The Role of Genetics in Cardiovascular Disease: Arrhythmogenic Cardiomyopathy

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ABSTRACT

Arrhythmogenic cardiomyopathy (ACM) is a heritable cardiomyopathy characterized by frequent ventricular arrhythmias and progressive ventricular dysfunction. Risk of sudden cardiac death is elevated in ACM patients and can be the presenting symptom particularly in younger individuals and athletes. This review describes current understanding of the genetic architecture of ACM and molecular mechanisms of ACM pathogenesis. We consider an emerging threshold model for ACM inheritance in which multiple factors including pathogenic variants in known ACM genes, genetic modifiers, and environmental exposures, particularly exercise, are required to reach a threshold for disease expression. We also review best practices for integrating genetics – including recent discoveries – in caring for ACM families and emphasize the utility of genotype for both management of affected individuals and predictive testing in family members.

Introduction

Arrhythmogenic cardiomyopathy (ACM) is a rare cardiomyopathy (prevalence 1/5,000) characterized by frequent sustained ventricular arrhythmias [average 10.6%/year(1)], progressive ventricular dysfunction [deterioration in right ventricular fractional area change average 0.7%/year(2)], and a high risk of sudden cardiac death [1-2% annual mortality]). Patients typically present between ages 12-50 with symptoms associated with arrhythmias, although pediatric and elderly cases have been described(3). Pathologic features of ACM include fibrofatty myocardial replacement, apoptosis, and inflammation. When the major subform of ACM, arrhythmogenic right ventricular cardiomyopathy (ARVC), was first described(4) the authors speculated it was a developmental abnormality of the right ventricular musculature. Soon, however, evidence emerged that ARVC clustered in families and was heritable. This review focuses on current understanding of the genetic basis of ACM and the interplay of genetic and environmental factors in its pathogenesis. We also discuss how best to utilize genetics – including recent discoveries – in caring for ACM families.

ARVC, in which the right ventricle is disproportionately affected, is diagnosed per 2010 Task Force Criteria (TFC) which incorporate major and minor criteria for repolarization, depolarization, and structural abnormalities, arrhythmias, and genotype/family history(5). Biventricular and left-dominant forms, sometimes called arrhythmogenic left ventricular cardiomyopathy (ALVC), are increasingly recognized and the term arrhythmogenic cardiomyopathy has been coined to incorporate both phenotpyes. "Arrhythmogenic cardiomyopathy" has also been proposed as a term to describe a broad spectrum of both inherited and acquired cardiomyopathies that have ventricular arrhythmias as a prominent aspect of their clinical presentation(6). This expanded phenotype is beyond this scope of this review. Here we use ACM to describe ARVC, ALVC, and biventricular disease.

Genetic architecture

ACM is often characterized as a disease of the cardiac desmosome. The initial discovery that pathogenic variants (mutations) in the gene encoding plakoglobin (*JUP*) caused Naxos disease(7), a rare cardio-cutaneous autosomal recessive ACM, prompted rapid identification of pathogenic variants in each of the other cardiac desmosome genes (*PKP2*, *DSP*, *DSC2*, *DSG2)* in ACM cohorts. Cardiac desmosomes are specialized structures composed of proteins (cadherins, armadillo proteins, and plakins) responsible for adhesion of cardiomyocytes. **Table 1** summarizes genes associated with ACM, prevalence of variants among ACM cases, and genotype/phenotype associations.

Numerous pathogenic variants have now been reported in each desmosomal gene and approximately half of ACM patients have one or more desmosomal pathogenic variants(8) . Heterozygous truncating variants in *PKP2* are most common, particularly in ARVC, while cohorts that include ALVC patients are enriched for cases with *DSP* and *DSG2* variants. Pathogenic desmosomal variants are also frequently identified in patients with dilated cardiomyopathy(9), including 3.5% of cases in a recent large cohort(10)). Loss-of-function variants are prevalent and have the strongest evidence for pathogenicity (11) based on their significant over-representation in ACM cases in comparison to population databases (**Table 1**).

