



Discordant clinical features of identical hypertrophic cardiomyopathy twins

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Contributed by J. G. Seidman, January 11, 2021 (sent for review October 27, 2020; reviewed by Libin Wang and Cordula M. Wolf)

Hypertrophic cardiomyopathy (HCM) is a disease of heart muscle, which affects ~1 in 500 individuals and is characterized by increased left ventricular wall thickness. While HCM is caused by pathogenic variants in any one of eight sarcomere protein genes, clinical expression varies considerably, even among patients with the same pathogenic variant. To determine whether background genetic variation or environmental factors drive these differences, we studied disease progression in 11 pairs of monozygotic HCM twins. The twin pairs were followed for 5 to 14 y, and left ventricular wall thickness, left atrial diameter, and left ventricular ejection fraction were collected from echocardiograms at various time points. All nine twin pairs with sarcomere protein gene variants and two with unknown disease etiologies had discordant morphologic features of the heart, demonstrating the influence of non-hereditary factors on clinical expression of HCM. Whole genome sequencing analysis of the six monozygotic twins with discordant HCM phenotypes did not reveal notable somatic genetic variants that might explain their clinical differences. Discordant cardiac morphology of identical twins highlights a significant role for epigenetics and environment in HCM disease progression.

hypertrophic cardiomyopathy | identical twins | genetics

Hypertrophic cardiomyopathy (HCM) is characterized by left ventricular (LV) hypertrophy accompanied by nondilated ventricular chambers in the absence of other cardiac or systemic disease that would cause similar cardiac morphology (1). HCM is clinically recognized in ~1 in 500 individuals, and >70% of familial HCM patients carry a pathogenic or likely pathogenic variant in genes encoding cardiac sarcomere proteins (2, 3) (denoted as HCM variants). Most HCM variants alter thick filament proteins myosin heavy chain 7 (*MYH7*) and myosin binding protein C (*MYBPC3*). Other HCM variants in genes encoding thin filament proteins, such as troponin T (*TNNT2*), account for a minority of cases (4, 5).

HCM variants generally have high penetrance but produce variable expression of clinical manifestations (6–13). Some variant carriers manifest LV hypertrophy early in childhood, while others have normal LV wall thickness (LVWT) until the sixth or seventh decade of life. Hypertrophy can range from the upper limit of normal (LVWT = 12 mm) to massive (>30 mm) and can occur with minimal symptoms, advanced heart failure, or fatal arrhythmias (3, 10). Approximately 25% of patients die from HCM-related adverse events (14), including sudden cardiac death, thromboembolic stroke, and heart failure. Although patients with HCM variants have more adverse outcomes than patients with unknown causes for HCM (15, 16), the mechanisms accounting for diverse responses to variants within the same or different HCM genes are unknown.

A classic approach to defining the contribution of genetic and environmental factors to variable progression of human disorders is to compare clinical phenotypes in monozygotic (MZ) twins, who have identical genome sequences. Few case reports have evaluated MZ twins with HCM (17–22). Wang et al. (19) showed that a twin pair with a pathogenic sarcomere gene mutation (*MYH7* G768R) had similar LVWT but different amounts of fibrosis, measured by late gadolinium enhanced magnetic resonance imaging. Jansweijer et al. (22) examined interventricular septum thickness (IVSd) from 11 MZ twin pairs with HCM, including 5 pairs with sarcomeric variants, and found no significant heritability for IVSd in HCM. In contrast, a recent study demonstrated concordant morphologic findings and clinical course of identical twins with HCM, suggesting little environmental influence on clinical expression of HCM (20). Whether MZ twins with HCM variants have similar or different clinical courses remains uncertain. To more fully identify factors influencing HCM progression, we characterized longitudinal disease progression in 11 MZ twin pairs, including 9 with pathogenic sarcomere variants over 5 to 14 y.

Significance

Genetics, notably pathogenic genetic variants in sarcomere protein genes, play a major role in development of hypertrophic cardiomyopathy (HCM). However, degree of contribution from epigenetic and environmental factors in clinical presentation of HCM is currently unclear. We investigated phenotypic differences between identical twins with pathogenic sarcomere protein gene variants and demonstrated their discordant HCM presentation despite having virtually identical genomes. Our study underscores the important contribution of epigenetics and environment in disease progression in genetically diagnosed HCM patients.

