Original Article

# Care in Specialized Centers and Data Sharing Increase Agreement in Hypertrophic Cardiomyopathy Genetic Test Interpretation

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***Background***—Clinically impactful differences in the interpretation of genetic test results occur between laboratories and clinicians. To improve the classification of variants, a better understanding of why discrepancies occur and how they can be reduced is needed.

***Methods and Results***—We examined the frequency, causes, and resolution of discordant variant classifications in the Sarcomeric Human Cardiomyopathy Registry (SHaRe), a consortium of international centers with expertise in the clinical management and genetic architecture of hypertrophic cardiomyopathy. Of the 112 variants present in patients at >1 center, 23 had discordant classifications among centers (20.5%; Fleiss κ, 0.54). Discordance was more than twice as frequent among clinical laboratories in ClinVar, a public archive of variant classifications (315/695 variants; 45.2%; Fleiss κ, 0.30; $P<0.001$). Discordance in SHaRe most frequently occurred because hypertrophic cardiomyopathy centers had access to different privately held data when making their classifications (75.0%). Centers reassessed their classifications based on a comprehensive and current data summary, leading to reclassifications that reduced the discordance rate from 20.5% to 10.7%. Different interpretations of rarity and co-occurrence with pathogenic variants contributed to residual discordance.

***Conclusions***—Discordance in variant classification among hypertrophic cardiomyopathy centers is largely attributable to privately held data. Some discrepancies are caused by differences in expert assessment of conflicting data. Discordance was markedly lower among centers specialized in hypertrophic cardiomyopathy than among clinical laboratories, suggesting that optimal genetic test interpretation occurs in the context of clinical care delivered by specialized centers with both clinical and genetics expertise. **(*Circ Cardiovasc Genet*. 2017;10:e001700. DOI: 10.1161/CIRCGENETICS.116.001700.)**

**Key Words:** cardiomyopathy, hypertrophic ■ genetic counseling ■ genetic testing ■ registries



Hypertrophic cardiomyopathy (HCM) is an inherited cardiovascular disease characterized by left ventricular hypertrophy that occurs in the absence of pressure overload, systemic disease, or infiltrative processes. Individuals with HCM are at increased risk for adverse clinical events, including heart failure, atrial fibrillation, stroke, and sudden cardiac death.[1] Disease-causing sarcomere variants are identified in a third of HCM cases, with another 15% having an inconclusive genetic test result.[2] Genetic testing for HCM has become routine in centers specialized in the disease and is recommended in multiple medical guidelines.[3,4] Once a variant is identified through genetic testing, a variety of data points are reviewed, and an assessment is made as to the likelihood that the variant causes HCM.[5] This leads to a classification that the variant likely causes disease (pathogenic, likely pathogenic), is inconclusive (variant of uncertain significance), or is unlikely to cause disease (likely benign, benign). The primary benefits of genetic testing arise when a pathogenic or likely pathogenic variant is found, which can help in establishing a definitive diagnosis in the patient and in assessing risk of disease in healthy relatives.

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At the same time that genetic testing for cardiovascular diseases like HCM has become common practice, the complexities of interpreting such tests and the need for reliable and consistent standards for interpretation have become increasingly evident.

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Large-scale population sequencing data sets such as ExAC (Exome Aggregation Consortium, http://exac.broadinstitute.org) have demonstrated that rare variation is abundant in the genome, challenging the assumption that most rare variation causes severe Mendelian genetic disease and questioning the pathogenicity of thousands of specific variants.[6] Data sharing efforts such as ClinVar have also revealed challenges in current variant classification approaches.[7] ClinVar, a public repository of variant classifications submitted by clinical laboratories and researchers, has facilitated comparisons among laboratories, revealing that differences in interpretation are not uncommon. Many of these differences are clinically impactful; one laboratory may classify a variant as pathogenic prompting the clinician to use that variant in diagnostic evaluations and to assess risk in healthy relatives, while another laboratory calls it a variant of uncertain significance and as such it would not be used in clinical care. The frequency of differences in interpretation among laboratories has ranged from 12% to 53% in different studies.[7–11] These developments in the field have revealed the need for improved approaches to genetic test interpretation.

Efforts are underway to both resolve disagreements among laboratories and to improve genetic test interpretation guidelines to increase agreement and accuracy.[5,7,11,12] Within cardiology specifically, the Cardiovascular Domain Working Group of the ClinGen initiative is developing gene- and disease-specific variant interpretation guidance[7] (https://www.clinicalgenome.org/working-groups/clinical-domain/subgroups/cardiovascular/).

A better understanding of why disagreements in classification occur and how they can be resolved will aid efforts to improve variant classification strategies and guide clinicians in navigating the clinical implications of differences in interpretation. To gain such insights, we investigated the frequency, origins, and resolution of disagreements in variant classifications among centers specialized in HCM participating in the Sarcomeric Human Cardiomyopathy Registry (SHaRe, http://www.thesharegistry.org).

## Methods

### SHaRe

SHaRe is an international consortium that amalgamates deidentified patient-level data on inherited cardiomyopathies from established institutional data sets at participating centers. At the time of analysis, SHaRe contained clinical and genetic testing data on 4944 patients with HCM from the following centers: Stanford University, Brigham and Women's Hospital, University of Michigan, Erasmus University, and Careggi University. All centers have expertise in both the clinical management of HCM and comprehensive genetics evaluations, including family evaluations and interpretation of genetic testing.