A minority of ACM patients have pathogenic variants in non-desmosomal genes. These include founder variants in transmembrane protein 43 (*TMEM43* p.S358L) common in Newfoundland(12) and phospholamban (*PLN* p.R14del) predominantly found in the Netherlands(13). Variants in two area composita genes, cadherin-2 (*CDH2*) and α T-catenin (*CTNNA3)* have been reported in several ACM families with confirmation in larger cohorts underway. Evidence is building that truncating variants in Filamin C (*FLNC*) cause a highly arrhythmogenic ALVC (14,15). During the past year three novel ACM genes have been proposed. Variants in *TJP1* which encodes tight junction protein 1 were identified via

exome sequencing of an ACM family. Additional *TJP1* variants were subsequently detected in a several

patients in a multinational patient cohort(16). *ANK2* (ankyrin-B) was proposed as an ACM gene based

on identification of a rare variant segregating in a large family, confirmation of rare *ANK2* variants in

numerous ACM cohorts, and recapitulation of the phenotype in a mouse model(17). Finally, Poloni et al

identified a nonsense variant segregating in a family in a candidate gene, *TP63* which encodes p63

protein, a member of the p53 family of transcription factors previously associated with ectodermal

dysplasias(18).

Variants in genes associated with other cardiomyopathies and arrhythmia syndromes including

desmin (*DES*), titin (*TTN*), lamin A/C (*LMNA*), the ryanodine receptor (*RYR2*), *TGFβ3*, $Na_v1.5$ (*SCN5A*), and

several sarcomere genes have also been reported in ACM patients(3). However, some of these

associations may have been erroneous and based on initial underappreciation of the prevalence of rare

variants in the general population. A thorough review and classification of ACM genes is underway by

the ARVC Gene Curation Expert Panel under the auspices of ClinGen. ClinGen is a National Institutes of

Health-funded central resource that defines the clinical relevance of genes and variants(19).

Finally, many ACM patients have no detectable pathogenic variant – 36% of 501 probands in a

recent multinational cohort(8). These gene elusive patients may harbor pathogenic variants not yet

identified; possibly private mutations in genes not yet associated with ACM or variants in non-coding

regions of known ACM genes. Other patients may have variants identified but classified as variants of

uncertain significance. Unidentified variants or potentially pathogenic variants awaiting resolution are

most likely when the pedigree suggests familial disease. Most gene elusive patients have isolated

disease suggesting oligogenic or multifactorial inheritance(20) .

Molecular mechanisms

These genetic discoveries have greatly informed investigation of the molecular mechanisms of ACM. Nonetheless our understanding remains incomplete with proposed disease mechanisms (summarized in **Figure 1**) addressing only parts of the phenotype.

Recognition that desmosomal variants caused ACM led to an initial "desmosomal model" which speculated that impaired desmosomes responded to mechanical stress with cardiomyocyte detachment, death, and fibrofatty replacement(21). Cardiac specimens from ACM patients(22) and animal models(23)indeed show ultrastructural abnormalities of desmosome and the intercalated discs (ID). However evidence soon suggested the mechanism was more complex than mechanical failure.

Studies demonstrate that disruption of interactions between components of the ID can lead to the structural and electrical abnormalities recognized in ACM. A hallmark of ACM is redistribution of plakoglobin from the cell junctions to intracellular and nuclear pools(24). This pattern has been seen in cardiac tissue from ACM patients with and without desmosomal variants including in patients with Carvajal syndrome, and in *TMEM43* and *PLN*(13) variant carriers. Gap junction remodeling with reduced connexin 43 expression is thought to occur early in ACM development(24). Ion channel remodeling has been observed with hiPSC-CMs from ACM patients showing reduced immunoreactive signal for $Na_v1.5$(25) and a haploinsufficient plakophilin-2 murine model displaying abnormal sodium current(26). Abnormal calcium handling has also been implicated with studies showing altered regulation of transcriptional networks associated with calcium cycling in both human and murine myocardial samples(27,28)

