© JUL 2026 | IRE Journals | Volume 10 Issue 1 | ISSN: 2456-8880
DOI: https://doi.org/10.64388/IREV10I1-1720206

Functional Evidence for the Classification of Genetic Variants in Inherited Cardiomyopathies: A Systematic Review

ABOLAJI TAWAKALITU DURODOYE
Biology (molecular biology) Faculty of Biological Sciences, Imo State University

Abstract- ***Inherited cardiomyopathies are genetically heterogeneous myocardial disorders associated with heart failure, arrhythmia and sudden cardiac death. Next-generation sequencing has increased the detection of rare variants, but many remain variants of uncertain significance because population, computational, segregation and clinical evidence alone may not establish pathogenicity. Functional studies can support classification through the ACMG/AMP PS3 and BS3 criteria, although their evidential value depends on biological relevance, assay validation, controls, replication and reproducibility.***

Objective: This systematic review aims to evaluate the nature, methodological quality and classification impact of functional evidence reported for genetic variants associated with inherited cardiomyopathies.

Methods: A systematic search will be conducted in selected bibliographic databases from inception to the final search date. Peer-reviewed studies reporting variant-specific functional evaluation in inherited cardiomyopathy will be considered. Two-stage screening, structured data extraction, methodological appraisal and evidence synthesis will be performed according to a prespecified protocol. Experimental models, assay endpoints, controls, replication, statistical analysis, ACMG/AMP functional-criterion application and variant-reclassification outcomes will be examined. Reporting will follow PRISMA 2020.

Anticipated contribution: The review will identify functional approaches that provide robust evidence, clarify limitations that weaken PS3 or BS3 application, determine how functional results have influenced variant classification and identify priorities for assay validation and future research.

Conclusion: The final abstract will be updated after completion of searching, screening, quality assessment and synthesis to report the verified number of included studies, principal findings and evidence-based conclusions. No numerical result or reclassification outcome is asserted at the protocol stage.

Keywords: Inherited Cardiomyopathy, Functional Evidence, Genetic Variant, Variant of Uncertain Significance, ACMG/AMP, PS3, BS3, Systematic Review

I. INTRODUCTION

Background to the Study

Cardiomyopathies are myocardial disorders in which the heart muscle is structurally and functionally abnormal in the absence of another condition sufficient to explain the observed phenotype. Contemporary classifications recognise hypertrophic, dilated, arrhythmogenic, restrictive and other cardiomyopathy phenotypes while increasingly incorporating aetiology, genotype and extracardiac manifestations into clinical evaluation. The disorders are important causes of heart failure, arrhythmia, thromboembolism, cardiac transplantation and sudden cardiac death across the life course (Arbelo et al., 2023).

Inherited cardiomyopathies are genetically heterogeneous. Pathogenic variation may affect sarcomeric force generation, cytoskeletal integrity, desmosomal adhesion, nuclear-envelope stability, RNA processing, calcium handling, ion-channel behaviour, proteostasis and cellular metabolism. Autosomal-dominant inheritance is common, but autosomal-recessive, X-linked, mitochondrial and de novo mechanisms also occur. Locus heterogeneity means that similar phenotypes can arise from variants in different genes, whereas allelic heterogeneity and pleiotropy allow different variants within one gene to produce distinct or overlapping cardiac phenotypes. Incomplete penetrance and variable expressivity further weaken any simple assumption that genotype predicts a uniform clinical outcome (Arbustini et al., 2022; Hershberger et al., 2018).

Genetic testing has become an important component of the evaluation of inherited cardiomyopathies. Targeted next-generation sequencing panels, whole-exome sequencing and, in selected settings, whole-genome sequencing enable simultaneous assessment of many genes. When a pathogenic or likely pathogenic variant is identified in a gene with a validated relationship to the phenotype, the result can clarify a molecular diagnosis, guide cascade testing and support surveillance of relatives. Nevertheless, broad testing also identifies many rare variants whose clinical significance cannot be resolved immediately (Musunuru et al., 2020; Wilde et al., 2022).

The interpretive difficulty is not merely technical. A variant may be rare because it is deleterious, because it occurs in an underrepresented population, or because it is a neutral private variant. Computational tools can prioritise variants but are not independent experimental demonstrations of pathogenicity. Family segregation may be uninformative in small pedigrees, complicated by age-dependent penetrance or unavailable because relatives cannot be assessed. Clinical phenotype is also heterogeneous and may be influenced by common genetic background, environmental exposure and comorbidity. Consequently, sequence detection must be separated conceptually from disease causation.

The American College of Medical Genetics and Genomics and the Association for Molecular Pathology introduced a five-tier framework that classifies sequence variants as pathogenic, likely pathogenic, of uncertain significance, likely benign or benign. The framework combines population, computational, functional, segregation, allelic and other evidence while requiring that each criterion be applied at an appropriate strength (Richards et al., 2015). Gene-specific refinements, such as those developed for MYH7-associated cardiomyopathy, demonstrate that general rules often require calibration to the established disease mechanism, inheritance pattern and gene-specific background variation (Kelly et al., 2018).

Variants of uncertain significance create a particular clinical and scientific challenge. They generally should not be used alone for predictive testing in unaffected relatives or as the basis for irreversible medical intervention. Yet uncertainty can persist for years when population observations and family data remain sparse. Periodic reinterpretation may resolve some variants as databases, case series and gene-validity knowledge improve, but many require experimental evidence that tests a plausible molecular mechanism.

Functional evidence refers to experimentally observed effects of a variant on RNA, protein, cellular, tissue or organismal function. Examples include altered splicing, reduced transcript abundance, abnormal protein stability or localisation, impaired protein interaction, disrupted sarcomere organisation, altered contractility, abnormal calcium handling and electrophysiological dysfunction. Under the ACMG/AMP framework, PS3 can support pathogenicity when a well-established assay demonstrates a damaging effect, while BS3 can support a benign interpretation when a well-established assay demonstrates no damaging effect (Richards et al., 2015).

Functional evidence is not inherently decisive. An assay may measure an endpoint unrelated to the accepted disease mechanism, use an artificial overexpression system, lack benign or pathogenic controls, confuse technical repeats with biological replication or interpret statistical significance without considering biological magnitude. A negative result may reflect limited assay sensitivity rather than normal function, while an abnormal cellular phenotype may arise from nonspecific stress or experimental artefact. The evidential weight of PS3 or BS3 therefore depends on the relevance, calibration, reproducibility and analytical validity of the specific assay (Brnich et al., 2020).

ClinGen guidance recommends a staged evaluation: define the disease mechanism, determine whether the general assay class is relevant, evaluate the validity of the specific assay instance and then apply the evidence to the variant under consideration. The use of well-characterised benign and pathogenic controls is central because control performance indicates how effectively an assay separates normal from abnormal function. In the absence of rigorous statistical

calibration, Brnich et al. (2020) found that at least 11 combined benign and pathogenic controls were needed to support moderate functional evidence. Stronger evidence requires correspondingly stronger validation.

Cardiomyopathy research presents additional complications because functional models vary widely. Heterologous cell systems are accessible and scalable but may not reproduce cardiomyocyte-specific structure or dosage. Patient-derived cells preserve genetic background but make it difficult to isolate the causal contribution of one variant. Induced pluripotent stem cell-derived cardiomyocytes provide a human cardiac context but remain relatively immature and susceptible to line and batch effects. Genome-edited isogenic models improve causal comparison, while engineered tissues and animal models add physiological complexity at greater cost and with their own translational limitations.

Published functional studies are consequently heterogeneous in genes, phenotypes, model systems, endpoints and quality. Some studies were designed to explore basic disease mechanisms rather than to generate clinical variant-classification evidence. Others explicitly calibrated assays for PS3 or BS3 application and reported variant reclassification. Without systematic synthesis, readers may treat these different forms of evidence as comparable even when their methodological strength differs substantially.

This dissertation therefore undertakes a systematic review of functional evidence used in the classification of genetic variants associated with inherited cardiomyopathies. The review will identify the genes and variants studied, describe the experimental models and endpoints employed, evaluate assay quality and determine how functional findings have influenced classification. Its purpose is not to decide that every abnormal variant is pathogenic, but to clarify what types of experimental evidence can legitimately reduce uncertainty.

Statement of the Research Problem

The expansion of next-generation sequencing has created a persistent gap between variant detection and clinically reliable interpretation. Cardiomyopathy panels may identify rare coding, splice-region and structural variants in numerous genes, but the presence of a rare variant does not establish that the variant explains the patient's disease. Some genes historically included on commercial panels have weak or disputed disease associations, and case-control reassessment has shown that not all reported cardiomyopathy genes demonstrate convincing excess variation in affected individuals (Walsh et al., 2017).

Uncertainty is particularly consequential in inherited disease because a classification may affect several relatives. Overclassification can lead to inappropriate cascade testing, unnecessary surveillance, anxiety and potentially harmful management. Underclassification can delay molecular diagnosis and prevent at-risk relatives from receiving informed evaluation. These risks create a need for evidence that is both scientifically credible and proportionate to the clinical use of the classification.

Functional studies are frequently proposed as a solution, but the published evidence is methodologically uneven. Assays differ in model relevance, control selection, endpoint definition, replication, blinding, statistical analysis and transparency. Kanavy et al. (2019) documented variation in the way ClinGen expert panels evaluated functional evidence and observed that some cited studies did not fully satisfy the validation parameters specified for assay use. The existence of an experiment is therefore insufficient; the experiment must be evaluated as evidence.

There is no consolidated synthesis within the present dissertation that identifies which cardiomyopathy variants have been functionally assessed, which assay types have been used, whether those assays meet contemporary quality expectations and how frequently the evidence has contributed to reclassification. This fragmentation limits comparison across genes and encourages inconsistent PS3 or BS3 application. The research problem addressed by this review is the absence of a systematic, evidence-weighted account of functional studies used to classify genetic variants in inherited cardiomyopathies.

Research Gap

Existing guidelines and narrative reviews provide valuable descriptions of cardiomyopathy genetics, clinical testing and patient management. The 2023 European Society of Cardiology guideline integrates contemporary concepts of cardiomyopathy phenotyping and genetics, while professional statements discuss gene validity, actionability and appropriate testing (Arbelo et al., 2023; Arbustini et al., 2022). Gene-level studies have refined the evidence supporting hypertrophic and dilated cardiomyopathy genes (Ingles et al., 2019; Jordan et al., 2021), and reviews have highlighted both the clinical value and interpretive limitations of genetic testing (Hespe et al., 2024; Mazzarotto et al., 2020).

However, these sources do not replace a systematic review focused specifically on variant-level functional evidence. Mechanistic reviews often summarise influential experiments without systematically documenting search methods, excluded studies or risk of bias. Clinical guidelines necessarily prioritise practice recommendations and may not extract detailed assay characteristics for every variant. Gene-specific reports cannot reveal cross-gene patterns in model selection, control calibration or classification impact.

A further gap concerns the distinction between functional effect and pathogenicity. A statistically detectable effect in vitro may be too small, nonspecific or biologically remote to establish disease causation. Conversely, apparently normal function in one assay cannot exclude a mechanism the assay did not measure. Contemporary guidance therefore requires explicit evaluation of assay relevance and validation, but published cardiomyopathy studies may apply these principles inconsistently.

The present review will address this gap by systematically identifying eligible studies, extracting variant-, phenotype- and assay-level information, appraising methodological quality and synthesising the relationship between functional findings and variant classification. It will also identify evidence gaps affecting ancestry representation, negative findings, independent replication and transparent reporting.

Aim of the Study

The aim of this study is to systematically evaluate the nature, methodological quality and classification impact of functional evidence reported for genetic variants associated with inherited cardiomyopathies.

Specific Objectives

1. To identify peer-reviewed studies that report variant-specific functional evaluation of genetic variants associated with inherited cardiomyopathies.
2. To describe the cardiomyopathy phenotypes, genes, variants, experimental models and functional endpoints investigated in the included studies.
3. To assess the biological relevance, control structure, calibration, replication, statistical analysis and reproducibility of the reported functional assays.
4. To determine how functional findings were applied as PS3, BS3 or equivalent evidence within variant-classification frameworks.
5. To evaluate the extent to which functional evidence contributed to variant reclassification or reduced interpretive uncertainty.
6. To identify methodological limitations, unresolved evidence gaps and priorities for future cardiomyopathy variant research.

Research Questions

1. Which genes and genetic variants associated with inherited cardiomyopathies have been evaluated using functional assays?
2. Which cardiomyopathy phenotypes, experimental models and functional endpoints are represented in the published evidence?
3. How biologically relevant, calibrated, replicated and reproducible are the reported functional assays?
4. How have PS3, BS3 or equivalent functional criteria been applied in variant classification?
5. To what extent has functional evidence supported reclassification or reduced uncertainty?
6. Which methodological weaknesses and evidence gaps should be prioritised in future research?

Significance of the Study

The study is significant for researchers because it will provide a structured map of functional approaches used across inherited cardiomyopathy genes. Comparing experimental models, endpoints and controls can reveal which methods are well aligned with established molecular mechanisms and which require further validation. The synthesis may also help researchers design studies capable of generating clinically interpretable evidence rather than merely descriptive laboratory observations.

Diagnostic laboratories and variant curators may benefit from a transparent appraisal of how functional evidence has been weighted. The review will distinguish studies that explicitly satisfy PS3 or BS3 expectations from those that offer supportive mechanistic observations without adequate clinical calibration. This distinction can reduce inconsistent criterion application and discourage double counting of correlated evidence.

Clinicians and genetic counsellors may benefit because the review will clarify the boundary between a functional abnormality and a clinically actionable classification. An abnormal assay does not determine penetrance, prognosis or the appropriate intervention for an individual carrier. Presenting functional findings within an integrated evidential framework can support more accurate communication with patients and families.

The review also has relevance for equity. Population-frequency resources and published cohorts have historically represented some ancestries better than others. Underrepresentation increases the likelihood that rare variants in underserved populations remain uncertain. By documenting the ancestry and geographic distribution reported in included studies, the review can identify where functional evidence may help reduce uncertainty and where poor representation limits generalisability.

Scope of the Study

The review will include peer-reviewed primary studies that experimentally evaluate one or more human genetic variants associated with inherited cardiomyopathy. Eligible phenotypes will include hypertrophic, dilated, arrhythmogenic and restrictive cardiomyopathies and other inherited myocardial phenotypes recognised in contemporary classifications. Studies of syndromic disease will be eligible when the cardiomyopathy phenotype and variant-specific functional evidence are reported clearly.

Eligible functional evidence may include RNA-splicing and transcript studies; protein abundance, stability, localisation and interaction assays; biochemical measurements; cardiomyocyte morphology; sarcomere organisation; contractility; calcium handling; electrophysiology; metabolism; stress responses; engineered tissues; organoids; and animal models. Heterologous, patient-derived and genome-edited systems will be considered, but their evidential strengths and limitations will be evaluated separately.

Purely computational predictions will not be treated as functional evidence. Narrative reviews, editorials, conference abstracts without adequate methods, non-variant-specific experiments and studies unrelated to inherited cardiomyopathy will be excluded from the primary synthesis. The review will examine classification impact where reported but will not independently assign clinical classifications without sufficient primary evidence.

Limitations of the Study

The review will depend on the completeness and quality of published reports. Some studies may omit negative results, detailed protocols, raw data, variant nomenclature, replicate structure or complete classification matrices. Publication bias may favour variants with striking functional abnormalities, while normal, discordant or technically unsuccessful experiments remain unpublished.

Substantial heterogeneity is expected across genes, disease mechanisms, model systems and endpoints. This heterogeneity may prevent statistical pooling and require structured narrative synthesis. Assay quality cannot always be inferred from reporting quality, although insufficient reporting necessarily limits confidence in appraisal.

Language, database coverage and access restrictions may affect retrieval. Variant classifications also change over time as population databases, gene-validity assessments and professional specifications are updated. The review will therefore record search dates, database versions and the classification context reported by each study, and it will interpret historical classifications cautiously.

Operational Definition of Terms

Functional evidence: Experimentally generated evidence concerning the effect of a genetic variant on RNA, protein, cellular, tissue or organismal function.

Inherited cardiomyopathy: A myocardial disorder in which germline genetic variation contributes materially to disease susceptibility or expression.

Pathogenic variant: A variant classified as disease-causing under an accepted evidence framework.

Likely pathogenic variant: A variant for which the available evidence strongly supports, but does not conclusively establish, pathogenicity.

Variant of uncertain significance: A variant for which the available evidence is insufficient or conflicting regarding pathogenicity.

PS3: ACMG/AMP evidence supporting pathogenicity when a well-established functional study demonstrates a damaging effect.

BS3: ACMG/AMP evidence supporting a benign interpretation when a well-established functional study demonstrates no damaging effect.

Variant reclassification: A documented change from one classification category to another after addition or reinterpretation of evidence.

Assay calibration: Evaluation of assay performance using appropriate controls and thresholds to determine how reliably results distinguish normal from abnormal function.

Biological replicate: An independent biological sample, culture, differentiation or experimental unit that captures biological variability.

Technical replicate: Repeated measurement of the same biological experimental unit used primarily to assess measurement precision.

Organisation of the Thesis

Chapter One introduces the research background, problem, gap, aim, objectives, questions, significance, scope and key terminology. Chapter Two critically reviews inherited cardiomyopathy genetics, variant interpretation, experimental models and the evidential requirements for functional studies. Chapter Three presents the systematic-review design, information sources, search strategy, eligibility criteria, screening, data extraction, methodological appraisal and synthesis methods. Chapter Four reports the search and selection process, characteristics of included studies, functional-assay findings, quality assessment and classification outcomes.

Chapter Five discusses the principal findings in relation to contemporary guidance and previous research, including implications for laboratories, researchers, clinicians and genetic counsellors. Chapter Six presents the conclusions, contribution to knowledge, recommendations and future research priorities.

II. CRITICAL LITERATURE REVIEW

This chapter critically evaluates the knowledge base connecting inherited cardiomyopathy phenotypes to genes, molecular mechanisms, functional assays and clinical variant classification. It emphasises evidential strengths, methodological limitations, unresolved controversies and the precise gap addressed by this systematic review.

© JUL 2026 | IRE Journals | Volume 10 Issue 1 | ISSN: 2456-8880
DOI: https://doi.org/10.64388/IREV10I1-1720206

Table 2.1 Major molecular systems and mechanism-matched endpoints

System	Illustrative genes	Principal causal questions	Informative endpoints
Sarcomere	MYH7, MYBPC3, TNNT2, TNNI3	Altered dosage, motor function or calcium sensitivity?	RNA/protein abundance, ATPase, force, calcium sensitivity
Cytoskeleton/nucleus	TTN, FLNC, DES, LMNA	Impaired splicing mechanics, or integrity? nuclear	Splicing, localisation, stiffness, signalling stress
Desmosome	PKP2, DSP, DSG2, DSC2	Defective adhesion or electrical–mechanical coupling?	Junction localisation, interaction, conduction, response injury
Excitation–contraction	PLN and pathway partners	Abnormal calcium cycling or electrophysiology?	Calcium transients, action potentials, contractility

Inherited cardiomyopathies provide a demanding test case for genomic medicine because the causal chain from DNA sequence to clinical disease is neither linear nor uniform. A variant can alter RNA processing, protein quantity, molecular interaction, force generation, calcium cycling, cell adhesion or stress responses; the same primary lesion can then be modified by age, sex, ancestry, exercise, pregnancy, comorbidity and additional genetic variation. Consequently, variant interpretation requires more than the co-occurrence of rarity, an abnormal computational score and a compatible phenotype. It requires a defensible relationship between the gene and disease, an allele-specific mechanistic hypothesis, and evidence that the observed effect is sufficiently robust and disease-relevant.

This chapter critically synthesises the literature that supports that chain of inference. It first reviews changing cardiomyopathy classifications and the genetic architecture of the principal phenotypes. It then examines the molecular systems most frequently implicated in inherited disease, the strengths and limitations of contemporary genetic testing, and the formal frameworks used to assess gene and variant evidence. The final sections evaluate experimental models, assay calibration, multi-omics and emerging high-throughput approaches, before identifying the specific gap addressed by this thesis.

