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I, Randa E Newman, hereby submit this original work as part of the requirements for the degree of Master of Science in Genetic Counseling.

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Hypertrophic Cardiomyopathy Genotype Prediction Models in a Pediatric Population

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Hypertrophic Cardiomyopathy Genotype Prediction Models in a Pediatric Population

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By

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Abstract

Background: The Toronto Hypertrophic Cardiomyopathy (HCM) Genotype Score and Mayo HCM Genotype Predictor are risk assessment models recently developed to estimate a patient's likelihood of testing positive for a pathogenic variant causative of HCM. These models were developed from adult populations with HCM based on factors that have been associated with a positive genotype and have not been validated in external populations. The purpose of this study was to evaluate the overall predictive abilities of these models in a clinical pediatric HCM setting.

Methods and Results: A retrospective medical record review of 77 pediatric patients with gene panel testing for HCM between September 2005 and June 2015 was performed. Clinical and echocardiographic variables used in the developed models were collected and used to calculate scores for each patient. To evaluate model performance, the ability to discriminate between a carrier and non-carrier was assessed by area under the ROC curve (AUC) and overall calibration was evaluated by the Hosmer-Lemeshow goodness-of-fit statistic. The Toronto model and the Mayo model showed p values of 0.41 and 0.88, respectively, for model calibration. Discrimination assessed by AUC was 0.72 ($P < 0.001$) for the Toronto model and 0.65 ($P = 0.009$) for the Mayo model.

Conclusions: Our findings suggest that these models are useful in predicting a positive genetic test result in a pediatric HCM setting. They may be used to aid healthcare providers in communicating risk and enhance patient decision-making regarding pursuit of genetic testing.

Key Words: hypertrophic cardiomyopathy, pediatric HCM, genetic counseling, risk assessment

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Introduction

Hypertrophic cardiomyopathy (HCM) is among the most commonly inherited cardiovascular diseases, with an estimated prevalence of ~1:500 individuals (Maron, 2002). It is characterized by unexplained hypertrophy of the ventricles in the absence of an underlying condition or other cardiac disease, such as valvular heart disease or hypertension (Teekakirikul, Kelly, Rehm, Lakdawala, & Funke, 2013; Watkins, Ashrafian, & Redwood, 2011). HCM has a broad spectrum of phenotypic variability with respect to age of onset, degree of hypertrophy, symptoms, and other clinical manifestations. It is genetically heterogeneous and multiple pathogenic variants have been identified, with the majority of them inherited in an autosomal dominant manner.

Genetic testing for HCM has become progressively widespread since the discovery of the first HCM-associated gene identified in 1990 (Geisterfer-Lowrance et al., 1990). Contemporary testing allow for identification of pathogenic variants in more than 20 genes (Teekakirikul et al., 2013). Previous studies suggest that sarcomeric pathogenic variants can be identified in approximately 30-68% of adults with HCM (Andersen et al., 2009; Erdmann et al., 2003; Richard et al., 2003; Van Driest, Ommen, Tajik, Gersh, & Ackerman, 2005). Pediatric studies in particular have revealed overall genetic testing yields ranging from 21.3-53.2% (Kaski et al., 2009; Kindel et al., 2012; Morita et al., 2008; Nugent et al., 2005).

Clinical genetic testing for HCM can be useful in confirmation of disease etiology and risk stratification of family members through targeted variant testing. It is also valuable in identifying phenocopies of HCM (e.g., Fabry disease, Noonan syndrome, mitochondrial cardiomyopathies, etc.). Several academic and commercial laboratories offer gene panel testing for HCM, in which the cost varies considerably. Studies have suggested that implementing

genetic testing into the management of families with HCM is cost-effective when compared solely to a serial cardiac screening approach. Based on the impact of clinical and family care, as well as possible cost savings, it has been suggested that families with HCM be referred to specialized cardiac genetic clinics that can offer genetic counseling and testing (Ingles, McGaughran, Scuffham, Atherton, & Semsarian, 2012; Wordsworth et al., 2010). Published guidelines from multiple organizations support the use of genetic testing in probands with a clinical diagnosis of HCM (Ackerman et al., 2011; Gersh et al., 2011; Hershberger et al., 2009).

