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Association of Cardiomyopathy With *MYBPC3* D389V and *MYBPC3*$^{\Delta 25bp}$ Intronic Deletion in South Asian Descendants

Shiv Kumar Viswanathan, PhD; Megan J. Puckelwartz, PhD; Ashish Mehta, PhD; Chrishan J. A. Ramachandra, PhD; Aravindakshan Jagadeesan, BS; Regina Fritsche-Danielson, PhD; Ratan V. Bhat, PhD; Philip Wong, MBBS; Sangeetha Kandoi, MSc; Jennifer A. Schwanekamp, PhD; Gina Kuffel; Lorenzo L. Pesce, PhD; Michael J. Zilliox, PhD; U. Nalla B. Durai, MD; Rama Shanker Verma, PhD; Robert E. Molokie, MD; Domodhar P. Suresh, MD; Philip R. Khoury, PhD; Annie Thomas, PhD; Thriveni Sanagala, MD; Hak Chiaw Tang, MBBS; Richard C. Becker, MD; Ralph Knöll, MD, PhD; Winston Shim, PhD; Elizabeth M. McNally, MD, PhD; Sakthivel Sadayappan, PhD, MBA

IMPORTANCE The genetic variant *MYBPC3*$^{\Delta 25bp}$ occurs in 4% of South Asian descendants, with an estimated 100 million carriers worldwide. *MYBPC3* $^{\Delta 25bp}$ has been linked to cardiomyopathy and heart failure. However, the high prevalence of *MYBPC3*$^{\Delta 25bp}$ suggests that other stressors act in concert with *MYBPC3*$^{\Delta 25bp}$.

OBJECTIVE To determine whether there are additional genetic factors that contribute to the cardiomyopathic expression of *MYBPC3*$^{\Delta 25bp}$.

DESIGN, SETTING, ANDPARTICIPANTS South Asian individuals living in the United States were screened for *MYBPC3*$^{\Delta 25bp}$, and a subgroup was clinically evaluated using electrocardiograms and echocardiograms at Loyola University, Chicago, Illinois, between January 2015 and July 2016.

MAIN OUTCOMES AND MEASURES Next-generation sequencing of 174 cardiovascular disease genes was applied to identify additional modifying gene mutations and correlate genotype-phenotype parameters. Cardiomyocytes derived from human-induced pluripotent stem cells were established and examined to assess the role of *MYBPC3*$^{\Delta 25bp}$.

RESULTS In this genotype-phenotype study, individuals of South Asian descent living in the United States from both sexes (36.23% female) with a mean population age of 48.92 years (range, 18-84 years) were recruited. Genetic screening of 2401 US South Asian individuals found an *MYBPC3*$^{\Delta 25bp}$ carrier frequency of 6%. A higher frequency of missense *TTN* variation was found in *MYBPC3*$^{\Delta 25bp}$ carriers compared with noncarriers, identifying distinct genetic backgrounds within the *MYBPC3*$^{\Delta 25bp}$ carrier group. Strikingly, 9.6% of *MYBPC3*$^{\Delta 25bp}$ carriers also had a novel *MYBPC3* variant, D389V. Family studies documented D389V was in tandem on the same allele as *MYBPC3*$^{\Delta 25bp}$, and D389V was only seen in the presence of *MYBPC3*$^{\Delta 25bp}$. In contrast to *MYBPC3*$^{\Delta 25bp}$, *MYBPC3*$^{\Delta 25bp/D389V}$ was associated with hyperdynamic left ventricular performance (mean [SEM] left ventricular ejection fraction, 66.7 [0.7%]; left ventricular fractional shortening, 36.6 [0.6%]; $P < .03$) and stem cell–derived cardiomyocytes exhibited cellular hypertrophy with abnormal Ca^{2+} transients.

CONCLUSIONS AND RELEVANCE *MYBPC3*$^{\Delta 25bp/D389V}$ is associated with hyperdynamic features, which are an early finding in hypertrophic cardiomyopathy and thought to reflect an unfavorable energetic state. These findings support that a subset of *MYBPC3*$^{\Delta 25bp}$ carriers, those with D389V, account for the increased risk attributed to *MYBPC3*$^{\Delta 25bp}$.

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Supplemental content

Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: Sakthivel Sadayappan, PhD, MBA, Heart, Lung and Vascular Institute, Department of Internal Medicine, University of Cincinnati, 231 Albert Sabin Way, Cincinnati, OH 45267 (sadayasl@ucmail.uc.edu).

Hypertrophic cardiomyopathy (HCM) is a global genetic heart disease affecting approximately 600 000 people in the United States and 14.25 million people worldwide.[1] Hypertrophic cardiomyopathy, characterized by excessive left ventricular thickening, features of diastolic dysfunction, left ventricular outflow obstruction, arrhythmia, myocardial ischemia, mitral regurgitation, and sudden death,[2,3] is mainly caused by mutations in sarcomeric genes. Mutations in the thick filament protein-encoding genes *MYH7* and *MYBPC3* together constitute more than 80% of inherited cases.[4,5] *MYBPC3* encodes cardiac myosin binding protein-C (cMyBP-C), a vital protein for cardiac performance[6] and regulator of cardiac contractility in response to adrenergic stimulation.[7]

