

Meta-analysis of Penetrance and Systematic Review on Transition to Disease in Genetic Hypertrophic Cardiomyopathy

Running title: *Topriceanu et al.; Meta-analysis of penetrance in genetic HCM*

Constantin-Cristian Topriceanu, MD^{1,2,4}; Alexandre C. Pereira, MD, PhD¹; James C. Moon,
MD^{2,4}; Gabriella Captur, MD, PhD^{2,3,5}; Carolyn Y. Ho, MD¹

¹Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; ²UCL Institute of Cardiovascular Science, University College London, London; ³UCL MRC Unit for Lifelong Health and Ageing, University College London, London; ⁴Cardiac MRI Unit, Barts Heart Centre, West Smithfield, London; ⁵The Royal Free Hospital, Centre for Inherited Heart Muscle Conditions, Cardiology Department, Pond Street, Hampstead, London

Address for Correspondence:

Dr. Carolyn Y. Ho
Cardiovascular Division,
Brigham and Women's Hospital,
Harvard Medical School
75 Francis Street, Boston, MA 02115
Email: cho@bwh.harvard.edu

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Abstract

Background: Hypertrophic cardiomyopathy (HCM) is characterized by unexplained left ventricular hypertrophy (LVH) and is classically caused by pathogenic or likely pathogenic variants (P/LP) in genes encoding sarcomere proteins. Not all subclinical variant carriers will manifest clinically overt disease, as penetrance (proportion of G+ who develop disease) is variable, age-dependent, and not reliably predicted.

Methods: A systematic search of the literature was performed. We employed random effects generalized linear mixed model meta-analyses to contrast the cross-sectional prevalence and penetrance of sarcomere genes in two different contexts: clinically-based studies on patients and families with HCM versus population/community-based studies. Longitudinal family/clinical studies were additionally analyzed to investigate the rate of phenotypic conversion from subclinical to overt HCM during follow-up.

Results: 455 full text manuscripts were assessed. In family/clinical studies, the prevalence of sarcomere variants in patients diagnosed with HCM was 34%. The penetrance across all genes in non-proband relatives carrying P/LP variants identified during cascade screening was 57% (95% confidence interval [CI] [52,63]) and the mean age of HCM diagnosis was 38 years (95% CI [36, 40]). Penetrance varied from ~32% for myosin light chain (*MYL3*) to ~55% for myosin binding protein C (*MYBPC3*), ~60% troponin T (*TNNT2*) and troponin I (*TNNI3*), and ~65% for myosin heavy chain (*MYH7*). Population-based genetic studies demonstrate that P/LP sarcomere variants are present in the background population, but at a low prevalence of <1%. The penetrance of HCM in incidentally identified P/LP variant carriers was also substantially lower; approximately 11%, ranging from 0% in Atherosclerosis Risk in Communities to 18% in UK Biobank. In longitudinal family studies, the pooled phenotypic conversion across all genes was 15% over an average of ~8 years of follow up, starting from a mean age of ~16 years. However, short-term gene-specific phenotypic conversion varied between ~12% for *MYBPC3* to ~23% for *MYH7*.

Conclusions: The penetrance of P/LP variants is highly variable and influenced by currently undefined and context-dependent genetic and environmental factors. Additional longitudinal studies are needed to improve understanding of true lifetime penetrance in families and in the community, and to identify drivers of the transition from subclinical to overt HCM.

Key Words: hypertrophic cardiomyopathy; pathologic/like pathologic sarcomeric variants; penetrance; HCM age of onset.

Non-standard Abbreviations and Acronyms

ACMG = American College of Medical Genetics and Genomics

ARIC = Atherosclerosis Risk in Communities

CMR = cardiovascular magnetic resonance imaging

CSRP3 = cysteine- and glycine-rich protein

ECG = electrocardiogram

FHS = Framingham Heart Study

G+LVH⁻ = the absence of left ventricular hypertrophy in sarcomere or sarcomere-related variant carriers or subclinical hypertrophic cardiomyopathy

G+LVH⁺ = the presence of left ventricular hypertrophy in sarcomere or sarcomere-related variant carriers or overt hypertrophic cardiomyopathy

GLMM = generalized linear mixed-model

GWAS = genome-wide association study

HCM = hypertrophic cardiomyopathy

I² = Higgins I² statistic

JHS = Jackson Heart Study

LV = left ventricle

LVH = left ventricular hypertrophy

MYBPC3 = myosin binding protein C

MYH7 = β -myosin heavy chain

MYL2 or 3 = myosin light chain 2 or 3

MWT = maximal wall thickness

NT-proBNP

P/LP = pathogenic or likely pathogenic

PECO = Patient/Exposure/Comparator/Outcomes framework

RE = random-effects

TNNI3 = troponin I

TNNT2 = troponin T

TPMI = tropomyosin 1



Clinical Perspective

What is new?

- In clinical studies on patients and families with HCM, the prevalence of causal sarcomere variants was ~34%; the penetrance of HCM in relatives with pathogenic variants was ~57%.
- In general population studies, the prevalence of pathogenic variants in sarcomere genes was 50-fold lower (0.7%) and the penetrance in those incidentally identified as variant carriers was 5-6-fold lower (11%).
- In longitudinal family studies, the pooled phenotypic conversion across all genes was ~15% over an average of ~8 years of follow up, starting from a mean age of ~16 years.

What are the clinical implications?

- As penetrance is context-specific, different surveillance strategies may be appropriate for follow up of at-risk family members compared to healthy individuals from the general population who are incidentally found to carry sarcomere variants.
- A multidisciplinary approach encompassing both basic and clinical investigation is needed to improve our understanding of penetrance of sarcomere variants and the transition from subclinical to clinically overt hypertrophic cardiomyopathy.

Introduction

Multidisciplinary studies of patients and families with hypertrophic cardiomyopathy (HCM) have provided valuable insights establishing that variants in genes encoding the sarcomere apparatus or sarcomere-related proteins as an important cause of HCM^{1,2}, and highlighting the remarkable diversity and complexity of phenotypic manifestations, including age of onset, symptom burden, cardiac remodeling, prognosis, and even the penetrance (proportion of variant carriers that develop clinically overt HCM)³.

Pathogenic (P) or likely pathogenic (LP) variants associated with HCM are most commonly found in the core sarcomere genes, particularly myosin binding protein C (*MYBPC3*; ~40% of sarcomeric HCM), β -myosin heavy chain (*MYH7*; 30-40%), troponin T (*TNNT2*; 5-10%) and troponin I (*TNNI3*; 5-10%) (Table 1). Genetic testing identifies a P/LP variant (i.e., G+) in ~30-40% of all-comers with a clinical diagnosis of HCM and >60% in patients with familial disease⁴⁻⁷. However, not all variant carriers manifest clinically overt HCM (G+LVH–, herein referred to as subclinical HCM) and in those who do, penetrance is age-dependent with disease typically developing during late adolescence through early to middle adulthood^{8,9}. Longitudinal studies have attempted to estimate the proportion of G+LVH– who develop HCM and to estimate the rate of phenotypic conversion, however individual studies have been limited by small size and short duration of follow up.

Furthermore, the advent of unbiased genotyping in large scale general population studies has led to the incidental identification of P/LP variants associated with HCM in individuals who typically do not have a known diagnosis. When cardiac imaging is also available, the percentage of variant carriers who have unexplained LVH can be calculated to estimate penetrance. The

penetrance and clinical impact of P/LP variants differ between family/clinically-based studies and general populations studies, but these differences have not been systematically characterized.

To address these key questions, we performed meta-analyses to summarize current understanding of the prevalence and penetrance of sarcomeric P/LP gene variants, comparing findings from family/clinically-based studies to those reported in general population studies with accessible genetic and cardiac imaging data. Meta-analyses were additionally performed in longitudinal studies which followed up gene variant carriers to summarize the current knowledge regarding the transition from subclinical to clinically overt HCM.

Methods

Compliance with ethical standards

The data used in this manuscript is publicly available and ethics approval was not required.

Search Process

The review was conducted to fulfill the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) criteria on published peer-reviewed journal articles but also included pre-print archives and conference proceedings full papers. Our review questions were: (1) *What is the penetrance in cross-sectional studies and the phenotypic conversion in longitudinal studies of each sarcomeric or sarcomeric-related gene associated with HCM (as defined in Table 1)?*; (2) *Does the penetrance differ between family/clinically-based studies and population studies?*; (3) *What is the age of HCM onset for each gene associated with HCM?*; (4) *What is the prevalence of sarcomeric or sarcomeric-related P/LP variants in family/clinically-based HCM cohorts?*; (5) *What proportion of asymptomatic individuals with unexplained LVH from the general population also carry a P/LP variant?*; and (6) *What is known about the transition from*



G+LVH- to G+LVH+?. All English-language manuscripts available online through electronic indexing addressing any of the review questions were included.

A systematic search of Embase, PubMed, Scopus, Google Scholar, MedRxiv, and BioRxiv was used to identify relevant manuscripts published up to 1st of March 2023. Search items were defined using the PECO (Patient/Exposure/Comparator/Outcomes) framework: (P)= (“hypertrophic cardiomyopathy”, “HCM”, “LV hypertrophy”); (E)= (“pre-phenotypic”, “prephenotypic”, “non-hypertrophic”, “nonhypertrophic”, “pre-LVH”, “pre-clinical”, “preclinical”, “sub-clinical”, “subclinical”, “early disease”, “gene carrier”, “mutation carrier”, “gene positive”, “gene mutation”, “HCM carrier”); (C)= (“overt HCM”, “hypertrophic HCM”, “genotype positive HCM”, “phenotype positive HCM”); (O)= (“penetrance”, “phenotypic conversion”, “expressivity”, “age of onset”, “age of diagnosis”, “detection”, “prevalence”, “manifestations”, “phenotype”, “morphological”, “functional”, “dysfunction”, “transition to disease”). Instead of the generic word “gene”, individual genes from Table 1 were also included in the search queries. The PECO framework categories were combined using “AND”, while we grouped the variations within categories via “OR”. We excluded: (1) studies with <5 participants per gene, (2) studies exploring outcomes of interest in syndromic HCM or HCM phenocopies, (3) studies not using echocardiographic or cardiovascular MRI (CMR) based measurements of LV wall thickness to assess the presence of LVH, (4) studies which did not define or it was not possible to re-define overt HCM with the available data as a left ventricular maximal wall thickness (MWT) ≥ 15 mm in probands and MWT ≥ 13 mm in relatives, (5) studies that did not employ robust genotyping to assess mutation status, and (6) non-original research (e.g., reviews). If cohorts with overlapping membership were identified, the study with the highest sample size was included to minimize bias. As there are no validated tools to evaluate

study quality within the specific scope of this review¹⁰, a formal quality assessment was not pursued but relevant studies were critically appraised.