Many factors influence the yield of genetic testing for HCM such as family history and age of diagnosis. Several investigators have suggested that the generation of a patient-specific pre-test probability of a positive test result could aid healthcare providers in conveying appropriate genetic risk assessment. Additionally, there is the potential to enhance patient decision-making and inform pre-test genetic counseling. Groups from Toronto General Hospital and Mayo Clinic developed separate prediction tools to identify the probability that an individual patient with clinically diagnosed HCM would be genotype-positive. These published prediction models are known as the Toronto HCM Genotype Score and the Mayo HCM Genotype Predictor (Bos et al., 2014; Gruner et al., 2013)

Description of Models

Toronto HCM Genotype Score (Gruner et al., 2013) – The Toronto HCM Genotype Score was developed with the aim to construct a score identifying individuals with a high likelihood of having a positive HCM gene panel test result. The study sample consisted of 471 adult probands (average age 45.2 ± 15.9 years) with a clinical diagnosis of HCM. A retrospective medical record review was performed and data extraction variables were determined based on

factors from previous studies that demonstrated a significant association with a positive HCM genotype (Andersen et al., 2009; Binder et al., 2006; Girolami et al., 2006; Ho, 2010). Study participants prospectively underwent gene panel testing for HCM through a variety of commercial laboratories between July 2005 and November 2010. Overall, 163 of 471 adult probands tested positive for an HCM-associated variant, resulting in a 35% genetic testing yield.

Multivariate regression analysis revealed six independent variables as predictors for a positive HCM genotype. Regression coefficients for each variable were divided by the absolute value of the lowest regression coefficient in the model and then rounded to the nearest integer as risk weights. The sum of the points for each variable comprises a patient's genotype score. Each score corresponds to a specific probability of testing positive for an HCM-associated pathogenic variant and is referred to by the investigators as the Long Model (Table 1A). Specific probability for testing positive for a pathogenic variant causative of HCM can be calculated using the following formula: $P = 1 / (1 + e^{-[-1.614 + \text{risk score} \times 0.1922]})$. For further analysis, the Toronto group also created a Tertiles Long Model (Table 1B), in which they categorized specific genotype scores by relative risk groups – low, intermediate, or high.

Mayo HCM Genotype Predictor (Bos et al., 2014) – The Mayo HCM Genotype Predictor was published with a similar aim as the Toronto HCM Genotype score – to provide a simple and clinically applicable prediction score to determine a pretest probability of having a positive HCM gene panel. A total of 1053 unrelated patients (average age 44 ± 19 years) with a clinical diagnosis of HCM had gene panel testing for nine HCM-associated myofilament genes. Of the 1053 adult patients, 359 were found to have a pathogenic variant, resulting in an overall genetic testing yield of 34%.

Clinical data deemed relevant to the HCM diagnosis was collected from participants' medical records. Receiver operating characteristic analysis for continuous variables as well as univariate and multivariate analysis identified five positive predictors and one negative predictor with regard to an HCM-positive genotype (Table 2A). The cumulative score of the six variables are associated with the likelihood that an individual will test positive for an HCM-associated pathogenic variant. The probabilities range from 6% for those with a score of -1 to 80% for those with a score of 5 (Table 2B).

Significance

The predictive abilities of these models have not been tested outside of the adult populations from which they were developed. Therefore, it has not been established that they are generalizable to other populations with HCM. Furthermore, these models were developed primarily from adult populations and little is known about their performance in a pediatric population. In the present study, we tested the predictive abilities of the Toronto HCM Genotype Score and the Mayo HCM Genotype Predictor in a pediatric population. Our objective was to assess whether or not the genotype prediction tools had comparable predictive abilities in a study population consisting of patients less than 18 years of age. We elected to study a pediatric population because there are expected to be fundamental differences between children and adults associated with certain variables/markers used in the models. For example, based on age, children are expected to have a smaller body habitus and smaller heart size than adults; therefore, children and adults may have distinct differences in cardiac measurements associated with variables in the models (e.g., maximum left ventricular wall thickness [MLVWT] used in the Mayo HCM Genotype Predictor). Additionally, the presence of hypertension is a variable used in

both models and is less likely to be observed in children than adults. Finally, all pediatric patients are under the age used as a cut-off for assigning points in both models. By conducting this study, we intended to provide information regarding clinical usefulness of the HCM genotype prediction tools in a pediatric HCM setting.

