CONCORDANCE IN CLASSIFICATION OF HYPERTROPHIC CARDIOMYOPATHY VARIANTS IS MARKEDLY HIGHER AMONG EXPERT CENTERS THAN AMONG CLINICAL LABS

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of Master of Science in Genetic Counseling

By
Aisha Furqan
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CERTIFICATION OF APPROVAL

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DEDICATION

To those who refuse to give up: Malala Yousafzai and Muhammad Jibran Nasir.

“One child, one teacher, one book, one pen can change the world.” – Malala Yousafzai, Pakistani Nobel Prize laureate.

“Let us try and focus on making others happy by sharing their pain and burden and giving them hope for a better future as a tribute to those futures which we have failed to save.” – Muhammad Jibran Nasir, Pakistani civil rights activist.

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ABSTRACT

It has become increasingly clear that there are often marked differences in how variants in Mendelian disease genes are classified by different laboratories. Efforts to improve variant classification strategies will be aided by a thorough understanding of why differences in classifications exist. To gain such insights, we examined discordance in sarcomere variant classifications in SHaRe, a consortium of multiple international centers with expertise in cardiomyopathy genetics. We evaluated the frequency of disagreement in variant classifications between centers submitting to SHaRe and compared it to the frequency of disagreement in classifications among clinical laboratories submitting to ClinVar. The frequency of discordance in ClinVar was two and a half times higher than in SHaRe. We then assessed the severity of discordance as severe (i.e. (likely) benign vs. (likely) pathogenic), moderate (i.e. VUS vs. pathogenic or benign), or modest (i.e. VUS vs. likely benign or likely pathogenic). Sixteen of the 21 discordant variants from SHaRe were further analyzed for severity and reasons of discordance. The majority of discordant classifications in SHaRe were modest (9/16), while about half were moderate (159/314) in ClinVar. To identify the sources of discordance in SHaRe, we compared the data and rationale that each center used in making their classifications. Among the most frequent cause of discordance were differences in privately held data (testing lab –11/16; clinical center –10/16), in lit data used (8/16), and in interpretations of the same data (7/16). The less frequent discordance among cardiomyopathy genetics centers may be due to classification of variants by experts or due to more thorough follow-up of genetic findings including segregation analysis. Taken together, these data suggest that variant classification can be improved by evaluation at specialized centers. Our findings also underscore the importance of sharing of privately held data as this was a frequent cause of discordance.

CHAPTER I

INTRODUCTION

Problem Statement

Genetic counselors and geneticists ordering tests for their patients have increasingly become aware of difference in variant classifications among clinical laboratories. Discordance in variant classifications leads to differences in diagnosis and subsequently affects surveillance and treatment for the condition in patients and their family members.

According to a report published by the ClinGen initiative group, the discordance rate among unique variants (i.e. seen less than 10 times among ClinVar submitters) is ~17 percent in ClinVar (Heidi, 2015). In another recently published article, Pepin et al. (2015) identified discordance in variant classification of 27 out of 38 variants that were brought to their attention by genetic professionals consulting for heritable connective-tissue disorders.

In this study, we investigate the rates of discordance among sarcomere variant classifications in SHaRe, a consortium of multiple international centers with expertise in cardiomyopathy genetics, in order to understand why differences in classifications exist. Efforts to improve variant classification strategies will be aided by a thorough understanding of these reasons.

Overview Of The Disease

Hypertrophic cardiomyopathy (HCM) is characterized by unexplained left ventricular hypertrophy in the absence of pressure overload, systemic disease or

infiltrative processes. Histopathologic hallmarks of HCM include myocyte hypertrophy and disarray, microvascular remodeling, and fibrosis. HCM is present in approximately 1 in 500 individuals in the general population, and sarcomere mutations can be documented in approximately 32 percent of HCM cases (Alfares, 2015). Symptomatic patients with HCM commonly report effort intolerance associated with diastolic dysfunction and/or left ventricular outflow tract obstruction. HCM patients are at increased risk for adverse clinical events including overt heart failure, atrial fibrillation (causing both worsening of exertional symptoms and an increased risk for stroke), syncope, malignant ventricular arrhythmias and sudden cardiac death (SCD). SCD due to HCM is one of the most common non-injury related causes of death in young adults. Therefore, early diagnosis and continued surveillance and treatment can be life saving for many patients (Maron, 1995; Maron, 2002).

Genetics Of HCM

HCM is an autosomal dominant Mendelian disease caused by variants in the genes that encode the sarcomere, the fundamental unit of contraction in striated muscle. Disease penetrance is reduced (~57%) (Page, 2012). Disease severity and clinical course can be quite variable both within families and between families, even between those with the same mutation. The marked genetic heterogeneity and the high degree of variability in phenotypic expression have posed challenges in care for probands and their family members as well as in variant classification. The American College of Cardiology Foundation/American Heart Association practice guidelines include genetic testing using next-generation sequencing (NGS) as a reasonable

approach to the diagnosis of HCM (Gersh, 2011). Most cardiomyopathy panels include at least the following sarcomere genes: *ACTC1, MYBPC3, MYH7, MYL2, MYL3, TNNI3, TNNT2*, and *TPM1*.

Sarcomeric Human Cardiomyopathies Registry (SHaRe)

The SHaRe was created in 2014 in the wake of surge in sarcomeric variant data due to influx in NGS based genetic testing for cardiomyopathies (http://www.theshareregistry.org/). The aim of this registry is to provide a platform for cardiovascular geneticists and research-based cardiologists where detailed, longitudinal genetic and clinical data can be aggregated to allow critical analysis and comparisons of variant classification reports for discordant classifications among participating centers. SHaRe is populated by data from participants in already existing internal databases at HCM centers throughout the world. The data used in this study originated from the initial SHaRe contributors: Stanford University (STD), Brigham and Women's Hospital (BWH), University of Michigan (UMH), Erasmus University (Rotterdam, Netherlands) (ERA), and Careggi University (Florence, Italy) (FLO). SHaRe is a de-identified, longitudinal, centralized database of clinical, genetic, imaging and laboratory data from individuals and families with sarcomeric cardiomyopathies including HCM and dilated cardiomyopathy (DCM). For the purposes of this study, we will concentrate our attentions to genetic testing results and clinical phenotypes of HCM. As HCM does not have an ethnic, race, or gender bias, , enrollment of participants includes pediatric and adult patients of both genders and all ethnic races.

Aims Of The Study

To better understand factors underlying differences in variant classifications, the aims of this study are (1) to compare the rate of discordant classifications among HCM centers submitting to SHaRe to the rate of discordant classifications among clinical laboratories submitting to ClinVar, and (2) to compare the variant summary reports collected from SHaRe participating sites to assess sources of discordance. 3) Lastly, in an attempt to resolve the discordances, variant specific data was aggregated from all known sources in "variant summary reports," which will be used to create surveys for SHaRe participating sites in the later part of this study.

CHAPTER II

METHODS

Variant Ascertainment From Share

As of March 2015, 589 variants in eight sarcomere genes observed in HCM patients were ascertained from the SHaRe. For each variant observed at their respective site, SHaRe participating sites provided the following information: (i) de-identified patient ID, (ii) de-identified family ID, (iii) primary diagnosis (HCM), (iv) age of diagnosis, (v) left ventricular wall thickness (LVWT) in mm at the time of evaluation, (vi) proband status in family evaluation (i.e. primary patient or a relative of primary patient), (vii) variant information in p. and/or c. nomenclature, (viii) clinical laboratory employed to conduct genetic testing, (ix) genes tested or name of test panel used, and (x) variant classifications. Variants were classified as one of the following five tier categories: pathogenic (P), likely pathogenic (LP), variant of unknown significance (VUS), likely benign (LB), or benign (B). Classifications for variants submitted by more than one SHaRe participating sites were compared to examine concordance of interpretation.

Variant Ascertainment From ClinVar

To compare the frequency of disagreement in variant classifications between centers submitting to SHaRe and clinical laboratories submitting to ClinVar, variant classification data was requested from ClinVar for 8 sarcomere genes via personal e-mail communications with Donna Maglott (April 29, 2015). In response to our request, the ClinVar team uploaded a tvs file called “submitter_overview.tsv.gz” at

the following URL: ftp://ftp.ncbi.nlm.nih.gov/pub/clinvar/tab_delimited/special_requests/. This file was downloaded and unzipped using winRAR software (http://www.win-rar.com/), and the content was copied into a Microsoft Excel spreadsheet for analysis. This file contains a summary of each submission (SCV) with the following values:

Column header	Description	Example
Submitter	Name of the submitter	GeneDx
SubmitterID	How the submitter identified the record	GDX:762551|HPO:1638
SCV	The accession assigned to the submission	SCV000061989
Definition	How the allele was defined by the submitter	NC_000015.9:g.35082610A>G
Pref_name	ClinVar's default description	NM_005159.4(ACTC1):c.301G>A (p.Glu101Lys)
Gene	Symbol of the gene asserted to be involved	ACTC1, MYBPC3, MYH7, MYL2, TNNI3, TNNT2, and TPM1
ClinSig	Clinical significance	Variants classifications: pathogenic, likely pathogenic, variant of unknown significance,

		likely benign, or benign.
LastEval	Date last evaluated	
AssertionMethod	How the variant was evaluated	clinical testing, literature only
Submitted_Condition	The name submitted for the condition	Hypertrophic Cardiomyopathy
Calc_Condition	ClinVar's preferred name for the condition	Hypertrophic Cardiomyopathy
Description	Free text describing the interpretation	

In total, 2405 variants in 8 sarcomere genes, including ACTC1, MYBPC3, MYH7, MYL2, TNNI3, TNNT2, and TPM1, were submitted. Twelve submitters contributed to the data, including Laboratory for Molecular Medicine (LMM), OMIM, GeneDx, LabCorp, Blueprint Genetics, Children's Hospital of Eastern Ontario (CHEO), Invitae, University of Washington (CSER_CC_NCGL), Emory Genetics Laboratory, Agnes Ginges Centre for Molecular Cardiology, Genetic Services Laboratory, University of Chicago, and Neurogenetics Laboratory, Royal Perth Hospital. In order to focus our attentions on data submitted by clinical laboratories, submissions from OMIM (n =134), University of Washington (CSER_CC_NCGL) (n = 83), and Agnes Ginges Centre for Molecular Cardiology (n = 18) were excluded. The remaining variants were then stratified by single and

multiple submitters. Classifications for variants submitted by multiple submitters were then compared for discordance.

Identification Of Discordance And Assessment Of Their Severity

In both sets of aforementioned data, discordance in classification was defined as the difference in clinical interpretation of the variant that was submitted by more than one group (i.e. clinic or lab). Likely pathogenic versus pathogenic and likely benign versus benign classification differences were not considered discordant as they seldom have clinical significance. Frequency of discordance was calculated as a percentage of the number of variants with different classifications divided by total number of variants submitted by multiple SHaRe participating sites.

To assess the severity of the discordance, variant classifications provided by SHaRe participating sites and ClinVar submitters were categorized into following degrees of discordance:

i) Severe - the classification of the variant assigned differed by likely benign or benign versus likely pathogenic or pathogenic.

ii) Moderate - the classification of the variant assigned differed by VUS versus pathogenic or VUS versus benign.

iii) Modest - the classification of the variant assigned differed by VUS versus likely benign or VUS versus likely pathogenic.

Comparison Of Analysis Summary Reports To Identify Reasons For Discordance

To understand the reasons for discordant classifications among SHaRe participating sites, a summary of rationale for the classification for each discordant variant was requested from the participating sites that had observed the variant in question. For each variant, the various sites' rationales for classifications were compared to one other. Specific sources of discordance emerged including, differences in published data from journal articles, private lab data, internal SHaRe site data, population frequency data, *in silico* predications, and evolutionary conservation analyses. Differences in interpretation were assumed to be at play when the same data was used to render different classifications. Specific information about criteria used to render classifications was not provided by the SHaRe participating sites (e.g. ACMG guidelines, Seidman group's classification criteria (2012), etc.). Analysis of the sources of discordance could not be completed for one variant, p.Arg1606Cys, as classification rationale was not available.

Comprehensive Up-To-Date Review Of Data On Discordant Variants

Up-to-date variant summary reports were curated from eclectic sources to help resolve the classification discordances among SHaRe sites. Sources of curated data included: (i) case and control data from published literature, (ii) case and control data held by commercial laboratories, (iii) case and control data held by SHaRe participating sites, (iv) control data in online databases, (v) in silico model predictions, (vi) evolutionary conservation, and (vii) variants in nearby amino acids

associated with disease. From data sources i-iv, the following information was collected (if reported): (a) type of disease studied (b) number of cases with variant out of total patient population, (c) number of controls with variant out of total individuals not selected for disease and affiliated traits (e.g. sudden death), (d) names of genes analyzed, (e) ethnicity of the cases and controls and/or location of clinic or hospital where care was provided, (f) clinical phenotype of probands (including age, sex, left ventricular wall thickness (in mm) of cases, and/or any other clinical presentation, (g) family history or disease co-segregation data, and (h) additional variants along with their classification in ClinVar.

Published Data Extracted From Journal Articles

A set of key words were created to optimize web-based searches. The search keywords included: gene names (e.g. MYBPC3), c. nomenclature in various forms (e.g. c.565G, 565G), p. nomenclature in various forms (e.g. p.V189, V189, p.V189I, V189I, p.Val189, Val189, p.Val189Ile, Val189Ile), and SNP IDs (e.g. rs11570052). These keywords were searched in web-based search engines, including NCBI PubMed (www.ncbi.nlm.nih.gov/pubmed/), NCBI PubMed Central® (http://www.ncbi.nlm.nih.gov/pmc/), NCBI ClinVar (http://www.ncbi.nlm.nih.gov/clinva r/), and Google Scholar (https://scholar.google.com/).

Published And/Or Unpublished Data Derived From Clinical Laboratories

Major sources of internal laboratory data were included in variant summary reports from ClinVar submissions (http://www.ncbi.nlm.nih.gov/clinvar/), and

personal communications between SHaRe participating sites and laboratory personnel. Most frequent laboratory submitters to ClinVar included Laboratory for Molecular Medicine, GeneDx, Invitae, and Ambry Genetics. Variant summary reports from ClinVar were downloaded and searched for internal data. Last date of submission to ClinVar was noted.

Unpublished Data From SHaRe

SHaRe was populated by data from participating sites with already existing internal databases at Stanford University, Brigham and Women's Hospital, University of Michigan, Erasmus University (Rotterdam, Netherlands), and Careggi University (Florence, Italy). Each participant submitted genetic test results along with clinical phenotypes and family history (when available) for carriers of up to 120 variants in the registry. For the purpose of this study, only the variants that had discordant classifications among SHaRe participating sites were evaluated (n = 17). They employed a variety of commercial and in-house labs to conduct genetic testing ranging from PCR analysis of known single sites to Next-Gen sequencing of multi-gene panels (see results for details).

Published Control Data Derived From Exome Aggregation Consortium (ExAC) Online Database

ExAC was employed to estimate the frequency of the variant in the general population. The dataset includes ~60,706 unrelated individuals sequenced as part of various disease-specific and population genetic studies. A list of cohorts contributing to the dataset is provided on their website (http://exac.broadinstitute.org/faq). The

phenotype of these individuals is not publicly available. The dataset is comprised of multiple cohorts, some of which were recruited from the general population; others were enriched for common cardiovascular disease. Currently, the following populations are represented in the ExAC: African/African American, Latino, East Asian, Finnish, Non-Finnish European, South Asian, and Others. The dataset is comprised of multiple cohorts, some of which were recruited from the general population; others were enriched for common cardiovascular disease. None were recruited for rare Mendelian cardiomyopathy and in some cohorts such cases were excluded. For the purpose of this study, ExAC database was quarried using SNP IDs for each of the 17 variants in order to ascertain the allele frequency for an aggregate of all ethnicities as well as each ethnicity separately.

In Silico Model Predictions

The impact of non-synonymous variants (n = 16) was assessed in silico, using Polyphen-2 (Adzhubei, 2010) and SIFT (Sorts Intolerant From Tolerant) (Ng, 2003) programs. Variants were predicted to be ‘benign’, ‘possible damaging’, or ‘probably damaging’ by PolyPhen-2, and ‘tolerated’, ‘not tolerated’, or ‘deleterious’ by SIFT.

Evolutionary Conservation

The UCSC Genome Browser was utilized to evaluate the degree of conservation of individual residues and their neighboring amino acids across species (Kent, 2002). The species compared mainly included: human, rhesus, mouse, dog, elephant, chicken, X-tropicalis, zebrafish, and lamprey.

Disease-Associated Nearby Amino Acids

The Human Gene Mutation Database (HGMD, publically available version was utilized to search for codons nearby the variants of interest that might be associated with HCM (Stenson, 2003). Nearby variants along with the author's last name and year of publication that reported these variants were recorded.

Variant Summary Surveys

To investigate whether the SHaRe participating sites would agree on a variant classification if provided with the same set of curated variant data, surveys will be created for each of the seventeen discordant variants that include comprehensive up-to-date summaries of all available data on each variant.

Survey Questions And Methods Of Distribution

The surveys will be created and sent using Survey Monkey (https://www.surveymonkey.com), and will include a complete variant summary along with the following six questions:

1) Please note your SHaRe site
    a. Erasmus
    b. Florence
    c. Stanford
    d. Michigan
    e. Brigham & Women's
2) Please note your first & last name:
3) Please note your email address

4) Given the data reviewed above, how does your site classify the variant?
    a. Benign
    b. Likely benign
    c. Variant of uncertain significance
    d. Likely pathogenic
    e. Pathogenic
5) What is your rationale for this classification? Choose all that apply.
    a. Seen in multiple cases of HCM
    b. Sufficiently rare or absent in controls and/or general population samples
    c. Segregation data favors pathogenicity
    d. Not seen in enough cases of HCM
    e. Suspect the variant is a modifier
    f. Co-occurs with another variant too often
    g. Segregation data favors benign (i.e. failure to segregate)
    h. Too frequent in controls and/or general population samples
    i. in vivo evidence
    j. in vitro evidence
    k. in silico evidence
    l. Evolutionary conservation
    m. Insufficient ancestry-matched controls
    n. Other (please specify)

6) Any additional comments?

Finally, the variant surveys will be sent to the participating SHaRe sites that report the discordant variant in question within their site's data. For example, the variant summary report forMYH7 variant p.Asn1327Lys will be sent to three out of five SHaRe participating sites (i.e. University of Michigan, Stanford University, and Brigham and Women's Hospital), who had reported this variant in their patients.

CHAPTER III

RESULTS

Lower Rates Of Discordance Among Share Than In ClinVar

To date, 589 sarcomere variants have been submitted to SHaRe. Overall, discordance was noted in 21 out of 112 variants (~19%) in four genes that were submitted by more than one SHaRe center: MYBPC3 (n = 9), MYH7 (n = 6), TNNT2 (n = 1), and TPM1 (n = 1). Nineteen of the 21 discordant variants were missense mutations, while two were splice site variants. As of April 2015, 2,405 sarcomere variants had been submitted to ClinVar with 695 variants submitted by more than one clinical laboratory. Discordance was noted in 314 out of 695 (45%) variants in eight genes that were submitted by more than one ClinVar submitters: ACTC1 (n = 5), MYBPC3 (n = 112), MYH7 (n = 130), MYL2 (n = 7), MYL3 (n = 7), TNNI3 (n = 20), TNNT2 (n = 19), and TPM1 (n = 14).

Table 1

Summary of Discordant variants in ClinVar and SHaRe

	ClinVar n (%)	**SHaRe n (%)**
Total variants	2405	589
Variants submitted by >1 labs/sites	695	112
Discordant variants	314 (45.2%)	21 (18.75%)

Comparison Of Discordance Among Share Sites And ClinVar Submitters

Of the 21 variants in SHaRe, a variant summary report was requested for 16 variants from the following SHaRe centers: Brigham and Women's - twelve,

University of Michigan – fourteen, Careggi University (Florence, Italy) – nine, Stanford University – ten, and Erasmus University (Rotterdam, Netherlands) – five (table 2). The remaining five discordant variants were not analyzed in this study due to shortage of time and will be analyzed in a future study. Comparison of classifications revealed that the majority of discordant classifications in SHaRe were modest (9/16; ~56%), with 1/16 (~6%) severe and 6/16 (~37%) moderate. Comparison of classifications in ClinVar revealed about half were moderate (159 of 314; ~50%), 148 of 314 (~47%) were modest and 7 of 314 (~2%) severe (table 3).

Table 2

Severity of Discordance Among 21 Variants in SHaRe Participating Sites

Gene	Variant	BWH	FLO	ERA	STD	UMH	Discordance direction	Discordance severity
MYH7	p.Met982Thr (c.2945T>C)	VUS	LP			VUS	VUS vs. LP	Modest
MYBPC3	p.Arg810His (c.2429G>A)	VUS	LP		VUS	VUS	VUS vs. LP	Modest
MYBPC3	p.Gly531Arg (c.1591G>C)	VUS	LP				VUS vs. LP	Modest
MYBPC3	p.Ser217Gly (c.649A>G)	LB			VUS	LB	LB vs. VUS	Modest
MYBPC3	p.Val189Ile	LB			VUS	LB	LB vs. VUS	Modest

	(c.565G>A)							
MYH7	p.Arg1606Cys (c.4816C>T)			VUS		LP	VUS vs. LP	Modest
MYH7	p.Arg204His (c.611G>A)	VUS	VUS		LP	LP	VUS vs. LP	Modest
MYH7	p.Asn1327Lys (c.3981C>A)	LB			VUS	VUS	LB vs. VUS	Modest
TPM1	p.Glu192Lys (c.574G>A)	LP			VUS		VUS vs. LP	Modest
MYBPC3	c.927-9G>A	P		VUS	LP	P	VUS vs. LP vs. P	Moderate
MYBPC3	p.Gln998Glu (c.2992C>G)				VUS	B	B vs. VUS	Moderate
MYBPC3	p.Gly490Arg (c.1468G>A)		LP	P		VUS	VUS vs. LP vs. P	Moderate
MYH7	p.Lys1459Asn (c.4377G>T)		VUS	P	LP	VUS	VUS vs. LP vs. P	Moderate
MYH7	p.Thr1377Met (c.4130C>T)	VUS	P			LP	VUS vs. LP vs. P	Moderate
TNNT2	p.Arg278Cys (c.832C>T)	VUS	LP	P	LP	P	VUS vs. LP vs. P	Moderate

MYBPC3	p.Glu619Lys (c.1855G>A)	LB	LP			LB	LB vs. LP	Severe

Table 3

Comparison of Severity of Discordance Between ClinVar and SHaRe

Discordance severity	ClinVar n (%)	SHaRe n (%)
Modest	148 (47.1)	9 (56.25)
Moderate	159 (50.6)	6 (37.5)
Severe	7 (2.2)	1 (6.25)
Total	314	16

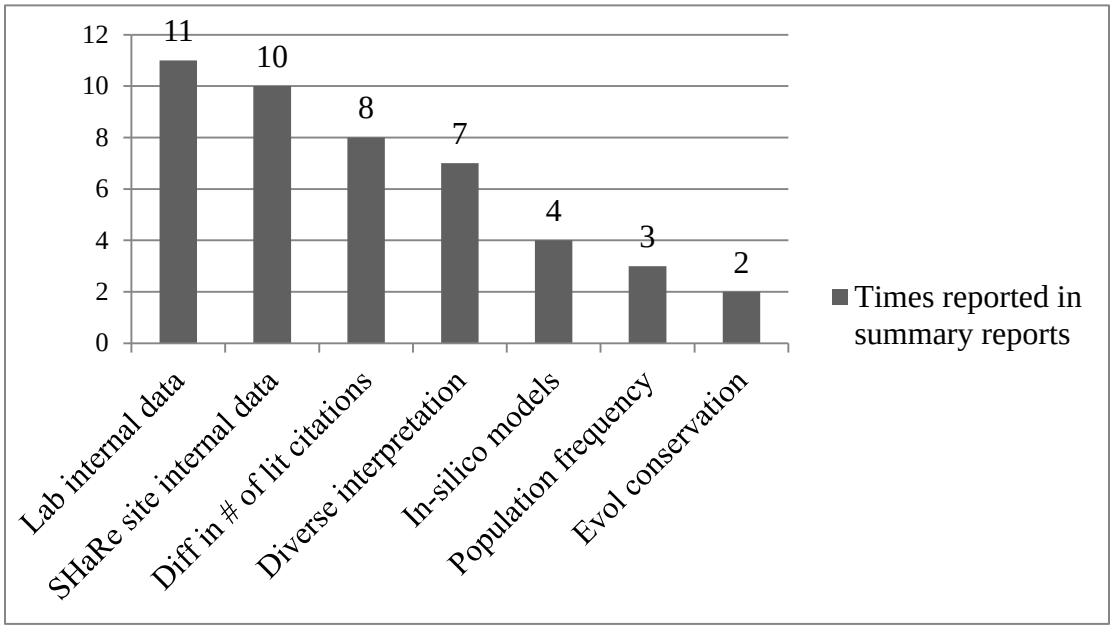
Reasons For Discordant Variant Classification Among SHaRe Sites

Comparison of rationales for variant classifications sent by SHaRe participating sites revealed that multiple factors contributed to discordance of classifications. The most common reason for discordance in classification was lack of access to privately held data including genetic testing lab internal data (11 of 16 variants) and SHaRe site internal data (10 of 16 variants). Additional sources of discordance included differences in evolutionary conservation analysis (2 of 16 variants) and population frequency data (3 of 16 variants) used, which were among the least common reasons for discordance. Furthermore, a difference in number of publications used was noted in 8 variants; a diverse interpretation of the same data was noted in 7 variants; a difference in *In silico* predictions was noted in 4 variants; a difference in interpretation of literature was noted in 3 variants; and a difference in

date of last review was noted in 3 variants (Figure 1). For detailed analysis of each variant, see text below.

Figure 1

Frequency of reasons for discordance among SHaRe participating sites



MYBPC3 - c.927-9G>A

Introduction

The c.927-9G>A (intron 11) variant in MYBPC3 has been detected in at least eight patient in four sites. In their most recent reviews of the classifications STD classified the variant as likely pathogenic, UMH and BWH classified the variant as pathogenic, while ERA classified the variant as a VUS. See Appendix A for details.

Literature

This variant has been reported in 3 publications in a total of 4 out of 640 HCM cases (published between 2010 and 05/2015). All of the four aforementioned SHaRe sites (or the clinical labs they employed for genotyping) utilized the three publications to classify the variant as either LP or P with the exception of ERA. They noted that the variant was “not described before (report from 2007)” and classified the variant as a VUS. This finding illuminates the necessity of re-classifying variants in frequent intervals as new published data becomes available.

Lab Internal Data

LMM reported more than 20 European ancestry individuals with HCM who had this variant.

Site Internal Data

BWH and STD reported the variant in two probands; UMH reported the variant in three probands; ERA reported the variant in one proband. None of the other sites reported additional site internal data. It is perhaps due to lack of any additional

probands who had this variant that ERA did not seek new information to re-classify this variant.

