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Diltiazem Treatment for Pre-Clinical Hypertrophic Cardiomyopathy Sarcomere Mutation Carriers

A Pilot Randomized Trial to Modify Disease Expression

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ABSTRACT

OBJECTIVES The study sought to assess the safety, feasibility, and effect of diltiazem as disease-modifying therapy for at-risk hypertrophic cardiomyopathy (HCM) mutation carriers.

BACKGROUND HCM is caused by sarcomere mutations and characterized by left ventricular hypertrophy (LVH) with increased risk of heart failure and sudden death. HCM typically cannot be diagnosed early in life, although subtle phenotypes are present. Animal studies indicate that intracellular calcium handling is altered before LVH develops. Furthermore, early treatment with diltiazem appeared to attenuate disease emergence.

METHODS In a pilot, double-blind trial, we randomly assigned 38 sarcomere mutation carriers without LVH (mean 15.8 years of age) to therapy with diltiazem 360 mg/day (or 5 mg/kg/day) or placebo. Treatment duration ranged from 12 to 42 months (median 25 months). Study procedures included electrocardiography, echocardiography, cardiac magnetic resonance imaging, and serum biomarker measurement.

RESULTS Diltiazem was not associated with serious adverse events. Heart rate and blood pressure did not differ significantly between groups. However, mean left ventricular (LV) end-diastolic diameter improved toward normal in the diltiazem group but decreased further in controls (change in *z*-scores, +0.6 vs. -0.5; $p < 0.001$). Mean LV thickness-to-dimension ratio was stable in the diltiazem group but increased in controls (-0.02 vs. +0.15; $p = 0.04$). Among *MYBPC3* mutation carriers, LV wall thickness and mass, diastolic filling, and cardiac troponin I levels improved in those taking diltiazem compared with controls. Four participants developed overt HCM, 2 in each treatment group.

CONCLUSIONS Pre-clinical administration of diltiazem is safe and may improve early LV remodeling in HCM. This novel strategy merits further exploration. (Treatment of Preclinical Hypertrophic Cardiomyopathy With Diltiazem; NCT00319982) (J Am Coll Cardiol HF 2015;3:180–8) © 2015 by the American College of Cardiology Foundation.

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Hypertrophic cardiomyopathy (HCM) is caused by mutations in sarcomere genes, most commonly cardiac β-myosin heavy chain (*MYH7*), myosin binding protein C (*MYBPC3*), and troponin T (*TNNT2*) (1–6). Most patients have normal longevity and manageable symptoms, but sudden cardiac death and heart failure are important features of disease (7).

The clinical diagnosis of HCM relies on identifying unexplained left ventricular hypertrophy (LVH), and management focuses on symptom palliation and risk stratification for sudden death. However, mutation carriers usually have normal left ventricular (LV) wall thickness until adolescence or later (8,9). Little is known about the pathways leading from sarcomere mutation to overt disease or adverse outcomes. Consequently, disease-modifying therapies have not yet been developed.

SEE PAGE 189

Studies in animal models of HCM indicate that sarcomere mutations trigger early dysregulation of intracellular calcium handling (10–12). These changes have been linked to the development of LVH and myocardial fibrosis. In these models, early diltiazem treatment decreased disease emergence, but late treatment could not reverse established HCM (11,13). We thus sought to determine the safety, feasibility, and efficacy of early diltiazem treatment in attenuating phenotypic progression of HCM, targeting at-risk sarcomere mutation carriers without LVH (NCT00319982).

METHODS

The institutional review and ethics boards of 3 collaborating medical centers approved the study protocol. All participants provided written informed consent or assent if they were younger than 18 years of age at enrollment.

STUDY DESIGN AND SUBJECTS. A double-blind, randomized, placebo-controlled pilot clinical trial was performed at Brigham and Women's Hospital (Boston, Massachusetts), Boston Children's Hospital (Boston, Massachusetts), and Royal Prince Alfred Hospital (Sydney, Australia). Eligible participants were at least 5 years of age, carried the pathogenic or likely pathogenic (14) sarcomere mutation presumed to cause HCM in their family, and had normal LV wall thickness (echocardiographic maximal LV wall thickness ≤12 mm in adults or *z*-score ≤3 in children <18 years of age). Major exclusion criteria included contraindications to diltiazem, concomitant treatment with cardioactive medications, impaired renal function (estimated glomerular filtration rate <60 ml/min/1.73 m^2), and pregnancy or lactation. Up to 4 relatives from a single family could participate.

STUDY PROCEDURES. Enrolled participants were randomly assigned in a 1:1 ratio to receive diltiazem or placebo. The Investigational Drug Service at Brigham and Women's Hospital performed block randomization using the website randomization.com and a block size of 4. Because of the influence of age on phenotypic expression, assignment was stratified by age (5 to <15 years and ≥ 15 years). Participants, parents, and all individuals involved in study procedures or data analysis were blinded to treatment assignment until after the database was locked.

