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Clinical and laboratory reporting impact of ACMG-AMP and modified ClinGen variant classification frameworks in *MYH7*-related cardiomyopathy

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PURPOSE: ClinGen provides gene-specific guidance for interpretation of sequence variants in *MYH7*. We assessed laboratory and clinical impact of reclassification by the American College of Medical Genetics and Genomics-Association for Molecular Pathology (ACMG-AMP) and ClinGen recommendations in 43 *MYH7* variants reported by a diagnostic laboratory between 2013 and 2017.
METHODS: Fifty-two proband reports containing *MYH7* variants were reinterpreted by original ACMG-AMP and ClinGen guidelines. Evidence items were compared across schemes and reasons for classification differences recorded. Laboratory impact was assessed by number of recommended report reissues, and reclassifications coded as clinically "actionable" or "equivalent." Available pedigrees were reviewed to describe projected cascade impact.
RESULTS: ClinGen produced a higher proportion of diagnostic classifications (65% of variants) compared with ACMG-AMP (54%) and fewer variants of uncertain significance (30% versus 42%). ClinGen classification resulted in actionable changes in 18% of variants with equal upgrades and downgrades from original report. ClinGen's revisions to PM1 and PS4 contributed to classification differences in 21% and 19% of variants respectively. Each classification change per proband report impacted, on average, 3.1 cascade reports with a further 6.3 first- and second-degree relatives potentially available for genotyping per family.
CONCLUSION: ClinGen's gene-specific criteria provide expert-informed guidance for interpretation of *MYH7* sequence variants. Periodic re-evaluation improves diagnostic confidence and should be considered by clinical and laboratory teams.

INTRODUCTION

Consistent and accurate interpretation of sequence variants is essential for effective clinical care for individuals with inherited cardiomyopathy and their families. Hypertrophic cardiomyopathy (HCM) is common and demonstrates considerable clinical and genetic heterogeneity[1,2], with presentations ranging from mild or asymptomatic disease to sudden cardiac death in the young. Pathogenic (P) or likely pathogenic (LP) variants in *MYH7* are identified in approximately a third of individuals with HCM[3,4], with rare or novel variants accounting for almost half of these[5]. Accurate and timely molecular characterization permits diagnostic security and may guide risk stratification and management[6].

Widespread utilization of next-generation sequencing (NGS) in HCM has uncovered substantial genetic variation and highlighted the importance of a standardized approach to variant interpretation. Determination of pathogenicity is a complex process involving distillation of various pieces of information from multiple sources that may change over time and are subject to variable interpretation and application. Laboratories and medical geneticists must be adaptive and implement strategies to stay abreast of new information as it becomes available and modify reporting practices to ensure clinicians and patients have up-to-date and clinically relevant information.

Revised consensus recommendations by the American College of Medical Genetics and Genomics-Association for Molecular Pathology (ACMG-AMP)[7] provide a framework for clinical interpretation of sequence variants that is now widely utilized[8]. Prior to implementation of these guidelines laboratories frequently employed bespoke or ad hoc classification practices that evolved over time and were inconsistent between laboratories. Additionally, availability of robust population data (Exome Aggregation Consortium[9] and Genome Aggregation Database)[10,11] has permitted improved discrimination between disease-causing variants and benign variation.

The 2015 ACMG-AMP guidelines were designed to be widely applicable, but several elements lack specificity[12,13], are applied discordantly,[14,15] and do not take into account disease-specific mechanisms. The Clinical Genome Resource (ClinGen)[16] aims to leverage expert knowledge of genetic variation and molecular underpinnings in specific disease groups to develop guidelines that take into account gene-specific information. In 2017, ClinGen's Inherited Cardiomyopathy Expert Panel published modified guidelines for interpretation of sequence variants in *MYH7*-associated HCM[17]. These recommendations provided increased specificity, clarified interpretation of particular ACMG-AMP evidence items, and resulted in expert curation and submission of 60 *MYH7* variants to the ClinVar database[18]. The pilot recommendations also provided impetus for similar guidelines in other disorders[19–21] (including recent refinement for dilated cardiomyopathy[22]) and further modification using phenotype-enhanced criteria[23]. There has been limited independent evaluation of the ClinGen *MYH7*-specific guidelines, although a recent study demonstrated a nonsignificant trend

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toward decreased variant of uncertain significance (VUS) burden in two HCM cohorts[23].

