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A comprehensive evaluation of myocardial fibrosis in hypertrophic cardiomyopathy with cardiac magnetic resonance imaging: linking genotype with fibrotic phenotype

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Aims In hypertrophic cardiomyopathy (HCM), attempts to associate genotype with phenotype have largely been unsuccessful. More recently, cardiac magnetic resonance (CMR) imaging has enhanced myocardial fibrosis characterization, while next-generation sequencing (NGS) can identify pathogenic HCM mutations. We used CMR and NGS to explore the link between genotype and fibrotic phenotype in HCM.

Methods and results One hundred and thirty-nine patients with HCM and 25 healthy controls underwent CMR to quantify regional myocardial fibrosis with late gadolinium enhancement (LGE) and diffuse myocardial fibrosis with post-contrast T_1 mapping. Collagen content of myectomy specimens from nine HCM patients was determined. Fifty-six HCM patients underwent NGS for 65 cardiomyopathy genes, including 36 HCM-associated genes. Post-contrast myocardial T_1 time correlated histologically with myocardial collagen content ($r = -0.70$, $P = 0.03$). Compared with controls, HCM patients had more LGE (4.6 ± 6.1 vs. 0%, $P < 0.001$) and lower post-contrast T_1 time (483 ± 83 vs. 545 ± 49 ms, $P < 0.001$). LGE negatively correlated with left-ventricular (LV) ejection fraction and outflow tract obstruction, whereas lower post-contrast T_1 time, suggestive of more diffuse myocardial fibrosis, was associated with LV diastolic impairment and dyspnoea. Patients with identifiable HCM mutations had more LGE (7.9 ± 8.6 vs. 3.1 ± 4.3%, $P = 0.03$), but higher post-contrast T_1 time (498 ± 81 vs. 451 ± 70 ms, $P = 0.03$) than patients without.

Conclusion In HCM, contrast-enhanced CMR with T_1 mapping can non-invasively evaluate regional and diffuse patterns of myocardial fibrosis. These patterns of fibrosis occur independently of each other and exhibit distinct clinical associations. HCM patients with recognized genetic mutations have significantly more regional, but less diffuse myocardial fibrosis than those without.

Keywords Hypertrophic cardiomyopathy • Magnetic resonance imaging • T_1 mapping • Myocardial fibrosis • Genotyping

Introduction

While hypertrophic cardiomyopathy (HCM) is defined by the presence of otherwise unexplained left-ventricular (LV) hypertrophy associated with non-dilated ventricular chambers,[1] precisely why the clinical manifestations and natural history of this condition are so diverse remains uncertain.

Advances in non-invasive tissue characterization with contrast-enhanced cardiac magnetic resonance (CMR) imaging have facilitated recognition of the importance of myocardial fibrosis in HCM. Late

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gadolinium enhancement (LGE) indicates a regional increase in myocardial collagen content,[2] and its presence and extent predict adverse outcomes.[3] However, LGE sequences cannot reliably evaluate the more diffuse patterns of myocardial fibrosis observed histologically in explanted hearts of HCM patients.[4] T_1 mapping is a novel technique to detect and quantify such diffuse myocardial fibrosis, and several methods have correlated with myocardial collagen content measured in histological specimens.[5,6]

Confirmation of a genetic basis for HCM was first described over two decades ago using linkage analysis[7] and, despite the subsequent identification of several disease-causing genes,[8] attempts to link genotype with phenotype have largely been unsuccessful. HCM patients possessing a recognized pathogenic HCM mutation are known to be younger at initial diagnosis,[9] exhibit more LV hypertrophy,[9] and are at a heightened risk of symptom progression and adverse cardiovascular outcomes,[10] however, clinically relevant associations with specific mutations have not been made. The development of next-generation sequencing (NGS) enables fast and cost-efficient testing of all known HCM genes to identify pathogenic mutations.[11]

In the present study, we performed a comprehensive evaluation of both diffuse and regional patterns of myocardial fibrosis in a typical cohort of HCM patients using contrast-enhanced CMR. Furthermore, we utilized NGS to explore links between genotype and phenotype in HCM.

Methods

Patient selection

All research was performed at the Alfred Hospital, Melbourne, Australia. One hundred and thirty-nine consecutive patients referred to our CMR department for the further evaluation of established asymmetric septal hypertrophy (ASH) or apical hypertrophy due to HCM were invited to participate. Asymmetric septal hypertrophy was defined as a maximum interventricular septum thickness $\geq$15 mm with a ratio of septal-to-lateral LV wall thickness of $\geq$1.3:1.0, while apical hypertrophy was defined as LV apical wall thickness $\geq$12 mm. Diagnosis of HCM required the absence of another condition that could cause the degree of hypertrophy observed.[1] Twenty-five asymptomatic subjects without cardiovascular disease or a family history of HCM formed a healthy control group.

Exclusion criteria included prior septal reduction therapy, coronary artery disease, chronic atrial fibrillation, contraindications to CMR, including pacemaker and defibrillator implantation, and significant renal dysfunction [estimated glomerular filtration rate (eGFR) $<$ 50 mL/min/1.73 m^2].

