



# South Asian-Specific *MYBPC3* $\Delta$ 25bp Deletion Carriers Display Hypercontraction and Impaired Diastolic Function Under Exercise Stress

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**Background:** A 25-base pair (25bp) intronic deletion in the *MYBPC3* gene enriched in South Asians (SAs) is a risk allele for late-onset left ventricular (LV) dysfunction, hypertrophy, and heart failure (HF) with several forms of cardiomyopathy. However, the effect of this variant on exercise parameters has not been evaluated.

**Methods:** As a pilot study, 10 asymptomatic SA carriers of the *MYBPC3* $\Delta$ 25bp variant ( $52.9 \pm 2.14$  years) and 10 age- and gender-matched non-carriers (NCs) ( $50.1 \pm 2.7$  years) were evaluated at baseline and under exercise stress conditions using bicycle exercise echocardiography and continuous cardiac monitoring.

**Results:** Baseline echocardiography parameters were not different between the two groups. However, in response to exercise stress, the carriers of  $\Delta$ 25bp had significantly higher LV ejection fraction (%) (CI:  $4.57 \pm 1.93$ ;  $p < 0.0001$ ), LV outflow tract peak velocity (m/s) (CI:  $0.19 \pm 0.07$ ;  $p < 0.0001$ ), and higher aortic valve (AV) peak velocity (m/s) (CI:  $0.103 \pm 0.08$ ;  $p = 0.01$ ) in comparison to NCs, and E/A ratio, a marker of diastolic compliance, was significantly lower in  $\Delta$ 25bp carriers (CI:  $0.107 \pm 0.102$ ;  $p = 0.038$ ). Interestingly, LV end-diastolic diameter (LVID<sub>dia</sub>) was augmented in NCs in response to stress, while it did not increase in  $\Delta$ 25bp carriers (CI:  $0.239 \pm 0.125$ ;  $p = 0.0002$ ). Further, stress-induced right ventricular systolic excursion velocity  $s'$  (m/s), as a marker of right ventricle function, increased similarly in both groups, but tricuspid annular plane systolic excursion increased more in carriers (slope:  $0.008$ ;  $p = 0.0001$ ), suggesting right ventricle functional differences between the two groups.

**Conclusions:** These data support that *MYBPC3* $\Delta$ 25bp is associated with LV hypercontraction under stress conditions with evidence of diastolic impairment.

**Keywords:** *MYBPC3*, South Asians, hypertrophic cardiomyopathy, ventricular diastolic dysfunction, stress echocardiography, 25bp deletion, DOSA study, myosin binding protein-C

## INTRODUCTION

Mutations in sarcomere genes are a common cause of familial hypertrophic cardiomyopathy (HCM), a prevalent cardiovascular genetic disorder that affects  $\sim$ 1 in 200–500 asymptomatic young adults in the United States (1–11). Mutations in *MYBPC3*, which encodes cardiac myosin-binding protein-C (cMyBP-C), a thick-filament cardiac muscle protein that regulates cardiac contractility (6, 7, 12–14), are associated with  $\sim$ 40% of all HCM cases (9, 15), many of which present late in life (16–22). HCM is characterized by asymmetric left ventricular (LV) thickening, diastolic dysfunction, heart failure (HF), and sudden cardiac death (SCD) (4, 23, 24). Affected individuals can remain asymptomatic or present with symptoms, such as dyspnea, angina, or palpitation (9), in early or late adulthood (11, 22). Heterogeneity in the phenotypic expression of disease from *MYBPC3* genetic variation requires an understanding of early features, optimal care, and longitudinal follow-up.

The prevalence of HCM may be affected by ancestry (4, 25), but the contribution of ancestry-based genetic variants in the pathogenesis of the disease is not yet well-established. Of the South Asian (SA)-specific variants, a polymorphic 25-base pair (bp) deletion in intron 32 ( $\Delta$ 25bp) of *MYBPC3* is present in 4–6% (26–28) of SA individuals and a risk allele for late-onset LV dysfunction, hypertrophy, and HF with multiple forms of cardiomyopathy, such as HCM with an odds ratio of  $\sim$ 7 (26, 29) (Figures 1A,B). Data have shown that asymptomatic *MYBPC3* $\Delta$ 25bp carriers are at risk of late-onset disease progression (16, 22, 26). This variant was defined as a risk allele based on a large case-control study from 6,273 individuals belonging to 107 ethnic populations across 35 Indian states and 2,085 individuals of 63 ethnic/racial groups from 26 countries, including all five continents (26, 30). Nonetheless, in recent studies, *MYBPC3* $\Delta$ 25bp was reported with incomplete penetrance, and the presence of additional genetic or non-genetic risk factors may predispose carriers to cardiomyopathies (26, 27, 31). Our recent investigation has revealed no significant difference in cardiac features between the carrier and non-carrier (NC) cohorts at baseline (27). However, several individual *MYBPC3* $\Delta$ 25bp carriers showed increased fractional shortening at baseline, and this hyperdynamic state is often seen as an early pathological finding in HCM (27). Harper et al. identified an enriched haplotype in SAs with HCM with both *MYBPC3* $\Delta$ 25bp and an associated variant, *MYBPC3* c.1224-52G>A (31). However, in Harper's study, only 134 subjects out of 5,394 HCM cases were defined as SAs, and only 17 carried the *MYBPC3* $\Delta$ 25bp variant. We also recently identified a co-segregating novel variant, D389V (*MYBPC3*<sup>D389V</sup>). It was observed in  $\sim$ 10% of *MYBPC3* $\Delta$ 25bp carriers and associated with hyperdynamic cardiac features on baseline echocardiography (27). These data have led to the hypothesis that the risk of HCM is not caused by the *MYBPC3* $\Delta$ 25bp allele alone, but rather conferred by additional rare, pathogenic variants present on the *MYBPC3* $\Delta$ 25bp haplotype. This possible coinheritance of additional risk alleles changes the interpretation of the role of the *MYBPC3* $\Delta$ 25bp in the development of LV dysfunction and HCM. It also means that the pathogenicity of the *MYBPC3* $\Delta$ 25bp

variant alone and in the presence of additional modifying risk factors needs further investigation and correlation, considering the growing number of SA carriers with the *MYBPC3* $\Delta$ 25bp variant, predisposing them to severe adverse events, such as SCD, even with the occult clinical phenotype (23).

*MYBPC3* gene variants, such as the *MYBPC3* $\Delta$ 25bp, are generally associated with late-disease onset (16, 22, 26). Herein, we continued genetic screening of the United States (US) SA general population for the presence of *MYBPC3* $\Delta$ 25bp and additional rare risk alleles using next-generation sequencing (NGS) technology. To determine the pathogenicity of the *MYBPC3* $\Delta$ 25bp variant, we conducted an exploratory pilot study and prospectively evaluated 10 asymptomatic male carriers of the *MYBPC3* $\Delta$ 25bp variant and 10 age-matched NCs for the changes in cardiac function under exercise stress using submaximal bicycle stress exercise echocardiography (BSE) and continuous cardiac monitoring. We hypothesized that asymptomatic *MYBPC3* $\Delta$ 25bp SA carriers have detectable subclinical risk factors under exercise stress conditions that predispose this group to develop LV hypercontractility and impaired relaxation. Our results suggest that *MYBPC3* $\Delta$ 25bp is consistent with its role as a risk allele for LV dysfunction and cardiomyopathy in SAs.