Clinical reporting of variants is probabilistic; the inherent uncertainty in test results has clinical and psychoemotional implications for the individual and the treating clinician. In particular, VUS have limited clinical utility and may result in confusion and anxiety for individuals and families[24,25]. Equally, misattribution of causation has the potential to impact negatively on clinical care or result in unnecessary investigations for unaffected family members. Confidence in genetic test results is influenced by recency of reporting and information available at the time of the report. Consequently, a gradient of confidence exists that brings into question many variants classified prior to endorsement of the ACMG-AMP guidelines and availability of population databases. Resultant uncertainty in assignment of pathogenicity in older reports furthermore complicates interpretation of current test results, which necessarily take into account previous classifications. Periodic review of previously classified variants with availability of new information and contemporary guidelines may improve diagnostic confidence and provides a mechanism for clarification of VUS[3,26,27]. Such review also permits iterative refinement of curation processes and enrichment of local and public clinical databases with up-to-date evidence and classifications.

This study evaluates the projected clinical and reporting impact of reclassification of *MYH7* variants in a cohort of individuals with cardiomyopathy, arrhythmia, and/or sudden death ascertained through diagnostic genetic testing undertaken in a single clinical laboratory. *MYH7* variants were re-evaluated by both unmodified ACMG-AMP criteria (using information currently available) and ClinGen's modified *MYH7*-specific guidelines, providing a basis for comparison of these two schemes and interrogation of the items most commonly responsible for changes in classification.

MATERIALS AND METHODS

Data collection

Proband reports issued through a clinically accredited (by the National Association of Testing Authorities [NATA]) laboratory in Victoria, Australia, between 2013 and 2017 were queried for reports of at least one *MYH7* variant (54 variants across 52 proband reports). These reports represented individuals who had undergone diagnostic testing using gene panels for cardiomyopathy, arrhythmia, and/or sudden death. Reports issued for cascade and predictive reasons were excluded. During this period, the laboratory performed testing by custom capture using Agilent Sure Select (Agilent Technologies, Santa Clara, CA, USA) followed by sequencing on an Illumina machine (MiSeq or HiSeq; Illumina, San Diego, CA, USA). Variant reports issued prior to availability of the 2015 ACMG-AMP criteria[7] were typically classified according to the body of evidence available at the time and in consideration of the interpretive categories defined in the earlier and less prescriptive ACMG recommendations[28]. Formal adoption of the ACMG-AMP guidelines occurred in early 2016. The query identified 52 proband reports that met criteria. These were reviewed and brief demographic details, indication for testing, genotype, and reported variant classification was recorded using a custom REDCap electronic data capture tool[29] hosted at Murdoch Children's Research Institute (Melbourne, Australia). The project received Human Research Ethics Committee approval (HREC/52933/RCHM-2019).

Curation and classification

All *MYH7* variants were curated and classified according to original unmodified ACMG-AMP criteria ("original ACMG") and ClinGen's *MYH7*-specific guidelines ("ClinGen"). The original ACMG-AMP scheme was applied strictly and where feasible was not informed by evidence arising primarily through the ClinGen paper[17]. For example, moderate (domain) evidence was rarely selected per the original ACMG-AMP scheme, as ClinGen introduced the ability to select moderate evidence (PM1) for missense variants occurring in the 181–937 amino acid residue hotspot[5,30]. Similarly, the original scheme does not define number of affected

probands required for strong clinical evidence (PS4) selection, so this was applied only where specific wording of the ACMG-AMP criteria was met. ClinGen-modified PS4 criteria requiring "probands with consistent phenotype" were interpreted to mean unrelated individuals with HCM, as reported in published medical literature, clinical databases (ClinVar[18]), or on review of the medical record, where accessible, by the curating physician. For variants previously classified by ClinGen, the curator was blinded to the ClinGen classification at the outset of curation, although this was typically uncovered as part of literature review and curation. Effort was made to apply ClinGen criteria agnostic of the previously assigned classification. ClinGen criteria were applied according to the original *MYH7*-specific guidelines[17], and did not incorporate revisions recommended subsequent to completion of curation (for example, proposed reduced weighting of absence/rarity PM2 criterion[31]).