In silico

BWH reported that the variant occurs in the conserved splice consensus sequence. LMM and GeneDx were also in agreement that the mutation creates a cryptic splice acceptor site, which is expected to result in an abnormal protein. In general, in silico predictions did not sway the variant classification among SHaRe sites.

Evolutionary Conservation

SHaRe sites did not utilized evolutionary conservation in making variant classifications.

Population Frequency

None of the sites reported seeing the variant with significant frequency in any of the large population cohorts (ESP and ExAC) or the controls used in published literatures.

Summary

The chief reason for discordance between ERA and three other SHaRe sites is because ERA has not re-classified this variant since 2007. Since 2010, four publications have reported this variant as disease causing in patients with HCM. This discordance is likely to be resolved by variant re-classification in the future unless conflicting data emerges.

MYBPC3 - p.Arg810His (c.2429G>A)

Introduction

p.Arg810His variant in *MYH7* has been detected in at least seven patients in four sites. In their most recent reviews of the classifications BWH, STD, and UMH classified the variant as VUS, while FLO classified the variant as likely pathogenic. See Appendix B for details.

Literature

This variant has been reported in 6 out of ~490 HCM cases in 5 publications (published between 2003 and 2011). One of the four aforementioned SHaRe sites (FLO) utilized one of the publications (Nanni et al, 2003) to classify the variant as likely pathogenic, while two of the four aforementioned SHaRe sites (BWH and STD) utilized at least four of the publications to classify the variant as VUS. This difference illustrates that new findings are used to refute the previously established classification that were based on a smaller number of cases with the variant. In addition to the difference in the number of publications, FLO did not take into account the findings from Nanni et al. (2003) that both of the individuals with the variants had additional variants (i.e. homozygous for the variant and compound heterozygous). Thus, indicating that interpretation of the data in published literature is also a cause of discordance in variant classifications among SHaRe sites.

Lab Internal Data

LMM reported 9 HCM cases that had this variant. They note that of the 9 individuals carrying this variant, 4 carried another likely or possibly significant

variant. GeneDx reported 14 HCM cases that had this variant. They report that of the 3 of 14 individuals carrying this variant, they also carried another likely or possibly significant variant, and 1 of 14 individuals carrying this variant also carried a VUS.

Site Internal Data

This variant is observed by FLO (n = 2), UMH (n = 2), BWH (n = 2), and STD (n = 1). One of the probands from FLO had an additional variant of unknown significance. No additional variants or co-segregation data was reported. Given the evidence from their summary reports, differences in site internal data was not a reason for discordance among SHaRe sites.

In silico

On one hand, STD, FLO and UMH used either Polyphen-2 and/or SIFT that predicts this variant to be possibly damaging or deleterious. It is evident that FLO relied on this information and classified the variant as likely pathogenic, while the other two sites were more cautious of this finding and classified the variant as a VUS. On the other hand, BWH reported that "computational prediction tools and conservation analysis do not provide strong support for or against an impact the protein," and rendered the variant as a VUS. In summary, in-silico models used and interpretation of their results might be one of the reasons why there is discordance in variant classification among different sites.

Evolutionary Conservation

Similar to the in-silico analysis, FLO relied on the amino acid conservation information and classified the variant as likely pathogenic, while the other sites were more cautious in considering this information as a cause of pathogenicity.

Population Frequency

All four SHaRe sites reported absence of the variant in any of the large population cohorts (ESP and ExAC) or the controls used in published literatures. FLO relied on the absence from large population cohorts and classified the variant as likely pathogenic, while the other sites were more judicious in considering this information as cause of pathogenicity.

Summary

The main reasons for discordance between the four sites are due to unique internal lab data, and difference in interpretation as well as number of literature (published data). BWH and UMH have access to LMM's unpublished internal data that showed 4 out of 9 individuals with additional pathogenic variants on the cardiomyopathy panel that led them to conclude that the variant is of uncertain significance. Since FLO does not have access to the lab internal data that shows additional variants, they classified the variant as likely pathogenic. Furthermore, a different interpretation of the study published by Nanni et al. in 2003 as evidence for pathogenicity in isolation rendered FLO to classify the variant as pathogenic. In addition, FLO is also missing additional published studies that have come out reporting HCM cases with additional variants supporting a VUS classification. Finally, the difference in reliance on computational prediction tools and conservation analysis was also a cause of variant classification discordance among FLO and the three other sites.

MYBPC3 - p.Gln998Glu (c.2992C>G)

Introduction

p.Gln998Glu variant in MYBPC3 has been detected in at least two patients at two SHaRe sites. In their most recent reviews of the classifications, UMH classified the variant as benign, while STD classified the variant as a VUS. See Appendix C for details.

Literature

This variant has been reported in 9 out of 1,502 HCM cases in 7 journal articles (published between 2004 and 2015). Most of these cases were of East Asian ancestry (n = 6). UMH did not provide a list of citations that they used to collect published data in their summary report, but since they employed LMM to conduct genetic testing, the number of citations (n = 4) can be inferred from LMM's variant summary report submitted to ClinVar (Oct 19, 2012). STD stated using the same four publications in addition to one more article that reported this variant in one Japanese case out of a total of six reported in published literature. Since the difference between the numbers of cases collected from published articles is small, the difference in number or interpretation of published data is not a reason for discordance among SHaRe sites.

Lab Internal Data

UMH that employed LMM for genetic testing do not report any internal lab data for this variant. STD stated that LMM had seen cases of HCM as well as DCM

in their lab. Since both UMH and STD had access to the same LMM data, lack of access to internal lab data is not a reason for discordance among SHaRe sites.

Site Internal Data

This variant is observed by UMH (n = 1) and STD (n = 1). UMH had observed a second, pathogenic mutation in MYBPC3 in their proband. They used this information as one of the reasons to classify the variant as benign. Since STD did not have access to this data prior to their variant classification, lack of access to internal site data could be a reason for discordance among SHaRe sites.

In silico

In their summary reports, both UMH and STD stated using in silico predictions by Polyphen (i.e. probably damaging) to classify the variant. In addition, UMH used SIFT that also predicted deleterious effects of the variant in their classification. Therefore, difference in *in silico* predictions is not a reason for discordance among SHaRe sites.

Evolutionary Conservation

In their summary report, STD stated using highly conserved status of the amino acid at this codon in their variant classification, while UMH did not mention evolutionary conservation analysis in their report. Therefore, evolutionary conservation could not be analyzed as a source of discordance. However, taken together with other pieces of evidence (i.e. population frequency; see below), evolutionary conservation cannot be deemed a major reason for discordance among SHaRe sites.

Population Frequency

In order to classify this as a benign variant, UMH stated using presence of this variant in 144 out of 27,500 chromosomes (0.5% in Latino and East Asian) from ExAC population frequency data, and in 6 of 128 (4.7%) of Mexican chromosomes from 1000 Genomes. On the other hand, STD stated using absence of this variant from NHLBI-GO Exome Sequencing Project (ESP) and presence of this variant in 2% of Asian and 2% of Hispanic individuals from 1000 Genomes to classify this variant as a VUS. Since ExAC contains larger population data for Latino and East Asian populations (most frequent ancestry of observed cases) and includes data from both ESP and 1000 Genomes Project, incorporating the population frequency data from ExAC by STD will likely resolve this discordance.

Summary

The chief reason for discordance between SHaRe sites is utility of ExAC population frequency data for Asian and Latino ancestry, while lack of access to internal SHaRe data might play a minor role in discordant classification of this variant.

MYBPC3 - p.Glu619Lys (c.1855G>A)

Introduction

p.Glu619Lys (c.1855G>A) variant in MYBPC3 gene has been detected in at least five patient in 3 SHaRe sites. In their most recent reviews of the classifications, UMH and BWH classified the variant as likely benign, while FLO classified the variant as likely pathogenic. See appendix D for details.

Literature

This variant has been reported in 5 out of 576 HCM cases in 4 journal articles published between 2009 and 2013 (see variant section # for details). Although both Careggi University and BWH utilized the same four publications to classify the variant, they rendered a different classification. UMH did not provide information regarding the publications used in their classification. This suggests that the published data is not a reason for discordance among SHaRe sites.

Lab Internal Data

UMH reported that GeneDx has seen the variant in multiple families with HCM and DCM. A third of these cases were of Ashkenazi Jewish ancestry and a third carried a second variant. BWH employed LMM to conduct genetic analysis, who reported at least three individuals who carried an additional disease-causing variant. These evidences provided by internal lab data against pathogenicity lead UHM and BWH to classify the variant as likely benign, which were not available to FLO.

Site Internal Data

This variant was observed by three SHaRe sites: FLO (n = 2), UMH (n = 1), and BWH (n = 2). Both of the probands observed by FLO carried an additional variant in MYH7 gene (c.976G>C; p.Ala326Pro), which is classified as VUS by LMM (Jul 29, 2011) and CHEO, and pathogenic by GeneDx (Jun 17, 2014) in ClinVar. In addition, FLO reported a relative of one of the probands with LVWT of 12 mm who carried this variant, which they reported as suggesting co-segregation of the variant with the disease. In this case, FLO is taking into account the "co-segregation" data as evidence of pathogenicity despite the observation of an additional pathogenic variant. Thus, site internal data is a reason for discordance among SHaRe sites.

In silico

UMH reported using PolyPhen and SIFT that predicted the protein to be benign or possibly damaging and deleterious, respectively. On the other hand, FLO reported a Polyphen prediction of "pathogenic" effects of the variation to render their classification of the variant as likely pathogenic. The reason for the difference in the predictions using PolyPhen could not be accessed. In general, difference in *in silico* predictions is not a source of discordance among SHaRe sites; however, for this variant, the *in silico* prediction might be at play.

Evolutionary Conservation

The variant occurs in the conserved splice consensus sequence. UMH and BWH did not report evolutionary conservation in their report, while FLO reported

amino acid being “conserved”. From this comparison, it is evident evolutionary conservation is not a reason for discordance among SHaRe sites.

Population Frequency

All three sites report low frequency of variant in general population as per ExAC online data. FLO has in part used this as evidence to render the variant likely pathogenic. Thus, population frequency could be considered a reason for discordance among SHaRe sites.

Summary

The chief reasons for discordance between SHaRe sites are due to testing lab internal data, site internal data and the differences in predictions from in silico models. In summary, despite the severity of discordance of this variant, it can be resolved by sharing of the private data along with discussion regarding interpretation of in silico models and low frequency in general population.

MYBPC3 - p.Gly490Arg (c.1468G>A)

Introduction

p.Gly490Arg in MYBPC3 has been detected in at least three patients in three SHaRe sites. In their most recent reviews of the classifications, UMH classified the variant as a VUS, while ERA and FLO classified the variant as pathogenic and likely pathogenic, respectively. See appendix E for details.

Literature

This variant has been reported in five out of ~ 868 HCM cases in 6 journal articles (published between 2004 and 2010), in two out of 63 cases of LVNC (Probst,

2011), and in one out of 312 cases of DCM (Hershberger, 2010). In addition, the variant has been reported in one individual unselected for HCM, who underwent whole exome sequencing (Ng, 2013), and in one individual from Framingham Heart Study who was also unselected for HCM but had increased LVWT (maximum LVWT > 13 mm) (Morita, 2006). In their summary reports, UMH stated ClinVar as their source of reported published data, which included HCM as well as non-HCM cases (one LVNC and one DCM) and FLO cited five published articles including Hershberger et al. (2010) that reported DCM cases, while ERA cited only one publication (van Driest, 2004) that reported HCM cases. Therefore, on one hand, UMH used the non-HCM cases as evidence against pathogenicity to classify the variant as a VUS, while FLO did not consider this as evidence against pathogenicity. This suggests a difference in either literature interpretation or in the use of classification criteria. On the other hand, observation of non-HCM cases in articles published after 2004, were not included in the analysis by ERA. This suggests that the difference in number of published data used was a cause of discordance among the SHaRe sites as new published data sheds more light into the pathogenicity of the variant.

Lab Internal Data

UMH used LMM's internal lab data that indicates HCM cases with a second, pathogenic variant as evidence against pathogenicity to classify the variant as VUS, while FLO and ERA rendered the variant disease causing in the absence of this data.

Therefore, lack of access to internal lab data is a reason for discordance among SHaRe sites.

Site Internal Data

This variant is observed by FLO (n = 1), ERA (n = 2), and UMH (n = 1). Three out of four of these probands carried a second pathogenic variant, while one proband cared for in ERA did not carry a second variant (information on analyzed genes was not provided). In addition, ERA repot that they "found [this variant] in 4 other Dutch HCM patients," that are used to classify the variant as pathogenic. Furthermore, FLO reported co-segregation of the variant with the disease in their proband. However, both affected relatives carried a second, pathogenic variant (MYBPC3 - p.Arg502Gln). It is interesting to note that ERA and FLO classified the variant as disease causing despite the findings of second pathogenic variant, while UMH uses this evidence to classify the variant as a VUS.

In silico

In their summary reports, UMH stated the amino acid change to be probably damaging (as predicted by PolyPhen) and tolerated (as predicted by SIFT), and FLO reported the change to be pathogenic, while ERA did not report in silico predictions. Therefore, difference in *in silico* predictions is not a reason for discordance among SHaRe sites.

Evolutionary Conservation

In their summary reports, FLO reported that Glycine is conserved across species, while other sites do not mention evolutionary conservation analysis in their

summary reports. In general, evolutionary conservation is not a source of discordance among SHaRe sites.

Population Frequency

In their summary reports, UMH and FLO reported the low frequency of the variant as reported in ExAC, while ERA did not report population frequency data. Therefore, difference in population frequencies is not a reason for discordance among SHaRe sites.

Summary

The chief reasons for discordance between SHaRe sites include lack of site internal data, lab internal data, literature citations, and differences in interpretation of co-segregation and second pathogenic variants. In summary, the discordance of this variant can be resolved by sharing of privately held data along with discussions regarding interpretation of co-segregation with second pathogenic variations.

MYBPC3 - p.Gly531Arg (c.1591G>C)

Introduction

p.Gly531Arg in MYBPC3 has been detected in at least two patients from two different SHaRe sites. In their most recent reviews of the classifications, BWH classified the variant as a VUS, while FLO classified the variant as likely pathogenic. See Appendix F for details.

Literature

This variant has been reported in 5 out of ~812 European HCM cases in 11 journal articles (published between 2006 and 2012). In their summary reports, BWH

noted nine publications that reported HCM as well as DCM cases, while FLO noted one publication that reports one HCM case. Since the number of DCM cases is used as evidence against pathogenicity by BWH, this difference in number of journal articles is a reason for discordance among SHaRe sites.

Lab Internal Data

BWH based their classification of VUS partly on evidence provided by LMM's internal data, who had observed this variant in more than 5 individuals with HCM (3 with c.1591G>A and 2 with c.1591G>C) including 2 individuals who carried a second pathogenic MYBPC3 variant. Since this information was not available to FLO, who classified the variant as likely pathogenic, prior to their last update of this variant, lack of access to internal lab data is a reason for discordance among SHaRe sites.

Site Internal Data

This variant is observed by BWH (n = 1) and FLO (n = 1). Both SHaRe sites noted co-segregation of the variant with the disease in affected relatives in the absence of second pathogenic variants. Since both sites have independent evidence of co-segregation from their respective patient populations, lack of access to internal site data is likely not a reason for discordance among SHaRe sites.

In silico

In their summary reports, BWH noted pathogenic prediction of amino acid change using LMM's in-house computational tool, and FLO also noted pathogenic

prediction using PolyPhen. Therefore, a difference in *in silico* predictions is not a reason for discordance among SHaRe sites.

Evolutionary Conservation

In their summary reports, BWH and FLO noted that the variant is conserved across species. Therefore, the difference in evolutionary conservation is not a reason for discordance among SHaRe sites.

Population Frequency

In their summary reports, BWH and FLO noted low variant frequency in general population as reported in ESP and ExAC, respectively. Therefore, the difference in population frequency is not a reason for discordance among SHaRe sites.

Summary

The chief reasons for discordance between SHaRe sites include lack of access to internal lab data and difference in number of published articles used. In addition, it is of note that FLO used evidence of co-segregation to classify the variant likely pathogenic in the absence of other evidence against pathogenicity, including second, pathogenic variants and presence in DCM cases, while BWH used these evidences to classify the variant as a VUS despite observing co-segregation in their patient's family. This suggests that differences in classification criteria may also be a source of discordance.

MYBPC3 - p.Ser217Gly (c.649A>G)

Introduction

p.Ser217Gly in MYBPC3 has been detected in at least four patients across three SHaRe sites. In their most recent reviews of the classifications, UMH and BWH classified the variant as likely benign, while STD classified the variant as a VUS. See Appendix G for details.

Literature

This variant has been reported in 2 HCM cases in 2 journal articles (published between 2009 and 2014). None of the SHaRe sites that observed this variant noted any publications in their summary reports. Therefore, the difference in number or interpretation of published data is not a reason for discordance among SHaRe sites.

Lab Internal Data

BWH and UMH based their likely benign classification of this variant partly on multiple pieces of evidence provided by LMM's internal data, who had observed this variant in more than 10 individuals with HCM or DCM and in one of 734 controls, and by GeneDx, who had observed that the variant failed to segregate in one family. Since STD classified this variant as VUS in the absence of this information, lack of access to internal lab data is a reason for discordance among SHaRe sites.

Site Internal Data

This variant is observed by UMH (n = 1), BWH (n = 2), and STD (n = 1). BWH and UMH based their classification of likely benign partly on their observation of a second, pathogenic variants in their probands (BWH - TNNI3 (p.Ser166Phe);

UMH - MYH7 (p.Asp1096Tyr)). On the other hand, while STD also observed additional variants in their probands, none were considered disease causing. Therefore, since the information about second, pathogenic variants from UMH and BWH was not available to STD prior to their last update of this variant (7/15/2013), differences in internal site data is a reason for discordance among SHaRe sites.

In silico

In their summary reports, BWH based their classification of likely benign partly on LMM's internal computational tool that predicted the variant to be benign, while STD and UMH noted using multiple in silico prediction tools with inconsistent prediction, including benign prediction by PolyPhen (HumVar) and LMM's internal computational tool, and probably deleterious and deleterious prediction by PolyPhen-2 and SIFT, respectively. Taken together, since all three SHaRe sites had evidence of a benign prediction, difference in *in silico* predictions is not a reason for discordance among SHaRe sites.

Evolutionary Conservation

In their summary reports, all three SHaRe sites indicated that the variant is not conserved across species. Therefore, differences in evolutionary conservation analysis is not a reason for discordance among SHaRe sites.

Population Frequency

In their summary reports, all three SHaRe sites indicated that the variant is present in 12 out of 12546 chromosomes in ESP data. However, UMH also included larger population data from ExAC in their report (248/109368 chromosomes; 177

South Asian). Taken together, the ExAC data may have contributed to UMH's likely benign classification as the highest frequency in ExAC (2%) is higher than the frequency in ESP (0.2%). Therefore, the difference in population frequency data is a reason for discordance among SHaRe sites.

Summary

The chief reasons for discordance between SHaRe sites are lack of access to internal site and lab data, which can be resolved by sharing privately held data, and differences in source of population data, which can be resolved by considering all possible public resources available for variant classification.

MYBPC3 - p.Val189Ile (c.565G>A)

Introduction

p.Val189Ile in MYBPC3 has been detected in at least five HCM patients in three SHaRe sites. In their most recent reviews of the classifications, UMH and BWH classified the variant as likely benign, while STD classified the variant as a VUS. See Appendix H for details.

Literature

This variant has been reported in 3 out of ~304 European ancestry HCM cases in three journal articles (published between 2006 and 2014). Only BWH noted one publication in their summary report (Girolami, 2006), while STD and UMH did not report any published cases. However, since the number of cases is relatively small, the difference in number of publications used is not a reason for discordance among SHaRe sites.

Lab Internal Data

To conduct genetic testing, UMH and BHW used LMM, who classified the variant as likely benign (2013; as per UMH and BHW summary reports), and STD used Ambry Genetics, who had not previously detected the variant in other patients and classified the variant as a VUS (2013; as per STD summary reports). None of the SHaRe sites noted using any internal lab data in their variant classification. Therefore, lack of access to internal lab data is not a reason for discordance among SHaRe sites.

Site Internal Data

This variant is observed by UMH (n = 1), BWH (n = 3), and STD (n = 1). BWH and UMH based their likely benign variant classification partly on their observation of a second, pathogenic variant in their probands (BWH - TNNT2 (p.Glu163del and p.Trp294Ter); UMH - MYBPC3 (p.Gly1248_cys1253Dup)). Since this internal data was available to STD prior to their variant classification (5/31/2013), lack of access to internal site data is a reason for discordance among SHaRe sites.

In silico

In their summary reports, STD noted possibly damaging effects of amino acid change using PolyPhen-2, while BWH and UMH did not note any *in silico* predictions. Therefore, a comparison of *in silico* predictions could not be made. However, taken together with other lines of evidence (see “site internal data” above) the difference in *in silico* predictions is not a likely reason of discordance among SHaRe sites.

Evolutionary Conservation

In their summary reports, STD noted that the variant is conserved across species although isoleucine is the reference amino acid in two species (African clawed frog and Medaka), while BWH and UMH did not provide evolutionary conservation analysis. Therefore, a comparison of the use of evolutionary conservation analysis could not be made. However, taken together with other lines of evidence (see "site internal data" above) the evolutionary conservation data is not a likely reason for discordance in variant classification.

Population Frequency

In their summary reports, all three SHaRe sites indicated that the variant is present in 26 out of 6830 European individuals in ESP data. Therefore, difference in population frequency data used is not a reason for discordance among SHaRe sites.

Summary

The chief reason for discordance between SHaRe sites is lack of access to internal site data, which can be resolved by sharing privately held data. In addition, a differences in classification criteria to classify a variant likely benign or benign based on population frequency might be a reason for discordance as all three sites had the same ESP data (0.38% European), yet rendered different variant classifications.

MYH7 - p.Arg204His (c.611G>A)

Introduction

p.Arg204His variant in MYH7 has been detected in at least one patient in 4 SHaRe sites. In their most recent reviews of the classifications, UMH and STD

classified the variant as likely pathogenic, while FLO and BWH classified the variant as a VUS. See Appendix I for details.

Literature

This variant has been reported in 6 out of ~1543 HCM cases in 5 publications (published between 2003 and 2014). Three out of four SHaRe sites cited Richard et al. (2013) in their variant summary reports, while UMH did not provide any literature sources in their variant summary report. Since the sites with known literature citations reported the same publication as their source of published data, the difference in published articles uses is not a reason for discordance among SHaRe sites.

Lab Internal Data

BWH based their classification of a VUS partly on LMM's internal data that noted this variant in 3 adults with HCM all of whom carried other disease-causing variants. Since this information is not available to STD, who classified the variant as likely pathogenic, differences in internal lab data is a reason for discordance among SHaRe sites.

Site Internal Data

This variant is observed by UMH (n = 1), FLO (n = 1), BWH (n = 1), and STD (n = 1). On one hand, UMH used co-segregation of the variant with the disease in their proband's daughter as one of the reasons to classify the variant as likely pathogenic. On the other hand, BWH used evidence of a second pathogenic variant in MYBPC3 (p.Ala181Cysfs*53; c.540_559del) as one of the reasons to classify the variant as a VUS. Since the SHaRe sites did not have access to their data prior to their

variant classification, differences in internal site data is a reason for discordance among SHaRe sites.

In silico

In their summary reports, STD and FLO noted prediction of possibly damaging (PolyPhen-2) and pathogenic (PolyPhen), respectively, while UMH and BWH noted LMM's in-house in silico prediction tool which predicts this variant to be benign. Therefore, difference in *in silico* predictions is a reason for discordance among SHaRe sites.

Evolutionary Conservation

In their summary reports, UMH and FLO noted amino acid position to be conserved, while STD noted amino acid position to be moderately conserved. Therefore, evolutionary conservation analyses used by SHaRe sites are not a reason for discordance among SHaRe sites.

Population Frequency

None of the sites reported seeing the variant in any of the large population cohorts (ESP and ExAC). Therefore, data from population frequency used by SHaRe sites is not a reason for discordance among SHaRe sites.

Summary

The chief reasons for discordance between the four sites are differences in internal site data, differences in internal lab data, and use of different in-silico predictions.

MYH7 - p.Arg1606Cys (c.4816C>T)

Introduction

p.Arg1606Cys variant in MYH7 has been detected in at least one patient in 2 SHaRe sites. In their most recent reviews of the classifications, UMH (n = 1) classified the variant as likely pathogenic, while ERA (n = 1) classified the variant as a VUS. ERA did not provide a summary report for this variant. Therefore, a comparative analysis of variant classifications could not be assessed. In brief, BWH noted absence of this variant in ESP data and probably damaging effects of amino acid change as predicted by Polyphen to classify the variant as likely pathogenic. No additional internal lab or site data was provided. See Appendix J for details.

MYH7 - p.Asn1327Lys (c.3981C>A)

Introduction

p.Asn1327Lys variant in MYH7 has been detected in at least one patient in 3 SHaRe sites. In their most recent reviews of the classifications, UMH and STD classified the variant as a VUS, while BWH classified the variant as likely benign. See Appendix K for details.

Literature

This variant has been reported in 2 out of 138 HCM cases in 3 journal articles (published between 2005 and 2012). In their summary reports, BWH and STD noted one HCM case reported in two publications (Houghs (2005), Jensen (2013)), while UMH did not report any published cases. However, since the number of cases is

relatively small, the difference in number of publications used is not a reason for discordance among SHaRe sites.

Lab Internal Data

In their summary reports, all three sites noted using LMM's internal data that identified this variant in at least 7 European ancestry individuals, 3 of whom have confirmed Ashkenazi Jewish ancestry, and that the variant failed to segregate with the disease in two families with affected individuals of unknown genetic etiology. BWH further noted that LMM had seen the variant "in Ashkenazi Jewish individuals with a prevalence that greatly exceeds that of HCM (5 of 282 chromosomes, 1.8%)". In addition, although no internal lab data was provided, UMH noted that GeneDx had classified the variant as pathogenic, and STD noted that LabCorp had classified the variant as likely pathogenic, which was partly used as a reason for their VUS classification. Taken together, lack of access to internal lab data is not a reason for discordance among SHaRe sites. However, a difference in classification criteria to classify a variant likely benign or being based on population frequency might be a reason for discordance as all three sites had the same allele frequency data of Ashkenazi Jewish ancestry from LMM, yet rendered different variant classifications.

Site Internal Data

This variant is observed by UMH (n = 1), BWH (n = 2), and STD (n = 2). STD and UMH based their VUS classification partly on their observation of a second, likely pathogenic variant in their probands (STD - MYBPC3 (p.Glu1179Lys); UMH - PRKAG2 (p.Val289Ala)). Although this internal data was available to BWH prior to

their variant classification, taken together with other lines of evidence (see “lab internal data” above) lack of access to internal site data is not a chief reason for discordance in variant classification.

