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Mid-Myocardial Fibrosis by Cardiac Magnetic Resonance in Patients with Lamin A/C Cardiomyopathy: Possible Substrate for Diastolic Dysfunction

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ABSTRACT

***Aims*: We sought to identify patterns of myocardial fibrosis *in vivo* in patients with lamin cardiomyopathy, and to determine its functional significance. *Methods and Results*: Eleven patients sharing the identical mutation in *LMNA* without contraindication to magnetic resonance were identified from a 1016-member pedigree. Eight autopsy hearts from deceased relatives were reviewed. Patients and age-matched controls underwent cardiac magnetic resonance that included measures of cardiac function and late gadolinium enhancement (LGE). LGE-CMR identified midmyocardial fibrosis of the basal interventricular septum in 5 of 11 *LMNA* patients that was identical to that seen in 6 autopsy specimens of related genotype-positive family members; this was not present in any of 11 controls. LGE-CMR was positive in the 5 oldest patients in the cohort, age 46 $\pm$ 6 years compared to 24 $\pm$ 10 years for LGE-negative subjects ($p = 0.003$). Systolic function was abnormal in 2 subjects, both with myocardial fibrosis. LGE-positivity distinguished patients with diastolic dysfunction by mitral inflow velocities from those with normal diastolic function; these patients also had significant left atrial enlargement compared to controls ($p < 0.05$). *Conclusions*: LGE-CMR can identify myocardial fibrosis under genetic control *in vivo* in patients with heritable cardiomyopathy similar in distribution to that observed at autopsy. Mid-myocardial fibrosis may form the substrate for diastolic dysfunction in these patients.**

INTRODUCTION

Over the past 5 decades, our institution has had the unique opportunity to define the natural history of progressive hereditary cardiac conduction and myocardial disease in a large kindred carrying a mutation in the lamin A/C gene (chromosome 1p1-1q1, *LMNA*) (1–4). Clinically, these patients develop atrial arrhythmias and atrioventricular (A-V) conduction abnormalities, and late-onset ventricular arrhythmias and myocardial dysfunction (Figure 1). Examination of 8 hearts of deceased family members at autopsy has demonstrated mid-myocardial fibrosis in all cases; chamber size showed atrial enlargement without significant ventricular dilatation (Figure 2) (3). *In vivo* detection with endomyocardial biopsy has been unsatisfactory due to the mid-myocardial location of fibrosis. Gadolinium-enhanced cardiovascular magnetic resonance (CMR) has demonstrated mid-myocardial fibrosis in patients with other forms of cardiomyopathy (5–8), suggesting that this technique might allow *in vivo* identification of myocardial fibrosis in *LMNA* patients.

We hypothesized that *LMNA* patients have myocardial fibrosis on late gadolinium enhancement (LGE) magnetic resonance imaging similar to that seen at autopsy, and that fibrosis produces

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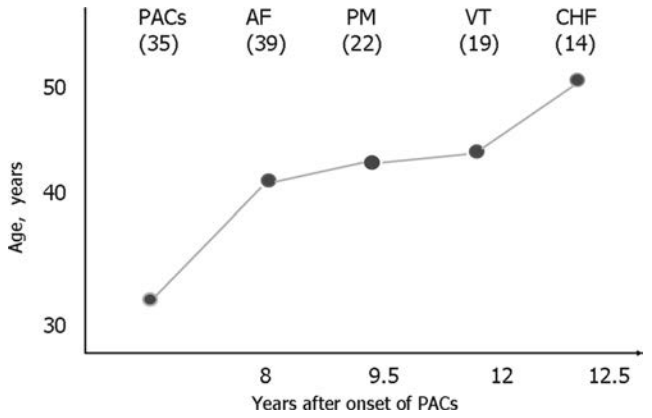


Figure 1. Natural history of cardiac manifestations in members of a family with *LMNA*-associated heritable cardiomyopathy with conduction abnormalities. Note that sudden cardiac death (SCD) has occurred in family members at various stages of cardiac manifestations. PACs = premature atrial contractions, Afib = atrial fibrillation, PM = pacemaker, VT = ventricular tachycardia, CHF = congestive heart failure.

measurable changes in cardiac function in the absence of overt symptoms of heart failure.

