Hypertrophy Regression With N-Acetylcysteine in Hypertrophic Cardiomyopathy (HALT-HCM)

A Randomized, Placebo-Controlled, Double-Blind Pilot Study

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Rationale: **Hypertrophic cardiomyopathy (HCM) is a genetic paradigm of cardiac hypertrophy. Cardiac hypertrophy and interstitial fibrosis are important risk factors for sudden death and morbidity in HCM. Oxidative stress is implicated in the pathogenesis of cardiac hypertrophy and fibrosis. Treatment with antioxidant N-acetylcysteine (NAC) reverses cardiac hypertrophy and fibrosis in animal models of HCM.**

Objective: **To determine effect sizes of NAC on indices of cardiac hypertrophy and fibrosis in patients with established HCM.**

Methods and Results: **HALT-HCM (Hypertrophy Regression With N-Acetylcysteine in Hypertrophic Cardiomyopathy) is a double-blind, randomized, sex-matched, placebo-controlled single-center pilot study in patients with HCM. Patients with HCM, who had a left ventricular wall thickness of ≥15 mm, were randomized either to a placebo or to NAC (1:2 ratio, respectively). NAC was titrated ≤2.4 g per day. Clinical evaluation, blood chemistry, and 6-minute walk test were performed every 3 months, and electrocardiography, echocardiography, and cardiac magnetic resonance imaging, the latter whenever not contraindicated, before and after 12 months of treatment. Eighty-five of 232 screened patients met the eligibility criteria, 42 agreed to participate; 29 were randomized to NAC and 13 to placebo groups. Demographic, echocardiographic, and cardiac magnetic resonance imaging phenotypes at the baseline between the 2 groups were similar. WSE in 38 patients identified a spectrum of 42 pathogenic variants in genes implicated in HCM in 26 participants. Twenty-four patients in the NAC group and 11 in the placebo group completed the study. Six severe adverse events occurred in the NAC group but were considered unrelated to NAC. The effect sizes of NAC on the clinical phenotype, echocardiographic, and cardiac magnetic resonance imaging indices of cardiac hypertrophy, function, and extent of late gadolinium enhancement—a surrogate for fibrosis—were small.**

Conclusions: **Treatment with NAC for 12 months had small effect sizes on indices of cardiac hypertrophy or fibrosis. The small sample size of the HALT-HCM study hinders from making firm conclusions about efficacy of NAC in HCM.**

Clinical Trial Registration: **URL: http://www.clinicaltrials.gov. Unique identifier: NCT01537926. (*Circ Res*. 2018;122:1109-1118. DOI: 10.1161/CIRCRESAHA.117.312647.)**

Key Words: acetylcysteine ■ cardiomyopathy, hypertrophic ■ death, sudden, cardiac ■ fibrosis ■ hypertrophy

Hypertrophic cardiomyopathy (HCM) is a hereditary disease of the myocardium caused mainly by mutations in genes encoding sarcomere proteins.[1] It is an important cause of sudden cardiac death in the young and a major cause of morbidity in older individuals, primarily because of cardiac arrhythmias and heart failure with preserved ejection fraction (EF).[1] HCM is clinically diagnosed by the presence of unexplained left ventricular (LV) hypertrophy and pathologically by the presence of myocyte disarray and interstitial fibrosis.[1]

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Novelty and Significance

What Is Known?

- Hypertrophic cardiomyopathy (HCM) is caused mainly by mutations in genes encoding sarcomere proteins.
- Cardiac hypertrophy and fibrosis—important determinants of clinical outcomes—are considered secondary to the activation of numerous trophic and mitotic pathways, including oxidative stress-responsive mechanisms.
- Studies in animal models of HCM have shown that cardiac hypertrophy and fibrosis are reversible on pharmacological interventions, including treatment with N-acetylcysteine (NAC)—a precursor to glutathione.

What New Information Does This Article Contribute?

- HALT-HCM (Hypertrophy Regression With N-Acetylcysteine in Hypertrophic Cardiomyopathy) is a double-blind, randomized, sex-matched, placebo-controlled study (n=42 patients) designed to test feasibility and determine effect sizes of NAC on indices of cardiac hypertrophy and fibrosis in patients with established HCM.
- Recruitment rate was slow, but retention and compliance rates were high.
- About two third of the participants had ≥1 pathogenic variants in genes known to cause HCM.
- Treatment with a high dose of NAC for 12 months had negligible-to-modest effect sizes on indices of cardiac hypertrophy and fibrosis in patients with established HCM.

HCM is an important cause of sudden cardiac death in the young and heart failure with preserved ejection fraction. There is no effective pharmacological treatment for reversing or preventing cardiac hypertrophy and fibrosis in patients with HCM. Pharmacological interventions in animal models of HCM have shown reversibility of cardiac hypertrophy and fibrosis on treatment with NAC. The findings in human patients, however, are notable for negligible or modest effect sizes on indices of cardiac hypertrophy and fibrosis. The small sample size of the study may have precluded firm conclusions about the efficacy of NAC in treatment of patients with HCM.

Nonstandard Abbreviations and Acronyms

CMR	cardiac magnetic resonance imaging
EF	ejection fraction
HALT-HCM	Hypertrophy Regression With N-Acetylcysteine in Hypertrophic Cardiomyopathy
HCM	hypertrophic cardiomyopathy
LV	left ventricular
NAC	N-acetylcysteine
NYHA	New York Heart Association

Cardiac hypertrophy is the quintessential clinical phenotype of human HCM and an important determinant of mortality, morbidity, and the risk of sudden cardiac death.[2–5] Likewise, interstitial fibrosis has been associated with clinical events, including cardiac arrhythmias, in HCM.[6–9] Current pharmacological therapies are not effective in preventing, attenuating, or reversing cardiac hypertrophy and fibrosis in HCM.

Genetic and pharmacological interventions in animal models of HCM have established the potential for reversibility of cardiac hypertrophy and fibrosis.[10–15] N-acetylcysteine (NAC) is a precursor to glutathione, which is the most abundant intracellular thiol pool against oxidative stress in the body. Treatment with NAC has been shown to reverse established cardiac hypertrophy and fibrosis in independent studies in animal models of HCM.[15–17] Treatment with NAC also attenuates cardiac hypertrophy induced by pressure overload, administration of angiotensin II, and altered myocardial metabolism.[18–20] The beneficial effects of NAC in models of cardiac hypertrophy and dysfunction are in accord with the important roles of oxidative stress-responsive signaling pathways, myocardial metabolism, and oxidative modification of myofilament proteins in the pathogenesis of cardiac hypertrophy.[17–20] Extensive clinical use and safety profile of NAC in humans and the results of the experimental studies in the animal models of HCM identify NAC as an attractive agent for potentially attenuating and reversing cardiac phenotypes in human patients with HCM. The purpose of this feasibility study, dubbed as HALT-HCM (Hypertrophy Regression With N-Acetylcysteine in Hypertrophic Cardiomyopathy), was to determine recruitment, accrual, retention and compliance rates, tolerability, safety, and side effects, as well as the effect sizes of NAC on the indices of cardiac hypertrophy and fibrosis in patients with HCM.

Methods

Institutional Review Board of the University of Texas Health Science Center approved the study. Each participant signed a consent form. Anonymized data that support the findings of this study are available from the corresponding author on reasonable request.

Eligibility Criteria

Eligibility criteria are described in Table 1. In brief, adult patients with an established diagnosis of HCM, defined as primary cardiac hypertrophy with an LV end-diastolic wall thickness of ≥15 mm on a 2-dimensional echocardiogram, nondilated LV cavity, and preserved LV systolic function, were considered eligible. Those allergic to NAC, a recent history of septal ablation or plan to undergo myectomy in the near future, known phenocopy conditions, and concomitant diseases were excluded. Patients with devices such as pacemakers and internal cardioverter/defibrillators and those expected to receive a device during the course of the study were excluded from the cardiac magnetic resonance imaging (CMR) part of the study.

Data Management

Research Electronic Data Capture tools, hosted at UTHealth, were used for collecting and managing data. Research Electronic Data Capture is a secure, web-based application designed to support data capture for research studies.[21]

Randomization

The study statistician prepared a block randomization schedule, which balanced the distribution of patients in the study arms 2:1 (NAC group versus placebo group) and with respect to patient sex. The randomization schedule was provided to the study pharmacist. The data manager regularly checked to ensure proper utilization of the randomization program.