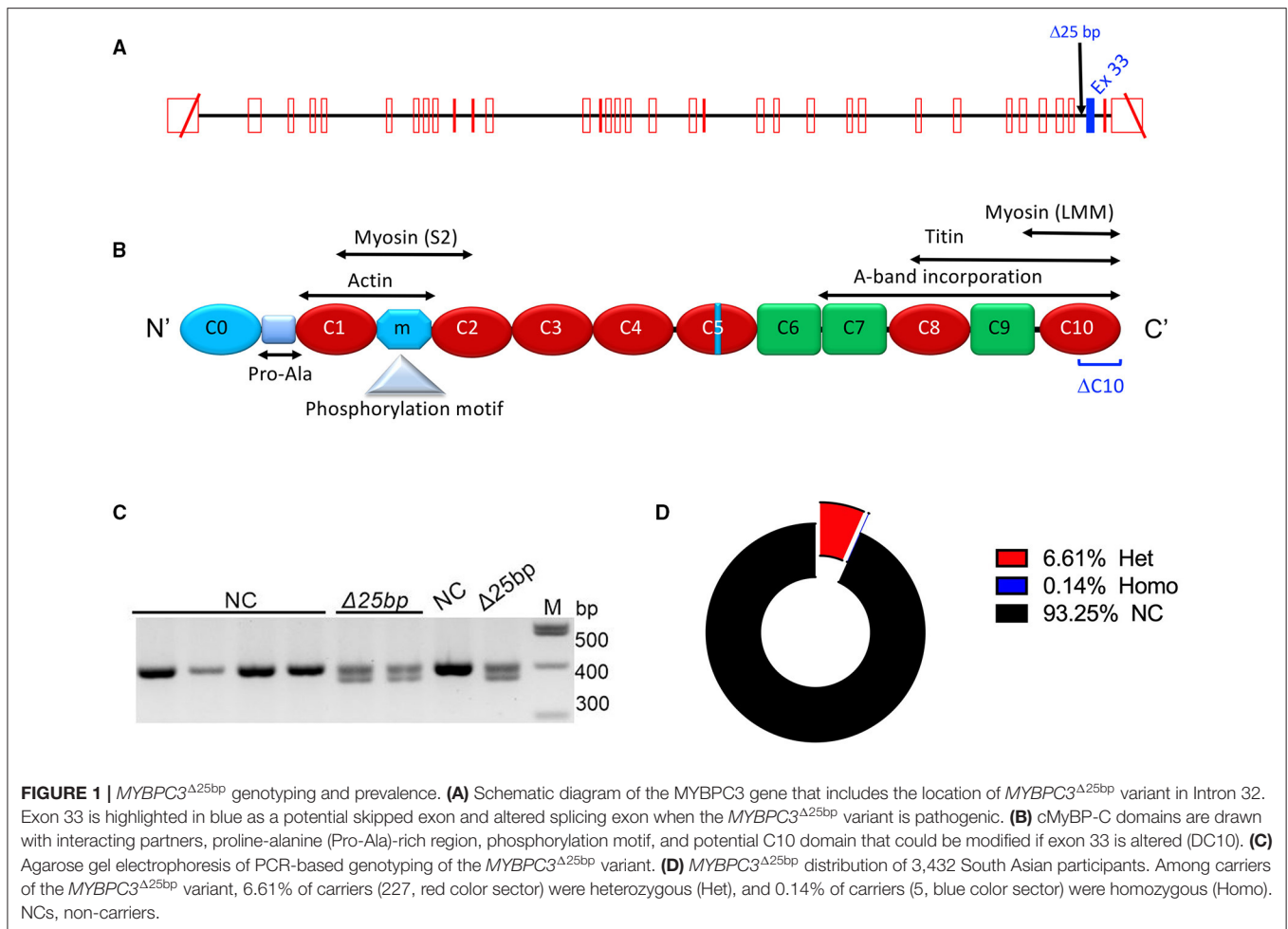
## METHODS

### Enrollment of Study Subjects: Prevalence and Genotype-Phenotype

This research study was reviewed and approved by the Institutional Review Board (IRB) of Loyola University Chicago and University of Cincinnati and was conducted in accordance with the Declaration of Helsinki. Subjects 18 years of age and older from US SA descendants from 9 countries, namely, India, Pakistan, Bangladesh, Sri Lanka, Nepal, Bhutan, Maldives, Afghanistan, and Myanmar, participated in the prevalence study (Loyola University Chicago IRB# LU207815 and 207359, Chicago, Illinois and University of Cincinnati IRB# 2016-7580, Cincinnati, OH, USA) and provided either blood or saliva samples through sample collection outreach events for detection of *MYBPC3* $\Delta$ 25bp variants. Additionally, carriers of *MYBPC3* $\Delta$ 25bp variants and age- and gender-matched NCs were recruited in the follow-up genotype-phenotype study (University of Cincinnati IRB# 2016-4948, Cincinnati, OH, USA) to perform ECG tracing and baseline transthoracic echocardiography (TTE) and BSE.

### Sample Collection and Genetic Screening of the Human *MYBPC3* $\Delta$ 25bp Variant

After giving written consent, blood or saliva samples of the US SA subjects were collected in community outreach events and initially screened for the detection of *MYBPC3* $\Delta$ 25bp variants. Blood samples (10 ml) were collected in ethylenediaminetetraacetic acid (EDTA) vacutainers (Catalog No. 367862; BD Bioscience, Woburn, MA, USA), and saliva samples (1–2 ml) were collected in sterile screw-cap transport 5-ml tubes. Saliva samples were transported in ice and stored at



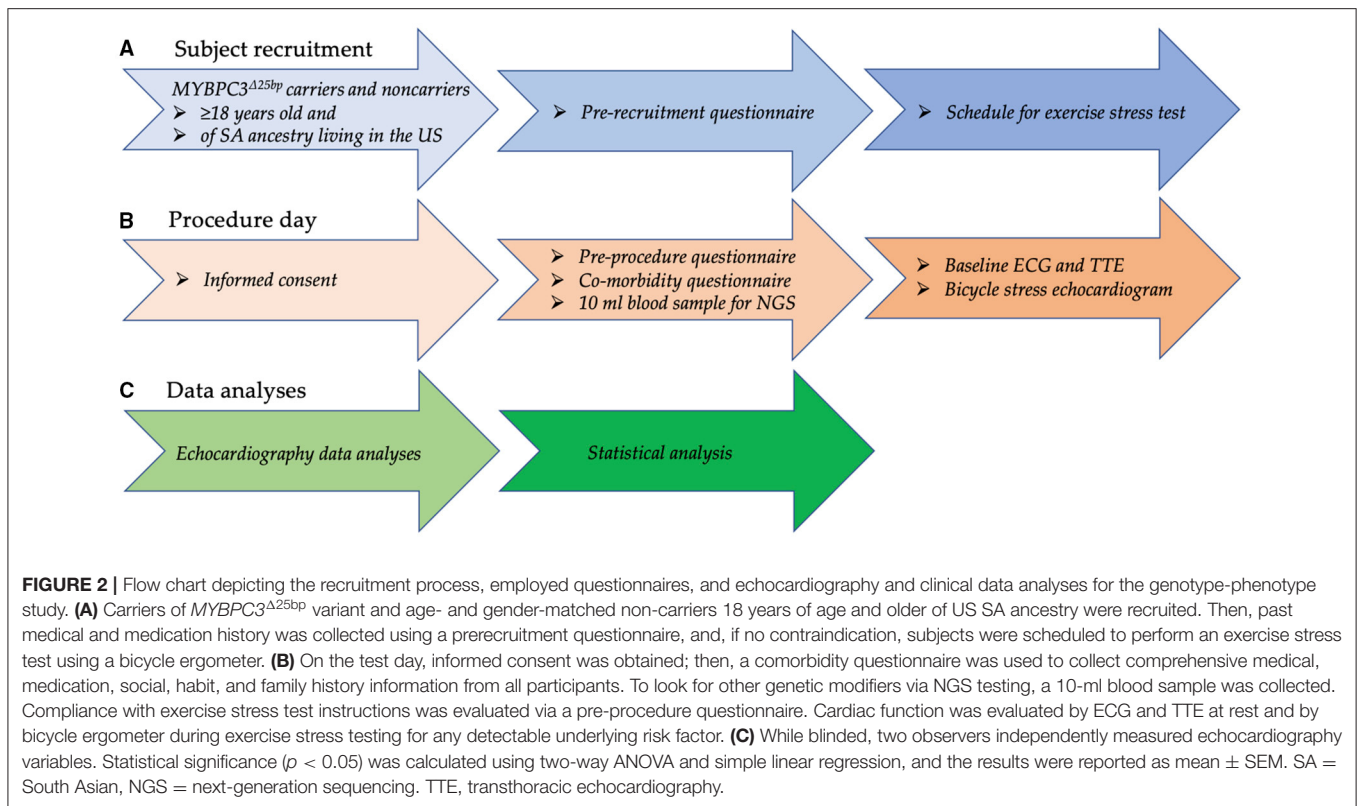
–20°C. Blood and saliva samples were used for human genomic DNA isolation using the QIAmp DNA Blood Mini Kit (Catalog No. 51106; Qiagen, Germantown, MD, USA). The polymerase chain reaction was used for genotyping of the *MYBPC3* $\Delta 25bp$  (rs36212066) variant with the forward 5'-GTT TCC AGC CTT GGG CAT AGT C-3' and reverse 5'-GAG GAC AAC GGA GCA AAG CCC-3' primer sequences on 2.5% agarose gel (27). After initial genotyping, we recruited carriers and NCs of the *MYBPC3* $\Delta 25bp$  variant for the follow-up genotype-phenotype study (27). After giving written consent, the recruited subjects provided an additional 10-ml blood sample for genotype confirmation and NGS analysis (27).