For both original ACMG-AMP and ClinGen schemes, items of evidence selected were coded and final classification recorded. Classifications by each scheme were compared with each other and with the initial ("report") classification. Where differences occurred, the reason for the classification change was coded and responsible evidence items annotated. Variants in which a classification change occurred ("report" and "ClinGen" classifications differed) were discussed in a multidisciplinary team meeting attended by genetic scientists, clinical geneticists, and genetic counselors to achieve consensus regarding final classification. Curation occurred between March 2019 and March 2020.

Laboratory impact was assessed on the basis of whether a report reissue was recommended ("no report change" or "report change"), and number of family members previously cascade tested was recorded. A report reissue was recommended in all cases where ClinGen and report classifications were discordant, and the direction of the change was coded. Classification changes were defined as clinically actionable if they occurred between VUS and LP/P categories, or between LP/P and benign (B) or likely benign (LB) categories. Classification differences between LP and P categories were considered clinically equivalent. Where classification changes occurred, clinical files were reviewed (as available; $n = 9$) to assess number of family members genotyped through the laboratory and expected clinical impact. Pedigrees were reviewed (where available; $n = 6$) to determine the number of untested first- and second-degree relatives available for cascade testing. Values were reported as mean number of individuals per family, with associated range. Descriptive statistics were generated using SPSS Statistics v26 (IBM, NY, USA).

RESULTS

Descriptive data

MYH7 variants were reported 54 times across 52 separate proband reports (with two instances of a report listing two *MYH7* variants in the same individual). These represented 43 unique variants, 37 (86%) of which were reported only once and 6 (14%) were recurrent within the call set. All reported variants were single-nucleotide variants. Forty (93%) of these were missense and 3 (7%) were splice site variants, consistent with the reported mutational spectrum of *MYH7*. Segregation information was not available for 74% of variants but 10 (19%) were inherited and 4 (7%) were apparently de novo (paternity assumed). The average number of variants listed per report, including those in other genes, was 2.1 (range 1–6).

In seven cases (14%) an amended report had previously been issued with an updated variant classification (for example, due to segregation or other new information). Where this occurred, both initial and most recent classifications were captured and the most recent classification used in all comparisons. One locally recurrent variant was classified differently on two separate reports creating an additional data point for comparisons between original report classifications and both ACMG-AMP/ClinGen classifications ($n = 44$). Thirteen (30%) of the unique variants have previously been curated by ClinGen[17].

Classification description and distribution

Application of ClinGen criteria resulted in a higher number of diagnostic classifications (LP or P) compared with original ACMG-AMP. The original ACMG-AMP scheme resulted in diagnostic

Table 1. Number of reports and number of *MYH7* variants by classification and scheme applied.

Scheme	Number of reports[a] (%)					Number of variants[b] (%)				
	B[c]	LB[c]	VUS	LP	P	B[c]	LB[c]	VUS	LP	P
Initial report			20 (37)	19 (35)	15 (28)			15 (35)	18 (42)	10 (23)
Original ACMG-AMP	0 (0)	7 (13)	17 (32)	21 (39)	9 (17)	0 (0)	2 (5)	18 (42)	17 (40)	6 (14)
ClinGen	6 (11)	1 (2)	13 (24)	16 (30)	18 (33)	1 (2)	1 (2)	13 (30)	16 (37)	12 (28)

ACMG–AMP American College of Medical Genetics and Genomics-Association for Molecular Pathology, *B* benign, *ClinGen* Clinical Genome Resource, *LB* likely benign, *LP* likely pathogenic, *P* pathogenic, *VUS* variant of uncertain significance.
[a]Total of 52 proband reports, two of which reported two *MYH7* variants ($n = 54$).
[b]Unique variants, with recurrent variants counted only once ($n = 43$).
[c]Reporting laboratory does not report variants of benign or likely benign classification.

classifications in 56% of reports and 54% of variants. Both initial report and the ClinGen scheme demonstrated LP/P outcomes in 63% of reports (65% of variants) although there were discrepant classifications in both directions. Classifications of pathogenicity by initial report, ACMG-AMP, and ClinGen schemes are summarized in Table 1, organized by reported variants ($n = 54$) and by unique variants ($n = 43$). A complete list of variants classified, and revised ClinGen criteria selected, is presented in Supplementary Data 1. The frequency with which individual evidence items were used to inform final classification is presented in Table 2 for both schemes.