Dysregulation of the Wnt and Hippo pathways are thought to contribute to cardiomyocyte death and adipogenesis in ACM(29). When plakoglobin translocates from desmosomes to the nucleus it competes with β-catenin for binding to the Tcf/Lef1 transcription factors leading to suppression of the Wnt pathway and an increase in adipogenesis(30). β-catenin cellular pools are modulated by glycogen

synthase kinase-3 beta (GSK3β), a suppressor of the Wnt signaling pathway. In a murine Dsg2 model,

inhibition of GSK3β reversed the abnormal remodeling and prevented cardiac dysfunction(31) The

Hippo/YAP pathway is also involved as activation of this pathway leads to phosphorylation and

inactivation of the Yes-associated protein (YAP), a transcription factor which interacts with β-catenin to

drive gene expression in the Wnt pathway(32). Dysregulation of microRNAs, an emerging area of ACM

research, have also been implicated in adipogenesis(33).

Finally, the role of inflammation in ACM pathogenesis is unresolved. Campuzano and colleagues

found that in 36 post mortem ACM hearts inflammatory infiltrates of T-lymphocytes were present in

those with severe forms of the disease with biventricular involvement but only in half of cases overall

(34). Autoimmunity has been recently proposed as a mechanism with autoantibodies to the cardiac

desmoglein-2 protein detected in the sera of 12/12 definite and 7/8 borderline ACM patients((35). The

authors propose that disruption of the desmosome results in release of masked epitopes of the

desmoglein-2 protein which stimulates an autoimmune response.

Inheritance

Inheritance of ACM is classically considered autosomal dominant with age-related, reduced

penetrance and variable expressivity. Some family pedigrees are consistent with this pattern. Others

are reminiscent of autosomal recessive inheritance. Naxos disease and Carvajal syndrome,

cardiocutaneous conditions that led to the identification of *JUP* and *DSP* respectively, are autosomal

recessive conditions. However, threads of evidence have emerged suggesting that many cases of ACM

are oligogenic or even multifactorial with both genomic and environmental factors contributing to

pathogenesis. The evidence supporting this contention is reviewed next.

Less prevalent disease in relatives of gene elusive ACM probands

Most ACM probands with familial disease have a detectable pathogenic variant (89% in a US / Dutch cohort(20) while isolated ACM cases are disproportionately gene elusive(36). In a study of cascade screening (37), first degree relatives in gene elusive families were less likely to meet TFC and six-fold less likely to experience a sustained ventricular arrhythmia than at-risk relatives in mutation positive families. Because only gene positive relatives in gene positive families were included while all first degree relatives were included in gene elusive families, a 2:1 gene positive: gene elusive ratio in phenotypic expression would be expected assuming autosomal dominant inheritance. The six-fold difference suggests that while a significant portion of gene elusive probands may harbor a yet undiscovered pathogenic variant gene elusive ACM on average is less heritable.

Reduced/incomplete penetrance and variable expressivity

Even among ACM families segregating a pathogenic desmosomal variant penetrance is substantially less than 100%. Quarta et al(38) found 34% of genotype-positive first-degree relatives had a definite diagnosis of ARVC and 27% a borderline diagnosis. Groeneweg et al reported 40% of 385 family members with a pathogenic variant detected via cascade screening met TFC(20). Similarly, Chivulescu et al recently found that 41% of genotype positive relatives met TFC and an additional 17% met one minor diagnostic criterion(2) . These estimates from ACM research centers likely represent a ceiling of penetrance. In contrast, a recent study(39) of a general clinical population showed 0.23% harbored a loss of function desmosomal variant. These patients had extremely low ACM penetrance (estimated at 6%) and were no more likely than age and sex matched controls to have ECG or echocardiography findings that met TFC. Another study showed substantial haplotype sharing in a multinational (Dutch, German, US) cohort of ACM families with *PKP2* variants indicating these variants are ancient founders and have persisted in the population for centuries (8). Given ACM has a high risk of sudden cardiac death beginning at puberty it would be anticipated that these variants would impair reproductive fitness and be gradually eliminated from the population (natural selection). Their

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persistence suggests the population penetrance and/or pathogenicity of ACM variants may be lower than expected with other factors playing a critical role in disease expression.