In silico

In their summary reports, STD noted possible damaging (HumVar score = 0.624) effects of amino acid change as predicted by PolyPhen-2, while UMH noted benign and tolerated effects as predicted by PolyPhen and SIFT, respectively. BWH noted an *in vitro* functional study by Wolny et al. (2013) that reported evidence that this variant may impact protein function. Taken together with other lines of evidence (see “lab internal data” above) the difference in *in silico* predictions is not a likely reason of discordance among SHaRe sites.

Evolutionary Conservation

In their summary reports, STD noted that the variant is conserved across species, while BWH and UMH did not provide evolutionary conservation analysis. Therefore, a comparison of use evolutionary conservation analysis could not be made. However, taken together with other lines of evidence (see “lab internal data” above) the evolutionary conservation data is not a likely reason of discordance among SHaRe sites.

Population Frequency

In their summary reports, UMH noted that the variant is present in 12 out of 57,990 (~0.02%) individuals from ExAC data; STD noted that the variant is present in 7 of 8,190 laboratory controls, published controls and individuals from publicly

available population datasets, including ESP and 1000 Genomes project; and BWH noted that the variant is present in 1 of 4,299 European American individuals from ESP. Taken together with other lines of evidence (see “lab internal data” above) the difference in population frequency is not a likely reason of discordance among SHaRe sites.

Summary

The chief reason for discordance between SHaRe sites is likely due to a difference in interpretation of population frequencies, as all three sites had the same allele frequency data of Ashkenazi Jewish ancestry from LMM, yet rendered different variant classifications.

MYH7 - p.Lys1459Asn (c.4377G>T)

Introduction

p.Lys1459Asn in MYH7 has been detected in at least four patients across four SHaRe sites. In their most recent reviews of the classifications, FLO and UMH classified the variant as a VUS, while STD classified the variant as likely pathogenic. Although BWH have observed the variant in their patient population, a summary report for this variant was not provided. ERA observed this variant in an individual with known familial mutation (LVWT = 9 mm), and classified the variant as pathogenic. See appendix L for details.

Literature

This variant has been reported in nine out of 4,057 HCM cases in eight journal articles published between 2004 and 2015. In their variant summary reports, STD

cited seven publications, while FLO and UMH used one publication (van Driest, 2004). ERA did not include published literature citations in their summary report. This suggests that the difference in number of publications used was a cause of discordance among the SHaRe sites as new published data sheds more light into the pathogenicity of the variant.

Lab Internal Data

STD noted in their summary report that LMM detected this variant in 3 out of >2000 Caucasian probands, while UMH noted in their summary report that in addition to the HCM cases LMM detected the variant in non-HCM cases, including 1 LVH, 1 DCM, and 1 individual with unknown diagnosis. Therefore, perhaps because both UMH and STD had access to parts of LMM's internal data, the presence of variant in LMM's non-HCM patients noted by UMH lead them to render the variant as a VUS, while STD rendered the variant as pathogenic in the absence of this information.

Site Internal Data

This variant was observed by FLO (n = 2), STD (n = 1), UMH (n = 2), and BWH (n = 1). One of the probands from FLO and one from UMH carried a second, pathogenic variant, which is used by these SHaRe sites as evidence against pathogenicity of this variant. As this information is not available to other SHaRe sites, lack of access to site internal data is a reason for discordance among SHaRe sites.

In silico

In their summary reports, three of four SHaRe sites (FLO, UMH, and STD) noted using PolyPhen's prediction of probably damaging, while ERA did not note any *in silico* predictions. Therefore, difference in *in silico* predictions is not a reason for discordance among SHaRe sites.

Evolutionary Conservation

FLO and STD SHaRe sites reported that the amino acids are highly conserved in their variant classification. The other two SHaRe sites (UMH and ERA) did not report evolutionary conservation analysis. In general, difference in evolutionary conservation analyses is not a reason for discordance among SHaRe sites.

Population Frequency

Three sites (FLO, UMH, and STD) reported using low frequency data from NHLBI's Exome Sequencing Project for their variant classification. ERA did not report any population frequency data. In general, difference in population frequencies used is not a reason for discordance among SHaRe sites.

Summary

The chief reasons for discordance between SHaRe sites included lack of incorporating latest published data, and lack of access to private lab and site data. Thus, this discordance can be resolved by ascertaining up-to-date published data and accessing the value of second pathogenic variants and observation the variant in non-HCM cases in both SHaRe as well as private lab data.

MYH7 - p.Met982Thr (c.2945T>C)

Introduction

p.Met982Thr in MYH7 has been detected in at least three patients in three SHaRe sites. In their most recent reviews of the classifications, UMH and BWH classified the variant as a VUS, while FLO classified the variant as likely pathogenic. See Appendix M for details.

Literature

This variant has been reported in 12 out of 1067 European ancestry HCM cases and 2 out of 485 European ancestry DCM cases in 8 journal articles (published between 2006 and 2015). In their summary reports, BWH noted seven publications that reported HCM as well as DCM cases, while FLO noted one publication that reports one HCM case; UMH did not note any published cases. Since the number of DCM cases is used as evidence against pathogenicity by BWH, this difference in number of journal articles is a reason for discordance among SHaRe sites.

Lab Internal Data

BWH and UMH based their classification of VUS partly on evidence in LMM's internal data that noted this variant in more than 14 individuals with HCM or DCM, and 3 of the individuals with HCM also carried other pathogenic variants. Since this information was not available to FLO, who classified the variant as likely pathogenic, prior to their last update of this variant, lack of access to internal lab data is a reason for discordance among SHaRe sites.

Site Internal Data

This variant is observed by UMH (HCM = 1), BWH (HCM = 2; DCM = 1), and FLO (HCM = 2). Since the DCM case observed in BWH was not available to FLO prior to their last update of this variant, who classified the variant as likely pathogenic, lack of access to internal site data is a reason for discordance among SHaRe sites. In addition, all five of the HCM cases also carried a second, pathogenic mutation, as did the DCM case. Both UMH and HCM used this information as evidence against pathogenicity, while FLO classified this variant as likely pathogenic. Since second, pathogenic variants can be evidence against pathogenicity, a difference in classification criteria used can also be a reason for discordance among SHaRe sites.

In silico

In their summary reports, BWH noted pathogenic prediction using LMM's in-house computational tool, and FLO also noted a pathogenic prediction using PolyPhen; UMH did not provide in silico model predictions. Therefore, the difference in *in silico* predictions is not a reason for discordance among SHaRe sites.

Evolutionary Conservation

In their summary reports, BWH and FLO noted that the variant is conserved across species; UMH did not provide evolutionary conservation analysis. Therefore, the difference in evolutionary conservation is not a reason for discordance among SHaRe sites.

Population Frequency

In their summary reports, BWH and FLO noted low variant frequency in general population as reported in ESP and ExAC, respectively; UMH did not note population frequency data in their summary report. Therefore, the difference in population frequency is not a reason of discordance among SHaRe sites. In addition, BWH noted, "The frequency in patients (0.26%; 14/5,340 chromosomes, LMM unpublished data) is similar to the frequency in the general population [0.26%; 88/33,370], suggesting a mild or even benign role." This insight into the frequency data was not noted by FLO in their summary report despite the use of similar frequency, although larger sample, data. Therefore, a difference in classification criteria is also a possible reason for discordance among SHaRe sites.

Summary

The chief reasons for discordance between SHaRe sites include lack of access to internal lab and site data, difference in number of published articles used, as well as difference in classification criteria pertaining to second pathogenic variants and use of population frequency data.

MYH7 - p.Thr1377Met (c.4130C>T)

Introduction

p.Thr1377Met in MYH7 has been detected in at least four patients in three SHaRe sites. In their most recent reviews of the classifications, FLO and UMH classified the variant as pathogenic and likely pathogenic, respectively, while BWH

classified the variant as a VUS. A variant summary report from FLO was not available for analysis. See appendix N for details.

Literature

This variant has been reported in 17 out of ~1950 HCM cases and 2 out of 269 unselected controls in 11 journal articles (published between 2003 and 2014). Both BWH and UMH provided information from the same five published articles. Therefore, differences in number or interpretation of published data are not a reason for discordance among SHaRe sites.

Lab Internal Data

BWH employed LMM to conduct genetic testing on their probands, who classified this variant as a VUS on ClinVar, while UMH employed GeneDx, who classified this variant as pathogenic on ClinVar. In their summary reports, both UMH and BWH stated using LMM's internal data, including 6 out of 3,250 HCM cases, and neither stated using internal data from GeneDx directly. Therefore, the difference in internal lab data is not a reason for discordance among SHaRe sites.

Site Internal Data

This variant is observed by UMH (n = 2), BWH (n = 1), and FLO (n = 1). None of the probands were reported to have additional variants or affected relatives that carried this variant. Therefore, the difference in internal site data is not significantly enough to be a reason for discordance among SHaRe sites.

In silico

In their summary reports, both UMH and BWH stated inconsistency in computational predictions (AlignGVGD, PolyPhen2, SIFT). Therefore, difference in *in silico* predictions is not a reason for discordance among SHaRe sites.

Evolutionary Conservation

In their summary reports, both UMH and BWH stated that the amino acid is conserved across species. Therefore, evolutionary conservation analyses are not a reason for discordance among SHaRe sites.

Population Frequency

UMH reported on the absence of the variant from the ExAC data, and BWH did not state using population database in their summary report. Therefore, a comparison analysis could not be performed.

Summary

In summary, both UMH and BWH have classified the variant based on the same information. However, UMH based their VUS classification on observing the variant in 2 out of 269 controls and inconsistency in computational predictions, while BWH based their likely pathogenic classification on observing the variant on >10 unrelated probands and absence from ExAC. Therefore, the discordance is likely due to classification criteria and not due to differences in sources of data.

TNNT2 - p.Arg278Cys (c.832C>T)

Introduction

The Arg278Cys variant in *TNNT2* has been detected in at least one patient in five sites. In their most recent reviews of the classifications, STD and FLO classified the variant as likely pathogenic, UMH and ERA classified it as pathogenic, while BWH classified it as a variant of unknown significance. See appendix O for details.

Literature

All 5 sites used Watkins et al. (1995) to achieve their current classifications. Watkins et al. reported this variant in a 17-year-old female who had normal left ventricular thickness yet had suffered a cardiac arrest; she was resuscitated. In the same family, the authors note that one family member survived a cardiac attack with no LVH and one person with abnormal LVH. The carrier status of these family members could not be confirmed from the study. FLO has used co-segregation from this study as one of the indicators to classify the variant as likely pathogenic, while other SHaRe sites do not report using segregation data from this study. In addition to difference in literature interpretation, there is a difference in the number of journal articles used by SHaRe sites to collect published data (BWH - 13, STD - 9, FLO - 2, ERA - 2, and UMH - none reported). Thus, literature interpretations as well as difference in number of publications used to collect data are a reason for discordance among SHaRe sites.

Lab Internal Data

BWH employed LMM to conduct genetic testing, who reported more than 15 European individuals with cardiomyopathy with this variant. However, 7 of 15 in lab's internal data carried a second likely pathogenic or pathogenic variant in another gene. This piece of internal data, which is not available to other sites, is the chief reason for BWH/LMM to sway their classification to a VUS. In addition, although no internal data was provided by GeneDx, UMH had aligned their classification of the variant with that of GeneDx (i.e. pathogenic).

Site Internal Data

This variant was observed by all five SHaRe sites: ERA (n = 3), FLO (n = 5), STD (n = 2), UMH (n = 2), and BWH (n = 4). After analyzing at least 3 major sarcomeric genes, FLO and BWH reported two probands with second variant and STD and UMH each reported one proband with a second variant. ERA did not report any probands with second pathogenic variant. All of the reported second variants were either classified as VUS or pathogenic. Since ERA do not have evidence of a second pathogenic variant in their probands, they have rendered this variant pathogenic. Furthermore, segregation was observed by FLO in their related patients, which is also taken into account by UHM to render their pathogenic classification. Of note, both of the affected members of this family also carried a second pathogenic variant that was not taken into account to classify the variant. Thus, site internal data is a reason for discordance among SHaRe sites as none of the sites shared their internal site data with the other sites.

In silico

In their summary reports, FLO, STD, and BWH/LMM reported the amino acid change to be damaging, while ERA and UMH did not report in silico predictions. In general, difference in *in silico* predictions is not a reason for discordance among SHaRe sites.

Evolutionary Conservation

In their summary reports, BWH/LMM report that the amino acid is not well conserved in evolution and 2 species (elephant and manatee) carry a cysteine at this position. FLO and STD reported the amino acid to be conserved in evolution, while the ERA and UMH did not cross species evolutionary analysis. Although it might not have a significant role in rendering variant classification, BWH/LMM might have used the conservation to put more weight on their VUS classification along with the lab's internal data.

Population Frequency

None of the sites reported seeing the variant with significant frequency in any of the large population cohorts (ESP and ExAC) or the controls used in published literatures. Thus, population frequencies are not a reason for discordance among SHaRe sites.

Summary

The chief reasons for discordance between the BWH and the other four sites are unique internal site data, internal lab data, difference in interpretation of literature, and evolutionary conservation.

TPM1 - p.Glu192Lys (c.574G>A)

Introduction

p.Glu192Lys variant in TPM1 has been detected in at least two patients in 2 SHaRe sites. In their most recent reviews of the classifications, STD classified the variant as a VUS (likely to cause disease), while BWH classified the variant as likely pathogenic. See Appendix P for details.

Literature

This variant has been reported in 7 out of 2564 HCM cases and 1 out of 63 Left ventricular non-compaction cases in 5 journal articles (published between 2008 and 2013). In their summary report, STD cited two journal articles: Fokstuen (2008) that reported one HCM case and Probst (2011) that reported one LVNC case. On the other hand, BWH cited two different journal articles: Ho (2009) that reported one HCM case and Deva (2013) that also reported one HCM case. Therefore, the difference in published data is a reason for discordance among SHaRe sites.

Lab Internal Data

BWH based their likely pathogenic classification of this variant partly on evidence provided by LMM's internal data, who had observed this variant in more than 10 individuals with HCM as well as in 3 affected family members. In their summary report, STD noted that GeneDx has not seen this variant in their patient population. Since STD classified this variant as a VUS in the absence of LMM's co-segregation data, lack of access to internal lab data is a reason for discordance among SHaRe sites.

Site Internal Data

This variant is observed by BWH (n = 1) and STD (n = 1). In their summary report, BWH reported using known TPM1 mutation indicating that a familial variant had been recognized, but no additional information on the affected relative was available. Therefore, this information cannot be analyzed as co-segregation. Neither of the SHaRe sites reported additional variants. Since both BWH and STD have similar internal data, lack of access to internal site data is likely not a reason for discordance among SHaRe sites.

In silico

In their summary reports, BWH noted pathogenic effects of amino acid change, while STD noted benign effects predicted by PolyPhen2. Therefore, a difference in *in silico* predictions is a reason for discordance among SHaRe sites.

Evolutionary Conservation

In their summary report, BWH stated using highly conserved status of the amino acid at this codon in their variant classification, while STD did not mention evolutionary conservation analysis in their report. Therefore, evolutionary conservation could not be analyzed as a source of discordance. However, taken together with other pieces of evidence (i.e. co-segregation; see below), evolutionary conservation cannot be deemed a major reason for discordance among SHaRe sites.

Population Frequency

In their summary reports, BWH and STD noted absence of this variant in large population frequency databases. Therefore, difference in population frequencies is not a reason for discordance among SHaRe sites.

Summary

The chief reasons for discordance between SHaRe sites include difference in literature citations used, lack of access to internal lab data, and use of different in-silico model predictions.

Summary Of Aggregated Data For Discordant Variants Among SHaRe Participating Sites

At least 297 HCM cases that carried one of -16 discordant variants in SHaRe were reported in published literature, internal lab databases, and SHaRe. Seventy seven of 297 carried an additional pathogenic variant and 22 had affected relatives with the same variant as the proband. For further details, see appendix A-P.

CHAPTER IV

DISCUSSION

Evaluation of frequency of disagreement in variant classifications revealed that the rate of discordance among ClinVar submitters (45%) was two and a half times higher than that among SHaRe participating sites (19%). This hike in discordance can be putatively attributed to multiple scientific and non-scientific factors. First, disease experts in clinical settings tend to do a more thorough follow-up of genetic findings including segregation analysis as compared to clinical labs that might not have the access to patient's relatives test results. Second, since the inception of SHaRe, it is likely that the sites corresponded with each other and resolved the discordances prior to submitting their classifications for some the variants that were initially discordant. In other words, the communication between SHaRe sites could have been better than the clinical laboratories, which could have aided in the reduced rate of discordance. Third, sampling error can also be at play given the large difference between the variants evaluated in ClinVar (n = 2,405) and in SHaRe (n = 589). In our future study, this sampling error can be resolved by analyzing the same set of 589 SHaRe variants in ClinVar. Fourth, the difference in rates of discordance might be attributed to the higher number of labs submitted to ClinVar (max 8 different submitters) as compared to SHaRe (max 5 different participants), as an increase in number of submitters also increases the likelihood of discordance among their classification. One way to access this hypothesis would be to analyze the number of submitters in concordant variants and compare them to the

number of submitters in discordant variants. Finally, a difference in submission of the last update of the variant reviews to ClinVar and to SHaRe might also play a role in difference in rate of discordance, as the further apart the dates of submission are the more likely they are to incorporate different data in their variant classification and more likely to be discordant. According to the data downloaded from ClinVar, dates of last review of the discordant variants dates as far back as January 2008. In sum, even though there is a possibility that the difference in rates of discordance is due to an artifact, the difference is large enough to recommend that engagement of expert centers for variant classification is vital to the process.

In order to bring to light the reasons for differences in classifications, we analyzed the variant summary reports from SHaRe sites which revealed three main reasons for discordance in variant classification. First, difference in internal lab and site data was the most prevalent reason for discordance noted in this study, highlighting the importance of data sharing among different sites . For example, the chief reason for discordance in classification of the variant with the most severe discordance (i.e. p.Glu619Lys in MYBPC3) was the difference in internal data, including HCM cases with additional pathogenic variants, DCM cases, as well as a high proportion of probands with Ashkenazi Jewish ancestry. The resolution for most of the discordances depends upon collaboration of data. Major efforts to share the data are underway by ClinGen to encourage laboratories to share their internal data and to resolve discordances in variant classification in light on the aggregate data.

Second, difference in use of published data also made a big difference in variant classifications. Most of these differences were due to outdated literature reviews. For instance, the splice site variant c.927-9G>A (intron 11) in MYBPC3 had discordant classification only because one of the SHaRe participating sites had not included any published data prior to 2007 in their summary report. Since literature searches require extensive man-hours, frequent updates of published data can be a challenge, especially for smaller clinical settings with limited human resources for manual curation. Thus, in order to resolve discordance caused by difference in published data, a systematic approach to collecting and interpreting literature derived data is required.

Third, diverse interpretations of the same set of data were inferred in seven of the 16 variants bringing to attention several areas of concern where consistent interpretation is needed for concordance in variant classification. In particular, a discussion regarding interpretation of second pathogenic variants, given that 3-5% of HCM cases carry more than one pathogenic variant, and definition of segregation data, given the reduced penetrance and variable expressivity of HCM, is warranted. In addition, two variants had discordant classifications in part due to difference in interpretation of population frequency data. Thus, standardization of evidence required to link a variant to disease causation in light of information on common variation across many populations is very much needed. Furthermore, two variants had discordant classifications in part due to difference in interpretation of non-HCM cases observed carrying the variant. In sum, efforts need to be made to custom tailor

the existing classification criteria recommended by ACMG according to the needs of specific conditions.

Other reasons for discordance, including differences in *in silico* model predictions, evolutionary conservation, and population frequency, were observed in lesser frequencies in this set of variants (i.e. less than 4 of 16).

Limitations

Limitations to findings in this study included the following factors: a small number of discordant variants were studied; internal lab data was not provided for all the labs (special requests were made for some variants); and where information on sources of data (e.g. use of published data, in silico models, etc.) was not provided by the SHaRe sites, we could not tell whether the SHaRe participating sites did not use that particular source of data, their significance was not appreciated, or the activity simply was not reported. The last two limitations can be eliminated by standardizing variant summary reports, which will also aid in easy identification of reasons for discordance and in so doing decrease the time to resolve discordances. In addition, establishing standards for contents uploaded on to databases is especially important at this stage in database development as genetic testing and sharing of large sets of data becomes more and more common place due to efforts by initiatives like ClinGen.

Future Studies

In a future study, the aggregated variant summary reports will be used as surveys to investigate if the SHaRe participating sites would agree on the same classification when they have the same set of data. This will provide insights on how

to resolve discordances and what are some of the data sources where classification criteria need to be adjusted to reach consensus.

CHAPTER V

CONCLUSION

In conclusion, this study underscores the importance of sharing private data and increased collaboration of cardiology experts in variant classification. The study also brings to light the need to create classification criteria custom tailored to the unique challenges of classifying variants associated with HCM. With an influx of genetic testing companies offering testing for cardiomyopathies as well as whole exome and genome sequencing, it is important to establish an atmosphere and platform for collaboration between clinical laboratories and disease experts to improve the quality of clinical care by way of improving the quality of genetic test interpretations.

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APPENDICES

APPENDIX A

VARIANT SUMMARY REPORT - MYBPC3 - c.927-9G>A

Summary

-Seen in at least 30 presumably unrelated cases of HCM (4 published, >26 unpublished).

-Four of >30 patients who had another variant:

-One patient with p.Arg502Trp in *MYBPC3* (pathogenic), with a severe phenotype and segregation consistent with two pathogenic variants.

-One patient with p.Arg278Cys (c.832C>T) in *TNNT2* (ClinVar: VUS by LMM, Blueprint, CHEO, Semsarian's group; pathogenic by GeneDx), with severe phenotype, no segregation data.

-One patient with p.Asn47Lys (c.141C>A) in *MYL2* (ClinVar: VUS by LMM, Blueprint; pathogenic by GeneDx), diagnosed 56yo with LVWT 24 mm.

-One patient with c.2864del in *MYBPC3* (not in ClinVar, not published, ERA classifies as pathogenic), diagnosed 54yo with LVWT 21 mm.

-There is moderate segregation data. In 5 families an additional affected relative carried this variant.

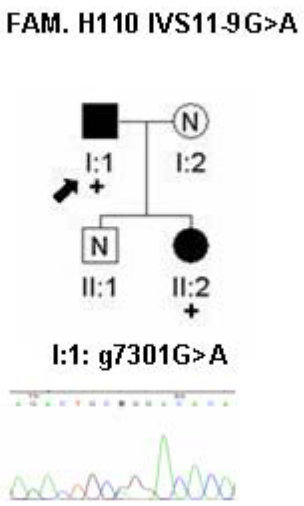
-In total, the variant has not been seen in at least 6326 and as many as 64,326 individuals from presumably unrelated published and database controls.

- Both in silico and in vitro data suggest that this variant causes aberrant splicing. Aberrant splicing has been confirmed in heart tissue from an HCM patient with

observation of abnormal intron inclusion and a premature termination codon downstream.

Published Cases

Rodriguez-Garcia (2010) reported this variant in 2 patients diagnosed with HCM cared for in Complejo Hospitalario Universitario A Coruña, Spain, who underwent analysis of 537 genetic variants of HCM disease genes - TNNT2, TNNI3, TPM1, MYL2, MYL3, ACTC, TTN, MYH6, MYLK2, MYO6, and TCAP. No phenotype information is provided for the two patients. Authors report 1 affected male has an affected daughter with this variant.



Crehalet (2012) reported this variant in 2 patients diagnosed with HCM cared for in Bron, Lyon, and Nantes (France) who underwent sequencing analysis of MYBPC3, MYH7, TNNT2, and TNNI3 genes. One of the patients was 12-year old boy with HCM and the other was a 30-year old man with HCM and atrial fibrillation.

Gruner (2014) reported this variant in one “G+/P-” patient cared for at the Tufts Medical Center or the Toronto General Hospital. The patient is a 27-year-old woman with a normal maximum left ventricular thickness of 11 mm and no LVOT obstruction was present. Her family history was not reported.

Laboratory Data

According to LMM’s report submitted to ClinVar on April 4, 2014, they observed this variant in >20 HCM probands of unknown ethnicity and 4 affected relatives.

According to GeneDx’s report submitted to ClinVar on May 12, 2014, they observed this variant in multiple HCM probands of unknown ethnicity.

SHaRe

4 sites have reported patients with this variant in SHaRe registry.

STD reported 2 probands of unknown ancestry with this variant after analyzing ACTC1, CAV3, GLA, LAMP2, MTTG, MTTI, MTTK, MTTQ, MYBPC3, MYH7, MYL2, MYL3, PRKAG2, TNNC1, TNNI3, TNNT2, TPM1, and TTR genes on the HCM panel by GeneDx. These two probands are likely among the multiple individuals reported in GeneDx variant summary report. One of these probands also had an additional variant in the MYBPC3 gene (c.1504C>T; p.Arg502Trp, which is classified as pathogenic by all four submitters in ClinVar). This patient has a severe phenotype with cardiac arrest as a teenager and burnt out disease at 20yo. Of note, her parent with c.927-9G>A has evidence of HCM. The other patient had p.Arg278Cys (c.832C>T) in TNNT2 (conflicting classifications in ClinVar), with a severe

phenotype including severe left ventricular hypertrophy requiring myectomy, ventricular tachycardia, and sufficient sudden death risk to warrant ICD.

UMH reported four probands with this variant after genetic testing at LMM or GeneDx, which included at least 8 sarcomeric genes (ACTC1, MYBPC3, MYH7, MYL2, MYL3, TNNI3, TNNT2, and TPM1). One of these patients also carries a missense variant in MYL2, p.Asn47Lys (c.141C>A), which is classified in ClinVar as a VUS by LMM and Blueprint and pathogenic by GeneDx. That patient was diagnosed at 56yo and has a max wall thickness of 24. The range of max wall thickness among these probands is 24-28 mm. These probands are likely among the multiple individuals reported in GeneDx and LMM's variant summary reports.

BWH reported four probands with this variant after analysis of at least 8 sarcomeric genes (ACTC1, MYBPC3, MYH7, MYL2, MYL3, TNNI3, TNNT2, and TPM1) at the LMM. In one of the families an additional affected relative carried this variant. The range of max wall thickness among these probands is 17-27 mm. None of these probands had an additional sarcomere variant. These probands are likely among the multiple individuals reported in LMM's variant summary reports.

ERA reported one proband of European ancestry with this variant. List of genes analyzed for this proband was not available. The patient was diagnosed at 64yo, had a left ventricular wall thickness of 21 and had an additional MYBPC3 variant, c.2864del.

Additional Data

This variant is located in flanking intronic regions of intron 11. According to Crehalet et al. (2012), the weak acceptor splice site of intron 11 was detected by Human Splicing Finder (HSF) which predicted the creation of a new acceptor site at position c.927-9, which is stronger than the wild type. In addition, HeLa cells transfected with the wild type sequence showed two different transcripts: a 410 bp normal product containing exon 12 and the 248 bp product corresponding to exon 12 skipping, as for the wild type minigene c.1090G, confirming that the weak acceptor site of intron 11 is not always recognized in HeLa cells. Furthermore, mutant minigene assay showed complete exon 12 skipping and the transcript lacking exon 12 would encode a truncated protein. Helms et al. (2014) confirmed altered splicing and generation of a premature termination codon by measuring PCR amplification and relative gene expression of *MYBPC3* in myocardium tissue of a patient with HCM carrying this variant.