Informed consent was obtained from all participants and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the Alfred Hospital Ethics Committee's guidelines.

CMR protocol

We performed CMR on all patients using a clinical 1.5-T scanner (Signa HD 1.5-T, GE Healthcare, Waukesha, WI, USA). All sequences were acquired during breath-holds of 10–15 s. Initially, a contiguous short-axis steady-state free precession cine stack [repetition time [TR] = 3.8 ms, echo time [TE] = 1.6 ms, 30 phases] was acquired, extending from the mitral valve annulus to the LV apex (8 mm slice thickness, no gap), to enable volumetric analysis of the LV using the summation of disc method.

Late gadolinium enhancement was evaluated 10 min after a bolus of gadolinium-diethylene triamine penta-acetic acid (DTPA) (0.2 mmol/kg BW Magnevist, Schering, Germany) to identify regional myocardial fibrosis using a T_1-weighted inversion recovery gradient echo technique [TR 7.1 ms, TE 3.1 ms, inversion time [TI] individually determined to null the myocardial signal, 8 mm slice thickness, acquisition matrix 256 $\times$ 192, number of acquisitions 2, inversion pulse every RR interval]. To enable accurate nullification of healthy myocardium, a TI optimization sequence was performed 8 min post-gadolinium administration with a fast gradient echo, inversion recovery, gated, multiphase acquisition, commencing at an inversion time of 150 ms, and increasing in 25 ms increments to 250 ms, in a mid-ventricular short-axis slice. LGE imaging was performed using standard long-axis views of the LV and a contiguous short-axis stack from the mitral valve annulus to the LV apex. Regional fibrosis was identified by LGE within the myocardium, defined quantitatively by a myocardial post-contrast signal intensity 6 SD above that within a reference region of remote myocardium (without LGE) within the same slice.[12,13] Late gadolinium enhancement quantity, expressed as a percentage of total LV mass, was calculated from all short-axis slices using the summation of disc method.

To evaluate diffuse myocardial fibrosis, a histologically validated post-contrast T_1 mapping sequence was used to cycle through acquisition of images obtained at an LV short-axis level over a range of inversion times, as described previously.[6] This adiabatic electrocardiogram-triggered, inversion recovery prepared, 2-dimensional fast gradient echo sequence employed variable temporal sampling of k-space (VAST)[14] (GE Healthcare). Ten images at the basal, mid, and apical LV short-axis levels were acquired sequentially at increasing inversion times, commencing 20 min after the bolus of gadolinium-DTPA (TI range 75–750 ms), and each over a series of 3–5 breath-holds using the following imaging parameters: TR 3.7 ms, TE 1.2 ms, 20° flip angle, 256 $\times$ 128 acquisition matrix, 36 $\times$ 27 cm field of view, 1.4 $\times$ 2.1 mm in plane resolution, 8 mm slice thickness, trigger delay 300 ms (2 R-R intervals were utilized if the trigger delay plus preparation pulse delay was longer than one R-R interval, and the trigger delay was reduced if the trigger delay plus preparation pulse delay was longer than two R-R intervals), and views per segment 24. In contrast to the commonly used Modified Look-Locker inversion recovery (MOLLI) technique, each preparation pulse was followed by a single readout phase to acquire each of the 10 images. Following acquisition, images were transferred to an external computer for analysis using a dedicated research software package with a curve fitting technique to generate T_1 maps (Cinetool, GE Healthcare). For each short-axis image, a region of interest (ROI) was manually drawn around the entire LV myocardium (excluding regions of LGE by visual assessment) to calculate post-contrast myocardial T_1 time (*Figure 1*). Post-contrast myocardial T_1 time was derived by calculating the mean T_1 time of all three short-axis levels. To account for the potential effects of renal function and time delay between contrast administration and image acquisition on gadolinium pharmacokinetics, correction values[15] were used to normalize post-contrast myocardial T_1 times to a matched state (eGFR = 90 mL/min/1.73 m^2, time from contrast administration to image acquisition = 20 min) for all three short-axis levels. To investigate the association between post-contrast myocardial T_1 time and the myocardial collagen content of septal myectomy specimens (see Histology), identical T_1 mapping methodology was used; however, the ROI was selected to include the likely site of surgical excision within the basal interventricular septum.

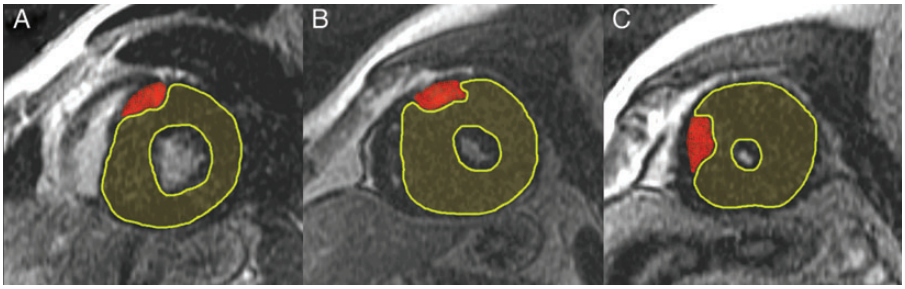

Figure 1: Calculating myocardial T_1 time in HCM. Post-contrast T_1 mapping image acquired at the basal (*A*), mid (*B*), and apical (*C*) ventricular short-axis level in a patient with HCM. The regions of interest to calculate T_1 time (demarcated by a yellow line) excluded areas of late gadolinium enhancement (shaded red).