Table 1. Eligibility Criteria

Inclusion criteria
Established diagnosis of HCM, defined as the presence of primary cardiac hypertrophy with an LVED wall thickness of ≥15 mm, a nondilated LV cavity, and preserved LV systolic function on a 2D echocardiogram
Exclusion criteria
Hypersensitivity to NAC
Individuals <18 y of age
Known phenocopy conditions
History of (with the last 6 mo) or planned transcatheter (alcohol) septal ablation or surgical myectomy
Patients who are likely to receive a pacemaker or an ICD implantation during the study period:
Patients with advanced atrioventricular block or severe bradycardia
Patients with serious ventricular arrhythmias
Known causal mutations but an LVED wall thickness of <15 mm
Patients with concomitant diseases
Coronary artery disease, defined as >70% luminal diameter stenosis in any of the major coronary arteries (if known)
Valvular heart diseases (more than mild aortic stenosis and mitral regurgitation)
Uncontrolled hypertension, defined as systolic blood pressure of ≥140 mm Hg and diastolic blood pressure of ≥90 mm Hg on medication (mean values of 3 measurements at rest)
Other significant medical problems, such as moderate-to-severe chronic renal failure (glomerular filtration rate <45 mL/min per 1.73 m^2), advanced liver disease, cancer, or other disabling conditions
Patients with active asthma
Pregnant women, nursing mothers, and those who plan pregnancy during the study period
Cardiac contraindications for MRI (for CMR studies only), including patients with devices such as pacemakers and ICDs

2D indicates 2-dimensional; CMR, cardiac magnetic resonance imaging; HCM, hypertrophic cardiomyopathy; ICD, internal cardioverter and defibrillator; LV, left ventricle; LVED, left ventricular end diastolic; MRI, magnetic resonance imaging; and NAC, N-acetylcysteine.

Dosing of NAC and Placebo

NAC was prepared by a compound pharmacy as 600 mg capsules along with identically appearing placebo capsules. Active and placebo capsules were coated to minimize the distinction between the 2 capsules based on odor and taste. The compound pharmacy labeled the study drug based on study identification number and randomization. NAC was administered at 600 mg orally every 12 hours for the first 3 months and then was increased to 1200 mg BID for an additional 9 months. Thus, the total duration of treatment with NAC or matching placebo was 12 months.

Compliance

Compliance with the study drug was determined by counting the number of unused pills in every visit and interviewing the participants.

Genetic Analysis

Exome sequencing was performed using Illumina sequencing platforms and analyzed by the investigators, as described.[22,23] In brief, the Sequence reads were mapped to the reference Human Genome version 19. Single-nucleotide variants and small/insertion deletion (indels) were called according to genome analysis tool kit best practices pipeline (http://software.broadinstitute.org/gatk/best-practices/). Functional annotation of the variant list for each exome was performed using ANNOVAR (annotate variation) software (http://annovar.openbioinformatics.org/). Rare variants in 19 genes (*ACTC1*, *ACTN2*, *CALR3*, *CSRP3*, *JPH2*, *MYBPC3*, *MYH7*, *MYL2*, *MYL3*, *MYOZ2*, *NEXN*, *PLN*, *PRKAG2*, *TCAP*, *TNNI3*, *TNNT2*, *TPM1*, *TTN*, and *VCL*), implicated in familial HCM (http://ghr.nlm.nih.gov/condition/familial-hypertrophic-cardiomyopathy#genes), were manually curated. Variants meeting the following criteria were considered pathogenic.

1. Minor allele frequency ≤0.001, as reported in the Genome Aggregation Database (http://gnomad.broadinstitute.org/); Combined annotation-dependent phred score, as a measurement of relative pathogenicity, of ≥10[24]; and
2. A minimum of 10 reads covering the candidate variant and the genotype quality of 99 (a Phred-scaled confidence that the genotype assignment is correct. It is equal to 1% error rate).

Clinical Evaluation

A medical history was obtained, New York Heart Association (NYHA) functional class was recorded, and physical examination was performed in every participant. The participants also completed a 6-minute walk test. A blood chemistry panel comprised of electrolytes, blood urea nitrogen, creatinine, liver enzymes, and lipids was obtained at the baseline and every 3 months until the completion of the study. Twelve-lead electrocardiograms were obtained in all participants at baseline and completion of the study.

Echocardiographic Imaging and Analysis

The patients were imaged in the left lateral decubitus position with an ultrasound system equipped with a multifrequency transducer. A complete echocardiographic study was performed per standard views. American Society of Echocardiography/European Association of Cardiovascular Imaging guidelines were applied to measure biplane LV volumes, mass, and EF, as well as left atrial maximum volume.[25] From the apical window, pulsed-wave Doppler was used to record mitral inflow for 3 to 5 cardiac cycles at the level of the mitral valve annulus and tips.[26] Pulmonary venous flow was recorded using color Doppler guidance. Tissue Doppler was applied to record mitral annular velocities at the septal and lateral sides of the annulus. The resulting annular velocities by pulsed-wave Doppler were recorded for 5 cardiac cycles at a sweep speed of 100 mm/s. Tricuspid regurgitation velocity was recorded by continuous wave Doppler from multiple windows, and the peak velocity was noted. Inferior vena cava diameter and its collapse, as well as hepatic venous flow, were recorded from the subcostal views.[27] Echocardiographic measurements were performed offline (Digisonics EC, Houston, TX) without knowledge of randomization groups, clinical, and CMR findings.

Mitral inflow from tips level was analyzed for peak early (E), and late (A) diastolic velocities, E/A ratio, and deceleration time of mitral E velocity. Mitral A duration was measured at the level of mitral annulus.[26] Pulmonary venous flow was analyzed for peak systolic velocity, peak diastolic velocity, peak amplitude, and duration of atrial reversal velocity (Ar) and the difference between Ar velocity duration and mitral A duration (Ar−A).[26] Mitral annulus early (e′) and late (a′) diastolic velocities were measured at septal and lateral mitral annulus, and E/e′ ratios were computed. Measurements were averaged for 3 cardiac cycles.

Cardiac Magnetic Resonance Imaging

In all CMR studies, 1.5-T whole-body imagers were used. LV cine imaging was performed using steady-state free-precession sequences in long and short axes with temporal resolution ≤40 ms. LV structural and functional measures included LV mass, end-diastolic volume, end-systolic volume, EF, and stroke volume based on contours of the endocardial and epicardial contours of the LV using CMR software (Circle Cardiovascular Imaging, Calgary, Canada). T1 mapping of the myocardium before and after 0.15 mmol/kg gadolinium contrast administration was performed. Details of the T1 mapping and CMR methods were as described previously.[28] Briefly, T1 mapping indices, including pre (native) and postcontrast T1× at

12 and 25 minutes, partition coefficient (λ), and extracellular volume fraction (100×λ×[1−hematocrit]), were assessed using a single breath-hold modified Look-Locker inversion recovery sequence.[29] Late gadolinium enhancement images were obtained at the same slice locations as the cine images in the short and long-axis imaging planes using fast-gradient echo imaging beginning 15 minutes after the administration of the gadolinium contrast agent. Myocardial scar was defined as focal late gadolinium enhancement either in 2 adjacent short-axis slices or in 1 short-axis and a long-axis image at a corresponding location. Focal late gadolinium enhancement was traced using QMass (version 7.2; Medis, Leiden, The Netherlands) to derive myocardial scar mass.

End Points

The study was a feasibility study, designed primarily to assess the recruitment, retention, and compliance rates, the side effects, and the effect sizes of NAC on echocardiographic and CMR indices of cardiac hypertrophy and fibrosis. Therefore, changes in LV mass index, ventricular wall thickness, LV EF on echocardiography, and CMR, as well as macroscopic interstitial myocardial fibrosis, as defined by delayed enhancement on CMR after gadolinium contrast administration, were included as secondary end points.

Statistical Analysis

Baseline characteristics of patients randomized to the 2 study groups were compared. Intention-to-treat analysis was applied to compare the clinical, echocardiographic, and CMR outcome variables. This was complemented with the per-protocol analysis to calculate the effect sizes of NAC. For continuous variables, normality of the distribution was tested by Shapiro–Wilk test. For continuous variables with normal distribution, groups were compared using 2 independent sample *t* tests. Continuous variables with non-normal distribution were analyzed with the Kruskal–Wallis test, and categorical variables were analyzed by the χ^2 test. Changes in outcome variables between the 2 time points were analyzed using longitudinal analyses (mixed models). In these models, interaction analysis was performed to determine whether the treatment effect varied by phenotype. The effect size of NAC on the echocardiographic and CMR indices was calculated as $(\Delta^{Placebo}-\Delta^{NAC})/SD^{Placebo}$, per Glass Δ method.[30] SAS 9.4 statistical package (SAS Institute, Inc) was used for data analyses.

Results

Recruitment, Retention, and Compliance

A total of 232 patients with HCM were screened during a period of 3 years of whom 85 met the eligibility criteria and 147 did not (Figure). The main reason for exclusion was an LV wall thickness of <15 mm (55 patients) on the screening echocardiogram (Figure). Twenty-eight of the 85 eligible individuals declined to participate, and 15 did not respond to the follow-up enrollment invitations. Forty-two participants (32 men and 10 women) signed informed consents; 29 were randomized to NAC and 13 to placebo groups. The baseline demographics of the study population in each group are shown in Table 2. There were no significant differences in ages, sexes, ethnic backgrounds, body mass indices, mean systolic and diastolic blood pressures, and NYHA functional classes or other clinical characteristics between the 2 groups at enrollment with the exception of a history of smoking, which was higher in the NAC group.

Twenty-four of 29 (83%) patients in the NAC and 11 of 13 (85%) in the placebo groups completed the 12-month duration of the study. The reasons for dropout were lost to follow-up (4 patients), unable to commit time (1 patient), non-study related surgical procedure requiring prolonged hospitalization (1 patient), and skin rash (1 patient). Demographics of patients who completed the study did not differ between the 2 groups. Compliance rate, determined by pill counting and patient interview in each visit, was high because the participants consumed 92±8% (range, 66%–100%) of the prescribed study drug.