### Clinical and Cardiac Function Evaluation at Baseline and Under Exercise Stress

Carriers of the *MYBPC3* $\Delta 25bp$  variant and age- and gender-matched NCs 18 years of age and older of US SA descendants were invited to participate in the follow-up genotype-phenotype study to undergo ECG and baseline TTE and BSE, as monitored by a team cardiologist (Figure 2). Recruited subjects for the follow-up study had no uncontrolled comorbidities and no significant differences in the frequency of hypertension, diabetes mellitus, dyslipidemia, and obesity between the two groups. The

subjects were instructed to abstain from alcoholic, caffeinated, tobacco products, and drugs, such as  $\beta$ -blockers (if applicable), for 24 h prior to stress testing. Echocardiographic images were captured by two cardiac sonographers of similar level and length of experience. Both subjects and observers, i.e., sonographers and team cardiologists, were blinded to *MYBPC3* $\Delta 25bp$  genotype results. Medical, medication, social, habit, and family history information of all participants were collected using a comorbidity questionnaire after giving written consent on the test day. Two additional forms, a prerecruitment questionnaire (PRQ) and a pre-procedural questionnaire (PPQ), were used for subjects who were scheduled for BSE. Before scheduling subjects for BSE testing, past medical and medication history was collected using the PRQ. Completed PRQs were then reviewed by a team cardiologist for any possible contraindications or medication modification. On the test day, subjects' compliance with BSE test instructions was evaluated using the PPQ.

Cardiac electrical activity was recorded using a standard 12-lead ECG with rhythm strips, and a Vivid E9 GE Healthcare instrument was used for TTE and BSE imaging using the 2-dimensional method in conjunction with color Doppler flow examination. Exclusion criteria for performing BSE included (1) acute myocardial infarction in the last 3 months; (2) ongoing



unstable angina; (3) known obstructive left main coronary artery stenosis; (4) recent stroke or transient ischemic attack in the last 3 months; (5) acute pulmonary embolism, pulmonary infarction, or deep vein thrombosis in the last 3 months; (6) moderate-to-severe aortic stenosis with velocity over 3 m/s; (7) hypertrophic obstructive cardiomyopathy with severe resting gradient of over 3 m/s; (8) uncontrolled cardiac arrhythmia with hemodynamic compromise; (9) advanced heart block; (10) decompensated HF; (11) active endocarditis; (12) acute myocarditis or pericarditis; (13) physical disability that would preclude safe and adequate testing; (14) pregnancy; (15) mental impairment with limited ability to cooperate; (16) history of severe hypertension (resting systolic blood pressures  $>200$  or diastolic blood pressures 110 mm Hg) or uncontrolled diabetes mellitus; and (17) uncorrected anemia, electrolyte imbalance or hyperthyroidism. Subjects were provided with the instructions for BSE preparation prior to the test that included (1) fasting for 4 h, (2) refraining from caffeinated and decaffeinated coffee and tea, colas, soft drinks, and chocolate for 24 h, and (3) refraining from smoking and other nicotine-containing products for 12 h.

Moreover, target heart rate (THR) was calculated for BSE as  $220 - (0.85 \times \text{subject's age})$ . Then, the subjects began cycling at a resistance of 15 Watts (W), increasing every 3 min by additional resistance of 15 W until the endpoint. Heart rate (HR), blood pressure, and oxygen saturation were monitored and documented at each minute of exercise. Endpoints of the test were one of the following: (1) achievement of THR; (2) significant angina; (3) significant shortness of breath; (4) significant ischemic changes, such as ST-segment elevations in ECG; (5) hypertension ( $>240$  mmHg systolic blood pressure/ 120

mmHg diastolic blood pressure); (6) hypotension  $>15$  mmHg decline in systolic blood pressure from baseline; (7) significant arrhythmia/aortic valve (AV) block; (8) new or worsening wall-motion abnormalities; (9) drop of oxygen saturation below 90%; (10) intolerable symptoms; (11) upon subject's request to stop; or (12) development of dynamic LV outflow tract (LVOT) gradient of  $>4.5$  m/s.

Finally, two observers independently measured echocardiography variables under the guidance of team cardiologists. The measurements of one observer were used for the analysis in this study, except LV ejection fraction (LVEF) for which the average value measured by two observers was used. The Biplane Simpson method was used to measure LVEF and LV fractional shortening.

## Next-Generation Sequencing

A panel of 174 genes related to cardiovascular diseases was DNA sequenced to identify the presence of any genetic variants using the TruSight Cardio Kit (Catalog No. FC-141-1011; Illumina, San Diego, CA, USA) on an Illumina MiSeq with 150-base pair paired-end reads, as described previously (27). Burrows-Wheeler alignment (BWA) and Genome Analysis Toolkit haplotype caller were applied as described in MegaSeq generate variant call files (VCFs) (32). Variants were excluded if they met any of the following criteria: biallelic balance  $>0.75$ ; quality score  $<30$ ; depth of coverage  $>360$ ; strand bias more than  $-0.01$ , and mapping quality zero reads  $\geq 10$ . Variant ranking and prioritization: variants were annotated using SnpEFF. HIGH and MODERATE variants were scored using Polyphen, Genomic Evolutionary Rate Profiling, and Sorting Intolerant



From Tolerant, to predict pathogenicity. All variants were ranked by minor allele frequency (MAF) based on data from public databases (ExAc). All variants were crosschecked against ClinVAR. The American College of Medical Genetics guidelines (ACMG) were used for determining pathogenicity (33). Sanger sequencing was applied for validation using individual primers.

## Statistical Analyses

The prevalence of genotype results was reported as a percentage. Descriptive statistics were reported in frequency tables to compare cardiac phenotype outcomes by genetic variants and by demographics. The chi-square statistics and unpaired *t*-test were used for categorical parameters and numerical variables at baseline, respectively. Regression analysis was used to compare differences in slopes between the groups, and a two-way ANOVA was performed to estimate the effect of exercise stress and genotype on cardiac phenotype continuous outcomes. All analyses were reported as mean  $\pm$  SEM at a significance level of 0.05, and 95% CIs and *p*-values were reported using GraphPad Prism (version 8.4.3).

## RESULTS

### Increased Prevalence of MYBPC3 $\Delta$ 25bp Variant Among US SAs

We previously reported the frequency of MYBPC3 $\Delta$ 25bp variant carriers to be 6% among the US SA population (27). Cumulative screening to date, which could be subject to ascertainment bias, estimated a slightly higher prevalence of MYBPC3 $\Delta$ 25bp at 6.75%. Of 3,432 participants, 232 (6.75%) were carriers of the MYBPC3 $\Delta$ 25bp variant, i.e., 227 (6.61%) heterozygous and 5 (0.14%) homozygous (Figures 1C,D).