Methods

Study Population

A retrospective medical record review was performed of patients seen at the Heart Institute at Cincinnati Children's Hospital Medical Center (CCHMC) from September 2005 to June 2015 with a clinical diagnosis of HCM and HCM gene panel testing. Patients were less than 18 years of age at the time of their clinical diagnosis and specimen collection date for genetic testing. Patients were excluded if there was no cardiac imaging accessible prior to a septal reduction therapy, if they had a genetic syndrome known to be associated with left ventricular hypertrophy (LVH), or if they had a congenital heart defect causative of LVH. The variables/markers used in the Toronto and Mayo models to calculate a genotype prediction score were collected for each patient. Study data were collected and managed using REDCap (Research Electronic Data Capture) hosted at CCHMC (Harris et al., 2009). REDCap is a secure, web-based application designed to support data capture for research studies. This study was approved by the CCHMC Institutional Review Board.

HCM Gene Panel Testing

Medical record review demonstrated that HCM gene panel testing was ordered through various commercial and academic laboratories. The number of genes analyzed ranged from 5 to

36. Variability was attributable to the laboratory used and time of testing. Patients were considered to have a positive HCM genotype if their test result was classified as pathogenic or likely pathogenic (i.e., disease-causing, likely disease-causing, and presumed pathogenic). A variant of uncertain significance (VUS) was not considered a positive test result. Benign, likely benign, or presumed benign variants were also considered negative. For the purpose of this study, variants were not reinterpreted; pathogenicity was based on each laboratory's analysis and interpretation at the time of testing.

Clinical Data

Relevant clinical information was collected from the medical records to calculate predictive genotype scores for each patient. For each parameter in both models, the respective authors' criteria were used (Tables 1A, 2A).

Echocardiograms performed closest to the date of specimen collection for genetic testing were used. If echocardiograms were unavailable, cardiac magnetic resonance imaging (cMRI) closest to the date of specimen collection for genetic testing was used. Morphological subtypes (e.g., sigmoid, reverse-curve, apical, and neutral) have been previously defined (Binder et al., 2006) and were interpreted jointly by two pediatric cardiologists. If a patient's morphological subtype did not fall into one of the previously mentioned categories, it was defined as "other".

Statistical Analyses

Descriptive statistics were used to summarize the demographics and clinical characteristics of the study population. Frequency (percentage) was reported for categorical variables; mean $\pm$ standard deviation was reported for continuous variables. In the present study,

the binomial marker of interest was a positive HCM genotype. Unpaired t-test was used to test the mean differences between individuals with positive genotypes and those with non-positive genotypes for continuous variables; Fisher's exact test was used for comparison of categorical variables. Genotype prediction scores for both models were calculated for each patient. Logistic regression was performed on scores for positive genotypes and the predicted positive genotypes from the logistic regression model were compared to the observed positive genotypes at each integer value of the scores. To evaluate model performance, discrimination was assessed by area under the ROC curve (AUC) and calibration was evaluated by the Hosmer-Lemeshow goodness-of-fit statistic. All statistical analyses were performed in R Version 3.2.1 (R Core Team, 2013).

Mayo HCM Genotype Predictor: MLVWT Marker

The Mayo HCM Genotype Predictor uses MLVWT as one of the clinical markers in their genotype prediction score. Individuals with a MLVWT of 20 millimeters (mm) or greater are assigned one point for that specific marker. Due to a smaller cardiac size in children, this particular measurement may not be reached even in children with severe hypertrophy. We acknowledged that for pediatric patients, the MLVWT z-score may be a more sensitive measurement that would capture children with significant hypertrophy who had an absolute measurement less than 20 mm. ROC analysis was performed to determine the cutoff value of MLVWT z-score associated with a positive genotype. The Mayo HCM Genotype Predictor using the cutoff value of MLVWT z-score (for simplicity, this will from hereon be referred to as the Mayo HCM Genotype Predictor [z-score]) was then analyzed as well as the original Mayo HCM Genotype Predictor using the 20 mm MLVWT cutoff (Mayo HCM Genotype Predictor [mm]).

Results

Study Population Demographic and Clinical Characteristics

A total of 78 patients met inclusion criteria for the present study. One patient had HCM gene panel testing with only three genes analyzed and a negative test result. For the purpose of this study, this patient was excluded from the analysis due to uncertainty of a true negative genetic test result, subsequently leading to a total study population of 77.