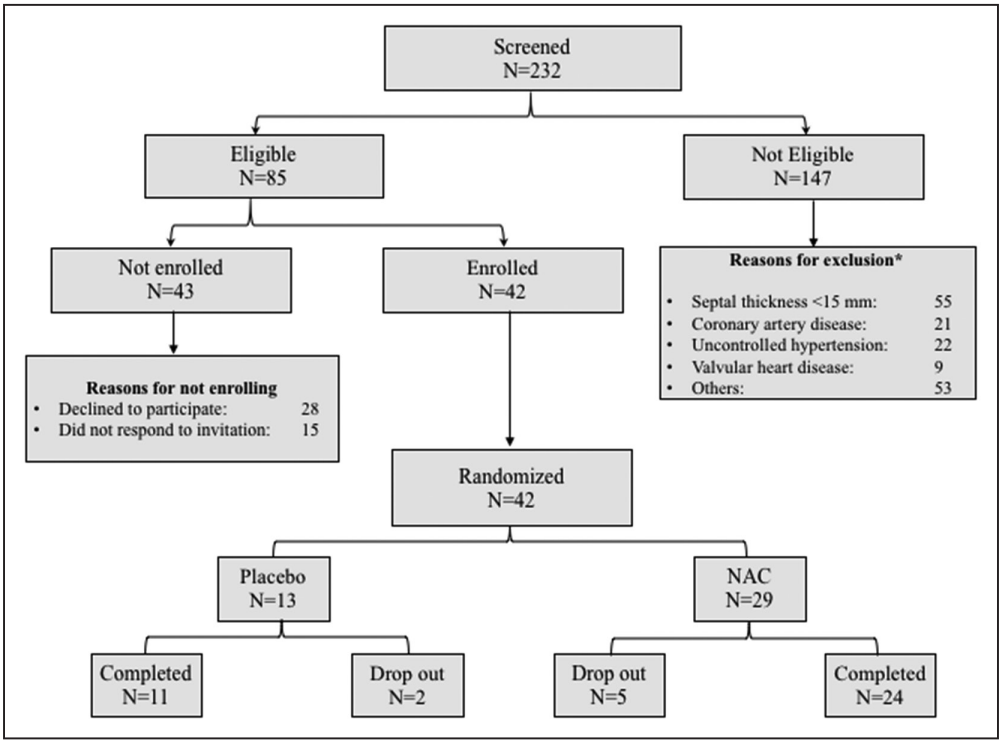

Figure. Consort chart showing screening, recruitment, and randomization process. NAC indicates N-acetylcysteine. *Some individuals had >1 exclusion criteria.

Biochemical laboratory values, including blood chemistry, kidney function, and liver function tests, did not significantly change in either of the study groups during the course of 12 months (data not shown).

Pathogenic Variants in the Known HCM Genes

Thirty-eight of the 42 participants in HALT-HCM study agreed to genetic testing by whole-exome sequencing. Variant analysis led to identification of 42, including 11 novel, (not reported in gnomAD database) pathogenic variants in 26 (68%) participants in 19 known genes considered to be causes of HCM (Table 3). *TTN* gene had the highest number of pathogenic variants (23, including 5 novel variants), likely reflective of its enormous size, and all were missense variants with a combined annotation-dependent score of >10. Eight pathogenic variants, including 2 indels, leading to frameshift and 1 splice junction variant, in the *MYBPC3* gene, were identified in 9 participants. Eleven participants had ≥2 pathogenic variants in the known HCM genes, including 1 individual, who had 4 pathogenic, including 3 novel, variants in the *TTN*, and 1 pathogenic variant in the *MYBPC3* genes.

Clinical Symptoms

Ten of 13 and 27 of 29 participants in the placebo and NAC groups, respectively, were in NYHA functional classes I or II (Online Table I). Only 5 patients in the study population were in class III and none in class IV (Online Table I). Distribution of patients among NYHA functional classes did not differ significantly between the placebo and NAC groups at the baseline and during the follow-up intervals (Online Table I). The change in NYHA functional class status of each patient during each follow-up interval is illustrated in Online Figure I. Frequency or severity of chest pain and palpitations did not change during the study period (data not shown). No patient experienced syncope or near syncope during the study period. All participants completed a 6-minute walk test without experiencing notable symptoms. There were no differences in the mean distance walked at the baseline and the follow-up time points between the 2 groups (Online Figure II).

Electrocardiographic Indices

There were no significant differences in the electrocardiographic findings, including indices of cardiac hypertrophy, at the baseline or on completion of the study (Online Table II).[31]

Echocardiographic Phenotypes

Indices of LV wall thickness, size, and function were not different between the placebo and NAC groups at baseline and on completion of the study (Table 4; Online Table III). Echocardiographic data in those who had baseline and 12-month studies were used to calculate the effect sizes. The effect sizes of NAC on indices of cardiac hypertrophy and function, including Doppler indices of LV function, were negligible to modest (Table 4). Likewise, when all patients who participated in the study were included in the analysis (intention-to-treat analysis), there were no discernible differences in echocardiographic or Doppler indices of LV size and function between the placebo and NAC groups in a model that included interaction between the study groups and the follow-up time (Online Table III).

Cardiac Magnetic Resonance Imaging

Data were available in 9 and 15 patients at the baseline in the placebo and NAC groups, respectively, and in 8 and 10 patients at both time points. Data in those who had the baseline and follow-up data were used to calculate the effect sizes of NAC on indices of cardiac hypertrophy, fibrosis, and fibrosis (late gadolinium enhancement). As shown in Table 5, no large effect sizes on cardiac mass, LV EF, LV midwall strain, and fibrosis were detected. Likewise, indices of LV wall thickness, cavity size, and function, as well as mass, were not significantly different between the 2 groups at baseline and on completion of the study in the intention-to-treat analysis (Online Table IV).

Adverse and Serious Adverse Effects

There was no adverse event. A total of 6 serious adverse events in 5 individuals were recorded in the study population, and all occurred in the NAC group (Fisher exact test, P=0.155). Two patients developed pneumonia requiring treatment with antibiotics, and 2 patients developed a cerebrovascular accident, including 1 who had prior episodes of cerebrovascular accidents and subsequently developed seizure. One patient developed chest pain, which was determined not to be of cardiac origin. None of the serious adverse events was judged to be the known side effect of the study drug.

Discussion

The results of HALT-HCM—a single-center randomized placebo-controlled feasibility study—were notable for a relatively slow accrual rate of patients with HCM but a high retention and compliance rates on enrollment. About one fifth of the 232 patients (42 patients) who were screened were recruited and randomized. The compliance and retention rates at 12 months were high, which are notable considering that most patients were asymptomatic or mildly symptomatic and did not have strong incentive to take the study medication and undergo testing. The recruitment, retention, and compliance rates in a single-center study of HCM, which is an uncommon disease, might reflect the referral basis of the study population. The findings also document the effect sizes of NAC on echocardiographic and CMR indices of cardiac hypertrophy and fibrosis, which were relatively modest. The study sample size did not provide sufficient power to determine efficacy of NAC in treatment of patients with HCM, except for detecting large changes in the indices of cardiac hypertrophy and fibrosis. Overall, the findings have implications for the design of future efficacy studies in patients with HCM.

The small sample size of the study prohibits from making firm conclusions about efficacy of NAC in HCM. Nevertheless, the effect sizes of NAC, administered for 1 year at a high dose, on the echocardiographic and CMR indices of cardiac hypertrophy and fibrosis were relatively small. The findings were concordant, regardless of whether the data were analyzed by the intention-to-treat or per-protocol approaches. Because the study was performed in patients with established cardiac hypertrophy and fibrosis, the findings might not pertain to evolving cardiac hypertrophy and fibrosis or prevention of the phenotype in HCM. Modest statistical differences in a few echocardiographic