A 25-base pair deletion within intron 32 of the *MYBPC3* gene (*MYBPC3*$^{\Delta 25bp}$) was previously described as a risk factor for cardiomyopathy[8] and heart failure[9] in South Asian individuals, with an odds ratio of 6.99 for cardiomyopathy in *MYBPC3*$^{\Delta 25bp}$ carriers.[10] Although this genetic variant was discovered and examined in South Asian individuals living in Asia, it is estimated that 100 million people carry the *MYBPC3*$^{\Delta 25bp}$ variant.[9] Genetically, *MYBPC3*$^{\Delta 25bp}$ is characterized by incomplete penetrance and variable expressivity.[8,9] *MYBPC3*$^{\Delta 25bp}$ is associated with a range of outcomes from asymptomatic normal hearts to diastolic dysfunction; hypertrophic, dilated, and restrictive cardiomyopathies with tachyarrhythmias[9]; and left ventricular dysfunction in the setting of coronary artery disease.[11,12] Cardiomyopathy features are increased when *MYBPC3*$^{\Delta 25bp}$ occurs in the presence of other sarcomere gene mutations,[8,13,14] explaining some of the variable expression seen with *MYBPC3*$^{\Delta 25bp}$. South Asian individuals have been the fastest-growing ethnic group in the world during the past decade, and defining genetic risk factors in this population has substantial clinical effect.[15] The high prevalence of *MYBPC3*$^{\Delta 25bp}$ in South Asian individuals exceeds the incidence of cardiomyopathy and heart failure,[8] indicating that *MYBPC3*$^{\Delta 25bp}$ on its own is not sufficient to cause cardiomyopathy and modifying factors add to its risk. Therefore, we undertook a systematic study of clinical and genetic screening to find *MYBPC3*$^{\Delta 25bp}$ carriers among South Asian individuals who migrated to the United States to better define the cardiovascular findings associated with *MYBPC3*$^{\Delta 25bp}$.

Key Points

Question Are there additional genetic factors that contribute to the cardiomyopathic expression of *MYBPC3*$^{\Delta 25bp}$ variant, which is found in nearly 100 million individuals worldwide?

Findings In this genotype-phenotype study, *MYBPC3*$^{\Delta 25bp}$ was found in 6% of 2401 South Asian individuals living in the United States, and genetic and phenotypic characterization of a subset identified distinct populations within this heterogeneous group. Specifically, 9.6% of *MYBPC3*$^{\Delta 25bp}$ carriers also had a novel *MYBPC3* variant, D389V, on the same single allele, and this subset had hyperdynamic findings on echocardiogram.

Meaning Additional genetic variants, specifically D389V, were associated with the variable cardiomyopathic findings linked to *MYBPC3*$^{\Delta 25bp}$.

Methods

Institutional Review Board Approval

This study was performed in accordance with the Declaration of Helsinki. All experimental protocols were approved by the institutional review board of the host institutes (Loyola University Chicago institutional review board [LU207815 and 207359], Chicago, Illinois, and SingHealth institutional review board [CIRB 2015/2521], Singapore).

Sample Collection and Genotyping

Participants were prospectively recruited and enrolled through community engagement at local venues. Saliva samples (3-5 mL) were collected in either sterile 15-mL tubes and frozen at −20°C or using the Saliva DNA Sample Collection Kit (Cat OG-500, DNA Genotek) and stored at ambient temperature. Blood samples for genotyping were collected in EDTA vacutainers (Catalog No. 367861; BD Bioscience), and DNA was isolated using the Blood DNA Isolation Kit (Catalog No. 51104; Qiagen). Polymerase chain reaction amplification of *MYBPC3*$^{\Delta 25bp}$ (rs36212066) used the forward primer 5′-GTT TCC AGC CTT GGG CATAGT C-3′ and reverse primer 5′-GAG GAC AAC GGA GCA AAG CCC-3′, using REDTaq PCR master mix (Sigma-Aldrich) and 2.5% agarose gel analysis. DNA samples for sequencing were isolated from 10 mL of peripheral blood, as described previously, using the Blood DNA Isolation Kit (Catalog No. 51104; Qiagen). Participants in the United States who were positive for the *MYBPC3*$^{\Delta 25bp}$ variant, their first-degree relatives, or random control individuals were contacted in a single-blinded manner to participate in the follow-up genotype-phenotype study at Loyola University Chicago (LU207377 and LU207813), for which they provided additional written informed consent. Participants were provided a questionnaire pertaining to ethnic background, health history, family history, comorbid conditions, and current medications. The questionnaire was completed on a voluntary basis, and questions were answered only to the extent that each participant was willing.