Histology

In a subgroup of patients with HCM who underwent septal myectomy within 4 weeks following CMR, histological quantification of myocardial collagen content was performed using surgically obtained specimens. Myectomy tissue specimens were fixed immediately in 10% buffered formalin, embedded in paraffin, routinely processed, and stained with picrosirius red to obtain contrast between myocardium and areas of collagen (*Figure 2*). At least 30 digital images per patient at 100× magnification were acquired from all myectomy specimens, with subendocardial and perivascular areas excluded from further analysis. An automated image analysis protocol (using an ImageJ macro[16]) determined myocardial collagen content, with myocardial collagen area expressed as a percentage of total myocardial area for each patient.

Echocardiography protocol

Echocardiography with a standard clinical protocol was performed immediately prior to CMR. Diastolic function was assessed by a combination of mitral inflow pattern (E to A ratio) and early mitral annular velocities (e′, measured at the septal and lateral aspects of the mitral annulus in the apical 4-chamber view). Mitral E/e′ was chosen as an index of LV diastolic function. In all subjects, peak LV outflow tract (LVOT) pressure gradients were measured with pulse wave Doppler both at rest and during Valsalva provocation. All measurements were made in accordance with the American Society of Echocardiography guidelines.[17]

HCM genetic mutation detection

A subgroup of HCM patients underwent clinical genetic testing after CMR for a panel of 65 cardiac disease genes using massively parallel sequencing approach on lymphocyte-derived DNA. Thirty-six genes associated with HCM were included in a targeted exon capture array (NimbleGen capture array or Agilent Sure Select), with the products sequenced on an Illumina HiSeq platform. All low-coverage regions (<15×) were re-sequenced by Sanger-sequencing. Data analysis was performed with a custom bioinformatics pipeline,[18] including the tools GATK for alignment, ANNOVAR[19] for annotation of variants and in-house scripts for analysis of splice effects. Variants were classified into five classes, including pathogenic or likely pathogenic mutations, based on an algorithm that incorporates reference to previous publications, locus specific databases, large population sequencing databases (dbSNP, 1000 Genomes, NIH Exome Variant Server), *in-silico* analysis of evolutionary conservation and physicochemical effects (GERP score, PhyloP, SIFT, Polyphen2, A_GVGD) and any available functional data.

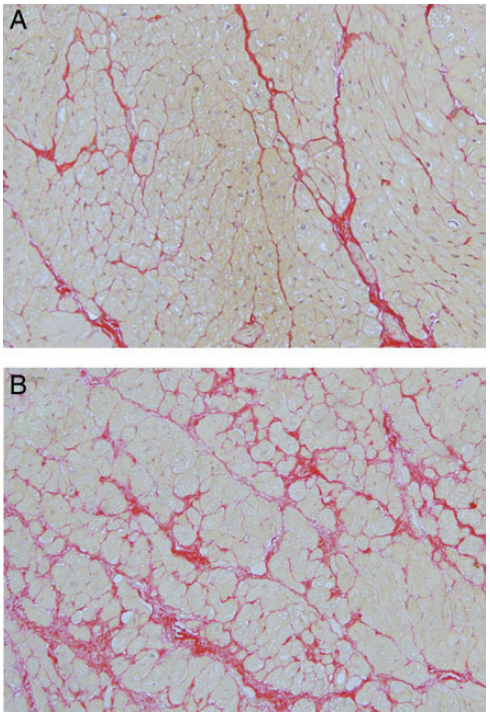

Figure 2: Myocardial tissue in HCM obtained by surgical myectomy. At 100 × magnification. Staining with picrosirius red identified collagen as red and myocytes as yellow. Myocardial collagen content was 7.5% (*A*) and 18.9% (*B*).

Final classifications were reviewed by an expert panel of clinicians and molecular geneticists blinded to patients' clinical data. Patients with pathogenic or likely pathogenic mutations were classified as gene-positive (G+), while remaining patients who had either no identifiable mutations or variants of uncertain significance (VUS) were considered gene-negative (G−).

Image analysis

CMR and echocardiographic images were interpreted by two experienced readers unaware of the subjects' clinical information and other

diagnostic test results. Endocardial and epicardial LV contours were drawn manually for each diastolic and systolic frame, excluding papillary muscles. CMR LV volumetric and LGE analysis was performed on a GE Healthcare Advantage Workstation 4.2 using ReportCARD 3.6 software.

