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RESEARCH LETTER

Patients With Hypertrophic Cardiomyopathy Deemed Genotype Negative Based on Research Grade Genetic Analysis

Time for Repeat Diagnostic Testing With Next-Generation Sequencing

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As a common cause of sudden cardiac death in the young, hypertrophic cardiomyopathy (HCM) is a heritable cardiovascular disorder affecting an estimated 1 in 500 individuals.[1] Genetic testing for HCM is a powerful tool which can reveal disease etiology and establish inheritance patterns. A positive test allows for variant-specific, cascade testing of at-risk family members, which can guide follow-up, lifestyle modifications, and reproductive counseling, while those who test negative for the familial variant may potentially discontinue clinical follow-up.[1,2]

With the advent of next-generation sequencing (NGS), mutation detection can now be achieved with significant reduction in cost and time. As such, NGS has improved the yield of genetic testing for HCM, with whole-genome sequencing representing the most comprehensive genetic test.[3] To further optimize the yield of genetic testing and to provide more thorough genetic counseling to patients and their families, phenotype-based genetic prediction scores were developed to identify HCM patients with the greatest likelihood of a positive genetic test result.[1,2]

Previously, 1053 unrelated patients with a clinical diagnosis of HCM underwent genetic testing for 9 HCM-associated myofilament genes. Of these, 694 (66%) remained genetically undiagnosed following denaturing high-performance liquid chromatography (DHPLC).[1] Next, the Mayo Clinic HCM Genotype Predictor Score was used to target 35 patients who remained genetically undiagnosed with the highest probability of a positive genetic test (genotype predictor score 4 [n=28] or 5 [n=7]; a priori, estimated yield of genetic test 83%–94%). Because of a lower phenotype risk score (especially those with scores −1 to 1; range ≈30%), the remaining genotype negative patients (n=664) have not been retested at this point.

All patients underwent either whole-exome sequencing (n=17) or whole-genome sequencing (n=18). Following NGS, all cases underwent gene-specific target analysis on the 10 most common HCM-associated sarcomere genes (*MYBPC3*, *MYH7*, *TNNT2*, *TNNI3*, *MYL2*, *MYL3*, *ACTC1*, *TPM1*, *TNNC1*, and *MYH6*) using Ingenuity Variant Analysis Software (Qiagen, Redwood City, CA). Only rare (minor allele frequency $<4\times10^{-5}$ in gnomAD) nonsynonymous and intronic variants located ≤20 bases from intron/exon boundaries were considered. The American College of Medical Genetics and Genomics guideline criteria were used to classify variants as pathogenic, likely pathogenic (LP), or variant of uncertain significance. Samples which remained negative underwent an expanded gene panel of 50 additional HCM-associated genes. This study was approved by the Mayo Clinic Institutional Review Board and all subjects signed informed consent. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Nearly half of the cohort was female (46%), the average age at diagnosis was 29±15 years, and the mean maximum left ventricular wall thickness was 24.9±5.7 mm. A reverse curve septal contour was

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Nonstandard Abbreviations and Acronyms

DHPLC	denaturing high-performance liquid chromatography
HCM	hypertrophic cardiomyopathy
LP	likely pathogenic
NGS	next-generation sequencing

observed in 83% of patients. The majority of patients had a family history of HCM (89%) or sudden cardiac death (77%). A history of hypertension was observed in 9% of patients.

Overall, 21/35 (60%) patients (5/7 [71%] with a genotype predictor score of 5 and 16/28 [57%] with a score of 4) had at least one rare, nonsynonymous, pathogenic, or LP variant identified (Table). Of these, 16 (46%) had at least one pathogenic or LP variant in *MYBPC3* (3 missense, 3 nonsense, 7 splice-error, 4 deletion/insertion variants). Of the 6 unique *MYBPC3* splice-error pathogenic or LP variants, 4 resided outside the canonical splice-site (first 2 nucleotides before or after an exon: c.1624+4A>T, c.3190+5G>A, c.3491-3C>G, and c.3330+5G>A). Additionally, 4 patients had a possible splice-error variant of uncertain significance residing outside the canonical splice-site: 3 patients with a MYBPC3-c.909-8T>A variant and 1 patient with MYBPC3-c.2735+5G>A. Two patients had a pathogenic missense variant in *MYH7* (6%). A pathogenic *MYL2* missense variant was identified in 2 patients (6%) and 1 patient (3%) had a pathogenic missense variant in *MYL3*. Four patients had multiple rare nonsynonymous variants. Our expanded panel identified one patient with an *HRAS*-p.G12V variant.

Surprisingly, 60% of our genetically undiagnosed (after previous non-NGS research grade investigations) HCM cases had a pathogenic or LP variant identified using NGS-based analysis. While the exact causes of these previous misses are currently unknown, common reasons for false DHPLC negatives include DHPLC instrument misses, user misses, or polymerase chain reaction design failures.[4] Of note, with the previous method, samples only proceeded to Sanger sequencing of the fragment of interest if the DHPLC results indicated that a variant was present. Previous studies have shown that, compared with complete direct sequencing of a gene, the sensitivity and specificity of DHLPC were 87.5% and 97.4%, respectively.[5]

The significantly lower cost and increased availability of high-fidelity sequencing techniques have significantly improved the yield of genetic testing for HCM. We recommend that all patients with a high clinical probability for sarcomeric HCM, including those who underwent research-based genetic testing using nondirect sequencing methods, such as DHPLC, should obtain a clinical genetic test for HCM.