Measuring Cardiac Function in US South Asian Individuals

Twelve-lead electrocardiograms with rhythm strips were performed at rest in a supine position using a MAC 5500 HD (GE Healthcare). Tracings were interpreted blinded to genotype. Two-dimensional (2-D) echocardiography and Doppler examinaton were performed from standard transthoracic windows using Acuson Sequoia (Siemens Medical Solutions) and Vivid 7 (GE Healthcare). Echocardiograms were interpreted by a reader blinded to genotype. Left ventricular (LV) internal diastolic diameter, end-diastolic thickness of the posterior wall and anterior interventricular septum were measured using 2-D echo. Left ventricular mass was determined using the Devereux formula and indexed for body surface area. Relative wall thickness was obtained using the formula (2 times posterior wall thickness in diastole) divided by LV diastolic dimension. Left ventricular ejection fraction (LVEF) and left ventricular

fractional shortening (LVFS) were calculated using Biplane Simpson method. Left ventricular diastolic function was evaluated using tissue Doppler velocities of mitral annulus as well as transmitral pulsed wave Doppler velocities. Medial and lateral tissue Doppler (e′) velocities were measured by placing the sample volume at the base of the interventricular septum and anterolateral wall in apical 4-chamber view immediately below the mitral annulus. Transmitral Doppler tracings were obtained by placing the sample volume in the left ventricle at the tip of the mitral valve leaflets. Early (E-wave) and late (A-wave) diastolic transmitral velocities, E to A ratio, and deceleration time were measured from transmitral Doppler tracings. Apical 4-chamber and 2-chamber views were used to determine left atrial area and length.

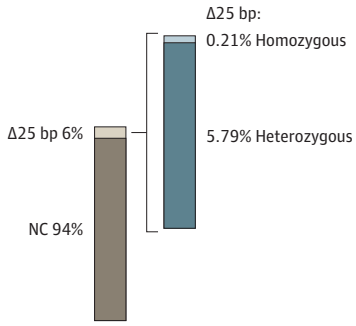
DNA Sequence Analysis

The TruSight Cardio Kit (Catalog No. FC-141-1011; Illumina) was used to sequence 174 cardiovascular disease genes (eTable 1 in the Supplement) on an Illumina MiSeq with 150-base pair paired-end reads. Sequences were aligned using the Burrows-Wheeler Aligner, and the GATK haplotype caller was applied to generate variant call files as described.[16] Variants were interpreted with SnpEff, and HIGH (stop gain/loss, splice site, frameshifts) and MODERATE (missense) effect variants were curated.[17] Variants were ranked by minor allele frequency based on data from ExAC.[18] Variant call files were filtered for variants in 46 genes specifically involved in cardiomyopathy, with an ExAC frequency of 0.01 or less (eTable 1 in the Supplement). The number of variants identified in each gene was normalized to cohort size, and *MYBPC3*$^{\Delta 25bp}$ carriers and noncarriers were compared. *TTN* variant comparisons were made with an unpaired *t* test. Fisher exact test was used to compare individual variants in carriers vs noncarriers. A 1-way analysis of variance was used to compare functional cardiac measures in noncarriers, *MYBPC3*$^{\Delta 25bp}$, and *MYBPC3*$^{\Delta 25bp/D389V}$ participant, followed by Tukey multiple comparisons test. Statistical analyses were performed using Prism 7 (GraphPad).

Generation of Human-Induced Pluripotent Stem Cells and Cardiomyocyte Differentiation

Genotype-positive and genotype-negative participants were recruited to generate induced pluripotent stem cells (iPSCs). Peripheral blood mononuclear cells were isolated from whole blood (5 mL) using Ficoll-Paque (GE Healthcare Life Sciences) and cultured in peripheral blood mononuclear cell medium. Peripheral blood mononuclear cells were transduced using CytoTune-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific), all performed according to the manufacturers' instructions. Colonies that appeared underwent clonal expansion and were maintained in feeder-free conditions.[19] Human iPSCs were differentiated into cardiomyocytes using an embryoid body-based protocol.[20] Briefly, iPSCs were dissociated into single cells and aggregated into embryoid bodies with stage-specific media changes for the next 8 days. Embryoid bodies were plated on gelatin-coated dishes on day 8 and maintained in embryoid body-2 medium (Dulbecco modified eagle medium/F12 with 2% fetal bovine serum).

Figure 1. Prevalence and Incomplete Penetrance of *MYBPC3*$^{\Delta 25bp}$ in South Asian Individuals From the United States



Genotype distribution of 2401 US South Asian participants. Among carriers of the *MYBPC3*$^{\Delta 25bp}$ variant, 5.79% (139, dark color sector) were heterozygous, and 0.21% (5, light color sector) were homozygous. bp Indicates base pair; NC, noncarrier.

Imaging and Protein Analysis of Ca^{2+} Transients

Imaging of Ca^{2+} transients was performed on isolated single CMs generated from human iPSCs and seeded on matrix-coated slides using the Ca^{2+} probe fluo-4-acetoxymethyl ester (Fluo-4AM). Signals were captured using a high frame rate Cascade EMCCD camera (Photometrics) and analyzed using MetaMorph software, version 7 (Molecular Devices).

Statistical Analysis

Results were reported as mean (SE) of independent experiments. The comparison between 2 groups was performed using the *t* test, whereas to compare multiple groups, 1-way analysis of variance followed by Kruskal-Wallis analysis was used. A 2-sided *P* value less than .05 was considered statistically significant.