Statistical analysis

All data are expressed as mean ± standard deviation (SD) unless otherwise indicated. For all comparisons, a *P*-value of <0.05 was considered significant, and all reported *P*-values are 2-tailed. Comparison of normally distributed variables utilized the unpaired Student's *t*-test; of non-parametric data, the Wilcoxon–Mann–Whitney test; and, of categorical data, the χ^2 test. Correlations of variables were determined by calculating the Pearson Product Moment. Multiple linear regression was used to determine the independence of correlations observed on simple linear regression, with all correlations with a *P*-value <0.10 entered into multiple linear regression analysis. Binary categorical variables were entered into the analyses using dummy coding. T_1 times were compared across groups using a one-way analysis of variance (ANOVA) with *post hoc* testing (Holm–Sidak method) to correct for multiple comparisons. All analyses were conducted using Stata software version 11.1 (StataCorp, College Station, TX, USA).

Results

Subject characteristics

A total of 139 patients with HCM from 133 unrelated families, and 25 healthy control subjects were included during the study period. General characteristics of both groups are presented in *Table 1*. The HCM and control groups had similar age, heart rate, systolic blood pressure, haematocrit, and eGFR, while the HCM group had significantly higher body mass index (BMI, 28.1 ± 5.2 vs. 24.1 ± 2.7 kg/m^2, $P < 0.001$).

Table 1 Subject characteristics

	Control ($n = 25$)	HCM ($n = 139$)	*P*-value
Age, year	47 ± 17	51 ± 14	0.2
Males, *n* (%)	18 (72)	91 (65)	0.4
BMI, kg/m^2	24.1 ± 2.7	28.1 ± 5.2	<0.001
Family history of HCM, *n* (%)	0 (0)	40 (29)	0.001
Dyspnoea, *n* (%)	0 (0)	96 (69)	<0.001
NYHA class I	25 (100)	43 (31)	—
NYHA class II	0 (0)	89 (64)	—
NYHA class III	0 (0)	7 (5)	—
NYHA class IV	0 (0)	0 (0)	—
Current medication, *n* (%)			
Beta-blocker	0 (0)	68 (49)	<0.001
Calcium channel blocker	0 (0)	29 (21)	<0.01
ACE-inhibitor	0 (0)	35 (25)	<0.01
ARB	0 (0)	12 (9)	0.11
Aldosterone antagonist	0 (0)	4 (3)	0.5
Heart rate, beats/min	62 ± 9	62 ± 11	0.9
Systolic blood pressure, mmHg	131 ± 18	130 ± 17	0.9
Haematocrit	0.42 ± 0.03	0.42 ± 0.03	0.7
eGFR, mL/min/1.73 m^2	86 ± 8	83 ± 10	0.07

HCM, hypertrophic cardiomyopathy; BMI, body mass index; NYHA, New York Heart Association; ACE, angiotensin-converting enzyme; ARB, angiotensin-receptor blocker; eGFR, estimated glomerular filtration rate.

Histological validation of post-contrast T_1 mapping in HCM

Septal myectomy was performed in nine HCM patients. Mean myocardial collagen content of myocardial specimens was 12.8 ± 5.3%. A significant correlation between post-contrast myocardial T_1 time and myocardial collagen content was observed ($r = -0.70$, $P = 0.03$) (*Figure 3*). Late gadolinium enhancement was not observed within the ROIs defining the presumed sites of surgical excision.

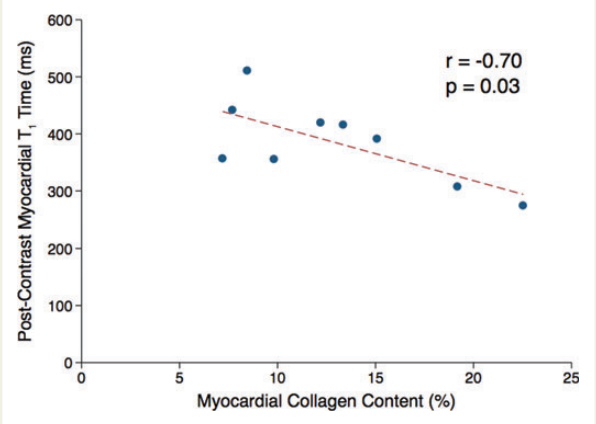

Figure 3: Relationship between myocardial collagen content and post-contrast myocardial T_1 time in HCM. Myocardial tissue obtained by surgical myectomy from nine patients was stained with picrosirius red and collagen content was calculated as a percentage of total myocardial tissue. A significant negative correlation was observed between myocardial collagen content and post-contrast myocardial T_1 time ($r = -0.70$, $P = 0.03$).

CMR and echocardiography data

CMR and echocardiography results are displayed in *Table 2*. CMR was completed in all patients and complete echocardiography results were available in 125 patients. Patients with HCM had higher LV ejection fraction (LVEF, 69 ± 7 vs. 60 ± 6%, $P < 0.001$) and LV mass indexed to BSA (85 ± 30 vs. 52 ± 9 g/BSA, $P < 0.001$) and their maximum wall thickness was 20 ± 4 mm.

Interpretable LGE images were obtained in 161 (98%) subjects. Late gadolinium enhancement was observed in 117 (86%) HCM patients and accounted for 4.6 ± 6.1% of LV mass. When present, LGE was most commonly observed at the point(s) of right-ventricular wall insertion (68%) or within the interventricular septum (44%). No patient demonstrated an ischaemic pattern of LGE.