### Absence of Second Genetic Variants by NGS

To assess the presence of any additional pathogenic variants, apart from the MYBPC3 $\Delta$ 25bp variant, NGS was performed on a 172 gene cardiovascular panel (TruSight Cardio Sequencing Kit, Illumina), and no pathogenic, or likely pathogenic, variants were identified in the present cohort (27). None of the 20 subjects included in the current study was found to carry the previously reported, potentially pathogenic MYBPC3<sup>-52</sup> allele (c.1224-52G>A) (31) or the novel MYBPC3<sup>D389V</sup> variant (27). We also did not identify any additional modifying loci in MYBPC3 as no other rare variants were identified in the coding region that occurred in more than one cohort subject. Lastly, among the 172 genes of the TruSight Panel, we only identified one pathogenic variant in one subject who carried an APOA4 variant in one of the 10 variant carriers.

### MYBPC3 $\Delta$ 25bp Was Associated With Hypercontraction Under Exercise

To test the hypothesis that asymptomatic MYBPC3 $\Delta$ 25bp US SA carriers have detectable subclinical risk factors under exercise stress conditions, we conducted an exploratory pilot study to evaluate asymptomatic MYBPC3 $\Delta$ 25bp US SA carriers compared to controls for subclinical cardiac changes under exercise stress

conditions (Figure 2). We prospectively studied 20 male subjects of US SA ancestry, including 10 carriers of the MYBPC3 $\Delta$ 25bp variant ( $52.9 \pm 2.14$  years) and 10 age- and gender-matched NCs ( $50.1 \pm 2.7$  years), for any changes in cardiac function at baseline prior to exercise and under exercise stress using bicycle exercise echocardiography and continuous cardiac monitoring (Tables 1, 2). The two groups (MYBPC3 $\Delta$ 25bp carriers and NCs) showed no significant difference in body surface area, body mass index, and baseline mean arterial pressure (MAP) and HR, and no difference in the frequency of comorbidities, such as hypertension, diabetes mellitus, and dyslipidemia (Table 1). Similarly, monitored exercise MAP and HR were similar in both groups (Table 2). All 20 subjects were able to complete at least 15 min of exercise at 75 W equivalent to 4.9 METs, while 4 subjects completed 30 min of exercise at 150 W equivalent to 8.5 METs.

While baseline echocardiography parameters were not different between the two groups (MYBPC3 $\Delta$ 25bp carriers and NCs) (Table 1), significant differences came to light under exercise stress condition in the following parameters: LV end-diastolic diameter (LVID<sub>dia</sub>), LVEF, LVOT and AV peak velocities (pv), and the ratio of early to late ventricular filling velocity (E/A ratio) (Table 2, Figures 3, 4). Stress-induced LVID<sub>dia</sub> augmented in NCs, while it did not increase in MYBPC3 $\Delta$ 25bp carriers with a significant difference between the two groups (CI:  $0.239 \pm 0.125$ ;  $p = 0.0002$ ), indicative of impaired relaxation and diastolic impairment. Strikingly, the estimated effect of exercise stress and genotype showed that MYBPC3 $\Delta$ 25bp carriers had significantly higher LVEF (%) (CI:  $4.57 \pm 1.93$ ;  $p < 0.0001$ ), higher LVOT pv (m/s) (CI:  $0.197 \pm 0.069$ ;  $p < 0.0001$ ), and higher AV pv (m/s) (CI:  $0.103 \pm 0.081$ ;  $p = 0.01$ ) in comparison to NCs. This was consistent with the findings that stress-induced LVID<sub>dia</sub> increase was significantly muted in carriers during exercise, as compared to NCs (CI:  $0.239 \pm 0.125$ ;  $p = 0.0002$ ). Further, E/A ratio, a marker of ventricular diastolic compliance, was significantly lower in carriers as compared to NCs (CI:  $0.107 \pm 0.102$ ;  $p = 0.038$ ) and the ratio of early transmitral peak velocity flow to early diastolic mitral annulus velocity (E/e' ratio), which showed a non-significant difference between the groups (CI:  $0.738 \pm 0.795$ ;  $p = 0.068$ ). Although stress-induced right ventricular systolic excursion velocity (s') was increased similarly in both groups, tricuspid annular plane systolic excursion (cm) increased more in carriers (slope:  $0.008$ ;  $p = 0.0001$ ) from the baseline, consistent with right ventricle functional differences. These findings are indicative of LV hypercontractility among asymptomatic carriers of the MYBPC3 $\Delta$ 25bp variant under exercise stress conditions and evidence of diastolic impaired relaxation at high workloads, suggesting that MYBPC3 $\Delta$ 25bp is an independent risk allele with subclinical pathology prior to late-onset LV dysfunction (16, 22) in the US SA population.

## DISCUSSION

The present study evaluated the exercise response in asymptomatic MYBPC3 $\Delta$ 25bp SA carriers to determine the presence of any subclinical features. Overall, our findings

**TABLE 1** | Demographics, baseline echocardiography parameters, and clinical characteristics of 20 South Asian subjects who participated in a bicycle exercise study.

Variable	Total	n	NC	n	MYBPC3 <sup>Δ25bp</sup>	n	P-value
	n (%), mean ± SEM		n (%), mean ± SEM		n (%), mean ± SEM		
Male (%)	20(100)	20	10(100)	10	10(100)	10	-
Age (years)	51.50 ± 1.71	20	50.1 ± 2.70	10	52.90 ± 2.14	10	0.427
BMI (kg/m <sup>2</sup> )	26.15 ± 0.611	20	26.23 ± 0.58	10	26.06 ± 1.11	10	0.893
BSA (m <sup>2</sup> )	1.95 ± 0.037	20	1.98 ± 0.04	10	1.91 ± 0.06	10	0.301
MAP (mmHg)	101.1 ± 2.00	20	98.70 ± 2.78	10	103.5 ± 2.82	10	0.244
HR (bpm)	74.65 ± 1.96	20	76.90 ± 2.42	10	72.40 ± 3.03	10	0.262
<b>Echocardiographic parameters</b>							
LVID <sub>dia</sub> (cm)	4.06 ± 0.10	20	3.97 ± 0.15	10	4.16 ± 0.13	10	0.376
LVID <sub>s</sub> (cm)	2.78 ± 0.08	20	2.69 ± 0.10	10	2.87 ± 0.14	10	0.331
LVEF (%)	53.65 ± 1.38	20	53.59 ± 1.81	10	53.70 ± 2.17	10	0.969
LVFS (%)	31.38 ± 0.99	20	31.55 ± 0.91	10	31.21 ± 1.82	10	0.867
IVS (cm)	0.94 ± 0.04	20	0.96 ± 0.07	10	0.93 ± 0.03	10	0.71
LVPW (cm)	0.88 ± 0.03	20	0.93 ± 0.03	10	0.83 ± 0.04	10	0.111
IVS/LVPW ratio	1.09 ± 0.05	20	1.04 ± 0.08	10	1.14 ± 0.05	10	0.351
LAV 4C Mod	31.75 ± 2.95	18	30.25 ± 4.69	8	32.95 ± 3.94	10	0.663
LVOT peak velocity (m/s)	0.79 ± 0.03	19	0.76 ± 0.05	9	0.82 ± 0.03	10	0.424
AV peak velocity (m/s)	0.98 ± 0.03	19	0.96 ± 0.06	9	1.01 ± 0.04	10	0.551
E/A ratio	1.15 ± 0.08	20	1.10 ± 0.13	10	1.20 ± 0.11	10	0.596
Average E/e' ratio	7.18 ± 0.46	20	7.20 ± 0.49	10	7.16 ± 0.82	10	0.973
Average e'/a' ratio	1.01 ± 0.08	20	0.90 ± 0.10	10	1.13 ± 0.13	10	0.204
RV s' (m/s)	0.097 ± 0.003	20	0.094 ± 0.004	10	0.09 ± 0.004	10	0.458
LV s' average (m/s)	0.07 ± 0.002	20	0.07 ± 0.003	10	0.07 ± 0.003	10	0.437
TAPSE (cm)	2.21 ± 0.12	19	2.40 ± 0.23	9	2.04 ± 0.10	10	0.166
<b>Clinical characteristics</b>							
HTN (%)	5(25)	20	2(20)	10	3(30)	10	>0.999
DM (%)	5(25)	20	3(30)	10	2(20)	10	>0.999
DLP (%)	8(40)	20	4(40)	10	4(40)	10	>0.999

Statistical significance ( $p < 0.05$ ) was calculated using unpaired t-test for numerical variables and Fisher's exact test for categorical variables.