After assignment, participants received sustained-release diltiazem (90 mg for adults and 1.5 mg/kg for children) or matched placebo capsules once daily. Dose was titrated over 2 to 4 weeks to a target daily dosage of 360 mg for adults and 5 mg/kg for children. Heart rate and blood pressure were monitored weekly during titration and at each visit during treatment. To maximize time on treatment, the duration of follow-up varied depending on enrollment date. Participants were treated for a minimum of 1 year and a maximum of 3 years; earliest enrolling participants received longest follow-up. Follow-up visits occurred at 6, 12, 24, and up to 36 to 48 months after enrollment. Participants between 8.5 and 16.5 years of age at enrollment underwent additional assessment 18 months after enrollment to capture potentially faster phenotypic progression during puberty. Participants enrolled in the first year had an additional follow-up visit 6 to 12 months after completing treatment.

At each study visit, participants underwent physical examination, 12-lead electrocardiography, transthoracic echocardiography, and serum biomarker analysis. Cardiac magnetic resonance (CMR) imaging was performed at enrollment and at the end of treatment. Participants or their caregivers were called every 4 months and asked about adverse events and changes in symptoms. Adherence to study medication was monitored by pill count (15).

ECHOCARDIOGRAPHIC ANALYSIS. Standard 2-dimensional, Doppler, and tissue Doppler images were obtained at each study visit. Cardiac dimensions and Doppler characteristics were recorded as the mean value of 3 cardiac cycles in accordance with the guidelines of the American Society of Echocardiography (16). If wall thickness was nonuniform, the

ABBREVIATIONS AND ACRONYMS

CMR = cardiac magnetic resonance

E′ = tissue Doppler mitral annular early relaxation velocity

HCM = hypertrophic cardiomyopathy

LGE = late gadolinium enhancement

LV = left ventricular

LVEDD = left ventricular end-diastolic diameter

LVEF = left ventricular ejection fraction

LVH = left ventricular hypertrophy

MYBPC3 = cardiac myosin binding protein C gene

MYH7 = cardiac β-myosin heavy chain gene

location and greatest dimension were recorded after visual assessment from multiple views. Echocardiographic measures in all participants were also converted to *z*-scores to adjust for differences in age and body size (17).

Left ventricular ejection fraction (LVEF) was calculated using Simpson's method (16,18). Standard metrics of diastolic function included peak early (E) and late (A) transmitral velocities, E/A ratio, and E-wave deceleration time (19). Early myocardial tissue Doppler relaxation velocities (E′) were measured at the lateral, septal, anterior, and inferior aspects of the mitral annulus. Global E′ velocity was determined by averaging these 4 values.

All echocardiographic images were analyzed offline by 2 investigators (C.Y.H. and N.K.L.) blinded to treatment assignment. Anticipating that some pediatric subjects might not be able to tolerate CMR imaging, echocardiography was designated as the primary modality to assess cardiac morphology and function.

CMR IMAGING. CMR images were acquired at enrollment and at the final visit on study medication with a 3.0-T system (Tim Trio, Siemens, Erlangen, Germany), or 1.5-T scanners (Achieva, Philips Healthcare, Best, the Netherlands, or HDX Excite II, General Electric, Milwaukee, Wisconsin). Images were acquired with both cardiac-gating and breath-holding. The standard protocol consisted of cine steady-state free precession imaging for LV function and LV mass (20). LV mass was derived by the summation-of-discs method after manual tracing of myocardial borders on short-axis cine images (21). LV wall thickness was measured in at least 4 sections: anterior and posterior septal, lateral, and inferior.

A late gadolinium enhancement (LGE) imaging protocol was used to detect focal myocardial fibrosis. A segmented inversion-recovery pulse sequence was started 10 to 15 min after the participant had received a 0.15 mmol/kg cumulative dose of gadolinium-diethylenetriamine penta-acetic acid (DTPA) (Bayer HealthCare Pharmaceuticals Inc., Wayne, New Jersey). Images were measured using a semiautomated gray-scale threshold technique using a cutoff of 6 standard deviations above the mean signal intensity (22). The quantity of LGE was expressed in grams and as a percentage of the total LV myocardial mass. All CMR analyses were performed using commercial software (QMassMR, version 7.4, Medis, Leiden, the Netherlands) by the same CMR physician (S.A.A.), blinded to treatment assignment.