Original report comparisons

Classification by strict ACMG-AMP criteria resulted in classification changes in 23 reports (43%) and 17 variants (39%). Ten report (19%) and variant (23%) classification differences represented clinically actionable changes. There were three instances (6% of reports and 7% of variants) where a new molecular diagnosis was made (VUS upgraded to LP/P) and seven cases (13% of reports and 16% of variants) where a molecular diagnosis was revoked (LP/P to VUS or benign). Concordance in classification between original report and ACMG-AMP/ClinGen schemes is summarized in Fig. 1.

Several factors contributed to differences in classification (Table 3). Classification changes in eight variants (47%) with ACMG-AMP criteria applied occurred due to availability of new information since original report issue (for example population data, clinical reports, segregation). Differences that reflected scheme application only, or differences in evidence weighting or scoring matrices that were independent of availability of new information, were considered "scheme-specific differences." These were a factor in classification changes for nine variants (53%) by ACMG-AMP criteria, of which eight were reported in 2015 or earlier, prior to formal implementation of the ACMG-AMP criteria locally.

Application of ClinGen criteria resulted in a classification change from 19 original reports (35%) of which eight changes (15%) were clinically actionable. These changes represented 13 variants (30%), 8 of which (18%) were clinically actionable changes. Of the actionable changes, a new molecular diagnosis was achieved in half (7% of probands, 9% of variants) and molecular diagnosis revoked in half. Thirteen variants included in this study were also classified in the paper that described the ClinGen scheme[17]; in all cases the classifications were concordant. Classification differences between the original report and the ClinGen scheme reflected specific elements of the scheme for seven variants (54%) and were attributable to new information emerging with passage of time in six variants (46%). There was a trend toward older reports being more likely to undergo a change in classification, although this

was not statistically significant (χ^2 1.7, $p > 0.5$). Forty-four percent of reports issued in 2013 had recommended classification changes, compared with 20% of reports issued in 2017 (Supplementary Data 3).

ACMG-AMP/ClinGen scheme comparison

ClinGen and original ACMG-AMP schemes were concordant in 35 proband reports (65%) and across 32 variants (74%). Of the 19 discrepant report classifications (representing 11 variants), a majority (79%) were clinically equivalent (between LP/P or between LB/B categories). The ClinGen scheme was more likely to achieve a "pathogenic" classification, upgrading six variants (14%) classified as LP by ACMG-AMP. In addition, four variants (9%) classified as a VUS per the original ACMG-AMP scheme were LP or P when classified by ClinGen criteria. Comparisons between ClinGen and ACMG-AMP schemes are summarized in Fig. 1c.

Differences between original ACMG-AMP and ClinGen classifications could be attributed to a small proportion of revised criteria (Supplementary Data 2). Use of gene-specific domain evidence (PM1) to assign an additional "moderate" criterion contributed to upgraded classifications in nine variants (82%). More precise and tiered definitions of variant prevalence in clinical cases (PS4) contributed to discordant classifications of eight variants (73%). The ClinGen scheme permitted downgrade of one variant due to a lower population allele frequency threshold for invoking the standalone benign criterion (BA1).

Impact of classification changes

Implications of proposed classification changes for cascade testing in family members were recorded by both number of genotyped individuals and number of individuals in whom testing might be of benefit on review of the family pedigree (where this was available; Table 4). Of the 19 proband reports with classification changes suggested by ClinGen criteria, 9 (47%) were issued in families where additional cascade or segregation testing had been undertaken. The total number of reports with suggested classification changes, including all probands and any cascade-tested family members, was 77 reports (41 positive for the reported variant, and 36 negative). The mean number of individuals genotyped per family, excluding probands, was 3.1 (range 0–40 individuals). In this group, six pedigrees were available, which identified an additional 38 first- and second-degree relatives potentially available for cascade testing (mean 6 individuals per family, range 0–12).

When stratified by classification changes that were clinically actionable (eight changes), four families had undergone cascade testing, representing 17 genotyped individuals (mean 2.1 individuals tested per family, range 1–4 individuals). Of these, ten were positive for the reported variant and seven were negative. Four of

Table 2. Frequency of original ACMG-AMP and revised ClinGen evidence items selected for 43 curated variants.

