ANNALS of Clinical and Translational Neurology
Open Access
AMERICAN NEUROLOGICAL ASSOCIATION

RESEARCH ARTICLE

Prevalence of heart disease in patients with mitochondrial abnormalities on skeletal muscle biopsy

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Funding Information
No funding information provided.

Received: 24 November 2020; Revised: 28 January 2021; Accepted: 4 February 2021

Annals of Clinical and Translational Neurology **2021; 8(4): 825–830**

doi: 10.1002/acn3.51327

Abstract

Objective: Mitochondrial DNA mutations are associated with an increased risk of heart disease. Whether an increased prevalence of cardiovascular disease is present in patients presenting with mitochondrial abnormalities on skeletal muscle biopsy remains unknown. This study was designed to determine the prevalence of cardiac conduction disease and structural heart disease in patients presenting with mitochondrial abnormalities on skeletal muscle biopsy. **Methods**: This is a retrospective cohort study of 103 patients with mitochondrial abnormalities on skeletal muscle biopsy who were referred for evaluation of muscle weakness at a single tertiary care referral center from 2012 to 2018. Of these patients, 59 (57.3%) had an electrocardiogram available and were evaluated for the presence of conduction disease. An echocardiogram was available in 43 patients (42%) who were evaluated for the presence of structural heart disease. The prevalence of cardiac disease was compared to control cohort populations (Framingham and the Atherosclerosis Risk in Communities, ARIC cohorts). **Results**: Mitochondrial abnormalities associated with cardiac conduction disease (defined as QRS duration $\geq$ 120 msec) were present in 8.9%, versus 2.0% ($p < 0.001$) in the Framingham population and 2.6% ($p = 0.003$) in the ARIC cohort. LV systolic dysfunction (LVEF $\leq$ 50%) was present in 11.6%, versus 3.6% ($p < 0.01$) in the Framingham and 3% ($p < 0.01$) in the ARIC populations. Left ventricular hypertrophy was present in 28.6%, versus 13.6% ($p < 0.02$) in the Framingham and 10.4% ($p < 0.001$) in the ARIC populations. **Interpretation**: Given the increased prevalence of cardiovascular disease, patients with mitochondrial abnormalities on skeletal muscle biopsy should undergo routine cardiac screening with physical exam, electrocardiography, and cardiac imaging.

Introduction

Mitochondrial myopathies (MM) are a broad group of disorders, characterized by muscle weakness and variable neurological manifestations.[1] A recent meta-analysis has identified an increased risk of cardiac abnormalities in patients with genetically diagnosed MM, with structural abnormalities present in 29% of patients and conduction abnormalities present in 39%.[2] Certain genetic mutations, including mitochondrial encephalopathy, lactic acidosis and stroke-like episodes (MELAS) and myoclonic epilepsy with ragged red fibers (MERRF) have more severe cardiac involvement.[2] Interestingly, another recent study found that the prevalence of arterial hypertension was also significantly higher in patients with MELAS and MERRF, affecting 66% and 61% of patients, respectively.[3]

The formal diagnosis of MM is complex and requires a combination of family history, genetic testing of the nuclear and mitochondrial genome, biochemical testing, and histologic analysis of muscle or cardiac tissue.[4] Skeletal muscle is commonly affected in mitochondrial diseases, and skeletal muscle biopsy is safe and readily

accessible for histologic analysis.[5] Diagnostic features of mitochondrial myopathy include the presence of positive succinate dehydrogenase (SDH) staining due to mitochondrial proliferation and subsarcolemmal accumulation from oxidative phosphorylation defects, or ragged red fibers (RRF).[5] Additionally, cytochrome c oxidase (COX) negative fibers at a frequency of >5% also suggest mitochondrial dysfunction.[5] Nonspecific findings on skeletal muscle biopsy are also commonly present and include increased glycogen or lipid accumulation and neurogenic atrophy.[5,6] Prior pathologic criteria have been developed to assist in the formal diagnosis of mitochondrial disorders, including the Walker and Sleigh criteria.[7,8] These criteria include major and minor criteria for the percentages of RRF and COX negative or SDH positive fibers, such as RRF > 1.04%, COX negative fibers > 3.46%, and SDH positive fibers > 0.89% using the Sleigh criteria.[7,8] Mitochondrial abnormalities (MA) are more common with aging, and can also occur as secondary effects of other myopathies, such as polymyositis, dermatomyositis, drug toxicity (especially statin use), or inclusion body myositis.[9] As the prevalence of cardiac disease in patients with solely MA without a formal diagnosis remains poorly characterized, we sought to examine the frequency of structural heart disease and cardiac conduction disease (CCD) in patients with MA.

