



UNIVERSITÀ
DEGLI STUDI
FIRENZE

FLORE

Repository istituzionale dell'Università degli Studi di Firenze

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

Questa è la Versione finale referata (Post print/Accepted manuscript) della seguente pubblicazione:

Original Citation:

Care in Specialized Centers and Data Sharing Increase Agreement in Hypertrophic Cardiomyopathy Genetic Test Interpretation / Furqan A., Arscott P., Girolami F., Cirino A.L., Michels M., Day S.M., Olivotto I., Ho C.Y., Ashley E., Green E.M., Caleshu C.. - In: CIRCULATION, CARDIOVASCULAR GENETICS. - ISSN 1942-325X. - ELETTRONICO. - 10:(2017), pp. 0-0. [10.1161/CIRCGENETICS.116.001700]

Availability:

The webpage <https://hdl.handle.net/2158/1181097> of the repository was last updated on 2020-01-03T16:10:40Z

Published version:

DOI: 10.1161/CIRCGENETICS.116.001700

Terms of use:

Open Access

La pubblicazione è resa disponibile sotto le norme e i termini della licenza di deposito, secondo quanto stabilito dalla Policy per l'accesso aperto dell'Università degli Studi di Firenze (<https://www.sba.unifi.it/upload/policy-oa-2016-1.pdf>)

Publisher copyright claim:

La data sopra indicata si riferisce all'ultimo aggiornamento della scheda del Repository FloRe - The above-mentioned date refers to the last update of the record in the Institutional Repository FloRe

(Article begins on next page)

Disclaimer: The manuscript and its contents are confidential, intended for journal review purposes only, and not to be further disclosed.

URL: <http://circgenetics-submit.aha-journals.org>

Title: Care in Specialized Centers and Data Sharing Increase Agreement in HCM Genetic Test Interpretation

Manuscript number: CIRCCVG/2016/001700

Author(s): Colleen Caleshu, Stanford University

Aisha Furqan, California State University

Patricia Arscott, University of Michigan Health System

Francesca Girolami, Careggi University Hospital

Allison L. Cirino, Brigham and Women's Hospital, Boston, MA

Michelle Michels, Erasmus Medical Center

Sharlene Day, University of Michigan

Iacopo Olivotto, Referral Center for Cardiomyopathies, Careggi University Hospital

Carolyn Ho, Brigham and Women's Hospital

Euan Ashley, Stanford University School of Medicine

Eric Green, MyoKardia

Disclaimer: The manuscript and its contents are confidential, intended for journal review purposes only, and not to be further disclosed.

Care in Specialized Centers and Data Sharing Increase Agreement in HCM Genetic Test Interpretation

Running title: Furqan: Reducing genetic test interpretation discordance

Aisha Furqan¹, Patricia Arscott², Francesca Girolami³, Allison L Cirino⁴, Michelle Michels⁵,
Sharlene M Day², Iacopo Olivotto⁶, Carolyn Y Ho⁴, Euan Ashley^{7,8}, Eric M. Green⁹, SHaRe
Consortium, Colleen Caleshu^{7,10}

1. California State University, Stanislaus, CA, USA

2. University of Michigan, Division of Cardiovascular Medicine, Ann Arbor, MI, USA

3. Genetics Unit, Careggi University Hospital Florence, Italy

4. Cardiovascular Division, Brigham and Women's Hospital, Boston, MA, USA

5. Department of Cardiology, Thoraxcenter, Erasmus Medical Center, Rotterdam, the
Netherlands.

6. Referral Center for Myocardial Diseases, Azienda Ospedaliera Universitaria Careggi,
Florence, Italy

7. Stanford Center for Inherited Cardiovascular Disease, Stanford, CA, USA

8. Division of Cardiovascular Medicine, Stanford Medical Center, Stanford, CA, USA

9. MyoKardia Inc., South San Francisco, CA, USA

10. Division of Medical Genetics, Stanford Medical Center, Stanford, CA, USA

Address for Correspondence:

Colleen Caleshu

Stanford Center for Inherited Cardiovascular Disease

Falk CVRC, 300 Pasteur Drive, Stanford, CA 94305-5406

Phone: 650-725-6273

Fax: 650-498-7452

Email: ccaleshu@stanfordhealthcare.org

Word count: 6345 (total); Journal Subject Terms: Genetics, Cardiomyopathy, Diagnostic
Testing, Hypertrophy

30 **ABSTRACT**

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

34 **Methods and results:** We examined the frequency, causes, and resolution of discordant
35 variant classifications in the Sarcomeric Human Cardiomyopathy Registry (SHaRe), a
36 consortium of international centers with expertise in the clinical management and genetic
37 architecture of hypertrophic cardiomyopathy (HCM). Of the 112 variants present in patients
38 at >1 center, 23 had discordant classifications between centers (20.5%, Fleiss' kappa
39 0.54). Discordance was more than twice as frequent among clinical laboratories in ClinVar,
40 a public archive of variant classifications (315/695 variants, 45.2%, Fleiss' kappa 0.30;
41 $p<0.001$). Discordance in SHaRe most frequently occurred because HCM centers had
42 access to different privately held data when making their classifications (75.0%). Centers
43 reassessed their classifications based on a comprehensive and current data summary,
44 leading to reclassifications that reduced the discordance rate from 20.5% to 10.7%.
45 Different interpretations of allele frequencies and co-occurrence with pathogenic variants
46 contributed to residual discordance.

47 **Conclusions:** Discordance in variant classification between HCM centers is largely
48 attributable to privately held data. Some discrepancies are due to differences in expert
49 assessment of conflicted data. Discordance was markedly lower among centers
50 specialized in HCM than among clinical laboratories, suggesting that optimal genetic test
51 interpretation occurs in the context of clinical care delivered by specialized centers with
52 both clinical and genetics expertise.

53
54 **KEY WORDS:** Genetic testing, hypertrophic cardiomyopathy, genetic counseling, genetic
55 test interpretation

INTRODUCTION

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 death¹. Disease-causing sarcomere variants are identified in a third of HCM cases with another 15% having an inconclusive genetic test result². 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 on genetic testing, a variety of data points are reviewed and an assessment is made as to the likelihood that the variant causes HCM⁵. 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, or 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.

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. Large-scale population sequencing datasets such as ExAC (Exome Aggregation Consortium, 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 variants⁶. Data sharing efforts like ClinVar have also revealed challenges in current variant classification approaches⁷. ClinVar, a public repository of variant classifications submitted by clinical laboratories and researchers, has facilitated comparisons between 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 between laboratories has ranged from 17-53% in different studies⁷⁻¹⁰. These shifts in the field have revealed the need for improved approaches to genetic test interpretation.

Efforts are underway to both resolve disagreements between laboratories and to improve genetic test interpretation guidelines to increase both agreement and accuracy^{5,7,11}. Within cardiology specifically, the Cardiovascular Domain Working Group of the ClinGen initiative is developing gene and disease specific variant interpretation guidance⁷ (<https://www.clinicalgenome.org/working-groups/clinical-domain/sub-groups/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.theshareregistry.org>).

METHODS

SHaRe is an international consortium that amalgamates de-identified patient-level data on inherited cardiomyopathies from established institutional datasets 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 (STU), Brigham and Women's Hospital (BWH), University of Michigan (UMH), Erasmus University (ERA), and

108 Careggi University (FLO). All centers have expertise in both the clinical management of
109 HCM and comprehensive genetics evaluations, including family evaluations and
110 interpretation of genetic testing. SHaRe centers assigned a classification to each
111 sarcomere variant present in their population based on both the interpretation provided by
112 the genetic testing laboratory that performed testing, as well as the center's judgment.