Evidence weight	ACMG-AMP criterion[7]	Times selected (%)	ClinGen criterion[17]	Times selected (%)
Pathogenic				
Very strong	PVS1	0 (0)		
Strong	PS1	0 (0)	PS1	0 (0)
PS2	2 (5)	PS2	2 (5)	
PS3	3 (7)	PS3	3 (7)	
PS4	18 (42)	PS4	16 (37)	
			PP1_strong	7 (16)
Moderate	PM1	0 (0)	PM1	28 (65)
	PM2	34 (79)	PM2	36 (84)
	PM3	0 (0)		
	PM4	0 (0)	PM4	0 (0)
	PM5	10 (23)	PM5	10 (23)
	PM6	5 (12)	PM6	5 (12)
			PVS1_mod	0 (0)
			PS4_mod	5 (12)
			PP1_mod	2 (5)
Supporting	PP1	15 (35)	PP1	5 (12)
	PP2	40 (93)		
	PP3	31 (72)	PP3	30 (70)
	PP4	0 (0)		
	PP5	16 (37)		
			PS4_supp	9 (21)
Benign				
Standalone	BA1	0 (0)	BA1	1 (2)
Strong	BS1	2 (5)	BS1	1 (2)
	BS2	0 (0)		
	BS3	0 (0)	BS3	0 (0)
	BS4	0 (0)	BS4	0 (0)
Supporting	BP1	0 (0)		
	BP2	0 (0)	BP2	0 (0)
	BP3	0 (0)		
	BP4	1 (2)	BP4	1 (2)
	BP5	3 (7)	BP5	3 (7)
	BP6	3 (7)		
	BP7	3 (7)	BP7	3 (7)

ACMG-AMP American College of Medical Genetics and Genomics-Association for Molecular Pathology, *ClinGen* Clinical Genome Resource.

the variants with actionable changes were suggested downgrades (LP to VUS or LB; molecular diagnosis revoked), representing nine genotyped individuals (five testing positive and four negative for the reported variant). The remaining four variants in this group were upgrades from VUS to LP or P (molecular diagnosis confirmed), impacting four genotype-positive reports and an additional four genotype-negative reports. Only two pedigrees were available for review in this group, identifying 12 additional first- and second-degree relatives potentially available for cascade testing (mean 6 individuals per family, range 0–12).