METHODS

We prospectively enrolled members of a large family with *LMNA* cardiomyopathy, of whom 348 members were genotyped as part of a longitudinal study. An identical frameshift mutation in *LMNA* had been identified in 56 family members at the time of enrollment.

Eleven genotype-affected family members without contraindication to magnetic resonance were available for study. Control subjects were healthy volunteers with no history of cardiomyopathy or skeletal muscle disorders. Written informed consent was obtained for participation in this Institutional Review Board-approved human subjects protocol.

Subjects without contraindication to MRI such as pacemakers, defibrillators, severe claustrophobia, ferromagnetic foreign body, or aneurysm clip underwent cardiac magnetic resonance (CMR). All CMR examinations were performed on a 1.5 Tesla clinical scanner (MAGNETOM Avanto, Siemens Medical Solutions, Malvern, Pennsylvania, USA) using a standard 12-channel cardiac coil. CMR protocol included cine imaging for ventricular volume and function quantification using a steady-state free precession (SSFP) pulse sequence (9) with the following typical acquisition parameters: matrix dimensions 192 × 256 pixels, field of view 360 × 400 mm, slice thickness 8 mm, interslice gap for contiguous short-axis cine acquisitions 2 mm, variable temporal resolution based on heart rate (typically 40–50 ms), TE 1.0 msec, flip angle 63°, and bandwidth 930 kHz/pixel. Measurement of left ventricular volumes and ejection fraction was done by semi-automatically tracing the endocardial and epicardial LV borders of the short axis cine images with dedicated segmentation software (Argus, Siemens Medical Solutions). Left atrial area was measured by drawing a contour excluding pulmonary venous ostia on the end-ventricular systole frame of a horizontal long-axis cine acquisition.

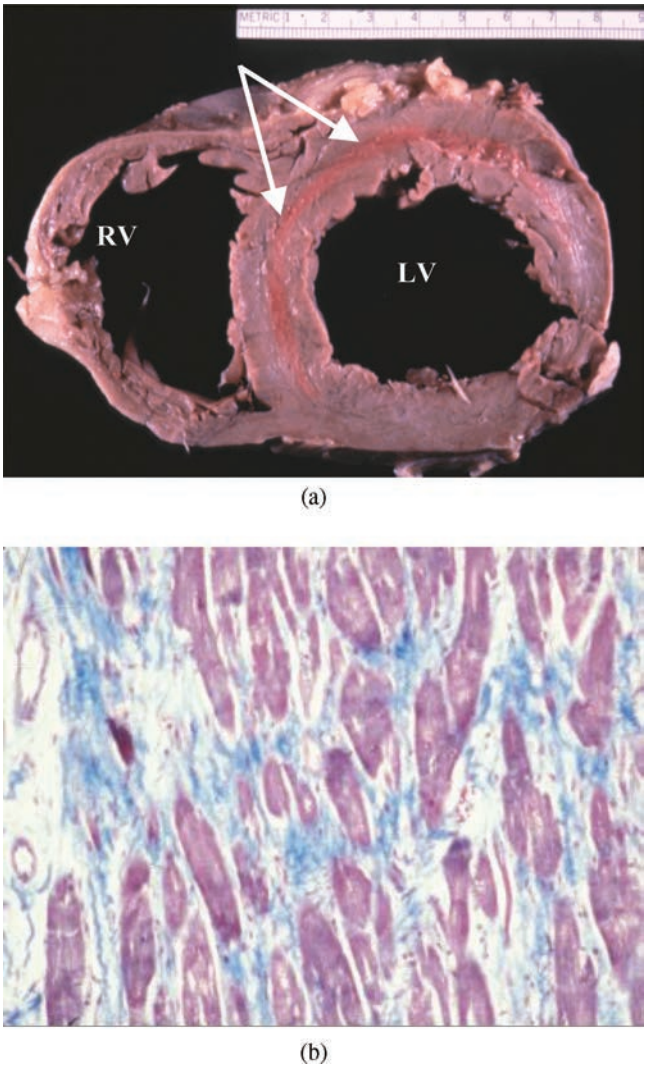


Figure 2. (a) Mid-septal fibrosis demonstrated at autopsy in a family member (Image reproduced with permission from Nature Genetics [3]. www.nature.com) (b) Masson's Trichrome stain of the left ventricular free wall obtained at autopsy of a Family OSU member with sudden cardiac death demonstrates extensive staining for collagen fibers (blue) consistent with fibrosis.