113 Variant data in eight sarcomere genes (*ACTC1*, *MYBPC3*, *MYH7*, *MYL2*, *TNNI3*,
114 *TNNT2*, and *TPM1*), were downloaded from the SHaRe database (March 2015). To set
115 the SHaRe data in context, we also examined discordant classifications in ClinVar, using
116 data on the same genes (downloaded April 2015). To focus on clinical laboratories,
117 ClinVar submissions from OMIM and research laboratories were excluded. ClinVar
118 submitters included in this analysis: Laboratory for Molecular Medicine, GeneDx, LabCorp,
119 Blueprint Genetics, Children's Hospital of Eastern Ontario, Invitae, University of
120 Washington, Emory Genetics Laboratory, Genetic Services Laboratory, University of
121 Chicago, Neurogenetics Laboratory (Royal Perth Hospital).

122 Any individual variant that was seen by more than one HCM center or clinical
123 laboratory had the potential to be classified discordantly. A variant was considered to have
124 discordant classifications if the classifications from two or more groups crossed a major
125 classification category (i.e. likely pathogenic/pathogenic vs. variant of uncertain
126 significance; likely pathogenic/pathogenic vs. likely benign/benign, variant of uncertain
127 significance vs. likely benign/benign). Classifications that differed only by degree of
128 confidence within the same major classification category were considered concordant (i.e.
129 likely pathogenic vs. pathogenic, likely benign vs. benign). The frequency of discordant
130 classifications was calculated by dividing the number of variants with discordant
131 classifications by the total number of variants with classifications by more than one group
132 (Figure 1). Fleiss' kappa was used to assess inter-rater reliability, a modification of
133 Cohen's kappa for three or more reviewers¹².

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

140 The reasons for discordance in SHaRe were assessed by comparing the rationale
141 provided by each SHaRe center to justify their classification of that variant. These data
142 was available for 20/23 discordant variants (the other three became concordant upon the
143 centers' review of their initial classification).

144 To assist in resolution of discordance among SHaRe centers, each center was
145 provided with up-to-date summaries of all available data on each discordant variant
146 reported by their center and asked to reassess their classification and provide a rationale.
147 To assess why discordance remained after these reclassifications we compared centers'
148 rationales for their final classification and examined the data available on each variant.
149 This included an assessment of the number of data points suggesting the variant may be
150 benign, which included co-occurrence with another likely pathogenic or pathogenic variant
151 in $>1\%$ of cases², presence in reference samples with $MAF > 0.00004$ ¹³, failure to
152 segregate, and occurrence with other phenotypes.

153

154 **RESULTS**

155 **Discordance in SHaRe is lower than in ClinVar**

156 Participants in SHaRe had 589 unique sarcomere variants, of which 112 were seen
157 by more than one center (Figure 1). Discordant classifications were present in 23 of these
158 variants (20.5%; Fleiss' kappa 0.54, 95% CI 0.38-0.69). To contextualize this rate of
159 discordance we compared it to the rate of discordance among clinical laboratories in

160 ClinVar. ClinVar contains 2,405 sarcomere variants, of which 695 variants were submitted
161 by more than one laboratory and 314 were discordant (45.2%; Fleiss' kappa 0.36, 95% CI
162 0.30-0.42; $p < 0.001$ for the comparison between SHaRe and ClinVar discordance). In both
163 ClinVar and SHaRe, most discordant classifications were clinically significant (SHaRe:
164 19/23, 82.6%; ClinVar: 229/314, 72.9%; $p=0.75$).

165 **Discordance is often due to lack of data sharing and outdated data**

166 Comparison of the rationale for initial variant classifications provided by SHaRe
167 centers revealed that most variants had more than one reason for discordance (mean 2.5,
168 standard deviation 1.4). The most common reason for discordance was differential access
169 to privately held data (15/20, 75%), from either the SHaRe center's clinical experience
170 (12/20, 60%) or the genetic testing laboratory's internal data (12/20, 60%) (Figure 2). This
171 most frequently involved co-occurrence of the discordant variant with another pathogenic
172 variant, suggesting the discordant variant may be benign. This occurred for 11/20
173 discordant variants (55%); in four of those cases that data was held only by a SHaRe
174 center, in another four it was held only by the laboratory that did the testing, and in the
175 remaining three it was held by both the testing laboratory and SHaRe center. For 7/20
176 discordant variants (35%) the SHaRe centers differed in their access to segregation data.
177 In five cases the segregation data was privately held by the SHaRe center, in two cases it
178 was held by the laboratory that did the genetic testing and in no cases was it held by both
179 the testing laboratory and the SHaRe center. Data that was not available to all SHaRe
180 centers included several types of data that suggest the variant could be benign: being
181 seen with inconsistent phenotypes (4/20, 20%), cases being from the same ancestry
182 without ancestry matched controls (1/20, 5%), and presence in reference samples (1/20,
183 5%). Consistent with the impact of differential access to data, more than half of the HCM
184 cases associated with the discordant variants were not publicly available; 125 cases were

185 published or publicly available while 193 were only available in a private dataset that not
186 all centers initially had access to (Table 1).

187 In some cases the centers disagreed in their classifications because the publicly
188 available data that they used differed, including citing different literature (9/20, 45%),
189 predictions from in silico models (5/20, 25%), population frequencies (3/20, 15%), and
190 assessments of evolutionary conservation (3/20, 15%) (Figure 2). This sometimes
191 occurred because the SHaRe centers' initial classifications were done at different times, so
192 some centers had classified the variant using data that was now outdated.

193 For nearly a third of discordant variants SHaRe centers cited one or more data point
194 that was identical, but was interpreted differently by different centers (Figure 2). For
195 example, for p.Gly490Arg (c.1468G>A) in *MYBPC3*, three sites were aware the variant
196 had been seen in cases that had another variant that was deemed pathogenic. One site
197 used that to reach a variant of uncertain significance classification while the other two sites
198 classified the variant as likely pathogenic or pathogenic despite that data.

199 **Partial resolution of discordance can be achieved through data sharing**

200 When SHaRe centers were asked to provide their rationale for their initial
201 classification to help illuminate sources of discordance, three centers changed initial
202 classifications based on review of the data the center already had on the variant in light of
203 their current approach to classifications. This resolved discordance for three of the 23
204 (13%) initially discordant variants (Figure 3A, Supplemental Table 1).

205 To resolve the remaining discordance in SHaRe, we compiled up-to-date
206 comprehensive summaries of the data on the 20 remaining discordant variants, including
207 both publically available data and data privately available to each center (Table 1). Each
208 SHaRe center was asked to review a detailed narrative summary of this data and provide
209 an updated classification for their discordant variants. This reduced discordance further,
210 from 23/112 initially (20.5%) to 12/112 (10.7%) (Figure 3). Nearly all of the remaining

211 discordant classifications were clinically significant (11/12, 91.7%). Most of these were
212 variant of uncertain significance vs. likely pathogenic (7/12, 58.3%) or pathogenic (2/12,
213 8.3%) (Table 1, Supplemental Table 1). Two were likely benign or benign vs. likely
214 pathogenic (2/12, 16.7%). For seven of the twelve variants that remained discordant, at
215 least one center changed their classification, yet that reclassification did not resolve
216 discordance (Supplemental Table 1). There were no reclassifications in the other five
217 variants.

