Genetics inMedicine | ORIGINAL RESEARCH ARTICLE

Results of clinical genetic testing of 2,912 probands with hypertrophic cardiomyopathy: expanded panels offer limited additional sensitivity

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Purpose: Hypertrophic cardiomyopathy (HCM) is caused primarily by pathogenic variants in genes encoding sarcomere proteins. We report genetic testing results for HCM in 2,912 unrelated individuals with nonsyndromic presentations from a broad referral population over 10 years.

Methods: Genetic testing was performed by Sanger sequencing for 10 genes from 2004 to 2007, by HCM CardioChip for 11 genes from 2007 to 2011 and by next-generation sequencing for 18, 46, or 51 genes from 2011 onward.

Results: The detection rate is ~32% among unselected probands, with inconclusive results in an additional 15%. Detection rates were not significantly different between adult and pediatric probands but were higher in females compared with males. An expanded gene panel encompassing more than 50 genes identified only a very small number of additional pathogenic variants beyond those identifiable in our original panels, which examined 11 genes. Familial genetic testing in at-risk family members eliminated the need for longitudinal cardiac evaluations in 691 individuals. Based on the projected costs derived from Medicare fee schedules for the recommended clinical evaluations of HCM family members by the American College of Cardiology Foundation/American Heart Association, our data indicate that genetic testing resulted in a minimum cost savings of about $0.7 million.

Conclusion: Clinical HCM genetic testing provides a definitive molecular diagnosis for many patients and provides cost savings to families. Expanded gene panels have not substantively increased the clinical sensitivity of HCM testing, suggesting major additional causes of HCM still remain to be identified.

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Key Words: cardiomyopathy; genes; genetics; genetics testing; hypertrophy

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is a heterogeneous genetic disorder with variable expressivity and incomplete penetrance that affects up to 1 in 500 individuals in the United States.[1,2] Clinical diagnosis of HCM is predicated on finding unexplained left ventricular hypertrophy; myofiber disarray and myocardial fibrosis are characteristic histological features. Dominant pathogenic variants in HCM genes (*ACTC1*, *MYBPC3*, *MYH7*, *MYL2*, *MYL3*, *TNNI3*, *TNNT2*, and *TPM1*) encoding sarcomere proteins that form the contractile apparatus of the heart are the most common cause of HCM. Additional genes, including *ACTN2*, *CSRP3*, *MYOZ2*, *NEXN*, *PLN*, *TNNC1*, and *TTR*, have been implicated but not definitively proven as causing disease.[3] Pathogenic variants in other genes (*GLA*, *LAMP2*, and *PRKAG2*) also cause left ventricular hypertrophy but result in metabolic or infiltrative disorders, distinct in their origin from HCM. Distinguishing these disorders is clinically important because enzyme replacement therapy can be considered to treat Fabry disease (*GLA* variants),[4–6] Danon disease caused by *LAMP2* variants is often rapidly progressive and fatal in young affected males,[7] and pacemakers are typically needed to treat the conduction disease associated with *PRKAG2* variants.[8]

American College of Cardiology Foundation/American Heart Association practice guidelines include genetic testing as a reasonable approach to the diagnosis of HCM.[9] The Clinical Laboratory Improvement Amendments–certified Laboratory for Molecular Medicine was the first laboratory to offer clinical HCM genetic testing, beginning in 2004. As technology has evolved from Sanger sequencing to array-based hybridization sequencing, and, recently, to next-generation sequencing (NGS), each strategy has been harnessed to sequence HCM genes.[3] Here we describe the results of gene-based diagnosis of HCM in unselected, prospective

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cases referred between 2004 and 2013. This study encompasses 2,912 unrelated probands and familial variant testing in 1,209 family members.

MATERIALS AND METHODS

Study population

All studies were performed under a waived-consent, institutional review board–approved protocol. Phenotypes and family histories that prompted HCM genetic testing, as well as demographic data (race, ethnicity, and country of origin), were obtained from the requisition form (**Supplementary Figure S2** online), which is normally completed by the referring health-care providers. The accuracy of these data is not comparable to the direct capture of primary clinical data but does represent reasonable capture of information to analyze clinical testing detection rates in various HCM demographic subpopulations typically referred for genetic testing. Among 3,459 probands initially referred for HCM genetic testing, we excluded individuals with left ventricular hypertrophy in the context of a clinical syndrome recognized before testing, such as Danon or Fabry disease,[9] and unaffected individuals with only a family history (**Figure 1**; **Supplementary Table S1** online).