The results were reported as mean ± SEM. NCs, non-carriers; MYBPC3<sup>Δ25bp</sup>, A 25-base pair deletion in cardiac myosin-binding protein C3; SEM, standard error of mean; BMI, body mass index; BSA, body surface area; MAP, mean arterial pressure; HR, heart rate; LVID<sub>dia</sub>, left ventricular internal diameter in diastole; LVID<sub>s</sub>, left ventricular internal diameter in systole; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; IVS, interventricular septum; LVPW, left ventricular posterior wall; LAV 4C Mod, left atrial volume four-chamber method of disc; LVOT, left ventricular outflow tract; AV, aortic valve; E/A ratio, ratio of early diastole transmitral peak velocity flow to late diastole peak velocity flow; E/e' ratio, ratio of early transmitral peak velocity flow to early diastolic mitral annulus velocity; e'/a' ratio, ratio of early diastolic mitral annulus velocity to late diastolic mitral annulus velocity; RV, right ventricle; s', peak systolic annular velocity; LV, left ventricle; TAPSE, tricuspid annular plane systolic excursion; HTN, hypertension; DM, diabetes mellitus; DLP, dyslipidemia.

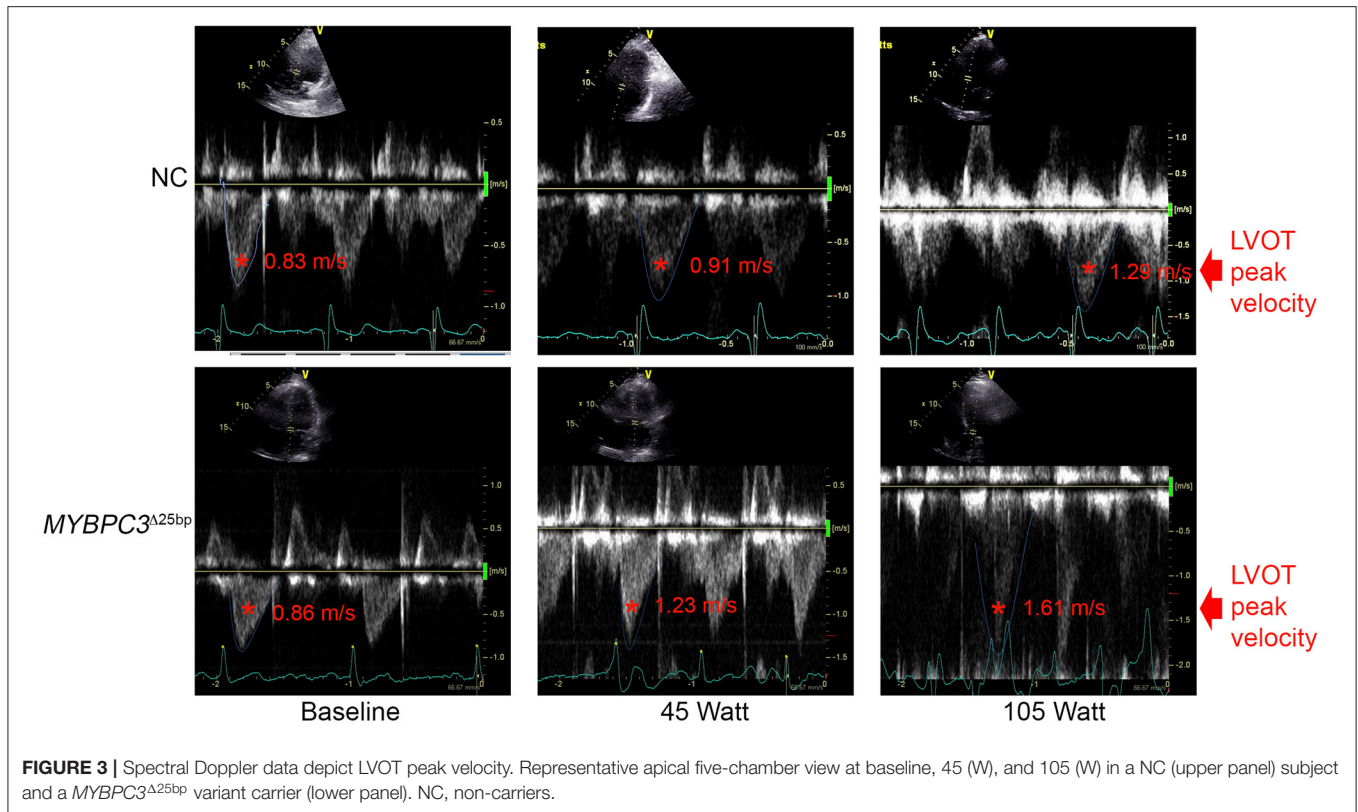
determined the presence of hyperdynamic manifestations under exercise conditions in asymptomatic MYBPC3<sup>Δ25bp</sup> variant carriers of US SA ancestry as compared to NCs who were presented with significantly higher EF, LVOT, and AV peak velocities, and impaired relaxation presented with a significant difference in the LVID<sub>dia</sub> and E/A ratio, but the non-significant difference in the average E/e' ratio. Hyperdynamic features and evidence of cardiac dysfunction were detected by echocardiography in MYBPC3<sup>Δ25bp</sup> carriers. These findings are also in line with the phenotype of genotype-positive individuals at stage 1 or those with non-hypertrophic HCM. These individuals can remain asymptomatic or present with subtle echocardiographic phenotype, such as diastolic dysfunction (34–36). In stage 2, however, individuals present with hypertrophy and LV hyperdynamic status (36), whereas MYBPC3<sup>Δ25bp</sup> variant carriers in the current study were non-hypertrophic, but did show hyperdynamic phenotype under exercise conditions.

As shown in the regression lines, NCs and carriers responded differently to the stress stage, the independent variable, as resistance increased. Starting at 75 W equiv. 4.9 METs, the regression line showed no increase in EF% in NCs after 15 min of exercise. This finding can be explained by the Frank-Starling law, which holds that an increase in workload and venous returns stretches myocardial muscle fibers and increases preload (end-diastolic volume), stroke volume, and, ultimately, cardiac output until maximum capacity is reached. However, the weak increase in LVID<sub>dia</sub> from the baseline, as indicated in the regression line for carriers (**Figure 4A**), could be a contributing factor in maintaining an upward trend in EF% in carriers after 75 w since lower LVID<sub>dia</sub> can contribute to lower LV diastolic volume (LVEDV) as resistance augments. However, further studies with an adequate number of subjects are needed to decisively explain this observed phenotypic feature. Collectively, then, these data suggest that exercise stress itself, as a secondary