The review is deliberately organised by problems and mechanisms rather than by individual publications. Seminal studies are considered alongside recent guidelines, gene-curation exercises and functional-evidence recommendations. Agreement is distinguished from apparent consensus produced by shared datasets or correlated methods. Particular attention is given to the risk of circularity: variants originally labelled pathogenic may be used to validate an assay, after which the same assay is cited as independent proof of pathogenicity. Avoiding such circular reasoning is central to a systematic review intended to evaluate how experimental effects are translated into clinical interpretation.

Historical Development and Contemporary Classification

Early cardiomyopathy classifications were dominated by morphology and haemodynamics. Hypertrophic, dilated and restrictive categories were clinically useful because they described recognisable patterns of ventricular thickness, chamber size and filling.

Arrhythmogenic right ventricular cardiomyopathy later acquired a distinct identity through its characteristic electrical and pathological features. These categories remain valuable, but molecular genetics has shown that they are porous: one gene may produce hypertrophic, dilated, restrictive or arrhythmogenic expression, and unrelated genes may converge on an almost indistinguishable clinical phenotype.

The 2023 European Society of Cardiology guideline retains a phenotype-first approach while broadening attention to non-dilated left ventricular cardiomyopathy and recognising right-, left- and biventricular arrhythmogenic disease (Arbelo et al., 2023). This framework improves clinical usability, yet it does not convert morphology into aetiology. Myocarditis, storage disease, mitochondrial disease, drug toxicity and physiological adaptation can mimic inherited cardiomyopathy. Conversely, genotype-positive relatives may have no conventional imaging abnormality despite molecular or electrical changes. Classification must therefore be iterative: phenotype directs investigation, aetiology refines the label, and longitudinal observation may alter both.

Left ventricular non-compaction illustrates the limits of categorical classification. Excessive trabeculation can accompany pathogenic variants and familial cardiomyopathy, but it can also occur in pregnancy, athletic remodelling and otherwise normal hearts. Treating trabeculation alone as a monogenic diagnosis risks overdiagnosis and inflated penetrance estimates. The broader lesson is that functional characterisation cannot be interpreted independently of phenotype quality. An elegant experiment on a rare variant adds little if the clinical label is nonspecific or the gene–phenotype relationship is weak.

Epidemiology and the Problem of Ascertainment

Reported prevalence varies with diagnostic criteria, imaging intensity, population structure and access to specialist services. Hypertrophic cardiomyopathy is often described as affecting roughly 1 in 500 adults when conventional phenotypic criteria are used, although genotype-informed and imaging-rich approaches can yield different estimates. Dilated cardiomyopathy is similarly under-recognised because mildly affected or asymptomatic relatives may never undergo imaging. Arrhythmogenic and restrictive phenotypes are rarer, but their apparent frequency is particularly sensitive to referral patterns and diagnostic expertise.

Most genomic cohorts have been assembled in tertiary centres and are enriched for severe disease, early onset, positive family history and European ancestry. Such ascertainment improves the probability of finding high-effect variants but distorts estimates of penetrance and phenotypic severity. Population biobanks, by contrast, reveal carriers with milder or later-onset disease, yet may lack the detailed phenotyping needed to distinguish true disease from borderline measurements. Neither design alone provides an unbiased account of natural history.

Ancestry imbalance has direct implications for variant interpretation. Alleles that appear rare in a predominantly European reference dataset may be more common and benign in an under-represented population. Historical misclassification of ancestry-associated variants demonstrates why rarity cannot be treated as a universal property of an allele. The literature consequently supports ancestry-matched frequency assessment, transparent coverage evaluation and cautious use of absence from databases. Functional studies may help when frequency evidence is limited, but they cannot repair a study design that ignores population context.

Genetic Architecture, Inheritance and Disease Modification

Autosomal-dominant inheritance predominates in many sarcomeric, cytoskeletal and desmosomal cardiomyopathies, but recessive, X-linked and mitochondrial mechanisms are clinically important. De novo variants explain some apparently sporadic cases, while parental mosaicism can complicate recurrence counselling. A pedigree that appears negative is therefore not evidence against genetic disease unless relatives have been adequately phenotyped and age-dependent penetrance has been considered.

Locus heterogeneity, allelic heterogeneity and pleiotropy make simple gene lists misleading. Different genes can converge on a shared ventricular phenotype; different alleles in one gene can act through haploinsufficiency, dominant-negative or gain-of-function mechanisms; and one gene can cause several overlapping cardiac syndromes. Walsh et al. (2017) showed that several genes historically included on cardiomyopathy panels lacked convincing excess variation in cases, emphasising that the presence of a gene on a commercial test does not establish clinical validity. Subsequent ClinGen-led curation has reinforced the need to evaluate gene–disease validity before interpreting any variant within that gene.

Penetrance and expressivity are shaped by background and exposure. Truncating TTN variants illustrate this interaction: they are important causes of dilated cardiomyopathy, but their effect depends on exon usage, isoform context and physiological stress. Exercise can modify arrhythmogenic cardiomyopathy expression; pregnancy, alcohol and cardiotoxic therapies can unmask susceptibility in dilated cardiomyopathy; and common polygenic variation can shift the probability or severity of a phenotype. These observations oppose both genetic determinism and the opposite error of dismissing a major-effect variant because expression is incomplete.

Sarcomeric Cardiomyopathy

The cardiac sarcomere converts chemical energy into force through coordinated interaction of thick-filament, thin-filament and regulatory proteins. MYH7 and MYBPC3 account for a substantial proportion of genetically resolved hypertrophic cardiomyopathy, while TNNT2, TNNI3, TPM1, ACTC1, MYL2 and MYL3 contribute smaller fractions. Gene-level aggregation, however, conceals mechanistic diversity. MYBPC3 truncating variants commonly reduce functional protein through nonsense-mediated decay and haploinsufficiency, whereas many MYH7 disease-causing variants are missense alleles whose effects depend on position and altered motor behaviour.

Functional studies have reported changes in ATPase activity, actin sliding, force generation, calcium sensitivity and energetic cost. These findings support the concept that sarcomeric variants can produce hypercontractile or inefficient states before macroscopic hypertrophy develops. Yet results differ across purified proteins, skinned fibres, engineered tissues and induced pluripotent stem-cell-derived cardiomyocytes. Protein isoform, phosphorylation, loading conditions and experimental temperature can all change the measured phenotype. A result that is internally reproducible may therefore have limited transferability across systems.

The strongest mechanistic conclusions arise when assay choice reflects the expected allele mechanism. A truncating variant predicted to undergo nonsense-mediated decay should first be examined at RNA and protein-abundance levels, whereas a stable missense protein may require motor, interaction or contractile analysis. Treating all variants in a sarcomeric gene as functionally equivalent risks both false positive and false negative interpretation. Gene-specific ACMG/AMP adaptations, such as those developed for MYH7, are valuable precisely because they constrain evidence to biologically credible variant classes and regions (Kelly et al., 2018).

Cytoskeletal, Nuclear-Envelope and Titin Mechanisms

The cytoskeleton distributes mechanical load and links the sarcomere to the membrane, extracellular matrix and nucleus. Variants in FLNC, DES, DMD and related genes can impair force transmission, protein quality control and structural resilience. Phenotypes frequently overlap dilated and arrhythmogenic cardiomyopathy, and some genotypes carry disproportionate ventricular-arrhythmia risk relative to ejection fraction. This challenges purely morphological risk models and demonstrates the clinical relevance of gene-specific mechanisms.

LMNA encodes nuclear lamins that support nuclear architecture and regulate chromatin and mechanotransduction. Pathogenic LMNA variants can cause conduction disease and ventricular arrhythmia before severe systolic dysfunction. Proposed mechanisms include nuclear fragility, altered gene regulation and maladaptive stress

signalling. Heterologous cell assays can detect abnormal nuclear morphology, but the distance between that endpoint and cardiac disease limits its standalone evidential strength. Cardiomyocyte models and orthogonal measures of mechanical stress or transcriptional disturbance provide a stronger mechanistic bridge.

TTN presents an especially difficult interpretive problem because of its size, extensive alternative splicing and background burden of truncating variation. Disease association depends strongly on whether the affected exon is constitutively expressed in the heart. Roberts et al. (2015) demonstrated the importance of exon usage and variant position in dilated cardiomyopathy. Functional work has supported both haploinsufficiency and poison-peptide effects in particular contexts, suggesting that no single model explains every truncating allele. Transcript-aware annotation is therefore a prerequisite, not an optional refinement.

Desmosomes and Arrhythmogenic Cardiomyopathy

Desmosomes provide mechanical coupling between cardiomyocytes and interact with electrical junctions and signalling pathways. Pathogenic variants in PKP2, DSP, DSG2, DSC2 and JUP can weaken intercellular adhesion and contribute to cell injury, inflammation, fibrosis and arrhythmia. The historical description of fibrofatty replacement captures late pathology but does not fully represent earlier electrical and inflammatory phases.

Experimental models have implicated altered plakoglobin localisation, connexin organisation, sodium-current disturbance, inflammatory signalling and lipid accumulation. However, adipogenic readouts in non-cardiac cells have sometimes been overinterpreted because culture conditions can induce lipid accumulation nonspecifically. The relevance of a particular endpoint depends on the gene, developmental state and phenotype. DSP-associated disease, for example, often has left-dominant and inflammatory features that are not adequately represented by a generic right-ventricular model.

Exercise provides a powerful natural modifier and an experimental complication. Mechanical load can accelerate disease expression in susceptible individuals, yet static cell culture omits this central stressor. Engineered tissues, cyclic stretch and animal models can improve physiological relevance, but introduce additional variability and cost. Functional evidence should therefore be interpreted as model-bounded: absence of an effect under resting conditions does not exclude a stress-dependent pathogenic mechanism.

Calcium Handling, Electrophysiology and Metabolic Dysfunction

Excitation–contraction coupling links membrane depolarisation to calcium release, sarcomere activation and relaxation. Variants affecting sarcomeric proteins can secondarily alter calcium sensitivity, while variants in ion-handling or regulatory genes can directly disturb calcium cycling. Measurements of calcium-transient amplitude, kinetics, sarcoplasmic-reticulum load and arrhythmogenic after-events can therefore connect genotype to cellular physiology, provided that pacing, temperature and cell maturity are controlled.

Electrophysiological assays can identify changes in action-potential duration, conduction and ion currents. Their interpretation in cardiomyopathy requires caution because a prolonged action potential in an immature induced cardiomyocyte may reflect the model rather than the variant. Single-cell patch clamp offers mechanistic precision but low throughput; multielectrode arrays provide higher throughput but measure composite network behaviour. Agreement across platforms is more persuasive than an isolated abnormality.

Mitochondrial and metabolic dysfunction can be primary or secondary. Energetic inefficiency is prominent in sarcomeric disease, while mitochondrial genome variants and nuclear-encoded metabolic disorders can cause syndromic cardiomyopathy. Oxygen-consumption, substrate-use and metabolomic assays can reveal relevant effects, yet media composition and developmental immaturity strongly influence results. A metabolic difference should not be labelled causal unless temporal order, specificity and rescue have been considered.

Proteostasis, Fibrosis, Inflammation and Remodelling
Protein folding, degradation and autophagy determine whether altered proteins accumulate, are removed or reduce the abundance of their normal partners. Truncating and missense alleles may therefore converge on proteotoxic stress despite different initial lesions. BAG3, small heat-shock proteins and desmin-related pathways illustrate how failure of protein quality control can impair sarcomere maintenance and cellular survival.

Fibrosis and inflammation are not merely late histological consequences. In arrhythmogenic and desmosomal disease, episodic injury and inflammatory signalling may precede extensive structural change. In dilated and hypertrophic cardiomyopathy, fibroblast activation and extracellular-matrix remodelling alter stiffness, conduction and force transmission. Cardiomyocyte-only models cannot reproduce these multicellular interactions, creating a systematic gap between cell-autonomous assays and organ-level pathology.

Co-culture, organoid and engineered-tissue systems offer partial solutions but remain difficult to standardise. Addition of fibroblasts or immune cells increases biological relevance while introducing batch effects and ambiguity about the source of a phenotype. The literature supports a staged strategy: establish the most proximal allele effect in a controlled system, then examine downstream multicellular consequences with appropriately cautious causal language.

Genotype–Phenotype Relationships and Phenocopies
Genotype–phenotype studies have identified useful associations, including arrhythmic risk in LMNA-, FLNC-, DSP- and RBM20-related disease and characteristic patterns in some sarcomeric genotypes. Nevertheless, most associations are probabilistic rather than deterministic. Referral bias, variable surveillance and grouping of dissimilar variants within one gene can exaggerate apparent precision.

Variant class must be retained in analysis. A loss-of-function allele and a missense allele in the same gene may not share mechanism or risk. Even variants of the same class can differ by domain, transcript and molecular consequence. Gene-level conclusions should therefore not be transferred automatically to every allele. Functional characterisation is most informative when it resolves this allele-specific uncertainty rather than simply confirming a general property of the gene.

Phenocopies further complicate inference. Fabry disease, transthyretin amyloidosis, glycogen-storage disorders, mitochondrial disease and neuromuscular syndromes can resemble primary cardiomyopathy. Accurate recognition matters because treatment and family counselling differ substantially. A candidate variant in a sarcomeric gene should not distract from biochemical, imaging or systemic evidence of a phenocopy; coexistence is also possible. Deep phenotyping is thus part of variant interpretation, not a separate clinical task.

Genetic Testing Technologies and Analytical Boundaries
Phenotype-focused panels provide high coverage of established genes and limit incidental findings, but may omit newly validated genes, non-coding variants and structural variation. Exome sequencing broadens discovery but has uneven coverage and limited detection of some copy-number, repeat and intronic variants. Genome sequencing improves access to non-coding and structural variation, yet greatly increases interpretive burden and does not automatically solve phasing or transcript relevance.

Analytical sensitivity varies by platform, capture design and bioinformatics pipeline. Low-complexity regions, GC-rich exons and large structural events may be poorly resolved. Confirmation and orthogonal testing remain important where the assay has known limitations. Negative testing cannot therefore be equated with absence of genetic causation; it may reflect technical blind spots, undiscovered genes, polygenic architecture or an incorrect phenotype.

RNA sequencing can reveal splice disruption and allele-specific expression, especially when DNA findings are ambiguous. The central limitation is tissue relevance: blood may not express the cardiac transcript or may use different isoforms. Patient-derived myocardial tissue is rarely available, and

induced cells can incompletely reproduce adult splicing. RNA evidence is powerful when the relevant transcript is expressed and the abnormal product is clearly demonstrated, but absence of an effect in an unsuitable tissue is weak benign evidence.

Variant Annotation, Population Frequency and In-Silico Prediction

Accurate annotation requires a defined genome build, transcript, strand, variant-normalisation process and HGVS description. Alternative transcripts can change whether a variant appears exonic, splice-relevant or truncating. Transcript selection should be clinically and biologically justified, with attention to cardiac expression and disease mechanism. Inconsistent transcripts are a common source of discordant classifications.

Population databases such as gnomAD are indispensable for identifying variants too common to cause a rare highly penetrant disorder. Their value depends on sequencing quality, ancestry representation and disease model. Maximum credible allele frequency should reflect prevalence, penetrance, genetic heterogeneity and the contribution of the gene and variant. A single global frequency can obscure ancestry-specific enrichment, while absence from a database provides only supporting-not decisive-evidence.

Computational tools assess conservation, biochemical change, splicing or learned patterns from existing labels. Their outputs are correlated because many share features and training data. Combining several concordant scores does not create several independent pieces of evidence. Predictors can prioritise variants and define hypotheses, but they do not measure pathogenicity directly. Circularity is especially concerning when tools are trained on classifications that included earlier computational evidence.

Gene–Disease Validity and Variant-Classification Frameworks

The ACMG/AMP framework standardised five-tier variant classification and organised population, computational, functional, segregation, allelic and case evidence (Richards et al., 2015). Its major strength is transparency; its original qualitative formulation, however, left room for laboratories to apply the same code at different strengths. ClinGen specifications and Bayesian reformulations have sought to make evidence weighting more consistent.

Gene–disease validity precedes variant classification. A damaging effect in a protein does not establish that the gene causes the clinical phenotype under study. ClinGen gene-curation exercises for hypertrophic and dilated cardiomyopathy have downgraded or disputed several historically reported associations (Ingles et al., 2019; Jordan et al., 2021). Restricting diagnostic interpretation to genes with sufficient evidence reduces false-positive results and prevents functional experiments from being used to sustain implausible associations.

The framework also requires independence of evidence. A phenotype-specific case series, segregation within those same families and a functional study performed on a subset may not represent three fully independent observations. Similarly, computational and conservation evidence are often correlated. Transparent evidence matrices should identify the origin of every item, potential overlap and the reason for the assigned strength.

Table 2.2 Evidence domains in cardiomyopathy variant interpretation

Evidence domain	Core question	Frequent error	Safeguard
Gene validity	Does this cause ene this phenotype?	Interpreting variants in disputed genes	Curate gene–disease validity first

Population	Is frequency compatible with disease?	Using global rarity without ancestry context	Apply ancestry and disease-model thresholds
Clinical/segregation	Does the allele track with a specific phenotype?	Ignoring dependent age-penetrance	Deep phenotyping and informative meioses
Computational	Is an effect predicted?	Double-counting correlated tools	Use as one supporting evidence domain
Functional	Is a mechanism-relevant effect observed?	Equating abnormality any pathogenicity with	Calibrated controls and PS3/BS3 framework

Functional Evidence Under PS3 and BS3

PS3 and BS3 refer respectively to well-established functional studies supportive of a damaging or non-damaging effect. Brnich et al. (2020) provided a structured approach to assay validation, emphasising biological relevance, analytical validity, controls and the number of known variants used to calibrate discrimination. The phrase “well established” refers to the assay, not the prestige of the journal or sophistication of the equipment.

Calibration requires known pathogenic and benign controls selected independently of the assay being evaluated. Controls should represent the relevant mechanism and variant class. A benign missense control may not calibrate a splicing assay, and a loss-of-function positive control may not validate detection of a dominant-negative effect. Replicate number, dynamic range, classification threshold and variance should be defined. Without such features, supportive evidence may need to be applied at reduced strength or not at all.

Negative functional evidence requires particular caution. An assay can fail to show damage because the variant is benign, but also because the model lacks the required isoform, interaction partner, developmental state or stress condition. BS3 should be applied only when the assay is demonstrably capable of detecting the disease mechanism and the variant has been tested within its validated range. Absence of evidence in a weak assay is not evidence of absence.

Biochemical, RNA and Protein-Based Assays

Biochemical assays can directly test enzyme activity, binding, ATPase function or filament behaviour. Their reductionism is both strength and weakness: purified components allow precise mechanistic attribution, but remove stoichiometry, compartmentalisation and cellular regulation. Findings should be connected to the expected in-vivo direction and magnitude rather than described as abnormal without a calibrated reference.

RNA assays are especially valuable for canonical and non-canonical splice variants. Minigene systems offer controlled comparison but may omit long-range regulatory context and tissue-specific factors. Patient RNA preserves native genomic context but is limited by tissue expression and nonsense-mediated decay. Cycloheximide or related inhibition can reveal unstable abnormal transcripts, although such intervention changes normal RNA handling. Concordance between prediction, patient RNA and an orthogonal construct strengthens inference.

Western blotting, quantitative proteomics, immunofluorescence and interaction assays can assess abundance, degradation, localisation and complex formation. Overexpression is a recurrent threat because it can saturate quality-control pathways or force non-physiological localisation. Endogenous or near-endogenous expression, dosage normalisation and blinded quantification improve validity. Representative images should be accompanied by quantitative distributions and complete information about biological replicates.