Genetic testing

Between 2004 and 2007, genetic testing was performed by polymerase chain reaction amplification of amplicons and Sanger dideoxy sequencing of a five-gene panel (*MYBPC3*, *MYH7*, *TNNI3*, *TNNT2*, and *TPM1*) that reflexed to an additional three-gene panel (*ACTC1*, *MYL2*, and *MYL3*) when the initial analyses were negative. In a subset of probands, two additional genes (*PRKAG2* and *LAMP2*) were sequenced. Between 2007 and July 2011 genetic testing was performed using the HCM CardioChip,[10,11] which included *GLA* in addition to all 10 genes listed above. Although a slightly reduced sensitivity for variant detection may have occurred while using the CardioChip technology, this was primarily abrogated by the continued use of parallel Sanger sequencing of the *MYBPC3* gene for all CardioChip cases, given the increased rate of insertions/deletions in this gene, which are known to be poorly detected.[12] Beginning in July 2011, genetic testing used NGS with either an HCM panel (18 genes, including the 11 mentioned above plus *ACTN2*, *CSRP3*, *MYOZ2*, *NEXN*, *PLN*, *TNNC1*, and *TTR*) or the Pan Cardiomyopathy Panel,[12] which initially encompassed 46 genes and subsequently 51 genes that cause or are implicated in HCM and other cardiomyopathies (dilated, arrhythmogenic, left ventricular noncompaction, and restrictive). For all tests described, all genes were sequenced to completion regardless of the identification of a variant in any one gene. Familial testing was performed by polymerase chain reaction amplification and Sanger sequencing of the relevant amplicon. All methods analyzed variants found in the coding regions and flanking intron boundaries ±12–15 bp. Other noncoding variants were generally not detected, except for the common East Asian deletion in intron 32 of *MYBPC3* (c.3628-41_3628-17del) and the cryptic splice-site variant in intron 4 of *GLA* (c.640-801G>A, also known as c.639+919G>A).

Variant assessment

Variants are reported using Human Genome Variation Society nomenclature guidelines (http://www.hgvs.org/mutnomen) and classified into one of five categories: pathogenic (P), likely pathogenic (LP), variant of uncertain significance (VUS), likely benign (LB), or benign (B) using Laboratory for Molecular

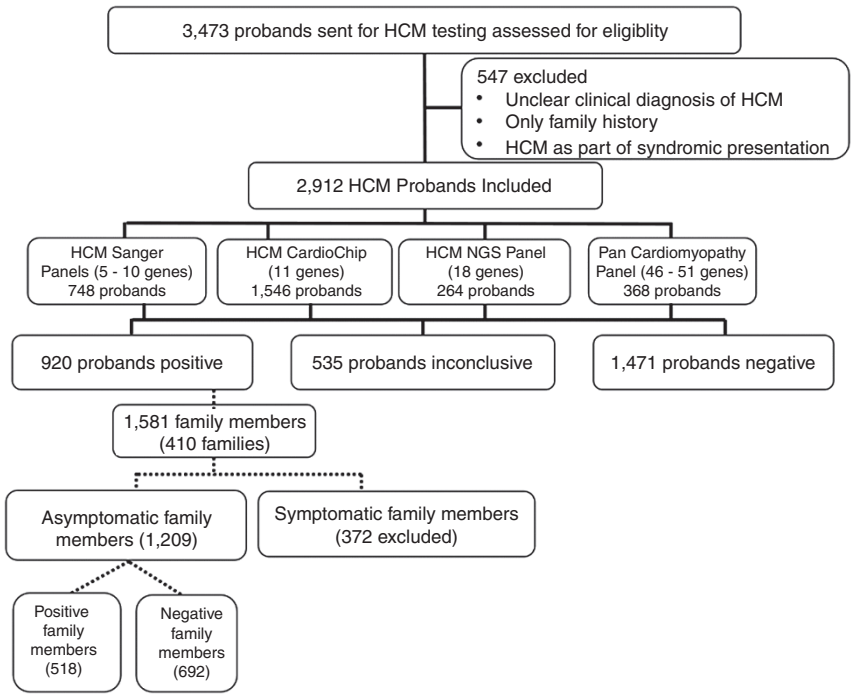

Figure 1 Breakdown of all hypertrophic cardiomyopathy (HCM) cases. NGS, next-generation sequencing.

Medicine classification criteria (**Supplementary Table S2** online). Details of the variant assessment methods and tools used have been described elsewhere.[13,14] Proband test results are reported as positive (≥1 P/LP variant), negative (only LB/B variants), or inconclusive (no P/LP variants and at least one VUS).

Variant database

All variants identified in the Laboratory for Molecular Medicine and reported here are provided in **Supplementary Table S7** online, along with clinical classifications. These were deposited into ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/),[15] including a summary of evidence used for classification, for unrestricted access to the community.

Cost analysis

The cost of familial variant testing is $400 per sample. Costs for clinical assessments were conservatively estimated as $150 (inclusive of cardiologist, electrocardiogram, and echocardiogram) based on Medicare rates. This is probably a substantial underestimate of costs compared with those of private insurers, but we were unable to get data from other sources. Cost of lifetime clinical screening was estimated for individuals grouped by their ages at the time of genetic testing (<12, 12–20, 21–50, 50–75, and >75 years old; **Figure 1**) and the expected total number of evaluations based on American College of Cardiology Foundation/American Heart Association clinical screening guidelines[9] until age 75 years. For example, at-risk family members younger than 12 years old were assigned 20 clinical visits (1 visit per year from 12 to 20 years and 1 visit every 5 years from age 21 to 75 years averaged across age groups), whereas individuals aged 50–75 years were assigned 3 visits. No clinical screens were assumed for individuals older than 75 years.