**TABLE 2 |** Clinical and bicycle exercise echocardiography parameters of 20 South Asian subjects.

Stress stage (W)	0		15		30		45		60		75		90		105		120		P-value	
	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM	n		
MAP (mmHg)	NC	98.70 ± 2.78	10	107.93 ± 2.37	10	110.1 ± 3.55	10	110.03 ± 3.73	10	109.2 ± 3.74	10	114.43 ± 2.94	10	114.75 ± 3.56	8	119.62 ± 4.96	8	117.33 ± 7.19	6	0.412
	MYBPC3 $\Delta$ 25bp	103.46 ± 2.82	10	109.92 ± 3.44	9	108.77 ± 2.74	9	112.53 ± 3.40	10	111.66 ± 4.34	10	112.76 ± 5.89	10	113.14 ± 6.31	9	123.44 ± 5.89	6	121.13 ± 4.57	5	
HR (bpm)	NC	76.90 ± 2.42	10	92.5 ± 1.8	10	98 ± 2.04	10	105.2 ± 2.4	10	112.5 ± 2.6	10	118 ± 2.02	10	125.75 ± 3.39	8	137.12 ± 4	8	146.83 ± 5.38	6	0.21
	MYBPC3 $\Delta$ 25bp	72.40 ± 3.03	10	91.2 ± 2.56	10	96.7 ± 2.92	10	103.5 ± 2.60	10	111.3 ± 3.34	10	119.5 ± 3.25	10	125.11 ± 3.63	9	132.83 ± 4.93	6	143.66 ± 4.69	6	
LVID <sub>dia</sub> (cm)	NC	3.97 ± 0.15	10	4.4 ± 0.08	9	4.52 ± 0.11	9	4.56 ± 0.11	9	4.29 ± 0.12	9	4.59 ± 0.15	7	4.57 ± 0.1	6	4.59 ± 0.12	5	4.44 ± 0.13	4	0.0002
	MYBPC3 $\Delta$ 25bp	4.16 ± 0.13	10	4.14 ± 0.13	10	4.17 ± 0.09	10	4.15 ± 0.08	9	4.18 ± 0.12	9	4.34 ± 0.15	7	4.32 ± 0.07	7	4.31 ± 0.16	6	4.01 ± 0.21	4	
LVID <sub>s</sub> (cm)	NC	2.69 ± 0.1	10	2.81 ± 0.07	9	2.77 ± 0.1	9	2.65 ± 0.09	9	2.47 ± 0.08	9	2.67 ± 0.05	7	2.53 ± 0.09	6	2.45 ± 0.09	5	2.36 ± 0.07	4	0.06
	MYBPC3 $\Delta$ 25bp	2.87 ± 0.14	10	2.68 ± 0.1	10	2.63 ± 0.07	10	2.48 ± 0.05	9	2.37 ± 0.1	9	2.54 ± 0.07	7	2.39 ± 0.07	7	2.39 ± 0.07	6	2.24 ± 0.12	4	
LVEF (%)	NC	53.59 ± 1.81	10	55.26 ± 1.60	10	58.14 ± 2.09	10	59.99 ± 2.17	10	61.53 ± 2.12	10	64.03 ± 2.27	10	62.86 ± 2.83	7	61.83 ± 3.05	7	61.37 ± 3.39	5	<0.0001
	MYBPC3 $\Delta$ 25bp	53.7 ± 2.17	10	58.26 ± 1.62	10	60.76 ± 1.69	10	63.07 ± 1.63	10	65.91 ± 1.71	10	67.65 ± 1.09	9	68.82 ± 1.48	8	70.19 ± 0.75	7	71.43 ± 0.58	4	
LVFS (%)	NC	31.55 ± 0.91	10	35.75 ± 0.95	9	38.79 ± 1.13	9	42.02 ± 1.03	9	42.19 ± 1.55	9	42.9 ± 1.81	7	44.62 ± 1.29	6	45.83 ± 2.33	5	46.39 ± 2.66	4	0.218
	MYBPC3 $\Delta$ 25bp	31.2 ± 1.82	10	35.98 ± 1.68	10	36.98 ± 1.41	10	40.15 ± 1.39	9	43.44 ± 2.14	9	41.24 ± 1.71	7	44.51 ± 1.9	7	43.72 ± 2.03	6	43.93 ± 1.53	4	
LVOT peak velocity (m/s)	NC	0.76 ± 0.05	9	0.94 ± 0.06	9	0.91 ± 0.04	9	0.97 ± 0.06	10	1.00 ± 0.07	10	1.06 ± 0.04	8	1.13 ± 0.03	6	1.18 ± 0.04	5	1.33 ± 0.11	4	<0.0001
	MYBPC3 $\Delta$ 25bp	0.82 ± 0.03	10	1.12 ± 0.06	10	1.08 ± 0.06	9	1.17 ± 0.06	10	1.34 ± 0.08	10	1.29 ± 0.07	10	1.36 ± 0.08	9	1.39 ± 0.07	7	1.49 ± 0.14	4	
AV peak velocity (m/s)	NC	0.96 ± 0.06	9	1.15 ± 0.06	9	1.09 ± 0.08	10	1.23 ± 0.09	9	1.30 ± 0.09	9	1.35 ± 0.07	8	1.43 ± 0.07	6	1.41 ± 0.13	4	1.61 ± 0.14	3	0.012
	MYBPC3 $\Delta$ 25bp	1.01 ± 0.04	10	1.2 ± 0.06	10	1.25 ± 0.08	8	1.31 ± 0.07	10	1.38 ± 0.07	10	1.45 ± 0.07	10	1.49 ± 0.08	8	1.6 ± 0.06	8	1.75 ± 0.07	4	
E/A	NC	1.10 ± 0.13	10	1.16 ± 0.06	10	1.15 ± 0.08	10	0.97 ± 0.06	9	1.10 ± 0.06	10	0.96 ± 0.09	9	1.05 ± 0.03	6	1.10 ± 0.04	6	1.15 ± 0.20	3	0.03
	MYBPC3 $\Delta$ 25bp	1.2 ± 0.11	10	0.9 ± 0.05	9	1.00 ± 0.09	9	1.01 ± 0.07	10	1.00 ± 0.1	9	0.96 ± 0.07	7	0.89 ± 0.09	5	0.77 ± 0.05	3	1.03 ± 0.27	2	
E/e' average	NC	7.20 ± 0.49	10	7.01 ± 0.50	9	7.31 ± 0.56	10	6.86 ± 0.63	8	7.25 ± 0.31	10	7.84 ± 0.63	7	8.38 ± 0.72	5	7.52 ± 0.40	6	8.81 ± 0.53	2	0.068
	MYBPC3 $\Delta$ 25bp	7.16 ± 0.82	10	7.44 ± 0.98	9	6.27 ± 0.74	7	7.32 ± 0.80	9	7.58 ± 0.87	7	6.84 ± 0.92	6	6.19 ± 0.89	4	6.62 ± 0.47	3	6.11 ± 0.84	2	
RV s' (m/s)	NC	0.095 ± 0.004	10	0.107 ± 0.007	10	0.109 ± 0.007	10	0.121 ± 0.006	8	0.130 ± 0.01	10	0.139 ± 0.009	9	0.16 ± 0.009	7	0.158 ± 0.01	7	0.172 ± 0.009	6	0.35
	MYBPC3 $\Delta$ 25bp	0.1 ± 0.005	10	0.12 ± 0.004	8	0.117 ± 0.005	8	0.131 ± 0.005	8	0.132 ± 0.008	8	0.139 ± 0.009	8	0.152 ± 0.009	6	0.157 ± 0.01	5	0.175 ± 0.014	3	
LV s' average (m/s)	NC	0.07 ± 0.004	10	0.08 ± 0.003	10	0.08 ± 0.004	10	0.08 ± 0.003	9	0.09 ± 0.003	10	0.101 ± 0.004	9	0.101 ± 0.006	8	0.114 ± 0.009	5	0.118 ± 0.014	3	0.557
	MYBPC3 $\Delta$ 25bp	0.07 ± 0.003	10	0.08 ± 0.002	9	0.08 ± 0.003	9	0.09 ± 0.004	9	0.09 ± 0.003	8	0.103 ± 0.005	9	0.106 ± 0.005	7	0.107 ± 0.004	6	0.112 ± 0.006	4	
TAPSE (cm)	NC	2.40 ± 0.23	9	2.34 ± 0.19	9	2.31 ± 0.13	9	2.42 ± 0.14	9	2.47 ± 0.16	9	2.53 ± 0.22	7	2.63 ± 0.13	6	2.44 ± 0.15	5	2.53 ± 0.13	4	0.065
	MYBPC3 $\Delta$ 25bp	2.04 ± 0.1	10	2.27 ± 0.13	10	2.38 ± 0.16	9	2.55 ± 0.13	10	2.68 ± 0.21	9	2.81 ± 0.1	7	2.52 ± 0.18	3	2.81 ± 0.23	6	3.71 ± 0.00	1	

Statistical significance ( $p < 0.05$ ) was calculated using two-way ANOVA. The results were reported as mean ± SEM. NCs, non-carriers; MYBPC3 $\Delta$ 25bp, A 25-base pair deletion in cardiac myosin binding protein C3; SEM, standard error of mean; MAP, mean arterial pressure; HR, heart rate; LVID<sub>dia</sub>, left ventricular internal diameter in diastole; LVID<sub>s</sub>, left ventricular internal diameter in systole; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; LVOT, left ventricular outflow tract; AV, aortic valve; E/A ratio, ratio of early diastole transmitral peak velocity flow to late diastole peak velocity flow; E/e' ratio, ratio of early transmitral peak velocity flow to early diastolic mitral annulus velocity; RV, right ventricle; s', peak systolic annular velocity; LV, left ventricle; TAPSE, tricuspid annular plane systolic excursion.