Additional MYBPC3 splice variants reported in association with HCM include: IVS2-1G>A, IVS6-2A>C IVS7+1G>A, IVS8+1G>A, IVS12-2A>G, IVS14-2A>G, IVS16-1G>A, IVS22+1G>A, IVS24+1G>A, IVS28+1G>A, IVS32+1G>A, and IVS33+1G>A.

The base is conserved across mammals with the exception of Cape golden mole (T).

Frequency In Controls, Large Cohorts Unselected For HCM

In total the variant has not been seen in at least 6326 and as many as 64,326 individuals from published controls and publicly available datasets that approximate the general population.

The variant is absent in European American and African American populations by the NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVS), which includes data on ~6,000 individuals of European or African American backgrounds. It is also not listed in the Exome Aggregation Consortium dataset (http://exac.broadinstitute.org/), which currently includes variant calls on ~64,000 individuals of European, African, Latino and Asian descent (as of April 22nd, 2015). We think that this dataset goes sufficiently far into introns to provide data on this variant, but we are awaiting confirmation of that from the ExAC group. This variant is currently listed in dbSNP: rs397516083. The variant was not observed in the following published control samples: Gruner, 2014 in 126 control individuals and Rodriguez-Garcia, 2010 in 200 control individuals.

APPENDIX B

VARIANT SUMMARY REPORT - MYBPC3 - p.Arg810His (c.2429G>A)

Summary

-Seen in at least 29 presumably unrelated cases of HCM (5 published, 24 unpublished).

-11 of 27 cases who had sequencing of at least MYH7, MYBPC3, and TNNT2 had another variant (10 had another sarcomeric variant, 1 had an FRDA variant).

-There is weak segregation data. In three families an additional affected relative carried Arg810His. In two other families an additional affected relative carried Arg810His in addition to another variant.

-In total, the variant has been seen in 4 of ~60,751 individuals from published controls and publicly available datasets that approximate the general population.

Published Cases

Nanni L et al. (2003) reported this variant in two patients diagnosed with HCM cared for in Rome who underwent analysis of MYH7, MYBPC3, and TNNT2. One of the two patients was homozygous for the variant and presented with dyspnea at age 39. He also showed severe hypertrophy (IVS 32 mm) and a LVOTO of 50 mmHg. Authors report a positive family history but no evidence was provided, and reported that he came from a closed community of a little town that may explain the homozygous genotype. The other patient, who also carries a second variant (MYBPC3-Arg820Gln) had moderate non-obstructive hypertrophy. Arg820Gln has

conflicting classifications in ClinVar: LMM – likely pathogenic, GeneDx – Pathogenic, and Molecular Genetics Diagnostic Laboratory – VUS.

Ackerman's group reported this variant in a patient with HCM. This cohort included 389 unrelated patients with HCM who were assessed for variants in eight HCM-associated genes (van Driest et al., 2004). The patient did not have any other sarcomere variants. However, the same group later reported a novel variant in FRDA gene (Arg40Cys) associated with Fredeich ataxia in this patient (Van Driest S et al. 2005). They searched for variants in FRDA in 389 patients with clinical HCM who were previously assessed for variants in eight HCM-associated genes. None of the 389 patients had a clinical diagnosis of Friedreich ataxia and the FRDA trinucleotide repeat expansion size was normal on both alleles. The double heterozygous patient was diagnosed with unequivocal and unexplained cardiac hypertrophy at 12 years of age but remained clinically asymptomatic until age 32. He underwent ICD placement due to recurrent ventricular fibrillation and received multiple appropriate shocks. He went on to develop left ventricular systolic dysfunction (ejection fraction 35%). No evidence of disease was found in any of his living first degree relatives. The authors propose that the combination of the MYBPC3 variant and the FRDA variant lead to his HCM. They argued that the young diagnosis, arrhythmic burden, and heart failure are all atypical for MYBPC3-associated HCM. They note no neurological involvement.

McKenna's group reported this variant in one out of 79 HCM patients with diagnosis under 13 years old recruited in the UK (Kaski et al. 2009). No individual

phenotype, genotype or ancestry information was reported. They sequenced 9 sarcomere genes (MYH7, MYBPC3, TNNI3, TNNT2, TPM1, MYL2, MYL3, ACTC, and TNNC1), the genes encoding desmin (DES), and the gamma-2 subunit of AMP kinase (PRKAG2). The paper states that a total of 5/79 patients were double heterozygotes in MYBPC3 gene, but it doesn't mention which variants in this gene were found in these individuals. Therefore, the variant zygosity of this patient is equivocal.

Maron and Semsarian reported this variant in two brothers with HCM (Maron et al. 2011). One of the brothers has two unaffected daughters (aged 10 and 12), who are heterozygous for this variant but had normal echocardiograms at that time. They do not note how many genes were analyzed, ancestry, or where the cases were recruited (though most likely Maron and Semsarian's clinics).

Laboratory Data

LMM's most recent data appears to be what they shared with the Michigan group in 2014. They identified this variant in 9 Caucasian HCM probands (out of >2,300 tested) and 2 affected relatives. They note that of the 14 individuals carrying this variant, 7 carried another likely or possibly significant variant. Given 3 of 5 published cases have another variant; this suggests that 4 of their 9 cases had another variant.

GeneDx has observed this variant in 14 presumably unrelated individuals of Caucasian ancestry (personal communication to Colleen Caleshu on 4/22/2015). Phenotype data suggestive of HCM was available on 11 of 14 index cases. Three out

of 14 probands had additional variants. Six family members were positive for Arg810His but most had no clinical data submitted. The only segregations were in two families, however in both the one additional relative with phenotype suggestive of cardiomyopathy had another variant classified as pathogenic (as did the probands in those families). Additional details on each of these cases are provided below.

SHaRe

4 sites have reported patients with this variant in SHaRe registry.

FLO reported two probands with this variant after analyzing ACTC1, MYBPC3, MYH7, MYL2, MYL3, TNNI3, TNNT2, TPM1 genes. One of these probands also had an additional variation in MYBPC3 gene (c.818G>A; p.Arg273His). Of note, while the cases reported by Nanni et al. (2003) also originate in Italy, it appears they are not the same probands since they have four distinct genotypes.

STD reported one probands with this variant after analyzing ACTC1, CAV3, GLA, LAMP2, MTTG, MTTI, MTTK MTTQ, MYBPC3, MYH7, MYL2, MYL3, PRKAG2, TNNC1, TNNI3, TNNT2, TPM1, and TTR genes on the GeneDx panel. This patient also had an MYH7 splice variant, that is a VUS (c.3726+6 C>T). This case is redundant with the GeneDx cases.

UMH reported two probands with this variant after analyzing ACTC (ACTC1), CAV3, GLA, LAMP2, MTTG, MTTI, MTTK, MTTQ, MYBPC3, MYH7, MYL2, MYL3, PRKAG2, TNNC1, TNNI3, TNNT2, TPM1, and TTR genes on GeneDx HCM panel. These cases are redundant with the GeneDx cases.

BWH reported two probands with this variant after analyzing 11 genes on LMM's HCM CardioChip panel (the exact gene names are currently not available). These cases are redundant with the LMM cases.

Additional Data

In silico analysis with PolyPhen-2 predicts the variant to be probably damaging and SIFT predicts the variant to be deleterious. The arginine at codon 810 is highly conserved across species, as are neighboring amino acids. Other variants have been reported in association with disease at this codon (R810L and R810Q) and nearby codons (G805S; K811R).

Frequency In Controls, Large Cohorts Unselected For HCM

Total the variant has been seen in 4 of ~60,751 individuals from published controls and publicly available datasets that approximate the general population.

The variant was reported online in 4 of 60,259 individuals in the Exome Aggregation Consortium dataset (http://exac.broadinstitute.org/), which currently includes variant calls on ~64,000 individuals of European, African, Latino and Asian descent (as of April 22nd, 2015). Specifically, the variant was observed in 4 of ~33,317 European individuals. The phenotype of those individuals is not publicly available. The dataset is comprised of multiple cohorts, some of which were recruited from the general population; others were enriched for common cardiovascular disease. Note that other variants with strong evidence for pathogenicity have been seen at similar frequencies in datasets like this so this does not necessarily rule out pathogenicity (Pan et al. 2012). This variant is currently listed in dbSNP:

rs375675796. The variant was not observed in the following published control samples: Nanni (2003) did not report this variant in 100 control individuals, Van Driest (2004) did not report it in 200 control individuals, Roncarati (2011) did not report it in 192 controls.

APPENDIX C

VARIANT SUMMARY REPORT - MYBPC3 - p.Gln998Glu (c.2992C>G)

Summary

-Seen in at least 35 presumably unrelated cases of HCM (9 published, 26 unpublished).

-Most published cases were East Asian (6/9).

-Multiple cases that had sequencing of at least *MYH7*, *MYBPC3*, and *TNNT2* had another variant.

-Both LMM and GeneDx report that multiple cases tested in their labs had additional variants, though they do not note the exact number.

- 39-year-old male with HCM and family history of HCM in both parents, carried another variant in *TNNI3* (p.Arg145Trp) (ClinVar: pathogenic by LMM, GeneDx; likely pathogenic by CHEO).

-A patient with unknown clinical information carried another variant in *MYH7* (p.Ile263Thr) (ClinVar: pathogenic by LMM and GeneDx).

-32-year-old patient with HCM, carried another variant in *MYBPC3* (p.Asp770Asn) (ClinVar: pathogenic by GeneDx; likely pathogenic by LMM, CHEO).

-36-year-old male with HCM carried another variant in MYBPC3 (IVS3 + 41 G/C) (ClinVar: not found; seen in two HCM cases in this publication both with a second variant)

-There is weak segregation data. In one family, an additional affected relative carried this variant, while two unaffected members of the same family carried this variant along with another variant.

-In total, the variant has been seen in 75 individuals (including 69 heterozygotes and 6 homozygotes) out of ~ 15,774 individuals from published controls and publicly available datasets that approximate the general population.

- Highest frequency is in Latinos: 83 heterozygotes and 4 homozygotes out of 922 individuals

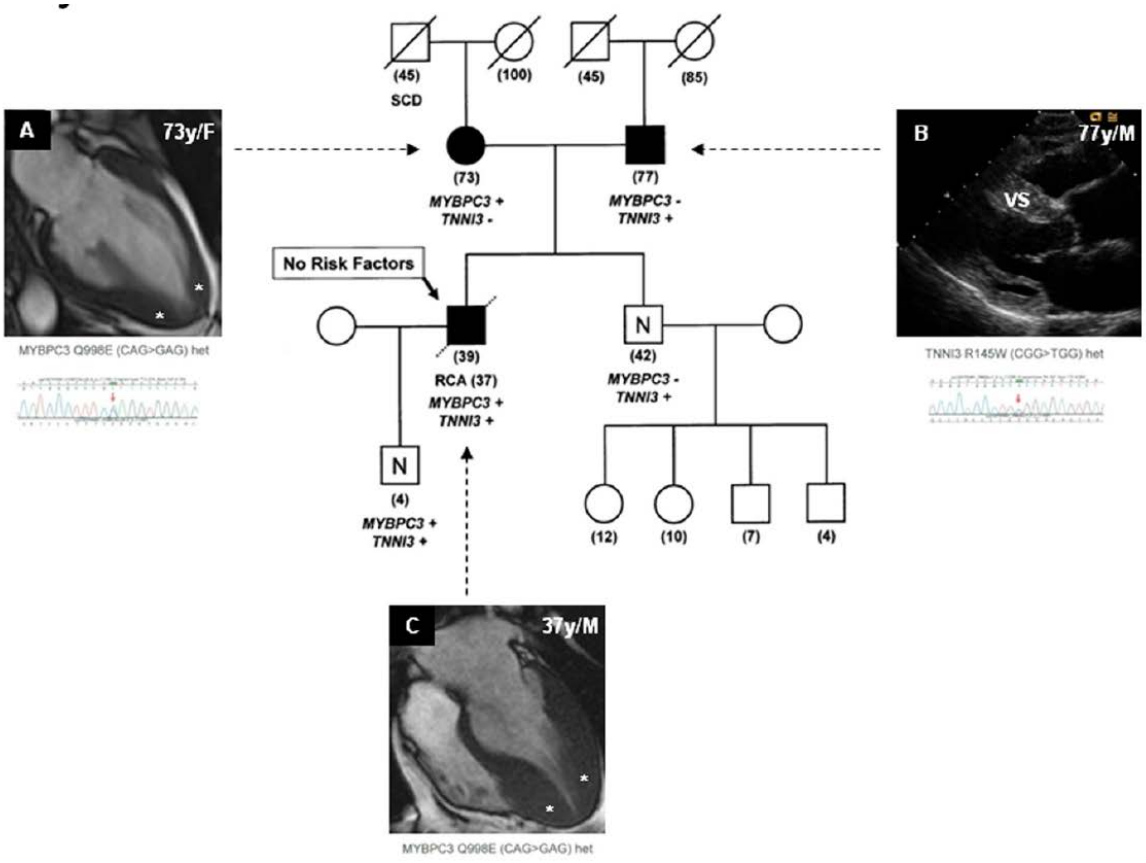
- East Asians: 41 heterozygotes and 2 homozygotes out of 1,510 individuals

Published HCM Cases

Van Driest et al. (2004) reported the variant in one of 389 unrelated individuals of unreported ancestry with HCM that were cared for at Mayo Clinic College of Medicine, Rochester, Minnesota. They sequenced 7 sarcomere genes (*MYH7, MYL2, MYL3, TNNT2, TNNI3, TPM1*, and *ACTC*). The patient did not have any additional variants.

In 2012, Maron et al. published a series of compound or double heterozygotes, and reported this variant in 1 proband as a double heterozygote with an additional variant in *TNNI3* gene. Cases were recruited from the Marons' and Semsarian's cohorts. The proband is a 39-year-old asymptomatic man of unreported ancestry previously diagnosed with non-obstructive HCM and hypertrophy confined to the apical region (25 mm). Proband inherited p.Gln998Glu variant from his mother, who was 73 years old with mild apical hypertrophy (15 mm) at the time of evaluation. He

inherited the *TNNI3* variant (p.Arg145Trp) from his father, who was 77 years old with non-obstructive HCM (anterior septum, 19 mm) with paroxysmal atrial fibrillation at the time of evaluation. The second variant is classified as pathogenic by LMM and GeneDx, and likely pathogenic by CHEO in ClinVar. The proband's 4-year-old son and 42-year-old brother are asymptomatic and have inherited both variants. Please refer to the pedigree for more details below.



Konno et al. (2006) reported the variant in 3 of 292 unrelated Japanese HCM patients. They only sequenced *MYBPC3* for their analysis, and did not report any additional variants.

Bahrudin et al. (2008) reported the variant in 1 of 30 Japanese patients with HCM. They only sequenced *MYBPC3* for their analysis, and did not report any additional variants.

Garcia-Castro et al. (2009) reported the variant in 1 of 120 European ancestry (Spain) HCM cases. They sequenced 5 sarcomere genes (*MYH7, MYBPC3, TNNT2, TNNI3,* and *TPM1*), and did not report any additional variants.

Maeda et al. (2009) reported the variant in one of 11 Japanese individuals with HCM. They sequenced 3 sarcomere genes (*MYH7, MYBPC3,* and *TNNT2*. The proband, 36-year-old male, carried another variant in MYBPC3 (IVS3 + 41 G/C), which was not found in ClinVar. The authors report the splice site variant in two HCM cases in this publication both with a second variant.

Berge et al. (2014) reported this variant in one out of 696 European ancestry patients from Norway diagnosed with HCM, who underwent analysis of the *MYH7, MYBPC3, TNNI3, TNNT2, MYL2,* and *MYL3* genes. The proband had an additional variant in *MYH7* gene (p.Ile263Thr), which is classified as pathogenic in ClinVar by LMM and GeneDx. No other phenotypic information was reported in the paper.

Chiou et al. (2015) reported this variant in one out of 38 Taiwanese patients diagnosed with HCM, who underwent analysis of *MYH7, MYBPC3, TNNT2, TNNI3, MYL2, MYL3, TPM1,* and *ACTC* genes. The proband had max wall thickness of 22 mm and no other variants were found in the aforementioned genes.

Published Non-HCM Cases

Lakdawala et al. (2012) report this variant in 1 out of 264 probands cared for in North America with dilated cardiomyopathy.

Laboratory Data

In LMM's variant summary report submitted to ClinVar (Oct 19, 2012), they identified this variant in 25 HCM individuals from 23 families of unreported ancestry. They note that multiple individuals with this variant carried another variant, and that they have seen it in multiple probands with HCM and DCM (personal conversation with Colleen Caleshu in October, 2011). The specific number of these probands not provided.

According to personal conversation with Colleen Caleshu in October, 2011, GeneDx has seen this variant in multiple probands with familial cardiomyopathy, however many of them had another pathogenic variant. A specific number of these probands not provided.

SHaRe

Two sites have reported patients with this variant in SHaRe registry.

UMH reported one proband with this variant after analyzing 11 genes on LMM's HCM CardioChip panel. The proband was diagnosed at the age of 32 with max wall thickness of 25 mm, and also had an additional variant in the *MYBPC3* gene, p.Asp770Asn (c.2308G>A), which is classified as likely pathogenic by LMM and CHEO and pathogenic by GeneDx in ClinVar. (June 19, 2015). This case is redundant with the LMM cases.

STD reported one proband with this variant after analyzing ACTC1, CAV3, GLA, LAMP2, MTTG, MTTI, MTTK MTTQ, MYBPC3, MYH7, MYL2, MYL3, PRKAG2, TNNC1, TNNI3, TNNT2, TPM1, and TTR genes on the GeneDx panel. The proband had a maximum wall thickness of 14 mm, and was not reported to have any additional variants in the tested genes. This case is redundant with the GeneDx cases.

Additional Data

In silico analysis with PolyPhen-2 predicts the variant to be "probably damaging" and SIFT predicts the variant to be "damaging." The glutamine at codon 998 is completely conserved, as are neighboring amino acids.

Other variants have been reported in association with disease at this codon: c.2993A>G (p.Gln998Arg) reported by Van Driest et al. (2004) is classified as a VUS by LMM in ClinVar, no other submitters; c.2992C>T (p.Gln998Ter) reported by Millat G et al. (2010) is classified as pathogenic by GeneDx in ClinVar, no other submitters. Another missense variant in a nearby codon has also been reported in association with HCM: p.Arg1002Gln reported by Seidman group online is classified as a VUS by LMM and GeneDx in ClinVar.

Frequency In Controls, Large Cohorts Unselected For HCM

In total, the variant has been seen in 72 heterozygotes and 6 homozygotes out of ~ 15,786 individuals from published controls and publicly available datasets that approximate the general population. The highest frequencies were observed in Latinos (83 heterozygotes and 4 homozygotes out of 922 individuals; allele frequency

= 91/1844) and East Asians (41 heterozygotes and 2 homozygotes out of 1510 individuals; allele frequency = 45/3020).

Large Public Cohorts

This variant is currently listed in dbSNP: rs11570112. As of June, 2015, the variant was reported online in 66 heterozygous and 6 homozygous individuals of 13,750 individuals in the Exome Aggregation Consortium dataset (http://exac.broadinstitute.org/), which currently includes variant calls on ~64,000 individuals of European, African, Latino and Asian descent. Specifically, the variant was observed in 82 heterozygous and 4 homozygous out of 500 Latino individuals, 39 heterozygous and 2 homozygous out of 688 of Asian individuals, 7 heterozygous out of 4107 of South Asian individuals, and 3 of 6532 European ancestry individuals. The phenotype of those individuals is not publicly available. The dataset is comprised of multiple cohorts, some of which were recruited from the general population; others were enriched for common cardiovascular disease.

Publications

Ng et al. (2013) reported this variant in three out of 402 research participants, not selected for arrhythmia, cardiomyopathy, or a family history of sudden death, who underwent whole exome sequencing (WES) analysis. The phenotype of those individuals is not available.

The variant was not observed in the following published control samples: 200 (100 black and 100 white) control individuals analyzed by Van Driest et al. (2004), 200 control individuals studied by Konno et al. (2006), 100 unrelated healthy

individuals studied by Bahrudin et al. (2008), and 100 control individuals studied by Chiou et al. (2015).

Laboratories

Additionally, the variant was not observed in GeneDx's sample of 556 Caucasian and African-American individuals, but was present in 1 of 56 individuals of Asian ancestry. They suspect that the control individual they observed this variant in may be the same individual reported in dbSNP (apparently because of ancestry). Familion/Transgenomic shared that they have seen the variant in 3 of their 422 healthy controls (two of Asian ancestry, the other Hispanic). They also note that several of the reported HCM cases are of Asian descent.

APPENDIX D

VARIANT SUMMARY REPORT - MYBPC3 - p.Glu619Lys (c.1855G>A)

Summary

-Seen in at least 10 presumably unrelated cases of HCM (5 published, 5 unpublished). Most were of European ancestry (4/10).

-The variant has also been observed with other phenotypes including DCM, LVNC and WPW.

-In 5 of 10 cases who had sequencing of at least MYH7, MYBPC3, and TNNT2 the patient had another variant.

-One proband carried a second variant in MYH7 (p.Arg663His) (ClinVar: pathogenic by LMM, GeneDx, Blueprint Genetics, and Invitae, and likely pathogenic by CHOE)

-One proband carried a second variant in MYBPC3 (p.Arg502Gln) (ClinVar: pathogenic by LMM, GeneDx, and Invitae)

- One proband carried a second variant in MYBPC3 (p.Leu1221fs) (ClinVar: not reported)

- Two probands carried a second variant in MYH7 (c.976G>C; p.Ala326Pro) (ClinVar: VUS by LMM (Jul 29, 2011) and CHEO, and pathogenic by GeneDx (Jun 17, 2014))

- GeneDx has seen the variant in multiple families with HCM and DCM; a third of these cases were of AJ; a third carried a second variant (as per UMH's variant summary report)

-There is weak segregation data. In one family a relative with mild hypertrophy (LVWT = 12 mm) also carried the variant. In one family with DCM one affected relative carried this variant.

- In total the variant has been seen in 45 of ~28,804 individuals from published controls and publicly available datasets that approximate the general population. The highest frequency is in individuals of European ancestry (41 of ~15,386 in ExAC (0.27%)).

Published HCM Cases

Frisso et al. (2009) reported this variant in 1 out of 39 European patients with HCM that were cared for at the Monaldi Hospital, Second University of Naples, Italy. Subjects underwent analysis of the MYH7, MYBPC3, TNNT2, TNNI3, TPM1, MYL2, MYL3, and ACTC genes. The proband is a 39-year-old female, who was diagnosed at the age of 12, with LVWT of 15 mm. This proband has a family history of HCM (details not provided). No additional phenotypic information or variants were provided by the authors.

Brito et al. (2012) reported this variant in two out of 77 European patients with HCM cared for in Lisboa, Portugal, who underwent analysis of MYBPC3, MYH7, TNNT2, TNNI3 and MYL2 genes. Both of the probands had LVWT of >15 mm. The first proband carried an additional variant in the MYH7 gene (p.Arg663His), which is classified as pathogenic by LMM, GeneDx, Blueprint Genetics, and Invitae, and likely pathogenic by CHOE in ClinVar. The second proband carried an additional variant in the MYBPC3 gene (p.Arg502Gln), which is

classified as pathogenic by LMM, GeneDx, and Invitae in ClinVar. Both of the probands had a family history of HCM (no details provided). No additional phenotypic information or variants were provided by the authors.

Kaseem et al. (2013) reported this variant in 1 out of 192 Middle Eastern patients with HCM that were cared for in Egypt, who underwent analysis of the MYBPC3, MYH7, and TNNT2 genes. The proband had an LVWT of ≥15 mm. No additional phenotypic information or variants were provided by the authors.

Marsiglia et al. (2013) reported this variant in 1 out of 268 Latino ancestry patients with HCM that were cared for in Brazil, who underwent analysis of MYH7, MYBPC3, and TNNT2 genes. The proband had LVWT >15 mm. This proband carried an additional variant in the MYBPC3 gene (p.Leu1221fs), which has not been reported in ClinVar. No additional phenotypic information or variants were provided by the authors.

Published Non-HCM Cases

Moller et al. (2009) reported this variant in 1 out of 31 dilated cardiomyopathy cases of European ancestry that were cared for in Copenhagen, Denmark. Subjects underwent analysis of MYH7, MYBPC3, TPM1, ACTC, MYL2, MYL3, TNNT2, CSRP3 and TNNI3 genes. The proband is a ~30-year-old male, required HTX 3 years after the onset of symptoms. This proband had an affected brother and an unaffected daughter who carry this variant. No additional phenotypic information or variants were provided by the authors.

Kaseem et al. (2013) reported this variant in 1 patient with Non-compaction LV cardiomyopathy without hypertrophy of Middle Eastern ancestry that was cared for in Egypt, who underwent analysis of MYBPC3, MYH7, and TNNT2 genes. No additional phenotypic information or variants were provided by the authors.

Laboratory Data

GeneDx did not report any internal data in their summary report on ClinVar (5/8/2014). However, according to UMH's variant summary report ascertained for SHaRe comparison study, GeneDx has seen the variant in multiple families with HCM and DCM. A third of these cases were of Ashkenazi Jewish ancestry and a third carried a second variant. This information could not be confirmed by GeneDx's variant summary report.

In LMM's summary report submitted to ClinVar (6/5/2014), they identified this variant in > 5 presumably unrelated cases of unreported ancestry with varying cardiomyopathies including DCM, HCM, LVNC and WPW.

SHaRe

Three sites have reported patients with this variant in the SHaRe registry.

BWH reported 2 probands. One of the probands was a female diagnosed at the age of 48 years, who had genetic testing using LMM's HCM Next Gen Subpanel. This proband carried an additional variant in GLA, LAMP2, and PRKAG2 genes (specific amino acid changes in these genes were not reported). The second proband was a female diagnosed at the age of 48 years. These probands are likely redundant with the LMM cases.

FLO reported 2 probands of European ancestry, who underwent the analysis of 3 "major" sarcomeric genes using Sanger sequencing. One of the probands was diagnosed at the age of 51 years, with max wall thickness of 15 mm. This proband carried an additional variant in the MYH7 gene (c.976G>C; p.Ala326Pro), which is classified as a VUS by LMM (Jul 29, 2011) and CHEO, and pathogenic by GeneDx (Jun 17, 2014) in ClinVar. The second proband was diagnosed at the age of 75 years, with max wall thickness of 20 mm. This proband also carried the same MYH7 variant as the first proband. A family member of the second proband also carried p.Glu619Lys and had mild hypertrophy (LVWT = 12 mm). These probands have not been reported previously.

UMH reported 1 proband, who underwent the analysis of genes on GeneDx's HCM panel. This proband was diagnosed at the age of 50 years, with max wall thickness of 14 mm, and has two unaffected relatives that carry this variant (LVWT of 10 and 9 mm). These probands are likely redundant with the GeneDx cases.