Genetic and environmental modifiers

Finally, both genetic and environmental modifiers are beginning to be identified. While initial high prevalence estimates (up to 21%) are artifacts of incorrect adjudication of missense variants, a sizable proportion (2-6%) of mutation positive ACM patients has a second pathogenic variant((20)). This is particularly common among ACM patients with *DSG2* and *DSC2* variants(40). Furthermore, there is strong evidence that exercise contributes to ACM pathogenesis. High intensity endurance exercise has been consistently shown to promote ventricular arrhythmias and worsen structural disease(41,42). Among carriers of desmosomal variants exercise increases penetrance and risk of incident arrhythmias(43). A recent study suggested exercise intensity predicted life-threatening ventricular arrhythmia independent of exercise duration(41). Studies have shown that gene elusive ACM patients with no family history have done the highest levels of exercise suggesting these patients may have a largely acquired ACM(44). It is unlikely, however, these patients have an entirely acquired condition. There are many high level endurance athletes and ACM is relatedly rare. The role of other environmental factors, many known to impact outcomes of other cardiovascular conditions including infectious burden, hypertension, and chronic pulmonary disease remain unknown but are theoretically likely to also influence ACM patient outcomes. Taken together this evidence strongly suggests a threshold model of ACM pathogenesis in which multiple hits, both environmental and genetic, are required for disease expression (**Figure 2**).

Genetics in the clinic

While ACM modifiers and mechanisms remain incompletely defined, genetic information is useful today in the management of ACM families. The next section reviews approaches to integrating

genetic information into ACM management. **Table 2** summarizes main points to be considered for genetic testing in a proband and subsequent cascade genetic testing of the family. A multidisciplinary team approach including cardiology, pathology, genetics, and genetic counseling expertise is optimal and patient-preferred(45) .

Diagnosis

Genetic testing is recommended for patients with ACM(6) Identification of a pathogenic desmosomal variant constitutes a major family history criterion toward diagnosis in the 2010 TFC. However, care must be taken in phenotyping, genetic test selection, and interpretation of genetic test results.

Patient selection: In a patient presenting with ventricular arrhythmias and structural abnormalities consistent with ACM a three generation pedigree should first be constructed. If a family has multiple affected members, the individual with the youngest presentation and/or most severe disease should ideally be tested first to the maximize detection of families in which more than one pathogenic variant is segregating. Genetic testing for individuals with a low probability of ACM is not advisable as any variant detected is likely to be of uncertain significance(45) .

Test selection: Genetic testing ranges from small disease-specific panels to exome and genome sequencing. The recently published Heart Rhythm Society Clinical Practice Recommendations for ACM(6) suggest a minimum list of genes to be included for ACM genetic testing and summarizes strengths and weaknesses of sequencing technologies. It is critical that the test chosen can detect copy number variants as medium to large deletions are relatively common in desmosomal genes (4% in a recent publication)(8). Currently most clinical genetic testing for ACM uses next generation sequencing panels. With increasing numbers of genes sequenced, the probability of detecting a variant of uncertain

significance increases much more rapidly that the probability of identifying a disease-causing variant (**Figure 3**).

Interpretation of results: The likelihood that a variant detected is associated with a patient's disease requires interpretation of 1) clinical characteristics of the patient, 2) strength of the gene:disease association, and 3) pathogenicity of the variant. Variant classification is based on standards set forth by the American College of Medical Genetics and Genomics / Association of Molecular Pathology(46). Class 4 and 5 variants (likely pathogenic and pathogenic) are those with sufficient evidence to guide clinical care. Strength of the association of the variant with ACM and the patient's phenotype influences interpretation. For example, detection of a pathogenic variant in *KCNH2* is unlikely to explain the phenotype of a patient with definite ACM who has significant structural disease but might for a patient with isolated arrhythmias. Reinterpretation of ACM variants should be done periodically as variant classification is evolving(45). In 2019 three professional position statements were published addressing variant reinterpretation and patient recontact in both the research and clinical setting(47-49) although more research to refine and standardize this process is needed.