### SHaRe and ClinVar Variant Data Sets

Variant data in 8 sarcomere genes (*ACTC1*, *MYBPC3*, *MYH7*, *MYL2*, *MYL3*, *TNNI3*, *TNNT2*, and *TPM1)* were downloaded from the SHaRe database (March 2015). Variants had been identified through genetic testing performed during routine clinical care of patients at SHaRe centers from 1992 to 2014. Classifications were arrived at through clinical care and were not reassessed for this study. The typical workflow for ordering and interpreting genetic test results used in SHaRe centers is displayed in Figure 1. At most SHaRe centers, this includes assessment of variant classifications by the clinical team, discussion of challenging classifications at multidisciplinary team meetings, and periodic reassessment of a subset of variants—such as when the patient returned to clinic or when predictive genetic testing is offered to a relative.

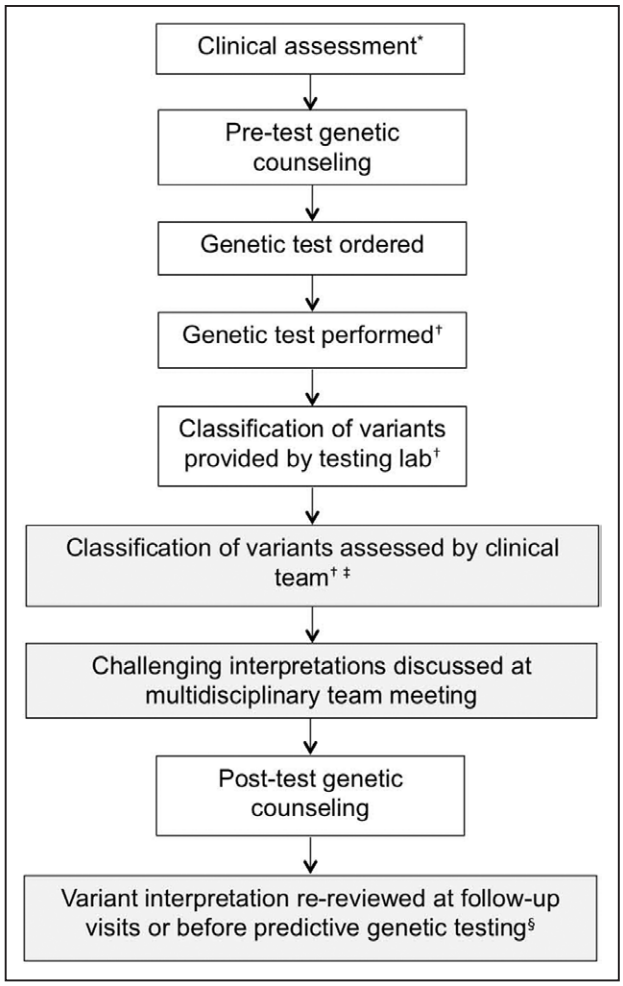


**Figure 1.** Genetic testing workflow in Sarcomeric Human Cardiomyopathy Registry (SHaRe) centers. Steps typically taken in genetic testing at SHaRe centers are shown. Test interpretation steps performed by clinical team shaded in gray. *Clinical assessment of evidence for hypertrophic cardiomyopathy (HCM) diagnosis and consideration of other genetic or nongenetic diagnoses, typically including cardiovascular phenotyping, medical history, 3 to 4 generation family pedigree. When needed, evaluation for alternate causes of left ventricular hypertrophy is performed such as metabolic work-up or testing for amyloidosis. †Careggi University: genetic testing is interpreted by geneticists who both staff the genetic testing laboratory and are part of the multidisciplinary team that sees patients in the HCM clinic. ‡Stanford University, Brigham and Women's Hospital, University of Michigan: variant classification assessed by cardiologist and genetic counselor. Erasmus University: clinical team does not assess variant classification. Clinical team's assessment of variant classification based on review of a range of available data types, guided by gene–disease expertise, without formalized classification criteria. In rare cases, SHaRe centers discuss classifications with one another (approximately once a year, within the setting of several hundred genetic tests a year). §Genetic test interpretation reconsidered by clinical team in a portion of cases at patient's follow-up appointments and before predictive genetic testing in at-risk relatives.

To set the SHaRe data in context, we also examined discordant classifications in ClinVar, using data on the same 8 sarcomere genes (downloaded, April 2015). To focus on clinical laboratories, ClinVar submissions from Online Mendelian Inheritance in Man and research laboratories were excluded. ClinVar submitters included in this analysis were the following: Laboratory for Molecular Medicine, GeneDx,

LabCorp, Blueprint Genetics, Children's Hospital of Eastern Ontario, Invitae, University of Washington, Emory Genetics Laboratory, Genetic Services Laboratory, University of Chicago, Neurogenetics Laboratory (Royal Perth Hospital).

## Discordance

Any individual variant that was seen by >1 HCM center or clinical laboratory had the potential to be classified discordantly (Figure 2). A variant was considered to have discordant classifications if the classifications from >2 groups crossed a major classification category (ie, likely pathogenic/pathogenic versus variant of uncertain significance; likely pathogenic/pathogenic versus likely benign/benign; variant of uncertain significance versus likely benign/benign). Classifications that differed only by degree of confidence within the same major classification category were considered concordant (ie, likely pathogenic versus pathogenic, likely benign versus benign).

We determined whether disagreements in classification were clinically significant, meaning they would impact medical care, such as diagnosis in the patient or use of predictive genetic testing for healthy at-risk family members. Discordance was considered clinically significant if it involved a likely pathogenic or pathogenic classification and any other classification (ie, likely pathogenic/pathogenic versus variant of uncertain significance or likely pathogenic/pathogenic versus likely benign/benign).