Author contributions: G.G.R., Y.K., C.E.S., and J.G.S. designed research; G.G.R., Y.K., A.C.P., J.I., M.W.R., and J.G.S. performed research; N.K.L., C.Y.H., S.D., C.S., B.M., D.Q., and E.M.G. contributed new reagents/analytic tools; G.G.R., Y.K., A.C.P., J.I., M.W.R., S.R.D., and J.G.S. analyzed data; and G.G.R., Y.K., C.E.S., and J.G.S. wrote the paper.

Reviewers: L.W., University of Maryland Medical Center; and C.M.W., German Heart Centre.

The authors declare no competing interest.

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This article contains supporting information online at <https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2021717118/-DCSupplemental>.

Published March 3, 2021.

Results

Among 11 twin pairs, monozygosity was confirmed by micro-satellite analysis (1 pair), whole genome sequencing (WGS; 6 pairs), placental pathology (1 pair), and medical record (3 pairs) (Table 1). Genetic analyses for HCM were prompted by clinical manifestations in nine twin pairs and by familial cascade testing in two twin pairs. We compared LVWT, left atrial (LA) size, and LV ejection fraction (LVEF), standard measures of clinical expression and severity of HCM (1), by retrospective analyses of echocardiograms and clinical cardiac records. Twins were considered discordant if maximal LVWT or LA size differed by $\geq 20\%$ (23, 24), or if absolute difference in LVEF $\geq 10\%$ was

noted between the two twins in at least two echocardiographic studies a year or more apart (25–27).

Nine twin pairs had HCM-causing variants in *MYBPC3* ($n = 6$), *MYH7* ($n = 2$), and *TNNT2* ($n = 1$) genes. The causes of HCM in two twin pairs, one of which tested negative for the sarcomeric gene panel and the other declined genetic testing, remain unknown. Seven individuals had cardiovascular comorbidities, including hypertension, hyperlipidemia, secundum atrial septal defect (status post percutaneous closure), and myxomatous mitral valve. These comorbidities, except for hypertension, were discordant between some twin pairs. Eleven individuals, inclusive of four twin pairs, had an implantable cardioverter

Table 1. Clinical characteristics of MZ twins

ID	Sex	Variant	Dx	Age of Dx	Monozygosity confirmation	Comorbidities [†]	ICD [‡]	AF	CHF	NYHA	LVOT obstruction	Interventions [‡]	F/U (y)	BMI
00554	M	MYBPC3: c.2373insG	Preclinical		Placental pathology	ASD	N	N	N	I	N	N	5	14.1
00555	M	(p.Trp792Valfs*41)	HCM	4.5		—	Y	N	N	I	N	N	5	14.6
00168	F	MYBPC3: c.1892delT	Preclinical		Medical record	—	N	N	N	I	N	N	6	25.8
00940	F	(p.Phe631Serfs*32)	Preclinical			—	N	N	N	I	N	N	6	28.5
PC13	F	MYBPC: c.927-9G > A	HCM	51	WGS	Hypothyroidism Myxomatous MV	N	N	N	II	Y	Myectomy (62)	14	24.4
PC14	F		HCM	52		—	N	N	N	I	N	N	14	26.5
GH1	F	MYBPC3: c.655G > C	HCM	21	WGS	—	N	N	N	I	Y	N	9	
GH2	F	(p.Val219Leu)	HCM	21		—	N	N	N	I	N	N	7	
OU3	F	MYBPC3: c.2735delG (p.Gly912Alafs*12)	HCM	46	WGS	HTN	Y	N	N	I	N	N	6	26.2
OU4	F		HCM	48		HTN	N	N	N	I	N	N	7	
ALH1	F	MYBPC3: c.772G > A	HCM	40	WGS	HTN	N	N	N	II	N	N	6	
ALH2	F	(p.Glu258Lys)	HCM	44		—	N	N	N	I	N	N	4	
NF111	F	MYH7: c.1012G > A (p.Val338Met)	HCM	14	WGS	—	Y	N	N	I	Y	N	7	26.6
NF112	F		HCM	14		—	Y	N	N	I	N	N	7	23.4
1212	F	MYH7: c.1988G > A (p.Arg663His)	HCM	39	Microsatellites	—	Y	Y	N	I	Y	AV node ablation (51)	7	24.8
1213	F		HCM	39		MR/TR HTN HLD DM CKD	N	Y	Y (66) EF = 40	IV	N	Pacemaker (63) MV and TV annuloplasty (68) LA appendage ligation (68) AV node ablation (68)	7	26.7
ZN1	F	TNNT2: c.275G > A (p.Arg92Gln)	HCM	14	WGS	—	Y	N	N	II	N	N	6	
ZN2	F		HCM	14		—	Y	N	N	II	N	N	6	
00872	F	Unknown	HCM	3	Medical record	—	Y	N	Y	III	N	N	5	
00925	F		HCM	17		NSVT	Y	N	Y	II	N	N	16	
00141	M	Unknown	HCM	15	Medical record	HTN	Y	N	N	I	Y	N	5	
00142	M		HCM	14		—	Y	N	N	I	Y	N	6	