Transmitral flow was measured using a retrospectively electrocardiographically gated, breathhold gradient-echo, phase-contrast sequence with a velocity sensitivity of 150 cm/s prescribed on the ventricular side of the mitral valve. The following scan parameters were typically used: matrix 132 × 192 pixels, field of view 275 × 400 mm, slice thickness 6 mm, bandwidth 355 kHz/pixel, temporal resolution based on heart rate, TE 2.0 ms, and flip angle 25°. Peak early (E) and late (A) filling velocities were recorded from diastolic portion of velocity-time curves generated by drawing a region of interest around the mitral valve

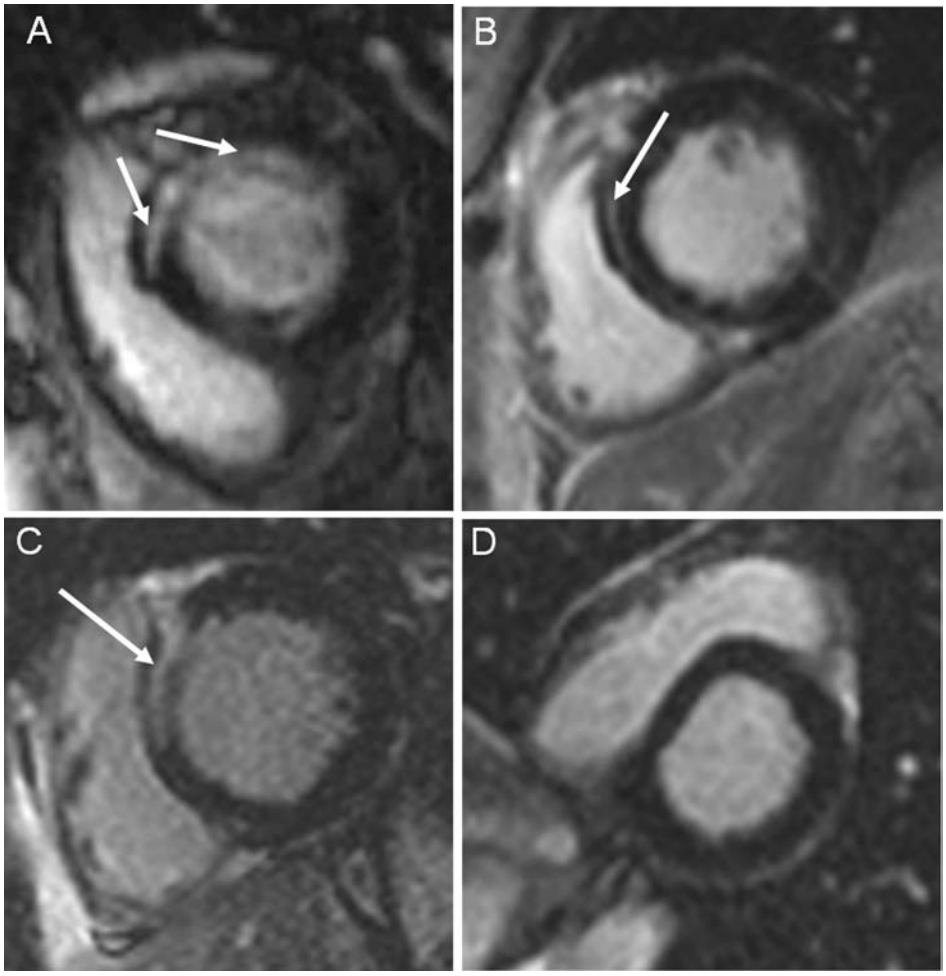


Figure 3. Mid-septal fibrosis identified as enhancement of the mid ventricular septum (arrows) on late gadolinium enhancement cardiac magnetic resonance imaging in the short-axis plane in three *LMNA* patients (a–c) compared with myocardium of a normal control subject (d) on late gadolinium enhancement magnetic resonance imaging.

on the phase-contrast mitral inflow images covering the cardiac cycle. Presence of diastolic dysfunction was defined in this relatively young population by a ratio of Mitral E velocity to Mitral A velocity $\leq$1:1.