The reasons for discordance in SHaRe were assessed by comparing the rationale provided by each SHaRe center to justify their classification of that variant. Data used by each center in classification was considered in terms of type or category of evidence, source of evidence, and whether that evidence was publicly available. These data were available for 20 of 23 discordant variants (the other 3 became concordant on the centers' updated review of their initial classification).

To assist in resolution of discordance among SHaRe centers, each center was asked to reassess their classification based on up-to-date summaries of all available data on each discordant variant. To assess why discordance remained after these reclassifications, we compared centers' rationales for their final classification and examined the data available on each variant. This included an assessment of the number of data points suggesting that the variant may be benign, which included co-occurrence with another likely pathogenic or pathogenic variant in >1% of cases,[2,5] presence in reference samples with minor allele frequency >0.00004,[5,13] failure to segregate,[5] and occurrence with other phenotypes not expected with a variant causing HCM.

## Statistical Analysis

We used Fisher exact test to compare discordance rates in SHaRe and ClinVar. Fleiss κ was used to assess inter-rater reliability, a modification of Cohen κ for >3 reviewers.[14] Analyses were performed with the use of the R statistical package.

# Results

## Discordance in SHaRe Is Lower Than in ClinVar

The SHaRe data set included genetic testing results for 2186 unrelated individuals with HCM, which yielded 589 unique sarcomere variants in 1145 unrelated individuals. Across SHaRe centers, 31.1% to 58.8% of unrelated patients with HCM had a sarcomere variant deemed pathogenic or likely pathogenic (mean, 43.9%; SD, 9.9%; Table I in the Data Supplement).

Of the 589 unique sarcomere variants in SHaRe, 112 (19.0%) were seen by >1 center (Figure 2). Discordant classifications were present in 23 of these variants (20.5%) with a Fleiss κ of 0.54 (95% confidence interval, 0.38–0.69). To contextualize this rate of discordance, we compared it to the rate of discordance among clinical laboratories in ClinVar. The ClinVar data set contained 2405 unique sarcomere variants, of which 695 variants were submitted by >1 laboratory and 314 were discordant (45.2%) with a Fleiss κ 0.36 (95% confidence interval, 0.30–0.42). Fewer classifications were discordant in SHaRe than in ClinVar (20.5% versus 45.2%; $P<0.001$). In both ClinVar and SHaRe, most discordant classifications were clinically significant (SHaRe: 19/23, 82.6%; ClinVar: 229/314, 72.9%; $P=0.75$).

## Discordance Is Often Caused by Lack of Data Sharing

Comparison of the rationale for initial variant classifications provided by SHaRe centers revealed that most variants had >1 reason for discordance (mean, 2.5; SD, 1.4). The most common reason for discordance was differential access to privately held data (15/20, 75%), from either the SHaRe center's clinical experience (12/20, 60%) or the genetic testing laboratory's internal data (12/20, 60%; Figure 3). This most frequently involved co-occurrence of the discordant variant with another pathogenic variant, suggesting that the discordant variant may be benign. This occurred for 11 of 20 (55%) discordant variants; in 4 of those cases that data were held

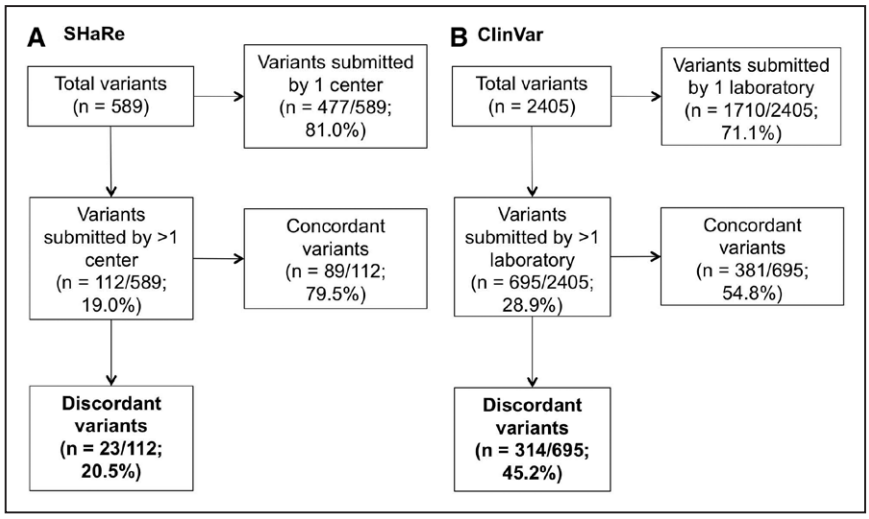


**Figure 2.** Assessment of discordance in Sarcomeric Human Cardiomyopathy Registry (SHaRe) and ClinVar. Variants for 8 sarcomere genes were downloaded from SHaRe (**A**) and ClinVar (**B**). Variants with classifications from >1 SHaRe center or ClinVar laboratory were identified. Classifications were compared across centers or submitters to assess discordance.