Table 2. Baseline Characteristics of the Study Population

	Placebo (±SD)	NAC (±SD)	*P* Value
n	13	29	
Men/women	10/3	22/7	0.94
Age, y (mean)	47.6 (15.1)	50.7 (15.0)	0.56
Height, cm (mean)	173.1 (11.2)	174.3 (11.4)	0.97
Weight, kg (mean)	89.3 (22.9)	92.6 (19.5)	0.71
BSA, m^2 (mean)	2.07 (0.32)	2.12 (0.27)	0.62
BMI, kg/m^2 (mean)	29.5 (5.4)	30.4 (5.4)	0.71
Ethnic, n (%)			0.57
Hispanic	1 (7.7)	4 (13.8)	
Non-Hispanic	12 (92.3)	25 (86.2)	
Race, n (%)			0.47
White	10 (76.9)	23 (79.4)	
Black	1 (7.7)	4 (13.8)	
Asian or Pacific Islander	2 (15.4)	1 (3.4)	
Mixed	0 (0.0)	1 (3.4)	
NYHA functional class, n (%)			0.29
I	5 (38.5)	16 (55.2)	
II	5 (38.5)	11 (37.9)	
III	3 (23.0)	2 (6.9)	
IV	0 (0.0)	0 (0.0)	
Medical history, n (%)			
Cardiac arrest	0 (0)	1 (3.4)	0.50
Atrial fibrillation	2 (15.4)	3 (10.3)	0.64
Other cardiac arrhythmias	8 (61.5)	19 (65.5)	0.80
Syncope	4 (30.1)	8 (27.6)	0.83
ICD implantation	3 (23.1)	11 (37.9)	0.34
Diabetes mellitus	0	3 (10.3)	0.23
Hypertension	5 (38.5)	12 (41.4)	0.86
Dyslipidemia	5 (38.5)	13 (44.8)	0.70
Ex-smoker and current smoking	1 (7.7)	13 (44.8)	0.02
History and physical examination			
Heart rate, bpm (mean)	67.2 (11.7)	62.4 (11.2)	0.14
Systolic blood pressure, mm Hg (mean)	121.2 (14.9)	126.0 (11.7)	0.30
Diastolic blood pressure, mm Hg (mean)	76.2 (10.8)	79.8 (8.3)	0.41
Chest pain/tightness, n (%)	5 (17.2)	6 (20.7)	0.23
Palpitations, n (%)	2 (15.4)	4 (13.8)	0.89
Systolic ejection murmur, n (%)	5 (38.5)	11 (37.9)	0.97
Pansystolic murmur, n (%)	1 (7.7)	4 (13.8)	0.57
Laboratory			
Total cholesterol, mg/dL (mean)	193.5 (54.5)	169.8 (36.5)	0.18
Triglycerides, mg/dL (mean)	128.6 (55.7)	170.9 (150.4)	0.87
Medication, n (%)			
β-Blockers	9 (69.2)	20 (69.0)	0.99
Statins	3 (23.1)	8 (27.6)	0.76
Antiarrhythmic drugs	1 (7.7)	3 (10.3)	0.79
RAAS drugs	2 (15.4)	9 (31.0)	0.29

Data are presented as mean±SD, unless specified. For comparison of categorical data, *P* value is based on χ^2 test or Fisher exact test. For comparison of continuous data, *P* value is based on Wilcoxon rank-sum test. BMI indicates body mass index; BSA, body surface area; ICD, internal cardioverter and defibrillator; NAC, N-acetylcysteine; NYHA, New York Heart Association; and RAAS, renin–angiotensin–aldosterone system.

indices, including tissue Doppler indices of LV function, were noted between the baseline and follow-up data in the placebo and treatment groups. However, these differences did not follow a concordant pattern with the related phenotypes and were not consistent across imaging modalities to suggest a clinical significance. Moreover, no changes in the clinical symptoms and exercise tolerance in a 6-minute walk test were observed, albeit most patients were asymptomatic or minimally symptomatic and did not have exercise intolerance. There was a higher number of adverse events in the NAC group, but the adverse events did not seem to be related to administration of NAC.

HALT-HCM was designed as a pilot feasibility study and not an efficacy study. It was designed to provide information on recruitment, retention, and compliance rates, as well as tolerability and the effect sizes of NAC on indices of cardiac hypertrophy and fibrosis, for designing future efficacy studies. The plan was to recruit 25 patients to the placebo and 50 patients to the NAC groups. However, only 42 patients participated in the 1-year-long HALT-HCM study. Approximately, half of the eligible patients, who were mostly asymptomatic or mildly symptomatic, declined to participate (Figure). Data collected from those who completed the 1-year study were used to determine estimates and 95% confidence intervals for the effect size of NAC on echocardiographic and CMR indices of cardiac hypertrophy, function, and fibrosis. These effect sizes were small (Tables 4 and 5). A post hoc analysis showed that the sample size of the study provided 66% power to detect 20% change (26 g/m^2) and 84% power to detect a 25% change (33 mg/m^2) in the mean LV mass index, when α is set at 0.05. The study did not have sufficient power to detect smaller changes, which might be more realistic, given the slow development of cardiac hypertrophy in HCM. Overall, the small sample size of the study on indices of cardiac hypertrophy and fibrosis in HCM suggest that designing and implementation of large-scale efficacy studies with NAC would be challenging.

HCM is a genetically and clinically heterogeneous disease. The spectrum of putative causal genes and mutations in the study participants were in accord with the known genetic heterogeneity of HCM.[1] The abundance of the pathogenic variants in *TTN* was notable but likely reflects the enormous

Table 3. Pathogenic Variants in the Participants in Known Hypertrophic Cardiomyopathy Genes

Gene	Variants With Reads ≥10, Genotype Quality=99	No. of Individuals	Gnomad AF	CADD Phred Score	ClinVar Clinical Significance
ACTC1	NM_005159:exon3:c.C299T: p.Pro100Leu	1	0.000 004	23.2	Uncertain significance
ACTN2	NM_001103:exon16:c.G1864A: p.Asp622Asn	1	0.0003	35	Conflicting interpretations of pathogenicity
MYBPC3	NM_000256:exon23:c.2373dupG: p.Trp792ValfsTer41	1	0.000 018	NA	W792Ter, W792R, pathogenic/likely pathogenic
MYBPC3	NM_000256:exon16:c.G1468A: p.Gly490Arg	1	0.0002	25.1	Conflicting interpretations of pathogenicity
MYBPC3	NM_000256:exon6:c.G655C: p.Val219Leu	1	Not listed	16.74	Pathogenic/likely pathogenic
MYBPC3	NM_000256:exon12:c.G1000A: p.Glu334Lys	1	0.0002	35	Conflicting interpretations of pathogenicity
MYBPC3	NM_000256:exon20:c.1928-2A>G	2*	Not listed	16.32	Pathogenic
MYBPC3	NM_000256:exon12:c.1028delC: p.Thr343fs	1	Not listed	NA	Pathogenic
MYBPC3	NM_000256:exon22:c.G2308A: p.Asp770Asn	1	0.000 016	35	Pathogenic/likely pathogenic
MYBPC3	NM_000256:exon16:c.G1484A: p.Arg495Gln	1	0.00 002	27.6	Pathogenic/likely pathogenic
MYH7	NM_000257:exon23:c.C2722G: p.Leu908Val	1	0.00 003	18.46	Conflicting interpretations of pathogenicity
MYH7	NM_000257:exon21:c.G2330C: p.Arg777Thr	1	Not listed	24.9	Pathogenic
MYL2	NM_000432:exon2:c.G37A: p.Ala13Thr	3*	0.0003	16.11	Conflicting interpretations of pathogenicity
TNNT2	NM_000364:exon10:c.C304T: p.Arg102Trp	3*	0.000 004	20.9	Pathogenic/likely pathogenic
TTN	NM_001267550:exon326:c.G78394A: p.Asp26132Asn	1	0.00 001 225	15.71	
TTN	NM_001267550:exon298:c.G58491A: p.Met19497Ile	1	Not listed	13.82	
TTN	NM_001267550:exon117:c.C31384A: p.Pro10462Thr	1	0.000 001 187	17.89	
TTN	NM_001267550:exon63:c.C18389G: p.Thr6130Arg	1	Not listed	12.97	
TTN	NM_001267550:exon300:c.C59315T: p.Pro19772Leu	1	Not listed	10.15	
TTN	NM_001267550:exon133:c.G32743C: p.Ala10915Pro	1	0.0 009 620	12.38	
TTN	NM_001267550:exon269:c.C50758G: p.Pro16920Ala	1	0.0 002 400	14	
TTN	NM_001267550:exon358:c.A106154C: p.Lys35385Thr	1	0.0 000 008 134	11.53	
TTN	NM_001267550:exon326:c.A74596G: p.Thr24866Ala	1	0.0 002 035	12.49	
TTN	NM_001267550:exon152:c.C34778T: p.Pro11593Leu	1	0.000 008 603	18.58	
TTN	NM_001267550:exon335:c.T90913C: p.Tyr30305His	1	0.0 001 468	16.06	
TTN	NM_001267550:exon358:c.C101281T: p.Arg33761Trp	1	0.00 009 394	16.42	
TTN	NM_001267550:exon345:c.G95743A: p.Ala31915Trp	1	Not listed	12.5	
TTN	NM_001267550:exon326:c.C85471T: p.Arg28491Cys	1	0.00 005 074	13.89	Uncertain significance
TTN	NM_001267550:exon318:c.G67318A: p.Gly22440Ser	1	Not listed	11.25	
TTN	NM_133379:exon46:c.T11289A: p.Asp3763Glu	1	Not listed	16	
TTN	NM_001267550:exon318:c.A67104C: p.Lys22368Asn	3*	0.000 049	10.46	
TTN	NM_001267550:exon326:c.G82810A: p.Gly27604Ser	1	0.0 004 783	23.8	p.Gly27604Arg: uncertain significance
TTN	NM_001267550:exon28:c.C5198T: p.Thr1733Met	1	0.0 002 640	11.69	Uncertain significance
TTN	NM_001267550:exon358:c.A103591G: p.Lys34531Glu	1	0.00 007 314	13.21	
TTN	NM_001267550:exon324:c.G68864C: p.Gly22955Ala	1	0.0 002 547	12.56	