SERUM BIOMARKERS ANALYSIS. Blood samples (serum and K3-EDTA plasma) collected at enrollment and at each study visit were processed within 1 h of phlebotomy and stored at -80°C before analysis. All assays were performed using commercial reagents by personnel blinded to clinical and genetic status of participants. The following markers were analyzed: carboxy-terminal propeptide of procollagen type I (Quidel Corporation, San Diego, California), amino terminal propeptide of B-type natriuretic peptide (Roche, Indianapolis, Indiana), and cardiac troponin I (supersensitive assay; Singulex, Atlanta, Georgia).

STATISTICAL METHODS. Patient characteristics are summarized as means and standard errors or counts, as appropriate. Standard errors rather than standard deviations are presented because a generalized estimating equation approach, using the Genmod procedure in the SAS statistical package (version 9.2, SAS Institute, Cary, North Carolina), was used to account for an exchangeable correlation structure within families. This assumption of equal correlation between any pair of family members is reasonable for the siblings that constitute almost all of our related participants, but may overestimate the correlation in the sole aunt-niece pair. This same model, additionally adjusted for age, sex, genotype, and baseline value, was used to analyze echocardiographic, CMR, and biomarker outcomes. The covariates in our models were chosen a priori on the basis of their potential roles as confounders or effect modifiers. Therefore, they were included in all models despite the risk of overfitting and the associated potential for unstable estimates and false positive or negative results. Interaction terms between treatment and age, sex, and genotype were analyzed to identify any responsive subgroups. For safety analyses, counts of patients with adverse events were compared between groups using Fisher's exact test.

As a pilot trial, all analyses were considered exploratory. Alpha was set at 0.05, and no adjustments were made for multiple testing. This trial was originally designed with global E′ as the primary outcome, and with the goal of detecting a 2 to 3 cm/s difference between the change in global E′ in controls and the change in global E′ in the diltiazem group. The final sample of 18 diltiazem and 20 placebo patients had a projected power of 83% to detect this difference, assuming a standard deviation of 2.5 cm/s. However, because of the small sample size, because early phenotypes of HCM are subtle and affect multiple pathways, and because the impact of treatment is unknown, the analysis plan was changed prior to data analysis. Rather than having a primary focus on E′, we consider the trial to be a pilot effort to explore a broad range of imaging and biomarker features. This modification maximizes the potential to detect phenotypic

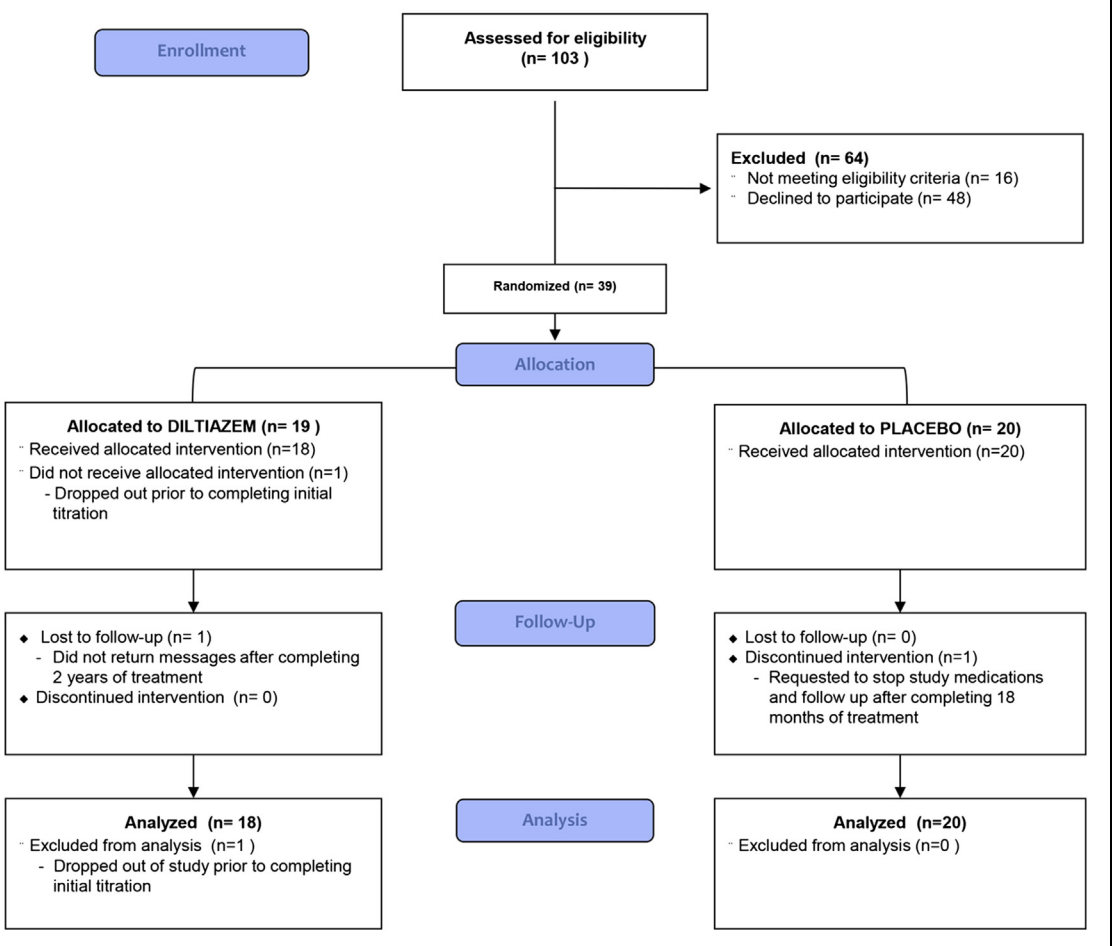
progression and treatment effect on complex disease biology.