Results

Prevalence of *MYBPC3*$^{\Delta 25bp}$ US South Asian Individuals

Most HCM-causing *MYBPC3* mutations are dominantly inherited; however, *MYBPC3*$^{\Delta 25bp}$ has a more complex effect because its population prevalence significantly exceeds that of HCM.[8,9] We randomly screened for the presence of *MYBPC3*$^{\Delta 25bp}$ among US South Asian individuals. During a 2-year period, we evaluated 2401 US South Asian participants from several cities in the United States. Using a polymerase chain reaction–based assay (eFigure 1A in the Supplement), the population frequency of the *MYBPC3*$^{\Delta 25bp}$ variant in US South Asian individuals was 6%, including both heterozygous (5.79%) and homozygous (0.21%) carriers (139 of 2401 and 5 of 2401, respectively) (**Figure 1**), as measured from a diverse age group (eFigure 1B in the Supplement). These values are higher than the previously reported prevalence of approximately 4.0% in South Asian individuals.[9] The variant allele frequency was 3.14% with a Fisher exact test *P* value of .03, indicating that the variant's distribution follows Hardy-Weinberg equilibrium.

Table. Sample Cohort, Clinical Risk Factors, ECG, and Echocardiographic Data

Variable	Noncarriers, Mean (SEM)	No.	Carriers, Mean (SEM)	No.	*P* Value[a]
Male, %	57.14	20	48.94	23	0.51
Female, %	42.86	15	51.06	24	
Age, y	45.1 (1.71)	35	47.6 (1.73)	47	.31
Blood pressure, mm Hg					
Diastolic	74.33 (1.78)	33	74.24 (1.73)	37	.93
Systolic	122.1 (2.29)	33	127 (2.64)	39	.12
Body surface index, %	1.81 (0.03)	35	1.81 (0.03)	42	.97
ECG characteristics[b]					
QTc	407.9 (3.38)	35	416.4 (3.96)	38	.10
Echocardiographic characteristics[b]					
LVEF, %	60.52 (1.19)	35	62.78 (1.08)	41	.28
LVFS, %	32.27 (0.81)	36	34.92 (0.81)	41	.04
LV mass index	63.32 (2.55)	36	66.74 (2.37)	42	.32
IVS, cm	0.91 (0.04)	36	0.89 (0.03)	42	.84
LVPW, cm	0.82 (0.02)	36	0.86 (0.03)	42	.11
RWT, cm	0.38 (0.01)	36	0.41 (0.01)	42	.21
LA Volume 4C mod, mL	38.22 (1.80)	36	36.52 (1.56)	37	.48
LA Volume index	24.9 (0.88)	32	24.05 (1.05)	40	.53
LVOT Gradient, mm Hg	4.24 (0.24)	36	4.33 (0.21)	40	.84
E/A Ratio	1.44 (0.07)	36	1.32 (0.07)	42	.16
E/e' Ratio	8.7 (0.43)	36	6.78 (0.34)	37	.49
Clinical presentation					
Family history, %	22.22	8	26.19	11	NA
Hypertension, %	13.89	5	26.19	11	NA
Diabetes	2.78	1	11.9	5	NA
Hyperlipidimic	13.89	5	16.8	7	NA

Abbreviations: ECG, electrocardiogram; E/A ratio, ratio of early transmitral flow to late flow owing to atrial contraction; E/e' Ratio, ratio of early transmitral flow to left ventricular early diastolic velocity; IVS, interventricular septal thickness; LA, left atrium; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; LVOT, left ventricular outflow tract; LVPW, left ventricular posterior wall thickness; NA, not applicable; QTc, corrected QT interval; RWT, relative wall thickness; 4C, four-chamber view.

[a] Statistical significance was calculated using unpaired t test or χ^2 test for categorical variables.

[b] Electrocardiogram and echocardiography measurements were taken under normal and conscious conditions.

Clinical Findings in US South Asian *MYBPC3*$^{\Delta 25bp}$ Carriers

To further characterize the association between the *MYBPC3*$^{\Delta 25bp}$ variant and the presence of cardiomyopathy in US South Asian individuals, cardiac phenotype was assessed using 12-lead electrocardiogram and echocardiogram in carriers (n = 47, 43 heterozygous and 4 homozygous) and noncarriers (n = 35) at a single time. Mean (SE) age at the time of study in noncarriers and carriers was 45.1 (1.71) years and 47.6 (1.73) years (*P* = .31), respectively (**Table**). Blood pressure was not significantly different between noncarriers and carriers (Table), and both noncarriers and carriers showed normal correlation between mean arterial pressure and body surface area (eFigure 1C in the Supplement). Comparing echocardiographic parameters indicative of HCM, such as relative wall thickness, intraventricular septal thickness, LV posterior wall thickness, and mitral inflow pattern (E/e' ratio) (eFigure 1D-G in the Supplement) revealed no significant differences between the groups. The percent LVFS (eFigure 1H in the Supplement), but not LVEF (eFigure 1I in the Supplement), showed a small but significant increase between *MYBPC3*$^{\Delta 25bp}$ carriers and noncarriers. Collectively, these data demonstrate that the *MYBPC3*$^{\Delta 25bp}$ variant alone does not elicit an overt HCM phenotype.