Abbreviations: ASD, atrial septal defect; AV, atrioventricular; CHF, congestive heart failure; CKD, chronic kidney disease; DM, diabetes mellitus; Dx, diagnosis; EF, ejection fraction; F/U, follow-up; HLD, hyperlipidemia; HTN, hypertension; ICD, implantable cardioverter, defibrillator; LVOT, left ventricular outflow tract; MR, mitral regurgitation; NSVT, nonsustained ventricular tachycardia; NYHA, New York Heart Association functional classification; TR, tricuspid regurgitation.

[†]No patient had a history of stroke, coronary artery disease, cardiac arrest, or inappropriate ICD firing.

[‡]Number in parenthesis indicates age at time of procedure.

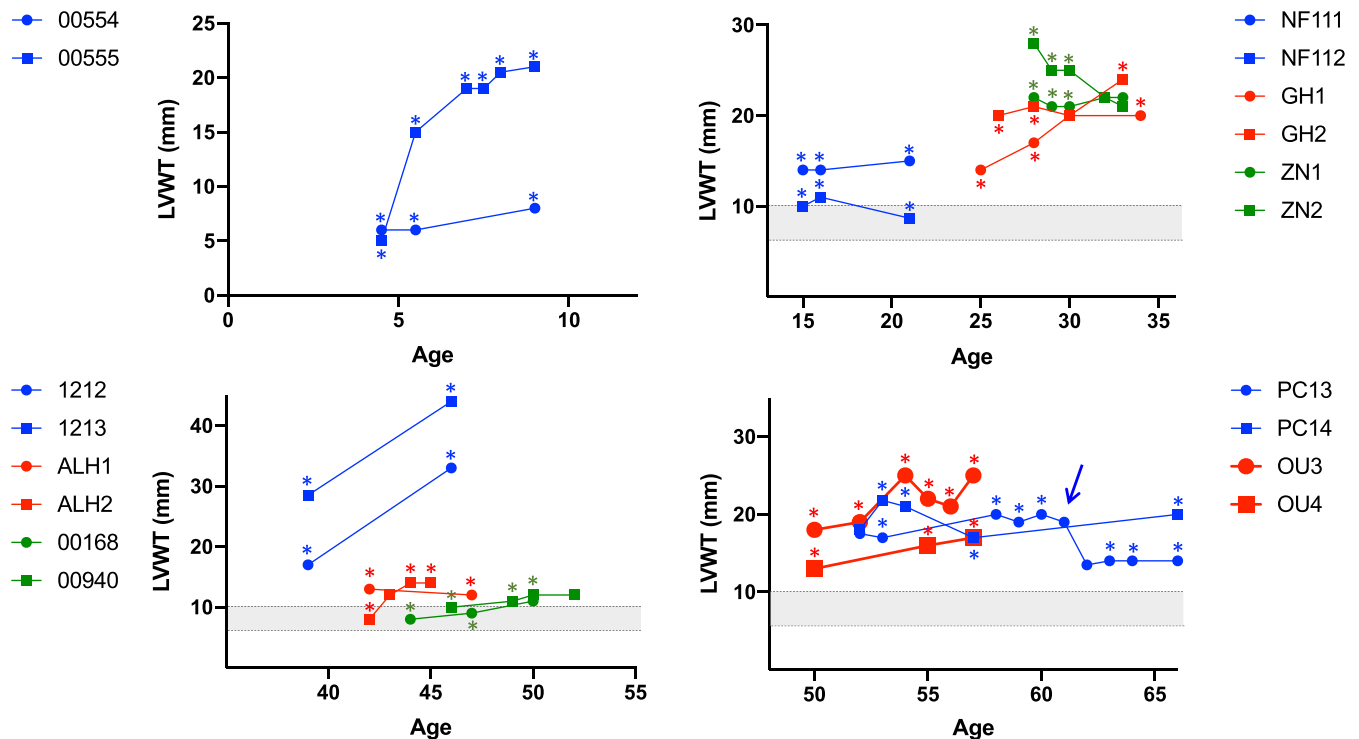


Fig. 1. LVWT of MZ twins with pathogenic/likely pathogenic sarcomere variants. Asterisks (*) represent at least 20% difference in LVWT between echocardiogram readings of each twin pair at each time point. First, echocardiograms of twin pairs taken in the same year were considered. If a subject's twin did not have an echocardiogram taken in the same year, then echocardiograms taken within 3 y were considered. Only LVWT measurements taken in the same region of the ventricle (septum) were considered. PC13 obtained myectomy at the age of 62, indicated by blue arrow. Dotted lines represent normal range of LVWT (43).

defibrillator for primary prevention or a pacemaker. Individual PC13 underwent myectomy at age 62 y to ameliorate symptoms of chest pain, exertional dyspnea, limited exercise capacity, tachycardia, and presyncopal events.

To determine if LVWT differences observed between identical twins reflected technical variation between different observers, we analyzed 2,317 HCM patients with HCM-causing genetic variants enrolled in the Sarcomeric Human Cardiomyopathy Registry (SHaRe), who have had one or more echocardiograms in a single year. The mean difference between the two echocardiograms performed in the same year was 1.11 ± 1.2 mm on the same individual ($n = 17,137$). In contrast, 54 paired LVWT measurements from 2 identical twins within 1 y differed by 4.75 ± 0.7 mm, which is significantly different ($P < 1e-16$) from the mean LVWT observed between measurements made in the same SHaRe subject.