Statistical Methods

All statistical analyses were performed in R 4.2.1 using the packages “meta” and “metafor”. A random-effects model meta-analysis using a generalized linear mixed model (GLMM) with binomial likelihood and logit link was employed to calculate the pooled penetrance (in cross-sectional studies) and the phenotypic conversion (in longitudinal studies), and their associated 95% confidence intervals (CIs), for each HCM-related gene (as per Table 1) and across all genes¹¹. Since the studies included individuals across various age groups, comprising both children and adults, our penetrance estimates aim to provide a representation of the penetrance across the life-course. The lack of individual participant data prevented the separation of age groups in most studies, leading to the inclusion of all age ranges in our analyses. In studies reporting only the number of included families, one proband per family was assumed to exist. When calculating the HCM penetrance, individuals who developed a different cardiomyopathy were excluded if LVH was absent. Participants with multiple pathogenic variants were excluded if their contribution could be separated. With regards to the cross-sectional penetrance in family/clinical studies, we provide the penetrance in the relatives carrying P/LP variants identified as part of cascade screening. Studies in which the contribution of probands towards the cross-sectional penetrance in relatives could not be separated were excluded from this analysis. For comparison, we also provide the cross-sectional penetrance across all carriers (i.e., both probands and relatives). However, the later estimate is biased especially since the number of probands varied between the studies. With regards to the longitudinal phenotypic conversion, any participant with prevalent disease (regardless of if proband or relative) was excluded. Studies in which the

contribution of those with prevalent disease towards phenotypic conversion could not be separated, were removed from this analysis. A random-effects (RE) meta-analysis model was employed to calculate the pooled mean age at baseline and follow-up duration in longitudinal studies evaluating phenotypic conversion.

In order to calculate the mean age of HCM onset across all genes and for each gene in all studies a random-effects meta-analysis was employed¹². Lastly, a random-effects GLMM model was used to derive the prevalence of sarcomeric or sarcomeric-related P/LP gene variants in clinical HCM cohorts with ≥ 200 genotyped participants. We also report the prevalence in the subgroup which used the American College of Medical Genetics and Genomics (ACMG) criteria for variant classification. When calculating the mean age of HCM onset or the prevalence of G+ in HCM cohorts, all carriers who had a confirmed diagnosis of HCM were included to minimize bias.

The reported penetrance, phenotypic conversion, age of HCM onset and prevalence of G+ in clinical HCM cohorts varied between the studies. However, some variation is expected to occur by chance (i.e., random measurement errors). The heterogeneity between studies was appraised via Cochran's Q p -value and Higgins I^2 statistics. The 95% CI of I^2 was also calculated. Either a Cochran's Q $p < 0.05$ or $I^2 \geq 50\%$ was interpreted as suggesting the presence of heterogeneity.

Meta-regression was employed to study potential covariates which might influence penetrance when: (1) there was evidence of heterogeneity in the meta-analysis, (2) there were $n \geq 5$ studies per analysis, and (3) the covariate variable was reported by the studies included in the meta-analysis. In this study it was feasible to explore age (as the mean age of the relatives), sex (as the percentage of males [males %]) and geography (the continent of the study participants) as

covariates. In instances where the origin of study participants was not explicitly stated, we used the continent corresponding to the senior author's institutional affiliation. Studies spanning multiple continents were excluded from the geographic meta-regression. To visually depict the trend of each covariate, meta-regression scatter bubble plots were generated for continuous covariates and box plots for categorical ones.

Small study effects is a phenomenon where studies with smaller sample sizes exhibit different (usually larger) effect sizes¹³. The most common reasons are publication, selective outcome reporting, and confounding bias. For meta-analyses with ≥ 10 studies, Egger's test¹⁴ was used to assess for small study effects and p -values < 0.05 were interpreted as potentially indicating their presence. Contour-enhanced funnel plots were also generated¹⁵, and the presence of asymmetry was interpreted as indicating the possibility of publication bias, small study effects, or methodological heterogeneity.

Lastly, we used a two-sample z test for independent variables which takes into account variance to compare two estimates for which the 95% CIs were available¹⁶.

Data and code availability

All the relevant data has been published in the manuscript and/or the supplementary publication material. The GLMM meta-analysis code template can be access on: <https://cran.r-project.org/web/packages/metafor/metafor.pdf>.

Results

Database searches identified 1734 articles. After screening the abstracts, 455 full text manuscripts were assessed, and 114 met inclusion criteria for quantitative analysis.

Supplementary Figure S1 presents the PRISMA flow chart. Cross-sectional family or clinically-

based studies that provided data on penetrance in proband relatives are presented in Supplementary Table S1. Longitudinal studies exploring phenotypic conversion in G+LVH– are presented in Supplementary Table S2. Studies providing only the age of HCM onset per sarcomere or sarcomere-related gene in HCM cohorts are presented in Supplementary Table S3. Studies with ≥ 200 genotyped participants providing the prevalence of gene variants in HCM clinical cohorts are presented in Supplementary Table S4.

Cross-sectional Prevalence and Penetrance: Family and Clinically-based Studies

Figure 1A summarizes study-specific and pooled prevalence of sarcomere variants in clinical HCM cohorts, examining 20,808 participants from non-overlapping cohorts with ≥ 200 genotyped participants. Using strict and standardized ACMG criteria for variant classification, genetic testing identified a P/LP variant in 34% (95% CI [29, 40]) of patients diagnosed with HCM (Figure 1B).

Focusing on family-based studies of kindreds with sarcomeric HCM, the pooled cross-sectional penetrance of all sarcomere or sarcomere-related gene variants in at-risk G+ relatives carrying the P/LP family variant (excluding probands) was 57% (95% CI [52,63]) (Table 2). Notably, penetrance differed from gene to gene ($I^2=55\%$, 95% CI [41,65]; Cochran's Q p -value <0.001), ranging from 32% for myosin light chain 3 (*MYL3*) to ~55% for *MYBPC3*, ~60% *TNNT2* and *TNNI3*, and ~65% for *MYH7*. If probands were included, the penetrance was higher ($p=0.013$) reaching 67% (95% CI [63,72]) with similar gene-to-gene variation (Supplementary Table S5). The mean age of diagnosis with HCM was 38 years (95% CI [36, 40]) across all genes, but it varied from ~33 years for *MYH7* to ~41 years for *MYBPC3*.

MYBPC3, *MYH7* and *TNNT2* were investigated further since they are the most prevalent genes associated with HCM. Results are presented in Table 2, and visually depicted in Figure 2

for *MYBPC3*, in Figure 3 for *MYH7*, and in Supplementary Figure S2 for *TNNT2*. The pooled penetrance for the 1024 relatives carrying *MYBPC3* P/LP variants identified during cascade screening was 55% (95% CI [49, 62]), and the mean age of HCM onset was 41 years (95% CI [39, 44]). However, the studies were heterogenous, and penetrance ranged from <40% to >80% (Figure 2A). For *MYH*, the pooled penetrance was 64% (95% CI [53,75]) across the 307 relatives with P/LP variants from the 17 family/clinically based studies. Although the absolute estimate was higher, there was no statistically significant difference in penetrance between *MYBPC3* and *MYH7* ($p=0.167$). The *MYH7* studies were also heterogenous, and penetrance ranged from 40% to 100% (Figure 3A). The mean age of HCM diagnosis in *MYH7* carriers was 33 years (95% CI [31, 35]). In relatives carrying P/LP variants of *TNNT2*, the penetrance was 62% (95% CI [44, 78]) at a mean age of diagnosis of 36 years (95% CI [31, 40]), although the studies were heterogeneous, and penetrance ranged from 25% to 100% (Supplementary Figure S2A).

There was no statistically significant difference in penetrance between *MYBPC3*, *MYH7*, *TNNT2*, *TNNI3*, myosin light chain 2 (*MYL2*) and tropomyosin 1 (*TPM1*) (~50-65%, all $p>0.05$). However, certain sarcomeric or sarcomeric-related genes may have a lower penetrance: ~32% for (*MYL3*) and ~38% for cysteine- and glycine-rich protein (*CSRP3*) albeit the sample sizes were smaller (i.e., $G+ n < 50$) (Table 2).

To explore sex as a source of heterogeneity (i.e., males and females having different penetrance rates or average age of HCM onset), meta-regression was employed. Sex-related differences in the overall or gene-specific penetrance were not observed when using the percentage of males as a covariate (Supplementary Table S6). A 1% increase in the included percentage of males was associated with an increase of 0.2 years (95% CI [0.1, 0.3]) in the mean age of HCM onset across all genes (Supplementary Table S6). We also evaluated whether

studies in which the relatives had a higher mean age at the time of the study were more likely to report a higher penetrance. Fewer than half of the studies reported age of relatives at the time of assessment, but using available data, the average mean age in relatives was 43.5 ± 10.6 years across all studies and genes. Using meta-regression, a 1-year increase in the average age was associated with a 1% (95% CI [0.1, 1.9]) increase in the reported penetrance (Supplementary Table S7).

We also employed meta-regression to explore whether the reported penetrance was influenced by geographic region. Across all genes, the highest observed penetrance was in studies conducted in Asia 68% (95% CI [54, 79]), followed by North American 60% (95% CI [52, 68]) and European studies 54% (95% CI [48, 60]). However, only the difference between Asian and European studies reached statistical significance ($p=0.047$) (Supplementary Table S8).

Cross-sectional Prevalence and Penetrance: General Population Studies

Population studies with adequate genotypic and clinical data were analyzed to estimate the prevalence and penetrance of HCM in individuals incidentally discovered to be carrying P/LP sarcomere gene variants in the community. These studies included Atherosclerosis Risk in Communities (ARIC)¹⁷, Framingham Heart Study (FHS)¹⁸, Jackson Heart Study (JHS)¹⁸ and UK Biobank^{19,20} (Table 3). Across all cohorts, 1397 individuals carried a P/LP sarcomeric gene variant amongst the 213,911 genotyped participants. This indicates a pooled prevalence of 0.7% for P/LP sarcomere variants in the general population.

The maximal LV wall thickness cut-off used to define LVH varied by study (MWT ≥ 12 mm in FHS and JHS, ≥ 13 mm in UK Biobank and ≥ 15 mm in ARIC) and granular individual-level details on LV wall thickness measures are not available. Using study definitions of LVH as indicating penetrance of HCM, the penetrance of P/LP sarcomere variants ranged from 0% in

ARIC (none of the 29 P/LP variant carriers, mean age 54.3 ± 6.1 years had a MWT ≥ 15 mm on echocardiography albeit only a subset had structural imaging¹⁷) to 18% in the UK Biobank (9 out of 49 G+ participants with MWT ≥ 13 mm on CMR at a mean age of $56. \pm 8.1$ years)¹⁹. The combined penetrance in FHS and JHS was 9% (2 out of 22 G+ individuals with MWT ≥ 12 mm on echocardiography)¹⁸. Given the non-standardized definitions of HCM, a meta-analysis was not formally performed. Overall, in the general population studies, the penetrance of sarcomere variants was 11% at a mean age of 55.8 ± 8.1 years.