Methods

Using a retrospective cohort study design, we reviewed all muscle biopsies taken at a single tertiary care referral center neurology clinic from 2012 to 2018 for mitochondrial abnormalities. Patients were evaluated in the Neurology Clinic for muscle weakness. Skeletal muscle biopsies were obtained when deemed appropriate by the referring neurologist following their clinic appointment.

Patients ($n = 103$) with evidence of MA on muscle biopsy were identified, and of these two biopsies had evidence of diabetes mellitus with MA, 15 biopsies with inflammatory changes along with MA, one biopsy with a lysosomal storage disorder with MA, six biopsies with lipid disorders with MA, and one biopsy with vasculitis changes with MA on pathologic analysis. Evidence of MA on muscle biopsy was defined by the presence of more than 1% of one or more of the following: increased ragged red fibers, increased COX-negative fibers, excessive succinate dehydrogenase (SDH) staining, and increased subsarcolemmal NADH positive staining, as evaluated by two experienced pathologists and confirmed by review from a neurologist. Muscle biopsies were read and evaluated in a clinical neuromuscular pathology laboratory and the diagnosis of MA was confirmed by three independent providers. Genetic testing was completed in 36 of the 103 patients from skeletal muscle tissue (mtDNA testing) or blood (whole-exome sequencing or mitochondrial panels). Of these, nine patients had genetically confirmed mitochondrial myopathy, typically from multiple mitochondrial deletions (Table 1). Interestingly, a history of diabetes mellitus and hypertension was present in five and seven of these patients, respectively, highlighting the high percentage of concomitant cardiovascular risk factors (Table 2). None of the nine patients exhibited a low ejection fraction, although this assessment is limited by the

Table 1. Baseline characteristics of 9 patients with mitochondrial myopathy supported by genetic testing.

Patient ID#	Genetic testing results	EF (%)	PR-interval (msec)	QRS-duration (msec)	QTc-interval (msec)	FHx	DM	HTN
#1	Negative WES, multiple mito deletions, heteroplasmy < 15%	60–65	154	104	438	–	Yes	No
#2	2.3 kb mtDNA deletion, heteroplasmy 89%	NA	138	82	439	–	Yes	Yes
#3	Heteroz. for 2 POLG pathogenic variants	59	191	101	459	–	Yes	Yes
#4	7.9 kb mtDNA deletion, heteroplasmy < 15%	60	162	84	436	–	No	Yes
#5	6.5 kb mtDNA deletion, heteroplasmy 20%	NA	NA	NA	NA	+	No	No
#6	6 kb mtDNA deletion, heteroplasmy 55%	60–65	162	94	406	–	No	No
#7	WES negative, m.7510T > C mutation in MT-TS1 gene, 62% heteroplasmy	59	156	102	444	+	No	Yes
#8	Multiple mito DNA deletions, <15% heteroplasmy, SPG7 Heteroz., VUS in MRPL3	NA	106	126	431	+	Yes	Yes
#9	Two mito DNA deletions (12.8 kb and 7.7 kb), <15% heteroplasmy.	60–65	174	112	463	+	Yes	Yes

–, negative; +, positive; FHx, family history of sudden cardiac death or arrhythmias; Heteroz., heterozygous; Mito testing, mitochondrial genome testing utilizing serum samples; Mito, mitochondrial; NA, not applicable; VUS, variant of unknown significance; WES, whole-exome sequencing.

Table 2. Frequency of QRS duration > 120 msec, PR prolongation > 200 msec, systolic dysfunction, left ventricular hypertrophy, and hypertension in patients with biopsy-proven MA, as compared with population frequencies obtained from the Framingham and ARIC cohort populations.