ARTICLE INFORMATION

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Table. Patient Genotype and Demographics

Patient ID	HCM score	Gene symbol	Transcript variant	Protein variant	ACMG variant classification	Sex	Age at diagnosis, y	MLVWT, mm	Obstructive HCM	Septal contour	Fam Hx of HCM	Fam Hx of SCD	Hx of hypertension
1	5	*MYBPC3*	c.2308G>A	p.D770N	P	F	22	25	Y	R	Y	Y	N
2	5	*MYBPC3*	c.3029_3030delAG	p.E1010fs*40	P	M	24	31	N	R	Y	Y	N
		MYBPC3	c.909-8T>A		VUS								
3	5	*MYBPC3*	c.3697C>T	p.Q1233*	P	F	31	28	Y	R	Y	Y	N
4	5	*MYL2*	c.173G>A	p.R58Q	P	F	24	34	N	R	Y	Y	N
5	5	*MYL3*	c.445A>G	p.M149V	P	F	17	23	Y	R	Y	Y	N
6	5					M	30	21	N	R	Y	Y	N
7	5					M	17	30	N	R	Y	Y	N
8	4	*HRAS*	c.35_36GC>TG	p.G12V	P	M	<1	8	Y	R	Y	Y	N
9	4	*MYBPC3*	c.655G>C	p.V219L	P	F	48	26	Y	R	Y	Y	N
10	4	*MYBPC3*	c.821+1G>A		P	F	33	23	Y	S	Y	Y	N
11	4	*MYBPC3*	c.1210C>T	p.Q404*	P	F	15	31	Y	R	N	Y	N
12	4	*MYBPC3*	c.1624+4A>T		P	M	27	24	Y	R	Y	N	N
13	4	*MYBPC3*	c.1624+4A>T		P	M	39	21	Y	R	Y	N	N
14	4	*MYBPC3*	c.2490dupT	p.H831fs*2	P	M	45	32	Y	R	Y	Y	N
15	4	*MYBPC3*	c.3124_3125insAA	p.T1042fs*5	P	F	22	16	Y	R	Y	Y	N
16	4	*MYBPC3*	c.3190+5G>A		P	F	45	33	N	R	Y	Y	N
17	4	*MYBPC3*	c.3330+5G>A		P	F	42	27	N	R	N	Y	N
		MYBPC3	c.3742G>A	p.G1248R	LP								
18	4	*MYBPC3*	c.3340_3342delACC	p.T1114del	LP	M	19	20	N	N	Y	Y	N
19	4	*MYBPC3*	c.3491-3C>G		LP	M	21	22	N	N	Y	Y	N
20	4	*MYBPC3*	c.3697C>T	p.Q1233*	P	F	18	21	Y	R	Y	N	N
		MYH6	c.5500C>T	p.R1834C	VUS								
21	4	*MYBPC3*	c.909-8T>A		VUS	M	43	20	N	A	Y	Y	N
22	4	*MYBPC3*	c.909-8T>A		VUS	F	11	31	Y	R	Y	N	N
		MYBPC3	c.3535G>A	p.E1179K	VUS								
23	4	*MYBPC3*	c.2737+5G>A		VUS	F	43	24	N	R	Y	N	N
26	4	*MYBPC3*	c.2995-1G>A		P	F	5	25	N	R	Y	N	N
24	4	*MYH6*	c.5393G>A	p.R1798Q	VUS	M	41	21	Y	R	N	Y	N
25	4	*MYH7*	c.3981C>A	p.N1327K	P	M	32	27	N	R	Y	Y	Y
27	4	*MYH7*	c.4258C>T	p.R1420W	P	M	5	25	Y	R	Y	N	N
28	4	*MYL2*	c.173G>A	p.R58Q	P	F	33	14	N	R	Y	Y	N
29	4	*MYL3*	c.307+274G>A		VUS	M	44	31	Y	R	Y	Y	Y
30	4					M	<1	24	Y	R	N	Y	N
31	4					M	26	30	Y	A	Y	Y	N
32	4					M	38	20	N	S	Y	Y	N
33	4					M	40	32	N	R	Y	Y	Y
34	4					M	44	24	N	R	Y	N	N
35	4					F	68	28	Y	R	Y	Y	N

A indicates apical; ACMG, American College of Medical Genetics and Genomics variant classification criteria[4]; HCM, hypertrophic cardiomyopathy; Hx, history; LP, likely pathogenic; MLVWT, maximum left ventricular wall thickness; N; neutral; P, pathogenic; R, reverse septal contour; S, sigmoidal septum; SCD, sudden cardiac death; and VUS, variant of uncertain significance.