LGE imaging was performed 5–10 minutes after intravenous administration of 0.2 mmol/kg gadolinium-DTPA using a single-shot inversion recovery SSFP sequence (10) as well as a segmented, breath-hold, phase-sensitive inversion recovery pulse sequence (11). Typical single shot LGE parameters were 120 $\times$ 192 matrix, TR 2.8 ms, TE 1.0 ms, flip angle 50°, BW 1000 Hz/pixel. Typical segmented breath-hold LGE scan parameters were as follows: 156 $\times$ 192 matrix, field of view 400 $\times$ 450 mm, slice thickness 8 mm, TR 850 ms, TE 4 ms, BW 130 kHz, flip angle 25°. Myocardial hyperenhancement on LGE images was defined as presence of myocardial signal intensity 2 standard deviations above the mean of normal myocardium.

Clinical severity of cardiomyopathy was assigned a stage based on clinical history and review of electrocardiographic data: Stage 0, no apparent cardiac involvement; Stage 1, atrial and ventricular premature beats; Stage 2, atrial fibrillation or supraventricular tachycardia; Stage 3, atrioventricular block; Stage 4, malignant ventricular arrhythmias; Stage 5, ventricular dysfunction.

Continuous variables were recorded as mean $\pm$ SD. Comparison of continuous variables between patients with versus those without myocardial fibrosis was performed using the Wilcoxon rank-sum test. P values $<$0.05 were considered significant.

RESULTS

Table 1 summarizes the clinical characteristics of the study population. Clinical, electrocardiographic, and imaging data are summarized in Table 2. Myocardial fibrosis by LGE imaging was present in 5 of 11 *LMNA* patients and none of the controls (Figure 3). The *LMNA* patients with hypertension (N = 2) had excellent BP control with medication, and neither had midwall enhancement on LGE. The pattern of myocardial enhancement with predominant involvement of the basilar interventricular

Table 1. Clinical characteristics of the study population

Variable	LMNA patients (n = 11)	Normal Controls (n = 11)
Age (mean ± SD), years	33.1 ± 13.2	37.1 ± 9.3
Female, %	63.6	45.4
Smokers, %	27.2	27.2
Hypertension, %	18.1	9.0
Diabetes, %	0	0
Caucasian, %	100	82.4

septum observed by CMR was similar to the distribution of fibrosis previously described by autopsy examination of hearts from related family members with identical *LMNA* mutation (Figure 4).

Figure 5 summarizes the quantitative CMR results comparing LGE-positive *LMNA* patients, LGE-negative *LMNA* patients, and controls. LV systolic function indicated by ejection fraction was normal (>50%) in all but 2 patients, both LGE-positive. Overall, *LMNA* patients had comparable LV ejection fraction compared to controls. There was no significant difference between LGE-positive and LGE-negative patients' LV end-systolic and end-diastolic volumes (40.6 ± 22.3 mL/m^2 vs. 29.8 ± 8.5 mL/m^2 and 79.8 ± 20.3 mL/m^2 vs. 72.3 ± 11.5 mL/m^2, respectively). LGE-positive patients had significant left atrial enlargement compared to LGE-negative patients and controls (21.6 ± 4.0 vs. 16.8 ± 3.5 vs. 16.3 ± 3.6 cm^2, $p < 0.05$ for LGE-positive patients vs. controls and $p = 0.07$ for LGE-positive patients vs. LGE-negative patients), similar to autopsy findings of atrial enlargement more than ventricular dilatation. Mitral E/A ratio was 1:1 in three LGE-positive compared to 1.8:1 ± 0.2 in LGE-negative subjects ($p = 0.02$, Figure 6). In a fourth LGE-positive patient, mital inflow velocities showed a pseudonormal pattern (E 0.6 m/s, A 0.4 m/s, Em 0.06 m/s) consistent with moderate diastolic dysfunction. PR interval was longer in LGE-positive patients compared to LGE-negative patients (236 ± 55 ms vs. 158 ± 30 ms, $p = 0.03$), consistent with older age at time of pacemaker requirement from natural history data (Figure 1).

Table 2. Clinical and structural characteristics of the *LMNA* study population. LGE = delayed myocardial enhancement imaging, which was performed only in patients who underwent MR examination.

