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ORIGINAL ARTICLE

Characterization of a Phenotype-Based Genetic Test Prediction Score for Unrelated Patients with Hypertrophic Cardiomyopathy

J. Martijn Bos, MD, PhD; Melissa L. Will, BS; Bernard J. Gersh, MB, ChB, DPhil; Teresa M. Kruisselbrink, MS, CGC; Steve R. Ommen, MD; and Michael J. Ackerman, MD, PhD

Abstract

Objectives: To determine the prevalence and spectrum of mutations and genotype-phenotype relationships in the largest hypertrophic cardiomyopathy (HCM) cohort to date and to provide an easy, clinically applicable phenotype-derived score that provides a pretest probability for a positive HCM genetic test result.

Patients and Methods: Between April 1, 1997, and February 1, 2007, 1053 unrelated patients with the clinical diagnosis of HCM (60% male; mean ± SD age at diagnosis, 44.4±19 years) had HCM genetic testing for the 9 HCM-associated myofilament genes. Phenotyping was performed by review of electronic medical records.

Results: Overall, 359 patients (34%) were genotype positive for a putative HCM-associated mutation in 1 or more HCM-associated genes. Univariate and multivariate analyses identified the echocardiographic reverse curve morphological subtype, an age at diagnosis younger than 45 years, a maximum left ventricular wall thickness of 20 mm or greater, a family history of HCM, and a family history of sudden cardiac death as positive predictors of positive genetic test results, whereas hypertension was a negative predictor. A score, based on the number of predictors of a positive genetic test result, predicted a positive genetic test result ranging from 6% when only hypertension was present to 80% when all 5 positive predictor markers were present.

Conclusion: In this largest HCM cohort published to date, the overall yield of genetic testing was 34%. Although all the patients were diagnosed clinically as having HCM, the presence or absence of 6 simple clinical/echocardiographic markers predicted the likelihood of mutation-positive HCM. Phenotype-guided genetic testing using the Mayo HCM Genotype Predictor score provides an easy tool for an effective genetic counseling session.

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Diagnosed as unexplained cardiac hypertrophy in the absence of inciting factors, such as uncontrolled hypertension and aortic stenosis, hypertrophic cardiomyopathy (HCM) is the most common heritable cardiovascular disease, with an estimated prevalence of 1 in 500 individuals.[1] Hypertrophic cardiomyopathy is the most frequent identifiable cause of sudden cardiac death (SCD) in young athletes and clinically has a heterogeneous presentation with varying degrees of hypertrophy, left ventricular outflow tract obstruction, ventricular septal morphology, and symptoms such as syncope and dyspnea.[2,3]

This phenotypic heterogeneity is matched by the marked heterogeneity in the underlying genotype. Currently, mutations in 9 genes that encode various cardiac myofilaments have been associated in the pathogenesis of sarcomeric/myofilament HCM, with most mutations being unique, individual variants. Clinical genetic testing (either institutionally provided or commercially available) is being used increasingly in the evaluation of patients with HCM and their family members.[4] Although specific genotype-based treatments for HCM are not yet available, a positive genetic test result confirms the etiology of the disease, potentially guides timing for the next follow-up evaluation, and enables mutation-specific confirmatory testing of the appropriate family members in accordance with the recent Heart Rhythm Society/European Heart Rhythm Association and American College of Cardiology Foundation/American Heart

From the Department of Molecular Pharmacology and Experimental Therapeutics, Windland Smith Rice Sudden Death Genomics Laboratory (J.M.B., M.L.W., M.J.A.), Division of Cardiovascular Diseases (B.J.G., S.R.O., M.J.A.), Division of Laboratory Genetics (T.M.K.), and Division of Pediatric Cardiology (M.J.A.), Mayo Clinic, Rochester, MN.