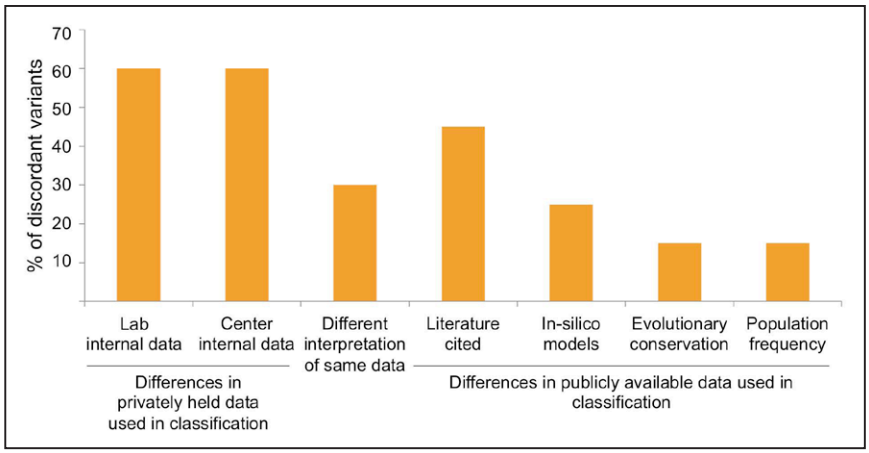


**Figure 3.** Reasons for initial discordance among Sarcomeric Human Cardiomyopathy Registry centers. Reasons for discordance were assessed by comparing the rationales for each center's classifications. Most variants had >1 reason for discordance with a mean of 2.5 reasons per variant.

only by a SHaRe center; in another 4, it was held only by the laboratory that did the testing; and in the remaining 3, it was held by both the testing laboratory and SHaRe center. For 7 of 20 discordant variants (35%), the SHaRe centers differed in their access to segregation data. In 5 cases, the segregation data were privately held by the SHaRe center; in 2 cases, it was held by the laboratory that did the genetic testing.

Data that were not available to all SHaRe centers included data that suggest the variant could be benign: being seen with inconsistent phenotypes (4/20, 20%, for example, dilated cardiomyopathy, Brugada, sudden infant death syndrome [Tables 1 and 2]), cases being from the same ancestry without sufficient ancestry matched controls (1/20, 5%), and presence in a genetic testing laboratory's internal reference samples (1/20, 5%).

Consistent with the impact of differential access to data, more than half of the HCM cases associated with the discordant variants were not publicly available; 125 cases were published or publicly available, while 193 were only available in a private data set that not all centers initially had access to (Tables 1 and 2).

## Discordance Because of Differences in Use of Publicly Available Data

In some cases, the centers disagreed in their classifications because the publicly available data that they used differed, including citing different publications (9/20, 45%), using different in silico models (5/20, 25%), population frequencies (3/20, 15%), and assessments of evolutionary conservation (3/20, 15%; Figure 3). This sometimes occurred because the

**Table 1. Variants With Discordance Resolved After Reassessment**

| Gene | Variant | Reclassification | Benign Evidence Count* | Unrelated HCM Cases | Other Phenotypes | Seen With LP/P Variant | Segregation | | Presence in Reference Samples | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Meioses Segregating | Meioses Failing to Segregate | Highest MAF | Population With Highest MAF |
| MYBPC3 | p.Gln998Glu (c.2992C>G) | VUS to LB/B | 2 | 35 | … | … | 1 | 0 | 0.09 | Latino (ExAC) |
| MYBPC3 | p.Ser217Gly (c.649A>G) | VUS to LB | 4 | 7 | DCM, SIDS | 3/7 | 0 | 2 | 0.012 | South Asian (ExAC) |
| MYBPC3 | p.Val189Ile (c.565G>A) | VUS to LB | 2 | 8 | … | 3/8 | 0 | 0 | 0.0043 | South Asian (ExAC) |
| MYBPC3 | p.Pro371Arg (c.1112C>G) | LP to VUS | 1 | 4 | … | 4/4 | 3 | 0 | 0 | … |
| MYBPC3 | c.927-9G>A | VUS to P | 0 | 30 | … | … | 5 | 0 | 0 | … |
| MYBPC3 | c.1224-2A>G | VUS to LP | 0 | 4 | … | … | 0 | 0 | 0 | … |
| TPM1 | p.Glu192Lys (c.574G>A) | VUS to LP | 0 | 18 | … | … | 0 | 0 | 0 | … |
| MYH7 | p.Arg1420Trp (c.4258C>T) | VUS to LP | 0 | 11 | … | … | 0 | 0 | 0.000015 | European (ExAC) |

Summary data on Sarcomeric Human Cardiomyopathy Registry discordant variants that became concordant after reassessment. B indicates benign; DCM, dilated cardiomyopathy; ExAC, Exome Aggregation Consortium; HCM, hypertrophic cardiomyopathy; LB, likely benign; LP, likely pathogenic; MAF, minor allele frequency; P, pathogenic; SIDS, sudden infant death syndrome; and VUS, variant of uncertain significance.

*Benign evidence types included co-occurrence with likely pathogenic or pathogenic variant, seen with inconsistent phenotype, failure to segregate, and higher minor allele frequency than expected for disease and gene. Note that comprehensive data was not gathered on the 3 variants that became concordant when the HCM centers reviewed the rationales for their initial classifications.