RESULTS

Variants and genes

Among 2,912 probands referred for HCM genetic testing, all were sequenced for *MYBPC3*, *MYH7*, *TNNI3*, *TNNT2*, and *TPM1*. In addition, 69% were also sequenced for three other genes (*ACTC1*, *MYL2*, and *MYL3*) encoding sarcomere proteins

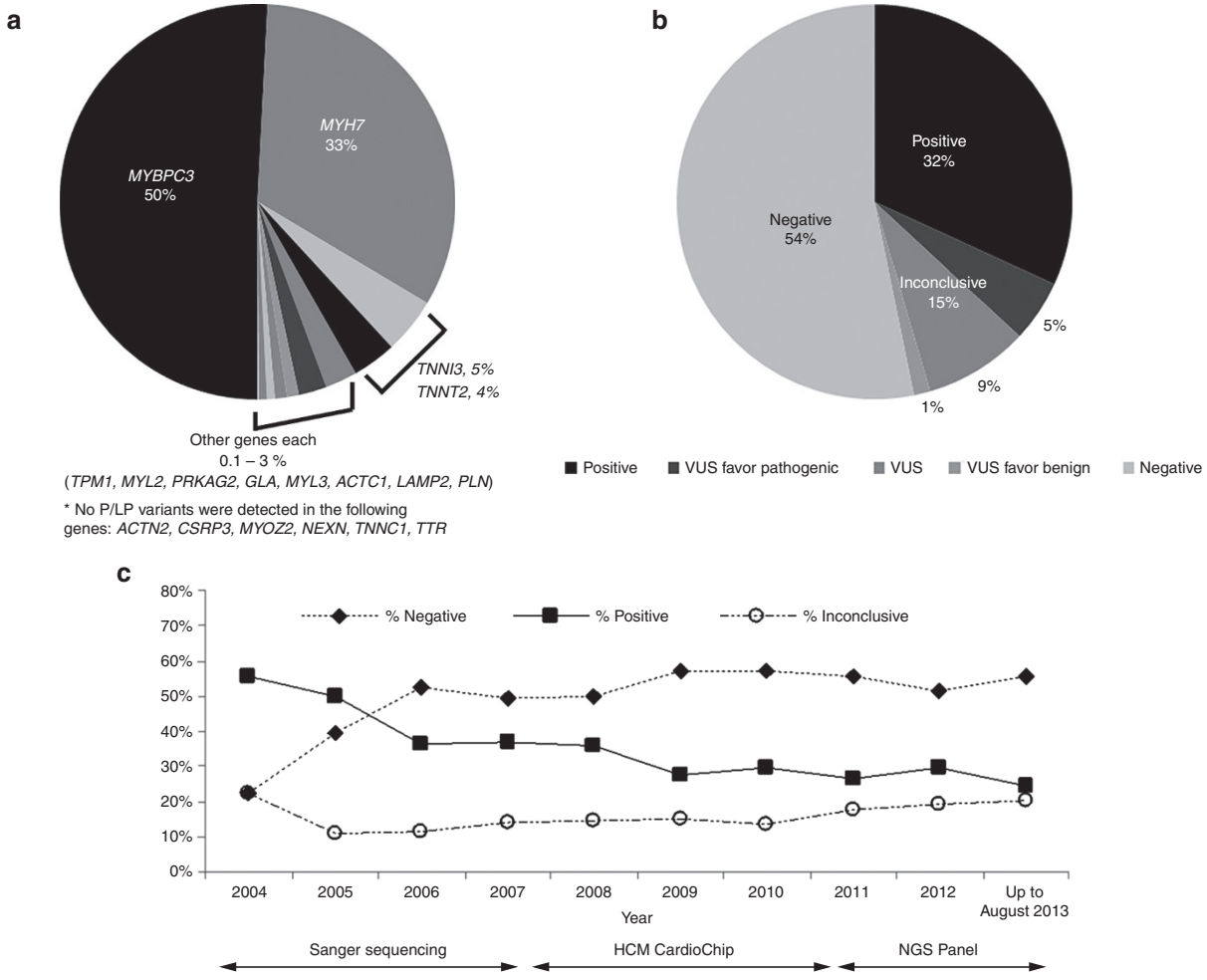

Figure 2 Hypertrophic cardiomyopathy (HCM) detection rates and gene contribution. (**a**) Breakdown of the relative contribution of each gene to all positive cases. For this analysis, only variants classified as pathogenic and likely pathogenic were included. (**b**) Detection rate for the entire HCM cohort broken down by overall result. (**c**) Detection rates stratified by year of testing. Detection rates dropped from 38 to 30% and then to 28% over a 9-year period (2004–2013), likely reflecting an evolving referral pattern. NGS, next-generation sequencing; VUS, variant of uncertain significance.