AF indicates allele frequency; CADD, combined annotation-dependent depletion; and NA, not available.
*Family members.

size of its main transcript, coding for ≈35 000 amino acids. Likewise, several individuals had multiple pathogenic variants in genes known to cause HCM, which is in keeping with the oligogenic basis of a subset of patients with HCM.[23] The variants were novel or rare (<1:1000 population frequency in the gnomeAD database) and were considered pathogenic. However, given the study participants were either sporadic cases or members of small families, causality of these variants in HCM could not be ascertained unambiguously. This is particularly the case for the pathogenic variants in the *TTN* gene because of the abundance of the nonsynonymous and truncating *TTN* variants in the general

Table 4. Echocardiographic Phenotypes (Per-Protocol Analysis)

	Placebo			NAC						
n	11			24						
M/F	9/2			18/6						
Age, y	49.35 (15.21)			50.18 (15.86)			*P* Value	$\Delta^{Placebo}-\Delta^{NAC}$* (95% CI)	SE	Effect Size
	Baseline	Follow-Up	Δ	Baseline	Follow-Up	Δ				
Body weight, kg	88.03 (23.30)	89.44 (22.70)	1.40	90.82 (16.91)	92.94 (17.96)	2.12	0.74	1.58 (–2.82 to 4.25)	2.16	0.11
BSA, m^2	2.05 (0.32)	2.07 (0.32)	0.02	2.1 (0.23)	2.12 (0.25)	0.02	0.93	0.00 (–0.04 to 0.05)	0.03	0.07
IVST, mm	18.00 (3.97)	17.82 (4.87)	–0.18	18.88 (4.59)	17.92 (3.3)	–0.96	0.57	0.78 (–0.78 to –3.53)	1.35	0.23
LVPWT, mm	13.55 (3.59)	13.27 (3.8)	–0.27	12.29 (2.69)	13.79 (1.96)	1.50	0.09	1.77 (–0.31 to 3.85)	1.02	0.53
Maximum wall thickness, mm	20.55 (3.24)	20.55 (4.11)	0.00	20.54 (4.42)	19.75 (2.92)	–0.79	0.47	0.79 (–2.97 to 1.39)	1.07	0.30
LVEDD, mm	39.36 (6.42)	39.91 (2.7)	0.55	39.38 (7.41)	39.83 (6.34)	0.46	0.97	0.09 (–5.37 to 5.20)	2.60	0.01
LVESD, mm	22.64 (5.16)	21.55 (3.8)	–1.09	22.13 (6.06)	21.88 (5.29)	–0.25	0.72	0.84 (–3.89 to 5.57)	2.32	0.14
EF, % (mean)	69.45 (6.53)	68.55 (5.17)	–0.90	69.23 (6.80)	69.44 (6.12)	0.22	0.59	1.12 (–3.00 to 5.24)	2.02	0.20
LA volume, mL	74.62 (27.34)	83.58 (27.83)	9.45	89.50 (26.7)	86.08 (31.63)	0.89	0.33	8.56 (–28.63 to 11.52)	9.72	0.49
LVM, g	292.80 (107.50)	290.44 (99.81)	–2.36	269.65 (94.17)	281.98 (81.75)	12.33	0.56	14.69 (–36.32 to 65.69)	25.10	0.23
LVMI, g/m^2	141.95 (50.14)	140.41 (47.27)	–1.55	128.48 (43.60)	132.92 (35.41)	4.45	0.63	5.99 (–19.10 to 31.10)	12.33	0.19
E, cm/s	79.04 (18.16)	77.55 (14.24)	4.19	70.53 (20.23)	74.79 (22.4)	3.81	0.85	0.38 (–10.95 to 10.19)	5.16	0.02
A, cm/s	71.00 (25.50)	64.29 (31.31)	1.58	63.04 (26.07)	61.36 (24.09)	–2.09	0.86	0.367 (–19.61 to 12.77)	7.77	0.20
E/A	1.28 (0.67)	1.40 (0.54)	0.09	1.27 (0.46)	1.38 (0.56)	0.13	0.94	0.04 (–0.67 to 0.75)	0.24	0.05
IVRT, ms	85.64 (12.85)	86 (18.77)	–2.00	82.25 (13.87)	87.79 (20.86)	2.14	0.59	4.14 (–16.19 to 24.48)	9.78	0.16
Septal Sa, cm/s	5.49 (1.89)	4.69 (1.79)	–1.38	5.16 (0.83)	4.26 (1.63)	–0.88	0.63	0.50 (–1.27 to 2.27)	0.83	0.39
Septal Ea, cm/s	4.49 (2.05)	5.17 (1.48)	–0.02	4.79 (1.42)	4.45 (1.61)	–0.04	0.48	0.02 (–1.68 to 1.64)	0.77	0.03
Lateral Sa, cm/s	5.13 (2.39)	4.53 (1.69)	–0.28	5.53 (1.51)	5.05 (1.84)	0.08	0.74	0.36 (–1.33 to 2.05)	0.78	0.23
Lateral Ea, cm/s	6.17 (2.10)	9.14 (3.27)	2.36	7.00 (2.16)	6.36 (2.35)	0.24	0.02	2.12 (–5.44 to 1.21)	1.53	0.64
E/e′ ratio	18.80 (9.14)	15.63 (6.33)	–0.47	15.26 (4.63)	18.97 (9.59)	1.88	0.21	2.35 (–5.40 to 10.10)	3.69	0.53
LVOT gradient at rest, mm Hg	10.45 (23.44)	15.73 (28.44)	5.27	14.81 (25.14)	15.75 (25.02)	1.62	0.65	3.65 (–22.05 to 14.75)	8.98	0.14
SAM: n (%)	1 (9.09)	3 (27.27)	2	3 (12.50)	5 (20.83)	2	NA	NA	NA	NA

All data are presented as mean (SD), unless specified. A indicates mitral valve inflow late velocities; BSA, body surface area; CI, confidence interval; E, mitral valve early inflow velocities; EF, ejection fraction; F, female; IVRT, isovolumic relaxation time; IVST, interventricular septal thickness; LA, left atrium; Lateral Ea, early diastolic mitral annulus velocity measured at lateral side of the mitral annulus; Lateral Sa, systolic mitral annulus velocity measured at lateral side of the mitral annulus; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVM, left ventricular mass; LVMI, left ventricular mass indexed to body surface area; LVOT, left ventricular outflow tract gradient; LVPWT, left ventricular posterior wall thickness; M, male; NA, not available; NAC, N-acetylcysteine; SAM, systolic anterior motion of the mitral leaflet; Septal Ea, early diastolic mitral annulus velocity measured at septal side of the mitral annulus; and Septal Sa, systolic mitral annulus velocity measured at septal side of the mitral annulus.

*Absolute value of $\Delta^{Placebo}-\Delta^{NAC}$.

population.[32] Consequently, the pathogenic variants in the *TTN* gene identified in the HALT-HCM population may not be the true causes of HCM in this population and might simply contribute or modify expression of the phenotype.[33,34] Nevertheless, a subgroup analysis in those carrying pathogenic variants in the *TTN* gene also showed no discernible effect of NAC on cardiac phenotype in HCM (Online Table V). Given the small number of the participants with each pathogenic variant and other genes, no additional gene-dependent analysis was performed.

The null findings of the study with regard to the effects of NAC on cardiac hypertrophy and fibrosis are in discord with the results of experimental studies in animal models of HCM, where treatment with NAC was highly effective in attenuating established hypertrophy and fibrosis.[15–17] Such conflicting findings also have been observed in studies of other pharmacological interventions in HCM, including studies with statins and inhibitors of the renin–angiotensin–aldosterone system, where the efficacy of these pharmacological agents in treatment of human patients with HCM has not been substantiated, despite the beneficial effects in animal models of HCM.[10,11,13,35] The differences in the results of experimental therapies in model organisms and clinical trials in humans are likely multifarious and not uncommon.[36–38] The

Table 5. Cardiovascular Magnetic Resonance Phenotypes (Per-Protocol Analysis)

	Placebo			NAC						
N	8			10						
M/F	7/1			7/3						
Age, y	50.1 (17.9)			48.9 (16.2)						
	Baseline	Follow-Up	Δ	Baseline	Follow-Up	Δ	*P* Value	$\Delta^{Placebo}-\Delta^{NAC*}$ (95% CI)	SE	Effect Size
MWT, mm	16.95 (5.66)	17.58 (4.01)	0.63	16.80 (4.77)	17.75 (6.05)	0.95	0.81	0.33 (–2.55 to 3.20)	1.36	0.12
LVEDV, mL	181.42 (43.85)	175.13 (47.92)	–6.29	180.9 (33.31)	179.0 (41.31)	–1.90	0.53	4.39 (–10.29 to 19.07)	6.92	0.32
LVESV, mL	78.20 (27.82)	79.25 (30.89)	1.06	75.7 (18.61)	77.2 (24.29)	1.50	0.93	0.45 (–9.78 to 10.67)	4.82	0.04
LVEF, %	57.48 (9.00)	55.00 (10.95)	–2.48	58.1 (7.11)	57 (8.03)	–1.10	0.50	1.38 (–2.81 to 5.56)	1.98	0.27
Mean LV midwall strain, %	–13.86 (3.73)	–15.86 (3.75)	–2.16	–15.22 (2.66)	–14.78 (2.37)	0.44	0.05	0.74 (–1.09 to 6.30)	1.25	0.08
Myocardial mass, g	207.83 (82.38)	221.29 (87.65)	–1.67	214.89 (97.68)	238.22 (99.72)	41.25	0.48	42.92 (–44.51 to 130.30)	43.32	2.83
EM, g	53.67 (45.03)	51.43 (25.53)	–5.17	29.67 (16.86)	38.00 (18.57)	7.00	0.32	12.17 (–13.98 to 38.31)	12.00	0.46
EV, %	22.50 (15.14)	22.29 (5.88)	–0.17	13.89 (5.25)	16.67 (7.84)	1.50	0.64	1.67 (–9.58 to 12.92)	5.16	0.14

All data are presented as mean (SD), unless specified. P values are per mixed model analysis. CI indicates confidence interval; EM, enhanced myocardium; EV, percent of myocardium that has scar (percentage EM/MM); F, female; LV, left ventricle; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; M, male; MWT, maximum wall thickness; and NAC, N-acetylcysteine.