All twins, with or without an HCM variant, shared classic asymmetry of the interventricular septum and had discordant LVWT over extended periods during this study (Fig. 1 and *SI Appendix, Fig. S1 and Table S1*). LVWT differences were typically evident early and maintained throughout longitudinal follow-up (5 to 14 y). Twin pair 00554/00555, with pediatric onset of HCM, had comparable LVWT at age 5 y, but over the ensuing 7 y the degree of hypertrophy markedly diverged (Figs. 1 and 2). As previously found in HCM cohorts (28, 29), LVWT of twin pairs 00554/00555 and 00168/00940 did not correlate with body mass index (BMI) (*SI Appendix, Fig. S2*) ($P = \text{NS}$, not significant).

Approximately 20% of HCM patients develop LA enlargement, a morphologic abnormality that conveys increased risk for atrial fibrillation (AF), embolic stroke, and reduced survival (30–33). Although LVWT differed between the all MZ twin pairs, LA diameters were discordant in only six of the nine twin pairs (ALH1/ALH2, OU3/OU4, PC13/PC14, ZN1/ZN2, 00168/

00940, 00554/00555) (Fig. 3 and *SI Appendix, Table S1*). Only one twin pair (1212/1213) developed AF, perhaps due to the overall young age of the twin cohort.

LVEF is typically normal or supranormal in HCM patients, and reduced LVEF can herald the onset of progressive dysfunction that results in heart failure. Four of seven twin pairs demonstrated discordance in LVEF (ALH1/ALH2, PC13/PC14, 00168/00940, 00554/00555) (*SI Appendix, Fig. S3*).

To investigate whether somatic genetic variants might account for these discordant phenotypes in MZ twins, we performed WGS analysis of six MZ twin pairs (ALH1/ALH2, GH1/GH2,

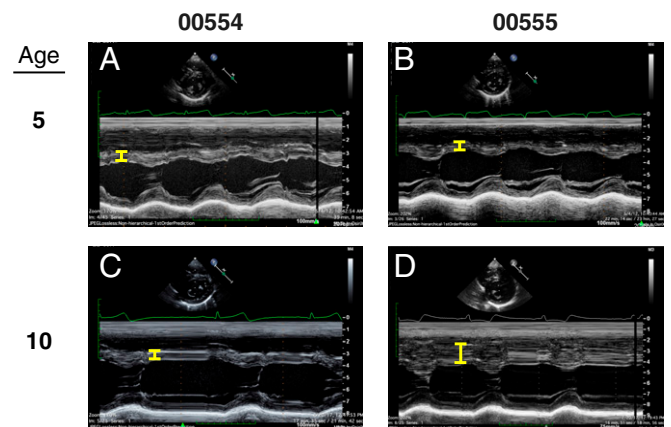


Fig. 2. Representative images of echocardiograms from twin pair 00554 and 00555 at age 5 y (A and B) and 10 y (C and D). Yellow bracket indicates LVWT measured in M-mode.