Additional Data

In silico analysis with PolyPhen-2 predicts the variant to be benign and SIFT predicts the variant to be deleterious. The Glutamic acid at codon 619 is moderately conserved across species. Elephant and chicken carry Glutamine and Asparagine instead of Glutamic acid, respectively. Neighboring amino acids are well conserved. No other variants have been reported in association with disease at this codon. Other variants reported in association with disease at nearby codons include: p.Pro608Leu

(van Driest, 2004), p.Asp610His (Olivotto, 2008), p.Ala627Val (Garcia-Castro, 2005).

Frequency In Controls, Large Cohorts Unselected For HCM

In total the variant has been seen in 45 of ~28,804 individuals from published controls and publicly available datasets that approximate the general population. This variant is currently listed in dbSNP: rs200352299.

The variant was reported online in 43 of ~27,404 individuals in the Exome Aggregation Consortium dataset (http://exac.broadinstitute.org/), which currently includes variant calls on ~64,000 individuals of European, African, Latino and Asian descent (as of April 22nd, 2015). Specifically, the variant was observed in 41 of ~15,386 European individuals (0.27%), 1 of ~1,767 Latino individuals, and 1 of ~4,781 South Asian individuals. The phenotype of those individuals is not publicly available. The dataset is comprised of multiple cohorts, some of which were recruited from the general population; others were enriched for common cardiovascular disease. None were recruited for rare Mendelian cardiomyopathy and in some cohorts such cases were excluded.

The variant was not observed in the following published control samples: 200 in Frisso (2009), 100 in Brito (2012), 100 in Moller (2009).

APPENDIX E

VARIANT SUMMARY REPORT - MYBPC3 - p.Gly490Arg (c.1468G>A)

Summary

-Seen in at least 11 presumably unrelated cases of HCM (5 published, 6 unpublished).

- 4 out of 11 of European ancestry; ancestry was not reported for 4 cases

-In 3 of 8 cases who had sequencing of at least *MYH7*, *MYBPC3*, and *TNNT2* another variant was present. All known second variants were in *MYBPC3*.

- 11-year-old proband with HCM (LVWT of 22 mm) carried another *MYBPC3* variant (p.Arg502Gln) (ClinVar: pathogenic by LMM, GeneDx, and Invitae).

- 15-year-old female with HCM (LVWT 10 mm) carried another *MYBPC3* variant (p.Phe305Profs*27) (ClinVar: not found; Calore et al. (2015) report it as a founder variant in Italians).

-One proband from unpublished LMM data had an additional disease causing variant; no clinical information is available.

-An 8-year-old proband with HCM (LVWT of 28 mm) carried another *MYBPC3* variant (p.Gln1233*) (ClinVar: pathogenic by LMM, GeneDx, CHEO, and Invitae); list of genes sequenced was not reported.

-There is weak segregation data. In 2 families an additional affected relative carried this variant. One of the two families had a second pathogenic variant.

-In total, the variant has been seen in 26 of ~ 62,360 individuals from published controls and publicly available datasets that approximate the general population. The

highest frequency is in European individuals: 21 heterozygotes out of 34,401 (0.06%).

-Variant found in one person who underwent exome sequencing (with mild hypertrophy), not selected for cardiac disorders or SCD.

-Variant seen in other cardiomyopathies: 1 DCM, 2 cases of LVNC..

Published HCM Cases

Van Driest et al. (2004) reported this variant in one out of 389 patients of unreported ancestry with HCM that were cared for in Mayo Clinic, Rochester, Minnesota, who underwent analysis of the *MYBPC3* gene.

Girolami et al. (2006) reported this variant in two out of 88 European patients with HCM that were cared for in Italy. Subjects underwent analysis of *MYBPC3*, *MYH7* and *TNNT2*. No other phenotypic information was reported in the paper.

Olivotto et al. (2008) reported this variant in one out of 203 European ancestry patients with HCM that were cared for at Azienda Ospedaliera-Universitaria Careggi, in Florence, Italy, and at Ospedale San Camillo, in Rome, Italy. Subjects underwent analysis of MYBPC3, MYH7, MYL2, MYL3, TNNT2, TNNI3, TPM1, and ACTC. This patient also carried a missense variant in MYBPC3 (p.Arg502Gln) that LMM, GeneDx, and Invitae classified as pathogenic in ClinVar. No other phenotypic information was reported in the paper. This proband is likely the same as one of the two probands reported by Girolami et al. (2006).

Millat et al. (2010) reported this variant in one out of 192 European patients with HCM that were cared for in France. Subjects underwent analysis of MYBPC3,

MYH7, TNNT2, TNNI3, 5 RAAS polymorphisms and TNF-a -308 G/A. No other phenotypic information was reported in the paper.

Morita et al. (2008) reported this variant in one out of 84 patients of unreported ancestry with isolated, unexplained left ventricular hypertrophy (wall thickness >2SD) diagnosed before 15 years of age. Subjects were drawn from Pediatric Cardiomyopathy Registry and underwent analysis of the MYH7, MYBPC3, TNNT2, TNNI3, TPM1, MYL2, MYL3, ACTC, PRKAG2, and LAMP2 genes. No other phenotypic information was reported in the paper.

Published Non-HCM Cases

Morita et al. (2006) reported this variant in one out of 50 unrelated European individuals with increased LVWT (maximum LVWT > 13 mm) that were part of the Framingham Heart Study. These individuals underwent analysis of the MYH7, MYBPC3, TNNT2, TNNI3, TPM1, MYL3, MYL2, and ACTC genes. No other phenotypic information was reported in the paper.

Hershberger et al. (2010) reported this variant in one out of 312 patients diagnosed with dilated cardiomyopathy, who underwent analysis of the MYBPC3, MYH6, TPM1, TNNC1, and TNNI3 genes. No other phenotypic information was reported in the paper.

Probst et al. (2011) reported this variant in two out of 63 European patients diagnosed with left ventricular noncompaction (LVNC), who underwent analysis of MYH7, ACTC1, TNNT2, TNNI3, MYL2, and MYL3, TPM1 and MYBPC3 genes. The first proband was tested “at age 70 because of dyspnea. Family screening

revealed that his asymptomatic 32-year-old son also” carried the variant. The second proband was tested "as a result of unclear syncope”. In the 2 individuals with the Gly490Arg mutation, the apex was not affected; only the mid-ventricular inferior and lateral wall".

Laboratory Data

In GeneDx’s variant summary report submitted to ClinVar (Jul 8, 2014), they note that they identified this variant in “several” HCM cases. No phenotypic or ancestral information was provided for the probands.

In LMM’s variant summary report submitted to ClinVar (Nov 16, 2010), they report they identified this variant in 4 HCM cases in 3 families of unknown ancestry. They note that of the 4 individuals carrying this variant, 2 carried a second variant sufficient to explain their disease. No phenotypic or ancestral information was provided for the probands.

SHaRe

Three sites have reported patients with this variant in SHaRe registry.

FLO reported one proband of European ancestry with this variant after analyzing 8 genes (presumably including sarcomere genes). Proband’s age of diagnosis is 11 years with max wall thickness of 22 mm. This proband also had an additional variant in *MYBPC3* gene (c.1505G>A; p.Arg502Gln), which is classified as "pathogenic" by LMM, GeneDx, and Invitae in ClinVar. This proband is redundant with Olivotto et al. (2008) proband. In addition, this proband has an affected family

member who carries both variants, and was diagnosed at 14.5 years of age, with a max wall thickness of 18 mm.

ERA reported two probands of European ancestry with this variant. List of genes analyzed for these probands was not available. One of these probands also had an additional variant in the MYBPC3 gene (c.913_914del; p.Phe305Profs*27). This frameshift variant was not found in ClinVar, however Calore et al. (2015) reported it as a deleterious, founder variant in Italians. The proband is a 15-year-old female with max wall thickness of 10 mm (data error?). The second proband is a 38-year-old female with max wall thickness of 10 mm (data error?). These probands do not appear to be reported elsewhere since none of the publications are from Netherlands, or in unpublished laboratory data since Rotterdam did not employ GeneDx or LMM to conduct testing.

UMH reported 1 proband with this variant after analyzing the genes on LMM's HCM Cardiochip 2008-2010 panel. The proband is an 8-year-old with LVWT of 28 mm. This proband has a second variant in the *MYBPC3* gene (c.3697C>T; p.Gln1233*), which is classified as "pathogenic" by LMM, GeneDx, Invitae, and CHEO in ClinVar. This case is redundant with the LMM cases.

Additional Data

In silico analysis with PolyPhen-2 predicts the variant to be probably damaging and SIFT predicts the variant to be deleterious. The arginine at codon 490 is highly conserved across species (with the exception of elephant), as are the neighboring amino acid. Other variants have been reported in association with disease

at this codon (p.Gly490Val) and nearby codons (p.Arg495Gly (Morita, 2008), p.Arg495Trp (Garcia-Castro, 2009)).

Frequency In Controls, Large Cohorts Unselected For HCM

In total, the variant has been seen in 26 of ~ 62,360 individuals from published controls and publicly available datasets that approximate the general population. Highest frequency was observed in European individuals (21 heterozygotes out of 34,401). This variant is currently listed in dbSNP: rs200625851.

Large Public Cohorts

The variant was reported online in 25 of ~60,191 individuals in the Exome Aggregation Consortium dataset (http://exac.broadinstitute.org/), which currently includes variant calls on ~64,000 individuals of European, African, Latino and Asian descent (as of April 22nd, 2015). Specifically, the variant was observed in 21 of ~ 33,285 European individuals. The phenotype of those individuals is not publicly available. The dataset is comprised of multiple cohorts, some of which were recruited from the general population; others were enriched for common cardiovascular disease. None of the cohorts recruited for rare cardiomyopathy and in some cases such phenotypes were excluded.

Publications

Ng et al. (2013) reported this variant in one out of 870 research participants, not selected for arrhythmia, cardiomyopathy, or a family history of sudden death, who underwent whole exome sequencing (WES) analysis. This participant “was a 56-year-old white male with a normal ECG and mild asymmetrical basal septal

hypertrophy (ECHO Septum 12 mm) and normal left ventricular ejection fraction (LVEF 65%) on ECHO. He had a history of elevated cholesterol, hyperuricemia, and renal cancer diagnosed at age 55, but no hypertension. His family history was significant for maternal grandparents dying from congestive heart failure, a sister diagnosed with cardiomyopathy in her 60s, a paternal cousin who died of congestive heart failure at age 68, and a paternal uncle who died at age 2." This individual had no primary reason for undergoing WES.

The variant was not observed in the following published control samples: 150 in Olivotto (2008), 180 in Probst (2011), 200 in Millat (2010), 200 in van Driest (2004), 1093 in Morita (2006), 100 in Girolami (2006), and 246 (186 Caucasian, 23 Yoruban, 19 Asian and 18 Hispanic) in Hershberger (2010).

APPENDIX F

VARIANT SUMMARY REPORT - MYBPC3 - p.Gly531Arg (c.1591G>C)

Summary

-Seen in at least 3 presumably unrelated cases of HCM (5 published, 4 unpublished). All cases were of European ancestry.

- The same amino acid change, via a different nucleotide change (c.1591G>A) has been seen in at least 6 presumably unrelated cases of HCM.

-In 1 of 10 cases who had sequencing of at least MYH7, MYBPC3, and TNNT2 the patient had another variant. The proband carried a second variant in MYBPC3 (p.Ala693fs) (ClinVar: not reported previously; LMM: variant “is sufficient to cause disease in isolation”)

-Seen in 1 DCM case

-There is weak segregation data. In 2 families, at least one additional affected relative carried this variant.

-In total, the variant has been seen in 2 of ~58,473 individuals from published controls and publicly available datasets that approximate the general population. Both were of European ancestry. In addition, 2 of 57,723 individuals in ExAC carry p.Gly531Arg (c.1591G>A) (same amino acid change, different nucleotide change).

Published HCM Cases

Garcia-Castro et al. (2009) and Coto et al. (2012) reported this variant (c.1591G>A) in 1 out of 150 European patients with HCM from their cohort in Spain,

who underwent analysis of MYH7, MYBPC3, TNNT2, TNNI3, and TPM1 genes. The proband is a 72-year-old female with LVWT of 20 mm, who presented with dyspnea, mitral murmur, and atrial fibrillation. No additional phenotypic information or variants were provided by the authors.

Girolami et al. (2006), Olivotto et al. (2008), and Donna et al. (2010) reported this variant (c.1591G>A) in 1 out of 203 European patients with HCM that were cared for in Italy, who underwent analysis of MYBPC3, MYH7, MYL2, MYL3, TNNT2, TNNI3, TPM1, and ACTC. The proband is a 54-year-old male with LVWT of 11 mm (data error?), who presented with long-standing persistent atrial fibrillation. No additional phenotypic information or variants were provided by the authors.

Millat et al. (2010) reported this variant (c.1591G>C) in 1 out of 192 European ancestry patients with HCM that were cared for in France, who underwent analysis of MYH7, MYBPC3, TNNT2 and TNNI3. This proband carried an additional variant in the MYBPC3 gene (p.Ala693fs), which has not previously been reported in ClinVar. However, according to LMM's summary report (11/14/2013), the variant "is sufficient to cause disease in isolation". No additional phenotypic information or variants were provided by the authors.

Waldmuller et al. (2011) reported this variant (c.1591G>A) in 1 out of 236 European ancestry patients with HCM that were cared for in Germany, who underwent analysis of MYH7 and MYBPC3 genes. 1 out of 652 DCM cases also carried the variant. No additional phenotypic information or variants were provided by the authors.

Olivotto et al. (2011) reported this variant (c.1591G>C) in 1 out of 61 European ancestry patients with HCM that were cared for in Italy, who underwent analysis of MYH7, MYBPC3, MYL2, TNNT2, and TNNI3 genes. The proband is a 27-year-old male with LVWT of 15 mm, who presented with dipyridamole myocardial blood flow of 3.1 ml/min/g. No additional phenotypic information or variants were provided by the authors.

Published Non-HCM Cases

Waldmuller et al. (2011) reported this variant in 1 out of 652 DCM cases of European ancestry that were cared for in Germany, who underwent analysis of MYH7 and MYBPC3 genes. No additional phenotypic information or variants were provided by the authors.

Laboratory Data

In LMM's summary report submitted to ClinVar (11/14/2013), they identified this variant in 4 (1 G>C; 3 G>A) HCM presumably unrelated cases of unreported ancestry and 2 affected relatives from the same family. They note that 2 carried another variant that could cause disease in isolation (no details were reported for these variants).

GeneDx did not report any internal data in their summary report on ClinVar (10/3/2014).

SHaRe

Two sites have reported patients with this variant in SHaRe registry.

BWH reported one proband of unreported ancestry, who underwent analysis of 11 genes using LMM's HCM CardioChip. This proband was diagnosed at the age of 33 years, with max wall thickness of 13 mm. This proband carried an additional variant in the MYL2 gene (c.243G>T; p.Val81Val), which is classified as likely benign by LMM (Jan 23, 2013) in ClinVar. One of the affected family members of this proband was diagnosed at the age of 17 with LVWT of 24 mm and the other affected family member was diagnosed at the age of 40 with LVWT of 13 mm. Neither of the family members carried the MYL2 variant. In addition, one individual with a family history of HCM carried this variant. No additional phenotypic information or variants were provided by the authors.

FLO reported one European ancestry proband, who underwent the analysis of 8, presumably sarcomeric, genes using Sanger sequencing. This proband was diagnosed at the age of 50 years, with max wall thickness of 18 mm. One affected family member was diagnosed at the age of 21 with LVWT of 16 mm. In addition, two of the family member that carried the variant did not have hypertrophy (LVWT of 11 mm) at ages 23 and 60 years. No additional phenotypic information or variants were provided by the authors.

Additional Data

In silico analysis with PolyPhen-2 predicts the variant to be probably damaging and SIFT predicts the variant to be deleterious. The Glycine at codon 531

is highly conserved with the exception of elephants, who lack sequence in relevant portions. Neighboring amino acids are not very well conserved. No other variants have been reported in association with disease at this codon. Other variants reported in association with disease at nearby codons include: p.Tyr525Ser (Girolami, 2006).

Frequency In Controls, Large Cohorts Unselected For HCM

Total the variant has been seen in 2 (both of European ancestry) of ~58,473 individuals from published controls and publicly available datasets that approximate the general population. This variant is currently listed in dbSNP: rs397515912.

The variant was reported online in 2 of 57,723 individuals in the Exome Aggregation Consortium dataset (http://exac.broadinstitute.org/), which currently includes variant calls on ~64,000 individuals of European, African, Latino and Asian descent (as of April 22nd, 2015). Specifically, the variant was observed in 2 of ~32,281 European individuals. Of note, the same amino acid change, due to a different nucleotide change (c.1591G>A) was reported in 2 additional individuals in ExAC and a synonymous variant at this codon was present in another 2 individuals. The phenotype of those individuals is not publicly available. The dataset is comprised of multiple cohorts, some of which were recruited from the general population; others were enriched for common cardiovascular disease. No cohorts recruited specifically for rare inherited cardiomyopathies and some excluded such cases.

The variant was not observed in the following published control samples: 100 in Girolami (2006), 150 in Olivotto (2008), 200 in Millat (2010), 150 in Olivotto (2011), and 150 in Coto (2012).

APPENDIX G

VARIANT SUMMARY REPORT - MYBPC3 - p.Ser217Gly (c.649A>G)

Summary

-Seen in >21 cases of HCM and DCM; at least 7 presumably unrelated probands had HCM (3 published, 4 unpublished), four of unreported ancestry, one Middle Eastern, one Indian

-LMM report >15 affected individuals with either HCM or DCM.

-5 of 7 HCM cases had another variant (3 likely pathogenic/pathogenic by at least one source; 1 likely benign /benign by at least one source; 1 VUS)

- 19-year-old female inherited a second variant in MYH7 gene from her affected father (c.1357C>T; p.Arg453Cys – ClinVar: pathogenic by LMM (Feb 17, 2014) and GeneDx (Jun 1, 2014), and likely pathogenic by CHEO)

- 52-year-old female proband with LVWT of 26 mm carried a second variant in TNNT2 (c.240C>G; p.Pro90Pro – ClinVar: likely benign by LMM (Mar 1, 2008))

- 20-year-old female proband with carried a second variant in MYBPC3 (c.2827C>T; p.Arg943Ter –ClinVar: pathogenic by CHEO, LMM (Dec 24, 2012) and GeneDx (Oct 9, 2014)) and a third variant TNNI3 gene (c.497C>T; p.Ser166Phe –ClinVar: VUS by LMM (Nov 26, 2008) and pathogenic by GeneDx (Apr 17, 2014)

- 40-year-old female proband with LVWT of 16 mm carried a second variant in MYBPC3 (c.2435A>G; p.Lys812Arg – ClinVar: VUS by CHEO)

- 52-year-old proband with LVWT of 18 mm carried a second variant in MYH7 (c.3286G>T; p.Asp1096Tyr – ClinVar: VUS by LMM (Aug 12, 2010) and pathogenic by GeneDx (Jun 11, 2014))

- One proband with no indication of HCM carried a second variant in MYBPC3 (c.3763G>A; p.Ala1255Thr – ClinVar: VUS by LMM (Jul 12, 2007) and pathogenic by GeneDx (Oct 16, 2014))

-Reported in two case of sudden infant death syndrome and four DCM cases

-In two families the variant was absent in affected family members.

-Seen in 249 (including 4 homozygotes) of ~57,006 individuals (published controls and publicly datasets). Highest frequency was in South Asian individuals with 169 heterozygotes and 4 homozygotes out of 7,279 individuals (2.3% heterozygotes).

Published HCM Cases

Roberts et al. (2010) reported this variant in one patient with HCM. The proband is a 19-year-old female who presented with a cardiac arrest and carried an additional variant in MYH7 (c.1357C>T; p.Arg453Cys), which is classified as pathogenic by LMM (Feb 17, 2014) and GeneDx (Jun 1, 2014) and likely pathogenic by CHEO in ClinVar. The MYH7 variant was inherited from her affected father and the p.Ser217Gly variant from her phenotypically normal mother (no additional information about mother's clinical testing was provided). The proband also has an affected sister and paternal aunt with the disease causing MYH7 variant neither of whom carried the p.Ser217Gly variant. For this proband, no additional phenotypic information or variants were provided by the authors.

Waldmüller et al. (2011) reported this variant in 1 out of 236 European ancestry patients with HCM that were cared for in Germany and underwent analysis of MYH7 and MYBPC3 genes. For this proband, no additional phenotypic information or variants were provided by the authors.

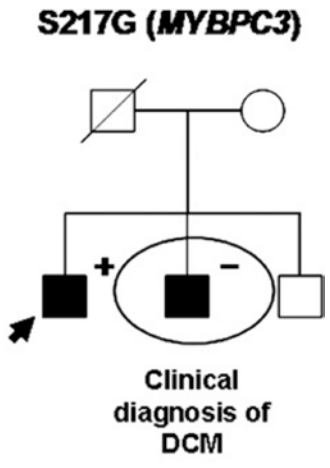
In an American Heart Association conference abstract, Da'as et al. (2014) reported this variant had been identified in one Middle Eastern patient with HCM cared for in Qatar. For this proband, no additional phenotypic information or variants were provided by the authors.

Published Non-HCM Cases

Brion et al. (2012) reported this variant in two out of 286 sudden infant death syndrome cases of European ancestry that were evaluated at the Institute of Legal Medicine of Zurich, Switzerland and the Institute of Forensic Medicine of the University of Bonn, Germany. Cases underwent analysis of 16 genes, including sarcomere genes. One case was a 5-month -old male, who presented with an upper respiratory tract infection some days before, and autopsy findings showed some mucus in the middle ear, bronchitis, subpleural petechiae and a microscopically normal heart. Cardiac hypertrophy was not reported by authors. The other case was a 17-week- old male, who had some symptoms of influenza two days before death. For these probands, no additional phenotypic information or variants were provided by the authors.

Lakdawala et al. (2012) reported this variant in one out of 264 dilated cardiomyopathy cases of multiethnic origins that were cared for in North America,

who underwent analysis of MYH7, TNNT2, TNNI3, TPM1, MYBPC3, ACTC, LMNA, PLN, TAZ, and LDB3 genes. The proband is a male with affected brother and unaffected parents. The variant was absent in his affected brother. For this proband, no additional phenotypic information or variants were provided by the authors. This case might be redundant with LMM/BWH cases.



Merlo et al. (2013) reported this variant in one out of 168 dilated cardiomyopathy cases of unreported ancestry that were cared for at the University of Colorado Cardiovascular Institute and the Cardiovascular Department of the University Hospital of Trieste, Italy. Subjects underwent analysis of MYH6, MYH7, MYBPC3, TNNT2 and TTN. For this proband, no additional phenotypic information or variants were provided by the authors.

Waldmüller et al. (2011) reported this variant in two out of 652 dilated cardiomyopathy cases of European ancestry that were cared for in Germany and underwent analysis of MYH7 and MYBPC3 genes. No additional phenotypic information or variants were provided by the authors.

Laboratory Data

In GeneDx's summary report submitted to ClinVar (9/29/2014), they did not report any internal case data.

In LMM's summary report submitted to ClinVar (9/24/2014), they note they have identified this variant in >15 affected individuals with either HCM or DCM of unreported ancestry. The variant was present in an unaffected parent of an individual with HCM who reported no further family history. For these probands, no additional phenotypic information or variants were provided by the authors. In addition, LMM stated in their summary report that GeneDx had seen this variant's failure to segregate with disease in one affected family member (GeneDx, personal communication with LMM).

SHaRe

Four sites have reported patients with this variant in SHaRe registry.

BWH's reported two probands. One of the probands, who underwent analysis of 11, including 8 sarcomeric, genes using LMM's HCM CardioChip, was diagnosed at the age of 52 years, with max wall thickness of 26 mm. This proband carried an additional variant in TNNT2 (c.240C>G; p.Pro90Pro), which is classified as likely benign by LMM (Mar 1, 2008) in ClinVar. The second proband, who underwent genetic testing using a Transgenomic/PGX/Familion panel of unreported genes, was a female diagnosed at the age of 20 years. This proband carried an additional variant in MYBPC3 gene (c.2827C>T; p.Arg943Ter), which is classified as pathogenic by CHEO, LMM (Dec 24, 2012) and GeneDx (Oct 9, 2014) in ClinVar. The second

proband also carried a third variant in TNNI3 gene (c.497C>T; p.Ser166Phe), which is classified as a VUS by LMM (Nov 26, 2008) and pathogenic by GeneDx (Apr 17, 2014). For this proband, no additional phenotypic information or variants were provided by the authors. The first proband is redundant with LMM cases.

FLO has one European ancestry proband with this variant whose phenotype is noted as “no clinical finding”. The subject underwent analysis of 8 sarcomeric genes. This proband also contains another variant in the MBPC3 gene (c.3763G>A; p.Ala1255Thr), which is classified as a VUS by LMM (Jul 12, 2007) and pathogenic by GeneDx (Oct 16, 2014) in ClinVar. This case has not been reported previously.

STD reported one proband of Indian ancestry, who underwent analysis of MYBPC3, MYH7, TNNI3, TNNT2, and TPM1 using Correlagen Labs. This proband was a female diagnosed at the age of 40 years, with max wall thickness of 16 mm. This proband carried an additional variant in MYBPC3 (c.2435A>G; p.Lys812Arg), which is classified as a VUS by CHEO in ClinVar. This proband had one affected relative who also carries the p.Ser217Glyvariant. The proband also carried a third variant in TNNI3 gene (c.25-8T>A), which is classified as benign by LMM (Oct 28, 2006) and GeneDx (Aug 28, 2012) in ClinVar. For this proband, no additional phenotypic information or variants were provided by the authors. This case has not been reported previously.

UMH reported one proband of unreported ancestry, who underwent genetic testing using a GeneDx HCM panel. This proband was diagnosed at the age of 52 years, with max wall thickness of 18 mm. This proband carried an additional variant

in the MYH7 gene (c.3286G>T; p.Asp1096Tyr), which is classified as VUS by LMM (Aug 12, 2010) and pathogenic by GeneDx (Jun 11, 2014) in ClinVar. For this proband, no additional phenotypic information or variants were provided by the authors. This case has not been reported previously as GeneDx does not report any cases in their summary report on ClinVar.

Additional Data

In silico analysis with PolyPhen-2 predicts the variant to be benign and SIFT predicts the variant to be deleterious. The Serine at codon 217 and neighboring amino acids are moderately conserved across species. No other variants have been reported in association with disease at this codon. Other variants reported in association with disease at nearby codons include: p.Ser212Arg (Olivotto, 2008), p.Ala216Thr (Fokstuen, 2008), p.Val219Leu (van Driest, 2004), p.Val219Phe (Millat, 2010).

Frequency In Controls, Large Cohorts Unselected For HCM

In total the variant has been seen in 249 (including 4 homozygotes) of ~57,006 individuals from published controls and publicly available datasets that approximate the general population. Highest frequency was in South Asian individuals with 169 heterozygotes and 4 homozygotes out of 7,279 individuals (2.3% heterozygotes). This variant is currently listed in dbSNP: rs138753870.