Table 3 describes the demographic and clinical characteristics of our study population. In summary, there were more males ($n=57$, 74%) than females, the mean age at time of genetic testing was 9.39 ± 5.98 years, and the mean MLVWT was 15.1 ± 7.7 mm. The average MLVWT z-score was 4.07 ± 2.33 and the mean ratio of maximal wall thickness to posterior wall thickness (MWTH:PWTH) was 1.70 ± 0.97 . More than one third of our study population had a sigmoid morphological subtype based on echocardiographic imaging. A positive family history of HCM was present in one quarter of the population ($n=19$, 25%). Most patients were of Caucasian race ($n=61$, 79%) and 18.2% were African-American ($n=14$). Two patients classified their race as other (2.6%).

Significant Predictors of a Positive Genotype

In the present study, the significant predictors of a positive HCM genotype (Table 3) were family history of HCM ($P = 0.029$), MLVWT ($P = 0.010$), MLVWT z-score ($P = 0.0013$), and MWTH:PWTH ($P = 0.042$).

Genetic Testing Yield

In the present pediatric HCM cohort, 38 of 77 (49%) patients tested positive for at least one pathogenic variant. In this subset of patients with a positive HCM genetic test result, the majority had a single pathogenic variant identified in MYBPC3 (n=16, 42%) and MYH7 (n=10, 26%), respectively. Another 21% of patients with a positive genetic test result had a single pathogenic variant in ACTC1, PRKAG2, TNNT, or TPM1 (n=8). In addition, 4 patients (11%) had pathogenic variants in multiple genes, with all 4 having at least one pathogenic variant in MYBPC3 (Table 4). Appendix A lists all variants deemed pathogenic/disease-causing or likely/presumed pathogenic/disease-causing based on the respective laboratory's interpretation at the time of genetic testing.

Mayo HCM Genotype Predictor (z-score)

ROC analysis was performed to determine a cutoff value of MLVWT z-score for a positive genotype. The analysis revealed the optimal MLVWT z-score value to be 4.2 (AUC=0.7, sensitivity = 57.9%, specificity = 76.9%, Figure 1).

Model Performance

The predicted and observed positive genotypes were compared for the Toronto HCM Genotype Score Long Model (Figure 2A). Model calibration assessed with the Hosmer-Lemeshow goodness-of-fit test showed a p value of 0.41. Discrimination assessed by AUC was 0.72 ($P < 0.001$). When the Tertiles Long Model with relative risk groups was used (Figure 2B), the model demonstrated much improved calibration (Hosmer-Lemeshow goodness-of-fit test, $P = 1$) and slightly lower model discrimination (AUC = 0.69; $P < 0.001$).

Figure 2C demonstrates the correlation between predicted and observed positive genotypes for the Mayo HCM Genotype Predictor (mm). The Hosmer-Lemeshow goodness-of-fit statistic gave a p value of 0.88 and model discrimination assessed by AUC was 0.65 ($P = 0.009$). When the 20 mm MLVWT marker was replaced with the optimal z-score of 4.2 determined from our study population, the Mayo HCM Genotype Predictor (z-score) again showed a relevant fit (Hosmer-Lemeshow goodness-of-fit statistic $P = 0.73$, Figure 2D). The AUC of 0.71 ($P < 0.001$) indicated a higher level of model discrimination than the Mayo HCM Genotype Predictor (mm), but both values were significantly different from 0.5.

Model Comparison

The Mayo HCM Genotype Predictor (mm) and the Mayo HCM Genotype Predictor (z-score) were compared to see if there was a distinct difference in how they discriminated between a pathogenic variant carrier and a non-carrier (Figure 3). It suggested that the model using the optimal z-score cutoff value of 4.2 performed significantly better in our study population ($P = 0.036$). However, when both variations of the Mayo HCM Genotype Predictor were compared to the Toronto HCM Genotype Score there was no significant difference in discrimination (Figure 3). This finding suggests that the Mayo HCM Genotype Predictor (mm) and the Mayo HCM Genotype Predictor (z-score) performed similarly to the Toronto HCM Genotype Score.