218 To gain insight into why discordance was not completely resolved despite the
219 SHaRe centers having access to the same data, we examined the data gathered on the
220 20 discordant variants the centers were asked to re-assess (Table 1). We also compared
221 HCM centers' rationales for their final classifications. Note that complete data on rationale
222 for final classifications was only available for 11 of 12 variants that remained discordant.

223 Among the variants that reached concordance, none of the variants reclassified to
224 likely pathogenic or pathogenic had evidence suggesting they may be benign. In contrast,
225 all of the variants reclassified to likely benign or benign had evidence suggesting they may
226 be benign, with a mean of 2.7 types of benign evidence per variant. The variants that
227 remained discrepant had a mean of 1.8 types of benign evidence per variant, suggesting
228 the data on these variants was more conflicted and did not point as clearly towards a
229 benign or pathogenic classification.

230 Notably, in nearly two-thirds of variants that remained discordant at least one center
231 remarked in their rationale that they suspect the variant is a modifier (7/11, 63.6%).
232 Consistent with this, these variants had features typically associated with modifying
233 variants; most of these variants had co-occurred with a pathogenic variant (9/12, 75%) and
234 were present in reference samples (11/12, 91.7%). This contrasts to the variants with
235 resolved discordance in which a minority had co-occurred with a pathogenic variant (3/8,
236 37.5%) and only half had been seen in reference samples (4/8, 50.0%). Among the

237 variants that were seen in references samples, the mean minor allele frequency was
238 higher for those with resolved discordance (0.11, three of four reclassified to (likely)
239 benign) than those with unresolved discordance (0.0021). It is also notable that 8 of 11
240 variants with resolved discordance were missense while all variants with unresolved
241 discordance (12/12) were missense, consistent with greater challenges in classifying
242 missense variation and their potential role as modifiers.

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

248

249 **DISCUSSION**

250 As genetic testing for inherited cardiovascular conditions such as HCM has become
251 common place, there has been increasing awareness of the complexity of genetic test
252 interpretation and the not infrequent occurrence of clinically impactful differences in the
253 classification of variants. While prior studies have examined differences in interpretation
254 among laboratories, the current study dissects differences in the interpretations used by
255 clinical centers, where genetic testing data is translated into patient care. Our data show
256 disagreements in classification are far less frequent within the setting of specialized HCM
257 centers with expertise in disease management, phenotypic and family assessment, and
258 understanding of the genetic architecture of HCM.

259 The initial rate of disagreement in variant classification among SHaRe centers
260 (20.5%) is at the lower range of the rate of disagreement among laboratories reported to
261 date (17-53%)⁷⁻¹⁰ and is less than half that seen in ClinVar for the same set of sarcomere
262 genes (45.2%) (Figure 3C). Furthermore, half of disagreements among HCM centers were

263 resolved via sharing of comprehensive up to date data. The rate of discordance in SHaRe
264 after efforts to resolve disagreements, 10.7%, is the lowest yet reported. These data
265 suggest that the complexities of genetic test interpretation are best addressed within the
266 context of specialized centers leveraging the benefits of data-sharing. Consistent with our
267 finding that discordance is lower when genetic testing occurs in the context of a
268 specialized center, professional societies have recommended that genetics evaluations for
269 heritable cardiomyopathies be carried out in such specialized centers⁴.

270 The lower rate of discordance among HCM centers as compared to clinical
271 laboratories could have several different origins, including selection of patients for genetic
272 testing, benefits of comprehensive family-based genetic evaluations, and application of
273 expertise in HCM. The patients included in SHaRe all have clear diagnoses of HCM. This
274 is in contrast to the sample of patients who undergo genetic testing at clinical laboratories,
275 which includes those with clear diagnoses as well as those with borderline or questionable
276 diagnoses. Prior studies in various genetic conditions have shown that the yield of genetic
277 testing is lower in patients referred to clinical laboratories than in studies of patients with
278 firm diagnoses^{14,15}. The current study demonstrates that not only is yield lower in the
279 heterogeneous samples seen in the clinical laboratory setting, but discordance is higher.
280 The comprehensive genetics evaluations provided by HCM centers is another potential
281 sources of lower discordance among those centers. The genetic evaluations performed by
282 these centers goes beyond genetic testing on the index patient to include analysis of 3-4
283 generation pedigrees, phenotyping and genotyping of family members, and expert
284 assessment for genocopies and phenocopies. The impact of these evaluations is evident
285 in the effect that data generated by individual centers had on classifications and
286 discordance. Centers' classifications arose not only from the data provided by the genetic
287 testing laboratory or published in the literature but from the centers' own clinical
288 evaluations, such as segregation analyses performed by the center. The lower rate of

289 discordance among HCM centers may also arise from application of these centers'
290 expertise in disease-specific genetic variation. In their 2015 guidelines on sequence
291 variant interpretation, the American College of Medical Genetics and Genomics specifically
292 pointed to the need for gene-disease specific classification criteria⁵. Prior studies have
293 demonstrated the impact of gene-disease expertise on variant classification. Comparing
294 variant classifications by a laboratory specialized in connective tissue disorders to other
295 laboratories, Pepin et al (2015) found that in a third of cases the other laboratory failed to
296 factor in key aspects of protein structure and function that significantly impact
297 classification¹⁶. Amendola et al (2016) found that differences in gene-disease expertise
298 contributed to differences in classification among laboratories in the Clinical Sequencing
299 Exploratory Research (CSER) consortium⁹. Thus the gene-disease expertise of
300 specialized HCM centers along with the comprehensive genetics evaluations they perform,
301 and the selection of patients for genetic testing may all contribute to optimization of genetic
302 test interpretation and minimization of discordance.

303 Our data also speak to the importance of data sharing, both between clinicians and
304 laboratories regarding an individual case and between individuals laboratories or centers
305 and the broader community. The most frequent reason for discordance within SHaRe was
306 lack of data sharing at the point of initial classification; the majority of classification
307 differences occurred, at least in part, because SHaRe centers had access to differing
308 private datasets. Data-sharing is particularly critical for a disease like HCM that is
309 characterized by such marked genetic heterogeneity; 56% of variants found on HCM
310 genetic testing by one laboratory were seen in just a single family². This makes it
311 challenging for any one laboratory or center to accumulate enough data to determine the
312 appropriate classification for such variants, particularly when they are missense. Data-
313 sharing efforts like SHaRe, ClinVar, and gnomAD (Genome Aggregation Database,
314 <http://gnomad.broadinstitute.org/>, formerly ExAC) are shifting variant classifications and

315 impacting clinical care⁶. There is debate about whether it should be mandatory for the
316 ordering clinician to share clinical data with the laboratory and the laboratory to share data
317 with the community through efforts such as ClinVar. Our data suggests that such sharing
318 would improve variant classifications and have clinical benefit.