RESULTS

PARTICIPANTS. Of 103 sarcomere mutation carriers screened between July 2006 and June 2010, 16 were ineligible and 48 declined participation, primarily because of concerns about taking daily medication or keeping study visits. Thirty-nine people (38% of those screened) were enrolled (**Figure 1**). All participants tolerated titration to target dose, although 1 withdrew from the study for personal reasons before completing titration. Thus, 38 participants, 18 in the diltiazem group and 20 controls, are included in analysis. Of the 38, 7 participants (5 to 18 years of age at baseline) declined CMR imaging; 3 others declined intravenous cannula insertion to administer gadolinium contrast.

Baseline characteristics of the treatment and placebo groups were generally similar (**Table 1**). A listing of the specific sarcomere mutations present in the study cohort is provided in the Online Table. For the overall study cohort, the mean age was 15.8 ± 8.6 years (range 5 to 39 years of age), 58% were female, and all were healthy and had no cardiovascular symptoms or concomitant illnesses. Of the 28 participants who underwent gadolinium-contrast CMR imaging, none had LGE at baseline. Twenty-nine families were represented, of which 7 families had more than 1 relative enrolled, including 1 set of 4 siblings, 1 set of 3 siblings, 4 sets of 2 siblings, and 1 aunt-niece pair. Of the 17 related subjects, 10 were assigned to placebo and 7 to diltiazem.

FIGURE 1 Diagram of Participant Flow



Participant flow and dispositions in a randomized trial of hypertrophic cardiomyopathy sarcomere mutation carriers treated with diltiazem (25).

TABLE 1 Baseline Characteristics of the Study Participants

	Diltiazem (n = 18)	Placebo (n = 20)
Age, yrs	14.1 ± 1.7	17.3 ± 2.1
Female/male	11/7	11/9
Causal gene		
MYH7	10	11
MYBPC3	6	6
TNNT2	2	3
Heart rate, beats/min	71 ± 4	73 ± 4
SBP, mm Hg	104 ± 3*	113 ± 2
DBP, mm Hg	63 ± 2	66 ± 2
Max LVWT, mm	8.1 ± 0.4	8.1 ± 0.35
Max LVWT *z*-score	0.7 ± 0.4	0.2 ± 0.2
LVEDD, cm	4.2 ± 0.2	4.3 ± 0.15
LVEDD *z*-score	-1.5 ± 0.2	-1.5 ± 0.3
LV thickness/dimension ratio	1.76 ± 0.05	1.63 ± 0.10
LVESD, cm	2.4 ± 0.1	2.4 ± 0.1
LVESD *z*-score	-2.7 ± 0.31	-2.7 ± 0.4
Echo LVEF, %	70 ± 1	70 ± 2
Global E', cm/s	14.7 ± 0.6	14.2 ± 0.5
E/E'	6.0 ± 0.3	5.6 ± 0.2
LA diameter, cm	3.1 ± 0.2	3.2 ± 0.1
LA *z*-score	-1.2 ± 0.2	-1.1 ± 0.1
TR maximal velocity, m/s	2.0 ± 0.1	1.9 ± 0.1
CMR LA volume, ml	57.1 ± 5.1	59.9 ± 7.0
CMR LA volume index, ml/m^2	38.0 ± 3.1	34.4 ± 2.5
CMR LV mass, g	83.8 ± 10.0	80.3 ± 8.8
CMR LV mass index, g/m^2	49.9 ± 3.8	46.6 ± 3.4
Troponin I, pg/ml	2.2 ± 0.3	3.2 ± 0.8
NT-proBNP, pg/ml	64.6 ± 15.4	97.1 ± 34.7
PICP, mcg/l	130 ± 8	118 ± 9

Values are mean ± SE or n. *p < 0.01 comparing participants treated with diltiazem and placebo; otherwise no significant differences identified.