Discussion

The present study is the first evaluation of the predictive abilities and usefulness of the Toronto HCM Genotype Score and the Mayo HCM Genotype Predictor in an external population and specifically a pediatric population, to our knowledge. Overall, both models fit well to our

pediatric HCM cohort. The Toronto Long Model showed suitable discrimination between a pathogenic variant carrier and a non-carrier. The Toronto Tertiles Long Model using relative risk groups showed slightly lower model discrimination than the original Toronto Long Model. The Mayo HCM Genotype Predictor (mm) also demonstrated slightly lower discrimination, but was still valuable when applied to our study population. In the present study, we established a useful alternative for our pediatric HCM cohort by replacing the mm marker with an optimal MLVWT z-score value generated from our study population. When the Mayo HCM Genotype Predictor (z-score) was applied, the model discriminated between a carrier and a non-carrier significantly better than the Mayo HCM Genotype Predictor (mm). This finding was expected since children can have qualitatively severe hypertrophy less than an absolute measurement of 20 mm due to inherently smaller cardiac size. Patients with a positive HCM genotype in our population had an average MLVWT less than the 20 mm cutoff used as a predictor of a positive HCM genotype in the original Mayo model. Based on this finding, it may be more desirable to replace the MLVWT 20 mm marker with a MLVWT z-score of 4.2 when utilizing the Mayo HCM Genotype Predictor in a pediatric setting.

On a larger measure, when both variations of the Mayo model were compared to the Toronto model, they performed similarly in our study population. From a clinical perspective, this finding suggests that Toronto HCM Genotype Score and the Mayo HCM Genotype Predictor are both useful in providing predictive risk counseling in the setting of pediatric HCM.

Several of our findings regarding genetic testing yield are consistent with previous adult and pediatric studies. The present study population had an overall genetic testing yield of 49%, which is higher than what is reported by the Toronto and Mayo groups. Most pathogenic variants were identified in MYBPC3 and MYH7, supporting the majority of shared causes of familial

HCM in children and adults. In addition, 5% of our total study population had multiple pathogenic variants with at least one pathogenic variant identified in MYBPC3. This is consistent with what has been previously reported (Ingles et al., 2005; Richard et al., 2003; Van Driest et al., 2004).

Previous studies also suggest that sigmoid septal morphology is more common in patients diagnosed with HCM at an older age and whose HCM may not necessarily have a genetic etiology (Binder et al., 2006; Niimura et al., 2002). However, in our study population of pediatric patients less than 18 years of age, sigmoid morphology was the most prevalent subtype (35% overall) with no statistically significant difference when comparing patients with positive versus negative HCM genotypes. This result is consistent with the Toronto group's findings, which revealed a higher proportion of sigmoid septal morphologies than previously described.

In the present study, the significant predictors of a positive HCM genotype were family history of HCM, MLVWT, MLVWT z-score, and MWTH:PWTH. Furthermore, personal history of hypertension was not necessarily a negative predictor for a positive HCM genotype. This suggests that, although the models ultimately proved useful in the present study population, the generation of a similar model that is more specific to pediatric patients with HCM may be appropriate.

In conclusion, we present findings which suggest that the Toronto HCM Genotype Score and the Mayo HCM Genotype Predictor can be usefully applied in a clinical pediatric HCM setting. The purpose of this study and seemingly previous studies is not to preclude patients from moving forward with genetic testing for HCM if they have a low probability of testing positive based on these models. Instead, the models may be used to aid cardiologists, geneticists, and

genetic counselors in providing more detailed risk counseling for patients and families, and enhance patient decision-making regarding pursuit of gene panel testing for HCM.

Limitations

The results of the present study are based on a relatively small sample size of 77 referred to a single pediatric institution; therefore, our population may not be representative of all pediatric patients with HCM. Furthermore, a larger sample size would likely even the distribution of genotype scores, providing more generalizable results. Specifically, we had only a small number of patients who had a genotype score of 4 or 5 using the Mayo HCM Genotype Predictor (mm). Further validation of the models in a pediatric population is recommended to better understand the models' applicability. In addition, the models have yet to be tested in external adult populations and there remains a need to verify their generalizability in populations similar to ones in which they were developed. Another potential limitation of our study is that we did not reinterpret the pathogenicity of variants reported by the different laboratories used at the time of genetic testing. Variant classification has the potential to change over time as more individuals consent to genetic testing and wider information becomes available from population based genetic data and variant functional studies.