319 A subset of discordance in SHaRe (13%) was resolved simply by the center
320 reconsidering the data they already had on the variant in light of current knowledge and
321 classification practices. An additional 35% of discordance was resolved by review of
322 current up to date data. This underscores the importance of ongoing re-review of variant
323 interpretations. Similarly, Das et al (2014) found that re-review of the pathogenic, likely
324 pathogenic, and uncertain variants in their HCM center lead to clinically impactful
325 reclassifications in 10% of their patients with variants¹⁷. The vast majority of laboratories
326 do not consistently re-review variants, typically only doing so if they observe the variant
327 again. Given the marked genetic heterogeneity in HCM this means that it may be many
328 years before a variant is re-reviewed and a substantial subset will never be re-reviewed.
329 Yet both our data and prior studies^{17,18} show that re-review can lead to changes in
330 classification that impact medical care in an appreciable subset of variants.

331 Despite basing revised variant classifications on identical data, 10.7% of variants
332 seen by more than one center in SHaRe remained discordant. This residual discordance
333 appears to be attributable to differences in expert opinion when the available data are
334 subjective and conflicted, particularly whether the variant is sufficiently rare and whether it
335 is seen too often in tandem with a pathogenic variant. Further pointing to the impact of
336 differences in expert opinion is our observation that a third of initial discordance in SHaRe
337 was at least partially attributable to differing interpretations of the same data. It is possible
338 that some of the residual discordance could be resolved by agreeing upon and using
339 identical classification criteria, such as cut offs for rarity and co-occurrence. Work is
340 underway within the ClinGen Cardiovascular Domain Working Group to develop disease

341 and gene specific guidance on matters like rarity and co-occurrence⁷
342 (<https://www.clinicalgenome.org/working-groups/clinical-domain/sub->
343 [groups/cardiovascular/](https://www.clinicalgenome.org/working-groups/clinical-domain/sub-groups/cardiovascular/)). These guidelines will be informed by insights into rarity of
344 pathogenic variation for a given gene and disease provided by analyses of large disease
345 and reference datasets¹³ and frequency of co-occurrence in large disease cohorts². While
346 such guidelines will undoubtedly improve variant interpretation, they may not completely
347 resolve discordance; Amendola et al (2016) found that even when laboratories used
348 identical classification criteria and identical data, discordance remained in a substantial
349 subset of variants⁹. Another possible explanation for the unresolved discordance in SHaRe
350 is suggested by the fact that in nearly two-thirds of variants that remained discordant at
351 least one center suspected the variant was a modifier. This is a class of variation that is
352 poorly understood and is not accounted for in existing classification guidelines. Given the
353 limitations of our current knowledge and guidelines, experts sometimes need to make
354 judgment calls in interpreting variant data and as such a certain amount of discordance will
355 remain due to differences in expert opinion. Moreover, discordant interpretation of clinical
356 data is not unique to genetic testing. Comparable rates of disagreement have been
357 reported across a range of medical specialties and tests including assessment of
358 ventricular tachycardia on ECGs (22%)¹⁹, subtyping of sarcoma on histopathology
359 (27%)²⁰, and assessment of wall motion abnormalities on dobutamine stress tests (15%)²¹.
360 Such data has led some authors to recommend routine second opinions for some medical
361 tests²². Data sharing via efforts like ClinVar is now allowing for a passive form of second
362 opinion, in which laboratories and clinicians can check ClinVar to see how other groups
363 classify a variant.

364

365 **Clinical implications:**

Discordance in the classification of variants from DNA sequencing data occurs both between genetic testing laboratories and between clinical centers. Most of the disagreements in classification in both SHaRe and ClinVar would affect clinical management of the patient and/or family, highlighting the importance of the current challenges with variant interpretation. This raises the question of how clinical teams should be handling variant classifications, particularly when there are disagreements in classification. Concerns regarding these differences 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 testing²³. In many ways this parallels how these teams often handle cardiac testing; in addition to reviewing reports for cardiac imaging they also look closely at the primary imaging data and interpret them independently. The need for periodic re-review of classifications suggested by our data and prior studies may provide further justification for clinicians taking greater responsibility for ongoing genetic test interpretation, since such re-review is currently not common practice among laboratories^{17,18}. Finally, the greater level of agreement among HCM centers than among clinical laboratories suggests genetic testing for HCM is best done in the setting of an expert center.

383

384 **CONCLUSION**

Disagreement in the interpretation of genetic test results exists among genetic testing laboratories and clinical HCM centers. Moreover, most of these disagreements are of sufficient magnitude to impact clinical utilization of the test results. The majority of disagreements are due to privately held or outdated data, which can be ameliorated by increased data sharing and periodic re-review 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

392 in HCM management and genetic testing was significantly lower than that seen between
393 clinical genetic testing laboratories. These findings highlight the important benefits that can
394 be achieved when expertise in disease management and family evaluations is combined
395 with expertise in genetic interpretation.

396 **ACKNOWLEDGEMENTS**

397 We thank James Signorovitch for statistical consultation.

398 **FUNDNG:**

399 The SHaRe consortium is funded by an unrestricted research grant from MyoKardia
400

401 **CONFLICT OF INTEREST DISCLOSURE:**

402 Colleen Caleshu: Consultant, Advisor – Recombine; Advisor – Invitae; Advisor –
403 Phosphorous.

404 Eric Green: Employee of and owns shares in MyoKardia, Inc.

405 Euan Ashley: Ownership interest, Advisor – Personalis Inc, Advisor - SequenceBio

406 Aisha Furqan, Patricia Arscott, Francesca Girolami, Allison L Cirino, Michelle Michels,

407 Sharlene M Day, Iacopo Olivotto, Carolyn Y Ho: None

408 **REFERENCES**

- 409 1. Maron BJ, Casey S a, Poliac LC, Gohman TE, Almquist a K, Aeppli DM. Clinical
410 course of hypertrophic cardiomyopathy in a regional United States cohort. *JAMA*.
411 1999;281:650–655.
- 412 2. Alfares AA, Kelly MA, McDermott G, Funke BH, Lebo MS, Baxter SB, et al. Results
413 of clinical genetic testing of 2,912 probands with hypertrophic cardiomyopathy:
414 expanded panels offer limited additional sensitivity. *Genet Med*. 2015;17:880–888.
- 415 3. Gersh B, BJ Maron, RO Bonow, JA Dearani, MA Fifer, MS Link, et al. 2011
416 ACCF/AHA guideline for the diagnosis and treatment of hypertrophic
417 cardiomyopathy. *J Thorac Cardiovasc Surg*. 2011;142:e153–e203.
- 418 4. Ackerman MJ, Priori SG, Willems S, Berul C, Brugada R, Calkins H, et al.

HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). *Heart Rhythm*. 2011;8:1308–39.

5. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405–423.
6. Lek M, Karczewski K, Minikel E, Samocha K, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016;536:285–91.
7. Rehm HL, Berg JS, Brooks LD, Bustamante CD, Evans JP, Landrum MJ, et al. ClinGen - The Clinical Genome Resource. *N Engl J Med*. 2015;372:2235–42.
8. Balmana J, Digiovanni L, Gaddam P, Walsh MF, Joseph V, Stadler ZK, et al. Conflicting interpretation of genetic variants and cancer risk by commercial laboratories as assessed by the prospective registry of multiplex testing. *J Clin Oncol*. 2016;34:4071–4078.
9. Amendola LM, Jarvik GP, Leo MC, McLaughlin HM, Akkari Y, Amaral MD, et al. Performance of ACMG-AMP Variant-Interpretation Guidelines among Nine Laboratories in the Clinical Sequencing Exploratory Research Consortium. *Am J Hum Genet*. 2016;98:1067–1076.
10. Amendola LM, Dorschner MO, Robertson PD, Salama JS, Hart R, Shirts BH, et al. Actionable exomic incidental findings in 6503 participants: Challenges of variant classification. *Genome Res*. 2015;25:305–315.
11. Garber KB, Vincent LM, Alexander JJ, Bean LJH, Bale S, Hegde M. Reassessment of Genomic Sequence Variation to Harmonize Interpretation for Personalized Medicine. *Am J Hum Genet*. 2016;99:1140–1149.