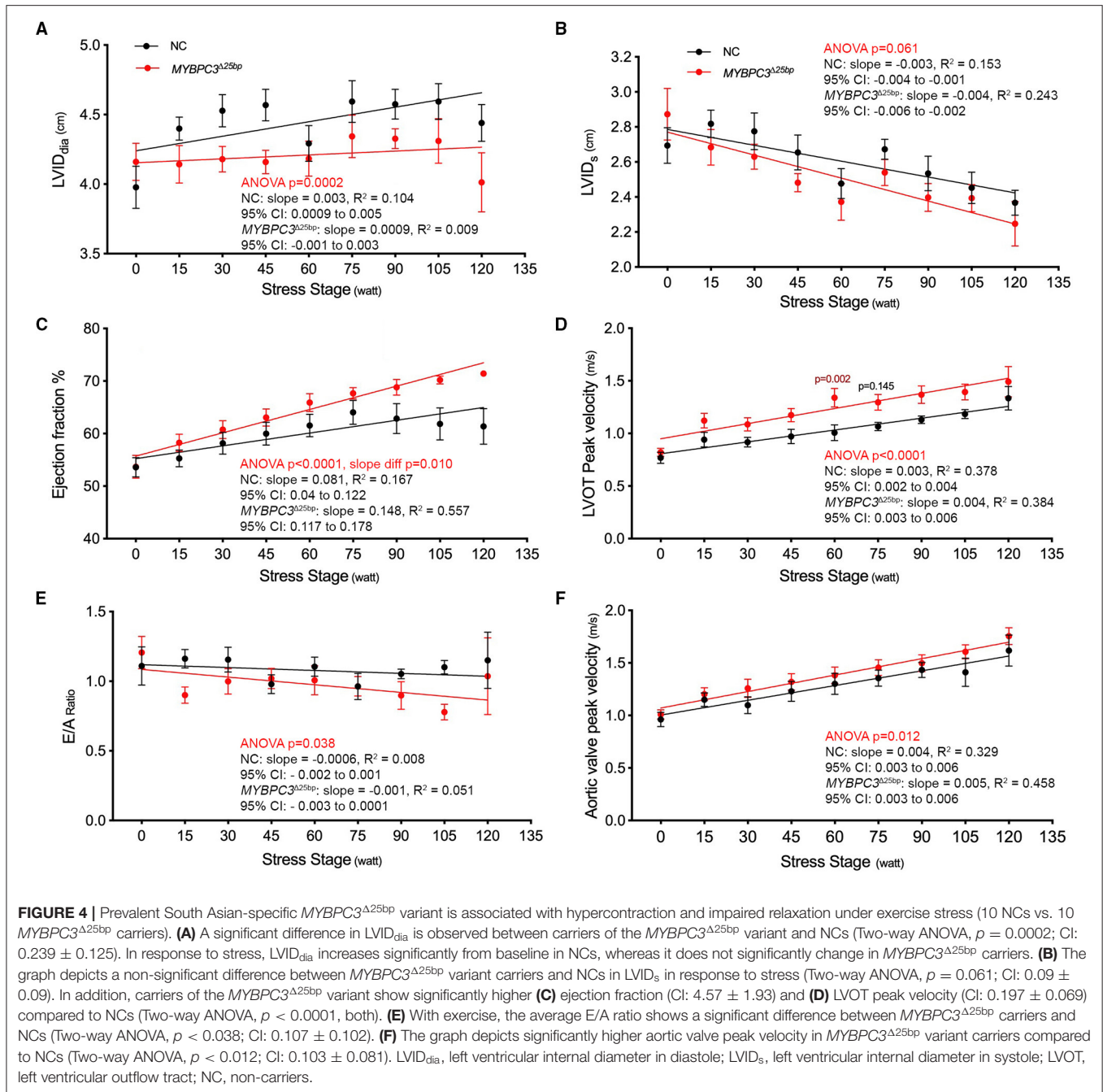
risk factor, potentially triggered hyperdynamic phenotype in asymptomatic MYBPC3 $\Delta$ 25bp carriers, as compared to NCs, considering that the hemodynamic parameters of MAP and HR did not differ between the two groups (MYBPC3 $\Delta$ 25bp variant carriers and NCs).

Previous studies have examined the distribution (26, 27, 37, 38) and clinical correlations (26, 27, 31, 39) of the MYBPC3 $\Delta$ 25bp variant, noting, importantly, that most of these studies were conducted in individuals of SA ancestry. In the first report (2003), the MYBPC3 $\Delta$ 25bp variant was initially detected in two affected Indian families. Then, it was reported in 16 of 229 unrelated healthy SA individuals and present in 3.8% of the general population, confirming variability in disease penetrance (29). In the next case-control study with a multi-ethnic population of 6,273 individuals in the SA Diaspora, the carrier frequency of the MYBPC3 $\Delta$ 25bp variant was reported to be 4% (26). Simonson et al. (38) and Bashyam et al. (37) reported 8 and 6%, respectively, in their studies. In 2018, we determined 6% prevalence in a sample taken from individuals of SA ancestry living in the United States (27). However, MYBPC3 $\Delta$ 25bp is largely associated with incomplete penetrance and delayed onset (26, 27, 29). In the current study, we identified a slightly higher prevalence at 6.75%, but given the nature of our study, this result may have been influenced by ascertainment. Collectively, these data support that MYBPC3 $\Delta$ 25bp is a common variant and that its distribution is associated with ethnicity (4, 25). Furthermore, our interpolation indicates that a prevalent SA-specific MYBPC3 $\Delta$ 25bp variant predisposes an estimated ~100

million people of SA ancestry worldwide (26) to such adverse cardiac events as cardiomyopathies, arrhythmias, HF, and SCD.

Previously, we reported no significant difference in clinical characteristics, ECG, or echocardiographic parameters between US SA carriers of the MYBPC3 $\Delta$ 25bp variant and NCs at rest, except for LV fractional shortening (27), which was slightly higher in carriers as compared to NCs ( $p = 0.04$ ), suggesting a minimal effect of the MYBPC3 $\Delta$ 25bp variant on the development of HCM phenotype. Compared to the current study, prior analyses included more individuals ( $n = 47$  carriers) who were younger (47.6 years) and, importantly, included both men and women (27). Both sex inclusion and age may have reduced the ability to detect a hyperdynamic state, which we discovered upon exercise stress. Many affected individuals could also remain asymptomatic until late adulthood (16, 22). Therefore, early detection of any subtle clinical phenotype and/or secondary contributing risk factors triggering symptoms in the carrier population, as we have suggested in our findings, is potentially relevant clinically. In the prior study, we also identified a second co-segregating novel variant (D389V) identified in ~10% of MYBPC3 $\Delta$ 25bp carriers associated with hyperdynamic cardiac features on echocardiogram as a potential secondary modifying factor for HCM (27). In this current investigation, however, no MYBPC3 $\Delta$ 25bp subjects with the D389V variant were included, meaning that the findings herein cannot be attributed to D389V. As previously noted, Harper et al. separately identified a haplotype in SAs with HCM with both the MYBPC3 $\Delta$ 25bp variant and a potentially pathogenic variant, MYBPC3 c.1224-52G>A





(31). None of the members of our *MYBPC3* $\Delta 25bp$  cohort were, in fact, found to carry the variant identified by Harper et al. Altogether, we propose that *MYBPC3* $\Delta 25bp$  is still a valid risk variant in the etiology of HCM and that it arises from a secondary risk factor, namely, exercise stress, and manifests as hypercontraction and impaired relaxation associated with late-onset LV dysfunction.