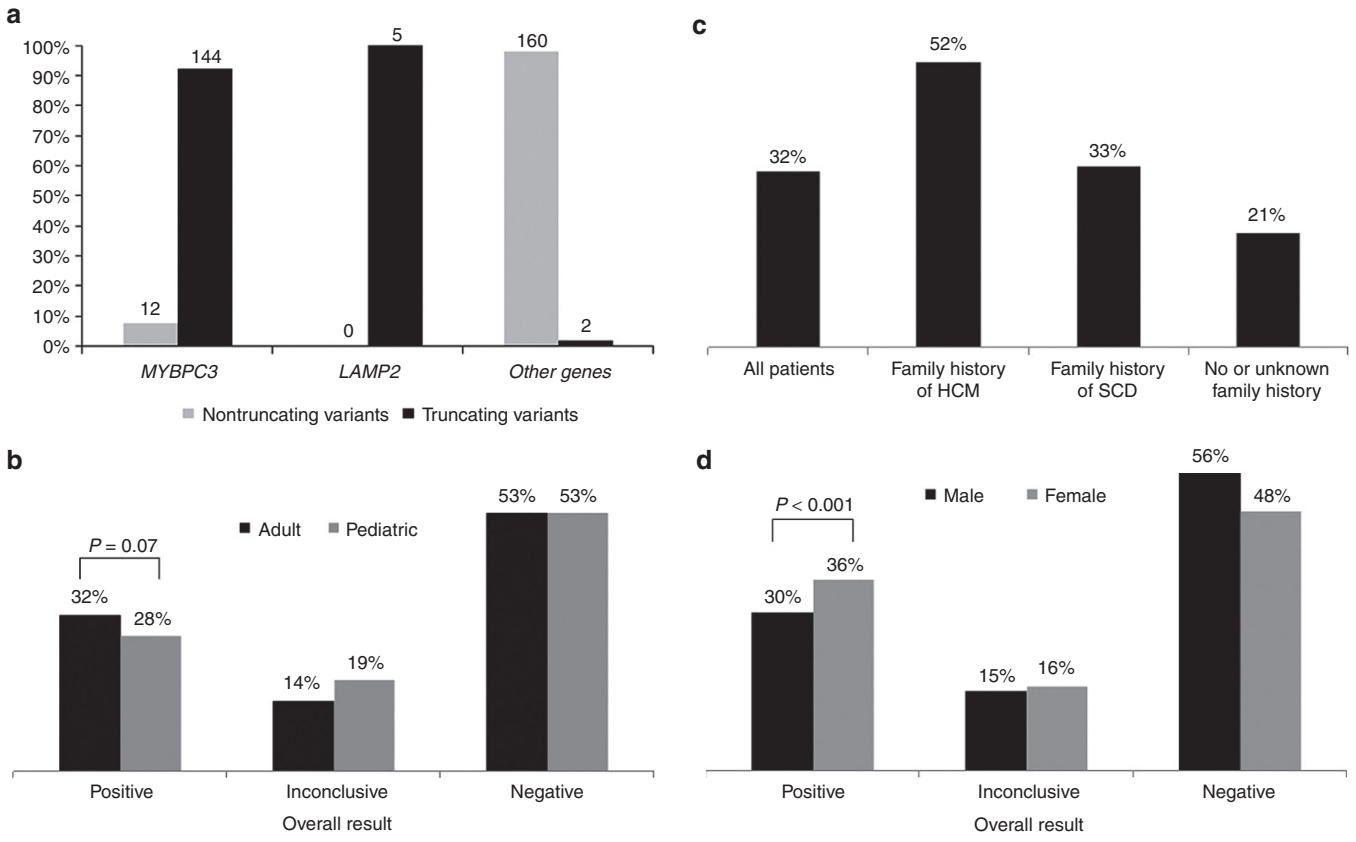

Figure 3 Result stratificatiion by variant type and demographics. (**a**) Comparison of the *MYBPC3* gene versus *LAMP2* versus all other contributing genes (*ACTC1*, *ACTN2*, *CSRP3*, *GLA*, *MYH7*, *MYL2*, *MYL3*, *MYOZ2*, *NEXN*, *PLN*, *PRKAG2*, *TINNI3*, *TNNC1*, *TNNT2*, *TPM1*, and *TTR*) in terms of the types of pathogenic and likely pathogenic variants identified in probands. Nontruncating variants include missense variants (amino acid substitutions) and in-frame amino acid insertions and deletions. Truncating variants include nonsense, frameshift, and canonical splice variants (±1,2). (**b**) Distribution of positive, negative, and inconclusive results in adult and pediatric age groups. Thirty-seven cases were excluded because of unavailable age. (**c**) Breakdown of detection rates based on reported family history. (**d**) Distribution of positive, negative, and inconclusive results in males as compared with females. Sixty-one cases were excluded because of unavailable sex. *P* values for all comparisons are noted in **Supplementary Table S1** online. HCM, Hypertrophic cardiomyopathy; SCD, sudden cardiac death.

and 22% were sequenced for both sets above plus 10 additional HCM genes (*ACTN2*, *CSRP3*, *GLA*, *LAMP2*, *MYOZ2*, *NEXN*, *PLN*, *PRKAG2*, *TNNC1*, and *TTR*). The remaining 9% were sequenced for only five genes encoding sarcomere proteins (excluding *ACTC1*, *MYL2*, and *MYL3*) because a pathogenic variant was identified from the initial five-gene panel (**Figure 1**).

We classified the 972 variants identified in the proband cohort as P (n = 194), LP (n = 129), VUS (n = 411), LB (n = 145), or B (n = 93). Among all variants, 56% were not previously reported or listed in online variant databases; these represented 45% of the P/LP, 65% of the VUS, and 56% of the LB/B categories. All variants annotated for pathogenic clinical classification and numbers of times identified are provided in **Supplementary Table S7** online and are available in ClinVar.