**Table 2. Variants With Discordance Unresolved After Reassessment**

| Gene | Variant | Remaining Discordance | Benign Evidence Count* | Unrelated HCM Cases | Other Phenotypes | Seen With LP/P Variant | Segregation | | Presence in Reference Samples | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Meioses Segregating | Meioses Failing to Segregate | Highest MAF | Population With Highest MAF |
| MYBPC3 | p.Glu619Lys (c.1855G>A) | LP vs LB | 3 | 10 | DCM, LVNC, WPW | 4/10 | 1 | 0 | 0.0013 | European (ExAC) |
| MYH7 | p.Met982Thr (c.2945T>C) | LP vs B | 3 | 19 | DCM, increased LVWT, SCD with dilatation | 7/19 | 1 | 0 | 0.0013 | European (ExAC) |
| MYH7 | p.Asn1327Lys (c.3981C>A) | VUS vs LB | 2 | 14 | … | 2/12 | 1 | 0 | 0.018 | Ashkenazi (LMM) |
| MYH7 | p.Lys1459Asn (c.4377G>T) | LP vs VUS | 3 | 15 | Ebstein's, Brugada | 2/9 | 1 | 0 | 0.00051 | European (ExAC) |
| MYH7 | p.Arg1606Cys (c.4816C>T) | LP vs VUS | 0 | 2 | … | … | 0 | 0 | 0.0000077 | European (ExAC) |
| MYH7 | p.Arg204His (c.611G>A) | LP vs VUS | 1 | 20 | … | 2/20 | 1 | 0 | 0 | … |
| MYBPC3 | p.Arg810His (c.2429G>A) | LP vs VUS | 2 | 29 | … | 5/27 | 5 | 0 | 0.00006 | European (ExAC) |
| MYBPC3 | p.Arg1002Gln (c.3005G>A) | LP vs VUS | 2 | 3 | DCM, giant RA, and arrhythmia | … | 1 | 0 | 0.00012 | East Asian (ExAC) |
| MYBPC3 | p.Gly531Arg (c.1591G>C) | LP vs VUS | 1 | 3 | … | 1/3 | 2 | 0 | 0.000031 | European (ExAC) |
| MYBPC3 | p.Gly490Arg (c.1468G>A) | LP vs VUS | 2 | 10 | … | 4/8 | 2 | 0 | 0.00045 | Finnish (ExAC) |
| TNNT2 | p.Arg278Cys (c.832C>T) | P vs VUS | 2 | 51 | DCM | 5/47 | 8 | 0 | 0.0016 | Other (ExAC) |
| MYH7 | p.Thr1377Met (c.4130C>T) | P vs VUS | 0 | 25 | … | … | 0 | 0 | 0.000029 | European (pooled) |

Summary data on Sarcomeric Human Cardiomyopathy Registry discordant variants that remained discordant. B indicates benign; DCM, dilated cardiomyopathy; ExAC, Exome Aggregation Consortium; HCM, hypertrophic cardiomyopathy; LB, likely benign; LP, likely pathogenic; LVNC, left ventricular noncompaction; LVWT, left ventricular wall thickness; MAF, minor allele frequency; P, pathogenic; RA, right atrium; SCD, sudden cardiac death; VUS, variant of uncertain significance; and WPW, Wolff–Parkinson–White.

*Benign evidence types included co-occurrence with likely pathogenic or pathogenic variant, seen with inconsistent phenotype, failure to segregate, and higher minor allele frequency than expected for disease and gene. Note that comprehensive data was not gathered on the 3 variants that became concordant when the HCM centers reviewed the rationales for their initial classifications.

SHaRe centers' initial classifications were done at different times, so some centers had classified the variant using information that was now outdated.

For nearly a third of discordant variants, SHaRe centers cited >1 data points that were identical but were interpreted differently by different centers (Figure 3). For example, for p.Gly490Arg (c.1468G>A) in *MYBPC3*, 3 sites were aware that the variant had been seen in multiple cases in conjunction with another variant that was deemed pathogenic. One site used that to reach a variant of uncertain significance classification, while the other 2 sites classified the variant as likely pathogenic or pathogenic despite that data (Tables 1 and 2,; Table II in the Data Supplement).

## Partial Resolution of Discordance Can Be Achieved Through Data Sharing

When SHaRe centers were asked to provide their rationale for their initial classification, 3 centers changed initial classifications based on review of the data the center already had on the variant, in light of their current approach to classifications. This resolved discordance for 3 of the 23 (13%) initially discordant variants (Figure 4A; Table II in the Data Supplement).

To attempt to resolve the remaining discordance in SHaRe, we compiled up-to-date comprehensive summaries of the data on the 20 remaining discordant variants, including both publically available data and data privately available to each center (Tables 1 and 2). Each SHaRe center was asked to review a detailed narrative summary of this data and provide an updated classification for their discordant variants. This reduced discordance further, from 23 of 112 initially (20.5%) to 12 of 112 (10.7%; Figure 4).

## Unresolved Discordance

Nearly all of the remaining discordant classifications were clinically significant (11/12, 91.7%). Most of these were variant of uncertain significance versus likely pathogenic (7/12, 58.3%) or pathogenic (2/12, 8.3%; Table 2; Table II in the Data Supplement). Two were likely benign or benign versus