CMR = cardiac magnetic resonance; DBP = diastolic blood pressure; E′ = tissue Doppler early myocardial relaxation velocity; LA = left atrium; LVEDD = left ventricular end-diastolic diameter; LVEF = left ventricular ejection fraction; LVESD = left ventricular end-systolic diameter; Max LVWT = maximal left ventricular wall thickness; *MYBPC3* = cardiac myosin binding protein C gene; *MYH7* = cardiac β-myosin heavy chain gene; NT-proBNP = N-terminal pro-B-type natriuretic peptide; PICP = C-terminal propeptide of procollagen type I; SBP = systolic blood pressure; *TNNT2* = troponin T; TR = tricuspid regurgitation.

SAFETY AND ADVERSE EVENTS. Median treatment duration was 756 days in the diltiazem group and 755 days in controls (overall treatment duration ranged from 369 to 1,280 days) (Table 2). Mean systolic blood pressure did not change significantly in either treatment group; participants in the diltiazem group had a minor decrease in heart rate, consistent with drug effect (Table 3). No participants requested or required discontinuation of study medication for safety concerns, adverse events, side effects, or intolerance.

One participant in the diltiazem group was lost to study follow-up after 2 years. One control participant withdrew after 18 months of treatment. Adherence to the protocol, assessed by pill counts, averaged 83% in the diltiazem group and 90% in controls (p = 0.08) (Table 2).

No serious adverse events were reported. Twenty-two subjects reported 52 mild adverse events possibly related to diltiazem (Table 2). Nonlimiting dyspnea, lightheadedness, and gastrointestinal upset were most frequently described. In the diltiazem group, 5 adverse events were related to 1 young female subject whose systolic blood pressure was <90 mm Hg. At enrollment, she was 9 years of age and had a blood pressure of 95/51 mm Hg. After titration, her blood pressure was 90/60 mm Hg. During treatment, her systolic blood pressure varied between 86 and 89 mm Hg. These values are normal for her age and size, but triggered a pre-defined adverse event to capture SBP <90 mm Hg. She was asymptomatic, pursued vigorous activities without difficulty, and so continued in the study.

Shortness of breath and exertional dyspnea were more frequent in the diltiazem group (9 events vs. 1 event, p = 0.03). However, 5 of these 9 events occurred in 2 participants, a 12-year-old girl and a 17-year-old boy. Both had mild and inconsistent symptoms that resolved spontaneously.

RESPONSE TO TREATMENT. We assessed changes in values (difference between final and baseline values) to gauge treatment response or disease progression over the course of the trial (Table 3). There was no significant change in systolic blood pressure or heart rate over time between treatment groups. Among imaging and biomarker characteristics, only changes in mean LV end-diastolic diameter (LVEDD) differed significantly between groups. At baseline, participants in both groups had below-average LV cavity size (mean LVEDD *z*-score = -1.5). During treatment, LVEDD *z*-scores improved slightly toward normal in 14 of 18 participants treated with diltiazem (78%) but in only 5 of 20 placebo-treated participants (25%). Collectively, controls showed a further small decrease in mean cavity size during treatment (0.53 decrease in LVEDD *z*-score; p < 0.001) (Table 3). Neither baseline heart rate nor change in heart rate affected this difference between the changes in LVEDD (data not shown). Although the treatment groups had comparable LV wall thickness after treatment, the posterior wall thickness-to-LVEDD ratios indicate differences in LV remodeling. At baseline, LV thickness-to-dimension ratios were similar (Table 1). With treatment, the ratio remained stable in the diltiazem group but increased significantly in controls (+0.15 ± 0.06; p < 0.001) (Table 3). Our original primary outcome, Global E′, showed small declines in both groups, with no significant difference between them (p = 0.75) (Table 3).

Hypertrophic cardiomyopathy is typically characterized by small LV cavity size; therefore, the increase in LVEDD in diltiazem-treated participants may

reflect a beneficial treatment response (Figure 2A). Among 29 participants who received additional follow-up approximately 1 year after study medications were stopped, LV cavity size decreased after diltiazem was discontinued (Figure 2B).

TREATMENT INTERACTIONS. We examined baseline features that might affect the expression of sarcomere mutations, including age, sex, and underlying genotype. Anecdotal reports have suggested that disease expression can be earlier or more pronounced in males and during puberty (3,8,23,24). In females receiving diltiazem, E′ velocity improved more than in controls (+0.63 vs. -0.32 cm/s; p = 0.049; interaction with sex p = 0.02). Additionally, diltiazem-treated participants <15 years of age showed a slight decrease in LV thickness-to-dimension ratio (-0.10), whereas controls had a slight increase (+0.22; p < 0.001; interaction with age, p = 0.02).