LMNA Patient No.	Age, years	Cardiac Disease†	PR interval, ms	LGE	LVEDVI, mL/m^2	LVEF, %
2	30	1	170	Negative	81	54
3	31	1, 2	200	Negative	60	52
4	35	1	140	Negative	89	52
5	22	0	110	Negative	65	62
6	16	0	165	Negative	76	72
7	10	0	160	Negative	63	62
1	36	1, 3	260	Positive	59	64
8	37	1, 2, 4	180	Positive	89	49
9	51	1, 2, 3	220	Positive	66	61
10	48	1, 2, 3, 5	320	Positive	110	30
11	48	1, 2	220	Positive	105	68

†0: no apparent cardiac manifestations, 1: atrial and ventricular premature complexes; 2: atrial fibrillation or supraventricular tachycardia; 3: atrioventricular block; 4: malignant ventricular arrhythmias; 5: left ventricular systolic dysfunction.

DISCUSSION

In this work, we demonstrated myocardial fibrosis using *in vivo* cardiac magnetic resonance in patients with *LMNA* HC-CMD that appeared virtually identical to that found at autopsy in related family members. We also discovered abnormal left ventricular diastolic function that paralleled the presence of mid-myocardial enhancement on LGE-CMR.

LGE imaging is arguably the most significant advance in CMR in the last decade, allowing for noninvasive detection of myocardial fibrosis with an accuracy matched only by TTC staining of the explanted heart and not feasible with other cardiac imaging modalities. While originally developed to identify extent of infarcted tissue after myocardial infarction (12), subsequent studies have shown this technique's utility in identifying myocardial fibrosis in dilated cardiomyopathy (5), hypertrophic cardiomyopathy (6), Chagas cardiomyopathy (13) and arrhythmogenic right ventricular cardiomyopathy (14). This work extends the investigation of non-ischemic cardiomyopathies using LGE-CMR to *LMNA* cardiomyopathy, with more extensive validation of imaging findings to autopsy evidence of myocardial fibrosis. The localization of scar to the mid-myocardium provides the first possible *in vivo* link between myocardial substrate and diastolic dysfunction in this group, substrate which may also be responsible for the later-onset systolic dysfunction characteristic of *LMNA* cardiomyopathies.

While LGE has been well-described in patients with dilated cardiomyopathy, lamin cardiomyopathy is not typically a disease of predominant ventricular dilatation. Rather, patients present with more atrial than ventricular enlargement as was confirmed in this work. Furthermore, this work adds to our understanding of genotype-phenotype interactions unlike most prior investigations of LGE in cardiomyopathy that did not have a single gene mutation common to the affected patients studied. Finally, this work presents the largest published experience to date of autopsy correlation, albeit in related family members, of a genetic myocardial and conduction system disease to corroborate LGE findings.

The results of this work build upon a recent report of abnormal myocardial tissue Doppler and strain recordings in a cohort of *EDMD2* patients that notably lacked myocardial fibrosis by CMR, leading the authors to conclude that diastolic dysfunction precedes scarring (15). Serial assessment in both populations would help clarify the mechanistic sequence of events, as would discovery of possible additional genetic factors that influence loss of myocyte viability. Direct histopathological assessment in *EDMD2* cardiomyopathy would also enhance further understanding of *EDMD2* and elucidate additional distinguishing features of these two diseases.