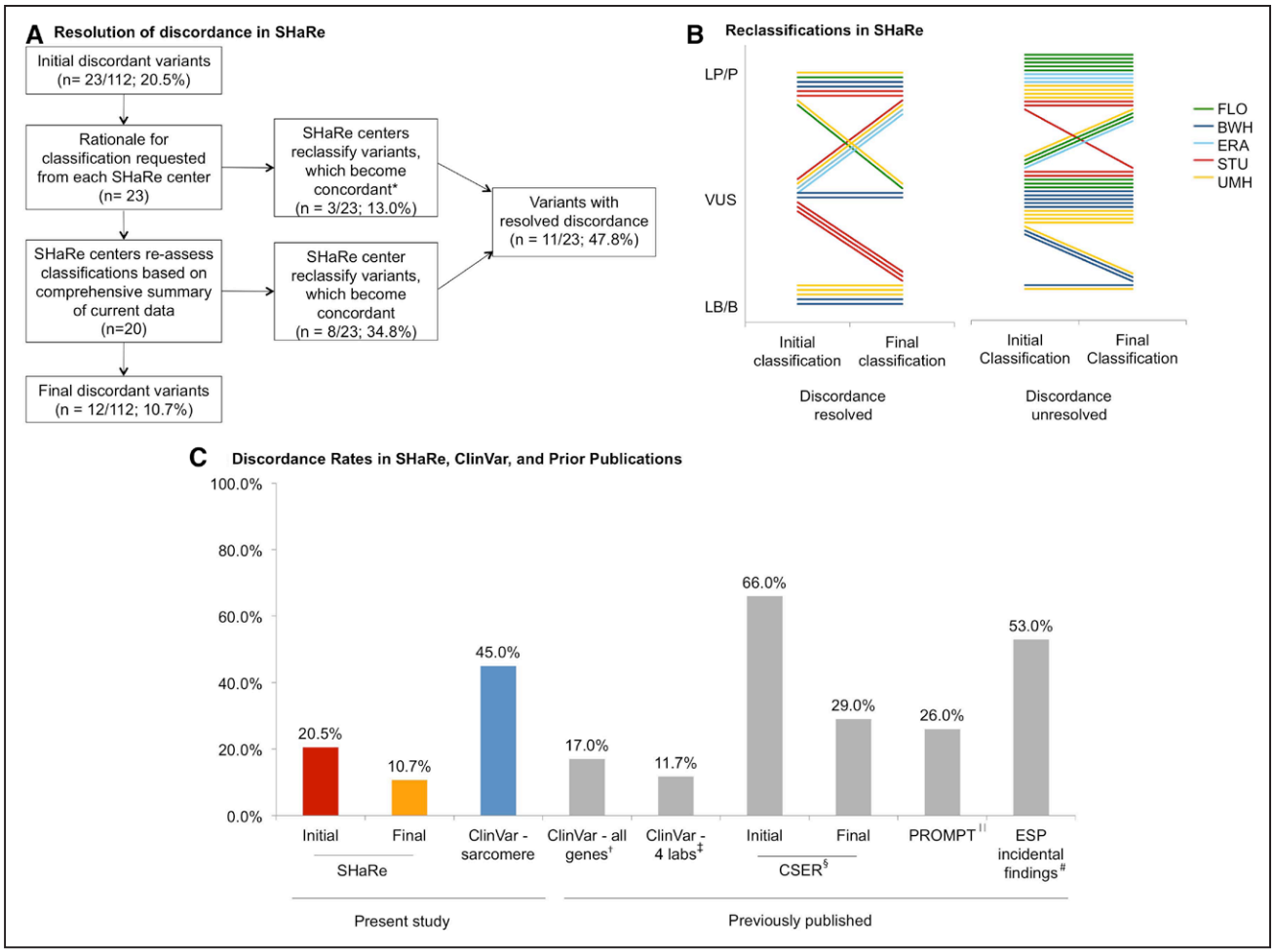


**Figure 4.** Reclassification of Sarcomeric Human Cardiomyopathy Registry (ShaRe) variants and resolution of discordance. **A**, Process of reclassification of variants and (partial) resolution of discordance in SHaRe. *When SHaRe centers reassessed the rationale for their initial classification, 3 centers changed their classification given their current classification methods. **B**, Initial and final classifications in SHaRe, shown for variants that became concordant after reclassification (**left**) and those that remained discordant (**right**). Each line represents 1 center's initial and final classifications of 1 variant. Centers are designated by line color (see legend). **C**, Discordance rates from the present study and previously published studies. †Overall discordance in ClinVar among all laboratories, across all genes.[7] ‡Discordance across all genes in ClinVar between 4 genetic testing laboratories (Ambry, GeneDx, Laboratory for Mole cular Medicine, and University of Chicago).[11] §Discordance in classification of selected variants studied by Clinical Sequencing Exploratory Research Consortium (CSER), before and after efforts to reduce discordance.[9] ‖Discordance rate among clinical laboratories on variants in cancer genes submitted to Prospective Registry of Multiplex Testing (PROMPT).[8] #Discordance among reviewers of potentially actionable incidental findings in Exome Sequencing Project (ESP).[10] B indicates benign; LB, likely benign; LP, likely pathogenic; P, pathogenic; and VUS, variant of uncertain significance. SHaRe centers: Stanford University (STU), Brigham and Women's Hospital (BWH), University of Michigan (UMH), Erasmus University (ERA), and Careggi University (FLO).

likely pathogenic (2/12, 16.7%). For 7 of the 12 variants that remained discordant, at least 1 center changed their classification, yet that reclassification did not resolve discordance (Table II in the Data Supplement). There were no reclassifications in the other 5 variants.

## Reasons for Unresolved Discordance

To gain insight into why discordance was not completely resolved, despite the SHaRe centers having access to the same data, we examined the data gathered on the 20 discordant variants the centers were asked to reassess (Tables 1 and 2). We compared the data on resolved and unresolved variants. However, given that the total number of variants was small (12 unresolved, 8 resolved), the data were not sufficiently powered to perform statistical tests of these comparisons. As such, these comparisons should be interpreted with caution and considered exploratory. We also compared HCM centers' rationales for their final classifications. Note that complete data on rationale for final classifications was only available for 11 of 12 variants that remained discordant.

Among the variants that reached concordance, none of the variants reclassified to (likely) pathogenic had benign evidence, while all of the variants reclassified to (likely) benign had benign evidence, with a mean of 2.7 (SD, 1.2) types of benign evidence per variant (Table 1). The variants that remained discrepant had a mean of 1.8 (SD, 1.1) types of benign evidence per variant, suggesting that the data on these variants were more conflicting and did not point as clearly toward a benign or pathogenic classification (Table 2).