HCM is most commonly caused by mutations in *MYH7* and *MYBPC3*. The clinical manifestations of *MYH7* mutations have been postulated to be more severe (3,8). We analyzed *MYBPC3* and *MYH7* mutation carriers to assess for potential interaction between treatment and underlying genotype. Mean maximal LV wall thickness *z*-score decreased by 0.02 in diltiazem-treated *MYBPC3* mutation carriers but increased by 2.6 in controls (p = 0.01) (Table 4). Similarly, LV mass as assessed by CMR decreased in diltiazem-treated *MYBPC3* mutation carriers but increased in controls (p < 0.001). The E/E′ ratio, which reflects LV filling pressure, decreased significantly more in diltiazem-treated *MYBPC3* mutation carriers than in controls (p = 0.001). Finally, serum cardiac troponin I levels decreased in diltiazem-treated *MYBPC3* mutation carriers while it increased in controls (p = 0.01). There was no suggestion of beneficial change in these parameters in diltiazem-treated *MYH7* mutation carriers.

DEVELOPMENT OF HCM. Four unrelated participants, 2 in each treatment group, had substantial increases in LV wall thickness during follow-up, leading to a diagnosis of HCM. All carried pathogenic mutations: *MYH7* Arg719Gln (2 unrelated participants; female, 9 years of age at baseline, and male, 12 years of age at baseline), MYH7 Arg663Cys (male, 17 years of age at baseline), and troponin T Arg92Trp (male, 17 years of age at baseline). None of these subjects reported symptoms or change in exercise capacity throughout the study.

TABLE 2 Treatment Duration, Adherence, and Adverse Events Possibly Related to Study Medication

	Diltiazem (n = 18)	Placebo (n = 20)	p Value
Length of treatment, days	756 [736-818]	755 [735-1,099]	0.85*
Adherence	83 (10.8)	90 (6.6)	0.08*
Participants reporting adverse events	10	12	1.0†
Number of adverse events	29‡	23	0.37§
Most common adverse events			
Shortness of breath/exertional dyspnea	6/9‖	1/1	0.03§
Lightheadedness or orthostasis	3/5	5/6	0.92§
Nausea or gastrointestinal symptoms	1/1	4/4	0.19§
Fatigue	1/1	2/2	0.62§
Headache	0/0	1/2	0.34§
Chest pain	3/3	1/1	0.25§

Values are median [interquartile range], % (SD), or n/N (number of participants/number of events) unless otherwise indicated. *Wilcoxon rank sum test. †Fisher's exact test. ‡5 events occurred in a 9-year-old girl with a baseline blood pressure of 95/51 mm Hg and intermittent, asymptomatic recordings of systolic blood pressure of 86 to 89 mm Hg during follow-up. §Chi-square trend test, allowing for multiple events per participant. ‖5 events with self-limited symptoms occurred in 2 participants.

DISCUSSION

We tested a novel, genotype-guided, disease-modifying intervention in individuals at risk for developing inherited cardiomyopathy, before clinical diagnosis with disease. This unique strategy involved treating at-risk sarcomere mutation carriers with the intention of changing the natural history of HCM by starting therapy before potentially irreversible changes in cardiac structure and function develop.

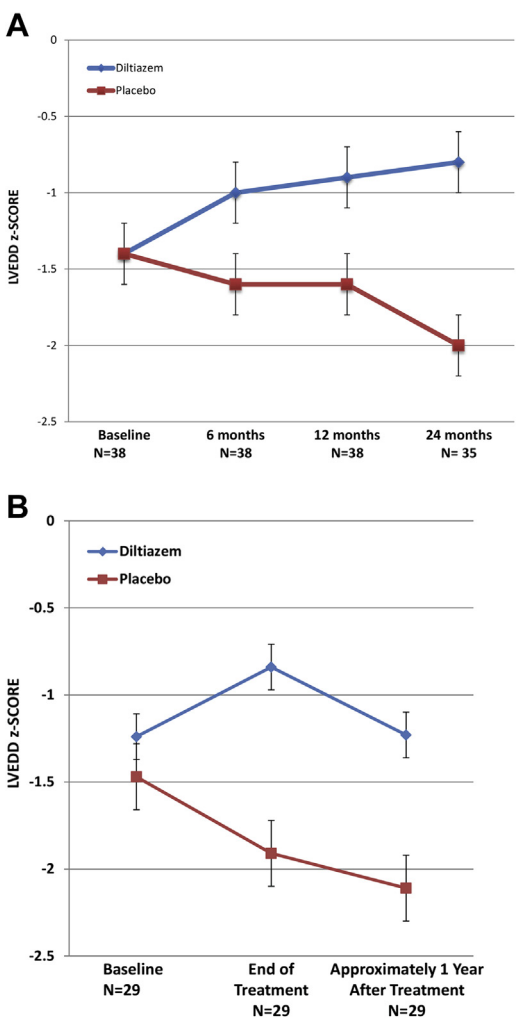
TABLE 3 Effect of Diltiazem Treatment on Hypertrophic Cardiomyopathy Sarcomere Mutation Carriers