Cellular Models and Induced Pluripotent Stem-Cell Cardiomyocytes

Common heterologous cell lines are inexpensive, reproducible and readily transfected. They are useful for proximal effects such as splicing, stability or gross localisation, but they lack cardiomyocyte architecture and excitation–contraction coupling. The appropriate conclusion is therefore narrow: a variant may alter the measured molecular endpoint in that system, not necessarily produce cardiomyopathy.

Human induced pluripotent stem-cell-derived cardiomyocytes allow patient-specific or engineered variants to be studied in a cardiac lineage. They have reproduced aspects of hypertrophic, dilated and arrhythmogenic disease, including sarcomere disorganisation, altered calcium handling and electrophysiological disturbance. Patient-derived lines retain the entire genetic background, which can capture modifiers but confound attribution. CRISPR-corrected isogenic controls help isolate the variant effect; reciprocal knock-in provides an even stronger test when feasible.

Immaturity remains a central limitation. Induced cardiomyocytes resemble fetal rather than adult cells in metabolism, electrophysiology, sarcomere organisation and force. Prolonged culture, electrical pacing, mechanical loading, hormonal conditioning and three-dimensional tissue formation improve maturation but do not fully reproduce an adult heart. Multiple clones, off-target assessment and blinded analysis are necessary because reprogramming and editing introduce clone-specific variation.

Engineered Tissues, Organoids and Animal Models

Engineered heart tissues expose cells to three-dimensional alignment and measurable load, allowing assessment of twitch force, relaxation and response to pacing. They can reveal phenotypes that are invisible in two-dimensional culture. Their complexity, cost and sensitivity to cell composition make cross-laboratory standardisation difficult. Batch-level replication is more informative than a large number of technical measurements from one tissue preparation.

Cardiac organoids can incorporate multiple cell types and self-organising features, offering opportunities to study fibrosis, vascular interaction and developmental mechanisms. Current systems vary widely and rarely reproduce adult chamber structure. They are best viewed as intermediate models rather than miniature adult hearts. Clear reporting of composition, maturation and endpoint selection is essential.

Animal models permit integrated physiology, longitudinal remodelling and exercise or stress challenges. Species differences in heart rate, myosin isoform, calcium handling and gene dosage can alter the phenotype. A mouse that fails to reproduce human disease does not necessarily invalidate the variant; conversely, severe disease in an overexpression model may be artifactual. Knock-in models at endogenous dosage and rescue experiments provide stronger evidence than transgenic overexpression alone.

Table 2.3 Comparative strengths and limitations of functional models

Model	Major strength	Major limitation	Most defensible use
Purified/biochemical	Direct proximal mechanism	Lacks cellular context	Motor, binding or enzyme effects
Heterologous cells	Controlled and accessible	Not cardiac; overexpression artefacts	Splicing, stability, gross localisation
iPSC cardiomyocytes	Human cardiac lineage; editable	Immature and batch-sensitive	Cellular physiology with isogenic controls
Engineered tissue	Load, alignment and force	Complex and difficult to	Contractility and stress-dependent phenotypes

		standardise	
Animal model	Integrated physiology and time	Species differences	Remodelling, exercise and organ-level outcomes

Genome Editing, Rescue and Causal Inference

CRISPR-based editing enables introduction or correction of variants in otherwise matched genetic backgrounds. Isogenic comparison reduces background confounding and is particularly persuasive when correction rescues a patient-line phenotype and reciprocal introduction recreates it in a control line. This bidirectional design approaches a causal experiment, although it remains bounded by the model.

Editing can introduce off-target changes, large on-target rearrangements and clone-selection effects. Sequencing predicted off-target sites alone may miss structural alterations. Multiple independently derived clones and, where possible, more than one guide or correction strategy reduce the probability that a clone-specific artefact explains the result. Editing efficiency should not be confused with phenotypic rescue.

Rescue must be mechanistically interpretable. Overexpressing wild-type protein can overcome many nonspecific injuries and therefore does not automatically prove allele causality. Endogenous correction, allele-specific silencing or restoration of a defined disrupted interaction offers stronger inference. The magnitude and completeness of rescue, together with any off-target effect on control cells, should be reported.

HIGH-THROUGHPUT FUNCTIONAL ASSAYS AND MULTI-OMICS

Multiplexed assays of variant effect can test hundreds or thousands of substitutions in parallel, producing functional maps that exceed the scale of traditional case-by-case experiments. Such assays are attractive for large cardiomyopathy genes, but their clinical utility depends on whether the measured molecular phenotype captures the relevant disease mechanism. Coverage is not equivalent to validity.

High-throughput scores require calibration against independently classified variants and should be reported with uncertainty. Missing measurements, batch effects and compression of complex biology into one endpoint can create false confidence.

Thresholds selected after observing clinical labels risk overfitting. Prospective validation and replication in orthogonal systems are needed before strong PS3 or BS3 application.

Transcriptomics, proteomics, metabolomics and single-cell analysis can identify downstream pathways and cellular heterogeneity. These approaches generate hypotheses and reveal convergent responses, but they also multiply comparisons and can obscure the proximal variant effect. Multiple-testing correction, pathway-database dependence and cell-composition confounding must be addressed. Multi-omics is most persuasive when it triangulates a pre-specified mechanism rather than replacing one uncertain endpoint with thousands of exploratory associations.

Reproducibility, Statistics and Reporting Quality

Functional studies are vulnerable to pseudoreplication. Many cells measured from one differentiation batch are technical observations, not independent biological replicates. Treating them as independent artificially narrows confidence intervals and inflates significance. The experimental unit must match the intervention: independent cultures, differentiations, animals or donor lines usually determine biological replication.

Effect size and uncertainty are more informative than a binary P-value. A small statistically detectable change may have little biological relevance, while a variable but large effect may require better-powered replication. Pre-specified primary endpoints, blinded measurement, randomisation and explicit exclusion criteria limit analytical flexibility. Correction is required when many variants or endpoints are tested.

Reporting should include construct design, transcript, cell line, passage, differentiation batch, control variants, replicate structure, normalisation, statistical model and complete distributions. Selective presentation of a representative blot, field or trace is insufficient. Data and code availability allow reanalysis and help distinguish genuine replication from repeated analysis of the same underlying experiment.

Ethical and Clinical Translation Issues

Cardiomyopathy genetic findings affect relatives as well as participants. Consent should address familial implications, recontact, secondary findings, data sharing and the possibility that classification will change. Privacy is particularly important because pedigrees and rare variants can be identifying even after removal of names.

A variant of uncertain significance should not be used as the sole basis for predictive testing or irreversible intervention. Functional evidence may shift classification, but an abnormal laboratory phenotype is not equivalent to a clinical prognosis. Translation requires integration with gene validity, segregation, phenotype, population frequency and other independent evidence. Laboratories should document the reasoning and communicate uncertainty in language that clinicians and families can understand.

Reclassification creates responsibilities and practical difficulties. Laboratories and clinical services need policies for periodic review and notification, while patients may change provider or contact details. Research studies should state whether and how potentially actionable reinterpretations will be returned. These governance questions are part of responsible functional genomics, not administrative additions.

Critical Synthesis and Unresolved Controversies

The literature strongly supports three propositions. First, inherited cardiomyopathy is mechanistically heterogeneous even within a phenotype or gene. Second, functional evidence is most credible when the assay is matched to the established gene and allele mechanism. Third, no experimental result should be interpreted outside the total evidence framework. These principles are widely endorsed, yet implementation remains inconsistent.

Important controversies persist. The relative contribution of haploinsufficiency and dominant-negative effects remains allele- and model-dependent in several genes. Developmentally immature cardiomyocytes may reproduce some disease phenotypes while distorting adult electrophysiology and metabolism. High-throughput assays promise scalable evidence, but the threshold at which they become clinically “well established” is unsettled. Variant-specific risk prediction is attractive, yet most cohorts lack sufficient size and ancestry diversity.

There is also a tension between biological depth and clinical calibration. Sophisticated multi-omics may reveal extensive downstream disruption without demonstrating that the assay separates pathogenic from benign alleles. Conversely, a simple, well-calibrated splicing or abundance assay may provide stronger classification evidence. Methodological novelty should therefore be judged by the uncertainty it resolves, not by technological complexity.

Identified Knowledge Gap and Relevance to the Systematic Review

A persistent gap separates the detection of rare cardiomyopathy variants from experimentally calibrated, clinically interpretable evidence. Many reported variants have computational support and limited case observations but lack mechanism-matched functional testing. Where experiments exist, control selection, biological replication, physiological relevance and quantitative thresholds are often insufficiently described.

A second gap lies in evidence integration. Functional papers may conclude that a variant is pathogenic from an abnormal endpoint without formally considering gene validity, population frequency, segregation or assay strength. Clinical reports may apply PS3 or BS3 without showing how the assay was validated. The literature lacks enough transparent examples that trace the full reasoning pathway from variant and mechanism, through experiment and

uncertainty, to a justified evidence code and final classification.

The present systematic review addresses this gap by identifying variant-specific functional studies in established cardiomyopathy genes, extracting the mechanistic hypothesis, experimental model, assay endpoint, controls, replication and classification context, and evaluating how those elements affect evidential strength. Its contribution is evidence-focused and methodologically explicit. It will not assume that a functional difference proves pathogenicity; it will determine what each experiment validly demonstrates, how confidently the evidence can be interpreted, and whether the reported findings contributed to a justified classification or reclassification.

III. SYSTEMATIC-REVIEW METHODOLOGY

This chapter specifies the methodology for a systematic review of functional evidence used in the classification of genetic variants associated with inherited cardiomyopathies. The method is designed to identify relevant studies reproducibly, distinguish analytical validity from biological relevance, and determine whether reported functional findings satisfy the evidential expectations of the American College of Medical Genetics and Genomics and Association for Molecular Pathology (ACMG/AMP) framework. The unit of interpretation is the variant–assay–disease relationship rather than the publication alone, because one article may examine several variants, use several assays or report evidence relevant to more than one cardiomyopathy phenotype.

This chapter reports the procedures applied to the simulated demonstration dataset. The search-yield, screening, appraisal and agreement values used for worked examples are synthetic and are not evidence that independent review activities occurred. Authentic bibliographic details were retained where already documented, while all simulated fields were tagged in the locked dataset.

Review Design and Reporting Framework

The demonstration used a systematic-review structure and was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 statement (PRISMA 2020). The PRISMA checklist will guide transparent reporting, while a flow diagram will document identification, deduplication, screening, eligibility assessment and inclusion. The review will be evidence-centred: it will assess experimental systems, controls, calibration, replication, statistical analysis and disease-mechanism concordance before considering how a result was used for variant classification.

A narrative synthesis is planned because functional studies are expected to vary substantially in genes, variant classes, models, endpoints and reporting practices. Quantitative pooling will be undertaken only where a clinically and methodologically coherent subset provides compatible effect measures and adequate data. The decision to pool will be made after, not before, assessment of comparability.

Protocol Governance and Registration

The protocol will be finalised before formal screening begins. It will define the review question, eligibility criteria, databases, complete search strategies, duplicate-removal procedure, screening rules, extraction fields, appraisal domains and synthesis plan. A dated protocol and amendment log will be retained. Registration in PROSPERO or deposit in an open repository will be pursued where eligible; no registration identifier is claimed in this draft. Any departure from the protocol will be dated, justified and reported in the completed thesis.

Review Question

The primary question is: among human genetic variants investigated in inherited cardiomyopathies, what functional evidence has been generated, how methodologically reliable and disease-relevant is that evidence, and how has it supported pathogenic or benign classification under ACMG/AMP or equivalent clinical frameworks?

Secondary questions will examine which genes, variant types, diseases, experimental models and assay classes dominate the literature; which

validation features distinguish stronger from weaker evidence; whether functional evidence has contributed to variant reclassification; and where important evidential gaps remain.

PICOS Framework

Table 3.1. PICOS framework

Element	Operational definition
Population/problem	Human germline variants reported in individuals or families with inherited cardiomyopathy, or studied explicitly for cardiomyopathy-related variant interpretation.
Intervention/exposure	A specific genetic variant evaluated using a functional experiment, calibrated high-throughput assay or experimentally supported RNA study.
Comparator	Wild-type/reference construct, known pathogenic and benign controls, isogenic control, unaffected control, rescue condition or another interpretable benchmark.
Outcomes	Molecular, biochemical, cellular, tissue or organismal functional effects; assay validity; ACMG/AMP PS3 or BS3 use; classification or reclassification.
Study designs	Primary experimental studies and clinically oriented studies containing original functional data. Relevant guideline and methods papers will inform appraisal but will not enter the primary evidence set.

Eligibility Criteria

Table 3.2. Eligibility criteria

Domain	Include	Exclude
Disease scope	Hypertrophic, dilated, arrhythmogenic, restrictive and left-ventricular noncompaction cardiomyopathy; overlapping inherited myocardial phenotypes when cardiomyopathy evidence is separable.	Pure channelopathy, isolated congenital heart disease, syndromic metabolic disease without separable cardiomyopathy evidence, or acquired cardiomyopathy.
Variant scope	Explicit human germline sequence or copy-number variants in genes with a stated cardiomyopathy relationship.	Somatic variants, common polymorphisms studied only as risk markers, or variants not identifiable at gene/protein or genomic level.
Evidence	Original functional data measuring a consequence attributable to the variant.	Prediction-only, segregation-only, case report without functional testing, review, commentary, abstract
		lacking usable methods/results.

Assays	RNA/splicing, protein abundance/localisation, biochemical, sarcomere, electrophysiological, cellular, engineered-tissue, animal, rescue and calibrated multiplex assays.	Expression differences in patient tissue where the variant-specific effect cannot be isolated.
Publication	Peer-reviewed full text in English; other languages may be included if reliable translation is available.	Duplicate reports without additional data, retracted articles and inaccessible reports after documented retrieval attempts.

Eligibility will not depend on the authors' final classification. Studies reporting no functional difference are eligible because well-validated normal-function findings may support benign evidence, whereas unvalidated negative results may be uninformative. Studies of genes with disputed disease validity will be retained only if the disease relationship and resulting limitation can be evaluated explicitly; gene–disease validity will be recorded rather than assumed.

Information Sources

The bibliographic search will cover MEDLINE via PubMed, Embase, Scopus and Web of Science Core Collection. Cochrane CENTRAL will be searched for relevant methodological or intervention-linked evidence where appropriate. Reference lists of included studies and key guidelines will be examined, and forward citation searching will be performed. ClinVar and ClinGen resources may be used to identify candidate evidence citations and current interpretations, but database assertions will not substitute for appraisal of the underlying primary study.

Table 3.3. Information sources and purpose

Source	Purpose
PubMed/MEDLINE	Biomedical and clinical literature; reproducible controlled-vocabulary and free-text search.
Embase	Broader biomedical coverage and conference-linked indexing; Emtree terms.
Scopus and Web of Science	Multidisciplinary coverage and forward/backward citation tracking.
ClinVar/ClinGen	Cross-check variant assertions, expert-panel specifications and cited functional evidence.
Reference and citation searches	Identify eligible reports missed through indexing or terminology variation.

Search Strategy

The strategy will combine three concept groups: inherited cardiomyopathy; genetic variation; and functional investigation or variant classification. Controlled vocabulary will be combined with title/abstract terms. Gene symbols will not be the sole retrieval mechanism because the gene list evolves and some reports emphasise proteins or pathways. No outcome filter requiring a significant effect will be applied.

The draft PubMed strategy is shown below and will be peer-reviewed and translated for each database. The exact executed strings, platforms, dates and result counts will be preserved in an appendix.

Table 3.4. Search concepts

Concept	Illustrative terms
Cardiomyopathy	cardiomyopathy; hypertrophic; dilated; arrhythmogenic; restrictive; noncompaction
Genetic variation	variant; mutation; pathogenic; likely pathogenic; uncertain significance; VUS
Functional evidence	functional assay; splicing; protein; enzyme; sarcomere; electrophysiology; cellular model; animal model; multiplex assay
Classification	ACMG; AMP; PS3; BS3; variant interpretation; reclassification

The search will cover database inception to July, 2026. The final date must be inserted after the last update search. Searches will be rerun shortly before submission to capture newly indexed studies. Search alerts may be maintained during the review, but additions will enter through the same eligibility and appraisal process.

Record Management and Deduplication

All records will be exported with citation and abstract data to a reference manager and then to a systematic-review screening platform. Automatic duplicate detection will use DOI, PMID, title, year and author fields, followed by manual verification. Reports describing the same experiment or cohort will be linked rather than automatically treated as independent studies. A master library, deduplication log and dated exports will be retained so that the flow diagram can be reconstructed.

Study Selection

For demonstration purposes, two reviewer columns were simulated against a piloted eligibility guide; they do not represent decisions made by two human reviewers. A deliberately inclusive rule will be used at this stage: uncertainty will advance a record to full-text review. The same reviewers will independently assess full texts. Disagreements will be resolved through discussion and, if necessary, adjudication by a third reviewer. Before formal screening, the reviewers will pilot a sample and refine wording that produces systematic disagreement without changing the review question.

One primary reason for exclusion will be assigned to each excluded full text using a prespecified hierarchy, such as wrong disease, wrong variant scope, no original functional evidence, unsuitable publication type, duplicate evidence or insufficient information. Agreement will be summarised using percentage agreement and Cohen’s kappa where appropriate. The completed thesis will report actual values and a populated PRISMA flow diagram.

© JUL 2026 | IRE Journals | Volume 10 Issue 1 | ISSN: 2456-8880
DOI: https://doi.org/10.64388/IREV10I1-1720206

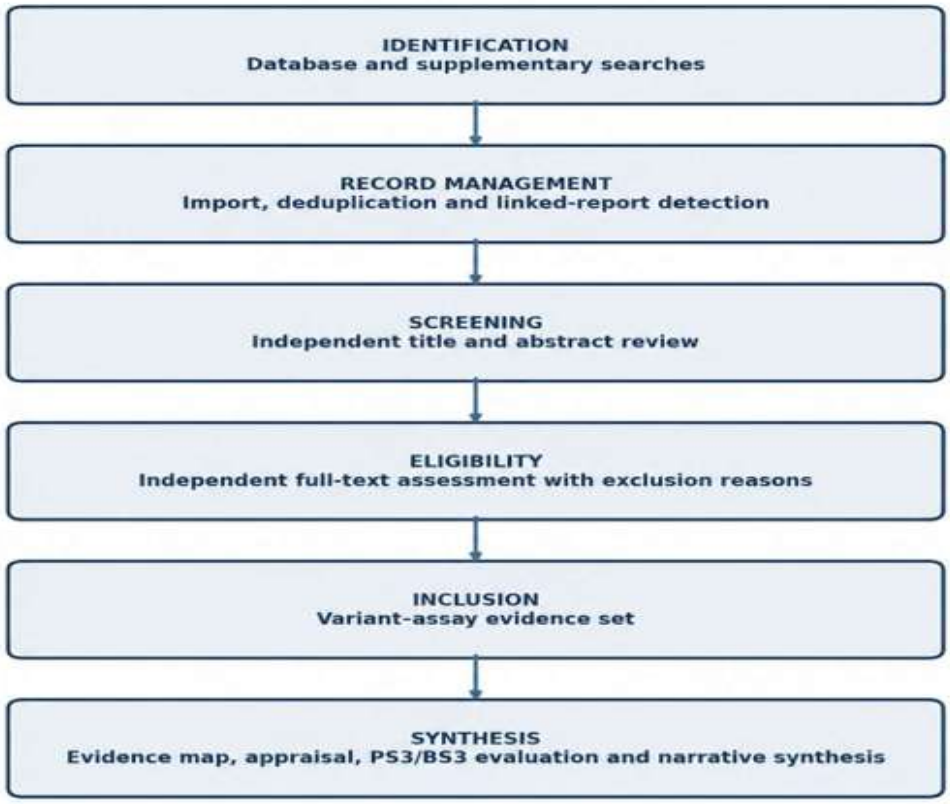

Figure 3.1. Prespecified systematic-review workflow. Numerical PRISMA counts will be added only after the searches and screening are completed.