DISCUSSION

Reclassification of previously reported *MYH7* variants with the benefit of current information and best practice improves diagnostic confidence and simultaneously contributes to a dynamic evidence base that informs future classification. ClinGen's modified *MYH7* criteria provide a structured and gene-specific framework for interpretation of sequence variants that, in our cohort, produced a higher proportion of diagnostic reports and fewer VUS compared with standard ACMG-AMP guidelines. Comparison of evidence items selected across both schemes
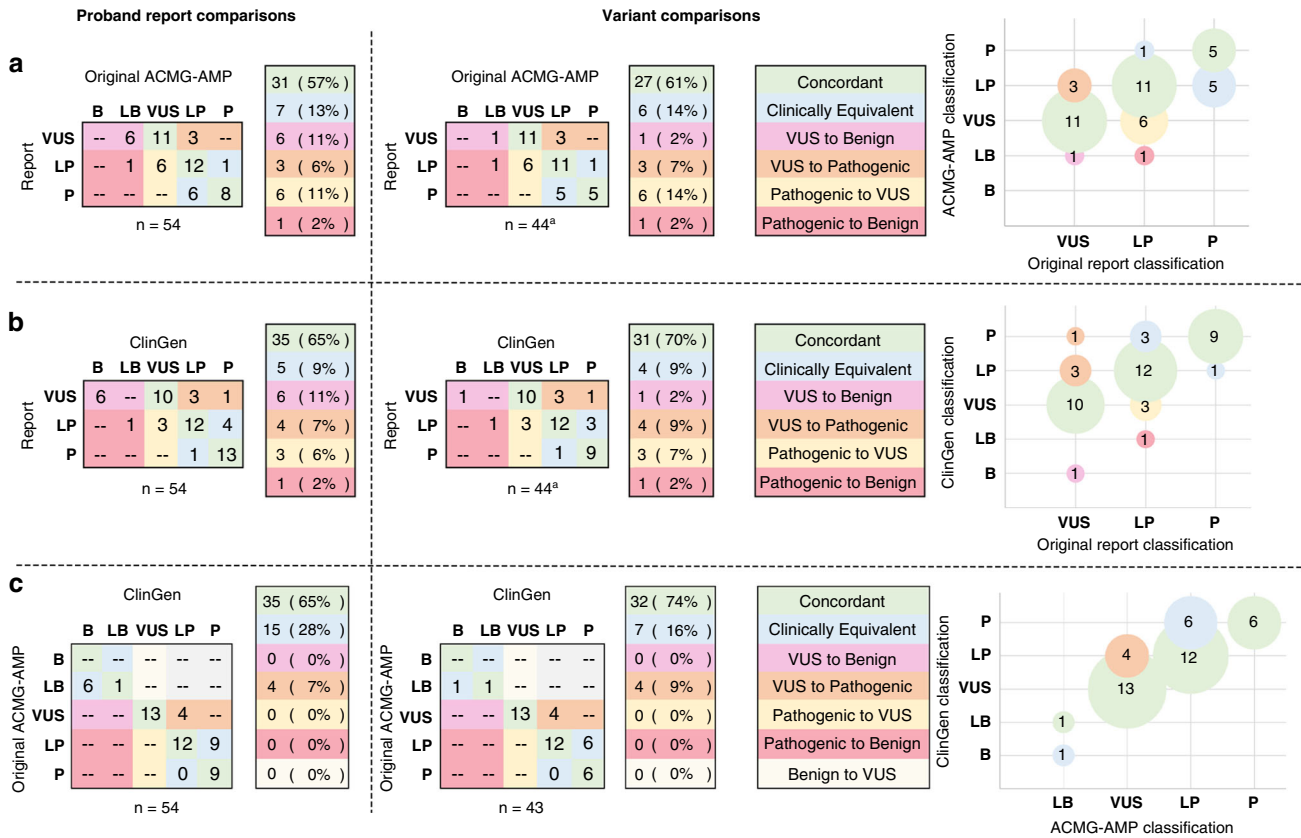
Fig. 1 Variant classification comparisons across original report, ACMG and ClinGen schemes. Comparison of (**a**) original report versus American College of Medical Genetics and Genomics (ACMG-AMP) classification, (**b**) original report versus ClinGen classification, and (**c**) ACMG-AMP versus ClinGen classification, organized by number of proband reports (left grid) and by variants (right grid). Bubble plots denote the respective comparisons with area of the bubble proportional to the number of variants. B benign, LB likely benign, LP likely pathogenic, P pathogenic, VUS variant of uncertain significance. [a]Represents 43 unique variants; however, one locally recurrent variant was classified differently on two separate initial reports, necessitating an additional comparison point ($n = 44$).

Table 3. Factors contributing to classification differences between original report and original ACMG-AMP/ClinGen schemes.

Contributory factors	Number of variants (%)	
Variants with classification change	Report vs. ACMG-AMP[17]	Report vs. ClinGen[13]
Variant classified by expert group	3 (18)	4 (31)
New clinical frequency data	4 (24)	2 (15)
New population frequency data	2 (12)	2 (15)
New functional/domain data	0 (0)	4 (31)
New segregation data	2 (12)	4 (31)
Scheme-specific differences	9 (53)	7 (54)

ACMG-AMP American College of Medical Genetics and Genomics-Association for Molecular Pathology, *ClinGen* Clinical Genome Resource.

largely supports the rationale behind ClinGen's revisions and provides insights into factors influencing classification differences.

Inherent uncertainty attached to "old reports" is anxiety-provoking for reporting scientists, clinicians, and their patients, especially when one considers the cascade clinical actions (and inactions) that result from declaration of a causative variant in a

family. There is limited literature regarding the psychological impact of variant reclassification in inherited cardiomyopathy. Qualitative reports suggest that upgraded classifications are more likely to be met with relief (owing to higher confidence in the cause of the condition), while individuals with downgraded variants are more likely to experience worry, frustration, and disappointment at the loss of diagnostic security[32]. In addition, disclosure of a changed classification may impact decision-making in areas of reproductive planning, uptake of medical advice, lifestyle choices, and family communication[32]. Careful counseling, including discussion around uncertainty and the possibility of future reclassification, should occur in both the pre- and post-test settings.