Genetically Distinct Subgroups Among *MYBPC3*$^{\Delta 25bp}$ Carriers

Because population reports of *MYBPC3*$^{\Delta 25bp}$ carriers suggest incomplete penetrance and an increased frequency among bona fide HCM patients,[9] we evaluated the possibility of other cardiomyopathy variants existing in *MYBPC3*$^{\Delta 25bp}$ carriers using targeted sequencing on 72 participants. A panel of 174 cardiovascular disease–associated genes was sequenced and evaluated for protein-altering variants with moderate and high effect, as interpreted by snpEff.[17] Forty-six genes from this panel are specifically linked to cardiomyopathy. We analyzed protein-altering variants including nonsense and nonsynonymous changes in only these 46 cardiomyopathy genes (eTable 1 in the Supplement). Protein-altering variant numbers were normalized to cohort size; the *MYBPC3*$^{\Delta 25bp}$ carrier group had a 1.4-fold excess of rare variants compared with the noncarrier group; this difference was not significant in the context of the small sample size. The genes with variants are depicted in eFigure 2A in the Supplement, with rare being defined as a global ExAC minor allele frequency of 0.01 or less. *MYBPC3*$^{\Delta 25bp}$ carriers had 4.8 rare protein-altering variants per individual, while noncarriers had 3.4, consistent with a distinct genetic background between *MYBPC3*$^{\Delta 25bp}$ carriers and noncarriers (eTables 2-3 in the Supplement).

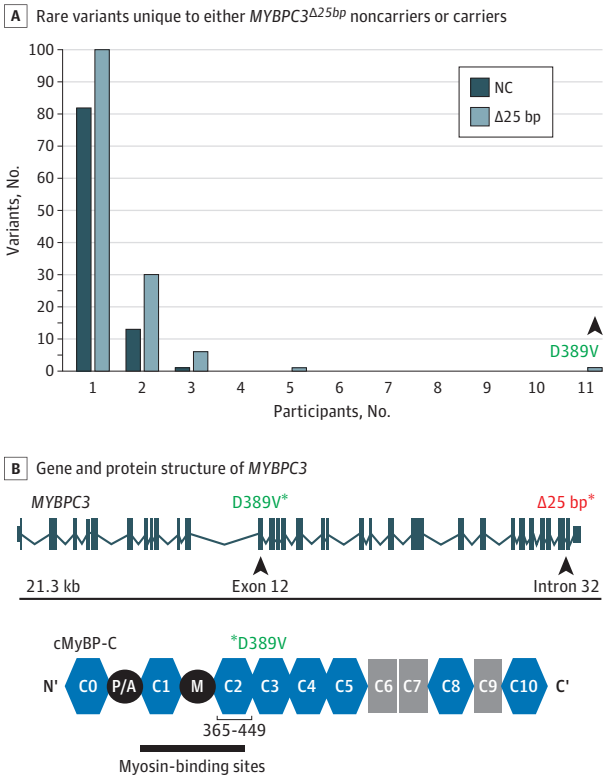
We separately considered protein-altering variants in *TTN*, which encodes the giant protein titin. *TTN* truncating mutations are linked to heart failure and cardiomyopathy.[21,22] *TTN* is also uniquely enriched with an extraordinarily high degree of missense variants of unclear functional impact. We reasoned that the signature of *TTN* variation could be used to identify subgroups within the cohort of *MYBPC3*$^{\Delta 25bp}$ carriers. *MYBPC3*$^{\Delta 25bp}$ carriers had an excess of rare *TTN* variants (3.2

counts), compared with noncarriers (2.4 counts) per participant (eFigure 2B in the Supplement). Using the South Asian (SAS) population frequencies from ExAC,[18] population frequency differed for *TTN* variants in *MYBPC3*$^{\Delta 25bp}$ carriers compared with the noncarrier group. The mean (SE) ExAC_SAS frequency of *TTN* variants in *MYBPC3*$^{\Delta 25bp}$ carriers was 0.010 (0.0009) compared with 0.005 (0.001) in noncarriers ($P < .001$) (eFigure 2C in the Supplement), suggesting a distinct genetic landscape between *MYBPC3*$^{\Delta 25bp}$ carriers and noncarriers. When using the Non-Finnish European ExAC population frequencies, we found no difference between carriers and noncarriers for *TTN* variants. In addition, when evaluating other cardiomyopathy genes, we saw no significant difference between the carriers and noncarriers using ExAC_SAS frequencies. Thus, the distinct *TTN* landscape in *MYBPC3*$^{\Delta 25bp}$ carriers is both population- and gene-specific (eFigure 2 in the Supplement). Because *TTN* truncating variants are highly enriched in dilated cardiomyopathy,[23] we separately evaluated *TTN* truncating variants. Two *TTN* truncating variants were identified in the cohort: a frameshift variant in a *MYBPC3*$^{\Delta 25bp}$ carrier and an early termination variant in a noncarrier, both of which are part of exon 48 of the Novex-3 transcript and unlikely to be important for the pathogenesis of cardiac dysfunction, suggesting that *TTN* truncating variants do not account for differences between *MYBPC3*$^{\Delta 25bp}$ carriers and noncarriers. Overall, the *TTN* genetic signature in *MYBPC3*$^{\Delta 25bp}$ carriers marks population stratification within the analyzed US South Asian cohort.