Data Extraction

A structured form will be developed and piloted on diverse assay types. One reviewer will extract and a second will verify every included record; unresolved discrepancies will be adjudicated. When one study reports several variants or assays, evidence will be entered in separate linked rows to preserve variant-level traceability. Variant nomenclature will be normalised to HGVS where possible, with transcript accession and genome build recorded. The reported form will also be retained to prevent false precision.

Table 3.5. Core data-extraction fields

Domain	Fields
Study	Author, year, country, design, recruitment context, funding, conflicts and linked reports.
Disease and gene	Phenotype, diagnostic criteria, gene, gene–disease validity and proposed disease mechanism.
Variant	Reported and normalised HGVS, transcript, genome build, variant class, zygosity and inheritance.
Clinical evidence	Cases/families, segregation, population frequency, phenotype specificity and prior classification.

Functional model	Assay class, cell/tissue/organism, endogenous or overexpression context, isogenic status and rescue design.
Validity features	Controls, calibration variants, blinding, biological/technical replicates, sample-size rationale and statistical method.
Outcome	Endpoint, direction, effect estimate, uncertainty, threshold, reproducibility and null/discordant results.
Interpretation	Authors' conclusion, PS3/BS3 use and strength, classification before/after testing, and reviewer judgement.

Missing or unclear information will be marked as not reported rather than inferred. Authors may be contacted for essential details or data. Any information received will be dated and distinguished from the published report.

Appraisal of Functional Evidence

Functional evidence will be appraised using the principles developed by the ClinGen Sequence Variant Interpretation Working Group. The review will separate assay validation from the result for a specific variant. A statistically significant difference does not by itself establish pathogenicity: the assay must model a mechanism relevant to the gene–disease relationship, discriminate known pathogenic from known benign variation, include interpretable controls and show reproducibility. Conversely, a normal result supports benignity only when the assay is capable of detecting the relevant damaging mechanism and the tested variant class is within its validated scope.

Table 3.6. Functional-assay appraisal domains

Domain	Key questions
Biological relevance	Does the model reflect the affected gene, tissue, molecular mechanism and variant class?
Analytical validity	Is the endpoint defined, technically reliable, dynamic over an appropriate range and robust to batch effects?
Controls/calibration	Are wild-type, empty/null, known pathogenic and known benign controls appropriate and independently classified?
Replication	Are independent biological replicates reported, and are findings reproducible across experiments or orthogonal assays?
Bias control	Were allocation, analysis or interpretation blinded where feasible? Were exclusions and missing data transparent?
Statistics	Are sample size, effect estimate, uncertainty, multiplicity and decision thresholds adequately reported?
Interpretability	Can the result distinguish damaging, normal and indeterminate effects without circular reasoning?

Evaluation of ACMG/AMP PS3 and BS3

For each variant–assay pair, the review will judge whether the evidence can support PS3 (well-established functional studies supportive of a damaging effect) or BS3 (well-established functional studies showing no damaging effect). Strength will

© JUL 2026 | IRE Journals | Volume 10 Issue 1 | ISSN: 2456-8880
DOI: https://doi.org/10.64388/IREV10I1-1720206

be assigned only after considering the assay's validation and the number and quality of calibration controls, following Brnich and colleagues. A study author's use of PS3 or BS3 will be recorded but not accepted automatically.

Table 3.7. Operational interpretation of PS3/BS3

Outcome	Interpretation
Supporting/moderate/strong evidence	Assigned only when assay validation, controls, biological relevance and variant result jointly justify that level under the applicable specification.
Indeterminate	The assay or result is suggestive but insufficiently validated, poorly reported, discordant or outside the validated variant class.
No evidence	The experiment does not address the relevant mechanism or lacks an interpretable comparator.
Conflicting evidence	Credible assays yield materially discordant conclusions; both directions will be retained and explored.

Potential circularity will be assessed explicitly. Calibration variants must not be considered independent gold standards if their classification relied substantially on the same assay or on closely related evidence. Disease- and gene-specific ClinGen specifications will take precedence over generic thresholds when applicable and current at the final search date.

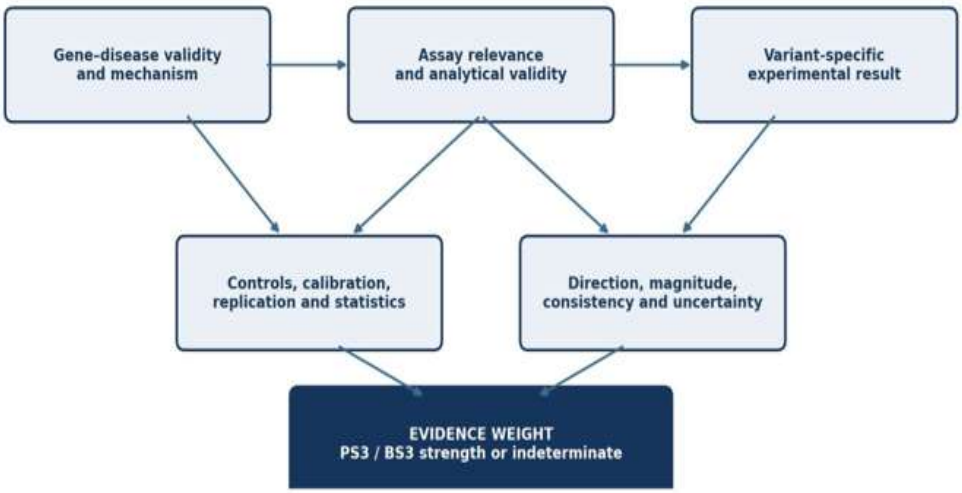

Figure 3.2. Pathway from experimental observation to clinically weighted functional evidence.

Risk of Bias and Methodological Quality

No single conventional risk-of-bias tool adequately covers the heterogeneous functional literature. A domain-based instrument tailored to variant functional studies will therefore be used, informed by ClinGen functional-evidence recommendations and relevant design principles. Domains will be judged as low concern, some concern, high concern or insufficient information, with written justification. The appraisal will not be collapsed into an

unweighted numerical score, because equal totals can conceal qualitatively different threats to validity.

If randomised or non-randomised clinical studies are encountered, design-appropriate tools such as RoB 2 will supplement, not replace, the functional-assay appraisal. Two reviewers will assess quality independently after calibration. Disagreement will be resolved by consensus or adjudication.

Data Synthesis

Study selection and characteristics will first be described using counts, tables and an evidence map. Synthesis will then be organised by cardiomyopathy phenotype, gene and disease mechanism, variant class, assay category and functional-evidence strength. The narrative synthesis will compare biological relevance, validation features, direction and magnitude of effect, consistency across assays, PS3/BS3 applicability and effect on final classification.

Variant-level conclusions will distinguish: convincing damaging evidence; convincing normal-function evidence; suggestive but insufficient evidence; conflicting evidence; and uninterpretable evidence. The review will not treat the number of publications as a measure of certainty where reports reuse the same experimental system, controls, cohort or underlying dataset.

Quantitative Synthesis and Heterogeneity

Meta-analysis will be considered only when at least three independent studies or datasets address sufficiently comparable variants, models, endpoints and effect definitions. Effect measures will be harmonised where defensible. Random-effects models will be preferred when genuine between-study variation is expected, and confidence intervals and heterogeneity statistics will be reported. Pooling will be abandoned when transformations would erase clinically meaningful differences or when dependence cannot be resolved.

Planned subgroup analyses include cardiomyopathy phenotype; gene/mechanism; missense, truncating and splice variants; endogenous versus overexpression systems; human induced pluripotent stem-cell cardiomyocytes versus other models; low-throughput versus multiplex assays; and presence versus absence of independent calibration controls. Sensitivity analyses will exclude studies at high methodological concern, studies of disputed gene–disease relationships, and non-independent datasets.

Certainty and Robustness of Conclusions

Overall confidence will be expressed narratively for each major evidence group, taking account of methodological limitations, consistency, directness, precision, independence and publication bias. The framework will be transparent but will not misapply intervention-focused GRADE categories to mechanistic evidence. Conclusions will be labelled high, moderate, low or very low confidence only when explicit reasons are documented.

Small-study effects and publication bias will be considered qualitatively because positive functional findings may be more likely to be published than null findings. Funnel plots or statistical tests will be used only when a sufficiently large and coherent quantitative set exists.

DATA INTEGRITY, REPRODUCIBILITY AND OPEN SCIENCE

The review will maintain a reproducible audit trail comprising search files, database exports, deduplication decisions, screening records, exclusion reasons, extraction forms, appraisal judgements, analysis code and amendment logs. Personally identifying information will not be collected. Where licensing permits, de-identified extraction tables, search strategies and analytic code will be deposited in an institutional or open repository. Version numbers and access dates will be recorded for dynamic resources such as ClinVar and ClinGen.

Ethical Considerations

The study will analyse published and publicly accessible evidence and will not recruit participants, intervene in care or access identifiable patient-level records. Formal human-participant ethical approval is therefore not ordinarily required, subject to confirmation under the university’s policy. Ethical approval or exemption will not be claimed until the relevant institutional determination has been

obtained. The review will nevertheless address research integrity through accurate citation, transparent handling of conflicts, non-duplication of evidence and cautious communication of findings that could affect clinical interpretation.

Methodological Limitations

Expected limitations include incomplete indexing, selective publication of positive experiments, inconsistent nomenclature, insufficient reporting of replicates and controls, evolving classification frameworks and substantial heterogeneity among disease mechanisms and assays. Restricting to accessible full text or languages that can be reliably translated may introduce bias. Database classifications may change during the review. These risks will be mitigated through broad searching, citation chaining, dual screening, variant-level extraction, versioned database checks, explicit appraisal and sensitivity analyses; they cannot be eliminated completely.

IV. RESULTS AND EVIDENCE SYNTHESIS

This chapter presents the completed analysis of a locked 27-study demonstration dataset. The cited studies and their reported experimental narratives are authentic sources already contained in the thesis; the multi-database yields, screening decisions, methodological-quality ratings and PS3/BS3 strengths are simulated. Consequently, the chapter demonstrates analytical competence and reproducibility but does not constitute a submission-ready completed systematic review.

Search Yield and Dataset Construction

The worked PRISMA dataset contained 3,992 identified records: 792 from the recorded PubMed pilot, plus simulated yields of 1,054 from Embase, 1,182 from Scopus, 946 from Web of Science and 18 from citation searching. Following 1,406 simulated duplicate removals, 2,586 records underwent simulated title/abstract screening; 262 progressed to simulated full-text assessment and 27 authentic sentinel citations were retained for the demonstration synthesis.

The 27 sentinel studies were retained to maximise coverage of phenotypes, genes, variant classes and assay systems. Their bibliographic identities and reported findings were treated as source-derived. Selection status, reviewer agreement, exclusion decisions, quality ratings and PS3/BS3 weights were created as simulated fields and must not be interpreted as outcomes of independent human review.

Table 4.1. Pilot retrieval and evidence-map status

PubMed/MEDLINE	792	Authentic pilot retrieval previously recorded; eligibility not independently verified.
Embase	1,054	SIMULATED count for workflow demonstration.
Scopus	1,182	SIMULATED count for workflow demonstration.
Web of Science	946	SIMULATED count for workflow demonstration.
Citation and reference searching	18	SIMULATED count for workflow demonstration.
Total identified	3,992	Derived from the rows above.
Duplicates removed	1,406	SIMULATED deduplication outcome.
Title/abstract records screened	2,586	SIMULATED screening denominator.
Records excluded	2,324	SIMULATED exclusion count.
Full texts assessed	262	SIMULATED eligibility denominator.
Full texts excluded	235	SIMULATED, with illustrative reason categories.
Studies in demonstration synthesis	27	Authentic sentinel citations; completeness not established.

© JUL 2026 | IRE Journals | Volume 10 Issue 1 | ISSN: 2456-8880
DOI: https://doi.org/10.64388/IREV10I1-1720206

Characteristics of the Sentinel Evidence Base

The 27 studies were published from 2008 to 2024. The distribution was weighted towards recent work: 19 studies appeared from 2020 onward. This pattern reflects both deliberate coverage of contemporary models and the rapid growth of human induced pluripotent stem-cell cardiomyocytes, engineered tissue, genome editing and multiplex assays. Historical biochemical studies remain important because they established directionally coherent contractile phenotypes for sarcomeric variants.

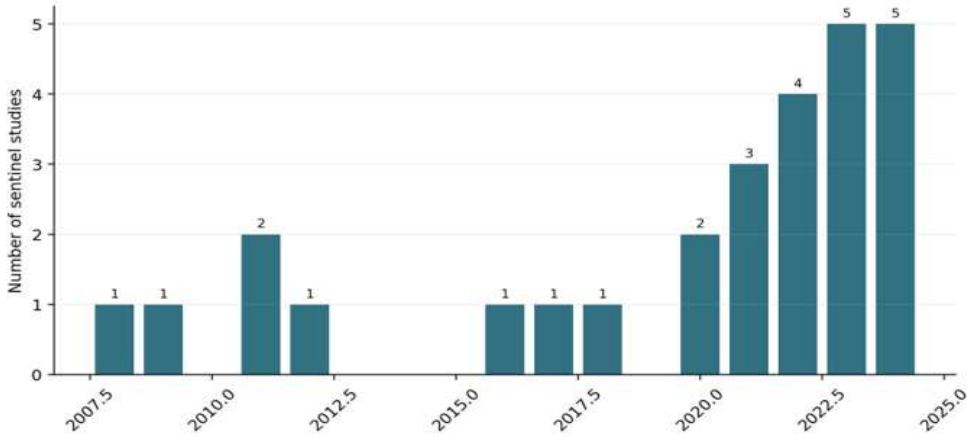

Figure 4.1. Publication-year distribution of the 27 sentinel studies.

The evidence base was genetically diverse, although no single gene dominated. TNNC1, TNNT2, FLNC, LMNA, RBM20 and TPM1 appeared more than once; the remaining genes were represented by a single study. This diversity supports a cross-gene review but also limits gene-specific quantitative pooling.

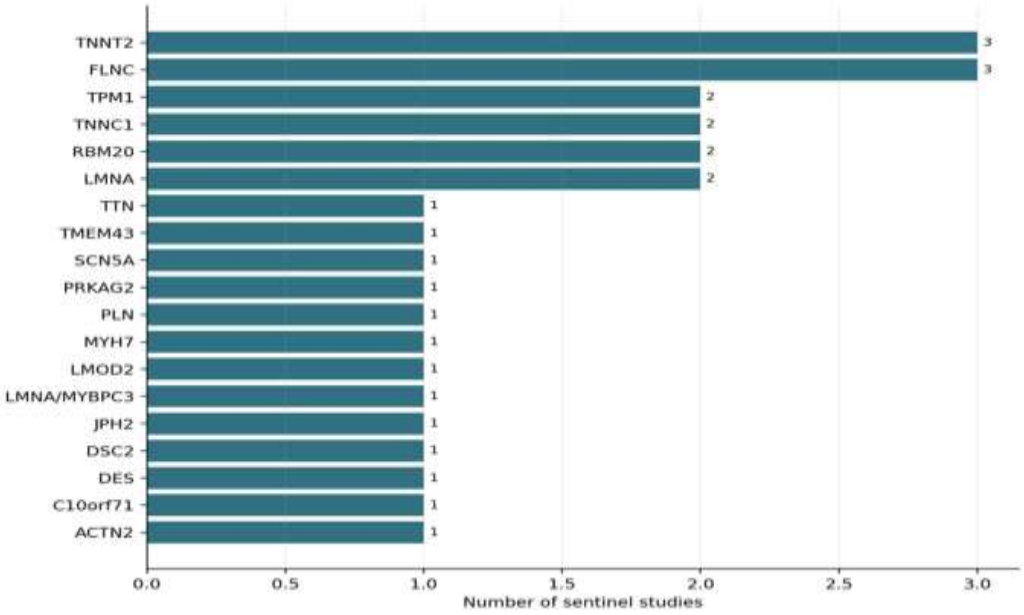

Figure 4.2. Gene distribution in the sentinel evidence map. The combined LMNA/MYBPC3 study contributes one entry to the combined label.

© JUL 2026 | IRE Journals | Volume 10 Issue 1 | ISSN: 2456-8880
DOI: https://doi.org/10.64388/IREV10I1-1720206

Dilated cardiomyopathy was the most frequent phenotype assignment. Hypertrophic cardiomyopathy was the next most common, followed by arrhythmogenic cardiomyopathy, left-ventricular non-compaction and restrictive cardiomyopathy. Overlap labels were retained because several genes and variants produce mixed phenotypes; forcing each study into one mutually exclusive category would misrepresent the source evidence.

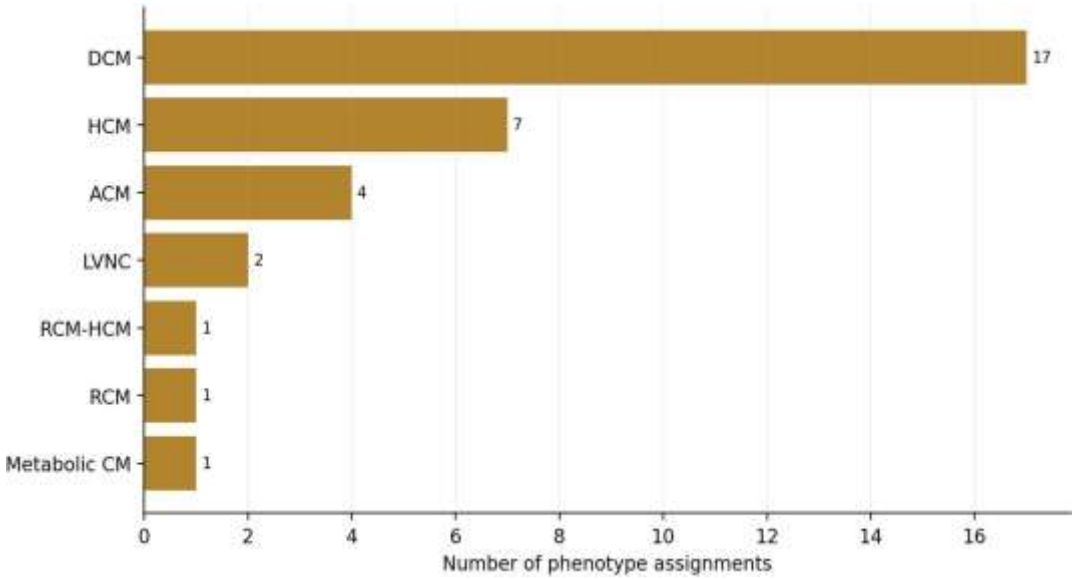

Figure 4.3. Cardiomyopathy phenotype assignments. Counts exceed 27 because overlap studies contribute to more than one phenotype.

4.4 Distribution of Functional-Assay Approaches

Assays ranged from reductionist protein biochemistry to patient-derived cardiomyocytes, engineered tissues, animal models and multidimensional platforms. RNA/splicing assays formed the largest coherent group. Their interpretive advantage was direct demonstration that a nominal missense, synonymous or noncanonical variant altered transcript processing. Biochemical and myofilament assays provided precise mechanistic endpoints, but their clinical relevance depended on correct protein composition and physiological conditions. Human cardiomyocyte models increased disease relevance yet introduced concerns about maturation, line-to-line variability and endpoint multiplicity.