The majority of positive test results were due to P/LP variants in the *MYBPC3* and *MYH7* genes (83%), with the other genes accounting for the remaining 17% (**Figure 2a**, **Supplementary Table S3** online). To consider whether gene size affected relative gene contribution, we examined variation per kilobase of DNA coding sequence across all 18 HCM genes. We also examined background variation in each gene using the National Heart, Lung, and Blood Institute Exome Sequencing Project data (http://evs.gs.washington.edu/EVS/; data downloaded in August 2013). After correcting for gene size, *MYBPC3* remained the largest source of P/LP variants per kilobase, despite reasonably similar background variation among the genes examined (**Supplementary Table S4** online).

The majority (53%) of P/LP variants across all HCM genes are not predicted to truncate the encoded protein. However, the types of variants that contributed to positive genetic test results differed for each gene. Truncating variants (e.g., nonsense, frameshift, ±1,2 splice) were found almost exclusively in *MYBPC3* and *LAMP2* and were rarely found in other genes (**Figure 3a**), as has been previously recognized.[16]

Variant detection rates

Genetic testing (**Figure 2b**) defined a P or LP variant in 917 probands (32%), was negative in 1,551 probands (53%), and was inconclusive (VUS identified) in 444 probands (15%). In recent years our laboratory began subclassifying VUSs into those that "favor a pathogenic role" and those that "favor a benign role," with the remaining being equivocal (see **Supplementary Table**

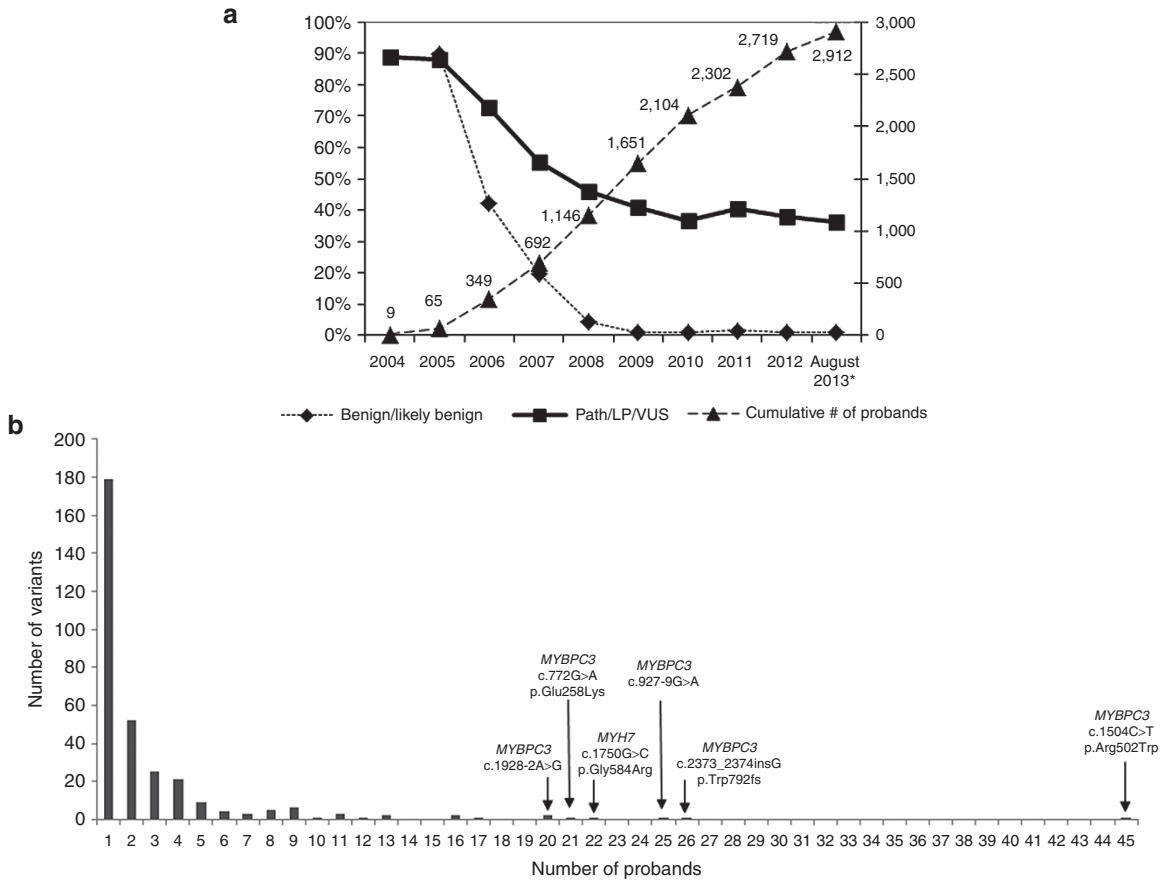

Figure 4 Novel and repeatedly observed variants. (**a**) Percentage of novel variants from among total variants identified in each year and divided by variant class. *Data from 2013 represent only cases collected until August 2013. (**b**) Histogram plot of all pathogenic and likely pathogenic variants distributed by the number of probands with each variant. For example, the p.Arg502Trp variant in *MYBPC3* was observed in 45 probands, whereas 179 variants were observed in only 1 proband.