A Novel Second Variant in *MYBPC3* Identified in *MYBPC3*$^{\Delta 25bp}$ Carriers

We found a novel *MYBPC3* missense single-nucleotide polymorphism in a subset of *MYBPC3*$^{\Delta 25bp}$ carriers encoding an A to T nucleotide change at chr11:47365100, producing a missense aspartic acid (D) to valine (V) variant at amino acid 389 in cMyBP-C (D389V) (**Figure 2**A and eFigure 3A in the Supplement). D389V was the only variant found to be significantly enriched in *MYBPC3*$^{\Delta 25bp}$ carriers and was not identified in noncarriers (Fisher exact test 0.0019, eTable 4 in the Supplement). D389V localizes in *MYBPC3* exon 12 at the interface between C2 domain of the cMyBP-C and the S2 domain of myosin (Figure 2B and eFigure 3B in the Supplement). This domain interface directly regulates the superrelaxed state of cardiac contraction.[24] The glutamic acid in position 389 of the protein has been conserved among various species (eFigure 3C in the Supplement). We next screened specifically for the D389V variant and determined its presence in 13 of 136 *MYBPC3*$^{\Delta 25bp}$ carriers (9.6%) and absence in 1488 US South Asian individuals who do not carry the MYBPC3$^{\Delta 25}$ allele (**Figure 3**A). D389V variant was carried in tandem on the same allele as *MYBPC3*$^{\Delta 25bp}$, as confirmed by family studies (eFigure 4A in the Supplement). D389V variant was never seen in the absence of *MYBPC3*$^{\Delta 25bp}$. Thus, it is likely that *MYBPC3*^{D389V} arose on the background of the *MYBPC3*$^{\Delta 25bp}$ allele. We reevaluated the echocardiographic findings, excluding those with confounding genetic variants or confounding comorbidities (eTable 5 in the Supplement). Left ventricular ejection fraction and LVFS were significantly increased in those carrying

Figure 2. Representation of *D389V* Rare Variant in *MYBPC3*$^{\Delta 25bp}$ Carriers



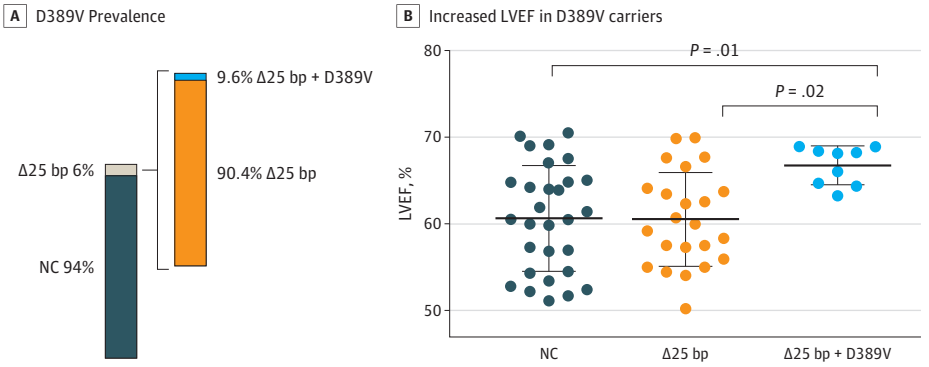
A, Sequencing was used to identify rare protein-altering variation in 46 cardiomyopathy genes. An excess of unique singleton rare variants occurred in both *MYBPC3*$^{\Delta 25bp}$ carriers (light bar) and noncarriers (dark bar). D389V (arrowhead) was the only variant present in more than 5 carriers and 0 noncarriers (NCs). B, The *MYBPC3* gene and cMyBP-C protein structure with the positions of D389V (green asterisks) and *MYBPC3*$^{\Delta 25bp}$ (red asterisk) indicated. Within the *MYBPC3* gene, these 2 variants are separated by 12 kilobases (kb) (top). D389V falls in the C2 domain of cMyBP-C in a region implicated in binding myosin heavy chain (bottom).

D389V plus *MYBPC3*$^{\Delta 25bp}$ (mean [SD] LVEF, 66.7% [0.7%]; LVFS, 36.6% [0.6%]; $P = .03$; n = 9) compared with *MYBPC3*$^{\Delta 25bp}$ carriers (mean [SD] LVEF, 61.7 [1.2], LVFS, 34.3 [1.2]) and noncarriers (mean [SD] LVEF, 60.6 [1.1]; LVFS, 32.3 [0.8]) (Figure 3B; eFigure 4B in the Supplement), consistent with a hyperdynamic state and similar to what has been described in early HCM.[25]

Human-Induced Pluripotent Stem Cell–Derived Cardiomyocytes Carrying D389V Recapitulated HCM-Like Phenotypes