	Patients with MA	Framingham Cohort population	ARIC Cohort population	Difference Framingham Cohort population (95% CI; *p*-value)	Difference ARIC Cohort population (95% CI; *p*-value)
QRS ≥ 120 msec	5/56 (8.9%)	169/8396 (2.0%)	377/14,478 (2.6%)	6.9% (1.8–17.2%; $p = 0.0003$)	6.3% (1.2–16.6%; $p = 0.0032$)
PR Prolongation (PR ≥ 200 msec)	7/56 (12.5%)	124/7575 (1.6%)	1351/14546 (9.3%)	10.9% (4.6–22%; $p < 0.0001$)	3.2% (−3.1 to 14.3%; $p = 0.4108$)
LV systolic dysfunction (EF ≤ 50%)	5/43 (11.6%)	340/9496 (3.6%)	71/2373 (3%)	8% (1.4–20.9%; $p = 0.0052$)	8.6% (2.0–21.5%; $p = 0.0014$)
Left Ventricular Hypertrophy	10/35 (28.6%)	676/4975 (13.6%)	598/5727 (10.4%)	15% (2.7–31.5%; $p = 0.0101$)	18.2% (5.9–34.7%; $p = 0.0005$)
History of Hypertension	47/103 (45.6%)	9623/22301 (43.2%)	3577/11061 (32.3%)	2.4% (−6.9 to 12%; $p = 0.6237$)	13.3% (4.0%-22.9%; $p = 0.0041$)

small sample size and lack of echocardiography in multiple patients. Unfortunately, many patients did not undergo genetic testing for financial or personal reasons and some patients were lost to follow-up.

The Framingham Heart Study and Atherosclerosis Risk in Communities (ARIC) cohorts served as control populations utilizing previously published data.[10–19] CCD was defined as a QRS duration ≥ 120 msec, or PR prolongation ≥ 200 msec while structural heart disease was defined as a left ventricular ejection fraction (LVEF) ≤50% or left ventricular hypertrophy (LVH) as assessed by 2-D echocardiography. LVH was defined as a left ventricular mass index (LVMI) of more than 115 g/m^2 in men or 95 g/m^2 in women, as recommended by current echocardiographic guidelines from the American Society of Echocardiography (ASE) according to the formula LV Mass (g) = 0.8{1.04[([LVEDD + IVSd + PWd]3 − LVEDD3)]} + 0.6.[20] Notably, the ARIC cohort (2017 study)[17] utilizes this current definition for LVH, whereas the Framingham cohort (1987 study)[16,18] utilized a higher LVMI cutoff of 131 g/m^2 for men and 100 g/m^2 for women. Of the 103 patients with MA, 43 patients (42%) had been evaluated with an echocardiogram utilizing 2D-measurements and analyzed for the presence of reduced left ventricular systolic function, and 35 patients (34%) had echocardiograms with mass index measurements completed and were evaluated for LVH. Of the 103 total patients, 59 patients (57.3%) had an electrocardiogram (EKG) completed and were evaluated for the presence of CCD. Of note, for most patients, cardiac measurements (EKG and echos) and muscle biopsy were performed within 12–24 months of each other. Included without cohort population comparisons are also chart reported histories of diabetes mellitus, coronary artery disease (CAD), and any family history of cardiomyopathies, arrhythmias, or sudden cardiac death (SCD) in the MA cohort. The chi-square test was used to compare categorical variables and the data are presented as mean differences in frequency with 95% confidence intervals. Statistical significance is defined as $p < 0.05$.

This study was approved by the Johns Hopkins School of Medicine institutional review board (#IRB00251040).

Results

We included all 103 patients (mean age 59.8 ± 1.4 years; 46% female) with evidence of MA on skeletal muscle biopsy from 2012 to 2018. A chart history of hypertension was present in 47 patients (45.6%), whereas 21 patients (20.4%) had a history of diabetes mellitus, and 20 patients (19.4%) had a history of CAD. A family history of cardiomyopathies, arrhythmias or SCD was present in 17 patients (16.5%). From our cohort, five of 56 patients (8.9%) had evidence of CCD (QRS ≥ 120 msec) and seven of 56 patients (12.5%) had evidence of PR prolongation (PR interval ≥ 200 msec) (Fig. 1). Notably, four patients in the MA cohort required implantation of a pacemaker for the presence of advanced conduction disease, and two patients had a history of ventricular tachycardia requiring an implantable cardioverter-defibrillator. The mean QTc interval in our MA cohort was 438 ± 26 msec, and when elevated was likely due to the presence of bundle branch block or delayed intraventricular conduction. For instance, the only patient with a QTc interval > 500 msec had an underlying QRS duration of 182 msec.