*Absolute value of $\Delta^{Placebo}-\Delta^{NAC}$.

differences, in part, may reflect extreme genetic heterogeneity of human patients with HCM, as opposed to the model organisms, where a single genetic mutation is introduced in a congenic background to induce the phenotype. Likewise, numerous other biological differences between humans and model organisms exist, including the confounding effects of the environmental factors and microbiome.[37] Finally, shortcomings of the study design in some of the experimental studies, such as introduction of unconscious biases in group assignment, data acquisition, and interpretation, might contribute to failure to extend the findings in the model organisms to humans.[36,39]

In conclusion, the results of the HALT-HCM—a pilot feasibility study—showed small effect sizes of treatment with a high dose of NAC for 12 months on indices of cardiac hypertrophy and fibrosis in patients with established HCM. A higher number of adverse events occurred in the NAC group, but these events did not seem to be related to administration of NAC. The small sample size of the study did not enable assessing efficacy of NAC. Data on recruitment, retention, compliance, and the effect sizes of NAC on indices of cardiac hypertrophy and fibrosis might provide guidance in designing efficacy studies in HCM.

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Disclosures

None.

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A Randomized Placebo Controlled Double Blind Pilot Study

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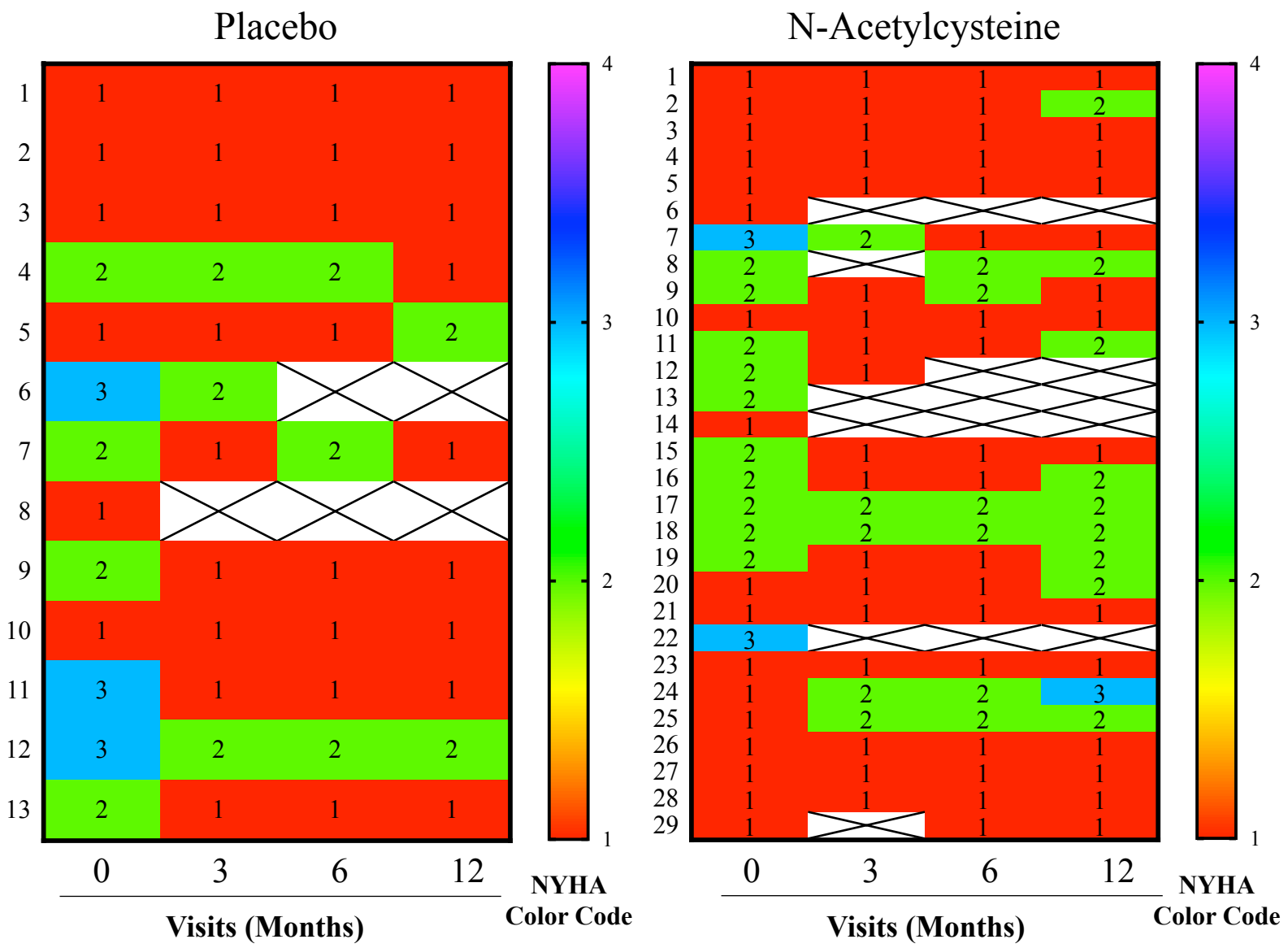
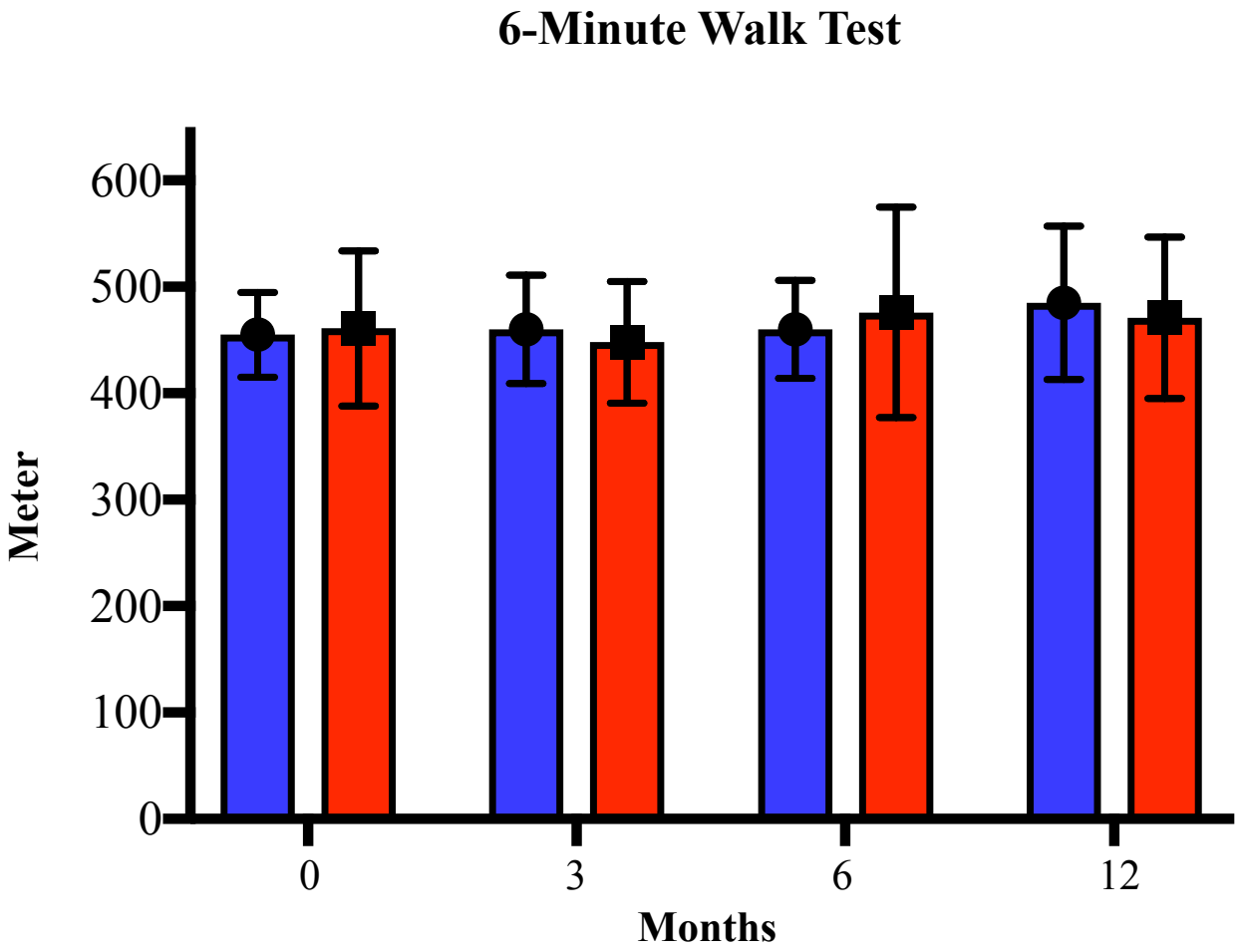


Online Figure I. Heat maps showing changes in New York Heart Association Functional Class in each individual at four time points. No significant difference was noted in any of the four time points in NYHA functional classes between the placebo and N-acetylcysteine groups. Each horizontal line indicate one individual. The number in each box indicates NYHA functional class. X indicates missing data.