Prognosis and management

Broadly, ACM patients who have a pathogenic variant have little difference in disease course from gene elusive cases(20). Nonetheless, knowledge of genotype can inform ACM management **(Table 1)**. ACM patients with multiple pathogenic variants have worse arrhythmia and heart failure outcomes(50,51). Patients with variants in *PLN* and *DSP* are particularly likely to have ALVC with the associated risk of heart failure(50). In patients initially diagnosed with dilated cardiomyopathy, detection of a desmosomal variant heralds a worse arrhythmic course and increased risk of sudden death independent of left ventricular ejection fraction(10). Finally, the Newfoundland *TMEM43* S358L founder variant is associated with very high penetrance and arrhythmic risk in males(12) while among *PLN* R14del carriers women have worse outcomes(13).

Cascade testing

Genetic testing is particularly useful in guiding cardiovascular screening and lifestyle management of relatives. After diagnosis of ACM in a proband initial cardiovascular screening is recommended for all first degree relatives age 10 or older including a 12-lead ECG, ambulatory ECG, and cardiac imaging(6). In families with a pathogenic variant, cascade genetic testing can identify which relatives require intensive longitudinal screening – suggested every 1-3 years depending on age, activity level, and phenotype(52,53). ACM-associated variants are rarely de novo(8). Therefore, not only children, but also parents, siblings, and grandparents are likely to be identified as variant carriers. Cascade genetic testing should be offered in the context of genetic counseling that includes discussion of medical, social, lifestyle, and insurance implications(6).

Endurance exercise is associated with penetrance and incident arrhythmias in family members with desmosomal variants(43). Decision-making for exercise participation for these patients is complex. In ACM there is the rare opportunity to reduce phenotypic expression of a genetic disease by avoiding frequent high-intensity endurance exercise. On the other hand, the indisputable benefits of exercise means that there are important tradeoffs. No exercise restriction is warranted in gene-negative members of these gene positive families(6), which can be a significant social and emotional benefit of genotyping for these individuals.

Molecular autopsy

In up to half of probands, ACM presents with sudden cardiac death. Genetic testing is recommended for ACM cases diagnosed post-mortem(6). The molecular autopsy is particularly useful for detecting ACM in decedents with limited structural disease. Recent cases series have reported ACM-associated variants in sudden cardiac death victims with reportedly normal cardiac structure(54).

ACM variants in the general population

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The increasing use of genetic sequencing in clinical medicine, research, and direct-to-consumer genetic testing is creating scenarios in which ACM-associated genetic variants are identified in people with neither ACM symptoms nor known family history. Recommendations for managing secondary genomic findings include reporting variants in *PKP2*, *DSG2*, *DSC2*, *DSP*, and *TMEM43*(55). Initial evidence suggests disease-risk in such individuals is substantially lower than in ACM families(39). We recently published a proposal for clinical surveillance in these cases that advocates a conservative approach as data emerges(56).

Future Directions

Although genes accounting for most familial ACM have been identified expanded sequencing efforts and new analytic approaches are likely to continue to identify rare or family-specific variants. Bioinformatic approaches that leverage population sequence data have promise to resolve variants in known ACM genes currently classified as VUSs. For instance, investigators recently identified gene regions in which non-truncating variants are significantly clustered in hypertrophic cardiomyopathy cases compared to controls resulting in an estimated 14-20% increase in cases with actionable variants(57). Meanwhile insights into the molecular basis of how desmosomal variants lead to ACM are expected to continue to inform drug development, clinical trials, and molecular diagnostic techniques(3).

Guidelines for cascade screening and early disease detection in ACM families are based on the presumption of autosomal dominant inheritance with age-related penetrance(6). Future research to better define genetic and environmental modifiers has promise to personalize risk prediction and screening. Multinational genome-wide association studies (GWAS) are actively being planned to identify genomic modifiers. For patients already diagnosed with ACM, assessing whether genotype can be integrated into recently developed arrhythmia risk stratification algorithms(58) may further increase

utility of genotype data in patient management. Simultaneously efforts to refine understanding of the

role of exercise including whether exercise guidance can be personalized by genotype are underway.

Conclusions

The past decade and a half of research has led to a solid understanding of the genetic

architecture, molecular mechanisms, and inheritance of ACM that can be used to inform care of ACM

patients and families (**Summary Figure**). Research underway now is expected to further refine the utility

of genetic data in caring for families with ACM.