T_1 mapping image quality was sufficient for analysis in 486 (99%) of 492 LV short-axis slices. Post-contrast myocardial T_1 time was significantly lower in HCM patients than healthy controls (483 ± 83 vs. 545 ± 49 ms, $P < 0.001$) (*Figure 4*). Post-contrast T_1 time of the LV blood pool was similar between both groups (308 ± 49 vs.

Table 2 Cardiac magnetic resonance imaging and echocardiography data

	Control (n = 25)	HCM (n = 139)	P-value
CMR data			
LVEDV indexed, mL/BSA	83 ± 13	80 ± 15	0.3
LVEF, %	60 ± 6	69 ± 7	<0.001
LV mass indexed, g/BSA	52 ± 9	85 ± 30	<0.001
Maximum septal thickness, mm	8 ± 2	20 ± 4	<0.001
Septal:lateral wall thickness	1.0 ± 0.1	2.3 ± 0.7	<0.001
LGE			
Presence, n (%)	0 (0)	117 (86)	<0.001
Quantity, % of LV mass	0	4.6 ± 6.1	<0.001
Post-contrast T_1 time, ms			
LV myocardium	545 ± 49	483 ± 83	<0.001
LV blood pool	306 ± 22	308 ± 49	0.8
Echocardiography data			
Left atrial volume indexed, mL/m^2	32 ± 9	48 ± 17	<0.001
Peak LVOT gradient, mmHg	4 ± 1	40 ± 45	<0.001
e′, cm/s	10.4 ± 3.3	7.2 ± 2.2	<0.001
E/e′ ratio	7.7 ± 2.4	12.3 ± 4.5	<0.001

HCM, hypertrophic cardiomyopathy; CMR, cardiac magnetic resonance; LVEDV, left-ventricular end-diastolic volume; BSA, body surface area; LVEF, left-ventricular ejection fraction; LGE, late gadolinium enhancement; LVOT, left-ventricular outflow tract.

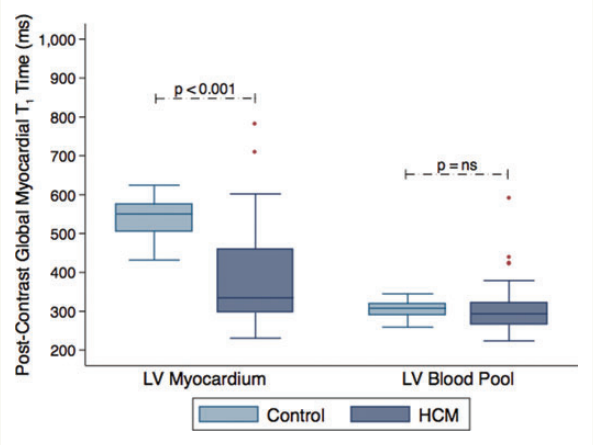

Figure 4: Post-contrast T_1 times of LV myocardium and blood pool in patients with HCM compared with healthy controls. Post-contrast myocardial T_1 times were significantly lower in patients with HCM compared with healthy controls, but blood pool T_1 times did not significantly differ (ns = non-significant).

306 ± 22 ms, P = 0.8), excluding altered contrast kinetics as a potential confounder to the observed differences in myocardial post-contrast T_1 times.

Factors associated with extent of LGE in HCM group

Significant correlations were observed between the extent of LGE and both LVEF (r = −0.52, P < 0.001) and peak LVOT pressure gradients (r = −0.32, P = 0.02), but not with E/e′ (r = 0.14, P = 0.3). Late gadolinium enhancement quantity did not significantly differ according to whether or not patients experienced dyspnoea (5.1 ± 6.9 vs. 3.4 ± 3.3%, P = 0.18).

Factors associated with post-contrast myocardial T_1 time in HCM group

As previously demonstrated,[20] post-contrast myocardial T_1 time negatively correlated with echocardiographic estimation of LV filling pressure (E/e′, r = −0.44, P < 0.001), and also with BMI (r = −0.35, P < 0.01), peak LVOT pressure gradient (r = −0.31, P = 0.02) and age (r = −0.28, P = 0.04) (*Table 3*). Post-contrast myocardial T_1 time was significantly lower in patients with dyspnoea compared with those without breathlessness (471 ± 78 vs. 505 ± 84 ms, P < 0.05). No significant correlations were observed between post-contrast myocardial T_1 time and LGE quantity (r = −0.13, P = 0.4), and T_1 time did not significantly differ according to whether LGE was present or absent (485 ± 84 vs. 474 ± 71 ms, P = 0.6). Following multivariate linear regression, only correlations between post-contrast myocardial T_1 time and BMI, and E/e′ remained statistically significant.

Linking HCM mutation status with phenotype

NGS to identify HCM mutations was performed in 56 HCM patients. There were 36 (64%) G+ patients with the following genes involved: MYBPC3 (17 patients); MYH7 (11 patients); MYH6 (3 patients); TNNI3 (3 patients); and TNNT2 (2 patients). G+ HCM patients were more likely to have a documented family history of HCM, but less likely to experience dyspnoea or receive beta-blocker therapy.

