.342	-	-	1.83 (0.89-3.75)	0.099	-	-
Age at baseline, years	1.01 (0.99-1.04)	0.306	-	-	0.99 (0.96-1.01)	0.332	-	-
Diabetes	7.39 (3.35-16.26)	<0.001	-	-	0.94 (0.40-2.19)	0.879	-	-
Genetic variant type								
Single, large-scale mtDNA	0.73 (0.32-1.66)	0.452			7.38 (3.16-17.23)	<0.001	4.92 (2.05- 11.79)	<0.001
m.3243A>G	6.19 (2.80-13.70)	<0.001	4.69 (1.97- 11.16)	<0.001	0.26 (0.08-0.85)	0.027	-	-
Other mtDNA point variants	0.11 (0.02-0.81)	0.030	-	-	0.22 (0.05-0.91)	0.037	-	-
Nuclear gene variants	0.00 (0.00-Inf)	0.987	-	-	0.43 (0.10-1.80)	0.248	-	-
Atrial fibrillation	1.38 (0.33-5.81)	0.663	-	-	3.27 (1.13-9.40)	0.028	-	-
Conduction defect	4.70 (2.14-10.33)	<0.001	2.63 (1.10-6.28)	0.029	16.13 (7.82-33.24)	<0.001	8.22 (3.67- 18.43)	<0.001
LV hypertrophy	6.60 (3.07-14.19)	<0.001	2.81 (1.23-6.39)	0.014	1.25 (0.56-2.81)	0.586	-	-
LV ejection fraction <50%	23.99 (11.51-49.98)	<0.001	11.43 (5.17- 25.26)	<0.001	6.52 (2.77-15.35)	<0.001	3.25 (1.27- 8.34)	0.014
Premature ventricular contractions	7.79 (3.32-18.30)	<0.001	4.46 (1.83- 10.88)	0.001	3.07 (0.93-10.14)	0.065	-	-

MACE = major adverse cardiac event; HR = hazard ratio; CI = confidence interval; mtDNA = mitochondrial DNA; LV = left ventricular