Disclosures

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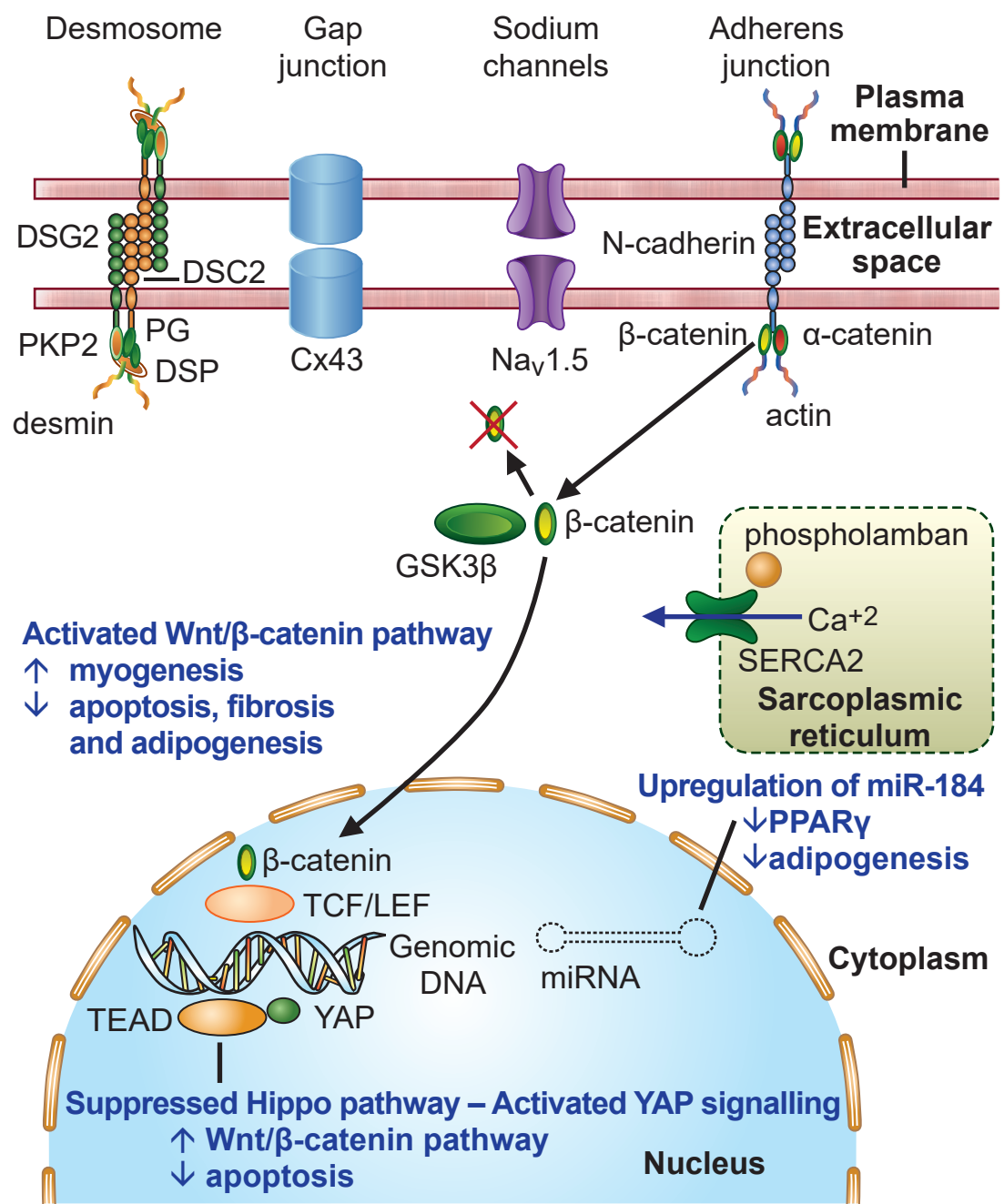
Figure Legends

Figure 1. Schematic representation of the area composita at the intercalated disc between neighboring (A) healthy and (B) ACM cardiomyocytes. Proposed ACM disease mechanisms are shown. *Abbreviations*: SERCA2, sarcoplasmic/endoplasmic reticulum calcium ATPase 2; TCF/LEF, T cell/lymphoid-enhancing transcription factors; TEAD, transcriptional enhancer factor TEF.