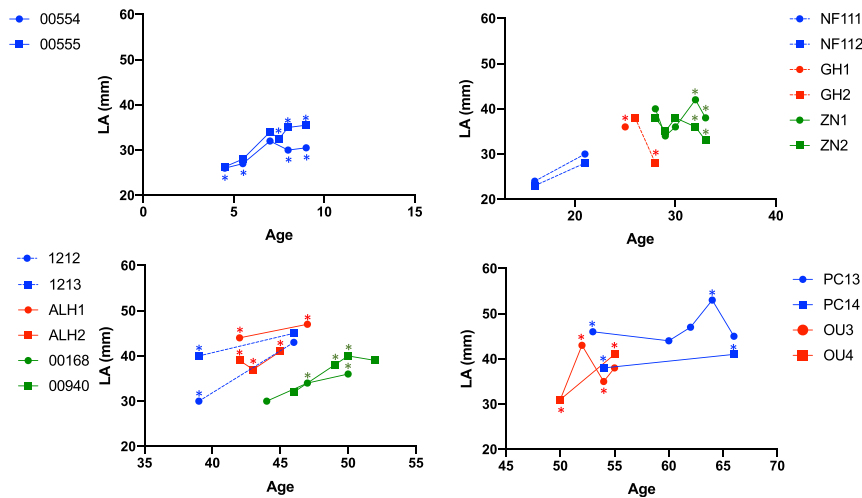


Fig. 3. LA diameter of MZ twins with pathogenic/likely pathogenic sarcomere variants. Asterisks (*) represent at least 20% difference in LA size between echocardiogram readings of each twin pair at each time point. First, echocardiograms of twin pairs taken in the same year were considered. If a subject's twin did not have an echocardiogram taken in the same year, then echocardiograms taken within 3 y of that echocardiogram were considered. Normal LA size (AP dimension) for females is 27 to 38 mm, and for males is 30 to 40 mm (43). Discordant twins are denoted by solid lines, concordant twins by dashed lines.

NF111/NF112, OU3/OU4, PC13/PC14, ZN1/ZN2) whose DNA was available for sequencing analysis. We did not identify any single nucleotide variants (SNV) or structural variants in HCM genes (*ACTC1*, *FHL1*, *GLA*, *MYBPC3*, *MYH7*, *MYL2*, *MYL3*, *PRKAG2*, *TNNI3*, *TNNT2*, *TPM1*) (5) or dilated cardiomyopathy genes (*ACTC1*, *BAG3*, *DSP*, *LMNA*, *MYH7*, *NEXN*, *PLN*, *TNNC1*, *TNNT2*, *TPM1*, *TTN*, *VCL*) (34) that differed between each twin pair. Furthermore, although our analysis identified a number of somatic variants in twin pairs, none of these somatic variants (coding, noncoding, or structural variants) were shared between any two or more twin pairs.

Discussion

Our analyses of cardiac morphology and function in 11 MZ HCM twin pairs, who were followed over 5 to 14 y, demonstrated LVWT discordance in all twin pairs. The discordant LVWT in all of the MZ twin pairs suggest that epigenetic and environmental factors, rather than background genetic variation, play a major role in hypertrophic remodeling.

In contrast to LVWT, LA dimensions were concordant in three of nine MZ twin pairs with HCM-causing variants. An appealing hypothesis to explain this greater degree of concordance is that LA size reflects impaired ventricular relaxation, which results directly from sarcomere dysfunction rather than environmentally controlled hemodynamic function. Four of seven MZ twin pairs with available LVEF data demonstrated discordance, suggestive of environmental factors influencing ventricular function in HCM in addition to its structural traits, such as LVWT. However, as LVEF is usually preserved or somewhat enhanced in HCM until late stages of the disease and only one twin pair was over 60 y of age, longer follow-up is needed to better assess the influence of genetic and nongenetic factors on ventricular function.