S2 online for subclassification rules). Subclassified VUSs were present in 190/444 of inconclusive cases; 150 were a VUS favoring a pathogenic role versus 40 that favored a benign role in the 18 tested HCM genes. The remaining 254 were not subclassified or were classified as equivocal.

More positive genetic tests were found in adult probands (32%) than in pediatric probands (age <16 years; 28%; P = 0.07; Figure 3b; **Supplementary Table S1** online). Among the positive pediatric age group, 20% (26/129) were younger than 2 years at the time of testing. Among adults, the average age of probands with a negative test result (44 years) was higher than that of those with a positive test result (39 years; $P < 0.01$).

We considered whether family history, age, sex, or race influenced positive genetic testing results (Figure 3c; **Supplementary Table S1** online). As expected, the highest detection rate of P/LP variants (52%) was observed in probands with a family history of HCM (491/938) or sudden cardiac death (33%, 134/402) compared with 21% of probands who did not have or did not specify these risk factors (343/1,637). Adult female probands with a positive family history of HCM had the highest detection rate (56%). Almost twice as many men (n = 1,837) than women (n = 993) were referred for genetic testing, but the detection rate of P/LP variants was significantly higher in women (36%) than men (30%; $P < 0.01$; Figure 3d; **Supplementary Table S1** online). However, more women (43%) than men (34%) had a family history of HCM ($P < 0.01$), which substantially increases the likelihood of positive genetic test results.

Positive genetic tests were also highest among white probands (34%, 644/1,896) and lowest among probands of Ashkenazi Jewish ancestry (19%, 17/90; **Supplementary Table S1** online). One variant (*MYBPC3* c.3190+1G>A) was reported twice in individuals with Ashkenazi Jewish ancestry, although no common pathogenic variant was found in this population. Detection rates in black and Asian populations were 23% (48/212) and 25% (46/184), respectively.

Probands with multiple variants

Among 2,912 probands, 8% (n = 234) had more than one P/LP variant or VUS: 0.6% (n = 18) had two or more P/LP variants (including homozygous variants and one proband with three P/LP variants), 5% (n = 147) had one P/LP variant plus at least one VUS, and 2.4% (n = 69) had two or more VUSs (**Supplementary Table S5** online). Ten of the 18 probands with two or more P/LP variants had compound heterozygous or homozygous variants in the same gene, including 5 probands with two or more *MYBPC3* variants. Eight of 18 probands had

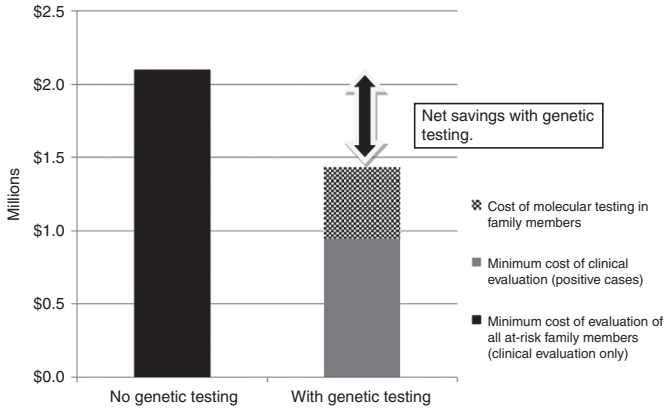

Figure 5 Comparison of genetic testing costs and estimated clinical screening costs when genetic testing is used or not used for determining risk status in unaffected family members. These data are based on actual numbers of unaffected family members tested over a 9-year period. Medical costs are conservatively estimated using the Medicare fee schedule.

two or three heterozygous variants in different genes. One proband with only clinical findings of HCM had P variants in both *MYH7* and *PKP2* that cause arrhythmogenic right-ventricular cardiomyopathy.[17] The average age (±SD) of probands referred for genetic testing tended to be younger among those with two or more P/LP variants (29 ± 3 years) compared with those with only one P/LP variant (39 ± 21 years; $P = 0.29$).

Population-specific variation characteristics

We found no statistically significant difference in the likelihood of finding a P or LP variant or a VUS between the adult (83% of the cohort) and pediatric (16% of the cohort) probands tested. However, the percentage of probands with a VUS as compared with a P/LP variant was higher in pediatric cases (19%) than in adult cases (14%). The larger numbers of VUSs in pediatric cases may reflect the greater proportion of *MYBPC3* missense variants in childhood-onset HCM[16] and the greater complexity in interpreting missense variants as compared with predicted null variants.

Probands with de novo variants

We previously identified de novo variants accounting for 36% of probands with pediatric-onset HCM and no family history of HCM.[16] To assess the frequency of de novo variants in this cohort, we first separated positive probands into pediatric (<16 years), adult, and unknown. We then assumed the variant was inherited in those with a reported family history of HCM or sudden cardiac death (71 of 131 pediatric probands and 472 of 764 adult probands), even if no familial testing was ordered. For those patients with no reported family history (60 pediatric probands and 292 adult probands), we identified pediatric and adult de novo rates of 62.9% (22/35) and 8.0% (4/50) (adults were 16, 20, 35, and 37 years at the time of testing), respectively, in those who had biparental testing (shaded areas of **Supplementary Figure S1** online). These rates do not, however, represent the overall disorder, given the exclusion of cases with family history and the bias in reduced parental testing when a family history is present. Therefore, we applied the observed rates in cases without a family history to the untested 25 pediatric and 242 adult probands without a family history (**Supplementary Figure S1** and **Table S6** online). We then combined the confirmed (22 pediatric, 4 adult) and estimated (16 pediatric, 19 adult) de novo cases divided by the total positive cohort (131 pediatric, 764 adult) to arrive at overall de novo rates of 28.8% in pediatric cases and 3.1% in adult cases. The pediatric rate is reasonably consistent with our 36% pediatric rate previously reported,[16] and the comparatively low adult rate is expected given that adult-onset disease has less impact on reproductive fitness and is therefore more likely to have a family history.