Figure 2. Threshold model of ACM pathogenesis highlighting impact of the interplay of genetic and environmental factors on inheritance pattern.

Figure 3. Predicted genetic test result in a proband with definite ACM. The probability of detecting a variant of uncertain significance increases more rapidly that the probability of identifying a disease-causing variant with increasing numbers of genes sequenced. *Abbreviations*: VUS; variant of uncertain significance; P/LP: pathogenic or likely pathogenic.

Summary Figure. Genetics of arrhythmogenic cardiomyopathy: From genes to patient care

A. Normal

Desmosome   Gap junction   Sodium channels   Adherens junction   **Plasma membrane**

DSG2

DSC2

Extracellular space

N-cadherin

PKP2   PG

DSP

Cx43   Na$_V$1.5   β-catenin   α-catenin

desmin

actin

GSK3β   β-catenin

phospholamban

Activated Wnt/β-catenin pathway
↑ myogenesis
↓ apoptosis, fibrosis and adipogenesis

Ca^{+2}
SERCA2
Sarcoplasmic reticulum

Upregulation of miR-184
↓PPARγ
↓adipogenesis

β-catenin
TCF/LEF
Genomic DNA
miRNA
Cytoplasm

TEAD   YAP

Suppressed Hippo pathway – Activated YAP signalling
↑ Wnt/β-catenin pathway
↓ apoptosis

Nucleus

B. ACM

Disruption of adherens junction

Autoimmunity to DSG2

PG

Disruption of desmosome

Gap junction and sodium channel remodelling

GSK3β

GSK3β

Supressed Wnt/β-catenin pathway
↓ myogenesis
↑ apoptosis, fibrosis and adipogenesis

↑phospholamban

? Ca^{+2}

Dysregulation of calcium handling

Downregulation of miR-184
↑PPARγ
↑adipogenesis

PG   β-catenin

P

Activated Hippo pathway – Supressed YAP signalling
↓ Wnt/β-catenin pathway
↑ apoptosis

Mendelian

↓

Oligogenic

↓

Multifactorial

Autosomal recessive ACM
(Carvajal, Naxos, other)