Exercise stress echocardiography is a standard clinical assessment and diagnostic armamentarium of HCM to assess

exercise-induced LVOT obstruction in patients with resting LVOT gradient  $< 50$  mm Hg (40). The only limitation is that not all patients with HCM and controls are able to perform stress exercises. Patients with HCM usually present with dynamic LVOT gradient, arrhythmias, and mitral regurgitation. However, for asymptomatic carriers with pathogenic sarcomere mutations, the exercise stress test is potentially an advantageous method for unmasking subclinical diseases, such as impairment in diastolic function and dynamic outflow tract obstruction in the absence

of a pathologically hypertrophied LV. The exercise stress test results presented here seem to have to accurately detect early changes in cardiac function, and the testing procedure was shown to be safe for enhancing and visualizing mid-range changes in cardiac function in variant carriers (41). Based on the results, asymptomatic MYBPC3 $\Delta$ 25bp carriers do have sublevel phenotype during exercise, which is masked under baseline conditions. These data further indicate that exercise testing may assist in risk stratification for the presence of any cardiac events as previously described (41–43).

Hypertension, diabetes mellitus, coronary artery disease, and other comorbid conditions were known to confound the phenotypic effects of cardiomyopathy and cardiac dysfunction. Initial studies have shown that the MYBPC3 $\Delta$ 25bp variant is associated with HCM (29), but then it was also determined to be associated with dilated and restrictive cardiomyopathies and HF (26). Later, it was determined that MYBPC3 $\Delta$ 25bp is significantly associated with LV dysfunction secondary to coronary artery disease (39, 44). After myocardial infarction, results from these studies have suggested that patients with MYBPC3 $\Delta$ 25bp show poor cardiac remodeling and recovery from the damage. This could be explained in several ways. First, cMyBP-C is sensitive to proteolysis during myocardial infarction and could be a potential earlier biomarker for heart attack (45–47). Thus, patients with MYBPC3 $\Delta$ 25bp may not have enough wild-type cMyBP-C turnover, resulting in haploinsufficiency (48). The failure to compensate for the loss of wild-type during myocardial infarction finally results in poor cardiac remodeling. Second, when MYBPC3 $\Delta$ 25bp carriers undergo myocardial infarction, it is possible that initial damage hinders quick recovery by the poison polypeptide effect (49). In the present study, we provided subjects with a questionnaire asking for information pertaining to their demographics, family history, medical history, and current and past medications. Using this as our guide, we then excluded any MYBPC3 $\Delta$ 25bp carriers who were presented with such secondary risk factors, thus eliminating them from participation in the stress echo study. This means that the outcome of the stress echo was based solely on the presence of MYBPC3 $\Delta$ 25bp and its effects on cardiac function. Collectively, results from the present study suggest LV hypercontractility under exercise stress conditions with evidence of diastolic stiffening and impairment among male asymptomatic MYBPC3 $\Delta$ 25bp carriers. As such, these results also suggest the presence of some subclinical pathology in MYBPC3 $\Delta$ 25bp carriers under exercise stress conditions. Based on the presence of HCM in transgenic mice expressing MYBPC3 $\Delta$ 25bp mRNA (49) and subclinical cardiomyopathy phenotype in carriers (26), MYBPC3 $\Delta$ 25bp can, therefore, be considered a potential risk allele for abnormal diastolic function and cardiomyopathy.

Complementary DNA sequencing of mRNA isolated from the biopsy of an MYBPC3 $\Delta$ 25bp-positive patient confirmed the presence of exon 33 skipping *in vivo* (26, 29). Exon 33 skipping results in the loss of 62 amino acids, resulting in a modified C10 domain of cMyBP-C (13). Exon 33 skipping also moves the stop codon to the 3' untranslated region (UTR), adding a novel 55 amino acids in this newly modified C10 domain at the carboxyl terminus (cMyBP-C $\Delta$ C10) (49). However, if the C10 domain is

modified, or truncated, then cMyBP-C will not properly localize in the sarcomere (50–52). The repercussions of this event can involve pathogenicity with the onset of HCM.

In fact, we have previously demonstrated that the presence of the MYBPC3 $\Delta$ 25bp variant could lead to exon 33 skipping during transcription (26, 29). We next determined that the MYBPC3 $\Delta$ 25bp variant in the presence of exon 33 skipping is pathogenic, both *in vitro* (46) and *in vivo* (49). Using a transgenic mouse model, we demonstrated that the expression of cMyBP-C $\Delta$ C10 is sufficient to cause HCM. Based on this observation, we propose that haploinsufficiency increases the disordered relaxed state (DRX) and decreases the super relaxed state (SRX) of myosin, leading to hypercontraction and HCM (53, 54). However, the molecular mechanism underlying these phenomena is yet to be validated in a humanized knock-in MYBPC3 $\Delta$ 25bp mouse model that shows normal cardiac function under baseline conditions (55). Thus, future studies will involve defining the pathogenicity of MYBPC3 $\Delta$ 25bp in a humanized knock-in mouse model in the presence of a comorbidity or acute insult.

## Study Limitation

This study is limited by its single-center nature, small cohort size, and inclusion of only men. Although HCM has equal distribution in both sexes (4, 56), women are more likely to remain undiagnosed (4); therefore, including female carriers and evaluating gender-specific manifestations under exercise stress conditions are strongly recommended. However, among those contacted for this study, male subjects were more willing to perform TTE and/or BSE, accounting for the disparity. We were also limited by the coronavirus disease-2019 (COVID-19), which resulted in a delay in recruitment and the need to follow health and safety protocols, such as wearing a mask during stress exercise. While the present study is valuable for its novel outcome, it is, by design, only a pilot study, so further, larger studies are needed.

## AUTHOR'S NOTE

We presented the current data at the American Heart Association Scientific Sessions on November 12, 2020 (abstract # 13481) entitled “South Asian-specific MYBPC3 $\Delta$ 25bp Intronic Deletion Carriers Demonstrate Hypercontractility and Impaired Diastolic Function Under Exercise Stress” (Circulation. 2020; 142:A13481; [https://doi.org/10.1161/circ.142.suppl\\_3.13481](https://doi.org/10.1161/circ.142.suppl_3.13481)).

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Prevalence study: Loyola University Chicago IRB# LU207815 and 207359, Chicago, Illinois and University

of Cincinnati IRB# 2016-7580, Cincinnati, Ohio. Genotype-phenotype study: University of Cincinnati IRB# 2016-4948, Cincinnati, Ohio. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

SB, SV, EM, and SS: conceptualization. SB, RS, SG, MP, KS, RRS, MK, DH, JR, and EM: data curation. SB, RS, SG, KS, MK, RJ, DH, JR, and EM: formal analysis. RB and SS: funding acquisition. SB, RS, SG, MP, MK, DH, JR, EM, and SS: investigation. SB, SV, MP, RJ, DH, JR, EM, and SS: methodology. DH, EM, and SS: project administration. RS, KS, RRS, RJ, DH, JR, RB, EM, and SS: resources. DH, EM, and SS: supervision. RJ, JR, and RB: validation. SB, MP, and SS: writing the original draft. SB, SS, MP, RJ, DH, JR, RB, EM, and SS: writing, reviewing, and editing. All authors contributed to the article and approved the submitted version.

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**Conflict of Interest:** SS provides consulting and collaborative research studies to the Leducq Foundation (CURE-PLAN), Red Saree Inc., Greater Cincinnati Tamil Sangam, Pfizer, Novo Nordisk, AstraZeneca, MyoKardia, Merck, and Amgen. EM serves as a consultant to AstraZeneca, Amgen, Pfizer, Tenaya Therapeutics, and Invitae. RB serves on scientific advisory boards for Janssen and Basking Biosciences and DSMB Committees for Ionis Pharmaceuticals, Akcea Therapeutics, and Novartis. These activities are unrelated to the content of this work. RRS has been a postdoctoral fellow of Amgen, starting from June 2019, and performs research at the University of Cincinnati.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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