In our cohort, five of 43 patients (11.6%) had evidence of a reduced LVEF, and 10 of 35 patients (28.6%) had LVH. Among patients with LVH, concentric hypertrophy was the most common finding, and only one patient

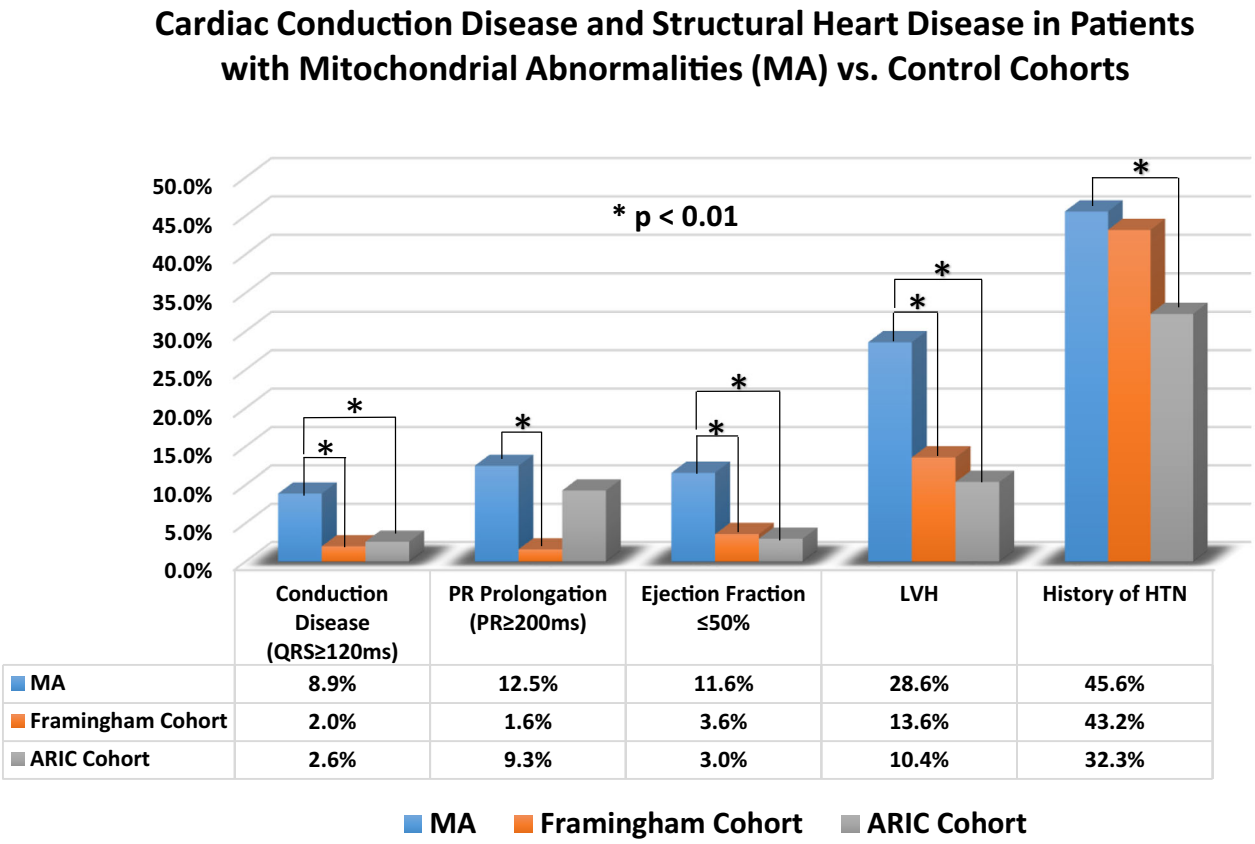

Figure 1. Graphical representation of the frequencies of conduction disease, PR prolongation, reduced ejection fraction (≤50%) and LVH in patients with MA (blue) compared with the Framingham (orange) and ARIC (gray) cohort populations. HTN, hypertension; LVH, left ventricular hypertrophy.

presented with asymmetric septal hypertrophy (ASH) defined as a septal to posterior wall ratio ≥ 1.3 (Table 3). Importantly, while hypertension may have contributed to the presence of LVH in some patients, our data suggest that LVH also occurred largely independently of hypertension, as four of 10 patients with LVH had no history of hypertension and the number of antihypertensive medications utilized by patients with LVH did not correlate

Table 3. Results of echocardiographic measurements of 10 patients with LVH, including relative wall thickness and classification of severity of hypertrophy.