Table 3 Predictors of post-contrast myocardial T_1 time in HCM group by simple and multiple linear regression

	Simple linear regression		Multiple linear regression	
	r	*P*-value	β	*P*-value
Demographic and clinical data				
Age	−0.28	0.04	−0.09	0.5
BMI	−0.35	<0.01	−0.29	0.02
Resting heart rate	0.03	0.8		
Systolic blood pressure	−0.15	0.3		
eGFR	0.07	0.6		
CMR data				
LVEF	−0.10	0.5		
LV mass indexed	−0.13	0.4		
Maximum LV wall thickness	−0.05	0.7		
Quantity of LGE	−0.13	0.4		
Echocardiography data				
Left-atrial volume indexed	−0.22	0.12		
Peak LVOT gradient	−0.31	0.02	−0.07	0.6
E/e′ ratio	−0.44	<0.001	−0.36	0.02

HCM, hypertrophic cardiomyopathy; BMI, body mass index; eGFR, estimated glomerular filtration rate; CMR, cardiac magnetic resonance; LVEF, left-ventricular ejection fraction; LGE, late gadolinium enhancement; LVOT, left-ventricular outflow tract.

HCM patients with a mutation also had significantly lower LVEF and peak LVOT pressure gradient and a trend toward lower E/e′ (*Table 4*).

CMR indices of regional and diffuse myocardial fibrosis differed according to HCM mutation status. Late gadolinium enhancement was observed in a greater proportion of G+ HCM patients than those without mutations (94 vs. 68%, $P < 0.01$), and LGE quantity was also significantly higher (7.9 ± 8.6 vs. 3.1 ± 4.3%, $P = 0.03$). Surprisingly, G+ HCM patients had higher post-contrast myocardial T_1 times compared with those without mutations (498 ± 81 vs. 451 ± 70 ms, $P = 0.03$), suggestive of less diffuse myocardial fibrosis in HCM patients with a recognized HCM mutation (*Figure 5*). When areas of LGE were included in the ROIs for post-contrast T_1 mapping, this significant difference was maintained (489 ± 92 vs. 436 ± 57 ms, $P = 0.02$).

Linking specific HCM mutations with phenotype

There were no significant differences in CMR or echocardiographic data according to specific HCM mutations. Specifically, G+ HCM patients with MYBPC3 or MYH7 mutations had similar LV dimensions, LGE quantity and location, and post-contrast T_1 times (*Table 5*).

Discussion

We performed a comprehensive evaluation of both diffuse and regional patterns of myocardial fibrosis in a cohort of patients with HCM using contrast-enhanced CMR, and investigated links between genotype and phenotype. Compared with healthy subjects, those with HCM manifest lower post-contrast myocardial T_1 times, suggestive of more diffuse myocardial fibrosis, and a greater quantity of LGE, consistent with more regional myocardial fibrosis. Furthermore, HCM patients with an identifiable genetic mutation had evidence of significantly more regional, but less diffuse, myocardial fibrosis, than patients without mutations. We did not demonstrate significant associations between individual HCM mutations and specific phenotypic characteristics.

What is the clinical significance of lower post-contrast myocardial T_1 times? HCM patients with dyspnoea had significantly lower T_1 times than those without breathlessness, and T_1 time correlated with both myocardial collagen content by histological analysis and estimated LV filling pressures by echocardiography. To our knowledge, this is the first study to document a link between abnormal T_1 myocardial signal, impaired LV physiology, and clinical symptoms in this patient group. Multiple studies performed by independent centres[5,6] have shown significant correlations between post-contrast T_1 time and the amount of fibrosis in human myocardial biopsy specimens. More diffuse myocardial fibrosis may result in increased passive LV stiffness, which contributes to diastolic dysfunction and resultant symptoms of dyspnoea. In a cohort of cardiac transplant recipients, we recently demonstrated a significant correlation between post-contrast myocardial T_1 time and invasively determined LV stiffness.[21] Further investigation using LV pressure–volume measurements, both at rest and with exercise, may support this hypothesis in patients with HCM. Whether post-contrast myocardial T_1 times also provide prognostic information will also be determined.

We found no association between post-contrast T_1 time and the presence or quantity of LGE. This differs from findings by Ho *et al.*[22] in which they found both a correlation between extracellular volume fraction (ECV) and LGE extent, and a higher ECV, suggestive of more diffuse fibrosis, in HCM patients with sarcomere mutations compared with healthy controls. There are several explanations for these differences. Firstly, the present study's HCM cohort was older and more symptomatic, had a lower proportion of MYBPC3 mutations but more MYH6 mutations, and had a higher number of G− HCM patients. Secondly, varying T_1 mapping methods and sequences characterize myocardial tissue differently. In the present study, we provided further histological validation of our VAST T_1 mapping technique in HCM. MOLLI sequences are sensitive to T_2, imaging parameters and heart rate, in addition to T_1 changes, due to the effect of multiple readouts following a single preparation pulse.[23,24] Also, while MOLLI sequences rely on the mathematical conversion of the measured T_1* to obtain a true T_1 value, 'single point' T_1 mapping sequences, such as the one employed in our study, measure true T_1 and are generally not sensitive to such T_2 effects. Lastly, in our genotype status comparison, all but one patient had multiple short-axis LV levels available to calculate T_1 time, whereas only a single mid-LV short-axis slice was used for T_1 mapping analysis in 18 patients of Ho *et al.*'s cohort reportedly due to technical difficulties.