Disclosures

The authors of this study disclose no conflicts of interest.

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Table 1A. Toronto HCM Genotype Score: Long Model

Variable	Risk Weight	Defined as
Age, years		Age
<20	0	
20-29	-1	
30-39	-2	
40-49	-3	
50-59	-4	
60-69	-5	
70-79	-6	
≥80	-7	
Female sex	4	Male or female
Arterial hypertension	-4	Blood pressure >140/90 mmHg during 2 separate clinic visits or if a patient was diagnosed and medically treated for arterial hypertension by his or her treating physician
Positive family history of HCM	6	Documented evidence of HCM in a family member of any relation or a highly convincing patient report
Morphology category I	5	Reverse-curve or neutral septal morphology (Binder et al., 2006)
Ratio MWTH:PWTH		Ratio of maximal wall thickness to posterior wall thickness
<1.46	0	
1.47-1.70	1	
1.71-1.92	2	
1.93-2.26	3	
≥2.27	4	
HCM = hypertrophic cardiomyopathy; MWTH = maximal wall thickness; PWTH = posterior wall thickness		

Table 1B. Toronto HCM Genotype Score: Tertiles Long Model

Toronto Genotype Score	Relative Risk Group	Probability of Positive HCM Genotype
-9 to -1	Low	~10%
0 to 6	Intermediate	30%
7 to 19	High	>60%

Table 2A. Mayo HCM Genotype Predictor

Marker	Points	Defined as
Age at diagnosis < 45 years	1	Age at diagnosis less than 45 years
MLVWT $\geq$ 20 millimeters	1	Maximum left ventricular wall thickness greater than or equal to 20 millimeters
Family history of HCM	1	Undefined (the present study defined this variable as documented evidence of HCM in a single family member of any relation)
Family history of SCD	1	Undefined (the present study defined this variable as documented evidence of SCD in a single family member of any relation)
Reverse-curve HCM	1	Previously defined (Binder et al., 2006)
History of hypertension	-1	Reported history of [previously untreated] hypertension
MLVWT = maximum left ventricular wall thickness; HCM = hypertrophic cardiomyopathy; SCD = sudden cardiac death		

Table 2B. Mayo HCM Genotype Predictor Risk Groups

Mayo HCM Genotype Score	Probability of Positive HCM Genotype
-1	6%
0	14%
1	19%
2	41%
3	60%
4	68%
5	80%

Table 3. Study Population Demographic and Clinical Characteristics

Characteristic	Total Number of Patients N=77	Positive Genotype N=38	Non-Positive Genotype N=39	P value
Male sex, n (%)	57 (74%)	27 (71%)	30 (77%)	0.61
Age at genetic testing, mean ± SD	9.39 ± 5.98	9.70 ± 5.59	9.08 ± 6.40	0.65
Caucasian race, n (%)	61 (79%)	33 (86.84%)	28 (71.79%)	0.18
Family history of HCM, n (%)	19 (25%)	14 (36.84%)	5 (12.82%)	0.029*
Family history of SCD, n (%)	23 (30%)	13 (34.21%)	10 (25.64%)	0.57
History of hypertension, n (%)	6 (8%)	2 (5.26%)	4 (10.26%)	0.70
MLVWT in mm, mean ± SD	15.1 ± 7.7	17.4 ± 8.7	12.9 ± 5.8	0.010*
MLVWT z-score, mean ± SD	4.07 ± 2.33	4.91 ± 2.45	3.24 ± 1.91	0.0013*
MWTH:PWTH, mean ± SD	1.70 ± 0.97	1.93 ± 0.97	1.48 ± 0.93	0.042*
Septal morphology, n (%)				Overall p value =0.44
Sigmoid	27 (35.06%)	11 (28.95%)	16 (41.03%)	
Reverse curve	17 (22.08%)	10 (26.32%)	7 (17.95%)	
Neutral	23 (29.87%)	13 (34.21%)	10 (25.64%)	
Apical	1 (1.30%)	1 (2.63%)	0 (0%)	
Other	9 (11.69%)	3 (7.90%)	6 (15.38%)	
Morphology category I, n (%)	40 (51.95%)	23 (60.53%)	17 (43.59%)	0.17
HCM = hypertrophic cardiomyopathy; SCD = sudden cardiac death; MLVWT = maximum left ventricular wall thickness; MWTH = maximal wall thickness; PWTH = posterior wall thickness; Morphology category I = neutral and reverse curve septal morphologies				