- 445 12. Fleiss JL. Measuring nominal scale agreement among many raters. *Psychol. Bull.*
446 1971;76:378–382.
- 447 13. Whiffin N, Minikel E, Walsh R, O'Donnell-Luria A, Karczewski K, Ing AY, et al. Using
448 high-resolution variant frequencies to empower clinical genome interpretation.
449 *bioRxiv*. 2016. 10.1101/073114.
- 450 14. Napolitano C, Priori SG, Schwartz PJ, Bloise R, Ronchetti E, Nastoli J, et al. Genetic
451 testing in the long QT syndrome: development and validation of an efficient
452 approach to genotyping in clinical practice. *JAMA*. 2005;294:2975–2980.
- 453 15. Kapplinger JD, Tester DJ, Salisbury BA, Carr JL, Harris-Kerr C, Pollevick GD, et al.
454 Spectrum and prevalence of mutations from the first 2,500 consecutive unrelated
455 patients referred for the FAMILION long QT syndrome genetic test. *Hear Rhythm*.
456 2009;6:1297–1303.
- 457 16. Pepin MG, Murray ML, Bailey S, Leistritz-Kessler D, Schwarze U, Byers PH. The
458 challenge of comprehensive and consistent sequence variant interpretation between
459 clinical laboratories. *Genet Med*. 2015;1–5.
- 460 17. Das K J, Ingles J, Bagnall RD, Semsarian C. Determining pathogenicity of genetic
461 variants in hypertrophic cardiomyopathy: importance of periodic reassessment.
462 *Genet Med*. 2014;16:286–293.
- 463 18. Aronson SJ, Clark EH, Varugheese M, Baxter S, Babb LJ, Rehm HL.
464 Communicating new knowledge on previously reported genetic variants. *Genet Med*.
465 2012;14:713–719.
- 466 19. Herbert ME, Votey SR, Morgan MT, Cameron P, Dziukas L. Failure to agree on the
467 electrocardiographic diagnosis of ventricular tachycardia. *Ann Emerg Med*.
468 1996;27:35–38.
- 469 20. Presant BCA, Russell WO, Alexander RW, Fu YS. Soft-Tissue and Bone Sarcoma
470 Histopathology Peer Review: The Frequency of Disagreement in Diagnosis and the

Need for Second Pathology Opinions The Southeastern Cancer Study Group
Experience. *J Clin Oncol*. 1986;4:1658–1661.

21. Paetsch I, Jahnke C, Ferrari VA, Rademakers FE, Pellikka PA, Hundley WG, et al.
Determination of interobserver variability for identifying inducible left ventricular wall
motion abnormalities during dobutamine stress magnetic resonance imaging. *Eur*
Heart J. 2006;27:1459–1464.

22. Al-Ibraheemi A, Folpe AL. Voluntary Second Opinions in Pediatric Bone and Soft
Tissue Pathology: A Retrospective Review of 1601 Cases From a Single
Mesenchymal Tumor Consultation Service. *Int J Surg Pathol*. 2016;24:685–691.

23. Reuter C, Grove ME, Orland K, Spoonamore K, Caleshu C. Abstract: Clinical
cardiovascular genetic counselors take a leading role in team-based variant
interpretation. *J Genet Couns*. 2016;25:1347–1472.

Disclaimer: The manuscript contents are
confidential, intended for journal review purposes
only, and not to be further disclosed.

494 **TABLES AND FIGURES:**

495 **Table 1. Discordant variants in SHaRe**

496 **A. Variants with Discordance Resolved**

497

Gene	Variant	Reclass- ification	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.1004G>C	VUS to LB	0	4			0	0	0	

498
499
500
501

MYH7	p.Arg1420Trp (c.4258C>T)	VUS to LP	0	11		0	0	0.000015	European (ExAC)
------	-----------------------------	-----------	---	----	--	---	---	----------	--------------------

B. Variants with Discordance Unresolved

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 v LB	3	10	DCM, LVNC, WPW	4/10	1	0	0.0013	European (ExAC)
MYH7	p.Met982Thr (c.2945T>C)	LP v B	3	19	DCM, increased LVWT, SCD with dilatation	7/19	1	0	0.0013	European (ExAC)
MYH7	p.Asn1327Lys (c.3981C>A)	VUS v LB	2	14		2/12	1	0	0.018	Ashkenazi (LMM)
MYH7	p.Lys1459Asn (c.4377G>T)	LP v VUS	3	15	Ebstein's, Brugada	2/9	1	0	0.00051	European (ExAC)

MYH7	p.Arg1606Cys (c.4816C>T)	LP v VUS	0	2		0	0	0.0000077	European (ExAC)	
MYH7	p.Arg204His (c.611G>A)	LP v VUS	1	20		2/20	1	0	0	
MYBPC3	p.Arg810His (c.2429G>A)	LP v VUS	2	29		5/27	5	0	0.00006	European (ExAC)
MYBPC3	p.Arg1002Gln (c.3005G>A)	LP v VUS	2	3	DCM, giant RA & arrhythmia		1	0	0.00012	East Asian (ExAC)
MYBPC3	p.Gly531Arg (c.1591G>C)	LP v VUS	1	3		1/3	2	0	0.000031	European (ExAC)
MYBPC3	p.Gly490Arg (c.1468G>A)	LP v VUS	2	10		4/8	2	0	0.00045	Finnish (ExAC)
TNNT2	p.Arg278Cys (c.832C>T)	P v VUS	2	51	DCM	5/47	8	0	0.0016	Other (ExAC)
MYH7	p.Thr1377Met (c.4130C>T)	P v VUS	0	25			0	0	0.000029	European (pooled)