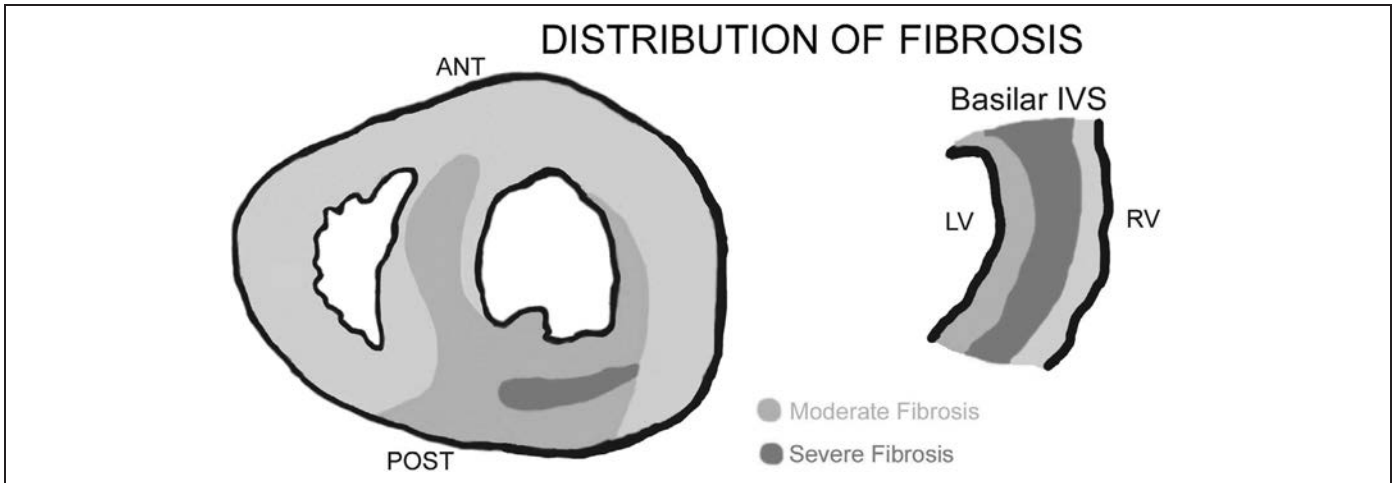


Figure 4. Distribution of fibrosis observed from review of autopsy data demonstrates predominant involvement of the basilar interventricular septum (IVS). ANT = anterior, POST = posterior, LV = left ventricle, RV = right ventricle.

Lamin A/C is a nuclear envelope protein whose mutations have been linked to cardiomyopathies with conduction defects but also a variety of other disease such as muscular dystrophies, progeria, and lipodystrophy (16–18). Cardiac involvement may occur in any of these disorders with variable onset and severity that is difficult to predict based on genotype alone (19). Appreciation of "overlapping phenotypes" suggests that direct myocardial assessment afforded by noninvasive CMR can establish the presence and extent of cardiac involvement in patients with laminopathies, facilitating earlier diagnosis and deployment of potentially life-saving therapies.

Mutations in *LMNA* account for a significant proportion of familial cardiomyopathy especially those with atrioventricular block (20), and an unknown percent of all cardiomyopathies. The cohort with *LMNA* mutation studied in this work was selected based on absence of devices such as pacemaker that