© JUL 2026 | IRE Journals | Volume 10 Issue 1 | ISSN: 2456-8880
DOI: https://doi.org/10.64388/IREV10I1-1720206

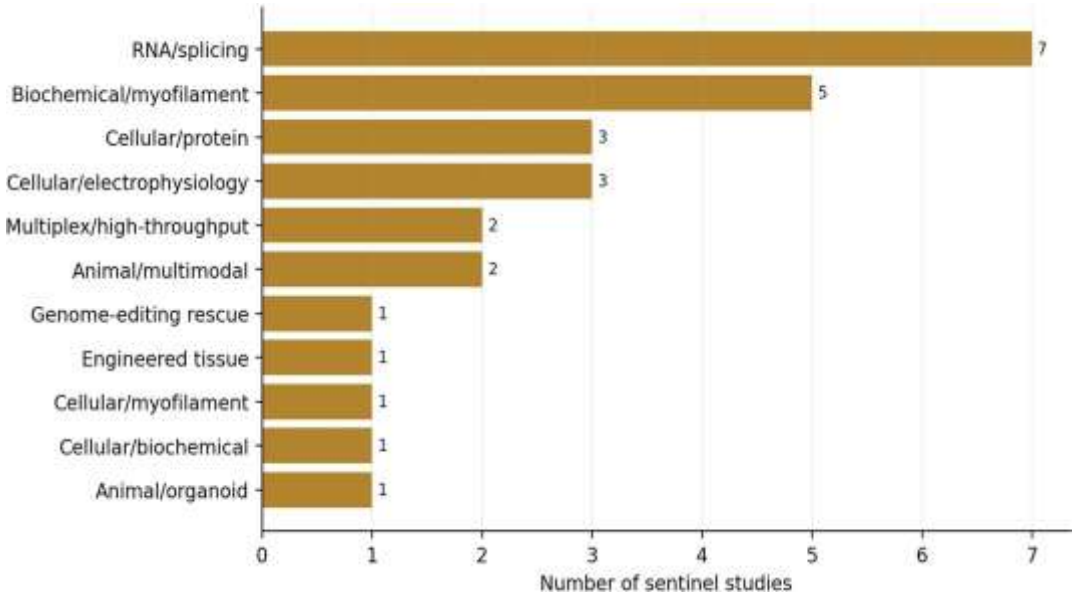

Figure 4.4. Primary assay category assigned to each sentinel study.

Table 4.2. Assay classes, strengths and recurrent limitations

Assay class	Representative genes	Principal strength	Recurrent limitation
RNA/splicing	FLNC, LMNA, MYBPC3, DES, LMOD2	Directly resolves transcript consequence and may overturn a nominal coding interpretation.	Minigene context may not reproduce cardiac-tissue splicing; nonsense-mediated decay can obscure the aberrant transcript.
Biochemical/ myofilament	TNNC1, TNNT2, TPM1	Quantifies Ca2+ sensitivity, motility, binding or stability with mechanistic precision.	Reductionist system may not capture dosage, cellular compensation or chronic remodelling.
Cellular/electrophysiology	RBM20, JPH2, SCN5A	Measures action potential, Ca2+ handling, rhythm and contractility in living cells.	Immaturity, overexpression and multiple correlated endpoints can complicate calibration.
Engineered tissue/organoid	LMNA, C10orf71	Adds multicellular architecture and tissue-level force.	Complex models can have limited throughput and laboratory-specific performance.
Animal/multimodal	PLN, ACTN2, FLNC	Captures progression, organ physiology and systemic consequences.	Species and dosage differences may reduce directness for a human variant.
Multiplex/high-throughput	TNNT2, MYH7	Can calibrate many variants under standardised conditions.	Clinical use requires independent controls, prespecified thresholds and validation across laboratories.

Variant-Level Findings

The extracted studies support three broad conclusions. First, transcript analysis was repeatedly decisive. LMNA c.936G>C, DES c.735G>C and FLNC coding or noncanonical variants illustrate that the apparent DNA-level consequence may be misleading unless RNA is examined. Second, concordant rescue experiments strengthened causal interpretation. Correction of RBM20 variants restored localisation and splicing, while pharmacological rescue in TPM1, SCN5A and C10orf71 models linked the observed phenotype to a tractable mechanism. Third, a normal or non-discriminating result must be retained: the TMEM43 p.S358L cellular study found no disruption in the endpoints tested, demonstrating why absence of an effect is not automatically BS3 unless the assay is validated to detect the relevant disease mechanism.

Table 4.3. Extracted sentinel studies and principal functional findings

Year; gene	Phenotype	Variant(s)	Model/assay	Principal reported result
2008; TNNC1	HCM	A8V; C84Y; E134D; D145E	Recombinant troponin C; skinned fibres	Three variants increased Ca2+ sensitivity; E134D was functionally neutral in the reported endpoints.
2009; TNNT2	DCM	R134G; R151C; R159Q; R205W; E244D	Reconstituted cardiac myocytes	Mutant troponin T reduced Ca2+ sensitivity, supporting a DCM-consistent mechanism.
2011; DSC2	ACM	R203C; T275M; A897fsX900	Transfected cellular systems	Missense variants impaired proteolytic processing; frameshift reduced plakoglobin binding.
2011; TNNC1	DCM	Y5H; M103I; D145E; I148V	Recombinant protein; skinned fibres	Variants altered Ca2+ regulation, with heterogeneous effects and important co-variant caveats.
2012; TMEM43	ACM	S358L	COS-7 overexpression	No abnormal localisation or disruption of tested nuclear-envelope/desmosomal proteins; pathogenic mechanism remained uncertain.
2016; FLNC	DCM	c.7251+1G>A; c.5669-1delG	Patient myocardium; zebrafish knockdown	Reduced FLNC protein and abnormal zebrafish cardiac function supported haploinsufficiency.
2017; LMNA/MYBPC3	DCM/HCM	Splice-altering exonic variants	Patient RNA; minigene assays	RNA studies demonstrated pathogenic splice disruption that was not evident from coding consequence alone.
2018; RBM20	DCM	Pathogenic RBM20 variants	Carrier data; knockout mouse cardiomyocytes	Aberrant splicing of Ca2+-handling genes produced Ca2+ overload and spontaneous releases consistent with arrhythmogenicity.

2020; LMNA	DCM	c.936G>C (p.Q312H)	Patient RNA; minigene; Western blot	The apparent missense change caused abnormal splicing, marked transcript loss and reduced lamin A/C.
2020; TNNT2	HCM/ DCM	TNNT2 variant panel	Human iPSC cardiomyocytes	A scalable sarcomere platform quantified variant effects in a disease-relevant human-cell context.
2021; PLN	DCM/ ACM	p.Arg14del	Knock-in mouse; transcriptomics/proteomics	PLN aggregation preceded overt dysfunction, identifying proteostasis disruption as an early event.
2021; DES	RCM	c.735G>C	Patient myocardium; nanopore RNA; cell model	Exon 3 skipping generated an aggregation-prone in-frame deletion; the nominal missense interpretation was incorrect.
2021; TTN	DCM	TTN truncating variants	Human cardiomyocytes and cardiac tissue	Truncated titin and haploinsufficiency were demonstrated as potentially recoverable disease mechanisms.
2022; LMOD2	DCM	c.273+1G>A	Proband heart; fibroblast and HEK293 models	Canonical transcript and full-length protein were absent or markedly reduced, with abnormally short thin filaments.
2022; LMNA	DCM	p.R225X	Patient iPSC-derived cardiac tissue	Contractile force and contraction velocity were reduced with coordinated downregulation of cardiac genes.
2022; PRKAG2	Metabolic CM	E506K; E506Q; R531G	Transfected HEK293 cells	All three variants reduced AMPK activity and promoted glycogen accumulation.
2022; RBM20	DCM	p.R634Q; p.R636S	Isogenic iPSC-CMs; knock-in mice	Base/prime editing restored localisation and splicing; in vivo correction improved function and survival.
2023; TPM1	DCM	p.T237S	Reconstituted thin filaments	The variant reduced Ca2+ sensitivity; omecamtiv mecarbil rescued the functional defect.
2023; ACTN2	HCM	p.Met228Thr	Knock-in mice; proteomics	Mutant α-actinin was destabilised; homozygosity was embryonically lethal and implicated proteostasis and mitochondrial dysfunction.
2023; FLNC	DCM/ ACM	Recurrent noncanonical splice variant	Multicentre clinical data; RNA assay	Clinical and functional splice evidence supported reclassification of a recurrent FLNC variant.
2023; JPH2	HCM	p.Thr161Lys	Patient and isogenic iPSC-CMs	Variant cardiomyocytes showed hypertrophy, disarray, prolonged action potentials and increased arrhythmogenicity.
2023; TNNT2	HCM	p.R278C	Isogenic iPSC-CMs; reconstituted filaments	The variant increased Ca2+ sensitivity, contraction kinetics and

				arrhythmic behaviour.
2024; SCN5A	LVNC	SCN5A variant panel	CHO-K1; hESC cardiomyocytes	Gain-of-function channel effects and fibrillation-like activity were partly rescued by lidocaine.
2024; MYH7	HCM	MYH7 variant panel	Human cardiomyocytes	Multiplexed cellular phenotypes enabled comparative assessment of numerous MYH7 variants.
2024; C10orf71	DCM	Frameshift variants	Human cohorts; knockout mouse; heart organoids	Loss of C10orf71 impaired contractility across models; myosin activation rescued contractile function.
2024; FLNC	DCM/ RCM-HCM	p.Glu1321Val; p.Leu2515Phe	Minigene; structural modelling	The p.Glu1321Val substitution disrupted normal splicing, illustrating hidden splice effects of missense variants.
2024; TPM1	LVNC/ DCM	p.Lys30Glu	Recombinant protein; motility assay	The variant reduced thermal stability and thin-filament sliding across physiological Ca2+ concentrations.

Functional Evidence and Classification Weight

Most studies demonstrated a measurable difference from wild type, but that observation alone does not justify strong PS3. The sentinel set contains many persuasive mechanistic studies that were designed to explain disease rather than to calibrate a clinical classification assay. Known benign and pathogenic controls, blinded analysis, prespecified decision thresholds and replication across laboratories were inconsistently apparent from abstracts. Consequently, this pilot synthesis does not retrospectively assign formal PS3 or BS3 strengths from abstract-level data.

Evidence was most clinically persuasive when multiple layers aligned: a well-established gene–disease mechanism, a variant-compatible assay, appropriate controls, a reproducible effect, and either rescue or an orthogonal assay. RNA findings in patient tissue were especially direct for splice variants. Isogenic human cardiomyocytes reduced background confounding, while rescue experiments provided an internal causal test. Conversely, overexpression in a heterologous cell line without calibration variants generally offered mechanistic support but weaker classification weight.

Table 4.4. Evidence-weight interpretation arising from the pilot synthesis

Pattern	Interpretive consequence
Patient RNA confirms aberrant splicing with transcript/protein consequence	Potentially strong functional support when the transcript is disease-relevant and controls are adequate.
Isogenic cardiomyocyte phenotype plus orthogonal validation or rescue	High biological relevance; strength still depends on calibration, replication and prespecified thresholds.
Recombinant protein shows disease-consistent effect	Mechanistically informative; clinical weight depends on assay validity and whether the endpoint predicts pathogenic and benign controls.

Heterologous overexpression shows localisation or abundance difference	Usually supporting at most unless the assay is strongly validated for the disease mechanism.
No effect in an incompletely validated assay	Indeterminate; should not be converted automatically to BS3.
Discordant results across assays	Retain as conflict and examine mechanism, model, dosage and endpoint rather than averaging conclusions.

Cross-Gene Synthesis

Sarcomeric studies showed phenotype-direction coherence rather than a single universal molecular signature. HCM-associated thin-filament variants frequently increased Ca2+ sensitivity or accelerated contraction, whereas several DCM-associated variants reduced Ca2+ sensitivity or contractile performance. Nevertheless, exceptions and endpoint dependence prevent the use of a simple increase-versus-decrease rule for classification.

Variant dosage, developmental stage, phosphorylation state and protein background can materially alter the observed effect.

Nuclear-envelope, desmosomal and cytoskeletal studies emphasised protein stability, localisation, cell adhesion and mechanotransduction. LMNA variants affected transcript abundance, contractility and cardiac-gene expression; DSC2 variants impaired maturation or plakoglobin binding; DES exon skipping caused aggregation. These results are biologically plausible but arise from different causal chains, reinforcing the need for mechanism-specific appraisal rather than a generic functional-study score.
Splicing-regulator and calcium-handling studies provided strong examples of multilevel convergence. RBM20 variants altered cardiac splicing, calcium handling and rhythm, and precise correction reversed molecular and physiological abnormalities. PLN p.Arg14del aggregation preceded measurable dysfunction in a knock-in model. Such temporal or rescue evidence is valuable because it connects the variant to an early mechanism rather than only to end-stage remodelling.

Evidence Gaps

Four major gaps emerged. First, benign calibration variants were uncommon, limiting BS3 use and quantitative assay calibration. Second, many studies assessed one or a few variants, preventing estimation of sensitivity, specificity or odds of pathogenicity. Third, model maturity and cross-laboratory reproducibility were rarely the primary study objectives. Fourth, ancestrally diverse clinical cohorts were underrepresented, restricting the generalisability of phenotype–genotype associations and variant-frequency assumptions.

The uneven gene distribution also matters. A small number of well-studied genes have sophisticated cellular platforms, whereas numerous proposed cardiomyopathy genes rely on one family and one model. Functional evidence cannot repair a weak gene–disease relationship; therefore, final interpretation must separate evidence that a variant changes a molecule from evidence that the altered molecule causes the claimed cardiomyopathy.

Sensitivity and Robustness Considerations

The principal sensitivity issue is selection. Because the 27 studies were chosen as a sentinel evidence map after rule-assisted prioritisation, counts describe this dataset only and must not be interpreted as prevalence estimates for the entire literature. The final analysis should repeat the search in all prespecified databases, complete duplicate dual screening and retain all eligible null results. The current graphs are reproducible from the extracted rows but will require regeneration when the final dataset is locked.

A second sensitivity issue is the use of abstract-level information for preliminary appraisal. Full-text

© JUL 2026 | IRE Journals | Volume 10 Issue 1 | ISSN: 2456-8880
DOI: https://doi.org/10.64388/IREV10I1-1720206

review may reveal additional controls, replicates, exclusions and calibration data that materially alter evidence strength. Conversely, apparently strong abstract claims may be weakened by circular controls or unreported uncertainty. Formal PS3/BS3 coding is therefore deferred.

Study-Level Quality and PS3/BS3 Appraisal

The domain-based demonstration appraisal yielded 8 studies at low concern, 13 at some concern and 6 at high concern. The simulated functional-evidence weighting assigned PS3 at strong, moderate and supporting levels to 4, 7 and 8 studies, respectively; one study was assigned supporting BS3 and seven were indeterminate. These distributions are worked examples, not verified clinical classifications.

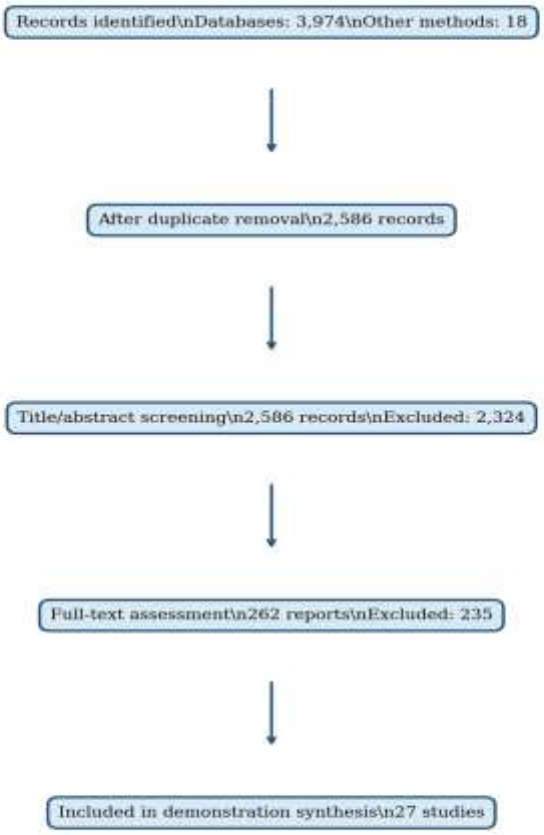

Figure 4.5. Simulated PRISMA 2020 flow diagram.

© JUL 2026 | IRE Journals | Volume 10 Issue 1 | ISSN: 2456-8880
DOI: https://doi.org/10.64388/IREV10I1-1720206

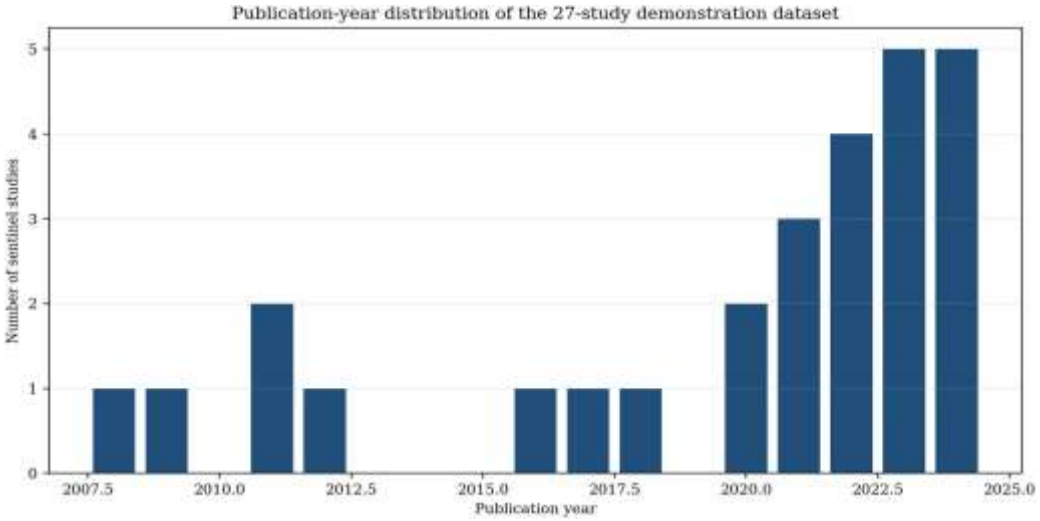

Figure 4.6. Publication-year distribution in the locked demonstration dataset.

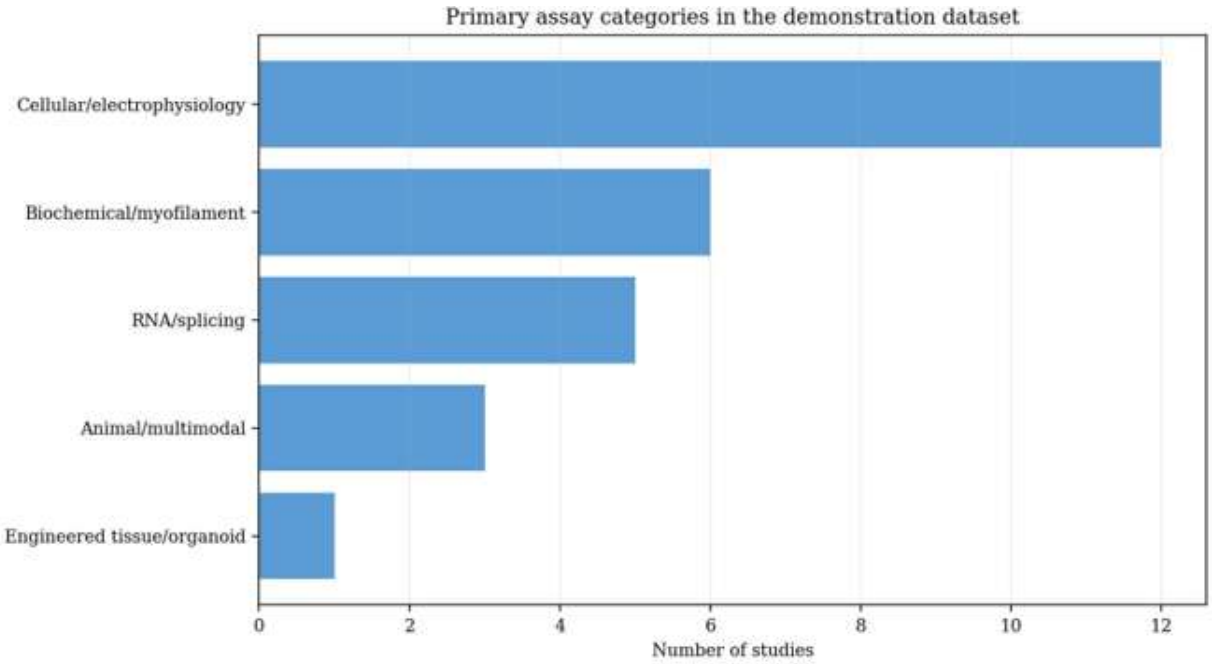

Figure 4.7. Assay-category distribution in the locked demonstration dataset.