Longitudinal Family and Clinical Studies: Phenotypic Conversion from Subclinical to Clinical HCM

Longitudinal family and clinical studies were analyzed to estimate the incidence and rate of phenotypic conversion from subclinical to clinical HCM. This was performed for individual sarcomere genes and across all genes. Phenotypic conversion was defined as the percentage of G+LVH- individuals who developed overt HCM (G+LVH+) during follow-up. As summarized in Table 4, across all studies, 146 of 524 (28%) at risk *MYBPC3*, *MYH7* and *TNNT2* variant carriers developed HCM in studies where a diagnosis was established during follow-up.

However, there was substantial variability between studies with Lorenzini *et al*²¹ reporting that 116 out of its 226 (51%) *MYBPC3*, *MYH7* and *TNNT2* carriers developed HCM (baseline age 14 ± 18 years and ~ 15 years of follow up), in contrast with only 30 out of the 298 (10%) carriers combined in the remaining studies (mean baseline age $\sim 16 \pm 11$ years and ~ 7 years of follow up). To account for the difference in size and participant characteristics of the studies, we used a random-effects model to estimate that pooled phenotypic conversion across these genes was 15% (95% CI [8, 27]) at a mean age at baseline of 16 years (95% CI [12, 20]), and average follow-up duration of 8 years (95% CI [6, 11]) such that the mean age at the end of follow-up was ~ 24

years (95% CI [18, 31]). Results were heterogenous ($I^2=81\%$, Cochran's Q p -value <0.001) and the phenotypic conversion rate among individual studies ranged from 0% to 67%. As most longitudinal studies did not report the mean age of HCM onset (Supplementary Table S2), we were unable to perform a meta-analysis.

Analyzable studies reported gene-specific phenotypic conversion of *MYBPC3* (n=9), *MYH7* (n=4) and *TNNT2* (n=4) (Table 4, Supplementary Table S2). During a mean follow-up of ~8 years starting from a mean age of ~20 years, 12% [6, 24] of the *MYBPC3* variant carriers developed HCM. In contrast, 23% [6,56] of younger *MYH7* variant carriers (mean age ~13 years at initial evaluation) were developed overt HCM over a similar follow up period of ~9 years. Phenotypic conversion in the 78 *TNNT2* variant carriers was 18% [5, 48] during a mean follow-up period of ~9 years, starting from an average age of ~12 years. Lorenzini *et al*²¹ reported phenotypic conversion rates of >40% for *MYBPC3*, *TNNT2* and *MYH7*. The higher rate of phenotypic conversion in their report was likely driven by the higher proportion of adults (~40%), longer follow-up (~15 years), and use of CMR to evaluate MWT which may be more sensitive in detecting LVH than echocardiographic measures. Lorenzini *et al*²¹ additionally reported a phenotypic conversion of 17% (95% CI [7, 39]) for *TNNI3* starting from a baseline age of 14 over 15 years of follow-up, but a meta-analysis could not be conducted due to the absence of additional studies with data on this gene. The differences observed in the phenotypic conversion rates between studies were not statistically significant. Forest plots of the random-effects GLMMs meta-analyses for *MYBPC3*, *MYH7* and *TNNT2* are shown in Figure 4.

Discussion

In this study, we performed meta-analysis to characterize the prevalence and penetrance of genetic variants that cause HCM in two different contexts: (1) clinically-based studies of families and patients with HCM and (2) general population studies where pathogenic variants were incidentally identified as part of unbiased genotyping. Findings are summarized in Figure 5. As expected, the prevalence of pathogenic and likely pathogenic variants in sarcomere genes was 50-fold higher, and the penetrance was 5-6 fold higher in HCM patients and relatives, compared to sarcomere variant carriers the general population. In phenotype-first clinically-based studies of ~21,000 genotyped patients with HCM, the prevalence of P/LP sarcomere variants was 37% (34% when using more rigorous ACMG criteria). Cross-sectional penetrance across all genes was 57% at a mean age of 44 years, with a mean age of diagnosis of 38 years. Penetrance ranged from ~32% for *MYL3* to ~55% for *MYBPC3*, ~60% *TNNT2* and *TNNI3*, and ~65% for *MYH7*. Genotype-first population-based studies of ~214,000 participants identified a low background prevalence of 0.7% for sarcomere variants in the general public. Notably, applying a phenotype-first approach to population studies yielded a higher prevalence with ~3% of the individuals with a CMR MWT ≥ 15 mm carried P/LP sarcomere variants in the UK Biobank¹⁹. Despite meeting criteria for classification as pathogenic or likely pathogenic, the penetrance of HCM was substantially lower (~11%) when variants were identified incidentally in the general population, assessed at a mean age of 56 years across all studies. Penetrance was estimated at ~11%, ranging from 0% in ARIC (assessed at age 54.3 ± 6.1 years) to 18% in the UK Biobank (assessed at 56.2 ± 8.1 years).

Analyzing longitudinal family-based studies, only 15% of G+LVH– relatives developed HCM during ~8 years of follow-up, starting at a mean age of ~16 years. Short-term phenotypic

conversion varied by gene, from ~25% for *MYH7*, ~20% *TNNT2* to ~10% for *MYBPC3*.

Similarly, the age of HCM diagnosis varied by gene as carriers of *MYBPC3* variants tended to be expressed ~10-years later than *MYH7*, *TNNT2* or *TNNI3* variant carriers. The meta-regression exploring the sources of heterogeneity suggested that studies including older carriers reported higher penetrance rates. This emphasizes the need for studies with extended follow up of subclinical HCM, continuing through at least middle age, to more accurately estimate lifetime penetrance and phenotypic conversion given the wide variation in the age at which clinically overt disease develops. Indeed, an important limitation of these studies is that the duration of follow up was relatively short and often ended prior to an age when features of clinically overt disease are most likely to emerge.

Penetrance of P/LP sarcomere variants in HCM cohorts versus the community



Although present at a low level in the general population, the clinical impact of sarcomere variants classified as P/LP differed in this context. Penetrance for HCM was low in the general population; estimated to be 11%. Because the penetrance of sarcomere variants in HCM, like other adult-onset genetic disorders, is heavily influenced by age, reported cross-sectional penetrance underestimates true lifetime penetrance as it merely captures the prevalence of the HCM phenotype at the time of the studies. However, assessments were performed at a mean age of 55.8 ± 8.1 years; an age when sarcomeric HCM would be anticipated to have developed in most variant carriers. Indeed, studies in clinical cohorts reported a mean age of diagnosis of 30-35 years for *MYH7*, *TNNT2*, and *TNNI3*; 40-45 years for *MYBPC3* and *TPM1*.

Sarcomere variants have been associated with subtle, often intra-normal, abnormalities in LV wall thickness when healthy variant carriers in the general population were compared to non-carriers using machine learning approaches on cardiac magnetic resonance images^{19,22}. We were

not able to apply these analyses in this study. Additionally, we did not assess the risk of heart failure and other adverse cardiac events in sarcomere variant carriers in the general population. Prior studies have indicated that adverse cardiac events were more prevalent in variant carriers than non-carriers in the population studies¹⁸. These findings highlight the importance of developing more precise definitions of penetrance and clinical disease. Incidentally identified pathogenic variants in HCM-associated sarcomere genes may not phenotypically silent even if criteria for HCM are not met. Although the anticipated penetrance of stereotypical HCM may be lower than in the family context, subtle abnormalities may result from sarcomere variants and may increase cardiac risk. While careful longitudinal screening to monitor for evolution to HCM is critical for at-risk members of families with HCM, less intense follow up for the emergence of HCM for individuals incidentally identified to carry P/LP sarcomere variants may be reasonable. However, it would be prudent to consider that they may be at heightened risk for developing other adverse cardiac events.

The difference in penetrance in population versus family studies emphasizes the presence of defined and undefined genetic and environmental factors associated with greater risk of developing HCM, including ethnicity²³, the presence of multiple P/LP variants²⁴, obesity²⁵ and hypertension²⁶. For example, the penetrance of HCM in *MYL3* carriers was 32% contrasting with the 65% penetrance observed in *MYL2* carriers, although both genes serve a similar molecular function. Claes *et al*²⁷ reported a penetrance of 89% in biallelic *MYL2* variant carriers, or in the presence of hypertension, or obesity, versus 36% in *MYL2* variant carriers without additional risk factors. Propensity matching all participants for all genetic and environmental HCM risk factors was not possible in this study but would have provided some clarity on whether the gene-specific estimates are truly different. Additionally, findings from large-scale genome wide association

studies (GWASs) in HCM suggest that genetic variants involved in myocardial growth, LV contractility, sarcomere organization, obesity and blood pressure regulation could influence HCM susceptibility, and even expressivity²⁸⁻³⁰.

Phenotypic Conversion and Transition to Clinically Overt HCM

In longitudinal family studies with an average follow-up period of ~8 years, from a mean age of ~16 years to ~24 years, the overall pooled phenotypic conversion was found to be 15%. There was gene-specific variation in short term phenotypic conversion, ranging from ~12% for *MYBPC3*, to ~18% for *TNNT2* and ~23% for *MYH7*, however differences in phenotypic conversion rates among specific genes were not found to be statistically significant. Importantly, most longitudinal studies enrolled participants who were young at baseline and the follow-up period concluded before reaching the typical mean age of HCM onset. As such, true penetrance and phenotypic conversion rates over a lifetime are underestimated.

The factors that either drive susceptibility or resilience to developing clinically penetrant HCM in individual sarcomere variant carriers are not understood. Prior studies have identified older age, male sex, and family history of HCM as risk factors for higher penetrance³¹⁻³³. A retrospective longitudinal study in 285 G+LVH- participants (from 156 families) suggested that an abnormal ECG quadrupled the risk of phenotypic conversion over ~15 years starting from a baseline average age of 14²¹. Another study suggested that progression to LVH in unrelated G+LVH- participants ~16 years of age, followed for a mean duration of 3 years, was more likely in those with longer mitral leaflets, lower global E' velocities and higher serum N-terminal pro-peptide of B-type natriuretic peptide³⁴. However, robust imaging and clinical biomarkers of *impending* progression of LVH and evolution to HCM are yet to be identified. Moreover,

inflection points where the natural course of the disease may be altered through interventions have not been elucidated.