In our cohort, the ClinGen *MYH7*-specific scheme resulted in a higher proportion of diagnostic (LP or P) classifications compared with original ACMG-AMP (65% vs. 54% of variants) and fewer VUS (30% vs. 42%). These findings are comparable with Mattivi et al., who demonstrated a nonsignificant reduction in VUS burden across two HCM cohorts[23]. The proportion of diagnostic classifications by variant did not differ considerably between original report (65%) and ClinGen schemes (65%), although notably these did not represent the same variants, as actionable changes were observed in 8 variants (18%) with a similar number of actionable upgrades (4) and downgrades (4). In this study, 39% of variants classified between 2013 and 2016 were reclassified compared with only 20% in 2017, suggesting that consideration be given to systematic review of *MYH7* variants classified before 2017 with contemporary guidelines.

Scheme-specific differences contributed to classification changes in just over half of reports for both original ACMG-AMP

Table 4. Cascade reporting impact of classification changes.

Reporting impact	All report changes (19)		Actionable changes (8)	
	No.	mean (range)	No.	mean (range)
Cascade testing	9 families cascade tested (47%)		4 families cascade tested (50%)	
Individuals genotyped (including proband)	77	4.1 (1–41)	17	2.1 (1–4)
Individuals genotyped (excluding proband)	58	3.1 (0–40)	9	1.1 (0–3)
Genotype-positive (including proband)	41	2.2 (1–17)	10	1.3 (1–2)
Genotype-positive (excluding proband)	22	1.2 (0–16)	2	0.3 (0–1)
Genotype-negative	36	1.9 (0–24)	7	0.9 (0–2)
Pedigrees available	6 pedigrees available (32%)		2 pedigrees available[a] (25%)	
Affected family members (including proband)	18	3.0 (1–7)	3	
Families cascade tested	5	83%	1	
1st degree relatives available for testing	12	2.0 (0–5)	4	
2nd degree relatives available for testing	26	4.3 (0–8)	8	
Total relatives available for testing	38	6.3 (0–12)	12	

[a]Descriptive statistics not reported due to small numbers.

and ClinGen schemes, suggesting that changes could not be accounted for by the emergence of new information alone. When new information contributed to reclassification by the ClinGen scheme, this was most frequently due to segregation data, clarification of functional/domain evidence, or expert group classification (each contributing to 30.8% of variant reclassifications). The latter two of these contributions flowed directly from the ClinGen initiative[17], further highlighting the important role of expert group consensus in clarifying variant pathogenicity.

Comparisons of individual evidence items selected in both schemes provided further insights into gene-specific disease mechanisms and rationales behind ClinGen's revisions (Table 2; Supplemental Data 2). In our cohort, PVS1 (null variant) was never selected per original ACMG-AMP criteria as all reported variants were missense or splice, consistent with the mutational spectrum of the condition and supporting removal of this criterion. The same can be said of removal of the PM3, BP1, and BP3 criteria (wrong mutational mechanism), and of PP4 (phenotype specificity) and BS2 (nonpenetrance), which do not apply in *MYH7*-associated cardiomyopathy. Conversely, ACMG-AMP permits selection of PP2 (missense variant in a gene with minimal benign missense variation) in every missense variant (93% of variants in our cohort), limiting its usefulness in discriminating between pathogenic and benign variation. Removal of this item did not have an impact on classification for any of the variants, further supporting its removal.

The revised item most commonly contributing to a classification difference was PM1 (functional/domain evidence). Clear definition of the requisite domain evidence in the ClinGen scheme allowed assignment of an additional "moderate" criterion in 28 variants (65%) and contributed to a classification change in 9 of these (21% of variants and 73% of those with classification changes). The unmodified ACMG-AMP PM1 criterion ("well-established functional domain") might now take into account domain evidence asserted in the ClinGen paper; however, our laboratory did not apply PM1 prior to availability of the revised guidelines, and as our study aimed to evaluate the impact of ClinGen revisions this item was not selected in the ACMG-AMP scheme in order to facilitate meaningful assessment of the impact of ClinGen's refined guidelines.