Large Cohorts Unselected For HCM

The variant was reported online in 244 (including 4 homozygotes) of 54,684 individuals in the Exome Aggregation Consortium dataset (http://exac.broadinstitute.org/), which currently includes variant calls on ~64,000

individuals of European, African, Latino and Asian descent (as of April 22nd, 2015). Specifically, the variant was observed in 64 of ~30,696 European individuals, and in 173 (including 4 homozygous) of ~7,279 South Asian individuals. The phenotype of those individuals is not publicly available. The dataset is comprised of multiple cohorts, some of which were recruited from the general population; others were enriched for common cardiovascular disease. However, none of the cohorts were comprised of individuals specifically recruited for having rare inherited cardiomyopathies and in some cases such individuals were excluded.

Laboratory Controls

In GeneDx's summary report submitted to ClinVar (9/29/2014), they note they identified this variant in 2 out of 368 presumably unrelated controls of unreported ancestry.

Published Controls

Ng et al. (2013) reported this variant in three out of 804 research participants, not selected for arrhythmia, cardiomyopathy, or a family history of sudden death, who underwent whole exome sequencing (WES) analysis. These individuals had no primary reason for doing WES.

The variant was not observed in the following published control samples: 150 in Merlo (2013) and 1000 in Brion (2012).

APPENDIX H

VARIANT SUMMARY REPORT - MYBPC3 - p.Val189Ile (c.565G>A)

Summary

-Seen in at least 8 presumably unrelated cases of HCM (3 published, 5 unpublished). 3 were known to be of European ancestry; ancestry was not reported for the rest.

-In 3 of 8 cases who had sequencing of at least MYH7, MYBPC3, and TNNT2 the patient had another variant.

-23-year-old proband with LVWT of 18 mm carried a second variant in TNNT2 (c.487_489delGAG; p.Glu163del) (ClinVar: pathogenic by LMM (Oct 8, 2012) and GeneDx (Sep 23, 2014), and likely pathogenic by CHEO (date of submission not provided online))

-8-year-old female proband with LVWT of 17 mm carried a second variant in TNNT2 (c.881G>A; p.Trp294Ter) (ClinVar: pathogenic by LMM (Nov 7, 2014) and GeneDx (Oct 22, 2014), and likely pathogenic by CHEO (date of submission not provided online))

-32-year-old proband with LVWT of 22 mm carried a second variant in MYBPC3 (c.3742_3759dup; p.Gly1248_Cys1253dup) (ClinVar: likely pathogenic by LMM (Nov 7, 2014) and pathogenic GeneDx (May 7, 2014))

-There is no segregation data.

-In total, the variant has been seen in 297 (4 homozygotes) of ~54,205 individuals from published controls and publicly available datasets that approximate the general population. The highest frequency was…

Published HCM Cases

Girolami et al. (2006) reported this variant in 1 out of 88 European patients with HCM that were cared for in Azienda Ospedaliero Universitaria Careggi and at the Ospedale San Camillo, Italy, who underwent analysis of MYBPC3, TNNT2 and MYH7 . The authors report this variant as a polymorphism. No additional phenotypic information or variants were provided by the authors.

Santos et al. (2012) reported this variant in 1 out of 80 European patients with HCM that were cared for in Lisbon, Portugal, who underwent analysis of 28 HCM-associated genes. The proband is a 19-year-old female, and has no family history of hypertrophic cardiomyopathy. No additional phenotypic information or variants were provided by the authors.

Gomez et al. (2014) reported this variant in 1 out of 136 European patients with HCM that were cared for in Asturias, Northern Spain, who underwent analysis of MYH7, MYBPC3, TNNT2, TNNI3, ACTC1, TNNC1, MYL2, MYL3, and TPM1. No additional phenotypic information or variants were provided by the authors.

Published Non-HCM Cases

Lakdawala et al. (2012) reported this variant in 1 out of 264 dilated cardiomyopathy cases of unreported ancestry that were tested by the LMM in Cambridge, Massachusetts. Subjects underwent analysis of at least MYH7, TNNT2,

TPM1, MYBPC3, and TNNI3 genes. No additional phenotypic information or variants were provided by the authors.

Millat et al. (2010) reported this variant in an unspecific number of individuals after analyzing MYBPC3, MYH7, TNNT2, and TNNI3. In this study, genomic DNA samples from control patients and from HCM patients with previously characterized genetic variants were used to determine the sensitivity of HRM analysis. From the data presented in the study, it is unclear if the variant is carried in a case or a control. Therefore, the data from this study cannot be including in the final tally of HCM cases carrying this variant. In addition, they report this variant as a SNP, which indicates that it has been seen in a large frequency in the population. No additional phenotypic information or variants were provided by the authors.

Millat et al. (2014) reported this variant in an unspecific number of individuals after analyzing MYH7, MYBPC3, MYL2, LMNA, SCN5A, TNNT2, TNNI3 and TPM1. In this study, genomic DNA samples from 75 HCM or DCM patients with previously characterized genetic variants were used to determine sensitivity and specificity of this NGS variant detection approach. From the data presented in the study, it is unclear how many of these probands carried this variant, and whether they had HCM or DCM. Therefore, the data from this study cannot be including in the final tally of HCM cases carrying this variant. No phenotypic information or additional variants were provided by the authors.

Laboratory Data

LMM did not report any internal data in their summary report on ClinVar (Jan 11, 2013).

The variant has not been detected in other patients at Ambry Genetics. CHEO did not report any internal data in their summary report on ClinVar (date of ClinVar entry not available online).

SHaRe

Three sites have reported patients with this variant in the SHaRe registry.

BWH's reported 3 probands. One of the probands, who underwent analysis of 11 genes (including sarcomeric genes) on LMM's HCM CardioChip, was diagnosed at the age of 23 years, with max wall thickness of 18 mm. This proband carried an additional variant in TNNT2(c.487_489delGAG; p.Glu163del), which is classified as pathogenic by LMM (Oct 8, 2012) and GeneDx (Sep 23, 2014), and likely pathogenic by CHEO (date of ClinVar submission not provided online) in ClinVar. The second proband was female and diagnosed at the age of 8 years, with max wall thickness of 17 mm. SHaRe data notes she underwent copy number variation analysis by LMM, however presumably she also had sequencing, given the identified variant. This proband carried an additional variant in TNNT2 (c.881G>A; p.Trp294Ter), which is classified as pathogenic by LMM (Nov 7, 2014) and GeneDx (Oct 22, 2014), and likely pathogenic by CHEO (date of ClinVar submission not provided online) in ClinVar. The third proband was a female diagnosed at the age of 35 years with max wall thickness of 10 mm, who underwent analysis of 5 sarcomeric genes on LMM's

hypertrophic cardiomyopathy panel A. No additional phenotypic information or variants were provided by the authors.

STD reported one proband, who underwent genetic testing at Ambry (genes not specified). This female proband was diagnosed with max wall thickness of 17 mm. No additional phenotypic information or variants were provided by the authors.

UMH reported one proband who underwent analysis of sarcomeric genes using LMM's HCM Cardiochip (2008-2010). This proband was diagnosed at the age of 32 years, with max wall thickness of 22 mm. This proband carried an additional variant in MYBPC3 (c.3742_3759dup; p.Gly1248_cys1253Dup), which is classified as likely pathogenic by LMM (Nov 7, 2014) and pathogenic GeneDx (May 7, 2014) in ClinVar. No additional phenotypic information or variants were provided by the authors.

Additional Data

In silico analysis with PolyPhen-2 predicts the variant to be benign and SIFT predicts the variant to be tolerated. The valine at codon 189 is conserved across vertebrae species, although isoleucine is the reference amino acid in two species (African clawed frog and Medaka). No other variants have been reported in association with disease at this codon. Other variants reported in association with disease at nearby codons include: p.Val178Met (Ho, 2009), p.Pro186Leu (Millat, 2010), p.Lys202Gln (Hershberger, 2010), p.Ser212Arg (Olivotto, 2008).

Frequency In Controls, Large Cohorts Unselected For HCM

In total the variant has been seen in 297 (4 homozygotes) of ~54,205 individuals from published controls and publicly available datasets that approximate the general population. This variant is currently listed in dbSNP: rs11570052.

Large Cohorts Unselected For HCM

The variant was reported online in 295 (4 homozygotes) of 53,470 individuals in the Exome Aggregation Consortium dataset (http://exac.broadinstitute.org/), which currently includes variant calls on ~64,000 individuals of European, African, Latino and Asian descent (as of April 22nd, 2015). Specifically, the variant was observed most in 202 (2 homozygotes) of ~30,071 European individuals, 22 of ~5,099 Latino individuals, 61 (2 homozygotes) of ~7,393 South Asian individuals, 4 of ~2,687 European (Finnish) individuals, and 3 of ~3,893 African individuals. The phenotype of those individuals is not publicly available. The dataset is comprised of multiple cohorts, some of which were recruited from the general population; others were enriched for common cardiovascular disease. No cohorts were intentionally composed of individuals with rare inherited cardiomyopathy and in some cases such individuals were excluded.

Publications

Ng et al. (2013) reported this variant in two out of 435 research participants of mixed ancestry, not selected for arrhythmia, cardiomyopathy, or a family history of sudden death, who underwent whole exome sequencing (WES) analysis. These individuals had no primary reason for undergoing WES.

The variant was not observed in the following published control samples: 200 in Girolami (2006) and 100 in Santos (2012).

APPENDIX I

VARIANT SUMMARY REPORT - MYH7 - p.Arg1606Cys (c.4816C>T)

Summary

-Seen in at least 2 presumably unrelated cases of HCM (unpublished).

-There is no segregation data.

-In total, the variant has been seen in 1 of ~64,870 individuals from published controls and publicly available datasets that approximate the general population.

Published Cases

This variant has not been reported in any published journal articles.

Laboratory Data

GeneDx (Feb 3, 2011) and LMM (Feb 3, 2011) reported this variant in ClinVar, however neither lab reported case data.

SHaRe

Two sites have reported patients with this variant in SHaRe registry.

UMH reported one proband of unreported ancestry, who underwent genetic testing using LMM's HCM Cardiochip (2008-2010) panel. This proband was diagnosed at the age of 59 years, with max wall thickness of 17 mm.

ERA reported one European ancestry proband. Number of genes analyzed is not noted in SHaRe. The proband is a 67-year-old female with LVWT of 15 mm.

Additional Data

In silico analysis with PolyPhen-2 predicts the variant to be probably damaging and SIFT predicts the variant to be not tolerated. The Arginine at codon 1606 is highly conserved across species, as are neighboring amino acids. No other variants have been reported in association with disease at this codon. Variants have been reported in association with disease at the following nearby codons: p.Glu1619Lys (Hershberger, 2008).

Frequency In Controls, Large Cohorts Unselected For HCM

In total the variant has been reported in 1 of ~64,870 individuals from published controls and publicly available datasets that approximate the general population. This variant is currently listed in dbSNP: rs200530211.

The variant was absent from the Exome Aggregation Consortium dataset (http://exac.broadinstitute.org/), which currently includes variant calls on ~64,000 individuals of European, African, Latino and Asian descent (as of April 22nd, 2015). The dataset is comprised of multiple cohorts, some of which were recruited from the general population; others were enriched for common cardiovascular disease. None were selected for rare inherited cardiomyopathies and in some cases those phenotypes were excluded.

Ng et al. (2013) reported this variant in 1 out of 870 research participants, not selected for arrhythmia, cardiomyopathy, or a family history of sudden death, who underwent whole exome sequencing (WES) analysis. These individuals had no primary reason for doing WES. The phenotype of that individual was not reported.

APPENDIX J

VARIANT SUMMARY REPORT - MYH7 - p.Arg204His (c.611G>A)

Summary

-Seen in at least 20 presumably unrelated cases of HCM (5 published, 15 unpublished).

-3 of 20 cases who had sequencing of at least MYH7 and MYBPC3 had another sarcomere variant:

-Late 50s female with HCM and fhx of CHF and sudden death, carried another MYH7 variant (classified as likely pathogenic by LMM)

-Late 30s female with HCM and fhx of sudden death, carried an MYBPC3 variant (frameshift variant, LMM classified as pathogenic)

-Woman diagnosed at 72yo with LVWT 15 mm and an MYL2 variant (GeneDx classifies as variant of uncertain significance)

-3 of 20 cases had an additional variant in a non-sarcomere gene:

-A 53yo male with LVWT 20 mm and a daughter with HCM carried a variant of uncertain significance in MTTG

-A patient carried a variant in MuRF2 that the authors hypothesize is a modifier

-Late 40s female with LVH and NSVT and fhx of “possible HCM/enlarged heart and sudden death, and carried a variant in PKP2 (-1 splice variant, LMM classified as likely pathogenic)

-There is weak segregation data. In at least one family an additional affected relative carried this variant.

-The variant was not observed in a total of ~61,161 individuals from published controls and publicly available datasets that were not selected for HCM.

Published Cases

Richard et al. (2003) reported this variant in one out of 197 patients diagnosed with HCM that were cared for in Paris, France, who underwent analysis of the MYH7, MYBPC3, MYL2, MYL3, TNNI3, and TNNT2 genes. This case is most likely redundant with a later report by the same group (Gandjbakhch et al., 2010).

Meyer et al. (2013) reported this variant in one out of 8 patients diagnosed with HCM that were cared for in Marburg, Germany, who underwent analysis of MYH7 and MYBPC3. No other phenotypic information was reported in the paper.

Berge et al. (2014) reported this variant in one out of 696 patients diagnosed with HCM that were cared for in Norway, who underwent analysis of the MYH7, MYBPC3, TNNI3, TNNT2, MYL2, and MYL3 genes. No other phenotypic information was reported in the paper.

Su et al. (2014) reported this variant in 2 patients diagnosed with HCM that were cared for in Beijing, China, who underwent analysis of MYH7, MYBPC3, TNNT2, TNNI3, MYL2, MYL3, TPM1, ACTC1, MuRF1, MuRF2, and MuRF3 genes. One of the two patients carries a second variant in MuRF2 gene (c.1516A>T; p.T506S). Authors propose that rare variants in MuRF2 genes increase the risk of

development of HCM and lead to more severe disease phenotypes, and thus may act as modifiers of the disease.

Laboratory Data

According to personal conversation with GeneDx (with Colleen Caleshu in May, 2015), they identified this variant in a total of ten individuals (see cases specifics below). Seven of nine had no other variants. Two individuals with HCM who carried another variant, one in the MTTG gene and the other in the MYL2 gene. They classify these as variants of uncertain significance, likely disease causing. The variant was also seen as a secondary finding of whole exome sequencing in a 2 year old male with no clinical findings of HCM.

LMM shared their experience. They have seen the variant in five probands with HCM and/or LVH. Two carried a likely pathogenic or pathogenic variant in another sarcomere gene (see specifics below) while one carried a likely pathogenic variant in PKP2. They also note that “the change to histidine (His) was predicted to be benign using a computational tool clinically validated by our laboratory. This tool's benign prediction is estimated to be correct 89% of the time (Jordan 2011).”

SHaRe

Four sites have reported patients with this variant in SHaRe registry.

FLO has one European ancestry proband with this variant after analyzing 111 genes (presumably including sarcomere genes). Proband’s age of diagnosis is 71 years with max wall thickness of 26 mm. No additional variants were reported in SHaRe on this proband. This proband does not appear to be reported elsewhere since

none of the publications are from Italy, or in unpublished laboratory data since Florence did not employ GeneDx or LMM to conduct testing.

UMH has one proband of unknown ethnicity with this variant who underwent analysis of ACTC (ACTC1), CAV3, GLA, LAMP2, MTTG, MTTI, MTTK, MTTQ, MYBPC3, MYH7, MYL2, MYL3, PRKAG2, TNNC1, TNNI3, TNNT2, TPM1, and TTR genes on GeneDx HCM panel. Proband's age of diagnosis is 51.5 years, with max wall thickness of 17 mm. No additional variants were reported in this proband. This variant was present in proband's daughter with HCM (diagnosis is 17 years with max wall thickness of 40 mm). This case is redundant with the GeneDx cases.

STD reported one proband of unknown ethnicity with this variant after analyzing ACTC1, CAV3, GLA, LAMP2, MTTG, MTTI, MTTK MTTQ, MYBPC3, MYH7, MYL2, MYL3, PRKAG2, TNNC1, TNNI3, TNNT2, TPM1, and TTR genes on the GeneDx panel. Proband's age of diagnosis is 49.6 years with Max wall thickness of 16 mm. No additional variants were reported in this proband. This case is redundant with the GeneDx cases.

BWH's reported one proband of unknown ethnicity with this variant after analyzing 11 genes on LMM's HCM CardioChip panel, which includes the sarcomere genes. Proband's age of diagnosis is 26 years with max wall thickness of 15 mm. This patient also carried a frameshift in MYBPC3 that LMM classified as pathogenic and a missense in MYBPC3 that LMM classified as a VUS. This case is most likely redundant with the LMM cases.

Additional Data

This variant substitutes a polar, positively charged amino acid (Arg) with a polar, positively charged amino acid (His). *In silico* analysis with PolyPhen-2 predicts the variant to be "possibly damaging" and SIFT predicts the variant to be "deleterious." The Arginine at codon 204 is moderately conserved across species, as are neighboring amino acids. It is highly conserved among mammals. Other variants have been reported in association with disease at this codon (c.610C>T (p.Arg204Cys) with classification as pathogenic by GeneDx in ClinVar, no other submitters; c.611G>T (p.Arg204Leu) with conflicting classifications in ClinVar) and nearby codons.

Frequency In Controls, Large Cohorts Unselected For HCM

The variant was not observed in ~61,113 individuals from published controls and publicly available datasets that approximate the general population.

This includes ~60,706 individuals in the Exome Aggregation Consortium dataset (http://exac.broadinstitute.org/), which currently includes variant calls on ~64,000 individuals of European, African, Latino and Asian descent (as of April 22nd, 2015). The phenotype of those individuals is not publicly available. The dataset is comprised of multiple cohorts, some of which were recruited from the general population; others were enriched for common cardiovascular disease. They were not selected for HCM and in some cases Mendelian heart disease was excluded. This variant is currently listed in dbSNP: rs397516260. The variant was not observed in the following published control samples: Richard (2003) did not report this variant in

100 control individuals, Su (2014) did not report this variant in 307 control individuals.

APPENDIX K

VARIANT SUMMARY REPORT - MYH7 - p.Asn1327Lys (c.3981C>A)

Summary

-Seen in at least 14 presumably unrelated cases of HCM (2 published, 12 unpublished).

-In 4 of 12 cases who had sequencing of at least MYH7, MYBPC3, and TNNT2 another variant was present.

- One proband carried a second variant in MYL2 (c.37G>A; p.Ala13Thr) (ClinVar: likely pathogenic by GeneDx (Aug 12, 2014) and a VUS by LMM (Mar 16, 2009))

- 66-year-old female proband carried a second variant in TNNI3 (c.373-10T>G) (ClinVar: benign by LMM (Apr 11, 2012) and GeneDx (Sep 25, 2012))

- 36-year-old female proband with LVWT of 25 mm carried a second variant in MYBPC3 (c.3535G>A; p.Glu1179Lys) (ClinVar: a VUS by LMM (Jul 1, 2014) and CHOE (date of submission not reported), and likely pathogenic by GeneDx (May 21, 2014))

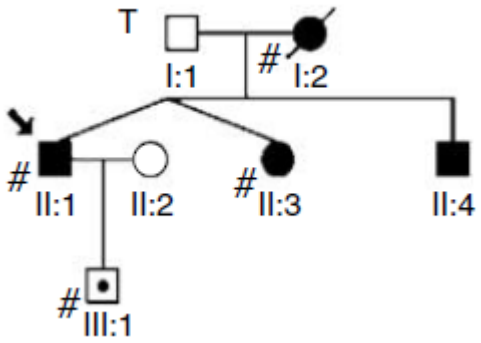
- 18-year-old proband with LVWT of 21 mm carried a second variant in PRKAG2 (c.866T>C; p.Val289Ala) (ClinVar: a VUS by GeneDx (Oct 4, 2012))

-There is weak segregation data. In one family, an additional affected relative carried this variant. Additional affected individuals in this family with more severe phenotype carried a variant in a different gene.

-In total, the variant has been seen in 18 of ~58,905 individuals from published controls and publicly available datasets that approximate the general population. Most were of European ancestry (12 of 32,593); 5 of 141 were Ashkenazi Jewish.

Published Cases

Hougs et al. (2005) and Jensen et al. (2013) reported this variant in 1 out of 92 European patients with HCM that were cared for in Copenhagen University Hospital in Copenhagen, Denmark, who underwent analysis of the MYH7, MYBPC3, TPM1, TNNT2, TNNI3, ACTC, MYL2, and MYL3 genes. The proband is a 46-year-old male with LVWT of 20 mm, who needed percutaneous transluminal septal myocardial ablation for treatment. This proband carried an additional variant in MYL2 gene (c.37G>A; p.Ala13Thr), which is classified as likely pathogenic by GeneDx (Aug 12, 2014) and a VUS by LMM (Mar 16, 2009) in ClinVar. He had a 49-year-old affected brother with LVWT of 14 mm, who carried the MYH7 variant, and was obese and hypertensive; his affected fraternal twin sister, who had LVWT of 23 mm, carried the MYL2 mutation, but did not carry the MYH7 variant; his deceased mother was affected, and was an obligate carrier for both of the variants; his 10-year-old unaffected son also carried both for the variants. No additional phenotypic information or variants were provided by the authors.



Miller et al. (2012) reported this variant in 1 out of 46 patients with HCM that were cared for in Cincinnati Children's Hospital Medical Center in Cincinnati, OH, who underwent analysis of MYH7, TNNT2, TPM1, MYBPC2, TNNI3, MYL2, MYL3, ACTC, TTR, TNNC1, CAV3, LAMP2, GLA, PRKAG2, MTTC, MTTI, MTTK, LMNA, ZAS/LDB3, DES, SGCD, PLN, ACTC1, TNNI2, TAZ, TTR, MTTL1, MTTQ, MTTH, MTTS1, MTTS2, MTND1, MTND5, and MTND6 genes. Ancestry of the patient was not reported. The study cohort included ~79% non-Hispanic Caucasian, 12 % Black, <2 % Asian, and 7 % Hispanic patients. No additional phenotypic information or variants were provided by the authors.

Laboratory Data

In LMM's summary report submitted to ClinVar (Oct 8, 2014), they note they have identified this variant in 9 presumably unrelated cases of HCM, of unreported ancestry. No additional variants were reported for these probands. The variant did not segregate with disease in 2 individuals from 2 families. They also report that the variant appears to be common amongst Ashkenazi Jewish individuals with a prevalence that greatly exceeds that of HCM (5/282 chromosomes, 1.8%; LMM

unpublished data); however no clinical information was available for these individuals.

GeneDx did not report any internal data in their summary report in ClinVar (Oct 3, 2014).

SHaRe

Three sites have reported patients with this variant in the SHaRe registry.

BWH's reported two probands. One of the probands was a female diagnosed at the age of 66 years, who underwent analysis of 18 genes using LMM's HCM CardioChip. This proband carried an additional variant in the TNNI3 gene (c.373-10T>G), which is classified as benign by both LMM (Apr 11, 2012) and GeneDx (Sep 25, 2012) in ClinVar. The second proband was diagnosed at the age of 18 years, with max wall thickness of 14 mm, who underwent analysis of 11 genes using LMM's HCM CardioChip. These cases are redundant with LMM cases.

STD reported two probands. One of the probands was a female diagnosed at the age of 36 years, with max wall thickness of 25 mm, who underwent genetic testing using Correlagen (list of genes tested was not provided). This proband carried an additional variant in MYBPC3 gene (c.3535G>A; p.Glu1179Lys), which is classified as a VUS by LMM (Jul 1, 2014) and CHOE (date of submission not reported), and likely pathogenic by GeneDx (May 21, 2014) in ClinVar. The second proband was diagnosed at the age of 46 years, with max wall thickness of 15 mm, who underwent genetic testing using Familion (list of genes tested was not provided). No additional phenotypic information or variants were provided by the authors.

UMH reported one proband, who underwent genetic testing with GeneDx's HCM panel. This proband was diagnosed at the age of 18 years, with max wall thickness of 21 mm. This proband carried an additional variant in the PRKAG2 gene (c.866T>C; p.Val289Ala), which is classified as a VUS by GeneDx (Oct 4, 2012) in ClinVar.

Additional Data

In silico analysis with PolyPhen-2 predicts the variant to be benign and SIFT predicts the variant to be tolerated. Functional studies suggest that this variant has a significant effect on alpha-helical content and stability in this region of the protein (Wolny, 2013). The asparagine at codon 1327 is highly conserved, as are neighboring amino acids. No other variants have been reported in association with disease at this codon. Other variants reported in association with disease at nearby codons include: p.Leu1297Val (Millat, 2010), p.Gln1334Ter (Hougs, 2005), and p.Thr1351Met (Girolami, 2006).

Frequency In Controls, Large Cohorts Unselected For HCM

Total the variant has been seen in 18 of ~58,905 individuals from published controls and publicly available datasets that approximate the general population. Five of 141 were Ashkenazi Jewish, while most were of European ancestry (12 of 32,593). This variant is currently listed in dbSNP: rs141764279.

The variant was reported online in 12 of 57,990 individuals in the Exome Aggregation Consortium dataset (http://exac.broadinstitute.org/), which currently includes variant calls on ~64,000 individuals of European, African, Latino and Asian

descent (as of April 22nd, 2015). Specifically, all of the individuals with the variant were of European ancestry (12 of ~32,143). The phenotype of those individuals is not publicly available. The dataset is comprised of multiple cohorts, some of which were recruited from the general population; others were enriched for common cardiovascular disease.

Ng et al. (2013) reported this variant in 1 out of 794 research participants of mixed ancestry, not selected for arrhythmia, cardiomyopathy, or a family history of sudden death, who underwent whole exome sequencing (WES) analysis. The phenotype of this individual is not reported. Of note, there is an over-representation of Ashkenazi Jewish individuals in this cohort (personal communication, L. Beisecker to C. Caleshu).

The variant was *not* observed in the following published control samples: 100 in Hougs (2005), 250 in Jensen (2013). The variant was observed in the following control samples: 1 out of 100 European and 324 multiple ethnicities in Kapplinger (2014), 5 out of 141 Ashkenazi Jewish in LMM (Oct 8, 2014, unpublished, reported in ClinVar).

APPENDIX L

VARIANT SUMMARY REPORT - MYH7 - p.Lys1459Asn (c.4377G>T)

Summary

-Seen in at least 15 presumably unrelated cases of HCM (9 published, 6 unpublished). Cases were of a range of ancestries (Confirmed European = 4 (2 from Spain; 2 from Italy); Spanish = 1; Brazilian = 3; unreported = 7).

-The variant was found in one case of Ebstein anomaly and in one case with Brugada syndrome.

-In 3 of 9 cases who had sequencing of at least MYH7, MYBPC3, and TNNT2 the patient had another variant.