Detection of metabolic cardiomyopathies

Approximately 3% of the positive probands had variants in *GLA*, *LAMP2*, and *PRKAG2*, which cause metabolic cardiomyopathies that mimic HCM. Because we excluded probands with syndromic manifestations (recognized before testing) that occur with pathogenic variants in *LAMP2* (Danon disease) and *GLA* (Fabry disease), these data reflect a minimum contribution by these genes to "unexplained" left-ventricular hypertrophy.

The X-linked *LAMP2* gene was sequenced in 2,451 probands without syndromic presentations, and P/LP variants were identified in 6 (0.2%), including 3 female probands. There were no differences in the ages of male (20, 21, unknown years) and female (14, 23, and 37 years) probands at the time of genetic testing. The X-linked *GLA* gene was sequenced in 2,165 probands, with P/LP variants identified in 9 (0.4%), representing a lower detection rate than the 1% reported by others.[18] *GLA* P/LP variants were identified in four men with an average age of 55 years (51, 53, 54, and 63 years), in four women with an average age of 63 years (48, 52, 72, 80 years), and in a proband of unspecified sex or age. Each of the *GLA* variants had been reported to cause classic Fabry disease, although two of these variants (p.Gly373Ser and c.640-801G>A) had been observed in patients with isolated cardiac manifestations.[19–23] *PRKAG2* was sequenced in 2,438 probands, and P/LP variants were identified in 9 probands (0.4%; 7 male and 2 female); the average age at testing was 11 years.

Evolution of testing

Between 2004 and 2013 we did not observe differences in the race distribution, age range, or sex ratio of probands referred for genetic testing, but we did observe a decreased frequency of family histories of HCM (48–41%). Applying our current stringent variant classification pipeline to all cases, we observed a decrease in the proportion of positive test results over time: 38% between 2004 and 2007, 30% between 2008 and July 2011, and 28% between August 2011 and August 2013 (**Figure 2c**). This shift presumably reflects changes in test ordering practice; testing has likely become more common among those with a less clear diagnosis of HCM, leading to lower detection rates. Alternatively, it may reflect a change in our own laboratory's case spectrum as additional laboratories have begun offering testing. Please see the Discussion for additional commentary.

Incremental yield using comprehensive gene panels

Testing 619 probands using the HCM NGS panel (18 genes) or the Pan Cardiomyopathy Panel (46–51 genes) (**Figure 1**) defined only one additional P or LP variant (in *PLN*) in the six additional HCM genes. Furthermore, when using the full Pan Cardiomyopathy Panel in a subset of the above (n = 202 probands), no probands with a primary causative variant outside the 18 HCM genes were identified; however, one proband was double heterozygous for both HCM-related (*MYH7*) and arrhythmogenic right-ventricular cardiomyopathy–related (*PKP2*) variants.

Private versus common variants

In our first 6 years of testing we observed a gradual reduction in the percentage of novel P/LP variants and VUSs being detected (**Figure 4a**). Over the past 4 years, however, that rate has stabilized at ~35–40%. After deploying preclassifications of variants commonly found in large population databases, only a small number of novel variants, primarily synonymous (silent) variants, can be classified as LB.

Consistent with the high rate of novel variant detection, our analysis shows that 56% (179/323) of variants were observed in only one family and can be considered "private" variants (**Figure 4b**). A total of 44% (142/323) of variants were observed more than once, although only 5% (17/323) appeared 10 times or more. The most common variants in our population were in the *MYBPC3* gene, including p.Arg502Trp,[24] which occurs in a known hotspot, and c.2373_2373insG (p.Trp792fs), a Dutch founder variant,[25] reported in 5% (45/917) and 3% (26/917) of all positive probands, respectively.

Medical cost savings

We conducted familial variant testing on family members of probands with a P/LP variant or a VUS. A subset of this testing was conducted on affected family members to evaluate segregation of the variant with disease, particularly for VUSs and sometimes for LP variants. However, most family members of probands with a P/LP variant were asymptomatic at the time of testing and were tested to determine their risk for disease. Of the 1,209 asymptomatic family members of a positive (P/LP) proband, 691 tested negative for the variant and no longer required cardiac evaluations recommended for high-risk family members (**Figure 1**). By examining the cost of genetic screening for a familial variant ($400) and comparing it with the costs of serial clinical evaluations as recommended by the American College of Cardiology Foundation/American Heart Association,[9] this screening saved an estimated $0.7 million (~$1,000.00/at-risk family member) when the cost of the clinical assessment was estimated at $150 per cardiac visit, based on Medicare rates (**Figure 5**). This is likely a gross underestimate of savings, however, given the large fraction of patients with private insurance. In addition, fewer than two unaffected family members per proband received testing, which is likely an underestimate of those in need of predictive testing, suggesting costs savings would be much higher with thorough familial testing.