Sex	Height (cm)	Weight (kg)	LVPW (cm)	IVS (cm)	LVEDD (cm)	LVMI (g/m^2)	RWT	IVS/LVPW	Hypertrophy classification	Number of HTN Meds
F	147	50.9	0.88	1.1	5.1	129	0.35	1.25	Severe eccentric	2
M	183	81.6	1.6	1.02	5.5	128	0.58	0.64	Mild concentric	1
F	163	60	0.8	0.88	5.7	110	0.28	1.1	Moderate eccentric	0
F	166	76.7	1.1	1.1	5.2	117	0.42	1	Moderate concentric	1
F	170	104.1	1.2	1.4	4.4	97	0.55	1.17	Mild concentric	0
F	160	47.1	1.3	1.4	3.5	113	0.74	1.08	Moderate concentric	0
F	160	43.8	1	1.5	3.8	117	0.53	1.5	Moderate concentric	3
F	155	72.1	1.1	1.1	5.2	125	0.42	1	Severe concentric	1
M	158	43.8	1.44	1.41	4.2	168	0.68	0.98	Severe concentric	0
M	188	143.9	1.07	1.32	6.3	124	0.34	1.23	Mild eccentric	1

F, female; HTN MEDS, antihypertensive medications; IVS, interventricular septum diameter; LVEDD, left ventricular end-diastolic diameter; LVMI, left ventricular mass index; LVPW, left ventricular posterior wall diameter; M, male; RWT, relative wall thickness.

with the degree of LVH (Table 3). However, the small number of patients with hypertension and LVH does not allow any conclusive statements about the causality of LVH independent of hypertension.

When compared to prior population cohort studies, a statistically significant difference was found in the frequency of CCD, reduced EF, and LVH. PR prolongation was only significantly higher when compared to the Framingham, but not the ARIC cohort, and a history of hypertension was significantly higher than the ARIC cohort, but not the Framingham population (Table 2, Fig. 1).

Discussion

The current case series is the largest study examining the prevalence of cardiovascular involvement in patients with solely MA and highlights increased rates of hypertension, CCD, and structural heart disease in these patients.

Limongelli et al. performed a similar, smaller cohort study of 32 patients with respiratory chain disease.[21] In their cohort, seven patients (22%) had intra-ventricular conduction abnormalities, one patient (3%) had first-degree atrioventricular block, and eight patients (25%) had cardiomyopathies.[21] Similar to this study, the study by Limongelli et al. included pathologic muscle biopsies on all patients supporting the diagnosis of MA.

A recent retrospective analysis of 260 patients with genetically proven mitochondrial syndromes reported a prevalence of hypertension of 41.5%. Of these, 85 patients (32.7%) were taking antihypertensive medications.[3] Similarly, in our cohort 47 patients (45.6%) had a chart history of hypertension, and 40 patients (38.8%) were taking antihypertensive medications, consistent with an increased prevalence of hypertension in this cohort. Importantly, the prevalence of hypertension was statistically higher in patients with MA compared to the ARIC cohort.[19] The pathophysiology of arterial hypertension in mitochondrial diseases remains poorly understood. Mitochondrial dysfunction leading to premature vascular aging and vascular endothelial dysfunction have been proposed.[3] Hypertension may be an independent risk factor for LVH, left ventricular systolic dysfunction, and conduction disease and may warrant regular screening and early treatment in this patient population. Despite the increased rate of cardiovascular disease in patients with mitochondrial syndromes, only ~50% of our patients with MA had EKGs or echocardiograms, indicating the relatively low rate of screening for cardiac disease in these patients.

Our study has several limitations. First, MA may result from normal aging and can also occur as secondary effects of other myopathies, such as polymyositis, dermatomyositis, drug toxicity (especially statin use), or inclusion body myositis.[9] As a result, CCD or structural cardiac abnormalities may not be related to the mitochondrial disorder, although a recent meta-analysis has found a higher prevalence of CCD and structural heart disease (39% and 29% respectively) than our study.[2] Second, there exists a potential for selection bias, as only patients with available EKG or echocardiographic data were included in this retrospective analysis which may falsely increase the number of abnormalities which were seen. Lastly, referral bias exists as patients from a tertiary referral center neurology clinic may not be representative of the general practice population. Prospective studies are necessary to determine both a more accurate estimate of the incidence and the natural progression of cardiac involvement in patients with MA.

Given the increased risk of hypertension, cardiac conduction disease, and structural heart disease, a muscle biopsy with associated MA may prompt practitioners to perform routine screening for cardiac disease. Multidisciplinary communication between pathologists, neurologists, and cardiologists would ensure that such biopsy findings are followed up appropriately.

Acknowledgment

This work was made possible, in part, through philanthropic support from Dr. Peter Buck and additional anonymous contributors.

Conflict of Interest

No conflict of interest has been declared by the authors of this manuscript.

Prior Submissions

There have been no prior submissions or publications of this study. An abstract of preliminary data from this study was presented electronically at the Heart Rhythm Society 2020 Conference in May 2020.

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