Change in Value (Final - Baseline)	Diltiazem (n = 18)	Placebo (n = 20)	p Value
Heart rate, beats/min	-4.9 ± 2.2*	2.0 ± 2.6	0.06
SBP, mm Hg	-1.4 ± 1.7	2.1 ± 1.8	0.15
DBP, mm Hg	-0.1 ± 1.1	2.7 ± 1.3*	0.08
Max LVWT, mm	0.9 ± 0.5	1.6 ± 0.6*	0.32
Max LVWT *z*-score	0.52 ± 0.44	1.4 ± 0.53*	0.22
LVEDD, cm	0.26 ± 0.06*	-0.02 ± 0.06	0.001
LVEDD *z*-score	0.60 ± 0.17*	-0.53 ± 0.16*	<0.001
LV thickness/dimension ratio	-0.02 ± 0.05	0.15 ± 0.06*	0.04
LVESD, cm	0.19 ± 0.05*	0.08 ± 0.06	0.14
LVESD *z*-score	0.61 ± 0.22*	-0.02 ± 0.27	0.05
Echo LVEF, %	-0.22 ± 0.86	-0.72 ± 0.95	0.72
Global E′, cm/s	-0.06 ± 0.27	-0.21 ± 0.42	0.75
E/E′	0.02 ± 0.17	-0.25 ± 0.15	0.29
LA diameter, cm	0.06 ± 0.09	0.10 ± 0.07	0.70
LA *z*-score	-0.02 ± 0.14	-0.01 ± 0.11	0.93
TR maximal velocity, m/s	0.02 ± 0.05	-0.07 ± 0.10	0.42
CMR LA volume, ml	4.5 ± 5.9	1.7 ± 6.1	0.73
CMR LA volume index, ml/m^2	-0.51 ± 2.6	0.88 ± 2.8	0.71
CMR LV mass, g	-3.7 ± 3.6	-7.5 ± 3.3*	0.49
CMR LV mass index, g/m^2	-0.96 ± 2.3	-4.2 ± 1.3*	0.25
Troponin I, pg/ml	0.22 ± 0.88	0.64 ± 0.56	0.72
NT-proBNP, pg/ml	22.3 ± 26.6	-17.3 ± 13.4	0.24
PICP, mcg/l	-2.9 ± 7.8	-21.3 ± 5.1*	0.08

Values are mean ± SE and are adjusted for age, sex, genotype, family relations, and baseline value. *Significant change over time (p < 0.05) within the designated treatment group.

Abbreviations as in Table 1.

FIGURE 2 Changes in LVEDD *z*-Scores Over Time in Study Participants



(A) Changes during treatment with diltiazem or placebo in the full cohort. The 2 treatment groups diverged over time (interaction between time and treatment, $p = 0.003$), with significant pairwise differences 12 ($p = 0.01$) and 24 ($p < 0.001$) months. **(B)** Changes during the year after the end of treatment with diltiazem or placebo in the 29 subjects with available follow-up. The 2 treatment groups again diverged over time (interaction between time and treatment, $p = 0.047$), with a significant pairwise difference at the end of treatment ($p = 0.005$). The pairwise difference 1 year after discontinuation of diltiazem was attenuated but remained significant ($p = 0.02$). Error bars are standard errors. LVEDD = left ventricular end-diastolic diameter.

Diltiazem was safely administered to this young, healthy population without detrimental effects. Mild adverse events were evenly distributed between treatment groups. Adherence to the study protocol was high, even in a potentially challenging cohort of adolescents.

Diltiazem may attenuate a longitudinal decrease in LV cavity size in HCM sarcomere mutation carriers. We suggest this result is notable because LVH with small LV cavity size is a distinctive feature of hypertrophic cardiomyopathy. Indeed, even in the absence of LVH, mutation carriers typically have relatively small LV cavities, reflected by the baseline mean LVEDD *z*-score of -1.5 in study participants. LV cavity size progressively improved toward normal in diltiazem-treated participants but progressively decreased in controls, without associated changes in heart rate or ejection fraction. The improvements were lost within a year after treatment was stopped. Additionally, the LV thickness-to-dimension ratio decreased slightly in the diltiazem group but increased significantly in controls. Otherwise, the treatment groups did not differ markedly.

We postulate these geometric changes may have a greater functional impact than reflected by simple linear measurements. A fundamental detrimental effect of hypertrophy in HCM is decreased LV compliance. The general assumption is that poor ventricular compliance in HCM is due primarily to myocardial abnormalities that result in increased stiffness. However, geometric effects are also important because wall stress is proportional to the product of pressure and the ratio of LV dimension and wall thickness. In HCM, the increase in LV thickness and concomitant decrease in LV cavity size reduce LV wall stress, and therefore higher diastolic pressures are required to sufficiently distend the ventricle. This purely geometric effect impacts filling, even if the muscle itself is normal. Increasing the LV dimension-to-thickness ratio (reducing the thickness-to-dimension ratio) improves ventricular compliance, independently of changing relaxation or myocardial compliance.