DISCUSSION

Analysis of 2,912 probands referred for clinical HCM genetic testing over 9 years identified a known etiology (P or LP variant) in 32% and a possible etiology (VUS) in an additional 15%. Clinical sensitivity for a known or highly likely etiology increased to 52% for patients with a positive family history of HCM. These detection rates are similar or somewhat lower than those found by prior studies of large HCM populations but are lower than detection rates from research genetic testing,[26] in part because of several factors. First, broader indications for clinical genetic testing have been adopted in recent years, including cases without a definitive diagnosis of HCM. For example, increased physician awareness of the manifestations of HCM and widespread, noninvasive cardiac imaging with extraordinary sensitivity to detect subtle degrees of hypertrophy may have lowered the threshold for diagnosing HCM and increased genetic testing. Second, the standard required by the Laboratory for Molecular Medicine for evidence of pathogenicity is high, including significant segregation data and/or functional data to classify variants as P or LP. Consistent with this, we observed fewer cases with multiple pathogenic variants compared to previous reports.[27–29] Third, the recent availability of exome and targeted resequencing data from large population cohorts has improved knowledge of the allelic variation that is clinically tolerated within HCM genes. For example, after we applied stricter evidence requirements for pathogenicity and incorporated the recently available population data (e.g., Exome Sequencing Project, 1000 Genomes Project), 68 variants changed from LP to VUS and 1 changed from LP to LB, whereas only 9 variants changed from VUS to LP. In addition, ~57 variants previously classified as VUSs were reclassified as LB, often based on newly available population frequency data. Earlier data on HCM variant reclassification have previously been reported.[30]

Notably, the application of NGS enabled simultaneous analyses of at least 18 HCM genes in more than 600 patients studied after 2011. However, broadening genetic testing to more comprehensive panels identified only a very small number of additional pathogenic variants beyond those identifiable by prior non-NGS tests. Indeed, only one proband had an additional pathogenic variant identified in a nonsarcomere gene included in the broader panel testing. As such, we suggest that current use of the extended cardiomyopathy gene panel is not useful for most patients with HCM and should be reserved for patients with atypical clinical phenotypes. A similar recommendation could be made for the anticipated increasing use of exome and genome sequencing approaches. In these cases initial data analysis could be confined to these same genes with reflex to the whole genomic data set only when health-care providers are trained to deal with the complexity of uncertain gene and variant findings that result from genomic testing.

The rate of novel variant detection throughout 9 years of clinical HCM genetic testing remained at 35–40%, with 56% of variants found uniquely in a single family. This observation implies that many pathogenic HCM variants arose recently under negative selective pressure. This is also consistent with

the high de novo variant rate of 28.8% in pediatric cases. These issues also underscore the complexity of genetic diagnosis; in addition to requiring sophisticated interpretive skills, family studies are critical during the first identification of each variant because it may never be seen again. Such diligence should reduce the percentage of inconclusive genetic testing results.

HCM testing enabled a large fraction of families to receive a precise diagnosis, including appropriate classification of 0.4% of the cohort with unrecognized Fabry disease who may benefit from enzyme replacement therapy. Gene-based diagnosis also accurately identifies preclinical carriers who benefit from appropriate clinical surveillance. At-risk relatives who test negative for the family's pathogenic variant no longer need routine echocardiographic and electrocardiographic screening, saving significant health-care costs and reducing concern for adverse outcomes. Using recommendations from the American College of Cardiology Foundation/American Heart Association and an estimated cost of $150 per clinical visit (based on current Medicare reimbursement rates), we projected ~$0.7 million total health-care cost savings for the families tested in our laboratory alone. Although a rigorous cost-effectiveness analysis is outside the scope of this study, our findings are consistent with two prior cost-effectiveness studies examining the clinical and economic utility of genetic testing for HCM.[31,32]

The inability to identify a genetic etiology in a substantial proportion of patients with HCM referred for clinical testing raises several important questions. Do variant-negative patients without family histories have HCM because of an undiscovered genetic or possibly somatic variant, or do they have an acquired cardiomyopathy? Were some variants missed in patients with HCM, such as pathogenic copy-number or regulatory variants in previously identified HCM genes? Do variant-negative patients with positive family histories have dominant or recessive variants in unidentified HCM genes? Or is family history in HCM an inaccurate indicator of genetic disease? Should HCM management guidelines be the same for patients with and without pathogenic variants? Discovering the answers to these questions is critical to enabling the full potential of genetic testing to be realized in diagnosing and treating HCM.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

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DISCLOSURE

Several authors (M.A.K., B.H.F., M.S.L., J.S., H.M.M., and H.L.R.) are employed by the Laboratory for Molecular Medicine, a nonprofit fee-for-service clinical laboratory performing cardiomyopathy testing. C.E.S. and J.G.S. are founders and owns shares in Myokardia Inc., a startup company that is developing therapeutics that target the sarcomere. The other authors declare no conflict of interest.

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