HCM patients with an identifiable mutation had evidence of significantly less diffuse myocardial fibrosis than patients without such a mutation and, supporting the link between T_1 time and diastolic function, also had significantly less dyspnoea and a trend towards lower estimated LV filling pressures. However, these same patients had

Table 4 Comparison of HCM patients according to HCM mutation status

	Genotype-positive ($n = 36$)	Genotype-negative ($n = 20$)	*P*-value
Demographic and clinical data			
Age, year	46 ± 14	51 ± 12	0.19
Males, *n* (%)	25 (69)	17 (85)	0.2
BMI, kg/m^2	27 ± 5	28 ± 4	0.5
Family history of HCM, *n* (%)	18 (50)	4 (20)	0.03
Dyspnoea, *n* (%)	22 (61)	19 (95)	0.01
Current medication, *n* (%)			
Beta-blocker	16 (44)	15 (75)	0.03
Calcium channel blocker	6 (17)	5 (25)	0.5
Resting heart rate, beats/min	62 ± 11	61 ± 14	0.6
Systolic blood pressure, mmHg	130 ± 18	127 ± 12	0.6
Haematocrit	0.42 ± 0.04	0.44 ± 0.03	0.18
eGFR, mL/min/1.73 m^2	81 ± 12	81 ± 13	0.8
Cardiac MRI data			
LVEDV indexed, mL/BSA	80 ± 13	81 ± 17	0.8
LVEF, %	67 ± 8	71 ± 7	<0.05
LV mass indexed, g/BSA	85 ± 27	87 ± 19	0.8
Maximum LV wall thickness, mm	20 ± 4	19 ± 3	0.4
LGE			
Presence, *n* (%)	34 (94)	13 (68)	<0.01
Quantity, % of LV mass	7.9 ± 8.6	3.1 ± 4.3	0.03
Location, *n* (%)			
RV insertion point(s)	25 (74)	7 (54)	0.17
Interventricular septum	22 (65)	7 (54)	0.4
LV apex	1 (3)	1 (8)	0.5
Other LV site	7 (21)	3 (23)	0.6
Post-contrast T_1 time, ms			
LV myocardium	498 ± 81	451 ± 70	0.03
LV blood pool	300 ± 32	316 ± 33	0.12
Echocardiography data			
Left atrial volume indexed, mL/m^2	49 ± 19	53 ± 17	0.4
Peak LVOT gradient, mmHg	28 ± 41	55 ± 49	0.03
e′, cm/s	0.07 ± 0.02	0.07 ± 0.02	0.5
E/e′ ratio	11.3 ± 4.3	13.9 ± 5.3	0.05

HCM, hypertrophic cardiomyopathy; BMI, body mass index; eGFR, estimated glomerular filtration rate; LVEDV, left-ventricular end-diastolic volume; LVEF, left-ventricular ejection fraction; LGE, late gadolinium enhancement; LVOT, left-ventricular outflow tract.

more regional myocardial fibrosis by LGE. Extent of LGE is a predictor of ventricular arrhythmia and sudden cardiac death in HCM[3] and, after medium-term follow-up, the presence of a pathogenic mutation has been associated with an increased risk of a combined endpoint that included cardiovascular death.[10] Increased quantities of LGE, reflective of more regions of potentially arrhythmogenic dense myocardial fibrosis, in our patients with pathogenic HCM mutations, may contribute to the worse prognosis previously observed in this subgroup. Long-term follow-up may strengthen this putative association.

The capability to non-invasively identify and measure separate patterns of myocardial fibrosis with distinct clinical implications has the potential to improve the management of this complex condition. For example, HCM patients with lower post-contrast myocardial T_1 times may benefit from targeted therapies aimed at regressing diffuse myocardial fibrosis, which may improve LV diastolic dysfunction. For patients with large quantities of LGE, an implantable cardioverter-defibrillator may be indicated for primary prophylaxis against ventricular arrhythmia and sudden death.

Despite our use of the latest cardiac imaging and genetic testing technologies, linking a specific genetic mutation with a particular phenotype remains elusive in HCM. Our HCM patients had comparatively thicker LV walls and a higher LGE prevalence than previously studied cohorts, yet only 64% exhibited an identifiable pathogenic mutation. After comparing patients with mutations in the most commonly identified genes, MYBPC3 and MYH7, no significant

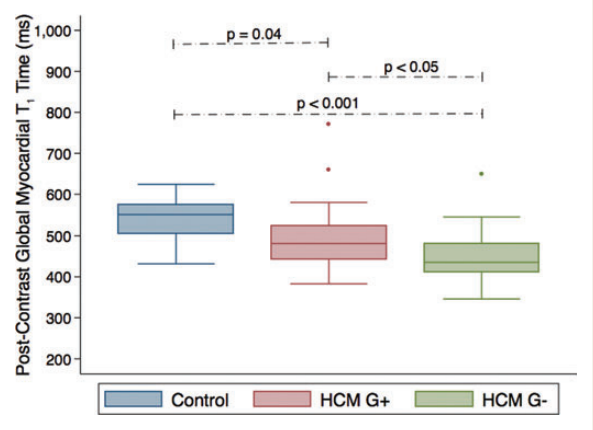

Figure 5: Post-contrast T_1 times of LV myocardium in patients with HCM, both with (G+) and without (G−) identifiable mutations, compared with healthy controls. Post-contrast myocardial T_1 times were significantly lower in patients with HCM compared with healthy controls, regardless of HCM gene status. Post-contrast myocardial T_1 times of the HCM G− group were significantly lower than those of the HCM G+ group. Comparison of groups was made using a one-way analysis of variance (ANOVA) with *post hoc* testing (Holm–Sidak method).