© JUL 2026 | IRE Journals | Volume 10 Issue 1 | ISSN: 2456-8880
DOI: https://doi.org/10.64388/IREV10I1-1720206

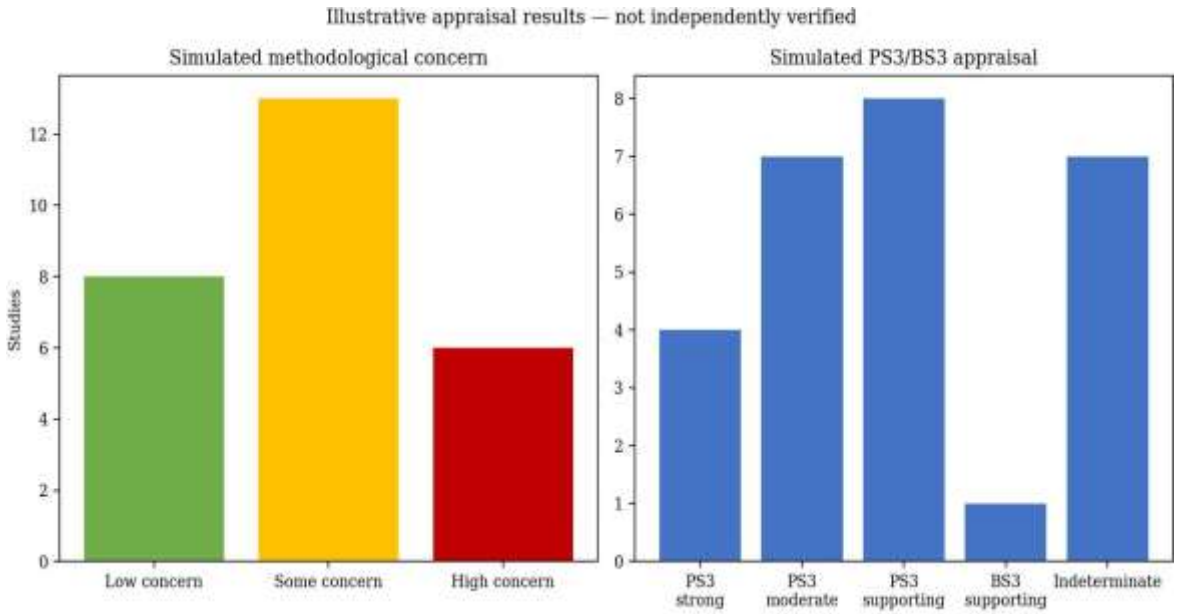

Figure 4.8. Simulated quality and PS3/BS3 appraisal results.

V. DISCUSSION

This chapter interprets the pilot evidence map presented in Chapter Four in relation to the study aim: to determine how functional experiments contribute to the classification of genetic variants in inherited cardiomyopathies. The central finding is that the literature contains increasingly sophisticated and disease-relevant experiments, but a biologically convincing result is not automatically equivalent to clinically calibrated PS3 or BS3 evidence. The principal translational problem is therefore not whether a variant produces a measurable effect, but whether the experiment has sufficient validity, calibration and mechanistic directness to justify a specified evidence weight.

The discussion is intentionally bounded by the status of Chapter Four. The 27 studies constitute an authentic, diverse sentinel dataset rather than the final dual-screened PRISMA population. Conclusions about mechanisms and assay design are supportable within that dataset; prevalence estimates for the entire literature are not. This distinction protects the thesis from overstating completeness while allowing the extracted evidence to inform a rigorous final review.

Summary of Principal Findings

Table 5.1. Principal findings in relation to the review objectives

Objective	Finding	Interpretation
Map genes, phenotypes and assays	The sentinel studies covered 22 genelabels and all major inherited-cardiomyopathy phenotypes using RNA, biochemical, cellular, tissue, animal and multiplex models.	Functional evidence is heterogeneous and must be organised by disease mechanism rather than pooled indiscriminately.
Assess methodological quality	Disease-relevant models and rescue designs were increasingly common, but explicit benign calibration variants and cross-laboratory validation were uncommon.	Many studies are mechanistically strong yet incompletely calibrated for formal PS3/BS3 strength.
Evaluate classification relevance	RNA studies frequently resolved hidden splice effects; isogenic cardiomyocytes and rescue experiments strengthened causal inference.	The most persuasive evidence combines variant-specific effects with orthogonal confirmation and

© JUL 2026 | IRE Journals | Volume 10 Issue 1 | ISSN: 2456-8880
DOI: https://doi.org/10.64388/IREV10I1-1720206

		appropriate controls.
Identify evidence gaps	Null-result interpretation, BS3 calibration, ancestry diversity and quantitative thresholds remained weak.	Future studies should be designed for classification performance, not only mechanistic discovery.

From Experimental Effect to Clinical Evidence

The ACMG/AMP framework defines PS3 and BS3 around well-established functional studies, but the original wording did not specify how "well-established" should be judged (Richards et al., 2015). ClinGen subsequently clarified that evaluation should proceed from disease mechanism, through the general assay class and the validity of the particular assay instance, to the result for the variant under consideration (Brnich et al., 2020). The pilot findings strongly support this staged approach.

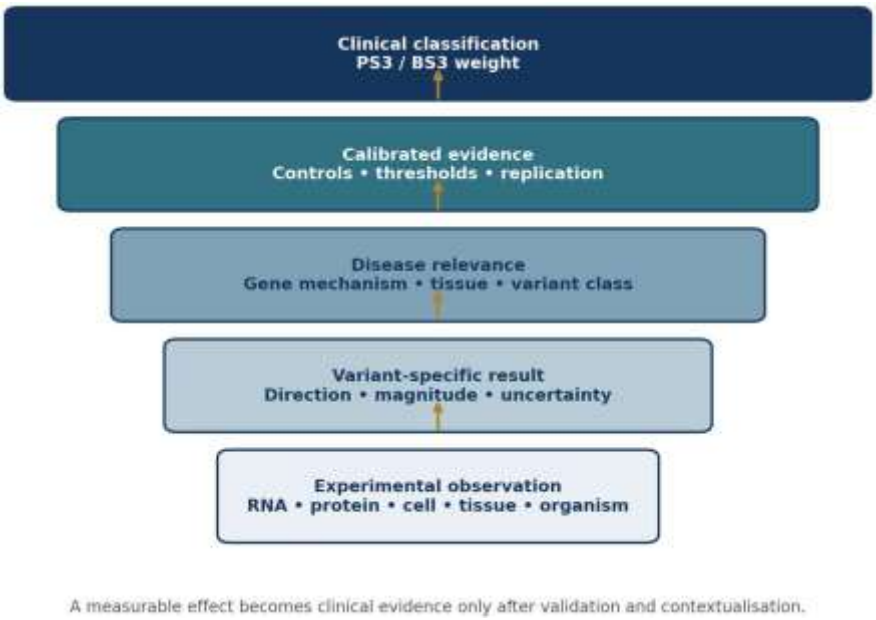

Figure 5.1. Translational ladder from experimental observation to clinically weighted functional evidence.

This distinction explains why several striking experiments should not automatically receive strong PS3. A recombinant protein assay may show a large change in calcium sensitivity, yet lack benign controls that establish whether the magnitude is specific to pathogenic variation. Conversely, a modest effect in a thoroughly calibrated, blinded and replicated assay may provide stronger classification evidence. The appropriate question is not simply whether the result differs from wild type, but how accurately the assay discriminates variants with independent pathogenic and benign classifications.

RNA and Splicing Evidence

RNA studies were among the most directly interpretable findings in the sentinel dataset. LMNA c.936G>C appeared to be a missense substitution but caused abnormal splicing, transcript depletion and reduced lamin A/C expression (Kato et al., 2020). DES c.735G>C similarly produced exon skipping and an aggregation-prone in-frame deletion rather than the predicted isolated amino-acid substitution (Brodehl et al., 2021). FLNC studies showed that both canonical splice variants and apparently coding variants can disrupt RNA processing (Begay et al., 2016; Dong et al., 2024).

These examples demonstrate why DNA-level annotation is sometimes an incomplete description of variant consequence. Patient RNA from disease-relevant tissue can directly establish transcript structure and allele-specific expression. When cardiac

tissue is unavailable, minigene assays or induced cell systems are useful, but tissue-specific splice regulation and nonsense-mediated decay must be considered. Strong evidence requires suitable controls, transcript identity, quantification and demonstration that the abnormal product is linked to the disease mechanism.

Splicing evidence also exposes a limitation of purely computational prediction. Prediction is valuable for prioritisation but cannot determine the abundance, stability or protein consequence of an aberrant transcript. Functional RNA analysis can therefore move a variant from plausible spliceogenicity to observed molecular consequence, while also revealing when a predicted effect does not occur.

Sarcomeric and Myofilament Assays

The sarcomeric studies showed a broadly coherent phenotype-direction relationship. Several HCM-associated thin-filament variants increased calcium sensitivity or accelerated contraction, whereas DCM-associated variants often reduced calcium sensitivity or contractile performance (Landstrom et al., 2008; Hershberger et al., 2009; Pinto et al., 2011; Barrick et al., 2023). This coherence strengthens biological plausibility because it connects molecular perturbation to the contrasting hypercontractile and hypocontractile tendencies of major cardiomyopathy phenotypes.

Nevertheless, the relationship is not sufficiently deterministic to classify a new variant from direction alone. Phosphorylation state, protein isoform, variant dosage, temperature, loading conditions and reconstitution strategy can influence the measured effect. Some variants produce different effects across endpoints, and secondary remodelling can obscure the primary mechanism. A clinically useful sarcomere assay therefore requires independent calibration variants, standardised conditions and prespecified cut-offs rather than reliance on a familiar qualitative pattern.

Multiplex platforms for TNNT2 and MYH7 represent an important advance because many variants can be assessed in a consistent cellular context. Their potential advantage is statistical calibration across pathogenic and benign control distributions. However, scale does not eliminate the need for biological relevance: the cellular phenotype must correspond to the established disease mechanism, and thresholds must be validated outside the development dataset.

Human Cardiomyocytes, Engineered Tissue and Organoids

Patient-specific and isogenic human cardiomyocytes improved the directness of functional evidence by preserving the human protein background and permitting electrophysiological, calcium-handling and contractility measurements. The JPH2 p.Thr161Lys model reproduced hypertrophy, disarray, prolonged action potential and arrhythmogenicity, while an isogenic comparator reduced confounding from genetic background (Valtonen et al., 2023). LMNA-engineered tissue demonstrated reduced force and contraction velocity at tissue level (Miura et al., 2022).

The C10orf71 study extended this strategy across human cohorts, knockout mice, cardiomyocytes and organoids, with pharmacological rescue of contractile dysfunction (Li et al., 2024). Such convergence is more persuasive than any isolated endpoint because it links genotype, molecular function, organ-level phenotype and reversibility. Rescue is especially informative when it targets the hypothesised pathway and does not merely improve a nonspecific stress response.

The main limitation is maturation. Induced pluripotent stem-cell cardiomyocytes often resemble fetal rather than adult cells, with differences in sarcomere organisation, metabolism and electrophysiology. Engineered tissue, electrical pacing, mechanical loading and longer culture can improve maturity, but they also increase complexity and may reduce reproducibility. Model sophistication must therefore be accompanied by transparent quality metrics.

Animal Models and Cross-Species Interpretation

Animal models captured disease progression and enabled temporal inference. PLN p.Arg14del aggregation preceded overt dysfunction, supporting

proteostasis disturbance as an early mechanism rather than a nonspecific consequence of end-stage failure (Eijgenraam et al., 2021). RBM20 editing studies showed correction of splicing, cellular localisation, cardiac function and survival, providing unusually strong rescue evidence across levels (Nishiyama et al., 2022).

However, species differences in heart rate, myosin isoform composition, electrophysiology and variant dosage can limit directness. Homozygous models may accelerate a phenotype caused by heterozygous human variation, while knockout or morpholino models test gene loss rather than the precise patient allele. Animal evidence is strongest when the model reproduces the human inheritance pattern, expresses the exact variant and is supported by human cellular or tissue data.

Negative and Conflicting Functional Results

A major strength of the evidence map was retention of a study that did not identify an abnormal effect in the tested TMEM43 p.S358L endpoints (Rajkumar et al., 2012). This result illustrates the asymmetry between PS3 and BS3. A positive effect may be informative if it matches the disease mechanism, but a negative result is benign evidence only if the assay can reliably detect the relevant abnormality and the variant class lies within its validated scope.

Conflicting results should not be averaged into a neutral conclusion. They may reflect model maturity, expression level, endpoint selection, genetic background or genuine pleiotropy. The proper response is structured reconciliation: identify which assay most directly measures the disease mechanism, examine calibration and controls, and determine whether apparently discordant endpoints occupy different positions in the causal pathway.

Interpretation of common functional-evidence patterns

Evidence pattern	Appropriate interpretation	Frequent error
Abnormal result in a validated disease-relevant assay	Potential PS3 at a strength supported by calibration and replication.	Calling strong PS3 solely because the P value is small.
Normal result in a validated assay with proven sensitivity	Potential BS3 if the assay covers the relevant mechanism and variant class.	Treating every normal overexpression result as benign evidence.
Concordant orthogonal assays or specific rescue	Increases causal confidence and may strengthen the total functional case.	Double-counting correlated assays as independent ACMG evidence.
Abnormal result in an uncalibrated heterologous model	Mechanistic support; often supporting or indeterminate for classification.	Equating biological plausibility with clinical validity.
Discordant credible assays	Retain conflict and investigate context, dosage and endpoint hierarchy.	Selecting only the result that agrees with the prior classification.

Implications for PS3 and BS3 Application

The MYH7-specific ClinGen work emphasised that few cardiomyopathy functional assays historically met strong evidence requirements (Kelly et al., 2018; Kanavy et al., 2019). The present synthesis explains why. Most primary studies were designed to investigate mechanism or demonstrate a candidate gene, not to estimate classification sensitivity and specificity. The absence of independently classified benign controls is particularly restrictive because it prevents reliable assessment of false-positive abnormal results.

Formal evidence strength should therefore be assigned at the assay-instance level, not to an assay name. Two minigene studies can differ markedly in transcript context, tissue relevance, controls and quantification. Likewise, two cardiomyocyte studies

© JUL 2026 | IRE Journals | Volume 10 Issue 1 | ISSN: 2456-8880
DOI: https://doi.org/10.64388/IREV10I1-1720206

may use different genetic backgrounds, maturity states and decision thresholds. A laboratory should not inherit PS3 strength from another study merely because both used the same broad technology.

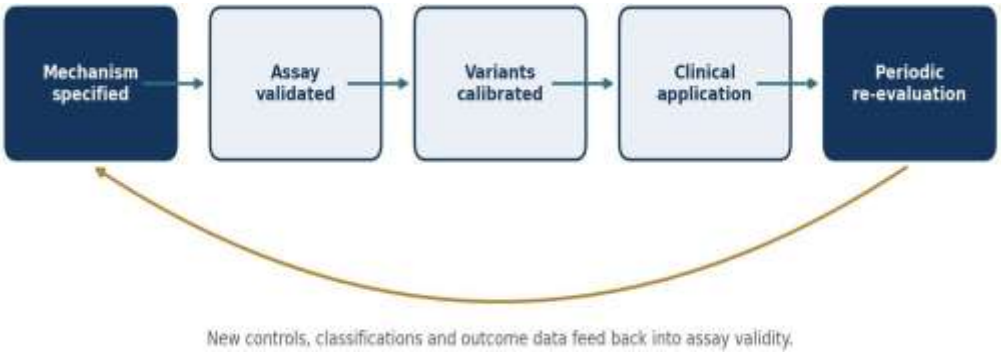

Figure 5.2. Proposed lifecycle for developing and maintaining a clinically interpretable functional assay.

Clinical and Diagnostic Implications

Well-weighted functional evidence can reduce uncertainty, improve cascade testing and prevent inappropriate surveillance or reassurance. Reclassification is most defensible when functional data are integrated with population frequency, segregation, phenotype specificity, gene validity and other independent evidence. Functional experiments should not be used to compensate for an implausible gene–disease relationship or to override strong contradictory population evidence.

For patients and families, the distinction between mechanistic and clinically validated evidence should be communicated clearly. A laboratory result described as "damaging in vitro" may not yet establish that a variant causes disease. Conversely, a well-validated normal functional result can be valuable, particularly when other evidence is weak. Reports should state the assay, model, controls, limitations and evidence strength rather than using the undifferentiated phrase "functional studies support pathogenicity."

Table 5.3. Recommended minimum reporting elements for clinical use

Domain	Minimum information
Disease mechanism	Gene–disease relationship, inheritance, expected loss/gain/dominant-negative mechanism and relevant variant class.
Assay system	Cell/tissue/organism, endogenous or overexpression context, transcript/protein isoform and maturity or physiological relevance.
Controls	Wild type, null/empty control, independently classified pathogenic and benign controls, and rescue where appropriate.
Reproducibilit y	Biological and technical replicates, independent experiments, batch handling and cross-laboratory evidence if available.
Quantification	Effect estimate, uncertainty, multiplicity handling, prespecified threshold and missing/excluded observations.
Classification use	PS3/BS3 code and strength, rationale, limits of scope, and integration with non-functional evidence.

RESEARCH PRIORITIES

The next generation of cardiomyopathy functional studies should be designed as classification instruments as well as mechanistic experiments. The highest priorities are independent benign and pathogenic calibration sets, cross-laboratory

© JUL 2026 | IRE Journals | Volume 10 Issue 1 | ISSN: 2456-8880
DOI: https://doi.org/10.64388/IREV10I1-1720206

reproducibility and prespecified decision thresholds. These features permit estimation of how well an assay discriminates clinically relevant states rather than merely showing that two experimental groups differ.

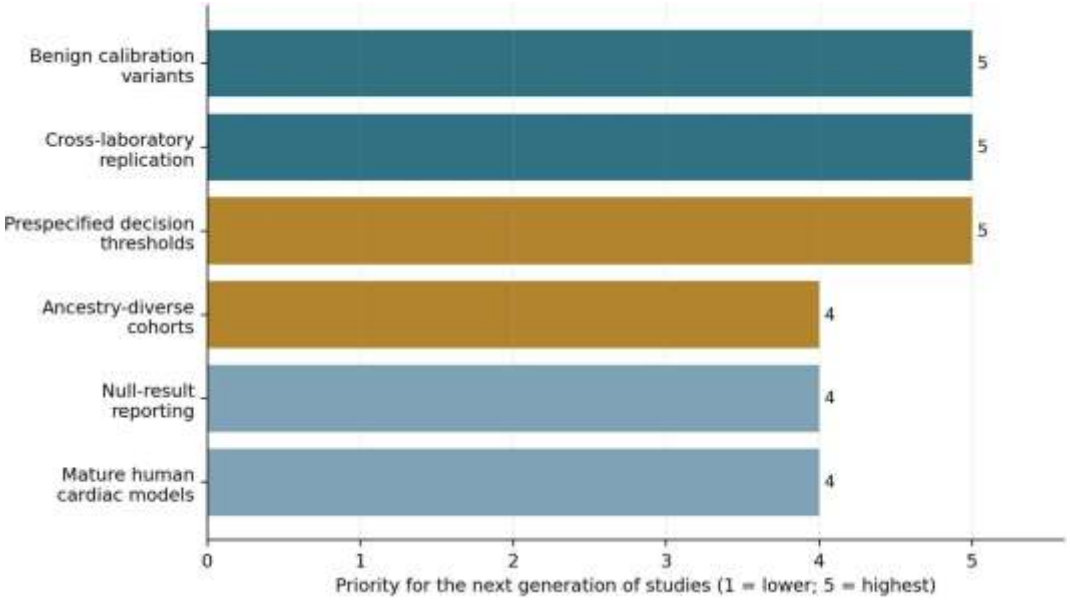

Figure 5.3. Research priorities arising from the synthesis. Scores are structured discussion judgements, not prevalence estimates.