Limitations

An inherent limitation of our meta-analysis is the high heterogeneity of the included studies and their lack of external validation. The heterogeneity likely relates to study-to-study differences in the underlying prevalence of genetic and environmental risk factors associated with the development of HCM. We attempted to identify sources of heterogeneity that may influence the reported penetrance through meta-regression using study-level covariates such as the percentage of males or the age of the relatives. However, this approach is prone to ecological fallacy as inferences made from group-level data might not accurately reflect individual-level relationships. In addition, the reported prevalence of P/LP variants is contingent on the criteria used for variant classification, which differed between studies and are somewhat subjective. This variability may impact population studies more than family studies where rare sarcomere variants are more likely to be truly pathogenic. Although these differences in classification might bias our meta-analytic estimates, independently reclassifying all variants using the ACMG criteria was not feasible due to the limited availability of variant data for individual participants. Most studies used echocardiography to measure LV wall thickness and diagnose HCM. Echocardiography may have a lower sensitivity in identifying LVH than CMR³⁵. We tried to obtain more accurate estimates by excluding participants which had multiple pathogenic variants, but most studies did not provide explicit information about the presence or absence of such complex genotypes in their participants. Lastly, we only included English-written manuscripts available online which could have biased our estimates and may decrease generalizability across different ancestries.

Future Directions

A multidisciplinary approach integrating basic and clinical investigation is needed to improve our fundamental understanding of the penetrance and early phenotypic manifestations of sarcomere variants, as well as the transition from subclinical to clinically overt HCM. Long term prospective study of subclinical HCM is specifically needed to provide: (1) better lifetime estimates of penetrance, (2) robust predictors that identify individuals most likely to develop penetrant disease, (3) more accurate characterization of the full phenotypic spectrum of HCM, including changes leading up to and spanning the transition from subclinical to clinically overt HCM. Additional genome wide association studies are also required to improve our understanding of genetic modifiers that convey susceptibility or resilience to developing phenotypic HCM. These insights will help to guide management and surveillance decisions in the clinical arena, provide crucial insights to suggest novel therapeutic targets, and provide a means to assess the efficacy of novel therapies intended to modify disease progression or prevent disease emergence.

Conclusions

The prevalence penetrance of sarcomere variants differs based on the underlying context. Although variants classified as pathogenic or likely pathogenic are present at a low level in the community, the likelihood of developing clinically overt HCM is 5-6 fold higher in studies on HCM cohorts and families compared to population studies. This highlights the presence of important but currently undefined genetic and non-genetic factors that influence the clinical impact of sarcomere variants. Because of the varying risk of developing HCM or other adverse cardiac outcomes, a more personalized approach to managing variant carriers is needed.

Different strategies may be appropriate for members of families with HCM versus healthy individuals who are incidentally found to carry sarcomere variants. Long-term prospective study of sarcomere variant carriers, in families and in the community, is needed to estimate true lifetime penetrance and to improve understanding of disease pathogenesis.



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Disclosures

The views expressed in this article are those of the authors who declare that they have no conflict of interest except for Dr. JC Moon who is the chief executive officer of Myocardium AI and has served on advisory boards for Genzyme and Sanofi.

Supplementary Material

Supplementary Tables S1-8

Supplementary Figures S1-2

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Table 1. Genes Associated With HCM.

Gene	Protein	Causal Association with HCM	Prevalence	Inheritance pattern	Constraint metrics		Other CM phenotypes
					Missense Z-score	Truncating pLI	
<i>MYBPC3</i>	Myosin-binding protein C	Definitive	30-40%	AD	1.45	0	-
<i>MYH7</i>	β -Myosin heavy chain	Definitive	10-30%	AD	3.93	0	ACM, DCM
<i>TNNT2</i>	Troponin T	Definitive	3-10%	AD	1.19	0	DCM, RCM
<i>TNNI3</i>	Troponin I	Definitive	3-10%	AD	1.28	0.1	DCM, RCM
<i>MYL2</i>	Myosin regulatory light chain 2	Definitive	<3%	AD or AR	0.4	0	-
<i>MYL3</i>	Myosin essential light chain	Definitive	<3%	AD or AR	0.76	0.09	-
<i>TPMI</i>	α -Tropomyosin	Definitive	<3%	AD	2.87	0	DCM
<i>PLN</i>	Phospholamban	Definitive	<3%	AD	0.62	0.45	-
<i>ACTC1</i>	α -Actin	Definitive	<1%	AD	4.52	0.74	DCM
<i>ALPK3</i>	α Protein Kinase 3	Definitive	<1%	AD or AR	0.13	0	-
<i>CSRP3</i>	Cysteine and glycine rich protein	Moderate	<1%	AD	-0.14	0	DCM
<i>JPH2</i>	Junctophilin	Moderate	<1%	AD	1.94	0	DCM
<i>TNNC1</i>	Troponin C	Moderate	<1%	AD	1.77	0.68	DCM
<i>TRIM63</i>	Muscle ring finger protein 1	Moderate	<1%	AD	-0.18	0	-

The genes were ordered based on the gene-HCM phenotype validity (as recorded in the Clinical Genome Resource), followed by prevalence, and then alphabetical order. The inheritance pattern of each gene was provided by the Online Mendelian Inheritance In Man compendium.

Constraint metrics reflect the tolerance to missense (Missense Z-score) or non-sense mutations (pLI). The higher the missense Z-score the greater the intolerance, while a non-sense pLI of 1 indicates complete intolerance. Metrics were provided by the Genome Aggregation Database.

ACM = arrhythmogenic cardiomyopathy; *AD* = autosomal dominant; *AR* = autosomal recessive; *CM* = cardiomyopathy. *DCM* = dilated cardiomyopathy; *G+LVH+* = left ventricular hypertrophy and a gene mutation associated with it co-exist; *HCM* = hypertrophic cardiomyopathy; *LVH+* = presence of left ventricular hypertrophy; *pLI* = probability of loss-of-function intolerance; *P/LP G+* = pathogenic or likely pathogenic gene mutation; *RCM* = restrictive cardiomyopathy.

Table 2. Penetrance and Age of Diagnosis in Familial HCM From Cross-sectional Studies.

Gene	Pooled penetrance in relatives carrying P/LP variants							Pooled age at diagnosis across all carriers					
	Number of Studies	Number of G+LVH+	Number of G+	Penetrance (95% CI)	Heterogeneity		Small study effects	Number of Studies	Number of G+LVH+	Age of Diagnosis, years (95% CI)	Heterogeneity		Small study effects
					I ² (95% CI)	Cochran's Q <i>p</i> -value	Egger's test <i>p</i> -value				I ² (95% CI)	Cochran's Q <i>p</i> -value	Egger's test <i>p</i> -value
All	70	1043	1924	57.4 (52.1, 62.5)	55 (41,65)	<0.001	0.053		4357	37.7 (35.9, 39.6)	94 (93, 95)	<0.001	0.467
<i>MYBPC3</i>	21	541	1024	55.4 (48.9, 61.8)	70 (53, 81)	<0.001	0.182	34	2350	41.0 (38.5, 43.5)	88 (85, 91)	<0.001	0.593
<i>MYH7</i>	17	188	307	64.3 (52.6, 74.5)	10 (0, 51)	0.733	0.078	31	1408	33.0 (30.6, 35.4)	82 (76, 87)	<0.001	0.133
<i>TNNT2</i>	11	141	228	62.4 (43.7, 78.0)	33 (0, 67)	0.139	0.915	12	292	35.8 (31.4, 40.2)	53 (9, 76)	0.016	0.317
<i>TNNI3</i>	5	50	115	60.3 (28.6, 85.2)	6 (0, 80)	0.375	N/A	5	113	34.9 (21.7, 48.1)	96 (94, 98)	<0.001	N/A
<i>MYL2</i>	2	30	44	64.8 (26.6, 90.4)	70 (10, 93)	0.067	N/A	N/A	N/A	N/A	N/A	N/A	N/A
<i>MYL3</i>	4	13	38	32.2 (9.1, 71.9)	17 (0, 87)	0.306	N/A	N/A	N/A	N/A	N/A	N/A	N/A
<i>TPM1</i>	3	43	98	48.6 (25.7, 72.2)	87 (65, 96)	0.003	N/A	3	31	40.3 (36.9, 43.8)	10 (0, 90)	0.504	N/A
<i>ACTC1</i>	3	18	26	69.2 (49.5, 83.8)	0 (0, 90)	0.639	N/A	2	35	35.1 (26.9, 43.3)	0 (0, 60)	0.390	N/A
<i>ALPK3</i>	2	10	20	50.0 (29.5, 70.6)	43 (18, 68)	0.185	N/A	2	65	34.2 (9.5, 77.9)	97 (93, 99)	<0.001	0
<i>CSRP3</i>	2	9	24	37.5 (20.7, 57.8)	36 (10, 62)	0.212	N/A	2	29	54.1 (49.4, 58.8)	0 (0, 60)	0.334	N/A

CI = confidence interval; N/A = not available. Other abbreviations as in Table 1.

Table 3. Prevalence and Penetrance in Large Scale General Population Studies.

Study	Sample size	Criteria for classifying variants as P/LP	Prevalence of P/LP, n (%)	Mean age when penetrance was evaluated \pm sd	Penetrance definition	Penetrance, n (%)
All	213911	ACMG and LMM	1397 (0.7%)*	55.8 \pm 8.1	Variable	11 (11.0%) [†]
Atherosclerosis Risk in Communities ¹⁷	9667	ACMG	29 (0.3%)	54.3 \pm 6.1	LV MWT \geq 15 mm on echocardiography	0 (0.0%) [‡]
Framingham Heart Study ¹⁸	1638	LMM	14 (0.9%)	58.3 \pm 9.2	LV MWT \geq 12mm on echocardiography	2 (9.1%) [§]
Jackson Heart Study ¹⁸	1963	LMM	8 (0.4%)	55.2 \pm 12.7		
UK Biobank with CMR data ¹⁹	21322	ACMG	49 (0.2%)	56.2 \pm 8.1	LV MWT \geq 13mm on CMR	9 (18.4%)
UK Biobank with questionnaire data ²⁰	200643	ClinVar and VKGL	1346 (0.7%)	56.2 \pm 9.6	HCM self-reporting	35 (2.6%)

* Only the UK Biobank study with the higher sample size was included.

[†] Only the UK Biobank study with CMR data was included.

[‡] A subset of carriers underwent echocardiography, but many did not have any structural imaging¹⁷.

[§] The results for disease penetrance were pooled across Framingham Heart Study and Jackson Heart Study by the original manuscript¹⁸.

ACMG-AMP = American College of Medical Genetics and Genomic; CMR = cardiovascular magnetic resonance imaging; LMM = Partners Healthcare Laboratory for Molecular Medicine; LV = left ventricle; MWT = maximal wall thickness; P/LP = pathogenic/likely pathogenic; sd = standard deviation; VKGL = Vereniging Klinische Genetische Laboratoriumdiagnostiek.

Table 4. Phenotypic Conversion of G+LVH- in Longitudinal Clinical Studies.