Table 4. Distribution of Pathogenic Variants by Gene

Gene	Yield among total study population, n=77 n (%)	Yield among patients with a positive HCM panel, n=38 (%)
ACTC1	2 (3%)	2 (5%)
MYBPC3	16 (21%)	16 (42%)
MYH7	10 (13%)	10 (26%)
PRKAG2	1 (1%)	1 (3%)
TNNT2	1 (1%)	1 (3%)
TPM1	4 (5%)	4 (11%)
Multiple (MYBPC3 plus another pathogenic variant)	4 (5%)	4 (11%)

Figure 1. Optimal MLVWT z-score for Mayo HCM Genotype Predictor

The cutoff value for MLVWT z-score for a positive genetic test result was determined by Receiver Operating Characteristic analysis. AUC = area under the curve.

Figure 2. Predicted (closed circle) versus observed (bars) positive genotypes at each score for (A) Toronto HCM Genotype Score: Long Model; (B) Toronto HCM Genotype Score: Tertiles Long Model; (C) Mayo HCM Genotype Predictor (mm); (D) Mayo HCM Genotype Predictor (z-score).

Figure 3. Receiver operating characteristic (ROC) curve and area under the curve (AUC) for Toronto HCM Genotype Score and Mayo HCM Genotype Predictor (mm, z-score). The grey line is the reference line.

Appendix A. Variants deemed pathogenic in our study population.

Gene	cDNA Level	Amino Acid Level	Variant Type	Quantity
ACTC1	c.694G>A	p.Ala232Thr	Missense	1
ACTC1	c.28C>A	p.Leu10Met	Missense	1
MYBPC3	c.1504C>T	p.Arg502Trp	Missense	5
MYBPC3	c.1343T>C	p.Phe448Ser	Missense	1
MYBPC3	c.1294G>A	p.Ala432Thr	Missense	1
MYBPC3	c.3811C>T	p.Arg1271Stop	Nonsense	1
MYBPC3	c.2373dupG	p.Trp792fs	Frameshift	1
MYBPC3	c.1624G>C	p.Glu542Gln	Missense	1
MYBPC3	c.3330+2T>G	-	Splice donor variant	2
MYBPC3	c.2374T>C	p.Trp792Arg	Missense	1
MYBPC3	c.1513_1515delAAG	p.Lys505del	Deletion	1
MYBPC3	c.3323A>C	p.Lys1108Thr	Missense	1
MYBPC3	c.2524G>A	p.Trp818Stop	Missense	1
MYBPC3	c.2441_2443delAGA	p.Lys814del	Deletion	1
MYBPC3	c.961G>A	p.Val321Met	Missense	1
MYBPC3	c.3330+5G>C	-	Intronic variant	1
MYBPC3	c.927-10C>T	-	Intronic variant	1
MYBPC3	c.927-2A>G	-	Intronic variant	1
MYBPC3	c.2308G>A	p.Asp770Asn	Missense	1
MYH7	c.1988G>A	p.Arg663His	Missense	1
MYH7	c.2717A>G	p.Asp906Gly	Missense	1
MYH7	c.2221G>A	p.Gly741Arg	Missense	2
MYH7	c.1208G>A	p.Arg403Gln	Missense	1

MYH7	c.4807G>A	p.Ala1603Thr	Missense	1
MYH7	c.2302G>A	p.Gly768Arg	Missense	2
MYH7	c.1207C>T	p.Arg403Trp	Missense	1
MYH7	c.2146G>A	p.Gly716Arg	Missense	1
MYL3	c.461G>A	p.Arg154His	Missense	1
PRKAG2	c.905G>A	p.Arg302Gln	Missense	1
TNNT2	c.832C>T	p.Arg278Cys	Missense	1
TNNT2	c.487_489delGAG	p.Glu163del	Deletion	1
TPM1	c.574G>A	p.Glu192Lys	Missense	1
TPM1	c.644C>T	p.Ser215Leu	Missense	1
TPM1	c.842T>C	p.Met281Thr	Missense	1
TPM1	c.637A>C	p.Lys213Gln	Missense	1