Desmosomal ACM

Familial gene elusive ACM
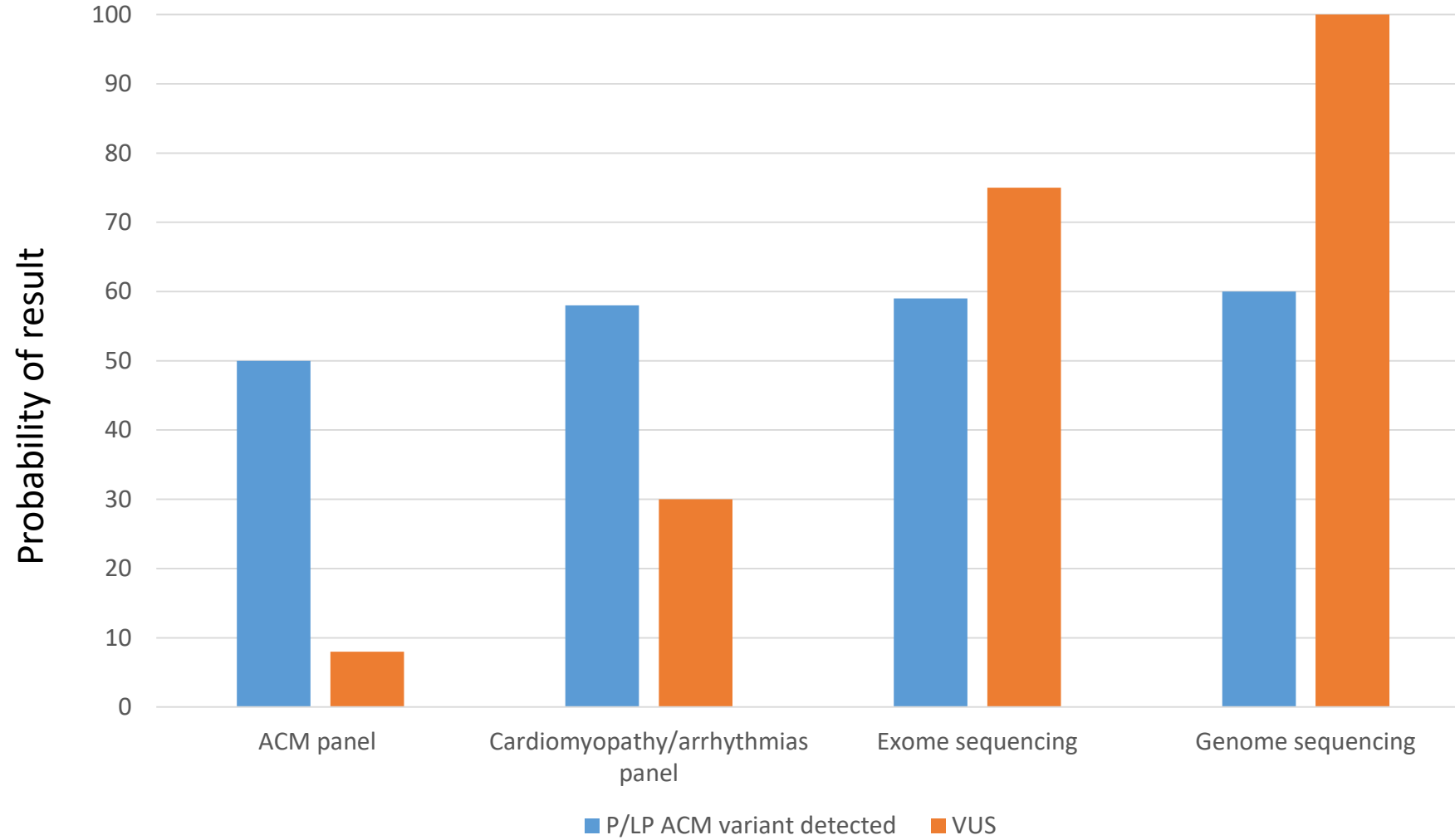
Isolated gene elusive ACM

**Threshold for
ACM onset**

Major ACM genes

Genetic Modifiers (multiple genes
with small individual effects)

Exercise

Predicted genetic test result in a proband with definite ACM

Genetics of Arrhythmogenic Cardiomyopathy (ACM)

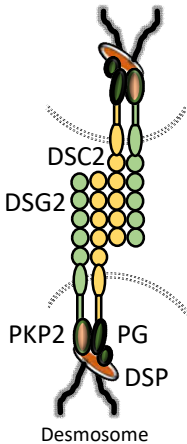## genes

encode desmosomal proteins

PKP2: 20-46%

DSP: 3-20%

DSG2: 3-20%

DSC2: 1-15%

JUP: 0-1%

DSC2
DSG2
PKP2 PG
DSP

Desmosome

Other ACM genes include:
DES, SCN5A, PLN, TTN, TMEM43, LMNA, FLNC

molecular mechanisms

Disease mechanisms

➤ Remodelling of the intercalated disc:

- desmosome
- gap junctions
- Na$^+$ channels
- adherens junctions

➤ Dysregulation of signalling pathways:

- Wnt/β-catenin pathway
- Hippo pathway

➤ Disruption of calcium homeostasis

➤ Downregulation of miR-184

➤ Autoimmunity to desmoglein-2

inheritance

Genetic and environmental factors	Inheritance pattern		
	Mendelian	Oligogenic	Multifactorial
Mutation in major ACM genes	■		
Genetic modifiers			
Exercise			■

+ → -

genetics in the clinic

Index ACM case: Full cardiovascular phenotyping

↓

Genetic counseling and sequencing of ACM genes (panel)

↓

Pathogenic or likely pathogenic variant

↓

Genetic counseling and cascade genetic testing 1st degree relatives (targeted sequencing)

↓

clinical evaluation in carriers

↓

inform about exercise; determine follow-up