Gene	Studies, n	G+ LVH+, n	G+, n	Pooled mean age at baseline	Pooled Phenotypic Conversion				Pooled mean length of follow up
					Phenotypic conversion % (95% CI)	Heterogeneity		Small study effects	
						I ² (95% CI)	Cochran's Q p-value	Egger's test p-value	
All	17	146	524	16.1 (12.3, 19.9)	15.2 (8.4, 27.1)	81 (71, 88)	<0.001	0.004	8.3 (6.2, 10.5)
<i>MYBPC3</i>	9	73	336	19.5 (11.0, 27.9)	12.4 (6.0, 23.6)	79 (60, 89)	<0.001	0.030	7.9 (5.0, 10.6)
<i>MYH7</i>	4	51	110	13.0 (11.2, 14.9)	22.6 (6.2, 56.1)	87 (70, 95)	<0.001	N/A	9.0 (3.9, 14.1)
<i>TNNT2</i>	4	22	78	12.4 (10.6, 14.1)	17.9 (5.0, 47.6)	66 (20, 88)	<0.001	N/A	8.7 (3.8, 13.7)

CI = confidence interval; N/A = not applicable. Other abbreviations as in Table 1.

Circulation

Figure Legends

Figure 1. Pooled prevalence of P/LP sarcomere gene variants in clinical HCM cohorts.

(A) Prevalence in cohorts with ≥ 200 genotyped HCM participants including probands. Smaller studies were excluded as they may introduce bias and potentially widen the confidence intervals, impacting the precision of our results. (B) Prevalence in studies which used the ACMG criteria for variant classification. Neubauer *et al* 2019² used the Oxford Genetics Laboratory criteria which closely follows the ACMG criteria, so it was included in this analysis.

ACMG = American College of Medical Genetics and Genomics; CI = confidence interval; GLMM = generalized linear mixed model; HCM = hypertrophic cardiomyopathy; P/LP = pathogenic/likely pathogenic; RE = random effects.



Figure 2. MYBPC3 penetrance in cross-sectional family/clinically-based studies.

(A) After excluding probands, cross-sectional penetrance (defined as the % of G+ with LVH) was calculated using a random effects GLMM meta-analysis, and the corresponding forest plot is shown. Overall, the pooled penetrance in G+ relatives in families with HCM who were identified as part of cascade screening was 55% (95% CI [49, 62]). (B) We explored whether this estimate could have been influenced by the tendency to publish only certain types of results (e.g., reporting a very high penetrance). This is not supported by the contour-enhanced funnel plot given its symmetry. (C) We explored whether the penetrance is influenced by sex (using males % as a covariate) and geography by study continent through meta-regression. Whilst the meta-regression bubble plot suggests that including more males was associated with reporting a higher penetrance, this association was not significant. (D) The reported penetrance was 56% (95% CI

[50, 62]) in studies from North America, 55% (95% CI [47, 64]) from Europe, and 59% (95% CI [46, 71]) from Asia. *P*-values for pairwise comparisons are provided and indicate similar estimates across these geographic regions.

G+ = sarcomere or sarcomere related pathogenic likely pathogenic variant carrier; *LVH* = left ventricular hypertrophy; *MYBPC3* = myosin binding protein C. Other abbreviations as in Figure 1.

Figure 3. MYH7 penetrance in cross-sectional family/clinically-based studies.

(A) After excluding probands, cross-sectional penetrance was calculated using a random effects GLMM meta-analysis, and the corresponding forest plot is shown. Overall, the pooled penetrance in *G+* relatives in families with HCM who were identified as part of cascade

screening was 64% (95% CI [53, 75]). (B) We explored whether this estimate could have been influenced by the tendency to publish only certain types of results (e.g., when reporting a very high penetrance). This is supported by the contour-enhanced funnel plot given its asymmetry.

(C) We explored whether the penetrance is influenced by sex (using males % as a covariate) and study continent through meta-regression. The meta-regression bubble plot does not suggest that including more males was associated with reporting a higher penetrance. (D) The reported penetrance did not differ significantly by geography as represented by study continents, with *p*-values > 0.05 for pairwise comparisons.

MYH7 = β -myosin heavy chain. Abbreviations as in Figures 1 and 2.



Figure 4. Phenotypic conversion for MYBPC3, MYH7 and TNNT2 genes in longitudinal studies.

After excluding probands, phenotypic conversion (defined as the % of G+ who developed LVH during longitudinal follow-up) was calculated using a random effects GLMM meta-analysis. The forest plots for (A) MYBPC3, (B) MYH7 and (C) TNNT2 genes are shown. Across all genes, the pooled phenotypic conversion was 15% over an average of ~8 years of follow up, starting from a mean age of ~16 years.

TNNT2 = troponin T. Other abbreviations as in Figures 1, 2 and 3.

Figure 5. Prevalence and penetrance of P/LP variants in family compared to population studies.

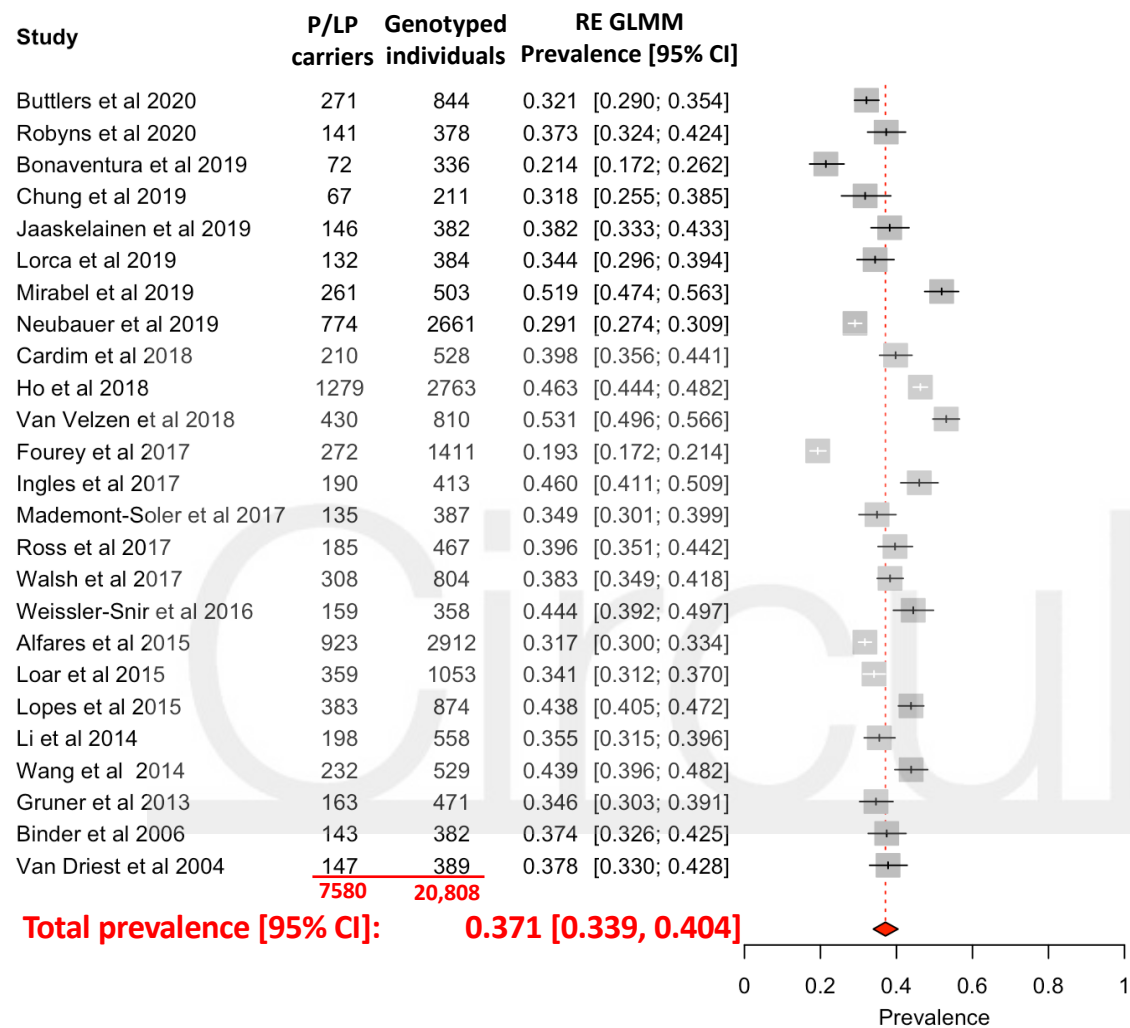


In clinical studies on patients and families with HCM, the prevalence of P/LP sarcomere variants was 34%, while the cross-sectional penetrance of HCM in relatives with P/LP was 57%. In contrast, in general population studies, the prevalence of P/LP variants in sarcomere genes was 50-fold lower (0.7%) and the cross-sectional penetrance in those incidentally identified as variant carriers was 5-6-fold lower (11%).

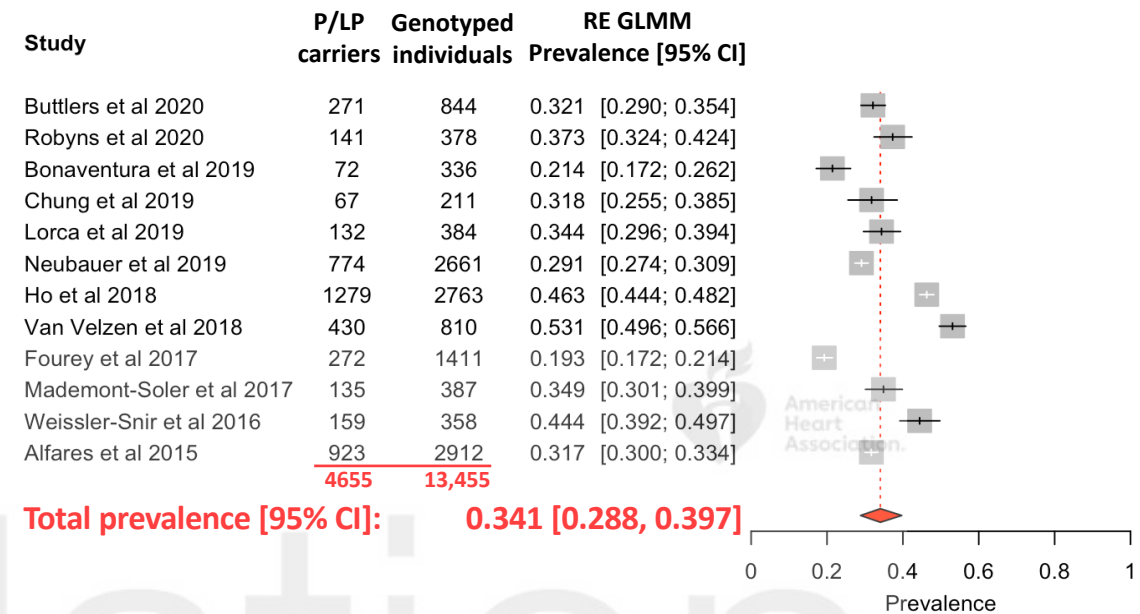
In longitudinal family studies, the pooled phenotypic conversion across all genes was 15% over an average of ~8 years of follow up, starting from a mean age of ~16 years. However, the phenotypic conversion in general population studies remains unknown.