Notably, for nearly two thirds of variants that remained discordant, at least 1 center remarked in their rationale that they

suspect the variant is a modifier (7/11, 63.6%). Consistent with this, these variants had features typically associated with modifying variants; most of these variants had co-occurred with a pathogenic variant (9/12, 75%) and were present in reference samples (11/12, 91.7%; Tables 1 and 2). This contrasts to the variants with resolved discordance in which a minority had co-occurred with a pathogenic variant (3/8, 37.5%) and only half had been seen in reference samples (4/8, 50.0%). Among the variants that were seen in reference samples, the highest minor allele frequency was higher for those with resolved discordance (mean, 0.027; SD, 0.043) than those with unresolved discordance (mean, 0.0021; SD, 0.0052). It is also notable that 8 of 11 variants with resolved discordance were missense, while all variants with unresolved discordance (12/12) were missense, consistent with greater challenges in classifying missense variation and their potential role as modifiers.

Examining the rationales that centers provided for their final classifications, 2 areas of disagreement occurred in over half of variants that remained discordant: differing assessments of whether the variant was sufficiently rare in reference samples (7/11 variants, 63.6%) and differing interpretations of how co-occurrence with another pathogenic variant affected classification (7/11 variants, 63.6%).

## Discussion

While prior studies have examined differences in the classification of variants among laboratories, the current study dissects differences in the interpretations used by clinical centers, where genetic testing data are translated into patient care. The initial rate of disagreement in variant classification among SHaRe centers (20.5%) is at the lower range of the rate of disagreement among laboratories reported to date (12% to 53%)[7–11] and is less than half that among laboratories in ClinVar for the same set of sarcomere genes (45.2%; Figure 4C). We were able to resolve nearly half of interpretation disagreements within SHaRe, leading to a final discordance rate of 10.7%, the lowest yet reported.

The lower rate of discordance among SHaRe centers compared with clinical laboratories could originate from differences in care provided by clinics specialized in HCM. These centers are staffed by cardiologists and genetic counselors (or geneticists) with deep expertise in the diagnosis and management of HCM and its genetic architecture. The lower rate of discordance among HCM centers may arise from application of this expertise to the interpretation of genetic tests. In their 2015 guidelines on sequence variant interpretation, the American College of Medical Genetics and Genomics specifically pointed to the importance of gene–disease–specific considerations.[5] Prior studies have identified lack of gene–disease expertise as a source of both inaccurate classifications and discordance in classifications.[9,15] In addition to their disease and gene expertise, the processes used by these centers in interpreting genetic tests may also contribute to the lower rate of discordance. This typically includes assessment of the variant classification by a cardiologist and genetics professional on the clinical team, discussion of challenging classifications at multidisciplinary team meetings, and periodic rereview of a subset of variants (Figure 1). Our findings also suggest that data generated by the HCM centers through comprehensive genetics evaluations is another potential source of lower discordance among those centers. Centers' classifications arose not only from the data provided by the genetic testing laboratory or published in the literature but from the centers' own clinical evaluations, such as segregation analyses performed by the center.

The lower rate of discordance among SHaRe centers may also be partially attributable to factors unrelated to the care provided by these centers. All the patients included in the SHaRe data set have clear diagnoses of HCM, whereas the larger sample of all patients who undergo genetic testing at clinical laboratories is heterogeneous. Consistent with prior studies[2,16,17] on a variety of genetic diseases, we see a higher genetic testing yield among patients with clear HCM diagnoses in SHaRe (45.5%) than has been reported in individuals who undergo HCM genetic testing in clinical laboratories (32%).[2] Our data reveal that not only is yield lower when diagnoses are not definitive, but discordance is higher. Another potential explanation for higher discordance among clinical laboratories is delay in updating classifications in ClinVar. Both our data and previously published data demonstrate that classifications change over time, and a portion of discordance is caused by outdated classifications.[11,12,18,19] Because of the somewhat onerous process of updating ClinVar, there is often a lag before revised classifications show up online, which contributes to discordance.[11] However, this is not necessarily unique to ClinVar. Variants in the SHaRe database were not rereviewed for this study, and some have not been evaluated since they were initially identified. Furthermore, the SHaRe data base is updated quarterly, and as such, delays in updates may also contribute to discordance among SHaRe centers.

Despite basing revised variant classifications on identical data, 10.7% of variants seen by >1 center in SHaRe remained discordant. This residual discordance seems to be attributable to differences in expert opinion when the available data are subjective and conflicted, particularly whether the variant is sufficiently rare to be a good candidate to cause HCM and whether it is seen too often in tandem with a pathogenic variant. It is possible that some of the residual discordance could be resolved by agreeing on and using identical classification criteria, such as cutoffs for rarity and co-occurrence. It is important to ensure that such classification criteria are sufficiently specific and detailed because discordance can arise when criteria are too vague.[9] The ClinGen Cardiovascular Domain Working Group is developing such disease- and gene-specific variant classification guidance[7] (https://www.clinicalgenome.org/working-groups/clinical-domain/subgroups/cardiovascular/). Another possible explanation for the unresolved discordance in SHaRe is suggested by the fact that in nearly two thirds of variants that remained discordant at least 1 center suspected the variant was a modifier. This is a class of variation that needs further study and is not accounted for in existing classification guidelines. Given the limitations of our current knowledge and guidelines, experts sometimes need to make judgment calls in interpreting variant data, and as such, a certain amount of discordance will remain because of differences in expert opinion. Moreover, discordant interpretation of clinical data are not unique to genetic testing. Comparable rates of disagreement have been reported across a range of medical specialties and tests, including assessment of ventricular tachycardia on ECGs (22% discordant),[20] subtyping

of sarcoma on histopathology (27%),[21] and assessment of wall motion abnormalities on dobutamine stress tests (15%).[22]