Table 5 Comparison of HCM patients according to specific HCM mutations

	MYBPC3 (n = 17)	MYH7 (n = 11)	*P*-value
CMR data			
LVEDV indexed, mL/BSA	81 ± 12	80 ± 14	0.9
LVEF, %	67 ± 6	69 ± 9	0.4
LV mass indexed, g/BSA	89 ± 27	86 ± 23	0.8
Maximum LV wall thickness, mm	20 ± 3	20 ± 3	0.7
LGE			
Presence, *n* (%)	16 (94)	10 (91)	0.7
Quantity, % of LV mass	8.7 ± 10.6	7.6 ± 7.9	0.8
Post-contrast T_1 time, ms			
LV myocardium	498 ± 61	469 ± 70	0.3
LV blood pool	307 ± 31	279 ± 32	0.04
Echocardiography data			
Left atrial volume indexed, mL/m^2	48 ± 21	52 ± 18	0.7
Peak LVOT gradient, mmHg	29 ± 43	44 ± 47	0.4
e′, cm/s	0.07 ± 0.02	0.07 ± 0.02	0.3
E/e′ ratio	10.8 ± 3.4	13.3 ± 6.0	0.17

HCM, hypertrophic cardiomyopathy; MYBPC3, myosin-binding protein C gene; MYH7, myosin heavy chain 7 gene; CMR, cardiac magnetic resonance; LVEDV, left-ventricular end-diastolic volume; BSA, body surface area; LVEF, left-ventricular ejection fraction; LGE, late gadolinium enhancement; LVOT, left-ventricular outflow tract.

phenotypic differences were observed. NGS enables faster, less expensive, and more comprehensive genetic testing than previous techniques and is likely to make the rapid assessment of larger numbers of HCM patients simpler and more cost-effective. However, determining which mutations are actually pathogenic will become increasingly challenging. We identified mutations currently classified as VUS in several patients. With co-segregation analysis, some of these VUS mutations may be reclassified as pathogenic and this could assist in better elucidating the roles of genetic and environmental factors in influencing phenotype. Also of importance will be the further study of G+ family members without LV hypertrophy, but who exhibit structural abnormalities, such as myocardial crypts, diastolic impairment, and LGE.[25,26]

Limitations

Hypertrophic cardiomyopathy patients with a defibrillator and at highest risk of sudden death were not included in this study. Further validation of T_1 mapping methods with myocardial tissue is necessary. Research correlating post-contrast T_1 mapping with myocardial fibrosis is limited by small patient numbers and limited specimens obtained by either endomyocardial biopsy[5,6] or surgical myectomy.[27] However, acquiring whole hearts for detailed collagen content quantification will remain difficult. Post-contrast T_1 times can be influenced by non-fibrotic myocardial tissue processes, such as myocardial oedema, and factors relating to gadolinium contrast pharmacokinetics, including dose of contrast, time from contrast administration to T_1 measurement, equilibrium kinetics, and renal clearance. In the present study, T_1 times were corrected for both eGFR and duration of time between contrast administration and image acquisition. While the development of this correction model[15] was limited by the absence of subjects with significant renal insufficiency, all participants in the present study had preserved renal function (ie. eGFR $\geq$50 mL/min/1.73 m^2). Furthermore, there were no significant differences in theoretical confounders of post-contrast T_1 time, such as haematocrit and heart rate, between the comparison groups and LV blood pool T_1 times did not significantly differ. ECV, an alternative method of extracellular matrix expansion quantification which incorporates native (pre-contrast) myocardial T_1 signal, blood pool T_1 time, and haematocrit,[28] was not performed in the current study as these methods had not been published at the time of study inception.

Conclusions

In HCM, contrast-enhanced CMR with T_1 mapping can non-invasively evaluate both regional and diffuse patterns of myocardial fibrosis. Greater quantities of LGE, consistent with more regional myocardial fibrosis, are associated with reduced LV systolic function and less outflow tract obstruction, whereas patients with lower post-contrast T_1 times, indicative of more diffuse myocardial fibrosis, have more LV diastolic impairment and symptoms of dyspnoea. Hypertrophic cardiomyopathy patients with an identifiable mutation have significantly more regional, but less diffuse myocardial fibrosis than those without. Further research utilizing advanced tissue characterization techniques and NGS may identify links between specific pathogenic mutations and particular HCM phenotypes and facilitate more individualized management strategies.

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