Secondary analyses suggested that *MYBPC3* mutation carriers may be more responsive to disease-modifying treatment with diltiazem than *MYH7* mutation carriers. Diltiazem-treated *MYBPC3* mutation carriers had significantly less increase in LV wall thickness and mass over time than controls. Diltiazem-treated *MYBPC3* mutation carriers also had lower serum levels of cardiac troponin I and a suggestion of improved LV filling pressures, as represented by a decrease in E/E′ values, compared with placebo-treated *MYBPC3* mutation carriers. These beneficial effects were not seen in diltiazem-treated *MYH7* mutation carriers. Further investigation is needed to better elucidate mechanisms that may govern genotypic differences in treatment response. Such advances may ultimately allow more targeted and personalized therapy.

TABLE 4 Change in Cardiac Measurements after 3 Years of Treatment With Diltiazem, by Genotype

Outcome	*MYH7* Carriers (n = 21)			*MYBPC3* Carriers (n = 12)			p for Interaction
	Diltiazem	Placebo	p Value	Diltiazem	Placebo	p Value	
Sample size (echocardiography), n	10	11		6	6		
Sample size (CMR), n	8	8		5	5		
Change in outcome measure							
Echo Max LVWT, mm*	+1.4 ± 0.60	+0.84 ± 0.70	0.49	-0.02 ± 0.60	+3.2 ± 0.84	0.001	0.003
Echo Max LVWT, *z*-score*	+0.92 ± 0.63	+0.78 ± 0.62	0.87	-0.02 ± 0.45	+2.6 ± 0.92	0.01	0.04
E/E′	+0.53 ± 0.20	-0.03 ± 0.17	0.04	-0.97 ± 0.15	-0.25 ± 0.17	0.001	<0.001
CMR LV mass,† g	-1.8 ± 4.4	-8.8 ± 3.7	0.24	-13.4 ± 3.4	+4.0 ± 4.2	<0.001	<0.001
CMR LV mass index,† g/m^2	-1.1 ± 3.1	-5.6 ± 1.6	0.21	-3.7 ± 2.3	+1.9 ± 1.4	0.008	0.01
Troponin I, pg/ml	+1.2 ± 1.2	-0.51 ± 0.59	0.24	-0.61 ± 0.70	+2.1 ± 1.1	0.01	0.02

Values are mean ± SE and are adjusted for age, sex, genotype, family relations, and baseline value. *Measured by echocardiography. †Measured by cardiac magnetic resonance (CMR) imaging.

E/E′ = mitral inflow E wave/E′; other abbreviations as in Table 1.

STUDY LIMITATIONS. This was a pilot, exploratory study to test a new approach to treating HCM. Firm conclusions about treatment efficacy cannot be drawn. The small number of participants and short follow-up duration are limitations of this study. We are unable to determine whether diltiazem may have more prominent effects if administered for longer periods or at higher doses. Additionally, the tools currently available to monitor treatment response and phenotypic progression are crude and may lack the resolution necessary to detect subtle changes in healthy young people. Identifying new, robust, quantitative and dynamic phenotypes will be key to better characterize early disease emergence, progression, and treatment effects. Furthermore, the penetrance of sarcomere mutations is variable, unpredictable, and not absolute. Some mutation carriers may not develop HCM or do so only at an advanced age. More precise understanding of the natural history of early HCM is critically needed to better understand the factors that drive disease evolution and adverse outcomes. Such knowledge will help guide the development of disease-modifying therapy and help direct such therapy to those at highest risk.

CONCLUSIONS

Genetic testing can identify individuals who carry sarcomere mutations which cause hypertrophic cardiomyopathy before they can be diagnosed with clinical disease. These at-risk mutation carriers may be targeted for therapies designed to modify the progression and emergence of disease. Furthermore, treatment can be started early in this population; at a time when disease-modifying therapy may be more likely to be successful. Intermediate-term use of diltiazem is safe and well-tolerated. This type of approach may be able to attenuate the phenotypic expression of HCM if given early in disease pathogenesis. Such strategies are worth exploring further in larger trials because of the potential to transform the care of delayed-onset genetic disease, such as HCM. Although there are many challenges to overcome, success with these tactics would allow management to evolve from a largely passive strategy of surveillance, symptom palliation and sudden death risk stratification to a proactive strategy of disease modification and ultimately, perhaps, disease prevention.

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APPENDIX For a supplemental table, please see the online version of this article.