ARIC = Atherosclerosis Risk in Communities. Other abbreviations as in Figures 1, 2, 3 and 4.

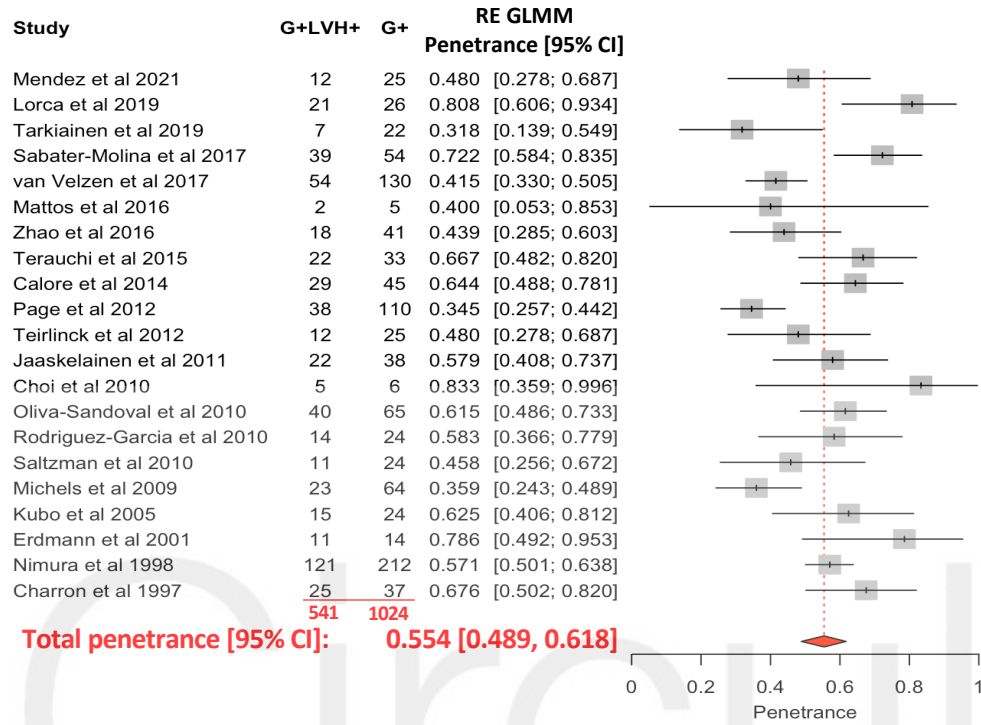
A. All criteria



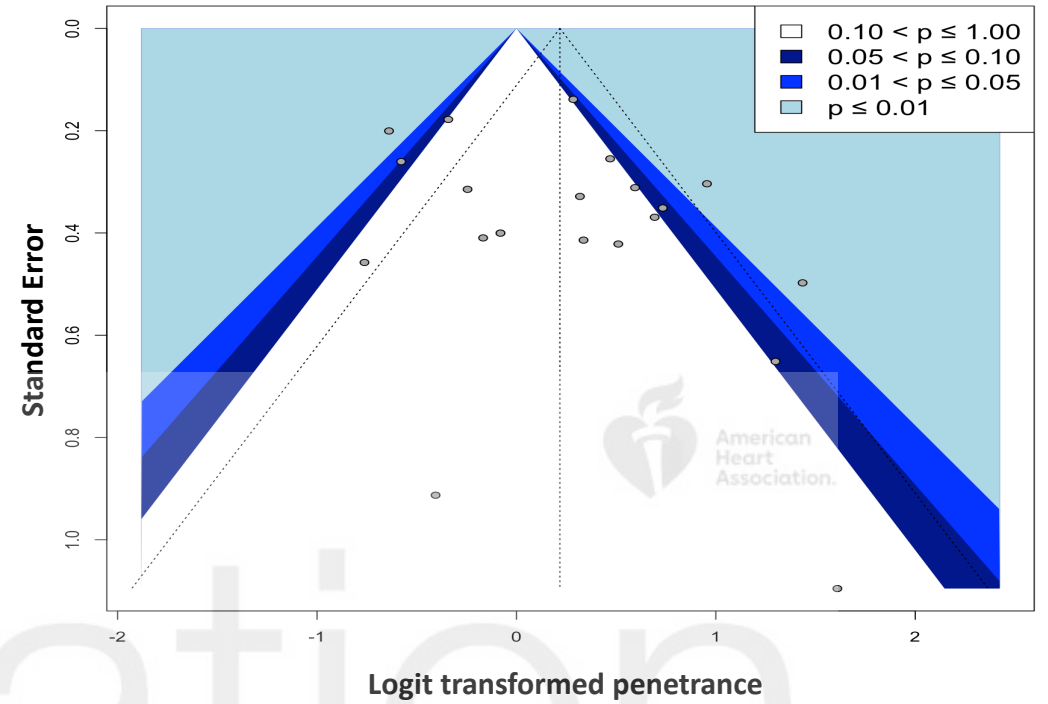
B. ACMG criteria



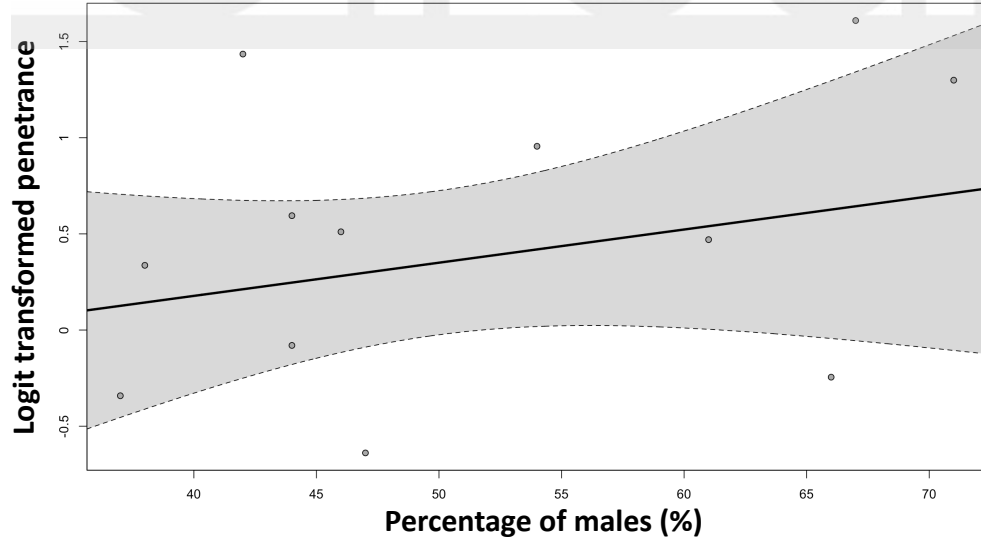
A. Meta-analysis: Forest plot



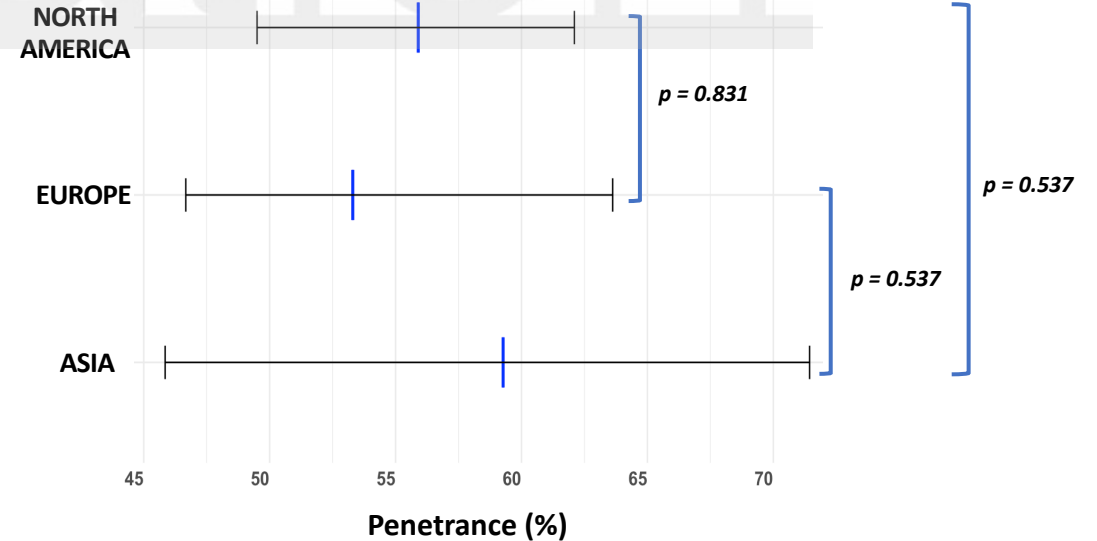
B. Meta-analysis: Contour-enhanced funnel plot



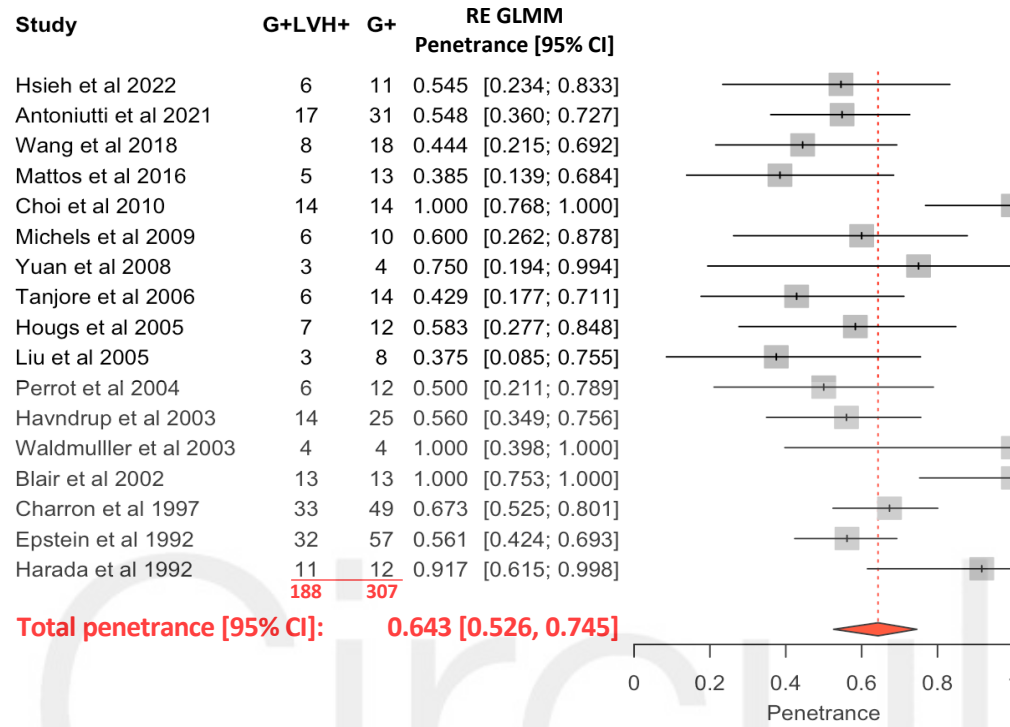
C. Meta-regression: Males (%)