-44-year-old proband with LVWT of 22 mm carried a second variant in the MYH7 gene (p.Met982Thr) (ClinVar: VUS by GeneDx and LMM and as likely pathogenic by CHEO)

-41-year-old proband carried a second variant in the MYH7 gene (p.Arg869His) (ClinVar: pathogenic by GeneDx and as a VUS by LMM)

-European proband had an additional variant in the MYH7 gene (p.Leu620Pro) that has not been previously reported in ClinVar, HGMD, ensemble, or ExAC

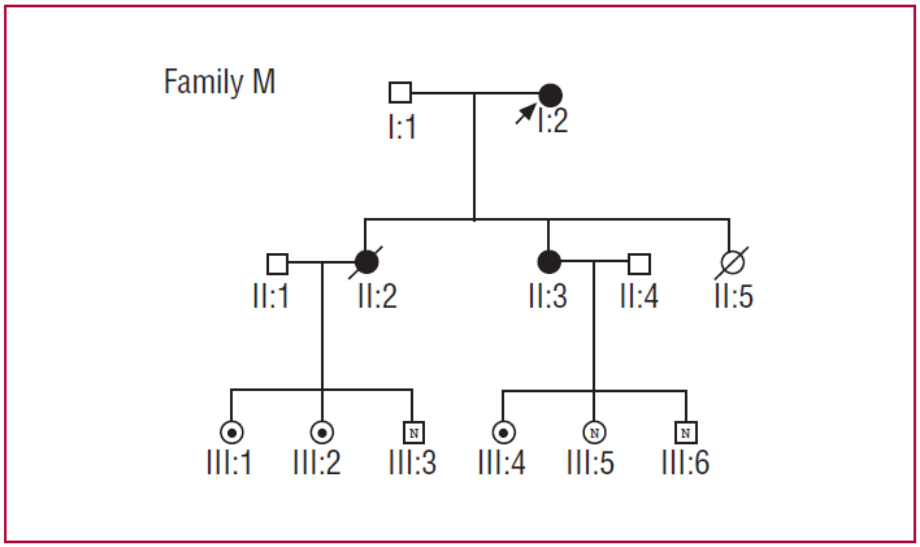
-There is weak segregation data. A 76-year-old female had a daughter with HCM who carried this variant.

-In total, the variant has been seen in 38 of ~63,667individuals from published controls and publicly available datasets that approximate the general population. The highest frequency was in European individuals (34 out of 33,923 (0.1%)).

Published HCM Cases

van Driest et al. (2004) reported this variant in one out of 389 HCM patients cared for in Mayo Medical Center's HCM Outpatient Clinic in Rochester, Minnesota. Ancestry was not reported. Subjects underwent analysis of the MYH7 gene. In addition, a separate report by the same group reported on the incidence of multiple variants in this cohort after analysis of 9 sarcomere genes and based on that it appears this patient had only this one MYH7 variant (van Driest et al. 2004). No additional phenotypic information was provided by the authors.

Laredo et al. (2006) reported this variant in one out of 128 patients with HCM cared for in Coruña, Spain. Subject underwent analysis of the MYH7 genes. The proband was a 76-year-old female with a maximum LVWT of 27 mm. One of her daughters had HCM with LVWT 15 mm and also carried the variant. Ancestry of the proband was not reported. Three additional family members were found to be carriers; they had a normal phenotype (and were in their thirties). No additional phenotypic information or variants were provided by the authors.



Garcia-Castro et al. (2009) and Coto et al. (2012) reported the variant in 1 of 150 European ancestry (Spain) HCM cases. They report no additional variants in the 5 sarcomere genes analyzed (*MYH7, MYBPC3, TNNT2, TNNI3,* and *TPM1*). The proband was a 56-year-old female who presented with dyspnea and had a family history of HCM.

Marsiglia et al. (2013) reported this variant in three out of 268 HCM cases, who underwent analysis of the MYH7, MYBPC3, and TNNT2 genes. Cases were cared for in Heart Institute, a University of São Paulo tertiary center, and other Brazilian cities, namely, Vitória, Manaus, and Recife. No additional phenotypic information or variants were provided by the authors.

Bos et al. (2014) reported this variant in two out of 1053 HCM cases that were cared for at Mayo Clinic in Rochester, Minnesota, who underwent analysis of the ACTC1, MYBPC3, MYH7, MYL2, MYL3, TNNC1, TNNI3, TNNT2, and TPM1 genes. Even though most of 1053 HCM cases were European ancestry, the ancestry

of the probands could not be determined from the publication as the cohort consisted of ethnically diverse cases (European ancestry (90%), African-American (1%), other (2%), and unknown or chose not to disclose (7%)). No additional phenotypic information or variants were provided by the authors. Note that these cases likely overlap with prior reports by Ackerman's group (ex. van Driest et al. 2004) and there is also a chance they overlap with internal cases from the clinical genetic testing labs.

Gomez et al. 2014 reported this variant in 1 out of 136 European ancestry patients with HCM that were cared for in Asturias, Spain. Subjects underwent analysis of MYH7, MYBPC3, TNNT2, TNNI3, ACTC1, TNNC1, MYL2, MYL3, and TPM1 genes. This proband had an additional variant in the MYH7 gene (p.Leu620Pro) that has not been previously reported in ClinVar, HGMD, ensemble, or ExAC. No additional phenotypic information or variants were provided by the authors.

Published Non-HCM Cases

Postma et al. (2011) reported this variant in one out of 141 western European patients with Ebstein anomaly that were cared for in The Netherlands, Germany, and United Kingdom, who underwent analysis of MYH7. No hypertrophy was noted in this proband. No additional phenotypic information or variants were provided by the authors. Of note, Ebstein's anomaly, often with noncompaction, has recently been associated with MYH7 variants (Budde et al. 2007, Postma et al. 2011, van Engelen et al. 2011).

Hertz et al. (2015) reported this variant in one out of nine European patients with Brugada syndrome that were cared for in Copenhagen, Denmark. Subjects underwent analysis of 34 genes (including sarcomeric genes). No additional phenotypic information or variants were provided by the authors.

Laboratory Data

LMM's data (as reported in Stanford's variant review) indicates that they have identified this variant in 3 presumably unrelated European individuals (out of >2000 tested). They note that one proband carrying this variant carried another pathogenic variant (specific variant not reported). No phenotypic information was provided for this proband. One of the individuals had borderline hypertrophy with an abnormal papillary muscle anatomy and LVOT obstruction. No information on diagnosis was available for the third individual.

GeneDx have not provided any internal data in the variant summary report submitted to ClinVar (Sep 8, 2014).

SHaRe

Five sites have reported patients with this variant in SHaRe registry.

BWH's reported one HCM case with this variant. They used Correlagen labs for genetic testing (analyzed genes not reported). Proband's age of diagnosis is 17 years with max wall thickness of 13 mm. This proband does not appear to be reported elsewhere since none of the publications are from Boston or in unpublished lab data since they did not use LMM to conduct testing.

ERA reported this variant in one European individual with family history of HCM. A diagnosis of HCM was not reported for this individual. The proband had LVWT of 9 mm. Names of genes analyzed were not reported. This case does not appear to be reported elsewhere since none of the publications are from Rotterdam or in unpublished lab data since they did not employ LMM to conduct testing.

FLO reported two probands with this variant after analyzing 8 genes (including sarcomeric genes). One of these probands also had an additional variant in MYH7 (c.2945T>C; p.Met982Thr), which is classified as a VUS by GeneDx and LMM and as likely pathogenic by CHEO in ClinVar. This proband was diagnosed at the age of 44 years with LVWT of 22 mm. The other proband was diagnosed at 52 with LVWT of 9 mm (data error?). These probands do not appear to be reported elsewhere since none of the publications are from Italy, or in unpublished laboratory data since Florence did not employ LMM to conduct testing.

STD reported this variant in 71-year-old female with HCM (LVWT of 17 mm). They used Familion for genetic testing (analyzed genes not reported). This proband does not appear to be reported elsewhere since none of the publications are from Palo Alto or in unpublished lab data since they did not use LMM for genetic testing.

UMH reported two HCM cases with this variant after using GeneDx's HCM panel (including sarcomeric genes) for one proband and a custom gene set for the other (specific genes and name of lab employed was not reported). The proband tested by GeneDx had an additional variant in MYH7 (c.2606G>A; p.Arg869His),

which is classified as pathogenic by GeneDx and as a VUS by LMM in ClinVar. The proband was diagnosed at the age of 41 years. The other proband was diagnosed at the age of 56 years with LVWT of 20 mm. These probands do not appear to be reported elsewhere since none of the publications are from Michigan or in unpublished laboratory data since they did not employ LMM to conduct testing.

Additional Data

In silico analysis with PolyPhen-2 predicts the variant to be probably damaging and SIFT predicts the variant to be deleterious. The Lysine at codon 1459 is highly conserved across species with the exception for western clawed frog, who do not have this peptide of the MYH7 protein. The neighboring amino acids are also highly conserved with the exception of Lamprey fish. No other variants have been reported in association with disease at this codon. Nearby codons associated with HCM include: p.Glu1455Ter (Girolami, 2010), p.Try1488Cys (Hougs, 2005).

Frequency In Controls, Large Cohorts Unselected For HCM

Total the variant has been seen in 38 of ~63,667individuals from published controls and publicly available datasets that approximate the general population. Most were of Europe ancestry (34 out of 33,923 (0.1%)).

The variant was reported online in 37 of 60,564 individuals in the Exome Aggregation Consortium dataset (http://exac.broadinstitute.org/), which currently includes variant calls on ~64,000 individuals of European, African, Latino and Asian descent (as of April 22nd, 2015). Specifically, the variant was observed in 34 of ~33,283 European individuals and 3 out of 5,779 Latino individuals. The phenotype

of those individuals is not publicly available. The dataset is comprised of multiple cohorts, some of which were recruited from the general population; others were enriched for common cardiovascular disease. Subjects were not recruited for rare inherited cardiomyopathies and for some cohorts such cases were excluded.

The Seidman group observed the variant in 1 of 1963 African American individuals from the Jackson Heart Study who underwent sequencing of eight sarcomere genes (Bick et al. 2012). They note the following about that individual's phenotype: 52-year-old with LVWT 11 mm, LVDD 4.64 cm, LAD 4.24 cm, fractional shortening 0.37, 1 physical cardiovascular risk factor (not specified).

This variant is currently listed in dbSNP: rs201307101. The variant was not observed in the following published control samples: 200 in van Driest (2004), 100 in Laredo et al. (2006), 150 in Coto (2012), 200 in Bos (2014), and 490 in Postma (2011).

APPENDIX M

VARIANT SUMMARY REPORT - MYH7 - p.Met982Thr (c.2945T>C)

Summary

-Seen in at least 19 presumably unrelated cases of HCM (9 published, 10 unpublished). Fourteen cases were of European ancestry.

-In 8 of 19 HCM cases who had sequencing of at least MYH7, MYBPC3, and TNNT2 had another variant:

- 13-year-old carried another variant in MYH7 (p.Asn696Ser) (ClinVar: pathogenic by GeneDx)

- 50-year-old carried another variant in MYBPC3 (p.Val219Phe) (ClinVar: likely pathogenic by CHOE)

- One proband carried another variant in MYH7 (p.His1494Leu) (ClinVar: VUS by LMM)

- 11-year-old female with LVWT of 33 mm carried another variant in MYBPC3 (p.Arg502Trp) (ClinVar: pathogenic by 3 labs)

-Female with LVWT of 15 mm carried another variant in MYBPC3 (p.Glu258Lys) (ClinVar: pathogenic by 4 labs)

- 23-year-old with LVWT of 39 mm carried another variant in MYH7 (p.Glu927Lys) (ClinVar: VUS by LMM, pathogenic by GeneDx, and likely pathogenic by CHOE)

-44-year-old with LVWT of 22 mm carried another variant in MYH7 (p.Lys1459Asn) (ClinVar: VUS by LMM and CHOE and pathogenic by GeneDx)

-~8 months old with LVWT of 18 mm carried another variant in MYH7 (p.Arg719Gln) (classified as likely pathogenic/pathogenic by 3 labs)

-Variant was also found in six cases of dilated cardiomyopathy, two individuals with increased LVWT unselected for HCM, and one case of SCD with a slight dilatation of the cavities noted on autopsy.

-There is weak segregation data available. FLO noted co-segregation in one affected relative.

-In total, the variant has been seen in 120 of ~ 61,377 individuals from published controls and publicly available datasets that approximate the general population. The highest frequency is in European individuals (93 of 36,614 (0.25%)).

Published HCM Cases

Millat et al. (2010) reported this variant in 2 out of 192 European patients diagnosed with HCM cared for in various cities in France. Subjects underwent analysis of MYH7, MYBPC3, TNNT2 and TNNI3 genes. Both of the probands contained additional variants. One was diagnosed at the age of 13 years and had an additional variant in MYH7 (c.2087A>G; p.Asn696Ser, which is classified as pathogenic by GeneDx in ClinVar). The second proband, diagnosed at 50 years old, had an additional variant in the MYBPC3 gene (c.655G>T; p.Val219Phe, which is classified as likely pathogenic by CHOE in ClinVar). No additional phenotypic information was provided by the authors.

Almaas et al. (2013) reported this variant in one out of 63 European patients diagnosed with HCM cared for in Norway, who underwent analysis of at least

MYBPC3, MYH7, MYL3, TNNI3, and TNNI2. No additional phenotypic information specific to this proband was provided by the authors.

Berge et al. (2014) reported this variant in four out of 696 European (Norway) patients diagnosed with HCM, who underwent analysis of MYH7, MYBPC3, TNNI3, TNNT2, MYL2, and MYL3 . The proband had an additional variant in MYH7 (p.His1494Leu), which is classified as VUS by LMM in ClinVar. No other phenotypic information specific to this proband was reported in the paper.

Glotov et al. (2015) reported this variant in 2 out of 38 European patients diagnosed with HCM cared for in Russia and Belarus. Subjects underwent analysis of ACTC1, MYBPC3, MYH7, MYL2, MYL3, TNNI3, TNNT2, TPM1, and CASQ2 genes. The first proband was diagnosed at the age of 41 years, and presented with IVS thickness of 22 mm, syncope, and slow VT. There was no family history of HCM. The second proband was diagnosed at the age of 33 years, and presented with IVS thickness of 19 mm. The proband was otherwise asymptomatic, had no family history of HCM, but did have a family history of sudden death in mother at age 42 (Personal Communication with Andrey Glotov on 6/29/15). No additional variants were reported for these two cases by the authors.

Published Non-HCM Cases

Morita et al. (2006) reported this variant in two out of 50 European individuals that were a part of Framingham Heart Study, who underwent analysis of the MYH7, MYBPC3, TNNT2, TNNI3, TPM1, MYL3, MYL2, and ACTC genes. Patients had increased LVWT, defined for the present study as maximum LVWT >13

mm. In addition, one of the probands had an echocardiographic pattern consistent with “burnt out” cardiac hypertrophy with a dilated left ventricle (LV diastolic diameter >56 mm) and enlarged atrium, whereas the other had a mildly enlarged left atrium and ECG voltages suggestive of HCM. These subjects were normotensive and had no history of treatment for hypertension. One of the probands had a family member who died suddenly.

Allegue et al. (2011) reported this variant in 1 out of 106 individuals with personal or family history of sudden death. Subjects were ascertained in Spain and underwent analysis of MYH7, MYBPC3, TNNI3, TNNT2, TPM1, TNNC1, ACTC, MYH6, MYL2, MYL3, TCAP, GLA, PRKAG2, TTN, MYLK2, MYO6, KCNQ1, KCNH2, and SCN5A. The proband was 22-year-old man who died suddenly. Reported phenotypic data is somewhat unclear. In the text they note “only a slight dilatation of the cavities” on autopsy. However, in a table, they note autopsy showed a “hypertrophied heart”. The study looked at 106 individuals that either had SCD (n = 37), recovered after SCD event (n = 12), and relatives of an unexplained SCD case (n = 57).

Van Spaendonck-Zwarts et al. (2013) reported this variant in 1 out of 418 European patients diagnosed with dilated cardiomyopathy cared for in Switzerland. Subjects underwent analysis of at least PLN, LMNA, MYH7, DES, TNNT2, TPM1, DMD, DMPK, SCN5A, SGCB, and TNNI3. No other phenotypic information specific to this proband was provided by the authors.

Merlo et al. (2013) reported this variant in 1 out of 67 European patients diagnosed with dilated cardiomyopathy cared for in Trieste, Italy. Subjects underwent analysis of MYH6, MYH7, MYBPC3, TNNT2 and TTN genes. No other phenotypic information specific to this proband was provided by the authors.

Laboratory Data

According to LMM data available on ClinVar (June 21, 2015), they identified this variant in 23 individuals from 16 families of unknown ancestry. At least 11 of these are confirmed to have HCM and 3 to have DCM by the report submitted to ClinVar (Sep 8, 2014). They note that 3 of the HCM cases carrying this variant also carried another pathogenic variant.

According to the report submitted to ClinVar by GeneDx, they have observed this variant in "multiple" cases of HCM "some of whom harbored disease-causing mutations in other genes associated with cardiomyopathy" (Sep 3, 2014).

SHaRe

Four sites have reported patients with this variant in the SHaRe registry.

BWH's reported two HCM cases with this variant after analyzing 11 genes on LMM's HCM CardioChip panel, which includes the sarcomere genes. One of these probands, 11 year old female with max wall thickness of 33 mm, also has an additional variant in MYBPC3 (c.1504C>T; p.Arg502Trp), which is classified as pathogenic by LMM, GeneDx and CHOE in ClinVar. The second proband, female with max wall thickness of 15 mm, also has an additional variant in MYBPC3 gene (c.772G>A; p.Glu258Lys), which is classified as pathogenic by LMM, GeneDx,

CHOE and Blueprint Genetics in ClinVar. In addition to the HCM cases, BWH also report this variant in a proband with dilated cardiomyopathy. This proband also carries an additional variant in the TTN gene (c.13589T>G; p.Ile4530Arg), which is classified as "VUS" by LMM in ClinVar. These cases are redundant with the LMM cases.

FLO reported two HCM cases with this variant after analyzing 8 genes (presumably including sarcomere genes). One of these probands, 23 year old with max wall thickness of 39 mm, had an additional variant in MYH7 gene (c.2779G>A; p.Glu927Lys), that LMM classified as VUS, GeneDx classified as pathogenic, and CHOE classified as likely pathogenic in ClinVar. The second proband, 44 year old with max wall thickness of 22 mm, also have an additional variation in MYH7 gene (c.4377G>T; p.Lys1459Asn) that LMM and CHOE classified as VUS, and GeneDx classified as pathogenic in ClinVar. These probands do not appear to be reported elsewhere since none of the publications are from Italy, or in unpublished laboratory data since Florence did not use GeneDx or LMM for genetic testing. In their summary report submitted for SHaRe discordance study, they noted cosegregation in one affected relative.

UMH reported one HCM case with this variant after testing with LMM's Pan Cardio panel, which includes the sarcomere genes. This was an ~8 month old with max wall thickness of 18 mm who also had an additional variant in MYH7 (c.2156G>A; p.Arg719Gln), which is classified as likely pathogenic/pathogenic by LMM, GeneDx and CHOE in ClinVar. Of note, a relative of this proband is also a

carrier for both of these variants and had normal max wall thickness of 5 mm. This case is redundant with the LMM cases.

STD reported 1 DCM case with this variant. The proband also had an additional variant in TPM1 gene (c.688G>A; p.Asp230Asn), which is classified as pathogenic by LMM and GeneDx in ClinVar.

Additional Data

In silico analysis predicts the variant to be benign, probably damaging, and deleterious according to PolyPhen-2, PolyPhen, and SIFT, respectively. The methionine at codon 982 is highly conserved across species, while the neighboring amino acid glycine at 980 is not well conserved. Other variants have been reported in association with disease at nearby codons.

Frequency In Controls, Large Cohorts Unselected For HCM

In total the variant has been seen in 120 of ~ 61,377 individuals from published controls and publicly available datasets that approximate the general population. The highest frequency is in European individuals (88 of ~ 33,370 European individuals in ExAC (0.26%). This variant is currently listed in dbSNP: rs145532615.

The variant was reported online in 110 of 60,706 individuals in the Exome Aggregation Consortium dataset (http://exac.broadinstitute.org/), which currently includes variant calls on ~64,000 individuals of European, African, Latino and Asian descent (as of April 22nd, 2015). Specifically, the variant was observed in 88 of ~ 33,370 European individuals (0.26%). The phenotype of those individuals is not

publicly available. The dataset is comprised of multiple cohorts, some of which were recruited from the general population; others were enriched for common cardiovascular disease. None of the studies recruited for rare inherited cardiomyopathy and some excluded such cases.

Andreasen et al. (2013) reported this variant in 3 out of 534 European population controls, who underwent analysis of two variants MYBPC3 (p.Val896Met) and MYH7 (p.Met982Thr). The control population consisted of men and women between the age of 55-75 years with no history of arrhythmias or other cardiac diseases.

Ng et al. (2013) reported this variant in 5 out of 870 research participants of mixed ancestry, not selected for arrhythmia, cardiomyopathy, or a family history of sudden death, who underwent whole exome sequencing (WES) analysis. These individuals had no primary reason for undergoing WES. Phenotype of those individuals is not reported.

The variant was observed in the following published control samples: 2 out of 2039 in Morita (2006), 3 out of 534 in Andreasen (2013). The variant was not observed in the following published control samples: 200 in Millat (2010), 21 in Glotov (2015), 300 in van Spaendonck-Zwarts (2013), and 150 in Merlo (2013).

APPENDIX N

VARIANT SUMMARY REPORT - MYH7 - p.Thr1377Met (c.4130C>T)

Summary

-Seen in at least 25 presumably unrelated cases of HCM (17 published, 8 unpublished). Most of the published cases (12/17) were of European ancestry.

-Three of 25 cases who had sequencing of at least MYH7 and MYBPC3 had another variant, however in these variants were likely benign or benign. No co-occurring pathogenic variants or VUS' were observed.

-There is no segregation data available.

-In total, the variant has been seen in 2 of ~61,955 individuals from published controls and publicly available datasets that approximate the general population. Specifically, the variant was seen in 2 of 96 European individuals with normal echocardiograms in one sample but not in other European individuals from a variety of other samples).

Published Cases

Richard et al. (2003) reported this variant in two out of 197 European patients diagnosed with HCM that were cared for in Paris, France. Subjects underwent analysis of the MYH7, MYBPC3, MYL2, MYL3, TNNI3, and TNNT2 genes. No additional phenotypic information or variants were provided by the authors.

Van Driest et al. (2004) reported this variant in four out of 389 patients of unreported ancestry diagnosed with HCM that were cared for in Mayo Medical

Center's HCM Outpatient Clinic in Rochester, Minnesota, who underwent analysis of the MYH7 gene. Bos et al. (2006) conducted an additional analysis on CSRP3, TCAP, HCM-associated exons (2, 3, 4, and 14) of TTN genes in the same set of 389 patients, and reported one of the four patients to carry an additional variation in CSRP3 gene (c.10T>C; p.Trp4Arg), which has been reported in association with dilated cardiomyopathy, but classified as likely benign by LMM in ClinVar (July 26, 2013). This proband is a European ancestry who was diagnosed at the age of 43 with LVWT of 18 mm, angina, dyspnea, pre-syncope, and underwent a myectomy. She also has a family history of HCM, but no segregation analysis was reported.

Fokstuen et al. (2008) reported this variant in 1 out of 38 European patients diagnosed with HCM that were cared for in Geneva, Switzerland, who underwent analysis of the MYH7, MYBPC3, TNNT2, TPM1, TNNI3, MYL3, MYL2, CSRP3, PLN, ACTC, TNNC1, and PRKAG2 genes. The Fokstuen group conducted an analysis of 130 genes in 2014 that reports a proband with this variant in addition to a variant in MYBPC3 (c.977G>A; p.Arg326Gln), which has been classified as benign/likely benign by 5 labs in ClinVar (June 22, 2015). This proband was 68 years old with LVWT of 18 mm and with family history of sudden cardiac death and end-stage heart failure.

Olivotto et al. (2008) reported this variant in one out of 203 European patients diagnosed with HCM that were cared for at Azienda Ospedaliera-Universitaria Careggi, in Florence, Italy, and at Ospedale San Camillo, in Rome, Italy, who underwent analysis of the MYBPC3, MYH7, MYL2, MYL3, TNNT2, TNNI3,

TPM1, and ACTC genes. No additional phenotypic information or variants were provided by the authors.

Millat et al. (2010) reported this variant in one out of 192 European patients diagnosed with HCM cared for in various cities of France. Subjects underwent analysis of MYH7, MYBPC3, TNNT2 and TNNI3 genes. No additional phenotypic information or variants were provided by the authors.

Sabater-Molina et al. (2013) reported this variant in one out of 115 patients diagnosed with HCM, who underwent analysis of MYH7 and MYBPC3. The ancestry of the proband could not be confirmed. However, most of the 243 total probands included in this study (~97%) were of European ancestry. No additional phenotypic information or variants were provided by the authors.

Witjas-Paalberends et al. (2013) reported this variant in two out of 57 European ancestry patients diagnosed with HCM cared for in The Netherlands, who underwent analysis of MYBPC3, MYH7, TPM1, TNNI3, and TNNT2 genes. One of the probands was a 58-year-old female with septal thickness of 20 mm, and the second proband was a 43-year-old male. No additional phenotypic information or variants were provided by the authors.

Berge et al. (2014) reported this variant in three out of 696 European ancestry Norwegian patients diagnosed with HCM, who underwent analysis of *MYH7, MYBPC3, TNNI3, TNNT2, MYL2,* and *MYL3*. No additional phenotypic information or variants were provided by the authors.

Helms et al. (2014) reported this variant in one out of 46 HCM patients. The patient underwent heart transplant. No additional phenotypic information or variants were provided by the authors.

Laboratory Data

In LMM's variant summary report submitted to ClinVar (Oct 20, 2011), they note that they identified this variant in 6 presumably unrelated HCM cases (Ancestry not reported, however >1950 out of >3250 tested are European ancestry).

GeneDx's report variant summary report submitted to ClinVar (Apr 10, 2014) does not mention if the variant has or has not been seen in their lab.

SHaRe

Three sites have reported patients with this variant in SHaRe registry.

BWH's reported one proband with this variant after analyzing 18 genes on LMM's HCM Panel, which includes the sarcomere genes. This proband, female with LVWT of 24 mm, had two additional variants, one in TNNI3 (c.373-10T>G) and one in TPM1 (c.120G>A; p.(=)) genes. Both are classified as likely benign by LMM. This case is most likely redundant with the LMM cases.

FLO reported one proband with this variant after analyzing 8 genes (presumably including sarcomere genes). Proband's age of diagnosis is 39.5 years with max wall thickness of 30 mm. No additional variants were reported in SHaRe on this proband. This proband might be redundant with the probands mentioned in Olivotto (2008) or Girolami (2006) as both are from Italy.