Online Figure II. Distance (meter) walked in 6 minutes by the study participants. There were no differences between the two groups in the distance walked in 6 minutes at the baseline and three follow up time points. Mean and SD data are shown.
Blue: Placebo; **Red: N-acetylcysteine**

ONLINE TABLE I

New York Heart Association Functional Class at the Baseline and Follow up Time Points

	Placebo (N=13)				**NAC (N=29)**			
	Baseline	3 months	6 months	12 months	Baseline	3 months	6 months	12 months
Class I	5	9	8	9	16	18	18	14
Class II	5	3	3	2	11	5	6	9
Class III	3	0	0	0	2	0	0	1
Class IV	0	0	0	0	0	0	0	0

ONLINE TABLE II

Electrocardiogram Phenotypes (Intention-to-treat analysis)

	Placebo					NAC					Interaction
	Baseline		12 Months		*p*	Baseline		12 Months		*p*	*p*
N	13		11			29		24			
M/F	10/3		9/2			22/7		18/6			
Age (years)	47.6 (15.1)		50.35 (15.2)			50.7 (15.0)		51.15 (15.8)			0.04
Body weight (Kg)	89.3 (22.9)		89.4 (22.7)		0.22	92.6 (19.5)		92.9 (18.0)		0.14	0.75
BSA (m2)	2.07 (0.33)		2.07 (0.32)		0.16	2.12 (0.27)		2.12 (0.25)		0.31	0.93
Heart rate (bpm)	13	66.2 (11.8)	11	60.4 (11.4)	0.06	29	62.4 (11.2)	24	61.1 (10.7)	0.49	0.14
Atrial Fibrillation/ Flutter (N)	13	0	10	0	NA	28	1	22	1	NA	NA
Paced rhythm (N)	13	0	10	1	NA	28	1	22	3	0.15	0.94
Conduction defect (N)	13	4	10	2	0.24	28	9	22	9	0.32	0.12
Pathological Q waves (N)	13	1	10	1	0.84	28	5	22	2	0.43	0.54
Left ventricular hypertrophy (N)	13	6	10	7	0.27	28	20	22	19	0.16	0.71
QT interval (mean)	13	444.1 (37.7)	10	452.6 (33.0)	0.18	29	458.4 (37.4)	24	448.8 (37.0)	0.12	0.053
ST & T abnormalities (N)	11	12	10	8	0.44	28	20	22	16	0.61	0.47
LVH Score - Estes	13	6.2 (4.0)	10	6.2 (3.8)	0.23	29	5.7 (3.0)	24	6.0 (2.9)	0.70	0.48

All data are presented as mean (SD), unless specified

Abbreviations: M/F: Male/Female; NAC: N-acetylcysteine; BSA: Body surface area; LVH: Left ventricular hypertrophy;

ONLINE TABLE III

Echocardiographic Phenotypes (Intention-to-treat analysis)

	Placebo					NAC					Interaction
	Baseline		12 Months		*p*	Baseline		12 Months		*p*	*p*
N	13		11			29		24			
M/F	10/3		9/2			22/7		18/6			
Age (years)	47.6 (15.1)		50.35 (15.2)			50.7 (15.0)		51.15 (15.8)			0.04
Body weight (kg)	89.3 (22.9)		89.4 (22.7)		0.22	92.6 (19.5)		92.9 (18.0)		0.14	0.75
BSA (m2)	2.07 (0.33)		2.07 (0.32)		0.16	2.12 (0.27)		2.12 (0.25)		0.31	0.93
Echocardiographic Phenotypes	N	Mean	N	Mean		N	Mean	N	Mean		
IVST (mm)	13	17.08 (4.35)	11	17.82 (4.87)	0.94	29	18.45 (4.30)	24	17.92 (3.30)	0.34	0.44
LVPWT (mm)	13	13.08 (3.48)	11	13.27 (3.80)	0.92	29	12.41 (2.67)	24	13.79 (1.96)	0.008	0.15
Maximum wall thickness (mm)	13	19.77 (3.56)	11	20.54 (4.11)	0.98	29	20.00 (4.25)	24	19.75 (2.92)	0.39	0.39
LVEDD (mm)	13	40.15 (6.34)	11	39.91 (2.70)	0.93	29	40.03 (7.52)	24	39.83 (6.34)	0.98	0.99
LVESD (mm)	13	23.31(5.25)	11	21.55 (3.80)	0.35	29	22.24 (5.77)	24	21.88 (5.29)	0.79	0.57
LVEF (%)-mean	13	69.56 (6.03)	11	68.55 (5.17)	0.56	29	69.59 (6.22)	24	69.44 (6.12)	0.95	0.61
LA Volume (ml)	11	69.47 (27.02)	9	83.58 (27.83)	0.11	25	87.85 (28.00)	19	86.08 (31.63)	0.96	0.28
LVM (g)	13	281.66 (103.04)	11	290.44 (99.81)	0.98	29	270.78 (94.98)	24	281.98 (81.75)	0.41	0.67
LVMI (g/m2)	13	136.78 (49.65)	11	140.41 (47.27)	0.98	29	127.67 (42.70)	24	132.92 (35.41)	0.49	0.73
E (cm)	10	79.17 (16.25)	11	77.55 (14.24)	0.85	28	71.85 (19.00)	23	74.79 (22.40)	0.10	0.81
A (cm)	10	70.61 (23.40)	11	64.29 (31.31)	0.78	27	66.06 (26.20)	22	61.36 (24.09)	0.48	0.81
E/A ratio	10	1.27 (0.61)	11	1.40 (0.54)	0.61	27	1.22 (0.44)	22	1.38 (0.56)	0.16	0.88
IVRT (msec)	12	84.42 (12.96)	9	86.00 (18.77)	0.70	19	82.84 (12.90)	19	87.79 (20.86)	0.38	0.71
Septal Sa (cm/s)	10	5.42 (1.79)	7	4.69 (1.79)	0.03	18	5.19 (1.09)	16	4.26 (1.63)	0.046	0.67
Septal Ea (cm/s)	10	4.63 (1.98)	7	5.17 (1.48)	0.54	19	4.65 (1.44)	15	4.45 (1.61)	0.72	0.59
Lateral Sa (cm/s)	9	5.38 (2.18)	7	4.53 (1.69)	0.43	19	5.56 (1.64)	14	5.05 (1.84)	0.60	0.68
Lateral Ea (cm/s)	9	6.92 (2.51)	7	9.14 (3.27)	0.06	18	7.09 (2.25)	14	6.36 (2.35)	0.50	0.04

E/e' ratio	9	18.48 (8.57)	8	15.62 (6.33)	0.66	22	16.87 (8.62)	19	18.97 (9.59)	0.30	0.32
LVOT Gradient at rest (mmHg)	13	8.85 (21.76)	11	15.73 (28.44)	0.46	25	13.12 (23.48)	22	15.75 (25.02)	0.69	0.63
SAM: N (%)	10	1 (10.0)	10	4 (40)	0.23	20	6 (25)	19	5 (26.35)	0.85	NA

All data are presented as mean (SD), unless specified

Abbreviations: NAC: N-acetylcysteine; M/F: Male/females; CI: Confidence interval; SE: Standard Error; BSA: Body surface area; IVST: Interventricular septal thickness; LVPWT: Left ventricular posterior wall thickness; LVEDD: Left ventricular end diastolic diameter; LVESD: Left ventricular end systolic diameter; LVEF: Left ventricular ejection fraction; LA: left atrium; LVM: Left ventricular mass; LVMI: Left ventricular mass indexed to body surface area; E: Mitral valve early inflow velocities; A: Mitral valve inflow late velocities; IVRT: Isovolumic relaxation time; Septal Sa: Systolic mitral annulus velocity measured at septal side of the mitral annulus; Septal Ea: Early diastolic mitral annulus velocity measured at septal side of the mitral annulus; Lateral Sa: Systolic mitral annulus velocity measured at lateral side of the mitral annulus; Lateral Ea: Early diastolic mitral annulus velocity measured at lateral side of the mitral annulus; LVOT: left ventricular outflow tract gradient; SAM: Systolic anterior motion of the mitral leaflet.