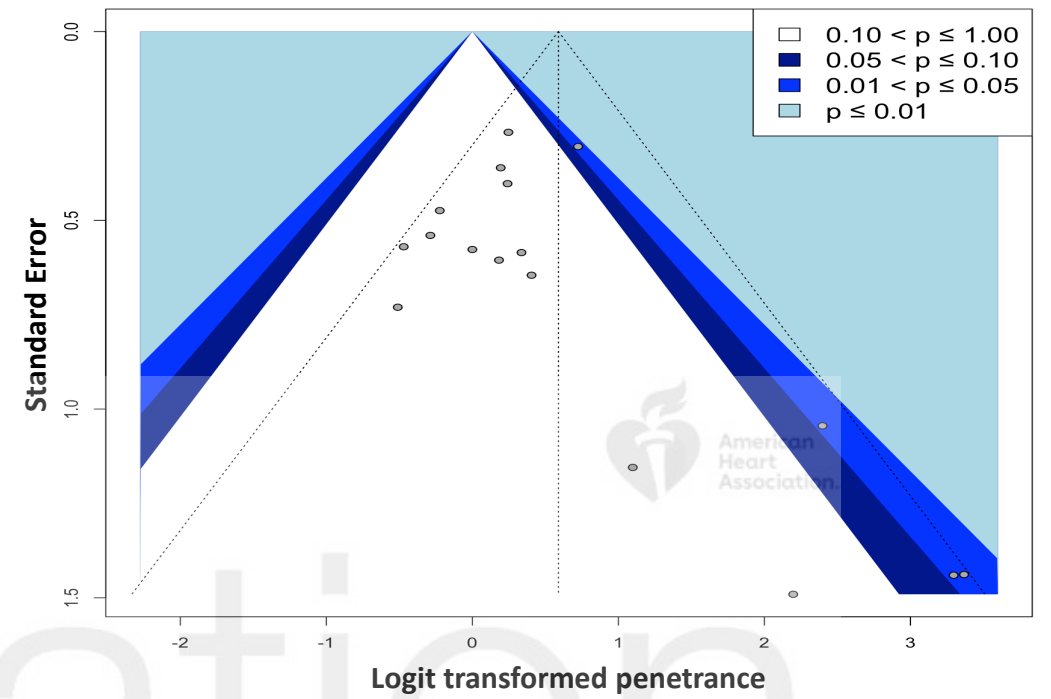
D. Meta-regression: Continent



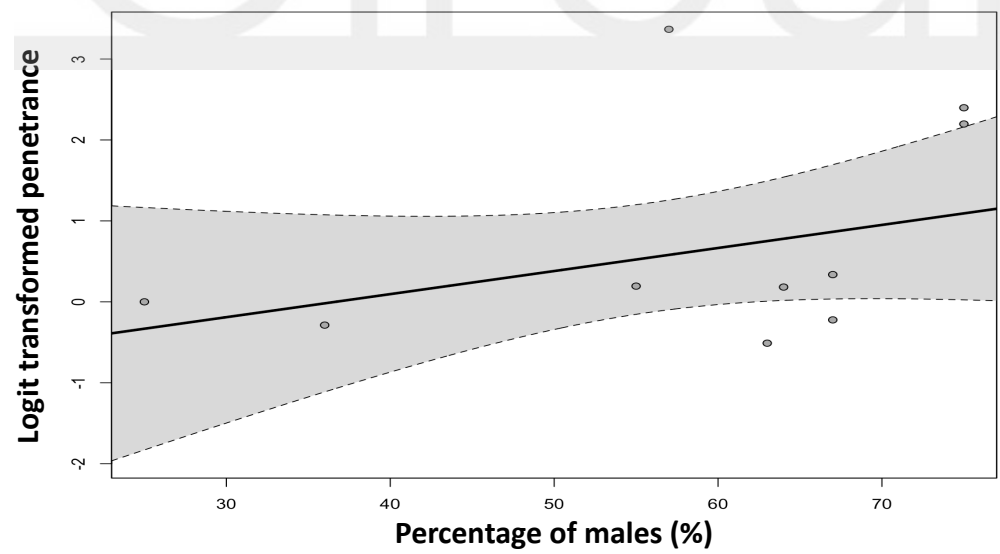
A. Meta-analysis: Forest plot



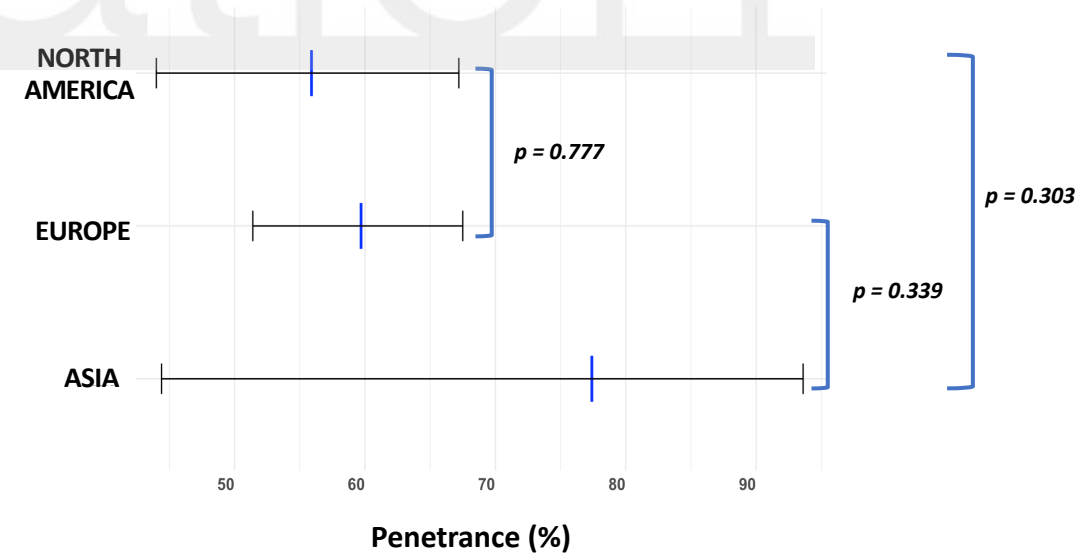
B. Meta-analysis: Contour-enhanced funnel plot



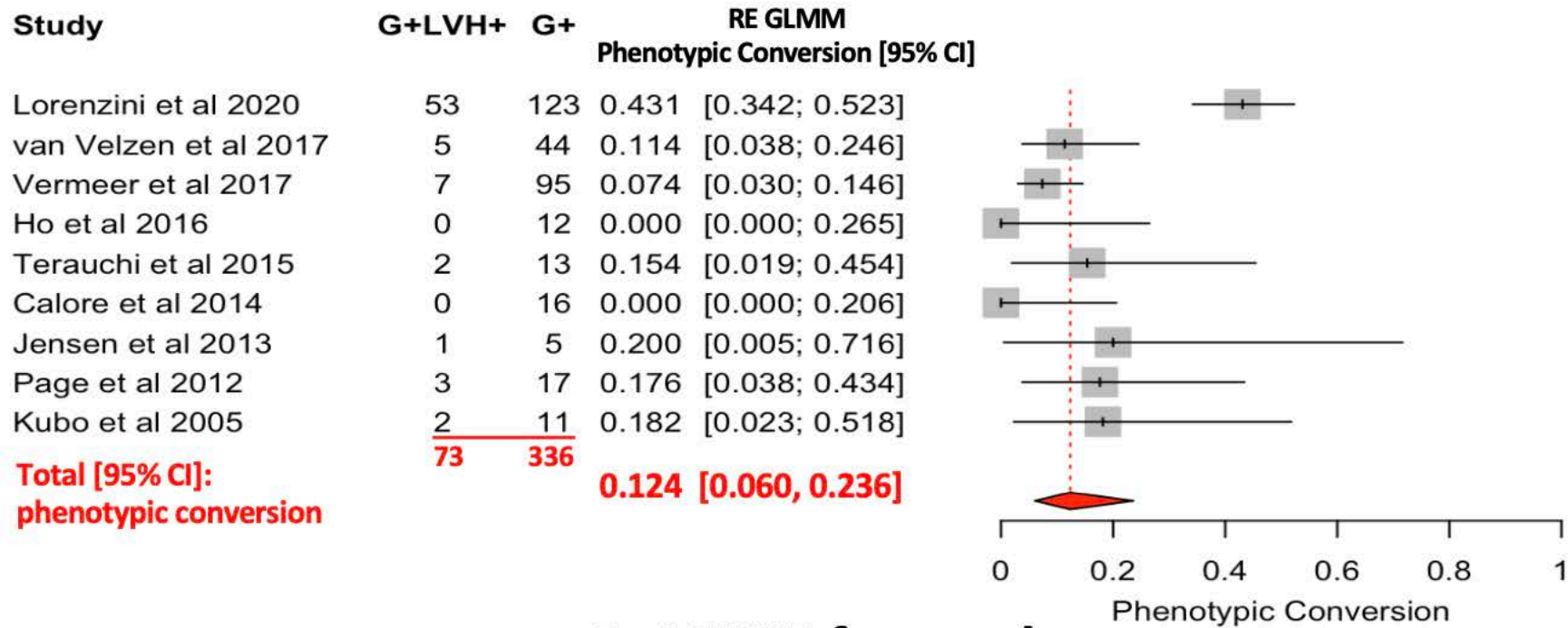
C. Meta-regression: Males (%)