Our finding that MZ twins with sarcomeric variants known to perturb structural traits in HCM have discordant phenotypes is consistent with the conclusions drawn from Jansweijer et al. (22), where the authors compared the first available measurement of IVSd of 11 MZ HCM twins. We followed the twin pairs for 5 to 14 y rather than a cross-sectional analysis to minimize sampling bias from analyzing one-time measurements and to study their clinical course over long periods of time. Nine of 11 twin pairs in the present study had pathogenic HCM variants identified,

compared to less than 50% of twin pairs with a known genetic mutation in Jansweijer et al. Identification of pathogenic sarcomeric variants ensures that subjects in the present study indeed have HCM rather than cardiomyopathy that mimics HCM, such as hypertensive cardiomyopathy or infiltrative cardiomyopathy. Furthermore, WGS analyses demonstrate that somatic mutation is unlikely to explain discordant phenotypes of the HCM MZ twins. The clinical course of HCM patients with pathogenic mutations affecting sarcomere genes is clearly impacted by epigenetic and environmental factors that might include microbial infection, diet, or exercise.

Limitations of our study include the retrospective nature of the analysis and possible interobserver variabilities of echocardiographic measurements. To minimize confounding from interobserver variation, we defined phenotypes to be discordant if maximal LVWT or LA size differed by $\geq 20\%$ or if absolute LVEF differed by $\geq 10\%$ based on published measurement variabilities (23–27). Furthermore, as noted in the results, echocardiographic measurements in discordant HCM twins were significantly greater than technical variation of the measurements in a single year from 2,317 HCM patients enrolled in SHaRe.

Our data infer a critical role for environmental factors in determining cardiac morphologic progression in HCM patients. Presumably, the consequences of different environmental factors are mediated through changes in the epigenome. Some of these epigenetic modulations will likely be identified through studies of small and large animal HCM models (35, 36). However, epidemiologic studies of HCM patients will be required to identify those environmental factors that mediate human HCM progression; information that will benefit future HCM patients.

Materials and Methods

MZ Twin Cohort. Data from 8 of the 11 twin pairs were acquired from SHaRe. Three additional twin pairs were subjects in studies approved by and carried out according to the Brigham and Women's Hospital (BWH) Human Subjects Committee. Clinical measures were obtained by review of each subjects' medical record as recorded in the SHaRe database or in medical records of BWH. All subjects gave informed consent to participate in research.

Next-Generation Sequencing and Variant Analysis. Genomic DNA extracted from peripheral blood sample was sequenced using Illumina HiSeq instruments. All sequencing reads were aligned to hg38 (GRCh38) using BWA-MEM

with the -Y option (BWA v0.7.15). SNVs and small indels were identified using the Genome Analysis Tool Kit (GATK; v4.1) Haplotype Caller tool (37). Structural variants were analyzed using Manta, Wham, and MELT as part of the GATK-SV pipeline (38). The SNVs were annotated using vcfanno (v0.3.2), dbSNP (build 151), the 1000 Genomes Project (phase 3), gnomAD (v3.0), SnpEff (v4.3t, annotation database GRCh38.86) (39), and dbNSFP (v3.5a). High-quality variants [pass GATK Variant Score Quality Recalibration (VSR) truth sensitivity threshold 99.5 for SNVs, 99.0 for indels), a minimum depth of 10, genotype quality ≥ 20 , and Phred-scaled quality score (QUAL) ≥ 30] were filtered for rare heterozygous variants [defined as minor allele frequency (MAF) $< 1.00e-04$] in gnomAD (40) and the 1000 Genomes Project (41). Loss-of-function variants (labeled by SnpEff as nonsense, canonical splice-site, frameshift indels, start loss, stop lost, and stop gained) and

damaging missense variants [deleterious by MetaSVM (42)] were considered potentially pathogenic to the disease.

Data Availability. All study data are included in the article and supporting information.

ACKNOWLEDGMENTS. We thank the patients and investigators, who participated in enrollment. This study was supported by the following funding: Department of Health and Human Services, NIH, National Heart, Lung, and Blood Institute (NHBLI) Grants 5R01HL084553, 5R01HL080494, and X01HL143310 (to C.E.S. and J.G.S.); Fondation Leducq 16-CVD 03 (to C.E.S. and J.G.S.); a John S. Ladue Fellowship at Harvard Medical School (to Y.K.); NIH, NHLBI 5T32HL007208 (to Y.K.); and a Sarnoff Cardiovascular Research Foundation Fellowship (to G.G.R.).

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