Association guidelines.[4,5] Furthermore, a genetic test result might lead to decreased frequency or even total discharge of clinical screening for genotype-negative, phenotype-negative relatives when an index case has a positive genetic test result, resulting in a significant decrease in screening-associated costs for the families and their insurance providers.

Studies during the past 2 decades have provided valuable insights on the yield of genetic testing, genotype-phenotype relationships, and have described clinical tools to aid cardiologists and genetic counselors about whether to order genetic tests. However, most of these studies have been limited by a small cohort size, early genetic data, and the absence of a full spectrum of variants in large populations of controls.[6-13] Herein, we present the prevalence of myofilament mutations and genotype-phenotype relationships in the largest cohort of unrelated patients with clinically diagnosed HCM tested to date. Furthermore, in this era of individualized medicine, we provide an easy-to-use, clinically applicable score to determine the likelihood of a positive genetic test result for patients presenting with HCM.

METHODS

Cohort

Between April 1, 1997, and February 1, 2007, 1053 unrelated patients diagnosed as having HCM were referred to Mayo Clinic for clinical evaluation and after obtaining written informed consent were enrolled in this study, which was approved by the Mayo Clinic Institutional Review Board. All the patients were evaluated by cardiologists who are HCM specialists and were diagnosed clinically as having HCM based on the presence of unexplained cardiac hypertrophy with maximum left ventricular wall thickness (MLVWT) of 13 mm or greater on echocardiography. Clinical data, such as age at diagnosis, symptoms, family history of HCM or SCD, and HCM-related interventions, were collected by review of the electronic medical record and were stored in a database blinded to the genotype. Echocardiographic septal contour was assessed as previously described and was categorized as reverse curve, sigmoid, apical, or neutral contour.[12] A common clinical finding, some patients in this cohort also had concomitant mild hypertension or a reported history of (previously untreated) hypertension. If present in the electronic medical record as a formal diagnosis, this was noted for these patients in the database. However, in these cases, the diagnosis of HCM was believed to be the appropriate diagnosis by experienced HCM specialists because the severity and extent of hypertrophy was out of proportion to the mild degree of concomitant hypertension.

Genetic Analyses

DNA was extracted from peripheral blood lymphocytes using the Purgene DNA extraction kit (Gentra Inc) and was stored at 4°C before genetic analysis. All the patients underwent comprehensive genetic testing for mutations in the 9 HCM-associated myofilament/sarcomeric genes (*ACTC1*-encoded cardiac actin, *MYBPC3*-encoded cardiac myosin binding protein C [MYBPC3], *MYH7*-encoded beta-myosin heavy chain [MYH7], *MYL2*-encoded regulatory myosin light chain, *MYL3*-encoded essential myosin light chain, *TNNC1*-encoded troponin C, *TNNI3*-encoded troponin I, *TNNT2*-encoded troponin T, and *TPM1*-encoded alpha tropomyosin. Genetic testing was performed using polymerase chain reaction (PCR), denaturing high-performance liquid chromatography (DHPLC) (WAVE; Transgenomic Inc), and direct DNA sequencing as described previously.[10,14] The reported mutation detection sensitivity of DHPLC is approximately 95%.[15-17] In short, each translated exon of the 9 myofilament genes was amplified by PCR, after which each amplicon was subjected to DHPLC. Abnormal DHPLC profiles were further processed and subjected to direct DNA sequencing to identify the nature of the nucleotide and possible amino acid substitution. The PCR and DHPLC methods and temperatures are available on request. Genetic variants predicted to alter the protein, such as missense mutations, in-frame and frameshift insertion/deletion mutations, canonical splice sites (±1-4), and nonsense mutations resulting in a premature truncation, were identified. The patient-identified variants were checked for their presence in (1) an internal panel of reference alleles derived from 200 ostensibly healthy controls and (2) more than 8000 publicly available exomes from the National Heart, Lung, and Blood Institute Exome Sequencing Project and the 1000 Genomes Project. Variants were considered HCM associated if they (1) were absent in all 8400