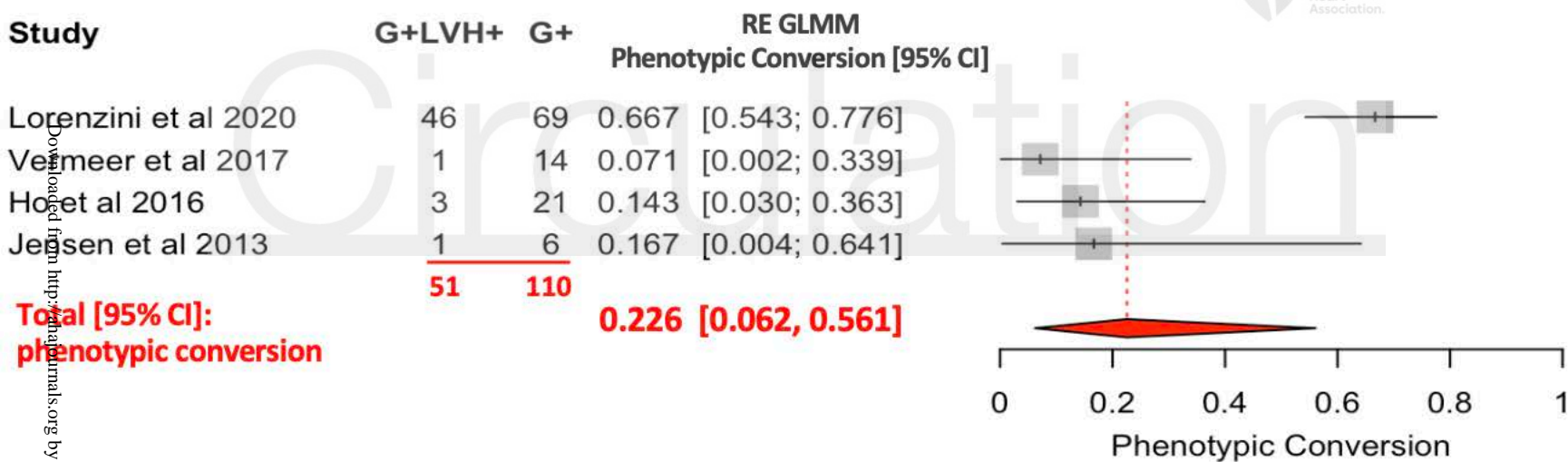
D. Meta-regression: Continent



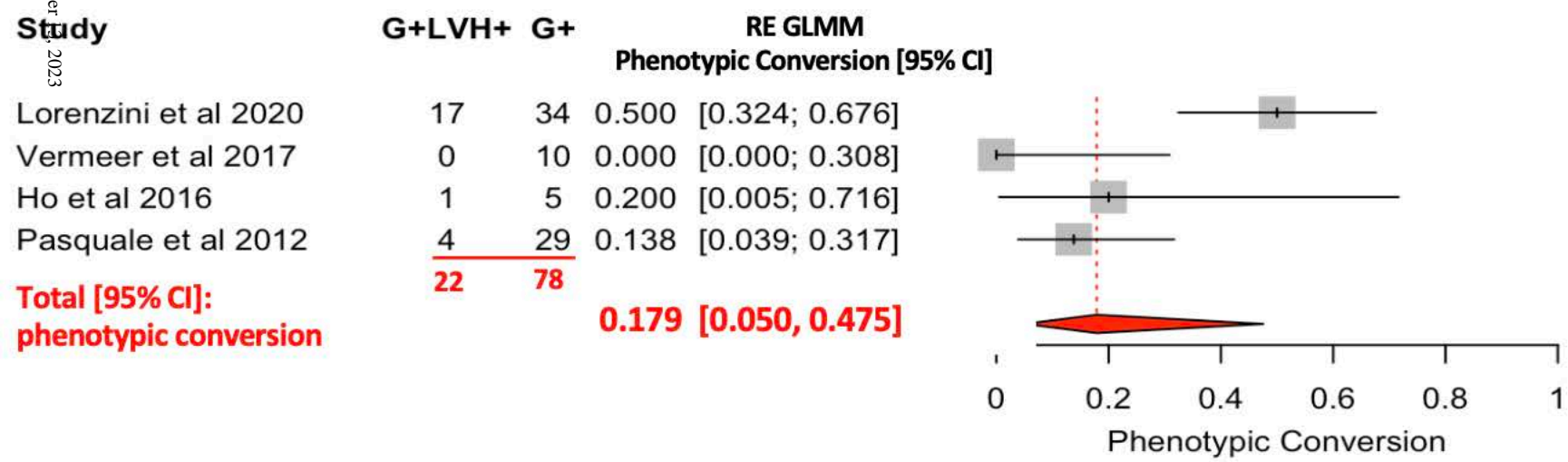
A. MYBPC3 forest plot



B. MYH7 forest plot



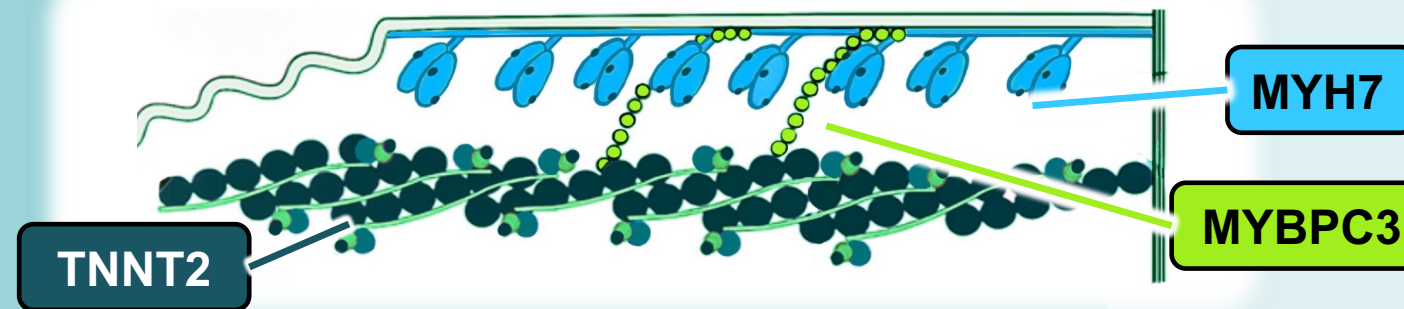
C. TNNT2 forest plot



Prevalence of Pathogenic and Likely Pathogenic Sarcomere Variants

Cross-Sectional Penetrance

Longitudinal Phenotypic Conversion

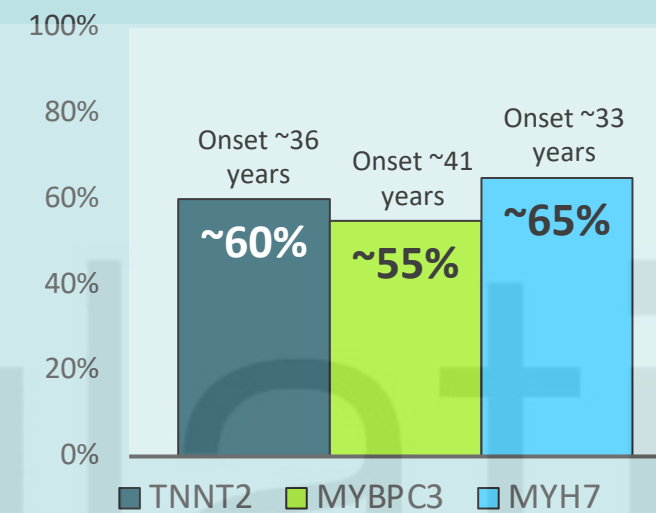


Family/Clinically-based Studies

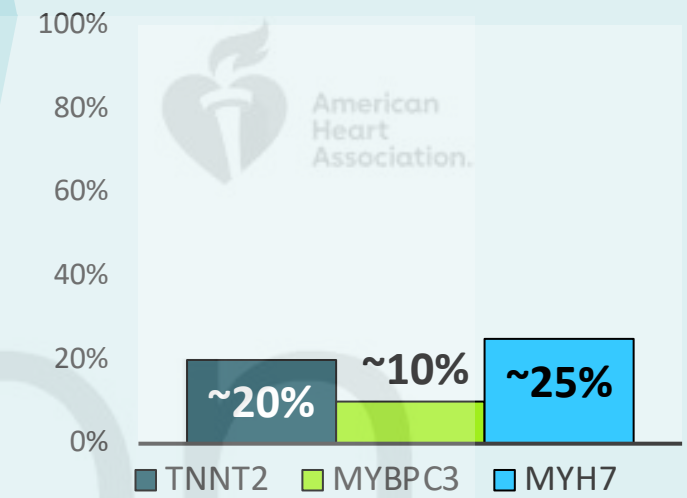


34%

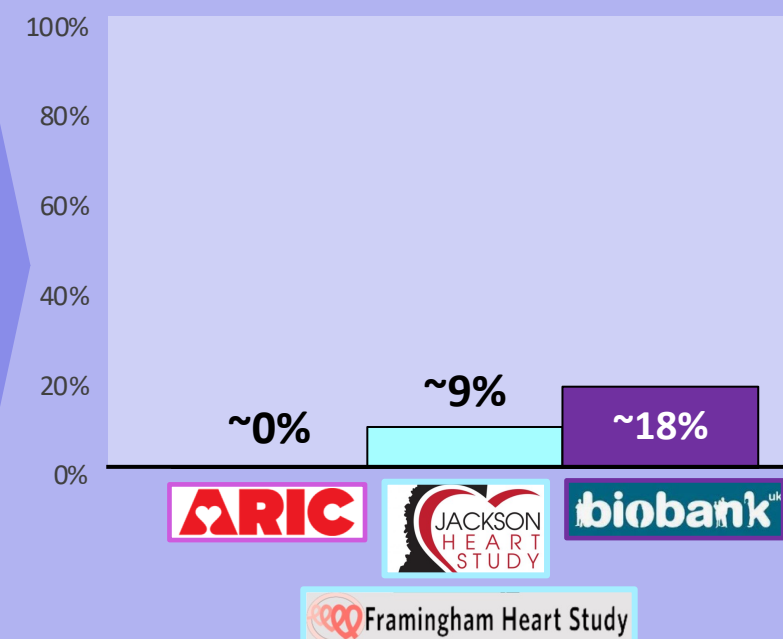
~60% of relatives with P/LP sarcomere variants were found to have HCM during cascade screening.



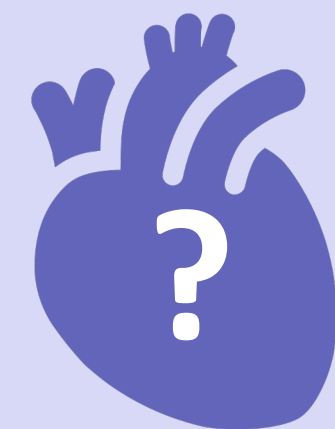
~15% over an average of ~8 years of follow up, starting from a baseline mean age of ~16 to ~24 years.



~10-15% of individuals in the community incidentally identified to carry P/LP sarcomere variants had evidence of HCM.



Unknown



Community/Population-based Studies



0.7%