Based on our data, we would suggest several strategies to improve genetic test interpretation and increase concordance among variant classifications from different groups, including data sharing, periodic reassessment of variant classifications, and comprehensive genetics evaluation by a multidisciplinary specialized team. The most frequent reason for discordance within SHaRe was that centers had access to different privately held data at the point of initial classification. Data sharing is particularly critical for a disease like HCM that is characterized by such marked genetic heterogeneity; 56% of the variants found on HCM genetic testing by 1 laboratory had been seen in only a single family.[2] This makes it challenging for any 1 laboratory or center to accumulate enough data to determine the appropriate classification for such variants. Data sharing efforts such as SHaRe, ClinVar, and Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org/, formerly ExAC) are shifting variant classifications and impacting clinical care.[6] While there is growing support for data sharing,[23] challenges and barriers remain, including concerns about privacy, intellectual property, and cost. While these challenges are real and need to be addressed, our data suggest that increased data sharing would improve the accuracy of variant classifications and thereby have clinical benefit. One aspect of care in SHaRe centers that could be leveraged to reduce the rate of discordance is periodic reassessment of variant classifications, which allows for changes to interpretations based both on emerging data and newly adopted methodologies for variant interpretation. We found that 13% of discordance in SHaRe was resolved simply by the center reconsidering the data they already had on the variant in light of current knowledge and classification practices. An additional 35% of discordance was resolved by review of new data that had come to light since the initial classification. Similarly, Das et al[19] found that rereview of selected classifications in their HCM center lead to clinically impactful reclassifications in 10% of their patients with variants. Routine reassessment of variant classifications can be onerous and has not historically been part of the services provided by clinical laboratories. Yet both our data and prior studies[18,19] show that rereview can lead to changes in classification that impact medical care for an appreciable subset of patients. Recent shifts in our understanding of just how common rare variation is in every human genome, the rapid rate of changes in genomics knowledge, and the trend toward reanalysis of exomes all point toward the need for laboratory business models to be revised to incorporate this service.[19,24,25] In the meantime, clinicians need to take responsibility for ensuring that periodic reassessment of variants occurs. This could be done by the clinician asking the laboratory to reassess the variant or, if the clinical team has the appropriate expertise, they could reassess it themselves. Last, our data suggest that discordance can be minimized by obtaining care in specialized centers that take an active role in initial and ongoing classification of variants. Consistent with this, professional societies have recommended that genetics evaluations for heritable cardiomyopathies be performed in such specialized centers.[4] A recent study of genetic counselors in cardiology found that concerns on differences in variant classifications among groups and a sense of responsibility for the clinical impact of the test interpretation have led many clinical cardiovascular genetics groups to start making their own assessments of variants received on clinical genetic testing.[26] In many ways, this parallels how these teams often handle cardiac testing; in addition to reviewing written reports from cardiac imaging, they also look closely at the primary imaging data and interpret this independently. There are currently too few cardiovascular genetics clinics to serve all families with HCM and related diseases. As long as that remains the case, we suggest that clinicians using HCM genetic testing outside of such centers do so within the context of a multidisciplinary team (genetics and cardiology), be cognizant of the lower yield and higher discordance when testing is performed on a case with a borderline clinical phenotype, request periodic rereview of variant classifications by the testing laboratory, consult databases such as ClinVar and gnomAD, and have a low threshold for referring uncertain or complex cases to specialized centers.

## Conclusions

Disagreement in the interpretation of genetic test results exists among genetic testing laboratories and clinical HCM centers. Minimization and resolution of such differences is critical because they are often of sufficient magnitude to impact clinical care and can thereby have a significant psychological impact on the patient and family.[27,28] The majority of disagreements are caused by privately held or outdated data, which can be ameliorated by increased data sharing and periodic rereview of currently available data. However, differences in expert assessment of complex data will continue to be a source of discordance for the near future. Notably, the discordance between centers with expertise in HCM management and genetic testing was significantly lower than that seen among clinical genetic testing laboratories. These findings highlight the important benefits that can be achieved when expertise in disease management and family evaluations is combined with expertise in genetic interpretation.

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## CLINICAL PERSPECTIVE

Hypertrophic cardiomyopathy (HCM) is an inherited disease of the cardiac muscle that is often caused by pathogenic variants in sarcomere genes. Genetic testing for HCM is recommended by multiple guidelines, primarily to assist in assessment of risk of HCM in healthy relatives and in ruling out related diseases, such as storage disorders. However, successful application of genetic testing requires careful and accurate classification of genetic variants. The present study found a lower level of disagreement in variant classification among multidisciplinary HCM centers than among genetic testing laboratories, suggesting that the expertise and clinical processes used within these centers may enhance variant classification. Disagreements in classification arose from outdated data, different privately held data sets, changes in classification criteria, and differences in interpretation of identical data. Genetic test interpretation can be improved through periodic rereview of classifications and the sharing of privately held data sets via repositories like ClinVar and multicenter national disease registries like the Sarcomeric Human Cardiomyopathy Registry (SHaRe). Clinicians using genetic testing in the care of patients and families with HCM should choose genetic testing laboratories that share their data, consider consultation with a specialized HCM center with genetics expertise, and pursue periodic reassessment of variant classifications.