502

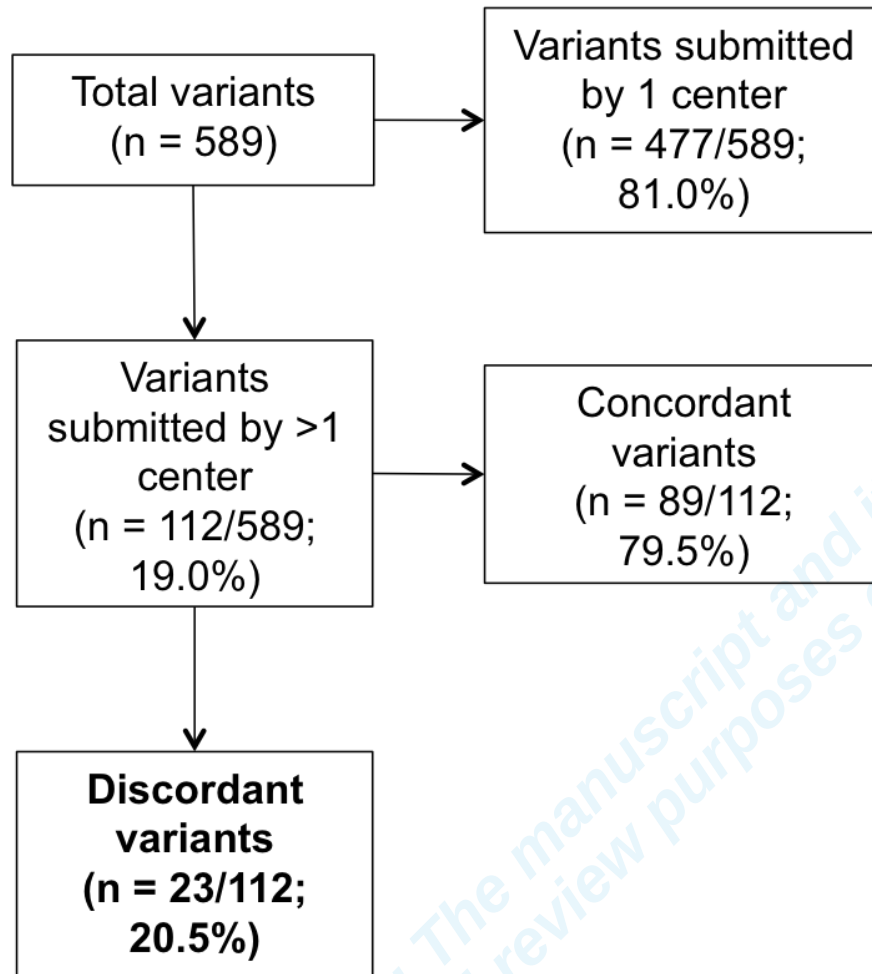
503 Summary data on SHaRe discordant variants that became concordant after reassessment (A) and those that remained discordant (B). VUS = variant of uncertain

504 significance, LP = likely pathogenic, P = pathogenic, LB = likely benign, B = benign. MAF = minor allele frequency. ExAC = Exome Aggregation Consortium

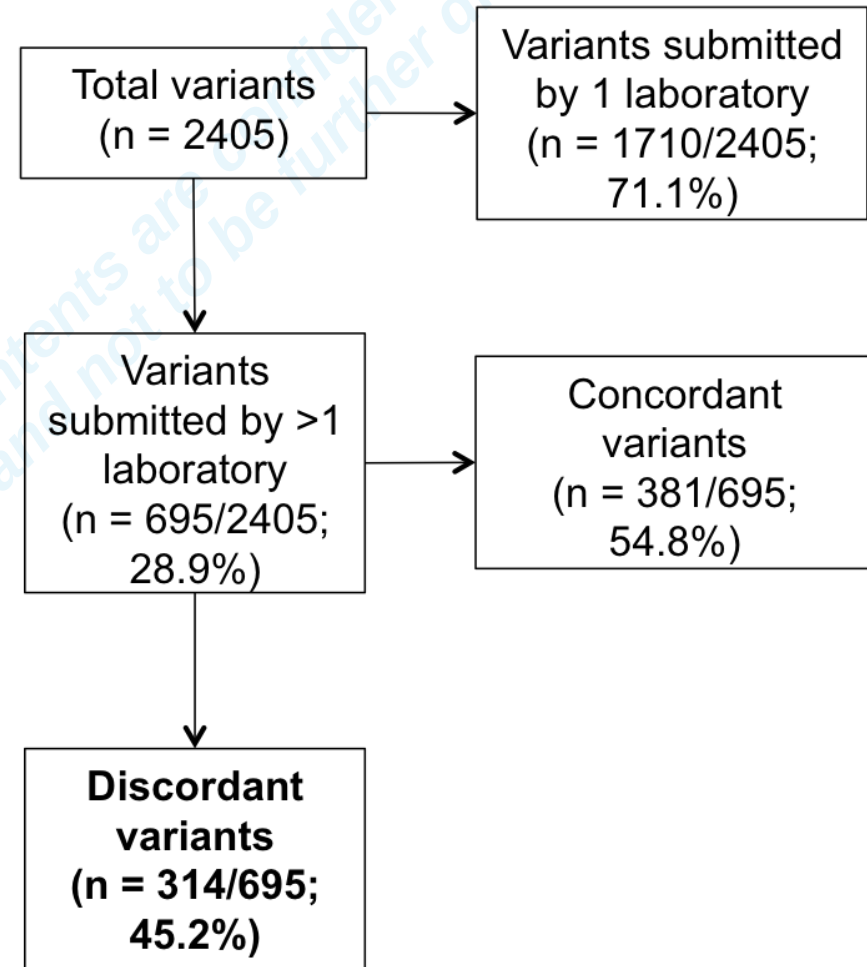
505 (exac.broadinstitute.org). DCM = dilated cardiomyopathy, SIDS = sudden infant death syndrome, LVNC = left ventricular non-compaction, WPW = Wolff-Parkinson-

506 White, LVWT = left ventricular wall thickness, SCD = sudden cardiac death, RA = right atrium. * Note that comprehensive data was not gathered on the three variants
507 that became concordant when the HCM centers reviewed the rationales for their initial classifications.