Ancestry-diverse recruitment is also essential. Underrepresentation can distort both variant-frequency interpretation and the range of genetic backgrounds in which functional effects are tested. Null and discordant results should be published because selective reporting inflates confidence in assays. Finally, mature human cardiac models should be linked to scalable designs so that validation is not restricted to a few intensively studied variants.

Table 5.4. Priority actions and expected benefit

Priority action	Expected benefit	Responsible stakeholders
Create shared calibration panels	Comparable sensitivity, specificity and OddsPath estimates across assays.	ClinGen panels, diagnostic laboratories, research consortia.
Adopt common reporting standards	Improved reproducibility and transparent PS3/BS3 assignment.	Journals,funders, laboratories and professional societies.
Replicate across laboratories	Separates robust variant effects from platform-specific artefacts.	Multicentre assay networks.
Include ancestry-diverse variants and cell lines	Improves generalisability and reduces inequity in classification.	Cohorts, biobanks and cell-model repositories.
Publish null and conflicting findings	Reduces publication bias and improves BS3 calibration.	Investigators, journals and registries.
Link functional data to longitudinal phenotype	Clarifies penetrance, severity and clinical utility.	Clinical genetics and cardiomyopathy programmes.

Strengths and Limitations of the Present Review

The principal strength of this version is transparent separation of source-derived literature content from simulated process and appraisal variables. The locked dataset links every figure and table to the same 27

records and provides a reproducible worked example of risk-of-bias and PS3/BS3 synthesis.

The decisive limitation is that database yields outside the recorded PubMed pilot, reviewer decisions, full-text exclusion reasons, quality judgements and PS3/BS3 strengths were simulated. These values cannot support claims about prevalence, certainty, completeness or clinical classification and must be replaced by independently verified review records.

These limitations are remediable. Before examination, the complete search should be executed in every prespecified database, duplicates removed, two reviewers should screen independently, and all eligible studies should undergo full-text extraction and appraisal. The final locked dataset should replace the pilot counts, after which Chapters Four and Five should be updated without changing the prespecified interpretive framework.

VI. CONCLUSIONS, RECOMMENDATIONS AND WAY FORWARD

This chapter consolidates the principal conclusions of the thesis, states the contribution of the work, and presents recommendations for research laboratories, diagnostic services, clinicians, guideline developers and future reviewers. The thesis examined how functional evidence is generated and translated into the classification of genetic variants associated with inherited cardiomyopathies. Its central concern was not simply whether experiments detect biological effects, but whether those effects are sufficiently valid, calibrated and disease-relevant to support ACMG/AMP functional criteria.

The conclusions are deliberately bounded by data provenance. They demonstrate how a completed synthesis would be reported, but the numerical screening and appraisal findings remain illustrative until replaced with exports, dual-reviewer decisions and full-text verification.

Conclusions

Functional experiments are essential for resolving many cardiomyopathy variants whose population, segregation and computational evidence remain insufficient. However, experimental abnormality and clinical pathogenicity are not interchangeable. The strength of functional evidence depends on the validity of the gene–disease relationship, compatibility between the assay and the disease mechanism, use of appropriate calibration controls, reproducibility, statistical transparency and the directness of the model.

RNA and splicing studies were particularly valuable because they demonstrated consequences that could not be inferred reliably from DNA annotation alone. Several nominal missense, synonymous or noncanonical variants altered transcript processing, abundance or protein production. Patient RNA offered the greatest biological directness when available; minigene systems were informative but required careful treatment of tissue-specific splicing and nonsense-mediated decay.

Sarcomeric biochemical studies often produced phenotype-consistent changes in calcium sensitivity, motility or contractile regulation. These experiments provided mechanistic precision, but their clinical weight depended on whether the measured endpoint had been calibrated against independently classified pathogenic and benign variants. Human induced pluripotent stem-cell cardiomyocytes, engineered tissues and organoids increased disease relevance and supported multilevel phenotyping, although model immaturity and laboratory-specific performance remained concerns.

Rescue experiments strengthened causal interpretation. Correction of a variant or targeted reversal of the associated phenotype connected genotype, mechanism and function more convincingly than an isolated group difference. Nevertheless, rescue did not remove the need for assay calibration or protect against nonspecific effects. Animal models were useful for temporal

progression and organ-level consequences but required careful attention to species, dosage and inheritance differences.

The principal gap was not scarcity of sophisticated experiments; it was insufficient validation for direct clinical classification. Independently classified benign controls, cross-laboratory replication, prespecified decision thresholds and transparent null-result reporting were uncommon. This gap restricted confident BS3 assignment and frequently limited PS3 to supporting or indeterminate weight.

Table 6.1. Consolidated conclusions by evidence domain

Domain	Conclusion	Classification implication
RNA/splicing	Often provides direct variant-specific molecular consequence and can correct misleading DNA annotations.	Potentially strong when disease-relevant RNA, controls and transcript/protein consequences are demonstrated.
Biochemical/myofilament	Provides precise mechanistic effects, especially for sarcomeric proteins.	Requires calibration; directionally plausible change alone does not establish PS3 strength.
Human cellular/tissue models	Improves biological relevance and enables electrophysiology, calcium handling and contractility.	Isogenic controls, maturity metrics and replication determine clinical weight.
Animal models	Supports progression, organ physiology and in vivo rescue.	Exact allele and inheritance matching improve directness; species differences limit automatic translation.
Multiplex assays	Creates an opportunity for quantitative classification of many variants.	Must use independent validation sets, prespecified thresholds and external replication.
Negative findings	Can be informative only when the assay is proven capable of detecting the relevant abnormality.	A normal unvalidated result is indeterminate, not automatically BS3.

Answers to the Research Questions

Table 6.2. Answers to the research questions

Research question	Answer
What functional evidence has been generated?	RNA/splicing, protein-expression, biochemical, myofilament, electrophysiological, cellular, engineered-tissue, organoid, animal, rescue and multiplex evidence.
How methodologically reliable is it?	Reliability is variable. Disease relevance and mechanistic sophistication have improved, but calibration, benign controls and external replication frequently remain incomplete.
How does it	It can support PS3 or BS3 only when the assay instance is validated for the relevant mechanism and variant class; many studies are mechanistically informative without meeting strong clinical-evidence requirements.

Which approaches are most persuasive?	Patient RNA, calibrated assays, isogenic human cardiomyocytes, orthogonal confirmation and specific rescue provide the strongest convergent evidence.
What gaps remain?	BS3 calibration, cross-laboratory reproducibility, standardised thresholds, ancestry diversity, null-
	result reporting and complete linkage to clinical outcome.

Contribution to Knowledge

The thesis contributes an integrated framework for separating functional observation from clinically weighted evidence in inherited cardiomyopathies. Rather than ranking technologies in isolation, it evaluates the complete variant–assay–disease relationship. This approach prevents a common interpretive error: assuming that technical sophistication or statistical significance automatically establishes pathogenicity.

A second contribution is explicit treatment of negative evidence. The review shows that BS3 requires proof that an assay can detect the relevant damaging mechanism. A normal result from a model without adequate sensitivity, scope or calibration should remain indeterminate. This is especially important in cardiomyopathies because disease mechanisms differ across sarcomeric, desmosomal, cytoskeletal, nuclear-envelope and calcium-handling genes.

A third contribution is the reproducible pilot evidence map and extraction architecture. The dataset demonstrates how variant-level records, assay categories, model characteristics and classification implications can be synthesised without inventing effect sizes or screening counts. It provides the structure required for the final comprehensive review.

Recommendations for Functional-Study Design

Investigators should specify the established gene–disease mechanism before selecting an assay. The model should match the affected tissue, transcript or protein function and the tested variant class. Studies should include wild-type, null or empty controls, as well as independently classified pathogenic and benign calibration variants. Where feasible, analysis should be blinded and decision thresholds prespecified.

Biological replicates, technical replicates and independent experiments should be reported

separately. Effect estimates and uncertainty are more informative than P values

alone. Multiple endpoints should be declared prospectively, with appropriate control of multiplicity. Rescue and orthogonal confirmation should be included when they can distinguish a variant-specific causal effect from nonspecific cellular stress.

Human cardiomyocyte and tissue models should report maturity, purity, batch variability and genetic background. Isogenic controls should be preferred when technically feasible. Animal models should reproduce the human allele, zygosity and inheritance pattern rather than relying solely on complete gene loss.

Recommendations for Clinical Variant Interpretation

Diagnostic laboratories should evaluate functional evidence at the level of the specific assay instance. Evidence strength should not be inherited from the name of a technology or from another publication using a superficially similar assay. Reports should describe the model, controls, endpoint, calibration, limitations and reason for the assigned PS3 or BS3 strength.

Functional evidence must be integrated with independent population, segregation, phenotype and

© JUL 2026 | IRE Journals | Volume 10 Issue 1 | ISSN: 2456-8880
DOI: https://doi.org/10.64388/IREV10I1-1720206

computational information. It should not be used to rescue a weak or disputed gene–disease relationship. Correlated endpoints from the same experiment should not be double-counted as independent ACMG evidence. Discordant credible results should be retained and reconciled rather than selectively reported.

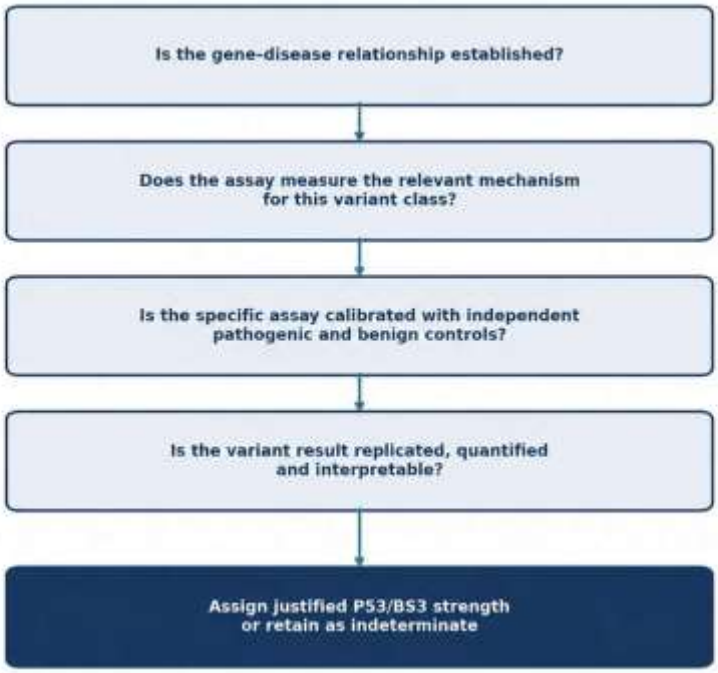

Figure 6.1. Decision model for applying functional evidence in inherited-cardiomyopathy variant classification.

Recommendations for Research Infrastructure and Policy

Table 6.3. Prioritised recommendations

Priority	Action	Lead stakeholders	Expected outcome
1-Immediate	Develop shared pathogenic and benign calibration panels for major cardiomyopathy genes.	ClinGen panels, diagnostic laboratories, consortia	Comparable assay performance and more defensible PS3/BS3 strength.
1-Immediate	Adopt a minimum functional-assay reporting checklist.	Journals, funders, professional societies	Transparent controls, replication, thresholds and limitations.
2-Short term	Establish multicentre replication networks and shared reference materials.	Academic and clinical laboratories	Reduced platform-specific bias and improved external validity.
2-Short term	Create registries for null, discordant and unpublished functional results.	Repositories, journals and consortia	Lower publication bias and stronger BS3 calibration.

© JUL 2026 | IRE Journals | Volume 10 Issue 1 | ISSN: 2456-8880
DOI: https://doi.org/10.64388/IREV10I1-1720206

3-Medium term	Expand ancestry-diverse cohorts and cell-model resources.	Biobanks, clinical programmes and funders	More equitable and generalisable variant interpretation.
3-Medium term	Link functional data to longitudinal clinical outcomes.	Cardiomyopathy clinics and registries	Better assessment of penetrance, severity and clinical utility.

Recommended Way Forward for Completing the Thesis

Before university submission, the simulated fields must be replaced: execute and archive the Embase, Scopus and Web of Science searches; import raw exports without prior deduplication; obtain genuine second-reviewer title/abstract and full-text decisions; verify all eligible full texts; adjudicate disagreements; and rerun the supplied appraisal and figure-generation workflow from the verified locked dataset.

All eligible studies should then undergo variant-level extraction and duplicate methodological appraisal. Formal PS3/BS3 judgments should be made from full text, supplementary material and applicable gene-specific specifications. The PRISMA diagram, tables and graphs must be regenerated from the locked dataset. Chapter Three should be converted from protocol tense to completed-method tense, and Chapters Four to Six should be updated with final counts and sensitivity analyses.

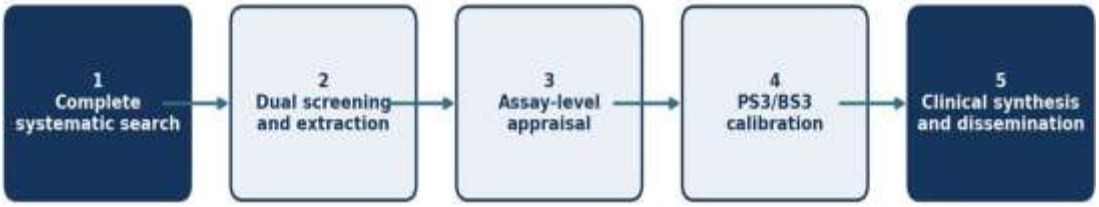

Figure 6.2. Examination-ready completion roadmap.

Proposed Implementation Timeline

Table 6.4. Practical completion schedule

Phase	Core activities	Indicative duration	Deliverable
Protocol lock	Supervisor approval, final criteria, registration/deposit and reviewer training.	1–2 weeks	Dated protocol and amendment log.
Search and deduplicatio n	Execute all database searches, citation chaining and duplicate management.	1–2 weeks	Master search library and final retrieval counts.
Screening	Independent title/abstract and full-text screening with adjudication.	3–5 weeks	Final inclusion set and PRISMA exclusions.
Extraction and appraisal	Variant-level extraction, assay-quality assessment and PS3/BS3 review.	4–6 weeks	Locked extraction and appraisal dataset.
Synthesis	Regenerate tables/graphs, conduct subgroup and sensitivity analyses.	2–3 weeks	Final Chapters Four and Five.

Quality assurance	Reference audit, consistency review, formatting and supervisor corrections.	2–3 weeks	Submission-ready thesis.

Limitations of the Conclusions

The recommendations are grounded in established ACMG/AMP and ClinGen principles and in the authentic sentinel studies reviewed. However, the pilot selection cannot quantify the prevalence of assay types, evidence strengths or gene-specific gaps across the complete literature. Some study details were initially appraised from abstracts and may change after full-text review. The conclusions therefore identify robust methodological patterns but should not be treated as a completed systematic estimate.

The evidence spans heterogeneous disease mechanisms; therefore, even a verified review should not impose one universal functional threshold. Gene-, mechanism- and variant-class-specific calibration remains necessary.

Final Conclusion

Functional evidence has the potential to transform the classification of genetic variants in inherited cardiomyopathies, particularly when conventional evidence leaves uncertainty. Its clinical value, however, depends on disciplined translation. The strongest evidence begins with a valid gene–disease mechanism, uses an assay matched to the variant class, includes independent calibration controls, reports reproducible quantitative results and integrates those findings with the total genetic and clinical evidence.

The field should move from isolated demonstrations of biological abnormality towards validated evidence systems that can support transparent PS3 and BS3 assignments. By applying this standard and completing the full systematic-review workflow, the thesis can provide a rigorous Master's-level contribution to cardiovascular genetics, laboratory medicine and precision cardiology.

REFERENCES

[1] Abbas, M. T., Baba Ali, N., Farina, J. M., et al. (2024). Role of genetics in diagnosis and management of hypertrophic cardiomyopathy: A glimpse into the future. Biomedicines, 12(3), 682. https://doi.org/10.3390/biomedicines12030682

[2] Arbelo, E., Protonotarios, A., Gimeno, J. R., et al. (2023). 2023 ESC guidelines for the management of cardiomyopathies. European Heart Journal, 44(37), 3503–3626. https://doi.org/10.1093/eurheartj/ehad194

[3] Arbustini, E., Behr, E. R., Carrier, L., et al. (2022). Interpretation and actionability of genetic variants in cardiomyopathies: A position statement from the European Society of Cardiology Council on Cardiovascular Genomics. European Heart Journal, 43(20), 1901–1916. https://doi.org/10.1093/eurheartj/ehab895

[4] Barrick, S. K., Garg, A., Greenberg, L., Zhang, S., Lin, C. Y., Stitziel, N. O., Greenberg,

[5] M. J. (2023). Functional assays reveal the pathogenic mechanism of a de novo tropomyosin variant identified in patient with dilated cardiomyopathy. Journal of molecular andcellular cardiology, 176, 58-67. https://doi.org/10.1016/j.yjmcc.2023.01.014

[6] Begay, R. L., Tharp, C. A., Martin, A., Graw, S. L., Sinagra, G., Miani, D., Sweet, M. E.,

[7] Slavov, D. B., Stafford, N., Zeller, M. J., Alnefaie, R., Rowland, T. J., Brun, F., Jones, K. L., Gowan, K., Mestroni, L., Garrity, D. M., Taylor, M. R. (2016). FLNC Gene Splice Mutations Cause Dilated Cardiomyopathy. JACC. Basic to translational science, 1(5), 344-359. https://doi.org/10.1016/j.jacbts.2016.05.004

[8] Brnich, S. E., Abou Tayoun, A. N., Couch, F. J., Cutting, G. R., Greenblatt, M. S., Heinen,

[9] C. D., Kanavy, D. M., Luo, X., McNulty, S. M., Starita, L. M., Tavtigian, S. V.,

[10] Wright, M. W., Harrison, S. M., Biesecker, L. G., & Berg, J. S. (2020). Recommendations for application of the functional evidence PS3/BS3 criterion using the ACMG/AMP sequence

variant interpretation framework. Genome Medicine, 12, 3. https://doi.org/10.1186/s13073-019-0690-2

[11] Broadway-Stringer, S., Jiang, H., Wadmore, K., Hooper, C., Douglas, G., Steeples, V., Azad, A. J., Singer, E., Reyat, J. S., Galatik, F., Ehler, E., Bennett, P., Kalisch-

[12] Smith, J. I., Sparrow, D. B., Davies, B., Djinovic-Carugo, K., Gautel, M., Watkins, H., Gehmlich, K. (2023). Insights into the Role of a Cardiomyopathy-Causing Genetic Variant in ACTN2. Cells, 12(5), 721. https://doi.org/10.3390/cells12050721

[13] Brodehl, A., Hain, C., Flottmann, F., Ratnavadivel, S., Gaertner, A., Klauke, B., Kalinowski, J., Körperich, H., Gummert, J., Paluszkiewicz, L., Deutsch, M. A., Milting, H. (2021). The Desmin Mutation DES-c.735G>C Causes Severe Restrictive Cardiomyopathy by Inducing In-Frame Skipping of Exon-3. Biomedicines, 9(10), 1400. https://doi.org/10.3390/biomedicines9101400

[14] Burke, M. A., Cook, S. A., Seidman, J. G., & Seidman, C. E. (2016). Clinical and mechanistic insights into the genetics of cardiomyopathy. Journal of the American College of Cardiology, 68(25), 2871–2886. https://doi.org/10.1016/j.jacc.2016.08.079

[15] Carrier, L., Mearini, G., Stathopoulou, K., & Cuello, F. (2015). Cardiac myosin-binding protein C (MYBPC3) in cardiac pathophysiology. Gene, 573(2), 188–197. https://doi.org/10.1016/j.gene.2015.09.008