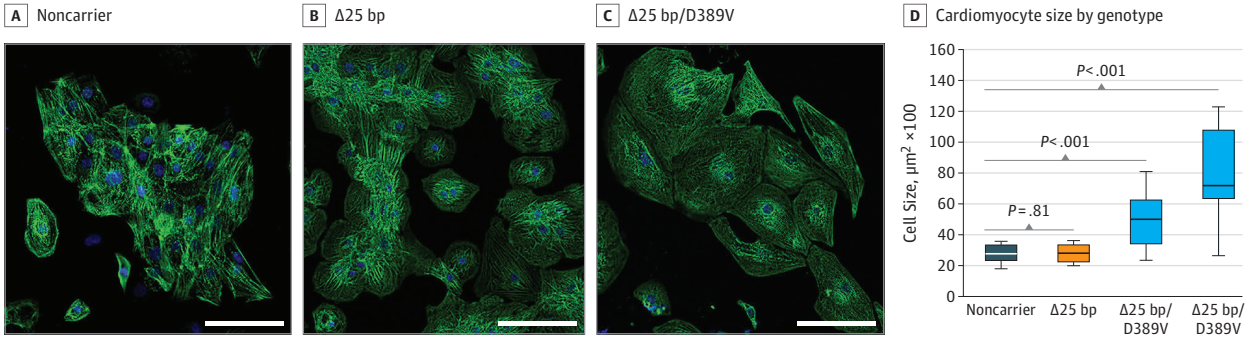
To better understand the cellular phenotype linked to *MYBPC3*$^{\Delta 25bp/D389V}$, we examined human cardiomyocytes derived from iPSCs. Strikingly, cardiomyocytes derived from 2 individuals with *MYBPC3*$^{\Delta 25bp/D389V}$ were significantly more hypertrophic compared with control cardiomyocytes and cardiomyocytes with *MYBPC3*$^{\Delta 25bp}$ alone (**Figure 4**A and B), consistent with a hypertrophic cellular phenotype. An increased frequency of arrhythmic cells as well as ectopic Ca^{2+} transients were observed, in cardiomyocytes with *MYBPC3*$^{\Delta 25bp/D389V}$ compared with *MYBPC3*$^{\Delta 25bp}$ alone or control cells (eFigure 4C and

Figure 3. Association of *MYBPC3*$^{\Delta 25bp}$ and *D389V* in Tandem on the Same Allele With a Hyperdynamic State



A, Prevalence of the D389V genotype as a subset of the *MYBPC3*$^{\Delta 25bp}$ carriers among all samples. B, Left ventricular ejection fraction was significantly elevated (66.7 [0.7]) in participants who carry *MYBPC3*$^{\Delta 25bp/D389V}$ compared with noncarriers and those with *MYBPC3*$^{\Delta 25bp}$ alone (1-way analysis of variance and Tukey post hoc test). bp Indicates base pair; NC, noncarrier.

Figure 4. Cellular Hypertrophy of Cardiomyocytes With *MYBPC3*$^{\Delta 25bp/D389V}$ Variants



Cardiomyocytes were differentiated from induced pluripotent stem cells (iPSCs) from (A) a noncarrier control individual, (B) a *MYBPC3*$^{\Delta 25bp}$ carrier (Δ25bp), and (C) a carrier of *MYBPC3*$^{\Delta 25bp/D389V}$ (*Δ25bp/D389V*). Cells stained for cardiac α-actinin (green) and nuclei (blue) were compared, and those with *MYBPC3*$^{\Delta 25bp/D389V}$ were larger than controls and *MYBPC3*$^{\Delta 25bp}$ alone. Scale bar: 100 μm. D, Graphic representation of cell size (n = 27 per cell line). One-way analysis of variance and Tukey post hoc test were used. bp Indicates base pair.

D in the Supplement). Collectively, these data show that the single *MYBPC3*$^{\Delta 25bp/D389V}$ variant allele is associated with a hyperdynamic, hypertrophic, and arrhythmogenic state, consistent with an HCM-like substrate.[25]

Discussion

The *MYBPC3*$^{\Delta 25bp}$ variant is carried by 5% to 6% of the large and diverse South Asian population.[9,26] Because this exceeds the incidence of heart failure, other factors, both genetic and environmental, were hypothesized to contribute to risk associated with *MYBPC3*$^{\Delta 25bp}$.[9,11,12] Armed with deep sequencing of additional cardiomyopathy genes from *MYBPC3*$^{\Delta 25bp}$ carriers and noncarriers, we identified a unique signature of *TTN* missense variation between *MYBPC3*$^{\Delta 25bp}$ carriers and noncarriers, suggesting discrete cohorts within the *MYBPC3*$^{\Delta 25bp}$ carrier group. The pathophysiologic significance of the *TTN* variation, if any, is not known. Other cardiomyopathy genes did not display this same pattern of enrichment in *MYBPC3*$^{\Delta 25bp}$ carriers compared with noncarriers, with the exception of *MYBPC3* itself.

Unexpectedly, we identified a novel variant in *MYBPC3* that cosegregates with the *MYBPC3*$^{\Delta 25bp}$ variant. This new variant, D389V (chr11:47365100A>T), was seen in 13 of 136 (9.6%) of *MYBPC3*$^{\Delta 25bp}$ carriers, putting prevalence of the *MYBPC3*$^{\Delta 25bp/D389V}$ allele at 1 in 200 South Asian individuals. *MYBPC3* variants have been described in combination with other genetic variants in the setting of dilated cardiomyopathy and heart failure, consistent with an oligogenic model of disease.[27-30] However, distinct from these previous studies, D389V and *MYBPC3*$^{\Delta 25bp}$ are found together on the same single allele, and D389V was never observed in the absence of *MYBPC3*$^{\Delta 25bp}$. D389V maps near the beginning of immunoglobulin-like domain C2 in cMyBP-C protein, a region that is known to directly interact with the neck region of β-myosin heavy chain (S2-region), effectively regulating the engagement and interaction of myosin heads with actin filaments.[31] In silico tools predict D389V to be highly disruptive to protein function owing to the exchange of the highly conserved charged amino acid for a nonpolar moiety.