A: SHaRe



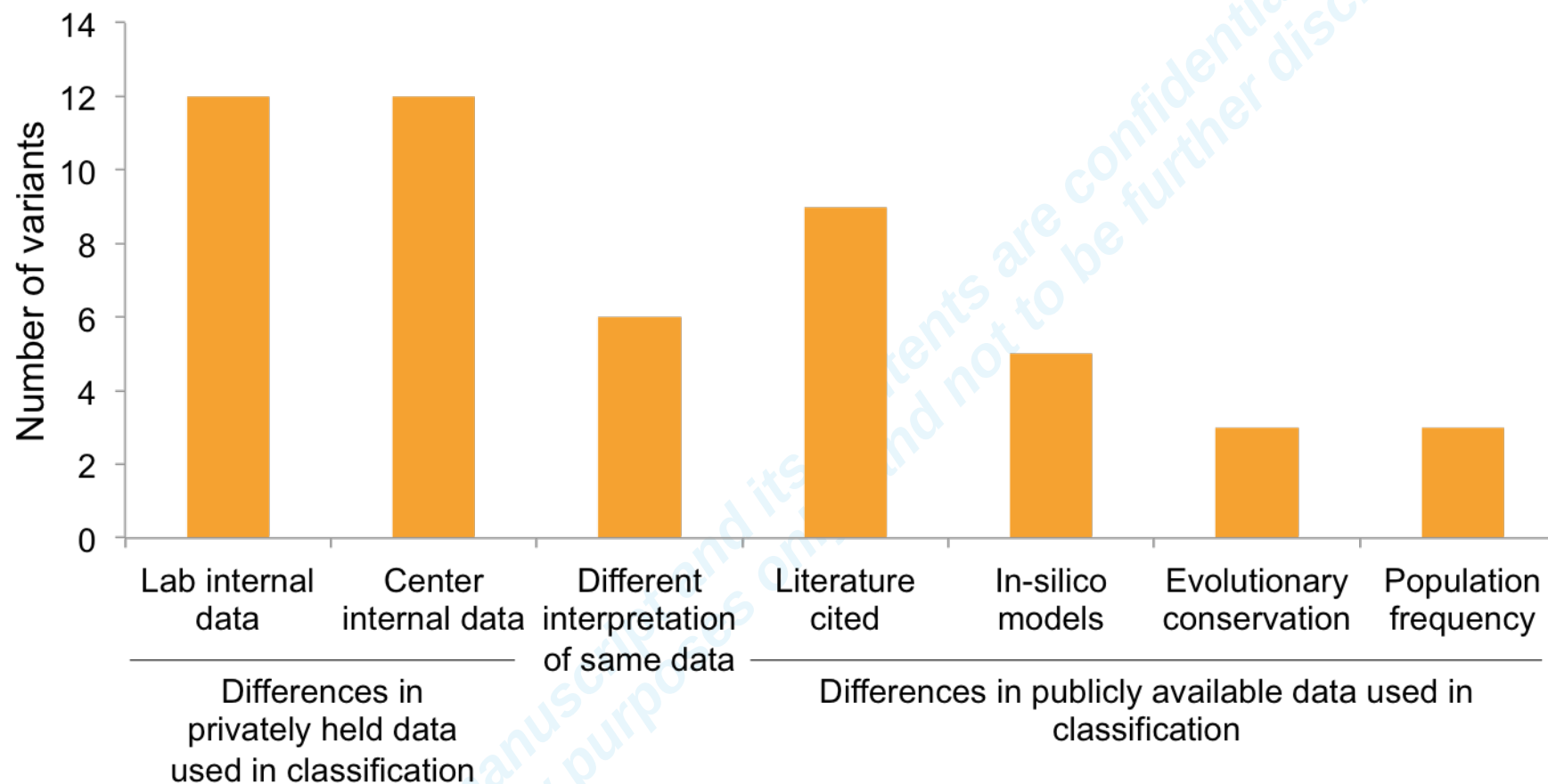
B: ClinVar



509 **Figure 1. Assessment of discordance in SHaRe and ClinVar.** Variants for 8 sarcomere genes were downloaded from SHaRe
510 (A) and ClinVar (B). Variants with classifications from >1 SHaRe center or ClinVar submitter were identified. Classifications were
511 compared across centers or submitters to assess discordance.
512

Disclaimer: The manuscript and its contents are confidential, intended for journal review purposes only, and not to be further disclosed.

513



514

515

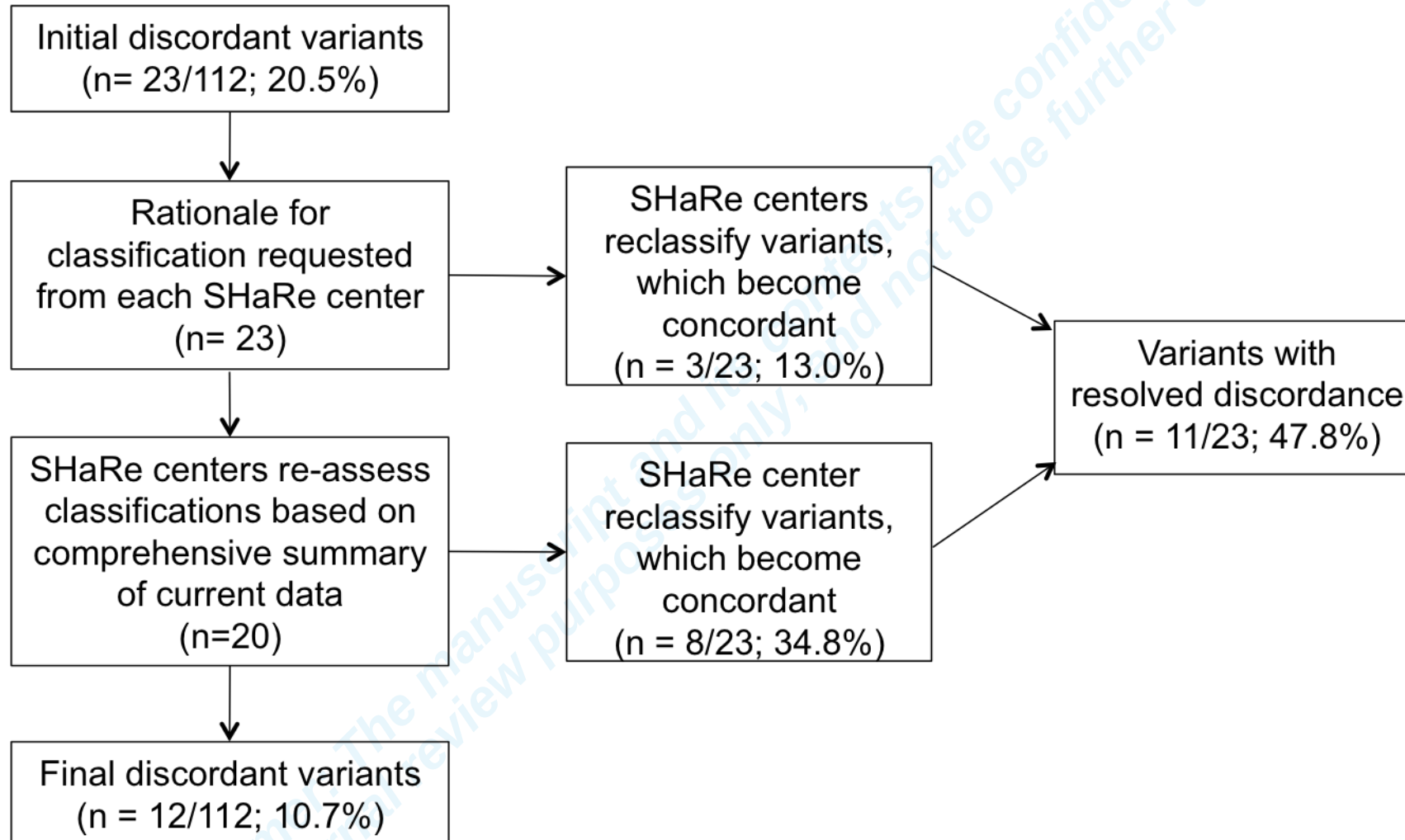
Figure 2. Reasons for initial discordance among SHaRe centers. Reasons for discordance were assessed by comparing the