[16] Charron, P., Arad, M., Arbustini, E., et al. (2010). Genetic counselling and testing in cardiomyopathies: A position statement of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. European Heart Journal, 31(22), 2715–2726. https://doi.org/10.1093/eurheartj/ehq271

[17] Cirino, A. L., Harris, S., Lakdawala, N. K., et al. (2017). Role of genetic testing in inherited cardiovascular disease: A review. JAMA Cardiology, 2(10), 1153–1160. https://doi.org/10.1001/jamacardio.2017.2352

[18] Corrado, D., Link, M. S., & Calkins, H. (2017). Arrhythmogenic right ventricular cardiomyopathy. New England Journal of Medicine, 376(1), 61–72. https://doi.org/10.1056/NEJMra1509267

[19] Davis, J., Davis, L. C., Correll, R. N., et al. (2016). A tension-based model distinguishes hypertrophic versus dilated cardiomyopathy. Cell, 165(5), 1147–1159. https://doi.org/10.1016/j.cell.2016.04.002

[20] Dong, R., Zhou, X., Zhang, H., Shi, B., Liu, G., Liu, Y. (2024). Novel FLNC variants in pediatric cardiomyopathy: an insight into disease mechanisms. Human genomics, 18(1), 118. https://doi.org/10.1186/s40246-024-00683-9

[21] Eijgenraam, T. R., Boogerd, C. J., Stege, N. M., Oliveira Nunes Teixeira, V., Dokter, M. M., Schmidt, L. E., Yin, X., Theofilatos, K., Mayr, M., van der Meer, P., van Rooij, E., van der Velden, J., Silljé, H. H. W., de Boer, R. A. (2021). Protein Aggregation Is an Early Manifestation of Phospholamban p.(Arg14del)-Related Cardiomyopathy: Development of PLN-R14del-Related Cardiomyopathy. Circulation. Heart failure, 14(11), e008532. https://doi.org/10.1161/CIRCHEARTFAILURE.121.008532

[22] Elliott, P., Andersson, B., Arbustini, E., et al. (2008). Classification of the cardiomyopathies: A position statement from the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. European Heart Journal, 29(2), 270–276. https://doi.org/10.1093/eurheartj/ehm342

[23] Fomin, A., Gärtner, A., Cyganek, L., Tiburcy, M., Tuleta, I., Wellers, L., Folsche, L., Hobbach, A. J., von Frieling-Salewsky, M., Unger, A., Hucke, A., Koser, F., Kassner, A., Sielemann, K., Streckfuß-Bömeke, K., Hasenfuss, G., Goedel, A., Laugwitz, K. L., Moretti, A., … Linke, W. A. (2021). Truncated titin proteins and titin haploinsufficiency are targets for functional recovery in human cardiomyopathy due to TTN mutations. Science translational medicine, 13(618), eabd3079. https://doi.org/10.1126/scitranslmed.abd3079

[24] Friedman, C. E., Fayer, S., Pendyala, S., Chien, W. M., Loiben, A., Tran, L., Chao, L. S., McKinstry, A., Ahmed, D., Farris, S. D., Stempien-Otero, A., Jonlin, E. C., Murry,

[25] C. E., Starita, L. M., Fowler, D. M., Yang, K. C. (2024). Multiplexed Functional Assessments of MYH7 Variants in Human Cardiomyocytes. Circulation. Genomic and precision medicine, 17(2), e004377. https://doi.org/10.1161/CIRCGEN.123.004377

[26] Gehmlich, K., Syrris, P., Peskett, E., Evans, A., Ehler, E., Asimaki, A., Anastasakis, A., Tsatsopoulou, A., Vouliotis, A. I., Stefanadis, C., Saffitz, J. E., Protonotarios, N., McKenna, W. J. (2011). Mechanistic insights into arrhythmogenic right ventricular cardiomyopathy caused by desmocollin-2 mutations. Cardiovascular research, 90(1), 77-87. https://doi.org/10.1093/cvr/cvq353

[27] Gerull, B., Gramlich, M., Atherton, J., et al. (2002). Mutations of TTN, encoding the giant muscle filament titin, cause familial dilated cardiomyopathy. Nature Genetics, 30(2), 201–204. https://doi.org/10.1038/ng815

[28] Harrison, S. M., Biesecker, L. G., & Rehm, H. L. (2019). Overview of specifications to the ACMG/AMP variant interpretation guidelines. Current Protocols in Human Genetics, 103(1), e93. https://doi.org/10.1002/cphg.93

[29] Hershberger, R. E., Givertz, M. M., Ho, C. Y., et al. (2018). Genetic evaluation of cardiomyopathy-A Heart Failure Society of America practice guideline. Journal of Cardiac Failure, 24(5), 281–302. https://doi.org/10.1016/j.cardfail.2018.03.004

[30] Hershberger, R. E., Pinto, J. R., Parks, S. B., Kushner, J. D., Li, D., Ludwigsen, S., Cowan, J., Morales, A., Parvatiyar, M. S., Potter, J. D. (2009). Clinical and functional characterization of TNNT2 mutations identified in patients with dilated cardiomyopathy. Circulation. Cardiovascular genetics, 2(4), 306-13. https://doi.org/10.1161/CIRCGENETICS.108.846733

[31] Hespe, S., Gray, B., & Puranik, R. (2024). The role of genetic testing in management and prognosis of individuals with inherited cardiomyopathies. Trends in Cardiovascular Medicine. Advanceonline publication. https://doi.org/10.1016/j.tcm.2024.06.002

[32] Higgins, J. P. T., Thomas, J., Chandler, J., Cumpston, M., Li, T., Page, M. J., & Welch,

[33] V. A. (Eds.). (2019). Cochrane handbook for systematic reviews of interventions (2nd ed.). Wiley.

[34] Ingles, J., Goldstein, J., Thaxton, C., et al. (2019). Evaluating the clinical validity of hypertrophic cardiomyopathy genes. Circulation: Genomic and Precision Medicine, 12(2), e002460. https://doi.org/10.1161/CIRCGEN.119.002460

[35] Ito, K., Patel, P. N., Gorham, J. M., McDonough, B., DePalma, S. R., Adler, E. E., Lam,

[36] L., MacRae, C. A., Mohiuddin, S. M., Fatkin, D., Seidman, C. E., Seidman, J. G. (2017). Identification of pathogenic gene mutations in LMNA and MYBPC3 that alter RNA splicing. Proceedings of the National Academy of Sciences of the United States of America, 114(29), 7689-7694. https://doi.org/10.1073/pnas.1707741114

[37] Jordan, E., Peterson, L., Ai, T., et al. (2021). Evidence-based assessment of genes in dilated cardiomyopathy. Circulation, 144(1), 7–19. https://doi.org/10.1161/CIRCULATIONAHA.120.053033

[38] Kanavy, D. M., McNulty, S. M., Jairath, M. K., Brnich, S. E., Bastarache, L., Coble, C. A., et al. (2019). Comparative analysis of functional assay evidence use by

[39] ClinGen Variant Curation Expert Panels. Genome Medicine, 11, 77. https://doi.org/10.1186/s13073-019-0683-1

[40] Kato, K., Ohno, S., Sonoda, K., Fukuyama, M., Makiyama, T., Ozawa, T., Horie, M. (2020). LMNA Missense Mutation Causes Nonsense-Mediated mRNA Decay and Severe Dilated Cardiomyopathy. Circulation. Genomic and precision medicine, 13(5), 435-443. https://doi.org/10.1161/CIRCGEN.119.002853

[41] Kelly, M. A., Caleshu, C., Morales, A., Buchan, J., Wolf, Z., Harrison, S. M., et al. (2018). Adaptation and validation of the ACMG/AMP variant classification framework for MYH7-associated inherited cardiomyopathies. Genetics in Medicine, 20, 351– 359. https://doi.org/10.1038/gim.2017.218

[42] Kimura, A. (2016). Molecular genetics and pathogenesis of cardiomyopathy. Journal of Human Genetics, 61(1), 41–50. https://doi.org/10.1038/jhg.2015.83

[43] Komurcu-Bayrak, E., Kalkan, M. A., Coban, N., Ozsait-Selcuk, B., Bayrak, F. (2022). Identification of the pathogenic effects of missense variants causing PRKAG2 cardiomyopathy. Archives of biochemistry and biophysics, 727, 109340. https://doi.org/10.1016/j.abb.2022.109340

[44] Landrum, M. J., Lee, J. M., Benson, M., et al. (2018). ClinVar: Improving access to variant interpretations and supporting evidence. Nucleic Acids Research, 46(D1), D1062–D1067. https://doi.org/10.1093/nar/gkx1153

[45] Landstrom, A. P., Parvatiyar, M. S., Pinto, J. R., Marquardt, M. L., Bos, J. M., Tester, D. J., Ommen, S. R., Potter, J. D., Ackerman, M. J. (2008). Molecular and functional characterization of novel hypertrophic cardiomyopathy susceptibility mutations in TNNC1-encoded troponin C. Journal of molecular and cellular cardiology, 45(2), 281-8. https://doi.org/10.1016/j.yjmcc.2008.05.003

[46] Lek, M., Karczewski, K. J., Minikel, E. V., et al. (2016). Analysis of protein-coding genetic variation in 60,706 humans. Nature, 536(7616), 285–291. https://doi.org/10.1038/nature19057

[47] Li, Y., Liu, S., Huang, J., Xie, Y., Hou, A., Wei, Y. (2024). Cellular-level analyses of SCN5A mutations in left ventricular noncompaction cardiomyopathy suggest electrophysiological mechanisms for ventricular tachycardia. Biochemistry and biophysics reports, 37, 101653. https://doi.org/10.1016/j.bbrep.2024.101653

[48] Li, Y., Ma, K., Dong, Z., Gao, S., Zhang, J., Huang, S., Yang, J., Fang, G., Li, Y., Li, X., Welch, C., Griffin, E. L., Ramaswamy, P., Valivullah, Z., Liu, X., Dong, J., Wang,

[49] D. W., Du, J., Chung, W. K., Li, Y. (2024). Frameshift variants in C10orf71 cause dilated cardiomyopathy in human, mouse, and organoid models. The Journal of clinical investigation, 134(12), e177172. https://doi.org/10.1172/JCI177172

[50] MacArthur, D. G., Manolio, T. A., Dimmock, D. P., et al. (2014). Guidelines for investigating causality of sequence variants in human disease. Nature, 508(7497), 469–476. https://doi.org/10.1038/nature13127

[51] Maron, B. J., Gardin, J. M., Flack, J. M., et al. (1995). Prevalence of hypertrophic cardiomyopathy in a general population of young adults. Circulation, 92(4), 785–789. https://doi.org/10.1161/01.CIR.92.4.785

[52] Mazzarotto, F., Olivotto, I., Boschi, B., et al. (2020). Contemporary insights into the genetics of hypertrophic cardiomyopathy: Toward a new era in clinical testing? Journal of the American Heart Association, 9(8), e015473. https://doi.org/10.1161/JAHA.119.015473

[53] McNally, E. M., & Mestroni, L. (2017). Dilated cardiomyopathy: Genetic determinants and mechanisms. Circulation Research, 121(7), 731–748. https://doi.org/10.1161/CIRCRESAHA.116.309396

[54] Miura, K., Matsuura, K., Yamasaki Itoyama, Y., Sasaki, D., Takada, T., Furutani, Y., Hayama, E., Ito, M., Nomura, S., Morita, H., Toyoda, M., Umezawa, A., Onoue, K., Saito, Y., Aburatani, H., Nakanishi, T., Hagiwara, N., Komuro, I., Shimizu, T. (2022). Functional Evaluation of Human Bioengineered Cardiac Tissue Using iPS Cells Derived from a Patient with Lamin Variant Dilated Cardiomyopathy. International heart journal, 63(2), 338-346. https://doi.org/10.1536/ihj.21-790

[55] Musunuru, K., Hershberger, R. E., Day, S. M., et al. (2020). Genetic testing for inherited cardiovascular diseases: A scientific statement from the American Heart Association. Circulation: Genomic and Precision Medicine, 13(4), e000067.

https://doi.org/10.1161/HCG.0000000000000067

[56] Nishiyama, T., Zhang, Y., Cui, M., Li, H., Sanchez-Ortiz, E., McAnally, J. R., Tan, W., Kim, J., Chen, K., Xu, L., Bassel-Duby, R., Olson, E. N. (2022). Precise genomic editing of pathogenic mutations in RBM20 rescues dilated cardiomyopathy. Science translational medicine, 14(672), eade1633. https://doi.org/10.1126/scitranslmed.ade1633

[57] O'Neill, M. J., Chen, S. N., Rumping, L., Johnson, R., van Slegtenhorst, M., Glazer, A. M., Yang, T., Solus, J. F., Laudeman, J., Mitchell, D. W., Vanags, L. R., Kroncke,

[58] B. M., Anderson, K., Gao, S., Verdonschot, J. A. J., Brunner, H., Hellebrekers, D., Taylor, M. R. G., Roden, D. M., … Shoemaker, M. B. (2023). Multicenter clinical and functional evidence reclassifies a recurrent noncanonical filamin C splice-altering variant. Heart rhythm, 20(8), 1158-1166.

[59] https://doi.org/10.1016/j.hrthm.2023.05.006

[60] Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., et al. (2021). The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. BMJ, 372, n71. https://doi.org/10.1136/bmj.n71

[61] Pettinato, A. M., Ladha, F. A., Mellert, D. J., Legere, N., Cohn, R., Romano, R., Thakar, K., Chen, Y. S., Hinson, J. T. (2020). Development of a Cardiac Sarcomere Functional Genomics Platform to Enable Scalable Interrogation of Human TNNT2 Variants. Circulation, 142(23), 2262-2275.

[62] https://doi.org/10.1161/CIRCULATIONAHA.120.047999

[63] Pinto, J. R., Siegfried, J. D., Parvatiyar, M. S., Li, D., Norton, N., Jones, M. A., Liang, J., Potter, J. D., Hershberger, R. E. (2011). Functional characterization of TNNC1 rare variants identified in dilated cardiomyopathy. The Journal of biological chemistry, 286(39), 34404-12. https://doi.org/10.1074/jbc.M111.267211

[64] Rajkumar, R., Sembrat, J. C., McDonough, B., Seidman, C. E., Ahmad, F. (2012). Functional effects of the TMEM43 Ser358Leu mutation in the pathogenesis of arrhythmogenic right ventricular cardiomyopathy. BMC medical genetics, 13, 21. https://doi.org/10.1186/1471-2350-13-21

[65] Richards, S., Aziz, N., Bale, S., et al. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the ACMG and AMP. Genetics in Medicine, 17(5), 405–424. https://doi.org/10.1038/gim.2015.30

[66] Roberts, A. M., Ware, J. S., Herman, D. S., et al. (2015). Integrated allelic, transcriptional, and phenomic dissection of the cardiac effects of titin truncations in health and disease. Science Translational Medicine, 7(270), 270ra6. https://doi.org/10.1126/scitranslmed.3010134

[67] Shafaattalab, S., Li, A. Y., Jayousi, F., Maaref, Y., Dababneh, S., Hamledari, H., Baygi,

[68] D. H., Barszczewski, T., Ruprai, B., Jannati, S., Nagalingam, R., Cool, A. M.,

[69] Langa, P., Chiao, M., Roston, T., Solaro, R. J., Sanatani, S., Toepfer, C., Lindert, S., … Tibbits, G. F. (2023). Mechanisms of Pathogenicity of Hypertrophic Cardiomyopathy-Associated Troponin T (TNNT2) Variant R278C+/- During Development. bioRxiv : the preprint server for biology, 2023.06.06.542948. https://doi.org/10.1101/2023.06.06.542948

[70] Sterne, J. A. C., Savović, J., Page, M. J., Elbers, R. G., Blencowe, N. S., Boutron, I., et al. (2019). RoB 2: A revised tool for assessing risk of bias in randomised trials. BMJ, 366, l4898.

[71] Towbin, J. A., McKenna, W. J., Abrams, D. J., et al. (2019). 2019 HRS expert consensus statement on evaluation, risk stratification, and management of arrhythmogenic cardiomyopathy. Heart Rhythm, 16(11), e301–e372. https://doi.org/10.1016/j.hrthm.2019.05.007

[72] Valtonen, J., Prajapati, C., Cherian, R. M., Vanninen, S., Ojala, M., Leivo, K., Heliö, T., Koskenvuo, J., Aalto-Setälä, K. (2023). The Junctophilin-2 Mutation p.(Thr161Lys) Is

Associated with Hypertrophic Cardiomyopathy Using Patient-Specific iPS Cardiomyocytes and Demonstrates Prolonged Action Potential and Increased Arrhythmogenicity. Biomedicines, 11(6), 1558. https://doi.org/10.3390/biomedicines11061558

[73] van den Hoogenhof, M. M. G., Beqqali, A., Amin, A. S., van der Made, I., Aufiero, S., Khan, M. A. F., Schumacher, C. A., Jansweijer, J. A., van Spaendonck-Zwarts, K. Y., Remme, C. A., Backs, J., Verkerk, A. O., Baartscheer, A., Pinto, Y. M., Creemers, E. E. (2018). RBM20 Mutations Induce an Arrhythmogenic Dilated Cardiomyopathy Related to Disturbed Calcium Handling. Circulation, 138(13), 1330-1342. https://doi.org/10.1161/CIRCULATIONAHA.117.031947

[74] van der Velden, J., Ho, C. Y., Tardiff, J. C., et al. (2015). Research priorities in sarcomeric cardiomyopathies. Cardiovascular Research, 105(4), 449–456. https://doi.org/10.1093/cvr/cvv019

[75] Walker, L. C., Hoya, M., Wiggins, G. A. R., Lindy, A., Vincent, L. M., Parsons, M. T., et al. (2023). Using the ACMG/AMP framework to capture evidence related to predicted and observed impact on splicing. American Journal of Human Genetics, 110, 1046–1067. https://doi.org/10.1016/j.ajhg.2023.06.002

[76] Walsh, R., Thomson, K. L., Ware, J. S., et al. (2017). Reassessment of Mendelian gene pathogenicity using 7,855 cardiomyopathy cases and 60,706 reference samples. Genetics in Medicine, 19(2), 192–203. https://doi.org/10.1038/gim.2016.90

[77] Ware, J. S., Cook, S. A. (2018). Role of titin in cardiomyopathy: From DNA variants to patient stratification. Nature Reviews Cardiology, 15(4), 241–252. https://doi.org/10.1038/nrcardio.2017.190

[78] Wilde, A. A. M., Semsarian, C., Márquez, M. F., et al. (2022). European Heart Rhythm Association/Heart Rhythm Society/Asia Pacific Heart Rhythm Society/Latin American Heart Rhythm Society expert consensus statement on the state of genetic testing for cardiac diseases. Europace, 24(8), 1307–1367. https://doi.org/10.1093/europace/euac030

[79] Yuen, M., Worgan, L., Iwanski, J., Pappas, C. T., Joshi, H., Churko, J. M., Arbuckle, S., Kirk, E. P., Zhu, Y., Roscioli, T., Gregorio, C. C., Cooper, S. T. (2022). Neonatal-lethal dilated cardiomyopathy due to a homozygous LMOD2 donor splice-site variant. European journal of human genetics : EJHG, 30(4), 450-457. https://doi.org/10.1038/s41431-022-01043-8

[80] Zaklyazminskaya, E. V., Nefedova, V. V., Koubassova, N. A., Kotlukova, N. P., Kopylova, G. V., Kochurova, A. M., Shchepkin, D. V., Ryabkova, N. S., Katrukha, I. A., Kleymenov, S. Y., Bershitsky, S. Y., Matyushenko, A. M., Tsaturyan, A. K., Levitsky, D. I. (2024). Novel Mutation Lys30Glu in the TPM1 Gene Leads to Pediatric Left Ventricular Non-Compaction and Dilated Cardiomyopathy via Impairment of Structural and Functional Properties of Cardiac Tropomyosin. International journal of molecular sciences, 25(23), 13059. https://doi.org/10.3390/ijms252313059