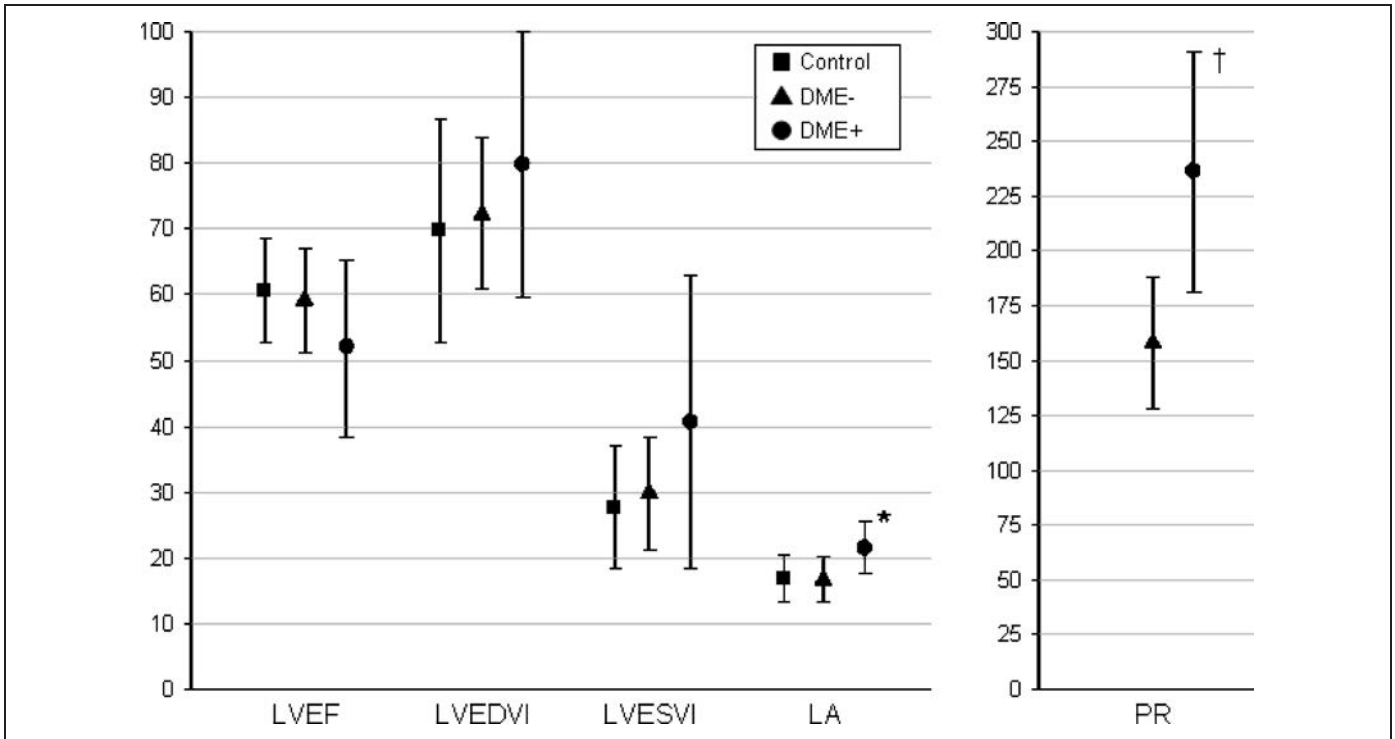
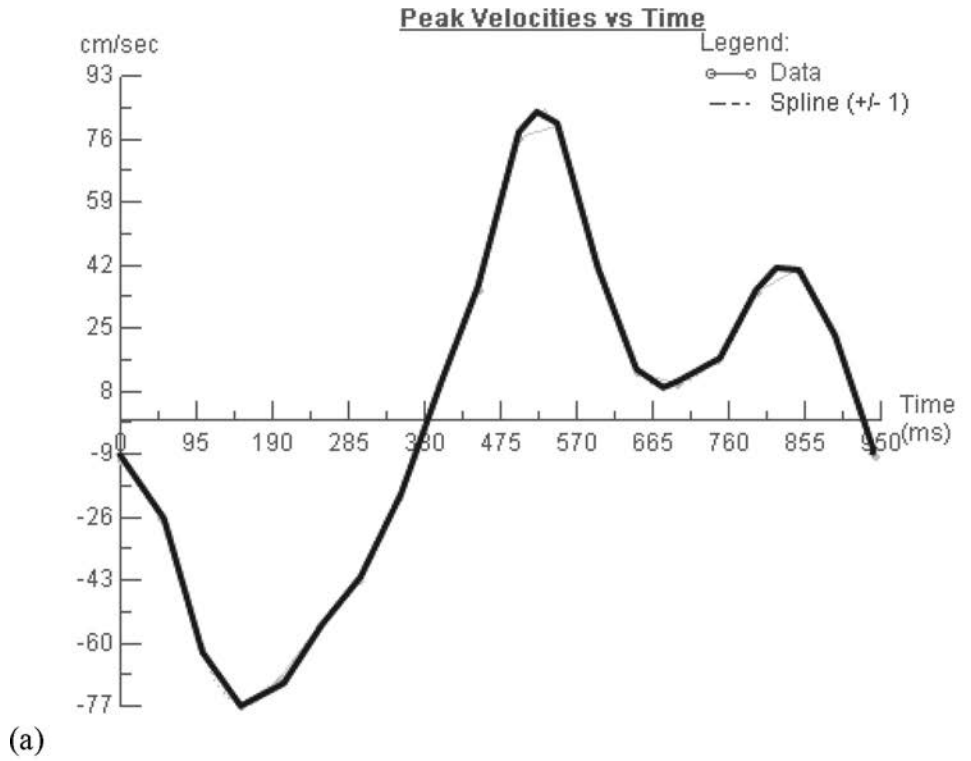
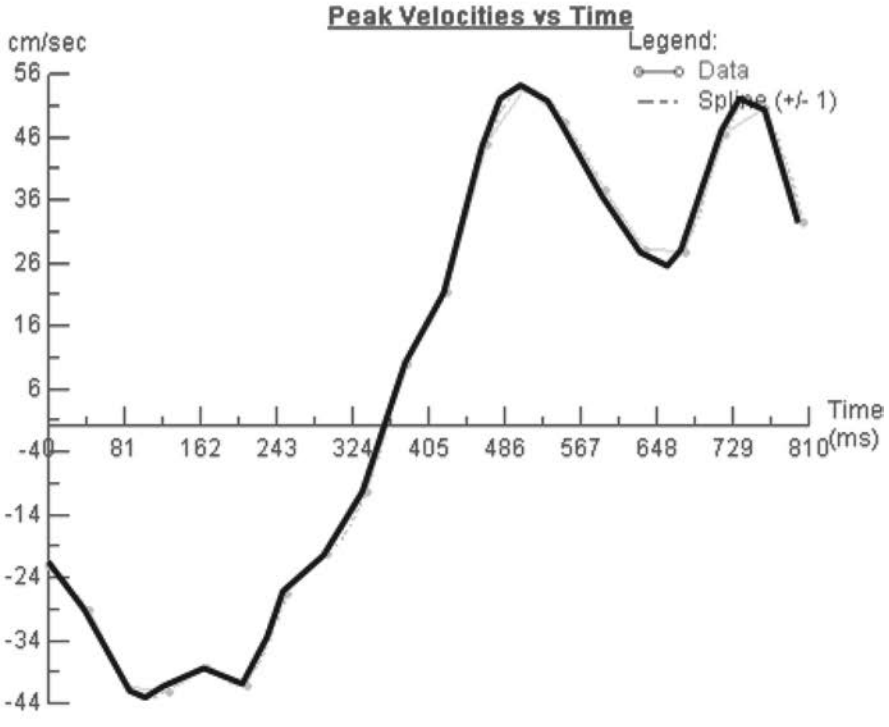