516

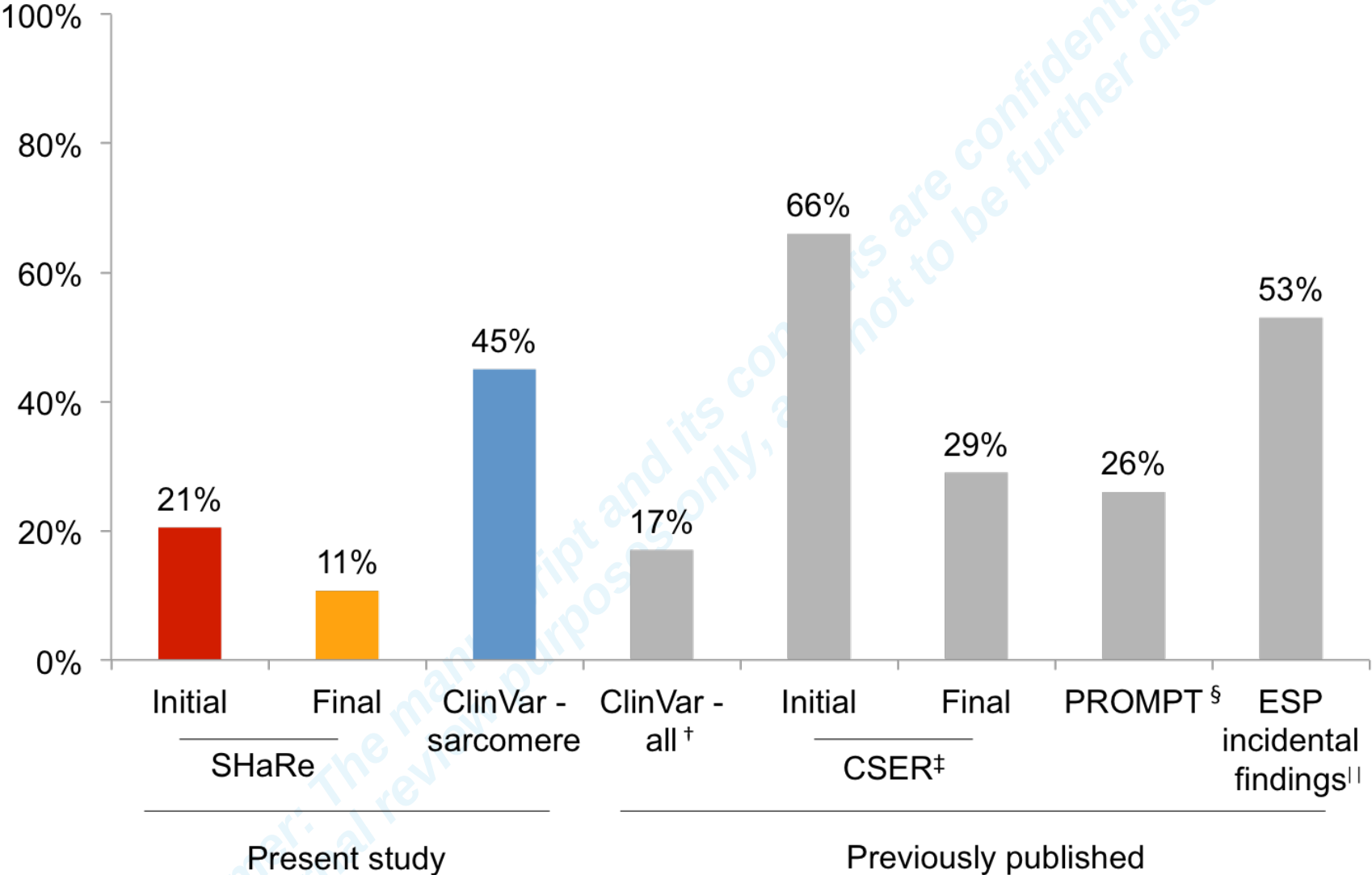
rationales for each center's classifications.

517

A: Resolution of discordance in SHaRe



C: Discordance Rates in SHaRe, ClinVar, and Prior Publications



520 **Figure 3. Reclassification of SHaRe variants and resolution of discordance. A:** Process of reclassification of variants and
521 (partial) resolution of discordance in SHaRe. *When SHaRe centers pulled their rationale for their initial classification, three centers
522 changed their classification given their current classification methods. **B:** Initial and final classifications in SHaRe, shown for
523 variants that became concordant after reclassification (left) and those that remained discordant (right). Each line represents one
524 center's classifications of one variant. Centers are designated by line color (see legend). **C:** Discordance rates in SHaRe and
525 ClinVar from the present study and previously published discordance rates. [†]Discordance in ClinVar across all genes⁷.
526 [‡]Discordance in classification of select variants studied by CSER (Clinical Sequencing Exploratory Research Consortium), before
527 and after efforts to reduce discordance⁹. [§]Discordance rate among clinical laboratories on variants in cancer genes submitted to
528 PROMPT (Prospective Registry of Multiplex Testing)⁸. ^{||}Discordance between reviewers of potentially actionable incidental findings
529 in ESP (Exome Sequencing Project)¹⁰. VUS = variant of uncertain significance, LP = likely pathogenic, P = pathogenic, LB = likely
530 benign, B = benign. SHaRe centers: Stanford University (STU), Brigham and Women's Hospital (BWH), University of Michigan
531 (UMH), Erasmus University (ERA), and Careggi University (FLO).

534 **Supplemental Table 1. initial and final classifications of discordant variants in SHaRe**

535 **A. Discordance resolved**

Gene	Variant	BWH		FLO		ERA		STU		UMH	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
MYBPC3	p.Val189Ile (c.565G>A)	LB	LB					VUS	LB	LB	LB
MYBPC3	p.Ser217Gly (c.649A>G)	LB	LB					VUS	LB	LB	B
MYBPC3	c.927-9G>A	P	P			VUS	P	LP	P	P	P
MYBPC3	p.Pro371Arg (c.1112C>G)	VUS	VUS	LP	VUS						
MYBPC3	c.1224-2A>G					VUS	LP	LP	LP		
MYBPC3	p.Asp605Asn	VUS	VUS							P	VUS

	(c.1813G>A)*										
MYBPC3	p.Ser858Asn	LP	VUS					VUS	VUS		
	(c.2573G>A)*										
MYBPC3	p.Gln998Glu							VUS	LB	B	B
	(c.2992C>G)										
MYH7	p.Arg1420Trp			P	LP					VUS	LP
	(c.4258C>T)										
TNNT2	p.Trp287*	LP	LP	VUS	P			P	P		
	(c.860G>A)*										
TPM1	p.Glu192Lys	LP	LP					VUS	LP		
	(c.574G>A)										

536

537 **B. Discordance unresolved**

Gene	Variant	BWH		FLO		ERA		STU		UMH	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
MYBPC3	p.Gly490Arg			LP	LP	P	LP			VUS	VUS

	(c.1468G>A)								
MYBPC3	p.Gly531Arg	VUS	VUS	LP	LP				
	(c.1591G>C)								
MYBPC3	p.Glu619Lys	VUS	LB	LP	LP			LB	LB
	(c.1855G>A)								
MYBPC3	p.Arg810His	VUS	VUS	LP	LP		VUS	VUS	VUS
	(c.2429G>A)								LP
MYBPC3	p.Arg1002Gln	VUS	VUS	P	LP				
	(c.3005G>A)								
MYH7	p.Arg204His	VUS	VUS	VUS	P		LP	LP	LP
	(c.611G>A)								
MYH7	p.Met982Thr	VUS	LB	LP	LP			VUS	B
	(c.2945T>C)								
MYH7	p.Asn1327Lys	LB	LB				VUS	VUS	VUS
	(c.3981C>A)								
MYH7	p.Thr1377Met	VUS	VUS	P	P			LP	LP

	(c.4130C>T)										
MYH7	p.Lys1459Asn			VUS	LP	P	LP	LP	VUS	VUS	VUS
	(c.4377G>T)										
MYH7	p.Arg1606Cys					VUS	LP			LP	VUS
	(c.4816C>T)										
TNNT2	p.Arg278Cys	VUS	VUS	LP	P	P	LP	LP	VUS	P	VUS
	(c.832C>T)										

538 The 23 variants in SHaRe with discordant classifications. Each center's initial and final classification is noted. VUS = variant of
539 uncertain significance, LP = likely pathogenic, P = pathogenic, LB = likely benign, B = benign. SHaRe centers: Stanford University
540 (STU), Brigham and Women's Hospital (BWH), University of Michigan (UMH), Erasmus University (ERA), and Careggi University
541 (FLO). *Discordance in these three variants was resolved when the centers reviewed their initial classifications and reclassified
542 these variants based on the data they already possessed (Figure 3A). The remaining variants were re-assessed based on review of
543 all data currently available to the authors.