UMH has two probands with this variant. One of the probands underwent analysis of ACTC (ACTC1), CAV3, GLA, LAMP2, MTTG, MTTI, MTTK, MTTQ, MYBPC3, MYH7, MYL2, MYL3, PRKAG2, TNNC1, TNNI3, TNNT2, TPM1, and TTR genes on a GeneDx HCM panel, while the other had a custom set of specific genes that are not provided in the SHaRe registry. The first proband's age of diagnosis is 11 years, with max wall thickness of 18 mm. The second proband's age of diagnosis is 13 years, with max wall thickness of 17 mm. This case is likely not reported previously since the number of cases seen by GeneDx was not provided in their summary report on ClinVar.

Additional Data

In silico analysis with PolyPhen-2 predicts the variant to be probably damaging and SIFT predicts the variant to be damaging. The Threonine at codon 1377 is highly conserved across species, as are neighboring amino acids. No other variants have been reported in association with disease at this codon. Following variants in the nearby codons have been associated with the disease: p.Arg1382Trp (Richard, 2003).

Frequency In Controls, Large Cohorts Unselected For HCM

In total the variant has been seen in 2 of ~34,106 European ancestry individuals from published controls and publicly available datasets that approximate the general population. The variant was absent in ~27,849 individuals of other ethnicities. This variant is currently listed in dbSNP: rs397516201.

The variant was absent in 60,682 individuals in the Exome Aggregation Consortium dataset (http://exac.broadinstitute.org/), which currently includes variant calls on ~64,000 individuals of European, African, Latino and Asian descent (as of April 22nd, 2015). Specifically, 33,357 of these individuals were of European ancestry. The phenotype of those individuals is not publicly available. The dataset is comprised of multiple cohorts, some of which were recruited from the general population; others were enriched for common cardiovascular disease.

Fokstuen (2008) reported this variant in 2 out of 96 European individuals with normal echos who were recruited in Germany. The variant was not observed in the following published control samples of European ancestry: 100 in Richard (2003), 100 in Van Driest (2004), 100 in Girolami (2006), 150 in Olivotto (2008), 200 in Millat (2010), and 103 in Kapplinger (2014). The variant was also absent in the following published control samples of non-European ancestry: 100 African Americans in Van Driest (2004) and 324 multiple ethnicities in Kapplinger (2014).

APPENDIX O

VARIANT SUMMARY REPORT - TNNT2 - p.Arg278Cys (c.832C>T)

Summary

-Seen in at least 51 presumably unrelated cases of HCM (26 published, >25 unpublished).

-most had European ancestry (at least 30 out of 51); ancestry for others was not reported.

-Also seen in DCM cases.

-14 of 47 cases who had sequencing of at least MYH7 and TNNT2 had another variant.

-5/14 are likely pathogenic/pathogenic, while 4/14 are VUS; 5/14 variant details were not available to confirm the classifications.

-60-year-old European (Spain) female with HCM had additional variant in MYBPC3 gene (p.Arg733His) (ClinVar: VUS by GeneDx)

-50-year-old European (Spain) female with HCM (LVWT of 22 mm) and her 2 affected family members carried additional variant in MYH7 (p.Asp928Asn) (ClinVar: likely pathogenic by LMM; pathogenic by GeneDx)

-14-year-old European (France) individual carried a second variant in MYBPC3 (p.Asp610His) (ClinVar: VUS by LMM)

-30.5-year-old HCM case (LVWT of 24 mm) had an additional variant in MYBPC3 (p.Asp560Thrfs*19) (classified as likely pathogenic by LMM)

-49-year-old female of unreported ancestry with HCM (LVWT of 35) had an additional variant in MYBPC3 (p.Arg1781His) (ClinVar: VUS by LMM)

-46.5-year-old European (Italy) proband with HCM (LVWT of 28 mm) and one affected family member carried a second variant in MYBPC3 gene (p.Thr1095Met) (ClinVar: not found; likely pathogenic by Florence).

-56-year-old European (Italy) proband with HCM (LVWT of 18 mm) carried a second variant in MYBPC3 (p.Lys814del) (ClinVar: not found; pathogenic by Florence)

-34-year-old female of unreported ancestry with HCM carried an additional variant in the MYBPC3 gene (IVS11-9G>A; c.927-9G>A) (Helms et al. (2014): pathogenic based on splice site functional studies)

-15-year-old of unreported ancestry with HCM (LVWT of 38) carried an additional variant in the in MYBPC3 gene (p.Ala848Gly) (ClinVar: VUS by GeneDx)

-There is some segregation data. In three families, four affected relatives (in addition to probands) carried this variant and one had SCD. In two other families, four affected relatives (in addition to probands) carried p.Arg278Cys in addition to another variant.

-In total, the variant has been seen in 40 of ~48,482 individuals (0.08%) from published controls and publicly available datasets that approximate the general population. Most were European ancestry (33 out of 28,255 individuals). In ExAC the highest MAF was 0.06% (33/26987 Europeans).

Published Cases

Watkins et al. (1995) first reported the variant in a 17-year-old female who had normal left ventricular thickness yet had suffered a cardiac arrest; she was resuscitated. The ethnicity of the proband was not reported. Probands included 16 from Europe, 4 from North America, 3 from Japan, and 1 each from China, Southeast Asia, and Pakistan. While this one case is somewhat concerning for an increased risk of sudden death conferred by this variant, other reported cases have had more typical HCM courses, consistent with the marked variable expressivity often seen in primary cardiomyopathies.

Elliott et al. (1999) reported the variant in a 57-year-old male of unreported ancestry with HCM who was treated at St. George's Hospital Medical School, London. The genes analyzed were not reported. The proband presented with syncope and dyspnea at the age of 54 years, and was found to have a septum of 12 mm, a left ventricular outflow tract (LVOT) gradient, and systolic anterior motion (SAM) of the mitral valve at 57 years. His grandfather had died suddenly at the age of 60 years. No additional variants were reported.

Van Driest et al. (2003) observed the variant in three of 389 unrelated patients of unreported ancestry diagnosed with HCM that were cared for at Mayo Clinic's HCM Clinic in Rochester, Minnesota. The cohort underwent an analysis of TNNT2, TNNI3, TPM1, and ACTC genes. The first proband, a male diagnosed with HCM at age of 54 years with no family history of HCM, presented with LVWT of 20 mm. The second proband, a male diagnosed with HCM at 31 years with family history of

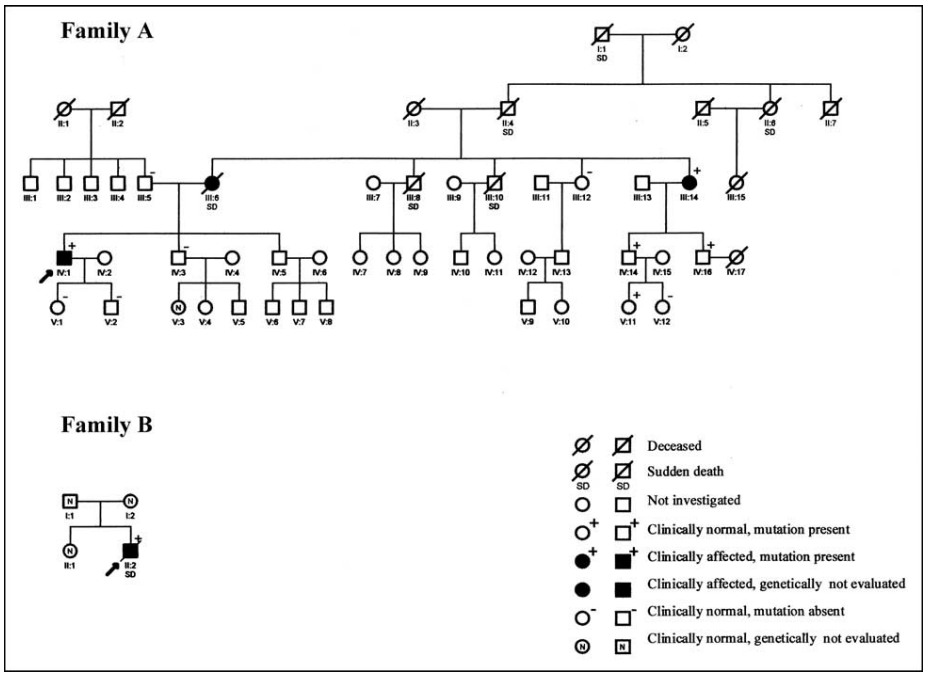
HCM, presented with angina, dyspnea, atrial fibrillation, LVWT of 15 mm, and needed a pacemaker. The third proband, a male diagnosed at age 69 year with no family history of HCM, presented with dyspnea, LVWT of 23 mm, and required a septal ablation. No additional variants were reported.

Garcia-Castro et al. (2003) reported this variant in a 60-year-old female, one of 30 European (Spanish) HCM cases who underwent analysis of the MYH7 and TNNT2 genes. She was diagnosed at age 49 with asymmetric septal hypertrophy and had a history of syncope, dyspnea, dizziness, palpitations, and an LVWT of 22 mm. In 2009, Garcia-Castro et al. reported an additional variation in this proband in the MYBPC3 gene (c.2198G>A; p.Arg733His), which is reported as a VUS by GeneDx in ClinVar (as of Aug 29, 2011). They reported that her double heterozygote daughter (40 years old) and granddaughter (6 years old) were “asymptomatic” (no mention of echo phenotyping). Proband’s older 52-year-old sister had mild hypertrophy (LVWT of 13 mm) and carried only p.Arg278Cys.

Torriceli et al. (2003) reported this variant in one of 150 unrelated HCM patients with European ancestry (Italian) that were cared for at Azienda Ospedaliera Careggi, who underwent the analysis of the MYH7, MYBPC3, TNNT2, and MYL2 genes. This was a 62-year-old male who had an LV thickness of 24 mm, needed percutaneous septal ablation and had no family history of hypertrophic cardiomyopathy. No additional variants were reported.

Theopistou et al. (2004) reported this variant in two probands with HCM from two families of European ancestry (Greek), who underwent analysis of the *TNNT2*

gene. In one family, the proband was diagnosed with HCM and a septum of 22 mm at 13 years of age. He died suddenly at 15. His sibling and parents all had normal echocardiograms, and did not have genetics evaluation. In the other family, the proband, 40-year-old male diagnosed at the age of 33 years with LVWT of 22, had family history of sudden death and HCM. His mother had sudden death (no genetics evaluation), and his maternal aunt, who was diagnosed with HCM at 64 years with LVWT of 15 mm, tested positive for this variant. Three members of his family with the variant had normal echocardiograms at 14, 38, and 42 years of ages. No additional variants were reported for either family.



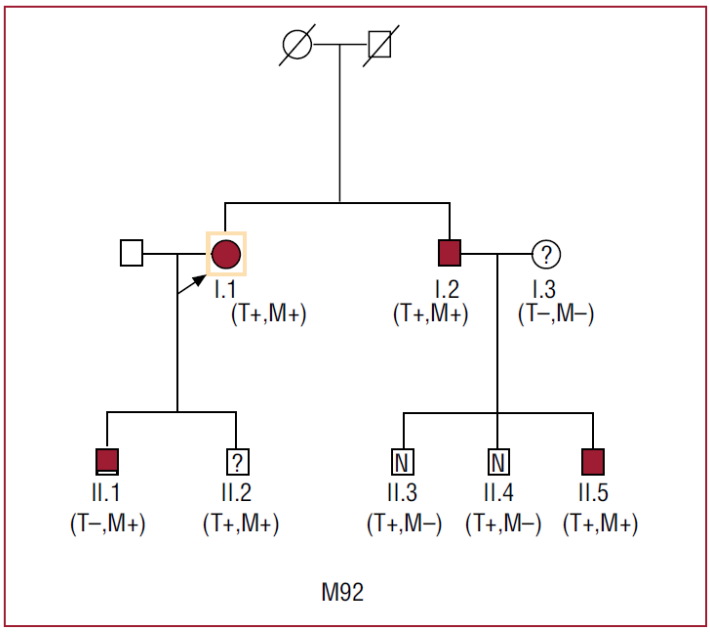
Ingles et al. (2005) reported this variant in one out of 80 Australian HCM cases. MYH7, MYBPC3, TNNT2, TNNI3, ACTC, MYL2, and MYL3 were evaluated and no other variants were found for this proband. No phenotypic information specific to this proband was provided by the authors.

Zeller et al. (2006) reported this variant in one of 30 European (German) HCM cases. No additional variants were found in any of the following genes: ACTC, ALP, CAPZB, CARP, DES, DMN, FKRP, FLT1, GJA1, JUP, LDB3, LMNA, MYBPC3, MYH7, MYOZ2, MYPN, NCK2, PLCG1, PXN, SGCD, TNNT2, TPM1, TPM2, TTID, and VEGF. No phenotypic information specific to this proband or additional variants were reported by the authors.

Kaski et al. (2009) reported this variant in one out of 79 cases with HCM. The ethnicity of the proband was not reported. No additional variants were found in any of the following analyzed genes: MYH7, MYBPC3, TNNI3, TNNT2, TPM1, MYL2, MYL3, ACTC, TNNC1, DES, and PRKAG2. The cohort comprised of 89.9% white, 3.8% Asian, 2.5% black, 2.5% Middle Eastern cases. All of the HCM cases were diagnosed under the age of 13 years. No phenotypic information specific to this proband was provided by the authors.

Gimeno et al. (2009) reported this variant in two probands with HCM from two families of European ancestry (Spanish). Of note, this report appears to be distinct from those by Garcia-Castro et al. The first proband, female diagnosed at the age of 50 years with LVWT of 22 mm, had two affected family members that carried this variant. Her brother was diagnosed at 18 years with LVWT of 22 mm who presented with palpitations and dyspnea, and her nephew (affected brother's son) was diagnosed at the age of 18 with LVWT of 40 mm who presented with presyncope. The following genes were analyzed in this family: TNNT2, MYH7, MYBPC3, TPM1, ACTC, TNNI2, TNNC1, MYL2, MYL3. All three of the affected members of

this family carried an additional variation in MYH7 gene (c.2782G>A; p.Asp928Asn), which is classified as likely pathogenic and pathogenic by LMM (Aug 26, 2014) and GeneDx (Mar 15, 2014) in ClinVar, respectively. Of note, the proband's affected son who carried the MYH7 gene variant, but did not carry the TNNT2 variant.



The second proband was a male diagnosed at the age of 59 years with 26 mm LVWT, persistent atrial fibrillation, and palpitations. No additional variants were reported for this proband. They report that his 33yo son carries the variant, has LVWT of 1.1 cm and a Brugada pattern on ECG.

Millat et al. (2010) reported this variant in 4 out of 192 unrelated HCM cases of European ancestry (French) who underwent analysis of MYH7, MYBPC3, TNNT2, and TNNI3 genes. One of the 4 variant carriers, diagnosed at the age of 14, also carried a second variant in the MYBPC3 gene (p.Asp610His), which is classified

as a VUS by LMM in ClinVar (Sep 28, 2011). No phenotypic information specific to this proband was provided by the authors.

Gruner et al. (2011) reported this variant in 1 out of 61 unrelated patients with apical HCM and 3 out of 365 unrelated nonapical HCM cases. Ethnicity of these probands was not reported, however the majority of the cohort had European ancestry (312/425). The proband with apical HCM was a 50-year-old male with maximal wall thickness of 16 mm and syncope. He has no family history of HCM or SCD. One of the 3 nonapical HCM probands had a family history of SCD. No additional variants were reported for these probands after analyzing the following genes: MYBPC3, MYH7, MYL2, MYL3, TNNT2, TNNI3, TPM1, ACTC, GLA, LAMP2, PRKAG2, and PRKAG2.

Millat et al. (2011) reported this variant in 1 out of 105 unrelated European (French) cases with dilated cardiomyopathy, who underwent analysis of the MYH7, TNNT2, TNNI3 and LMNA genes. The proband was a 69-year-old female with LVEDD of 65 mm, syncope, atrial fibrillation, and NYHA class III (LVWT was not reported). She has at least two family members diagnosed with DCM but no molecular data available on them.

Brito et al. (2011) reported this variant in a mother and daughter out of 77 unrelated European (Portuguese) cases with HCM, who underwent analysis of MYBPC3, MYH7, TNNT2, TNNI3 and MYL2 genes. In addition, there was a family history of sudden death in a first-degree relative (aged <50 years). No additional variants were reported in this family.

Nunez et al. (2013) reported this variant in one out of 104 European (Spanish and English) patients with sporadic HCM who underwent analysis of MYH7, MYBPC3, TPM1, TNNT2 and TNNI3 genes. No additional variants were reported in this proband. No phenotypic information specific to this proband was provided by the authors.

Laboratory Data

According to personal communication with GeneDx, they observed this variant in a patient with HCM, who had a frameshift variant in the MYBPC3 gene (c.927-9G>A) in addition to this variant, which is classified as pathogenic by Helms et al. (2014) based on splice site functional studies. This proband is the same as one of the Stanford probands.

LMM reported this variant in at least 15 (out of 5,160 tested) mostly European ancestry HCM probands. Seven out of these were reported as having additional likely pathogenic or pathogenic variants. Interestingly, they also reported this variant to be not well conserved in evolution, with 2 species (elephant, manatee) carrying a cysteine at this position.

SHaRe

ERA reported three probands of European ancestry with this variant. Names of genes analyzed were not available. The first proband was a 52-year-old female with LVWT of 8 mm (data entry error?); the second proband was a 56-year-old female with LVWT of 19 mm; the third proband was a 50-year-old female with LVWT of 16 mm. No additional variants were reported.

FLO reported five probands of European ancestry with this variant after analyzing 8 sarcomeric genes in 4 probands and 3 genes in 1 proband (specific names of the genes were not provided). The first proband, who was diagnosed at the age of 46.5 years with LVWT of 28 mm, had an additional variation in MYBPC3 gene (c.3284C>T; p.Thr1095Met), which is classified as likely pathogenic by Florence, but was not found in ClinVar (Jun 25, 2015). This proband also has an affected family member diagnosed at 71 years with LVWT of 30 who is a double heterozygote for the same variants. The second proband, who was diagnosed at the age of 56 years with LVWT of 18 mm, had an additional variation in the MYBPC3 gene (c.2440_2442del; p.Lys814del), which is classified as pathogenic by Florence, but was not found in ClinVar (Jun 25, 2015). The other three probands were diagnosed with HCM at the ages of 43, 43, and 66 years at LVWT of 30, 21, and 20 mm, respectively. Of note, since Torriceli et al. (2003) also reported 1 proband with this variant in their study from Tuscany, it is possible that they are the same probands.

STD reported two probands of unreported ethnicity with this variant after analyzing ACTC1, CAV3, GLA, LAMP2, MTTG, MTTI, MTTK, MTTQ, MYBPC3, MYH7, MYL2, MYL3, PRKAG2, TNNC1, TNNI3, TNNT2, TPM1, and TTR genes on the GeneDx panel. The first proband, a female diagnosed at the age of 34 years, has an additional variant in the MYBPC3 gene (IVS11-9G>A; c.927-9G>A), which is classified as pathogenic by Helms et al. (2014) based on splice site functional studies. The second proband with this variant was a female diagnosed at the age of 46 years.

In addition, a proband with family history of DCM was also tested positive for this variant. These probands are redundant with the GeneDx data.

UMH reported two probands of unreported ethnicity. The first proband, who was diagnosed at the age of 15 years with LVWT of 38, had an additional variation in the MYBPC3 gene (c.2543C>G; p.Ala848Gly), which is classified as a VUS by GeneDx in ClinVar (Jan 25, 2013). This proband was tested for following genes on the GeneDx HCM panel: ACTC1, CAV3, GLA, LAMP2, MTTG, MTTI, MTTK , MTTQ, MYBPC3, MYH7, MYL2, MYL3, PRKAG2, TNNC1, TNNI3, TNNT2, TPM1, and TTR. The second proband was diagnosed at the age of 70.5 with LVWT of 16 mm. These probands are redundant with LMM and GeneDx cases.

BWH's reported four proband of unreported ethnicity with this variant after analyzing 11 genes with LMM's HCM panel. The first proband, who was diagnosed at the age of 30.5 years with LVWT of 24 mm, had an additional variant in the MYBPC3 gene (c.1678del; p.Asp560Thrfs*19), which is classified as likely pathogenic by LMM. The second proband, who was a female diagnosed at the age of 49 years with LVWT of 35, had an additional variation in MYBPC3 gene (c.5342G>A; p.Arg1781His), which is classified as a VUS by LMM in ClinVar (Jan 13, 2013). The other two probands were diagnosed with HCM at the ages of 30.5 and 34 years at LVWT of 33 and 25 mm, respectively, and had no other variants in the analyzed genes. These cases are redundant with the LMM cases.

Additional Data

In silico analysis with PolyPhen-2 predicts the variant to be probably damaging. The arginine at codon 278 is not well conserved in evolution and 2 species (elephant, manatee) carry a cysteine at this position. Other variants have been reported in association with disease at this codon: p.Arg278Pro (Van Driest et al, 2003). In addition, the following variants in nearby codons have been associated with HCM: p.Arg286Cys (Richard, 2003), p.Arg286His (Van Driest, 2003). Functional studies by Yanaga et al. (1999) indicate that p.Arg278Cys causes an increase in Ca2+ sensitivity therefore increasing the contractility of the cell and inducing hypertrophy.

Frequency In Controls, Large Cohorts Unselected For HCM

In total the variant has been seen in 40 of 48,482 (0.08%) laboratory controls, published controls and individuals from publicly available population datasets.

The variant was reported online in 33 of 26,987 European ancestry individuals (0.12%) and 3 of 4,095 African-American individuals in the ExAC Browser dataset (as of 5/1/15). None were selected for rare inherited cardiomyopathies and in some cases those phenotypes were excluded. However, the cohorts that were merged to create this dataset were all either general population samples or samples recruited for common cardiovascular disease such as hypertension. The variant was not observed in 1818 European and 100 African ancestry individuals across the following published studies in presumably healthy controls: 100 in Watkins (1995), 200 in Van Driest (2003), 200 in García-Castro (2003), 150 in Torriceli (2003), 100 in Miliou

(2005), 150 in Ingles (2005), 168 in Zeller (2006), 200 in Kaski (2009), 200 in Millat (2010), 200 in Millat (2011), and 200 in Nunez (2013).

APPENDIX P

VARIANT SUMMARY REPORT - TPM1 - p.Glu192Lys (c.574G>A)

Summary

-Seen in at least 18 presumably unrelated cases of HCM (7 published, 11 unpublished) and one case of LVNC.

-No segregation data was available.

-In total, the variant was absent in ~60,896 individuals from published controls and publicly available datasets that approximate the general population.

-Most pathogenic variants in TPM1 gene are located in this region of the protein (i.e. it lays in the troponin T binding region)

Published HCM Cases

Deva et al. (2013) reported this variant in one out of 300 patients with HCM that were cared for in Toronto, Canada, who underwent analysis of unreported genes. Ancestry was not reported. The proband is a 33-year-old female who presented with reverse septal curvature morphologic phenotype and deep basal inferoseptal crypts on MRI. No additional phenotypic information or variants were provided by the authors.

Ho et al. (2009) reported this variant in one out of 40 patients with HCM that were cared for in Boston, MA, Minneapolis, MN, and Copenhagen, Denmark, who underwent analysis of at least the MYH7, MYBPC3, TNNT2, TNNI3, and TPM1 genes. Ancestry was not reported. This proband had LVWT of ≥12 mm. No additional phenotypic information or variants were provided by the authors.

Kapplinger et al. (2013) reported this variant in four out of 2,178 patients with HCM that were cared for in Mayo Clinic in Rochester, Minnesota or tested by Transgenomic Inc. Patients underwent analysis of the MYH7, MYL2, MYL3, MYBPC3, ACTC, TNNC1, TNNI3, TNNT2, and TPM1 genes. Patient specific ancestry was not reported. No additional phenotypic information or variants were provided by the authors.

Fokstuen et al. (2008) reported this variant in one out of 8 patients with HCM of unreported ancestry that were cared for in University College London Hospitals, London, United Kingdom. They performed analysis of HCM associated genes including: MYH7, MYBPC3, TNNT2, TPM1, TNNI3, MYL3, MYL2, CSRP3, PLN, ACTC, TNNC1, and PRKAG2. The aim of this study was to validate a DNA resequencing array. No additional phenotypic information or variants were provided by the authors.

Published Non-HCM Cases

Probst et al. (2011) reported this variant in one out of 63 LVNC cases of western European ancestry that were cared for in University Hospital Zurich, Switzerland, and the German Heart Institute Berlin, Germany, who underwent analysis of MYH7, ACTC1, TNNT2, TNNI3, MYL2, MYL3, TPM1 and MYBPC3 genes. The proband is a 55-year-old male, who presented with sudden chest pain, dyspnea, pronounced midventricular wall LVNC and increased right ventricular trabeculations. The proband's son had a normal echo and did not carry the variant. No additional phenotypic information or variants were provided by the authors.

Laboratory Data

GeneDx did not report any internal data in their summary report on ClinVar (10/28/2014).

In LMM's summary report submitted to ClinVar (4/10/2014), they note that they have identified this variant in at least 11 presumably unrelated cases of HCM and 3 affected family members (unclear if they are from the same family or different families). They do not report ancestry. They also note that a computational tool clinically validated by their laboratory predicts this variant to be pathogenic. This tool's pathogenic prediction is estimated to be correct 94% of the time (Jordan, 2011). No additional phenotypic information or variants were provided by the authors.

SHaRe

Two sites have reported patients with this variant in the SHaRe registry.

BWH reported one proband of unreported ancestry, who underwent the analysis of known mutation in TPM1 gene using LMM. This proband was diagnosed at the age of 6 years, with max wall thickness of 19 mm. No additional phenotypic information or variants were provided by the authors. This case is likely redundant with the case reported by Ho et al, and the LMM cases.

STD reported one proband of unreported ancestry, who had genetic testing using Familion labs (genes not noted). This proband was diagnosed at the age of 31 years, with max wall thickness of 16 mm. No additional phenotypic information or variants were provided by the authors.

Additional Data

In silico analysis with PolyPhen-2 predicts the variant to be benign and SIFT predicts the variant to be not tolerated. Glutamic acid at position 192 is highly conserved in mammals and across evolutionarily distant species. No other variants have been reported in association with disease at this codon.

Frequency In Controls, Large Cohorts Unselected For HCM

This variant is currently listed in dbSNP: rs199476315. In total the variant has not been seen in ~61,476 individuals from published controls and publicly available datasets that approximate the general population.

The variant was absent in 60,335 individuals in the Exome Aggregation Consortium dataset (http://exac.broadinstitute.org/), which currently includes variant calls on ~64,000 individuals of European, African, Latino and Asian descent (as of April 22nd, 2015). The phenotype of those individuals is not publicly available. The dataset is comprised of multiple cohorts, some of which were recruited from the general population; others were enriched for common cardiovascular disease.

The variant was not observed in the following lab control samples: 400 by Familion labs.

The variant was not observed in the following published control samples: 38 in Ho (2009), 96 in Fokstuen (2008), and 427 in Kapplinger (2013), 180 in Probst (2011).