Figure 5. Measurements of cardiac size and function from cardiac magnetic resonance and PR intervals in patients versus control subjects. $P < 0.05$ for left atrial area (LA, cm^2) in LGE-positive *LMNA* patients vs. controls and PR interval in LGE-positive vs. LGE-negative *LMNA* patients. LVEF = left ventricular ejection fraction (%), LVEDVI = left ventricular end-diastolic volume index (mL/m2), LVESVI = left ventricular end-systolic volume index (mL/m^2), and PR interval is expressed in milliseconds.



(a)



(b)

Figure 6. Mitral inflow velocity-time curve from a *LMNA* patient without (A) versus a *LMNA* patient with (B) myocardial fibrosis indicates diastolic dysfunction in the latter.

would preclude MR examination, thereby selecting patients with earlier stage myocardial disease. This may explain the minority of *LMNA* patients (45%) who exhibited LGE-positivity in this study. The patients in this study population also had mild PR prolongation compared to controls. The cause of AV block in *LMNA*-related cardiomyopathies has been postulated to be myocardial fibrosis near the region of the conduction system, though evidence of this process to date has been shown only at autopsy (1, 2). The midmyocardial fibrosis demonstrated on CMR is proximal to the compact AV node, which may be difficult to visualize with the 1.5T CMR technique used in this study. Myocardial scar does, however, provide a substrate for ventricular dysfunction and ventricular arrhythmias (21). Further studies are needed to extend these findings to the ventricular arrhythmias seen in *LMNA* cardiomyopathy, which were present in only one of the patients in this cohort.

CONCLUSIONS

This work showed that genotype-positive *LMNA* patients with heritable cardiomyopathy have midmyocardial fibrosis that can be detected noninvasively with CMR. The pattern of fibrosis was similar to that described at autopsy in family members with evidence of advanced myocardial disease prior to death. Myocardial fibrosis was the likely substrate for diastolic dysfunction in this cohort; patients without fibrosis had normal diastolic function. These findings are consistent with the myocardial disease that occurs in these patients characterized primarily by diastolic dysfunction early in the disease course, followed later by progressive systolic dysfunction. Given the high phenotypic variability of cardiac disease in the broad spectrum of *LMNA*-related disorders, further investigation of the role of CMR in relating myocardial phenotype to genotype are warranted.

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