ONLINE TABLE IV

CMR Phenotypes (Intention-to-treat analysis)

	Placebo					NAC					Interaction
	Baseline		12 Months		*p*	Baseline		12 Months		*p*	*p*
N	9		8			15		10			
M/F	7/2		7/1			12/3		7/3			
Age (years)	47.6 (18.4)		51.1 (17.9)			48.0 (14.8)		49.9 (16.2)			0.22
Body weight (Kg)	84.2 (17.1)		85.8 (16.4)		0.45	90.4 (18.1)		93.6 (17.7)		0.08	0.27
BSA (m^2)	1.98 (0.24)		2.03 (0.24)		0.30	2.08 (0.25)		2.11 (0.25)		0.21	0.47
CMR	N	Means	N	Means		N	Means	N	Means		
MWT (mm)	9	16.40 (5.54)	8	17.58 (4.01)	0.38	15	16.14 (4.11)	10	17.75 (6.05)	0.37	0.80
LVEDV (ml)	9	175.48 (44.71)	8	175.13 (47.92)	0.21	15	177.20 (36.43)	10	179.00 (41.31)	0.60	0.51
LVESV (ml)	9	76.28 (26.65)	8	79.25 (30.89)	0.80	15	73.67 (17.01)	10	77.20 (24.29)	0.73	0.93
LVEF (%)	9	56.98 (8.55)	8	55.00 (10.95)	0.20	15	58.27 (6.19)	10	57.00 (8.03)	0.30	0.48
Mean LV mid wall strain (%)	8	-14.8 (3.50)	7	-15.86 (3.74)	0.16	15	-15.32 (2.24)	10	-14.78 (2.37)	0.32	0.04
Myocardial Mass (g)	7	191.86 (86.27)	7	221.29 (87.65)	0.85	14	211.21 (85.10)	9	238.22 (99.72)	0.33	0.56
EM (g)	7	48.00 (43.75)	7	51.43 (25.53)	0.75	14	27.57 (15.37)	9	38.00 (18.57)	0.14	0.35
EV (%)	7	21.29 (14.19)	7	22.29 (5.88)	0.89	14	12.93 (5.10)	9	16.67 (7.84)	0.30	0.59

All data are presented as Mean (SD), unless specified

Abbreviations: CMR; Cardiac magnetic resonance; NAC: N-acetylcysteine; M/F: Male/Female; BSA: Body surface area; MWT: Maximum wall thickness; LVEDV: Left ventricular end diastolic volume; LVESV: Left ventricular end systolic volume; LVEF: Left ventricular ejection fraction; LV: Left ventricle; EM: Enhanced myocardium; EV: Percent of myocardium that has scar (percentage of EM/MM)

Online Table V

Echocardiographic Phenotypes in Participants with Pathogenic Variants in *TTN* Gene

	Placebo			**NAC**			**p**	**$\Delta^{Placebo}$-Δ^{NAC}* (95% CI)**	**standard error**	**Effect size**
N	6			6						
M/F	5/1			4/2						
Age (year)	49.40 (13.17)			53.888 (16.50)						
	Baseline	**Follow-up**	**Delta (Δ)**	**Baseline**	**Follow-up**	**Delta (Δ)**				
Body weight (Kg)	88.98 (18.50)	91.41 (17.56)	2.42	90.65 (16.48)	91.78 (18.44)	1.14	0.62	1.29 (-6.90, 4.32)	2.52	0.32
BSA (m2)	2.08 (0.28)	2.12 (0.28)	0.03	2.1 (0.25)	2.08 (0.26)	0.02	0.66	0.02 (-0.10, 0.07)	0.04	0.32
IVST (mm)	18.17 (2.64)	17.50 (3.02)	-0.67	20.33 (3.98)	19.33 (3.78)	-1.00	0.77	0.33 (-2.75, 2.08)	1.09	0.24
LVPWT (mm)	11.50 (3.21)	12.50 (4.72)	1.00	12.50 (1.64)	14.33 (2.66)	1.83	0.65	0.83 (-3.17, 4.84)	1.80	0.22
Maximum wall thickness (mm)	19.33 (3.44)	19.17 (4.49)	-0.17	23.00 (4.82)	20.50 (2.74)	-2.50	0.28	2.33 (-6.87, 2.207)	2.04	0.70
LVEDD (mm)	37.50 (7.87)	39.83 (3.1)	2.33	41.00 (6.26)	42.33 (5.78)	1.33	0.77	1.00 (-8.44, 6.44)	3.34	0.15
LVESD (mm)	20.67 (5.47)	21.17 (2.4)	0.50	24.67 (6.02)	22.50 (7.37)	-2.17	0.32	2,67 (-8.31, 2.98)	2.53	0.70
EF (%)-mean	72.15 (4.56)	68.92 (5.46)	-3.23	68.68 (9.37)	68.72 (7.20)	0.03	0.44	3.27 (-5.82, 12.35)	4.08	0.58
LA Volume (ml)	82.82 (31.57)	87.67 (33.45)	9.48	92.38 (25.13)	90.70 (37.03)	0.14	0.72	9.34 (-57.22, 38.54)	20.77	0.46
LVM (g)	233.69 (95.11)	264.49 (118.99)	30.80	323.70 (108.44)	327.65 (106.04)	3.96	0.56	26.86 (-126.80, 73.10)	44.85	0.43
LVMI (g/m2)	109.18 (37.46)	123.17 (52.44)	13.98	157.15 (52.72)	156.08 (44.13)	-1.07	0.46	15.05 (-59.00, 28.90)	19.73	0.51
E (cm/s)	80.03 (20.20)	84.38 (12.87)	4.35	79.38 (18.69)	84.92 (21.36)	5.53	0.90	1.18 (-18.52, 20.90)	8.84	0.07
A (cm/s)	68.10 (25.55)	66.78 (31.41)	-1.32	69.56 (23.29)	63.46 (20.05)	-6.10	0.62	4.78 (-25.70, 16.14)	9.34	0.24
E/A	1.34 (0.72)	1.49 (0.68)	0.14	1.30 (0.37)	1.50 (0.49)	0.19	0.91	0.05 (-0.96, 1.06)	0.44	0.05
IVRT (msec)	79.33 (8.89)	83.75 (12.07)	2.25	72.75 (111.00)	98.40 (29.53)	16.00	0.16	13.75 (-29.98, 57.48)	17.01	0.74

Septal Sa (cm/s)	6.45 (2.02)	5.12 (1.99)	-1.05	5.30 (0.57)	3.66 (1.15)	-1.55	0.65	0.50 (-3.20, 2.20)	0.97	0.42
Septal Ea (cm/s)	5.50 (2.45)	5.36 (1.71)	-0.15	4.57 (1.00)	3.44 (0.88)	-1.00	0.32	0.85 (-3.19, 1.50)	0.84	1.10
Lateral Sa (cm/s)	5.46 (2.37)	4.68 (2.05)	0.28	5.04 (1.64)	6.20 (2.26)	-0.05	0.30	0.33 (-2.70, 2.05)	0.85	0.31
Lateral Ea (cm/s)	6.66 (1.60)	9.28 (3.98)	2.70	5.97 (2.04)	7.90 (4.10)	1.20	0.36	1.50 (-9.51, 6.51)	2.89	0.41
E/e' ratio	17.28 (10.25)	15.82 (7.47)	0.18	17.15 (3.48)	27.10 (7.80)	11.37	0.02	11.19 (1.21, 21.16)	4.08	2.41
LVOT Gradient at rest (mmHg)	8.50 (20.82)	15.73 (28.44)	0.33	3.75 (7.75)	17.60 (34.54)	24.33	0.15	24.00 (-62.03, 110.0)	13.10	29.39
SAM: N	1	1	0	0	0	0	NA	NA	NA	NA

All data are presented as mean (SD), unless specified

* Absolute value of $\Delta^{Placebo}$-Δ^{NAC}

Abbreviations: NAC: N-acetylcysteine; M/F: Male/females; CI: Confidence interval; SE: Standard Error; BSA: Body surface area; IVST: Interventricular septal thickness; LVPWT: Left ventricular posterior wall thickness; LVEDD: Left ventricular end diastolic diameter; LVESD: Left ventricular end systolic diameter; LVEF: Left ventricular ejection fraction; LA: left atrium; LVM: Left ventricular mass; LVMI: Left ventricular mass indexed to body surface area; E: Mitral valve early inflow velocities; A: Mitral valve inflow late velocities; IVRT: Isovolumic relaxation time; Septal Sa: Systolic mitral annulus velocity measured at septal side of the mitral annulus; Septal Ea: Early diastolic mitral annulus velocity measured at septal side of the mitral annulus; Lateral Sa: Systolic mitral annulus velocity measured at lateral side of the mitral annulus; Lateral Ea: Early diastolic mitral annulus velocity measured at lateral side of the mitral annulus; LVOT: left ventricular outflow tract gradient; SAM: Systolic anterior motion of the mitral leaflet.