Several items in the revised ClinGen criteria have tiered weighting based on the volume of evidence available. The refined definition and tiered weighting of PS4 (prevalence in affected individuals) permitted additional assignment of pathogenicity criteria in 12 variants (28%) that would not otherwise have met the "strong" weighting by ACMG-AMP. This contributed to a classification change in eight variants (19%). Similarly, reweighted PP1 tiers (segregation evidence) allowed selection of an additional "strong" weighting in seven variants (16%) and "moderate" weighting in two variants (5%), but only contributed to an upgraded classification in two variants (5%). Our utilization of this criterion may have been limited by segregation testing being undertaken in only 26% of variants, thus the potential utility of the PP1 (and PS2; "de novo variant") items may be underrepresented. Members of the curating multidisciplinary team also valued the clarity these tiered items provided over their unmodified ACMG-AMP equivalents, which were frequently prone to subjective interpretation.

The revised population evidence threshold (PM2) permitted an additional moderate criterion in two variants (5%) and was responsible for the upgrade of one variant (2%). The ClinGen working group has recently proposed a revision to reduce weighting of PM2 from moderate to supporting evidence[31]. While this recommendation was not available at the time of study curation, it is recognized that a reduced PM2 weighting would remove one moderate piece of evidence for multiple variants, and therefore potentially result in a downgraded classification. Further study could evaluate the impact of this new proposal. ClinGen's revised benign criteria resulted in a classification downgrade in only one variant in our cohort (2%) compared with original ACMG-AMP criteria, owing to a lower permissible population allele frequency for selection of BA1.

We assessed the predicted clinical impact for the proband on the basis of whether a molecular diagnosis was confirmed where it was not previously, or was revoked. Classification by ClinGen resulted in an equal number of clinically actionable upgrades and downgrades, with actionable changes recommended in 18% of variants. The study cohort consisted of clinically affected individuals in whom diagnostic testing was undertaken. Seven individuals underwent testing due to sudden death, thus proband

clinical utility was minimal in these cases. Report changes were recommended in three individuals with sudden death, and only one of these was an "actionable" change. In the surviving probands, molecular confirmation (or revocation) of the diagnosis was unlikely to modify medical management or surveillance, but might preclude (or enact) further investigation for a cause.

Projected cascade impacts for family members were more considerable, as predictive testing informs the need for echocardiographic surveillance. In our cohort, each classification change in a proband report impacted, on average, an additional 3.1 cascade reports for genotyped family members with a further 6.3 first- and second-degree relatives potentially available for cascade testing per family. The latter projection relies on a number of assumptions, including that "untested" relatives were still alive since documentation of the pedigree and that they had not undergone subsequent testing in another laboratory. Interpretation of clinical impact was also limited by the relatively small number of reports in which a clinical file and pedigree were available for review, especially when stratified for actionable changes. This study relied largely on information available at time of referral for testing, which in most cases was several years prior to study curation. Additional assessment of clinical impact might involve survey of referring clinicians and families with review of contemporary clinical history and pedigree information.

Few studies have examined the laboratory and clinical impact of variant reinterpretation in inherited cardiomyopathy. Laboratories typically reclassify variants in a reactive and ad hoc fashion, usually following a new request from a referring clinician or identification of a previously classified variant in a new individual. Systematic or periodic reinterpretation has been perceived as potentially unfeasible, costly, and logistically challenging for laboratory services, with the responsibility of initiating re-evaluation considered to lie with the patient and clinician[27,33,34]. Reclassification may also warrant report reissue, an undertaking that presents logistical challenges for the reporting laboratory, particularly if the original report was issued some time ago, and may not attract remuneration.

Periodic re-evaluation of variants, with the benefit of new information and improved understanding of the genetic architecture of HCM, poses logistical and economic challenges for laboratories but may have wider public health benefits. Further evaluation is needed to understand the potential social and health economic gains that may ensue from a refined molecular diagnosis within a family. These might include escape from surveillance of unaffected genotype-negative individuals or availability of cascade testing in families where an upgraded classification has occurred. ClinGen's revised ACMG-AMP criteria provide clear expert-informed guidance for interpretation of sequence variants in *MYH7* and a proof-of-concept that will undoubtedly presage future evidence-based revisions across a variety of genes and disease groups.

DATA AVAILABILITY
Variant data and genomic coordinates are supplied in Supplementary Data 1 and are also available upon request to the corresponding authors.

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ETHICS DECLARATION

This project received Human Research Ethics Committee approval (HREC/52933/RCHM-2019) and site-specific approval through Murdoch Children's Research Institute and Royal Children's Hospital (Parkville, Victoria, Australia) in 2019.

COMPETING INTERESTS

ADDITIONAL INFORMATION

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