Limitations

This study specifically focused on 48 genes, mutations in which are known to cause cardiomyopathy. Systematic analysis of the additional sequenced genes could yield other cosegregating gene variations. The sample size in this study is relatively small,

but proportional considering South Asian individuals represent about 1.0% of the United States population.

Conclusions

MYBPC3$^{\Delta 25bp}$ in the context of D389V may be an important marker of cardiac health. *MYBPC3*$^{\Delta 25bp/D389V}$, especially when combined with additional cardiac stressors, could prove suitable substrate for cardiomyopathy development and heart failure. Specifically, those with single *MYBPC3*$^{\Delta 25bp/D389V}$ allele had increased LVEF. The hyperdynamic phase of HCM is recognized as an early feature of HCM, and despite an appearance of hyperperformance is one that is deceivingly energetically unfavorable.[25,32,33] At a cellular level, iPSC-derived cardiomyocytes with *MYBPC3*$^{\Delta 25bp/D389V}$ had hypertrophy and increased frequency of abnormal Ca^{2+} transients. Ca^{2+} hypersensitivity and hyperdynamic ventricles are viewed as an early compensation or adaptation for the unfavorable energetic state and altered myocyte signaling.[34] Importantly, this hyperdynamic state in HCM is viewed as a target for drug development and intervention.[35]

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Author Affiliations: Heart, Lung and Vascular Institute, Department of Internal Medicine, University of Cincinnati, Cincinnati, Ohio (Viswanathan, Kandoi, Schwanekamp, Becker, Sadayappan); Department of Cell and Molecular Physiology, Loyola University Chicago, Maywood, Illinois (Viswanathan, Jagadeesan, Kandoi, Sadayappan); Center for Genetic Medicine, Northwestern University, Chicago, Illinois (Puckelwartz, McNally); National Heart Research Institute Singapore (Mehta, Ramachandra, Wong, Shim); Cardiovascular Academic Clinical Program, DUKE-NUS Medical School, Singapore (Mehta); PSC and Phenotyping Laboratory, Victor Chang Cardiac Research Institute, Sydney, Australia (Mehta); Cardiovascular and Metabolic Disease Innovative Medicines and Early Development Unit, AstraZeneca Research and Development, Gothenburg, Sweden (Fritsche-Danielson, Bhat, Knöll); Cardiovascular and Metabolic Disorders Program, DUKE-NUS Medical School, Singapore (Wong, Shim); Department of Cardiology, National Heart Centre Singapore, Singapore (Wong, Tang); Department of Biotechnology, Indian Institute of Technology Madras, Chennai, Tamilnadu, India (Kandoi, Verma); Department of Public Health Sciences, Loyola University Chicago, Maywood, Illinois (Kuffel, Zilliox); Computation Institute, The University of Chicago, Chicago, Illinois (Pesce); Divison of Hematology and Oncology, University of Illinois at Chicago (Durai, Molokie); St Elizabeth Physicians Heart and Vascular, Florence, Kentucky (Suresh); Heart Institute, Cincinnati Children's Hospital, Cincinnati, Ohio (Khoury); Marcella Niehoff School of Nursing, Loyola University Chicago, Maywood, Illinois (Thomas); Department of Cardiology and Echocardiography and Cardiographics, Loyola University Chicago, Maywood, Illinois (Sanagala); Integrated Cardio-Metabolic Centre, Myocardial Genetics, Karolinska Institutet, University Hospital, Heart and Vascular Theme, Stockholm, Sweden (Knöll); Associate Editor for Translational Science, *JAMA Cardiology* (McNally).

Author Contributions: Drs Sadayappan and McNally had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Drs Viswanathan, Puckelwartz, Mehta, and Ramachandra contributed equally. Drs Knöll, Shim, McNally, and Sadayappan contributed equally as senior authors.

Concept and design: Viswanathan, Fritsche-Danielson, Bhat, Kuffel, Zilliox, Durai, Knoll, Shim, McNally, Sadayappan.
Acquisition, analysis, or interpretation of data: Viswanathan, Puckelwartz, Mehta, Ramachandra, Jagadeesan, Bhat, Wong, Kandoi, Schwanekamp, Kuffel, Pesce, Zilliox, Durai, Verma, Molokie, Suresh, Khoury, Thomas, Sanagala, Tang, Becker, Knoll, McNally, Sadayappan.
Drafting of the manuscript: Viswanathan, Puckelwartz, Mehta, Ramachandra, Kandoi, Kuffel, Durai, Khoury, Thomas, Knoll, Shim, McNally, Sadayappan.
Critical revision of the manuscript for important intellectual content: Viswanathan, Puckelwartz, Jagadeesan, Fritsche-Danielson, Bhat, Wong, Kandoi, Schwanekamp, Pesce, Zilliox, Durai, Verma, Molokie, Suresh, Khoury, Thomas, Sanagala, Tang, Becker, Knoll, McNally, Sadayappan.
Statistical analysis: Viswanathan, Puckelwartz, Jagadeesan, Pesce, Durai, Khoury, McNally, Sadayappan.
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Supervision: Jagadeesan, Wong, Zilliox, Durai, Verma, Suresh, Thomas, Knoll, Shim, McNally, Sadayappan.

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